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# Detection of *cfr*-carrying *Staphylococcus* spp. Isolates Recovered from Blood Cultures in a Spanish Hospital During a Phase III Clinical Trial of Topical Omiganan 1% Gel Versus 10% Povidone Iodine

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## ABSTRACT

**Objectives:** Omiganan, a topical cationic antimicrobial peptide, is being studied for prevention of catheter-related infections in a large, multicenter phase III trial, which included multiple surveillance cultures collected by a central laboratory. Phenotypic and genotypic characterizations were done on clinically significant isolates. We report the detection of two *cfr*-carrying *Staphylococcus* spp. isolated from a single patient during this trial. Experiments were conducted to evaluate the potential for in vivo genetic transfer of *cfr*.
**Methods:** *Staphylococcus* spp. isolates were tested for susceptibility by the CLSI broth microdilution methods. Identification was performed by Vitek 2. Isolates displaying linezolid MIC at ≥4 mg/L were screened for mutations at the 23S rRNA, L4 and L22 genes, followed by sequencing. The isolates were screened for *cfr*. Primers targeting the sequences previously detected in the *cfr*-carrying plasmid pSCFS3 were used to PCR map the *cfr* surrounding region. Gene location was accessed by Southern blot and hybridization. *S. aureus* isolate was submitted to *spa* and MLST typing.
**Results:** A 77-year-old male patient was hospitalized in the ICU due to community-acquired pneumonia and respiratory failure. During his ICU-stay he received broad-spectrum antimicrobial therapy, including linezolid. One *S. epidermidis* and one *S. aureus* were recovered on the 23rd and 26th hospital day from catheter-drawn and peripheral blood specimens, respectively. Isolates were susceptible to omiganan (MIC, 8 and 32 mg/L, respectively) and resistant to mupirocin, neomycin, ciprofloxacin, erythromycin, gentamicin and oxacillin. Both strains were resistant to chloramphenicol, clindamycin, linezolid, retapamulin and quinupristin/dalfopristin, consistent with *cfr*. Ribosomal target mutations were not detected. Plasmid analysis identified two plasmid bands in each isolate of c.a. 160- and 250-, and 145- and 190-kb. Hybridization signals with a *cfr*-probe were noted from the 250-kb and 190-kb bands. PCR mapping identified Δ*tnpB* downstream of the *cfr* in the *S. epidermidis*. Both isolates did not possess the *istAS* and *istBS* structures associated with the *cfr* mobility.
**Conclusion:** *cfr* were embedded in different size plasmids and both genes showed distinct surrounding sequences, suggesting diverse acquisition events. This report highlights the ability of staphylococci to acquire linezolid resistance and the potential for *cfr* dissemination, representing a serious threat against current Gram-positive antimicrobial agents.

## INTRODUCTION

Healthcare-associated infections (HAIs) are a common cause of morbidity and mortality in the United States (USA) and are among the most common adverse events in healthcare. In addition, HAIs may be caused by multidrug-resistant (MDR) organisms and this scenario is usually associated with prolonged hospitalization and increased costs, particularly for bloodstream infections (BSI) and ventilator-associated pneumonia.

Among antimicrobial resistance genes, *cfr* was originally described as conferring chloramphenicol resistance in *Staphylococcus* spp. recovered from clinical animal specimens. *cfr* encodes for a methyltransferase, which provides post-transcriptional methylation of the 23S rRNA at position A2503. This methylation effects the binding of at least five antimicrobial classes (phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A; so-called phLOPS$_A$), leading to a MDR phenotype. Recently, two reports described the detection of *cfr* in human staphylococcal clinical isolates recovered from patients in the USA and Colombia.

Among HAIs, central venous catheters are a major source of preventable BSI. The use of the antiseptics is among the currently recommended techniques to prevent local catheter site infections and thus, controlling the incidence of HAIs. Recently, omiganan pentahydrochloride (formerly MBI 226), a novel topical cationic peptide showed broad potential as a topical decontamination agent. Therefore, this peptide has been evaluated for preventing catheter-related infections in a Central Line Infection Reduction Study (CLIRS), a large multicenter phase III trial, where a total of 1,859 patients from 58 medical sites across the USA and Europe were enrolled.

