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Identification and Characterization of Linezolid-Resistant *cfr*-Positive *Staphylococcus aureus* USA300 Isolates from a New York City Medical Center

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The *cfr* gene was identified in three linezolid-resistant USA300 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates collected over a 3-day period at a New York City medical center in 2011 as part of a routine surveillance program. Each isolate possessed a plasmid containing a pSCFS3-like *cfr* gene environment. Transformation of the *cfr*-bearing plasmids into the *S. aureus* ATCC 29213 background recapitulated the expected Cfr antibiogram, including resistance to linezolid, tiamulin, clindamycin, and florfenicol and susceptibility to tedizolid.

The *cfr* multidrug resistance gene represents the first horizontally transferable resistance determinant for linezolid (LZD) (1). Methylation of the 23S rRNA base A2503 by the Cfr methyltransferase confers resistance to 6 classes of drugs that target the peptidyl transferase center in the 50S ribosomal subunit, i.e., phenicols, lincosamides, oxazolidinones, pleuromutilins, streptogramin A, and 16-member-ring macrolides (2, 3). Therefore, use of any one of these classes can select for retention of the *cfr* gene. The *cfr* gene has been found globally (4); in the United States, it has been identified in isolates from California (5), Ohio (6), Arizona (6), Utah (7), Michigan (8), Indiana (9), Missouri (7), Kentucky (8), Maryland (10), and Illinois (11). U.S. surveillance studies have shown that the frequency of LZD resistance among Gram-positive pathogens from 2004 to 2012 was <0.5%, and of these isolates, ~5 to 16% possessed the *cfr* gene (8, 12, 13).

Other than *cfr*, linezolid resistance has primarily been associated with rare, chromosomal mutations in genes encoding 23S rRNA or ribosomal proteins L3 and L4 (14, 15). While the novel oxazolidinone tedizolid (TZD) (16) is also impacted by these chromosomal mutations, it retains antimicrobial activity against LZD^r *cfr*-positive strains without chromosomal mutations due to structural features that increase its binding site affinity and reduce steric clash with the Cfr-modified A2503 residue compared to linezolid (17, 18).

(Portions of this work were presented at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy [19].)

A 2011 TZD surveillance study of 3,817 Gram-positive isolates (20) included *Staphylococcus aureus* strains 2823634, 2823586, and 2823605, which were collected over a 3-day period from 3 different patients at the New York Presbyterian Hospital/Weill Cornell Medical Center (New York, NY) (Table 1). These clinical isolates, *S. aureus* strain ATCC 29213, and transformants of the latter were cultured aerobically at 37°C on cation-adjusted Mueller-Hinton II agar (MHA; Becton Dickinson, Franklin Lakes, NJ) or in MH broth (MHB). MIC values were assessed by broth microdilution as previously described (21, 22); daptomycin MIC values were assessed in MHB supplemented with 50 mg/liter Ca^{2+}. Each of these three clinical isolates was resistant to LZD (MIC = 16 μg/ml) but susceptible to TZD, with an MIC value of 0.5 μg/ml (Table 2), equivalent to the *S. aureus* wild-type MIC_{90} for TZD (23), prompting further genotypic analyses of their relatedness and underlying resistance mechanisms.

The typing and relatedness of isolates were assessed by pulsed-field gel electrophoresis (PFGE). Chromosomal DNA was prepared in agarose plugs, digested with SmaI restriction endonuclease, and analyzed as previously described (24). A USA300 control strain was included as a reference. All 3 strains had USA300-like profiles, suggesting a common genetic background (Fig. 1). An additional group of 7 methicillin-resistant *S. aureus* (MRSA) isolates collected at this site (up to 2 months prior and 1

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TABLE 1 MRSA surveillance isolates analyzed in this study

Strain	Source	Isolation date (mo/day/yr)	Presence of *cfr*	Notes
2823634	Blood	3/11/2011	+	Recurrent bacteremia with probable hVISA,[a] not treated with linezolid
2823586	Blood	3/12/2011	+	Line-associated bacteremia, treated with linezolid for earlier MRSA bacteremia
2823605	Wound	3/13/2011	+	Line-associated bacteremia, treated with linezolid for earlier MRSA bacteremia
2823611	Urine	4/12/2011	−	Not treated with linezolid

[a] hVISA, heterogeneous vancomycin-intermediate *Staphylococcus aureus*.

