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Decreased-linezolid Susceptibility Due to *cfr* in a Community-associated Methicillin-resistant *Staphylococcus aureus* Clone (USA300)

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SENTRY ANTIMICROBIAL SURVEILLANCE

Abstract

Background: Cfr causes post-transcriptional methylation in 23S rRNA (A2503), conferring resistance to several antimicrobial classes, including oxazolidinones and lincosamides. We report the first clinical case of skin infection caused by a *cfr*-carrying *S. aureus* belonging to the USA300 MRSA clone.

Methods: Identification was performed by Vitek 2. Investigated isolate was tested for susceptibility by CLSI methods (M07-A8 and M100-S20). The strain was screened for *cfr* and mutations in the 23S rRNA, L3 and L4 proteins. Clonality was examined via PVL genes and SCC*mec*, PFGE, *spa* and MLST typing. Gene location was assessed by Southern blot and hybridization.

Results: A 56-year-old man with a long history of a chronic neurological condition characterized by optic neuritis, spasticity and neuropathic pain was seen in an emergency department (ED). The patient had been on chronic immunosuppressant therapy for over a decade. He also had active rheumatoid arthritis and a history of psoriasis among other chronic medical conditions. A *S. aureus* (1848L) was recovered from a skin lesion at the time of the ED visit. *S. aureus* exhibited elevated MIC results for linezolid (4 μg/mL), quinupristin/dalfopristin (2 μg/mL), retapamulin (32 μg/mL), chloramphenicol (>128 μg/mL) and clindamycin (>256 μg/mL). 1848L was PCR-positive for *cfr* and wildtype for screened ribosomal proteins. *S. aureus* showed a SCC*mec* type IV, was PVL-positive and the PFGE profile matched that of USA300-0114 (NRS384). In addition, the 1848L strain was t008 and ST-8. *cfr* was plasmid-located (32-kb).

Conclusions: This is the first case report of *cfr* in a *S. aureus* strain belonging to USA300, the predominant community-associated MRSA strain in the USA. The ability of USA300 isolates to disseminate and cause infections, coupled with the *cfr* mobility may jeopardize the empiric therapy of skin-structure infections across several classes.

Introduction

Cfr was originally described as a chloramphenicol resistance marker in *Staphylococcus sciuri* recovered from animal sources. Cfr causes post-transcriptional methylation in the 23S rRNA (A2503), modifying the target site for drugs belonging to several antimicrobial classes, including phenicols, oxazolidinones, pleuromutilins, lincosamides and streptogramin A compounds. Unlike other linezolid resistance mechanisms, which are associated to ribosomal mutation(s), *cfr* has been described to be plasmid-borne. In addition, *cfr* mobilization among clinical human pathogens has been recently reported.

S. aureus belonging to a single pulsed-field gel electrophoresis type (PFGE; USA300; multilocus sequence type [MLST] 8) has been the predominant cause of community-associated methicillin-resistant *S. aureus* (CA-MRSA) infections in the USA. Recently, USA300 isolates have also emerged as a cause of health-care associated infections in USA hospitals. Although these strains are usually resistant only to oxacillin and erythromycin, resistance to other antimicrobial agents has been observed. The emergence of resistance in USA300 strains will pose a challenge for treating both community- and health care-associated infections. Herein, we report the first clinical case of skin infection caused by a *cfr*-carrying *S. aureus* belonging to the USA300 MRSA clone.

Methods

Clinical isolate: *S. aureus* (1848L) was recovered from a skin lesion of a 56-year-old male outpatient during an emergency visit to a USA medical institution. The isolate was submitted to a central monitoring laboratory (JMI Laboratories, North Liberty, Iowa) as part of the 2009 SENTRY Antimicrobial Surveillance Program, according to defined protocols. Species identification was confirmed by standard biochemical tests and use of the Vitek® 2 System (bioMérieux; Hazelwood, Missouri).