During the CLIRS trial, multiple surveillance cultures were collected and processed by local laboratories. Clinically significant isolates were further phenotypic and genotypic characterized by a central laboratory (JMI Laboratory, North Liberty, IA). Here, we report the detection of two *cfr*-carrying *Staphylococcus* spp. isolated from a single patient during this trial. Experiments were conducted to evaluate the potential for in vivo genetic transfer of the *cfr* gene.

## MATERIALS AND METHODS

Bacterial Isolates and Clinical Cases. One *S. aureus* and one *S. epidermidis* were recovered from a single patient admitted to the Hospital Clinico San Carlos (Madrid, Spain) and enrolled in the CLIRS clinical trial. These isolates displayed multidrug resistance (MDR) phenotype.

*S. aureus* was recovered from a 77-year-old male patient, admitted in the hospital ICU on April 18, 2008 with severe respiratory failure requiring mechanical ventilation. He was diagnosed with community-acquired pneumonia due to ESBL-producing *Escherichia coli*. Prior clinical history included hypertension, hypercholesterolemia, and non - insulin - dependent diabetes mellitus, mild to moderate COPD and smoking history until 2002.

The patient clinical course was initially complicated by multiple organ failure (mainly respiratory, hemodynamic and renal) and broad-spectrum antimicrobial therapy including linezolid (7 days, 8,400 mg total) was initiated. Still under mechanical ventilation, a central venous line and an indwelling urinary catheter, *S. epidermidis* strain was recovered from a catheter-draw on the 23rd hospital day (HD). During the 26th HD, the MDR *cfr*-carrying *S. aureus* was cultured from a bronchial aspirate and a blood culture specimen. The patient received tigecycline and his clinical condition improved, being discharged from the ICU on July 8 and from hospital on July 15.

Antimicrobial Susceptibility Testing. Both *Staphylococcus* spp. isolates were tested for susceptibility by reference broth microdilution method using cation-adjusted Mueller-Hinton broth in commercially prepared and validated panels (TREK Diagnostics, Cleveland, OH), according to the Clinical and Laboratory Standards Institute (CLSI; M7-A7, 2006). The strains were tested against a variety of antimicrobial agents representing the most common antimicrobial classes and examples of drugs used for the empiric or directed treatment of the indicated pathogens.

Interpretation of MIC results was in accordance with published CLSI breakpoint criteria (M100-A18, 2008), except for retapamulin and mupirocin, which were interpreted according to Traczewski et al. (2008) and Deshpande et al. (2002), respectively. Tigecycline results were interpreted according to the breakpoints approved by the USA Federal Drug Administration (US-FDA; i.e. ≤0.5 mg/L for susceptibility with no resistant breakpoint criteria). Quality control (QC) strains utilized included *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212. All QC results were within CLSI (2008) specified ranges.

Molecular Screening for Resistance Mechanisms. Upon MDR phenotype displayed by both isolates, including linezolid, screening for mutations in the central loop of domain V region of 23S rRNA and presence of the Cfr-encoding gene were performed using standards PCR reactions, followed by sequencing. The primers used for 23S rRNA and *cfr* gene were as follows: SA23S-F – GCGGTCGCCTCCTAAAAG and SA23S-R – ATCCCG GTCCTCTCGTACTA; cfr-F2 – TGAAGTATAAAGCAGGTTGGGAGTCA; cfr-R2 – ACCATATAATTGACCACAAGCAGC.

