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Dissemination of the Same *cfr*-Carrying Plasmid among Methicillin-Resistant *Staphylococcus aureus* and Coagulase-Negative Staphylococcal Isolates in China

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Six *cfr*-harboring methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, which belonged to the same clone of sequence type 5 (ST5)-staphylococcal cassette chromosome *mec* element II (SCC*mec* II)-*spa* t311, were investigated in this study. Complete sequencing of a *cfr*-carrying plasmid, pLRSA417, revealed an 8,487-bp fragment containing a Tn*4001*-like transposon, *cfr*, *orf1*, and IS*Enfa4*. This segment, first identified in an animal plasmid, pSS-01, was observed in several plasmids from clinical coagulase-negative staphylococci in China, suggesting that the *cfr* gene, which might originate from livestock, was located in the same mobile element and disseminated among different clinical staphylococcal species.

Methicillin-resistant *Staphylococcus aureus* (MRSA), an important pathogen, is resistant not only to β-lactams but usually also to several other antibiotics. Linezolid is an important alternative for the treatment of infections with MRSA. Alterations in domain V of the 23S rRNA gene, most frequently the G2576T mutation, are the main mechanism contributing to linezolid resistance among staphylococcal isolates (1). In addition, mutations in ribosomal proteins L3 and L4 have been associated with decreased susceptibility to linezolid (2, 3). Staphylococci can also exhibit linezolid resistance by acquisition of the *cfr* gene, which was originally identified in a bovine *Staphylococcus sciuri* isolate in 2000 (4) and was subsequently detected in a clinical MRSA isolate in 2005 (5). So far, *cfr*-carrying staphylococci have spread worldwide, even causing several outbreaks (1).

In China, the *cfr* gene has been extensively detected, and its genetic environment has been well characterized in both Gram-positive and Gram-negative bacteria of animal origin (6). However, linezolid resistance had not been described in clinical staphylococci in China until the emergence of 17 isolates of methicillin-resistant coagulase-negative staphylococci (MRCoNS) in our hospital in 2011 (7). Soon, linezolid resistance in human clinical CoNS seems to have become an increasing problem in China. Several linezolid-resistant clinical isolates of *Staphylococcus capitis* ($n = 9$), *Staphylococcus cohnii* ($n = 6$), *Staphylococcus haemolyticus* ($n = 1$), *Staphylococcus epidermidis* ($n = 1$), and *Staphylococcus hominis* ($n = 1$) from Shenyang ($n = 3$), Beijing ($n = 3$), Hangzhou ($n = 10$), Rui'an ($n = 1$), and Nanjing ($n = 1$) have been reported (8–11). To date, there has been no report of linezolid-resistant MRSA (LRSA) of human origin in China. In the current study, the molecular epidemiology of six *cfr*-harboring MRSA isolates and the genetic environment of the *cfr* gene were investigated.

The 2nd Affiliated Hospital of Zhejiang University is a 2,000-bed comprehensive tertiary care hospital in Hangzhou, China. Six LRSA isolates were obtained from sputum samples from six patients in the neurology intensive care unit (NICU) between April and July 2013. All patients were suffering from cerebral hemorrhage accompanied by pulmonary infection. Other diagnostic samples from six patients with LRSA, including those from blood, cerebrospinal fluid, and feces, were negative for LRSA. No patient received linezolid therapy, except patient 6 was treated for 17 days during the prior month (Table 1). Patients with this organism were placed in isolation rooms under strict contact precautions. No new LRSA isolates have been identified since August 2013.

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TABLE 1 Clinical characteristics of patients with LRSA

Patient no.	Isolate	Collection date (mo/yr)	Sex	Age (yr)	Days of LRSA isolation/no. of days in ICU	Antibiotic therapy within 2 wk prior to LRSA isolation[a]	Outcome
1	LRSA417	4/2013	Male	66	16/50	TZP	Survived
2	LRSA422	4/2013	Male	65	3/13	None	Died
3	LRSA531	5/2013	Male	63	9/26	CMN, TZP	Survived
4	LRSA608	6/2013	Female	61	22/28	MOX, MEM, TEC	Survived
5	LRSA621	6/2013	Male	40	15/22	MEM, TZP, AK	Survived
6	LRSA726	7/2013	Male	45	33/58	AK, MEM, PB	Died

[a] TZP, piperacillin-tazobactam; CMN, cefminox; MOX, moxifloxacin; MEM, meropenem; TEC, teicoplanin; AK, amikacin; PB, polymyxin B.