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed by reference broth microdilution methods, according to the Clinical and Laboratory Standards Institute (CLSI; M07-A8, 2009) recommendations. Minimum inhibitory concentrations (MIC) interpretations were applied as described in M100-S20-U (CLSI, 2010). Retapamulin MIC results were interpreted according to microbiological parameters reported by Traczewski et al. (2008). Tigecycline MIC values were interpreted based on the breakpoint for *S. aureus* approved by the US Food and Drug Administration (FDA; Tygacil Product Package Insert, 2005). *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were concurrently tested for quality assurance purposes.

Evaluation of linezolid resistance mechanisms: Presence of *cfr* and mutations in the 23S rRNA, L3 and L4 ribosomal proteins were screened by PCR and sequencing. Amplicons were sequenced on both strands. Ribosomal proteins obtained were compared to those from wildtype *S. aureus* RN4220 using the Lasergene® software package (DNAStar; Madison, Wisconsin).

Plasmid analysis: Whole genomic DNA was prepared in 1% agarose blocks and partially digested with S1 endonuclease. DNA fragments were resolved by PFGE using CHEF-DR II (BioRad, Richmond, California) and transferred to a nylon membrane. Membrane was hybridized using a digoxigenin-labeled *cfr*-specific probe (Roche Diagnostics GmbH, Mannheim, Germany).

Molecular typing: The 1848L index strain was subjected to PFGE and the profile obtained was compared to USA clonal types using the GelCompar II software (Applied Math, Kortrijk, Belgium). *S. aureus* 1848L was further characterized by SCC*mec*, *spa*, and MLST typing. In addition, this strain was screened for Panton-Valentine Leukocidin (PVL; lukS-PV and lukF-PV) determinants.

Transfer of *cfr*-carrying plasmid: Plasmid extraction from 1848L was carried out by using the Plasmid MIDI kit (QIAGEN; Hilden, Germany). Plasmid transfer into wildtype *S. aureus* RN4220 was performed by electroporation using Micropulser (BioRad, Richmond, California). Probable transformants were selected using retapamulin (0.25 μg/mL). The presence of *cfr* among transformant colonies was confirmed by PCR.

Results

- A 56-year-old caucasian man with a long history of a chronic neurological condition characterized by optic neuritis, spasticity and neuropathic pain was seen in the emergency department. A *S. aureus* strain (1848L) was recovered from a skin lesion at the time of the emergency department visit.
- The patient had been on chronic immunosuppressant therapy for over a decade. He also had active rheumatoid arthritis and a history of psoriasis among other chronic medical conditions. Moreover, this patient had previous history of MRSA infections involving the right elbow (July 2007) and ear (December 2007).
- Strain 1848L exhibited elevated MIC results for linezolid (4 μg/mL), streptogramin combinations (2 − 4 μg/mL), pleuromutilins (≥32 μg/mL), chloramphenicol (>128 μg/mL) and clindamycin (>256 μg/mL; Table 1).
- *S. aureus* clinical strain 1848L demonstrated wildtype sequences for 23S rRNA (including G2576), L3 and L4, when compared to those from RN4220 strain (Table 1).
- S1 partial-digested genomic DNA revealed the presence of a single plasmid band (approximately 32-kb), which was transferred to RN4220 (Figure 1). 1848L and RN4220 (p1848L) strains were PCR-positive for *cfr* (Table 1) and hybridization signals were observed on plasmid bands from both strains.
- RN4220 (p1848L) transformant strain displayed elevated MIC values for the Cfr drug resistance markers (i.e. phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramins; Table 1). Resistance markers for other drugs were not observed.
- PFGE profile of 1848L matched that of USA300-0114 (NRS384; Figure 2). In addition, this strain possessed SCC*mec* type IV, was PVL-positive, *spa* type t008 and associated with ST-8.

Table 1. Antimicrobial susceptibility profile and molecular findings for *S. aureus* 1848L, RN4220 (p1848L) transformant and wildtype RN4220.