*cfr* Gene Location. Plasmid DNA was extracted using the Plasmid DNA Midi Kit (Qiagen GmbH, Hilden, Germany), separated on 1% agarose gel in TAE buffer on a Criterion Sub-cell GT system (Bio-Rad, Hercules, CA). Plasmid sizes were determined using plasmid bands from *Escherichia coli* NCTC 50192 as standard reference. Total DNA from clinical isolates was digested with I-Ceu-I and chromosomal and plasmid DNA bands were transferred onto a nylon membrane by Southern blot. Specific labelled probes for *cfr* and 16S rRNA were used for hybridization. Furthermore, primers targeting the surrounding sequences previously detected in the *cfr*-carrying plasmid pSCFS3 were used to PCR map the *cfr* embedded region.

Molecular Typing. *S. aureus* and *S. epidermidis* displayed the methicillin resistance phenotype and had their respective SCC*mec* elements characterized (I through VI) using a multiplex PCR strategy. In addition, *S. aureus* isolate was further characterized by multilocus (MLST) and singlelocus sequencing typing (*spa*). The *S. epidermidis* strain was also submitted to MLST typing.

## RESULTS

- The antimicrobial susceptibility profiles of the evaluated strains are shown in Table 1. Both isolates were resistant to mupirocin (low- and high-level), ciprofloxacin, erythromycin, gentamicin and oxacillin, and displayed omiganan MIC values of 8 and 32 mg/L.
- In addition, the isolates were non-susceptible to several antimicrobial agents, including clindamycin, chloramphenicol, linezolid, quinupristin/dalfopristin and retapamulin; phLOPS$_A$ resistance phenotype consistent with Cfr production.
- Both evaluated *Staphylococcus* spp. isolates harboured the Cfr-encoding gene and showed wild-type nucleotide sequences for 23S rRNA gene.
- Plasmid analysis identified two plasmid bands in each isolate of c.a. 160- and 250-, and 145- and 190-kb. Hybridization signals with a *cfr*-specific probe were noted from the 250-kb and 190-kb bands. No signals were observed from chromosomal DNA bands.
- PCR mapping detected Δ*tnpB* downstream of the *cfr* gene in the *S. epidermidis*. Both isolates did not possess the *istAS* and *istBS* genes located upstream of *cfr*, which were previously associated with *cfr* mobility.

**Table 1**. Antimicrobial susceptibility profile of *cfr*-carrying *S. aureus* and *S. epidermidis* recovered from a single patient enrolled in the CLIRS clinical trial in the Hospital Clinico San Carlos (Madrid, Spain).

| | MIC (mg/L) and Category[a] (Susceptible/Resistant) | | | |
|---|---|---|---|---|
| Antimicrobial agent | *S. aureus* | | *S. epidermidis* | |
| Oxacillin | >2 | Resistant | >2 | Resistant |
| Omiganan | 32 | - | 8 | - |
| Mupirocin | 16 | Resistant | >256 | Resistant |
| Daptomycin | ≤0.25 | Susceptible | ≤0.25 | Susceptible |
| Vancomycin | 1 | Susceptible | 2 | Susceptible |
| Ciprofloxacin | >4 | Resistant | >4 | Resistant |
| Erythromycin | >8 | Resistant | >8 | Resistant |
| Gentamicin | >8 | Resistant | >8 | Resistant |
| Retapamulin | 64 | Resistant | 32 | Resistant |
| Clindamycin | >256 | Resistant | >256 | Resistant |
| Chloramphenicol | 128 | Resistant | 128 | Resistant |
| Linezolid | 16 | Non-susceptible | 16 | Resistant |
| Rifampin | ≤0.5 | Susceptible | >2 | Resistant |
| Quinupristin/Dalfopristin | 16 | Resistant | 2 | Intermediate |
| Tetracycline | ≤2 | Susceptible | ≤2 | Susceptible |
| Tigecycline | 0.25 | Susceptible | 0.12 | Susceptible |
| Trimethoprim/sulfamethoxazole | ≤0.5 | Susceptible | >2 | Resistant |

a. Breakpoint criteria were those from CLSI, except for retapamulin and mupirocin, which were interpreted according to Traczewski et al. (2008) and Deshpande et al. (2002), respectively; tigecycline results were interpreted according to the breakpoints approved by the US-FDA (i.e. ≤0.5 mg/L for susceptibility with no resistant breakpoint criteria). - = no breakpoint established.