TABLE 2 MICs of *S. aureus* clinical isolates and ATCC 29213 *cfr*-bearing plasmid transformants thereof

Origin	Strain	Presence of *cfr*	MIC (μg/ml)[a]												
			TZD	LZD	TIA	FFC	CLI	ERY	GEN	OXA	CIP	TMP	TET	VAN	DAP
Clinical	2823611	−	0.5	2	0.5	8	0.13	16	>128	64	1	2	1	1	0.25
	2823634	+	0.5	16	>128	>128	>128	16	>128	64	1	1	1	1	0.25
	2823586	+	0.5	16	>128	>128	>128	16	>128	64	1	1	1	1	0.25
	2823605	+	0.5	16	>128	>128	>128	16	>128	64	1	1	1	1	0.25
Laboratory	29213	−	0.5	2	0.5	8	0.13	0.5	1	0.5	0.5	2	1	1	0.25
	29213(p2823634)	+	0.5	16	>128	>128	>128	0.25	1	0.25	0.5	4	1	1	0.25
	29213(p2823586)	+	0.5	16	>128	>128	>128	0.25	1	0.25	0.5	4	1	1	0.25
	29213(p2823605)	+	0.5	16	>128	>128	>128	0.25	1	0.25	0.5	4	1	1	0.25

[a] TZD, tedizolid; LZD, linezolid; TIA, tiamulin; FFC, florfenicol; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; OXA, oxacillin; CIP, ciprofloxacin; TMP, trimethoprim; TET, tetracycline; VAN, vancomycin; DAP, daptomycin.

month after) were also analyzed by PFGE, revealing an LZD^s isolate (2823611) with a nearly identical profile (Fig. 1) (Table 2). The other 6 MRSA isolates had PFGE profiles unrelated to this cluster of 4 isolates (data not shown).

The genetic basis of LZD resistance was determined by amplification and sequence analysis of genes encoding 23S rRNA, ribosomal proteins L3 (*rplC*) and L4 (*rplD*), and Cfr as previously described (25). Sequencing data were analyzed using Vector NTI Advance 11 software (Invitrogen, Carlsbad, CA). All three of the LZD^r isolates possessed the *cfr* gene and carried wild-type alleles for 23S rRNA, *rplC*, and *rplD*. These data and the 32-fold MIC value differential between TZD and LZD are consistent with previous reports for *S. aureus* possessing the *cfr* gene and lacking chromosomal resistance mutations (17, 25). The observed resistance to tiamulin, florfenicol, and clindamycin is also consistent with the presence of *cfr* (Table 2). As expected, the LZD^s 2823611 isolate was PCR negative for *cfr* and possessed wild-type alleles for all chromosomal genes sequenced.

To assess whether the *cfr* gene was plasmid borne, total plasmid DNA was isolated from each strain and transformed into *S. aureus* ATCC 29213 as previously described (26, 27). Putative *cfr*-positive transformant colonies that grew on MHA medium containing 5 μg/ml of tiamulin were confirmed through PCR amplification of the *cfr* gene. The antibiogram of these isogenic 29213 transformant strains matched the profile of the parent strains, providing further evidence that the *cfr* gene wholly accounted for the LZD^r phenotype observed (Table 2). Within the range of drugs tested, no additional drug resistance was conferred by the *cfr*-bearing plasmids (p2823634, p2823586, and p2823605). The consistency in MIC values for the non-Cfr-impacted drugs tested between the *cfr*-negative 2823611 isolate and the three *cfr*-positive isolates supports the possibility that this endemic hospital strain acquired the *cfr*-bearing plasmid.

