An oxazolidinone resistance mechanism (Cfr) was recently described in human isolates of Staphylococcus (18). Cfr causes posttranscriptional methylation of the 23S rRNA (A2503), affecting drugs belonging to several antimicrobial classes (10). cfr-carrying isolates recovered from human clinical specimens are still rare (4, 6); however, cases were reported in the United States (12), Colombia (18), and Spain (15). Here, we report the first cases of human clinical infections caused by Cfr-producing Staphylococcus species in Mexico and demonstrate evidence of interspecies cfr mobilization.

Three linezolid-resistant (MIC, 32 μg/ml) Staphylococcus isolates were submitted to a central monitoring laboratory (JMI Laboratories) as part of the SENTRY Antimicrobial Surveillance Program in 2009. These strains were collected from hospitalized patients at the Hospital Civil de Guadalajara. Staphylococcus cohnii (10842A) was found in a blood culture (August 2009) from a 30-year-old man admitted with multiple trauma. Staphylococcus epidermidis (12898A) was also recovered in blood (October 2009) from a 50-year-old female with bacteremia who was admitted with a diagnosis of Guillain-Barre syndrome. Both isolates were cultured within 48 h after patients had developed clinical signs of sepsis (i.e., systemic inflammatory response syndrome [SIRS]). The third organism was an S. epidermidis isolate (5873X) cultured (October 2009) from abdominal fluid in a 36-year-old male presenting with multiple trauma.

Bacterial identification was confirmed by 16S rDNA sequencing (3). Isolates were tested for susceptibility by the reference broth microdilution method (1). MIC interpretations were performed based on Clinical and Laboratory Standards Institute criteria (2), except for retapamulin MIC values (19). Quality control strains included Staphylococcus aureus ATCC 29213 and Enterococcus faecalis ATCC 29212 (2). Isolates were screened for cfr and mutations in the 23S rDNA as described previously (12). L3- and L4-encoding genes were PCR amplified (13), amplicons were sequenced on both strands, and putative proteins were compared with those from linezolid-susceptible S. epidermidis ATCC 12228 and S. cohnii ATCC 29974. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing were performed on S. epidermidis isolates (11, 14). After extraction (plasmid DNA minikit; Qiagen GmbH, Hilden, Germany), plasmid DNAs were digested (HindIII and XbaI), separated on a 1% agarose gel, and transferred onto a nylon membrane by Southern blotting (17). Membranes were hybridized using a cfr-specific probe (Roche Diagnostics GmbH, Mannheim, Germany).

Linezolid-resistant isolates had their identifications confirmed as S. epidermidis isolates (12898A and 5873X) and S. cohnii (isolate 10842A). Isolates were oxacillin resistant (MIC, >2 μg/ml) and exhibited elevated MICs for linezolid (32 μg/ml), quinupristin-dalfopristin (1 to 4 μg/ml), retapamulin (≥8 μg/ml), chloramphenicol (16 to 32 μg/ml), and clindamycin (≥64 μg/ml) (Table 1). Isolates were susceptible to tetracycline, tigecycline, daptomycin, and glycopeptides.

All strains were PCR positive for cfr and wild type for 23S rRNA and L4, except for S. cohnii, which showed L4 substitutions (Asn205Ser, Ala133Thr, and Val155Ile) (Table 2). L3 Ser158Tyr, Asp159Tyr, and Leu101Val mutations were noted in both S. epidermidis isolates, while Ser158Phe and Asp159Tyr were observed in S. cohnii. The L3 Leu101Val substitution was previously noted in a linezolid-susceptible clinical isolate (data on file, JMI Laboratories). However, Gly155 and Ala157 were previously implicated in disturbing linezolid binding (8, 9). Thus, due to the proximity of these amino acid substitutions to those found in this study, the L3 mutations coupled with cfr may act synergistically and possibly contribute to the elevated linezolid MIC results. An Asn158Ser mutation in L4 was previously detected in a linezolid-susceptible S. epidermidis strain (20). Therefore, since Val155Ile is close to Asn158 and the alterations found in L4 are not within a conserved region, they likely do not represent resistance mutations; however, additional experiments are needed.

The S. epidermidis isolates (12898A and 5873X) displayed...
identical PFGE profiles (data not shown) and were sequence type 23 (ST-23). S. epidermidis strains also demonstrated identical plasmid patterns, which were different from that of 10842A (Fig. 1). All strains showed similar hybridization signal patterns, suggesting similar cfr genetic contexts. These results indicate that recombination events may have mobilized the cfr gene into different plasmids, which were later acquired by these species, as previously proposed (7). However, additional experiments are required to determine the genetic elements responsible for these events.

Cfr-encoding genes are still rare among human isolates (4, 6), and the relevance of coagulase-negative staphylococci (CoNS) isolates in clinical specimens can be difficult to assess (5). Nevertheless, the CoNS role as pathogens has increasingly been recognized, especially among immunocompromised patients, with indwelling or implanted foreign bodies (5, 16). S. cohnii (10842A) and S. epidermidis (12898A) were cultured from the blood of patients associated with SIRS and were therefore considered clinically relevant. In addition, these staphylococcal species may, at the least, act as a reservoir for resistance gene determinants in this nosocomial environment.

FIG. 1. Plasmid profile analysis of cfr-carrying strains. (A) Lane λ represents 1-kb DNA ladder used as negative-control plasmid DNA (New England Biolabs, Ipswich, MA). Lanes 1, 2, and 3 represent S. cohnii 10842A, S. epidermidis 12898A, and S. epidermidis 5738X, respectively. Band patterns of uncut and HindIII- and XbaI-digested plasmid preparations. (B) Hybridization profiles with a cfr-specific probe. Horizontal arrows indicate hybridization signals. Lanes are as defined for panel A.

**REFERENCES**