	S. aureus MIC (μg/mL) [susceptibility category][a]		
Parameters	1848L (index strain)	RN4220 (p1848L)	RN4220
Antimicrobial agent			
Linezolid	4 [S]	4 [S]	1 [S]
Chloramphenicol	>128 [R]	128 [R]	4 [S]
Clindamycin	>256 [R]	>256 [R]	0.25 [S]
Virginiamycin M1	128	128	4
Virginiamycin	4	2	0.25
Quinupristin/dalfopristin	2 [I]	2 [I]	0.5 [S]
Retapamulin	32 [R]	16 [R]	0.06 [S]
Tiamulin	>64	>64	≤0.5
Tigecycline	0.12 [S]	0.12 [S]	0.12 [S]
Tetracycline	0.25 [S]	0.25 [S]	0.25 [S]
Vancomycin	0.5 [S]	1 [S]	1 [S]
Oxacillin	>2 [R]	≤0.25 [S]	≤0.25 [S]
Ciprofloxacin	0.5 [S]	0.5 [S]	0.5 [S]
Erythromycin	>2 [R]	≤0.25 [S]	≤0.25 [S]
Gentamicin	≤1 [S]	≤1 [S]	≤1 [S]
Trimethoprim/sulfamethoxazole	≤0.5 [S]	≤0.5 [S]	≤0.5 [S]
Molecular findings			
cfr	Positive	Positive	Negative
23S rRNA	WT	WT	WT
L3	WT	WT	WT
L4	WT	WT	WT

a. MIC interpretive criteria as published by CLSI M100-S20-U, when available. Retapamulin MIC results were interpreted according to the microbiological breakpoints proposed by Traczewski et al. (2008; i.e ≤0.5 μg/mL for susceptibility; 1 μg/mL for intermediate; and ≥2 μg/mL for resistant). Tigecycline susceptible breakpoint for *S. aureus* (≤0.5 μg/mL) was that approved by the US FDA. S, susceptible; I, intermediate; and R, resistant; WT, wildtype.

Figure 1. **A.** λ represents Lambda ladder PFGE marker also used as negative control (New England Biolabs, Ipswich, Massachusetts). S1 partial-digested genomic DNA of *cfr*-carrying 1848L (lane 1) and RN4220 transformant (p1848L; lane 2) strains. Lane 3 and 4 represent wildtype RN4220 and positive control, respectively. **B.** Hybridization signal profiles obtained with a *cfr*-specific probe. Horizontal arrow indicates plasmid bands and hybridization signals.

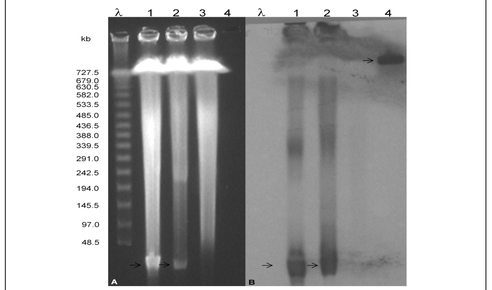
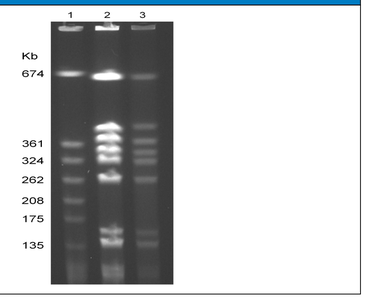


Figure 2. PFGE profiles of Cfr-producing *S. aureus* 1848L (lane 2) and reference NRS384 strain (USA300-0114; lane 3). Lane 1 represents *S. aureus* NCTC 8325 used as PFGE standard.



Conclusions

- This is the first case report of a plasmid-borne *cfr* in a *S. aureus* strain belonging to the epidemic USA300 clone, which has become the predominant community-associated MRSA strain in the USA.
- *Cfr*-carrying isolates from human clinical specimens are rare; however, the finding of *cfr* in a community strain may be significant, since it could jeopardize the empiric therapy for skin infections.

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