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First Report of *cfr*-carrying Staphylococcal Clinical Isolates in Mexico and Evidence of in vivo *cfr* Mobilization

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SENTRY ANTIMICROBIAL SURVEILLANCE

Abstract

Background: A recent oxazolidinone resistance mechanism, Cfr, was identified in staphylococci. We report the first cases of human clinical infections caused by Cfr-producing coagulase-negative staphylococci (CoNS) in Mexico and demonstrate evidences of interspecies *cfr* mobilization.

Methods: Three linezolid-resistant (MIC, 32 μg/mL) CoNS were recovered from patients at the Hospital Civil de Guadalajara (2009). Identification was confirmed by 16S rRNA. Isolates were tested for susceptibility by CLSI broth microdilution (M07-A8) and interpretive criteria (M100-S20). Strains were screened for *cfr* and mutations in the 23S rRNA-, L3- and L4-encoding genes. Clonality was assessed by PFGE and MLST. Gene location was performed by Southern blot and hybridization.

Results: *S. epidermidis* 12898A and *S. cohnii* 10842A were blood cultured and associated with sepsis, while *S. epidermidis* 5873X was recovered from abdominal fluid. Isolates were oxacillin-resistant (MIC, >2 μg/mL) and exhibited elevated MIC results for quinupristin/dalfopristin (1 – 4 μg/mL), retapamulin (≥8 μg/mL), chloramphenicol (16 – 32 μg/mL) and clindamycin (>64 μg/mL). Overall, isolates were susceptible only to tetracycline, tigecycline, daptomycin and glycopeptides. All strains were PCR-positive for *cfr* and wild-type for 23S rRNA and L4. L3 Ser158Tyr or Phe and Asp159Tyr mutations were detected. *S. epidermidis* strains showed identical PFGE (ST-23) and plasmid band profiles. This plasmid band profile was different from that of *S. cohnii*; however, all strains showed identical hybridization signal patterns.

Conclusions: Cfr-producing strains had L3 mutations adjacent to those (Gly155 and Ala157) previously associated with linezolid resistance. Identical hybridization profiles indicate that recombination events appear to have mobilized *cfr* and emphasize its potential for strain-to-strain dissemination.

Introduction

Linezolid is the only oxazolidinone approved for the treatment of complicated skin and skin-structure infections (cSSSI) and nosocomial pneumonia caused by Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Linezolid inhibits protein synthesis by interfering with the formation of the 70S initiation complex. Although, linezolid resistance remains rare, sporadic staphylococci and enterococci non-susceptible isolates have been detected and usually associated with mutations in 23S rRNA, mostly G2576T.

More recently, an oxazolidinone resistance mechanism was identified in staphylococci. This gene, named *cfr* (chloramphenicol-florfenicol resistance) encodes a protein that causes post-transcriptional methylation of 23S rRNA (in the position A2503) affecting drugs belonging to several antimicrobial classes, including phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A; so-called phLOPS$_A$ phenotype. *cfr*-carrying isolates recovered from human clinical specimens are still rare; however, cases were described in the USA, Colombia, Spain and Italy.

Here, we report the first cases of human clinical infections caused by Cfr-producing *Staphylococcus* spp. in Mexico and demonstrate evidences of interspecies *cfr* mobilization.

Methods

Bacterial isolates: Three coagulase-negative staphylococci (CoNS) isolates recovered from hospitalized patients at the Hospital Civil de Guadalajara during 2009 were submitted to a central monitoring laboratory (JMI Laboratories, North Liberty, Iowa, USA) as part of the SENTRY Antimicrobial Surveillance Program. Species identification was confirmed by 16S rRNA sequencing.

Susceptibility testing: Isolates were susceptibility tested against more than 25 antimicrobials by broth microdilution procedure described by the Clinical and Laboratory Standards Institute (CLSI; M07-A8, 2009) using validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Validation of the minimum inhibitory concentration (MIC) values was performed by concurrent testing of CLSI-recommended (M100-S20, 2010) quality control (QC) strains: *Enterococcus faecalis* ATCC 29212 and *S. aureus* ATCC 29213. Interpretation of MIC results was in accordance with published CLSI (M100-S20), except for retapamulin (Traczewski *et al.* [2008]).

Detection of linezolid-resistance mechanisms: Isolates were screened for *cfr* and mutations in the 23S rRNA-, L3- and L4-encoding genes by PCR. Amplicons were sequenced on both strands and putative proteins compared with those from linezolid-susceptible *Staphylococcus epidermidis* ATCC 12228 (GenBank accession number, AE015929) and *Staphylococcus cohnii* ATCC 29974.

Molecular typing: Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were performed on *S. epidermidis* isolates. Genomic DNA was prepared in agarose blocks and digested with Sma I (New England Biolabs; Beverly, Massachusetts, USA). Electrophoresis was performed on the CHEF-DR II (BioRad, Richmond, California, USA). Multilocus sequence typing (MLST) was carried out as described elsewhere.

Plasmid and hybridization analysis: After extraction (Plasmid DNA MIDI Kit; Qiagen GmbH, Hilden, Germany), plasmid DNA were digested (Hind III and XbaI), separated on 1% agarose gel and transferred onto a nylon membrane by Southern blot. Membranes were hybridized using a *cfr*-specific probe (Roche Diagnostics GmbH, Mannheim, Germany).