- Both staphylococcal isolates harboured SCC*mec* type I. In addition, *S. aureus* and *S. epidermidis* were associated with ST-228 (*spa* type 109) and ST-2, respectively.

## CONCLUSIONS

- Both *cfr*-carrying isolates showed a resistance phenotype to most clinically available antimicrobial agents, and were overall only susceptible to vancomycin, daptomycin (lipopeptide), tetracycline and tigecycline (glycocyclines).
- *cfr* genes were embedded in different size plasmid bands and both genes showed distinct surrounding sequences, suggesting diverse acquisition events and origins.
- *S. aureus* isolate was ST-228-I, which belongs to the Southern German clone. Isolates belonging to this clone have been detected in several European countries, such as Germany, Slovenia, Hungary, Belgium, Switzerland, Portugal, Italy and Spain.
- *S. epidermidis* was ST-2-I, which has been recently recognized as being widely disseminated and identified in several countries worldwide, such as Denmark, Italy, Iceland, Argentina, Mexico, Greece, Spain, Hungary, Colombia, Uruguay, Japan and Bulgaria.
- This report highlights the ability of staphylococci to acquire MDR and the potential for *cfr* dissemination in clinically predominant human clones, representing a serious threat against contemporary Gram-positive antimicrobial agents.

## SELECTED REFERENCES

1. Aires-de-Sousa M, Correia B and de Lencastre H (2008). Changing patterns in frequency of recovery of five methicillin-resistant *Staphylococcus aureus* clones in portuguese hospitals: surveillance over a 16-year period. *J. Clin. Microbiol.* 46:2912-2917.
2. Blanc DS, Petignat C, Wenger A, Kuhn G, Vallet Y, Fracheboud D, Trachsel S, Reymond M, Troillet N, Siegrist HH, Oeuvray S, Bes M, Etienne J, Bille J, Francioli P and Zanetti G (2007). Changing molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a small geographic area over an eight-year period. *J. Clin. Microbiol.* 45:3729-3736.
3. Clinical and Laboratory Standards Institute (2006). *M07-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - seventh edition.* Wayne, PA., CLSI.
4. Clinical and Laboratory Standards Institute (2008). *M100-S18. Performance standards for antimicrobial susceptibility testing. 18th informational supplement.* Wayne, PA., CLSI.
5. Deshpande LM, Fix AM, Pfaller MA and Jones RN (2002). Emerging elevated mupirocin resistance rates among staphylococcal isolates in the SENTRY Antimicrobial Surveillance Program (2000): correlations of results from disk diffusion, Etest and reference dilution methods. *Diagn. Microbiol. Infect. Dis.* 42:283-290.
6. Mendes RE, Deshpande LM, Castanheira M, DiPersio J, Saubolle MA and Jones RN (2008). First report of cfr-mediated resistance to linezolid in human staphylococcal clinical isolates recovered in the United States. *Antimicrob. Agents Chemother.* 52:2244-2246.
7. Miragaia M, Thomas JC, Couto I, Enright MC and de Lencastre H (2007). Inferring a population structure for *Staphylococcus epidermidis* from multilocus sequence typing data. *J. Bacteriol.* 189:2540-2552.
8. Traczewski MM and Brown SD (2008). Proposed MIC and disk diffusion microbiological cutoffs and spectrum of activity of retapamulin, a novel topical antimicrobial agent. *Antimicrob. Agents Chemother.* 52:3863-3867.
9. Wisplinghoff H, Ewertz B, Wisplinghoff S, Stefanik D, Plum G, Perdreau-Remington F and Seifert H (2005). Molecular evolution of methicillin-resistant *Staphylococcus aureus* in the metropolitan area of Cologne, Germany, from 1984 to 1998. *J. Clin. Microbiol.* 43:5445-5451.