The relatedness of the p2823634, p2823586, and p2823605 plasmids was investigated through single-primer PCR amplification (28) and sequence analysis of the immediate *cfr* gene flanking region using plasmid DNA reisolated from each of the 29213 *cfr*-positive transformants. Each plasmid possessed an identical 5.4-kb region containing the IS*21-558* element upstream of *cfr* and a truncated *tnpB* gene downstream (Fig. 2). This proximal *cfr* environment for the plasmids contained in these three isolates is 99.7% identical to that found in the pSCFS3 plasmid identified in German *S. aureus* and *Staphylococcus lentus* veterinary isolates from the early 2000s (AM086211) (29) and 100% identical to the pSA737 plasmid from the 2007 Ohio *S. aureus* isolate 004-737X (KC206006) (6, 30).

Although there is limited information on patient medical history, the use of LZD was a commonality for two patients with *cfr*-positive isolates. Because *cfr* has a low fitness cost (31) and can be selected for by any drug within the Cfr resistance spectrum, it is not unexpected that a *cfr*-positive isolate was recovered from a patient not receiving LZD therapy (Table 1).

This report is the first documentation of the *cfr* gene in clinical isolates from New York State. The presence of *cfr* within the epidemic USA300 genetic background suggests the possibility of further dissemination. Continued LZD resistance surveillance efforts that incorporate identification of *cfr* by PCR can readily monitor the frequency and location of strains carrying this gene. Armed with this information, appropriate drug selection strategies can be utilized to combat the potential spread of *cfr*.

Nucleotide sequence accession numbers. Sequences of the 5,415-bp *cfr* gene environments analyzed for p2823634, p2823586, and p2823605 were deposited into the NCBI database under GenBank accession numbers KJ819951, KJ819952, and KJ819953, respectively.

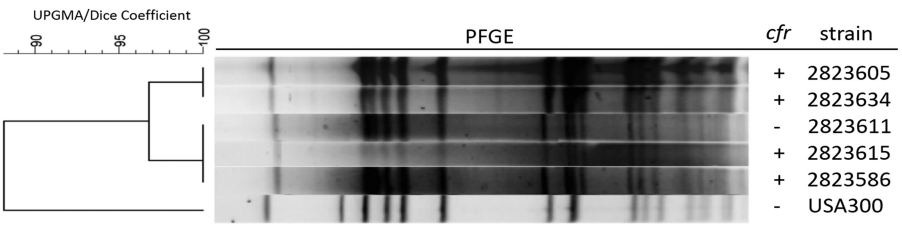

FIG 1 PFGE profiles of *cfr*-positive (+) and *cfr*-negative (−) isolates collected from the same hospital and a USA300 reference strain.

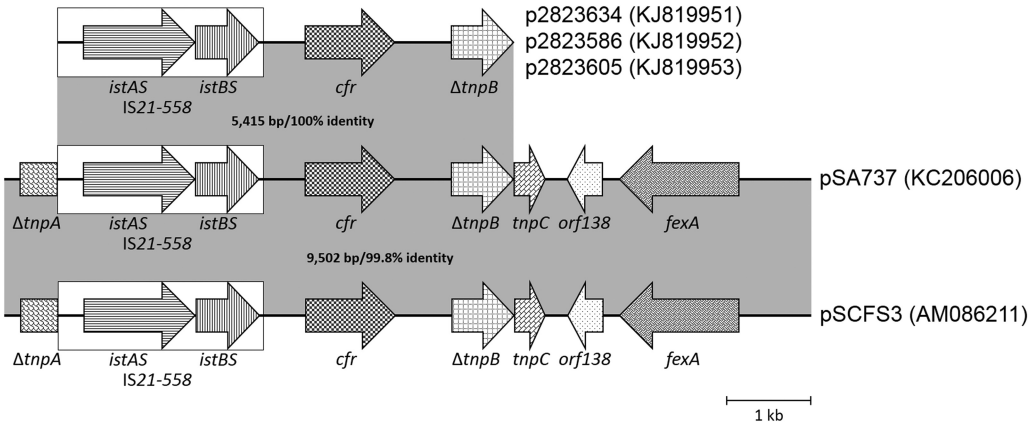

FIG 2 Schematic comparison of *cfr* gene environments found in p2823634, p2823586, and p2823605 with those previously described for *S. aureus* plasmids pSA737 (30) and pSCFS3 (29).

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