Results

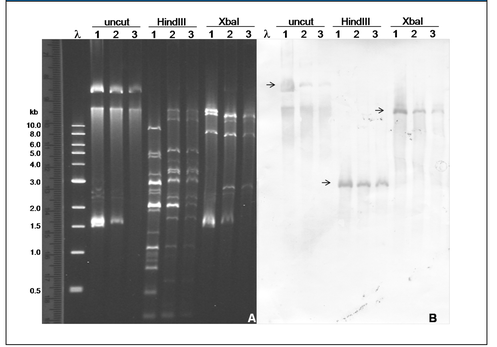
- Linezolid-resistant strains recovered from a Mexican hospital had the species identification confirmed as *S. epidermidis* (isolates 12898A and 5873X) and *S. cohnii* (isolate 10842A).
- *S. cohnii* was blood cultured (August/2009) from a 30-year-old man admitted with multiple trauma. *S. epidermidis* 12898A was recovered (October/2009) from the blood of a 50-year-old female admitted with a diagnosis of Guillain-Barre Syndrome. Both isolates were cultured within 48 hours after patients had developed systemic inflammatory response syndrome (SIRS). *S. epidermidis* 5873X was recovered (October/2009) from abdominal secretion of a 36-year-old male presented with multiple trauma.
- Isolates were oxacillin-resistant (MIC, >2 μg/mL) and exhibited elevated MIC results for linezolid (32 μg/mL), quinupristin/dalfopristin (1 – 4 μg/mL), retapamulin (≥8 μg/mL), chloramphenicol (16 – 32 μg/mL) and clindamycin (>64 μg/mL; Table 1). Isolates were susceptible to tetracycline, tigecycline, glycopeptides and daptomycin.
- All strains were PCR-positive for *cfr* and wild-type for 23S rRNA and L4. L3 aminoacid alterations Ser158Tyr, Asp159Tyr and Leu101Val were noted on both *S. epidermidis,* while Ser158Phe and Asp159Tyr were observed on *S. cohnii.*
- *S. epidermidis* 12898A and 5873X displayed identical PFGE profiles and were associated with sequence type (ST)-23.
- *S. epidermidis* strains also demonstrated identical plasmid band profiles, which were different from *S. cohnii* 10842A (Figure 1). However, all strains showed similar hybridization signal patterns, suggesting related *cfr* genetic contexts.

Table 1. Antimicrobial susceptibility profile and molecular findings among *cfr*-carrying *Staphylococcus* spp. recovered from clinical specimens of hospitalized patients in Guadalajara (Mexico).

Antimicrobial agent	MIC (μg/mL) [susceptibility category][a]		
	S. cohnii 10842A	*S. epidermidis* 12898A	*S. epidermidis* 5873X
Linezolid	32 [R]	32 [R]	32 [R]
Q/D[b]	4 [R]	2 [I]	1 [S]
Retapamulin	>8 [R]	8 [R]	>8 [R]
Chloramphenicol	32 [R]	16 [I]	16 [I]
Clindamycin	>64 [R]	>64 [R]	>64 [R]
Tigecycline	0.06 [S]	0.12 [S]	0.25 [S]
Tetracycline	≤0.12 [S]	2 [S]	1 [S]
Doxycycline	≤0.12 [S]	0.5 [S]	1 [S]
Daptomycin	0.25 [S]	0.5 [S]	0.5 [S]
Vancomycin	1 [S]	2 [S]	2 [S]
Teicoplanin	≤2 [S]	8 [S]	8 [S]
Oxacillin	>2 [R]	>2 [R]	>2 [R]
Ciprofloxacin	>4 [R]	>4 [R]	>4 [R]
Erythromycin	>2 [R]	>2 [R]	>2 [R]
Gentamicin	>8 [R]	>8 [R]	>8 [R]
T/S[b]	≤0.5 [S]	>2 [R]	>2 [R]
Molecular findings			
cfr	Positive	Positive	Positive
23S rRNA	WT	WT	WT
L3	Ser158Phe/Asp159Tyr	Ser158Tyr/Asp159Tyr/Leu101Val	Ser158Tyr/Asp159Tyr/Leu101Val
L4	Asn20Ser/Ala133Thr/Val155Ile	WT	WT

a. MIC interpretive criteria as published by CLSI M100-S20 . Retapamulin MIC results were interpreted according to parameters reported by Traczewski et al. (2008). S, susceptible; I, intermediate; R, resistant; and WT, wild-type.
b. Q/D=Quinupristin/dalfopristin, T/S=Trimethoprim/sulfamethoxazole.

Figure 1. Plasmid profile analysis of *cfr*-carrying strains. **A.** λ represents 1-kb DNA Ladder used as negative control plasmid DNA (New England Biolabs, Ipswich, MA, USA). 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 arrow indicates hybridization signals.



Conclusions

- The L3 Leu101Val substitution was previously detected in a linezolid-susceptible clinical isolate; however, mutations in Gly155 and Ala157 have been implicated on possibly disturbing the linezolid binding. Thus, the L3 amino acid alterations found in this study coupled with *cfr* may have acted synergistically and responsible for the elevated linezolid MIC results.
- *S. epidermidis* strains were clonally related and showed distinct *cfr*-carrying plasmid when compared to *S. cohnii.* However, a similar *cfr* hybridization signal pattern was noted, which indicates a related genetic context. These findings suggest that recombination events may have mobilized the *cfr* gene into different plasmids, which were later acquired by these species.
- The clinical relevance of CoNS has been uncertain. Nevertheless, the CoNS role as pathogens has increasingly been recognized, especially among immunocompromised patients, with indwelling or implanted foreign bodies.
- Isolates from this study were blood cultured from patients associated with SIRS, therefore considered clinically relevant. In addition, these staphylococcal species may, in any case, act as reservoir for resistance gene determinants in the nosocomial environment.

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