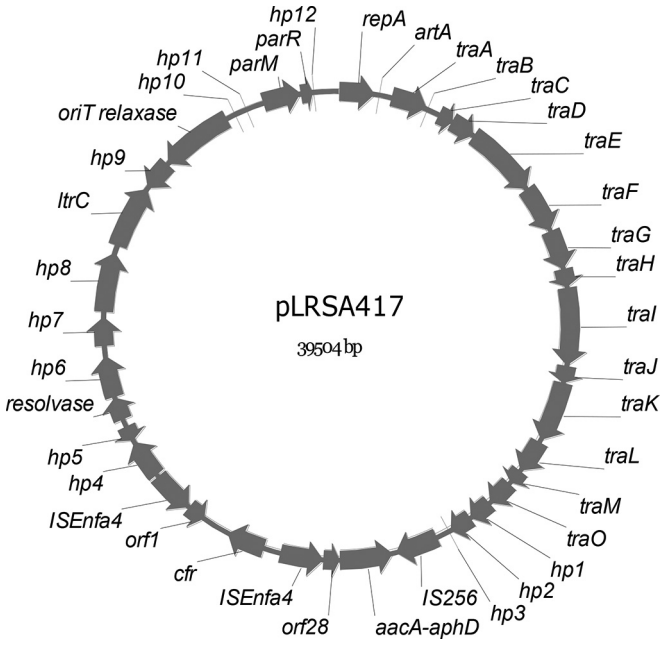

FIG 1 Genetic map of pLRSA417. Coding regions of >50 amino acids are represented by arrows indicating the direction of transcription, and the corresponding genes are annotated. *hp1* to *hp12* represent putative genes encoding hypothetical proteins.

The MICs were determined by Etest. The *cfr* gene and 23S rRNA mutations were examined by PCR and sequence analysis (5, 12). Molecular typing was performed by pulsed-field gel electrophoresis (PFGE) (13), multilocus sequence typing (MLST) (14), staphylococcal cassette chromosome *mec* element (SCC*mec*) typing (15), *spa* typing (16), and Panton-Valentine leukocidin (*pvl*) gene detection (17). The location of the *cfr* gene in six MRSA isolates was determined by S1-nuclease PFGE and Southern blot hybridization (18). Plasmid DNA was sequenced using the Illumina HiSeq 2000 platform, and reads were assembled using the CLC Workbench program (version 5.5; CLC bio, Aarhus, Denmark). The gaps between contigs were closed by primer walking. The putative open reading frames (ORFs) were identified using the FramePlot 4.0beta program. The Vector NTI program (Invitrogen, CA) was used for annotation of the DNA sequence. Twenty-six pairs of PCR primers (see Table S1 in the supplemental material) were designed based on the whole assembled sequence and were used for analysis of other *cfr*-carrying plasmids in this study.

Six *S. aureus* isolates showed similar susceptibility profiles, with a linezolid MIC of 8 μg/ml. All isolates were resistant to oxacillin, cefoxitin, chloramphenicol, clindamycin, ciprofloxacin, gentamicin, erythromycin, and tetracycline but were susceptible to vancomycin, teicoplanin, rifampin, tigecycline, and trimethoprim-sulfamethoxazole. All LRSA isolates were positive for the *cfr* gene, and no 23S rRNA mutations were detected. All isolates with indistinguishable PFGE band patterns belonged to the same clone of sequence type 5 (ST5)-SCC*mec* II-*spa* t311 and were negative for the *pvl* gene.

The *cfr* gene of six LRSA isolates was located on a plasmid (see Fig. S1 in the supplemental material). One *cfr*-carrying plasmid, pLRSA417, was sequenced, and a circular closed sequence of 39,504 bp was obtained (GenBank accession no. KJ922127). pLRSA417 consisted of 40 putative genes for deduced proteins of ≥50 amino acids (Fig. 1). PCR mapping demonstrated that another five LRSA isolates and five representative MRCoNS, which were isolated from our hospital (7), contained the same *cfr*-carrying plasmid.

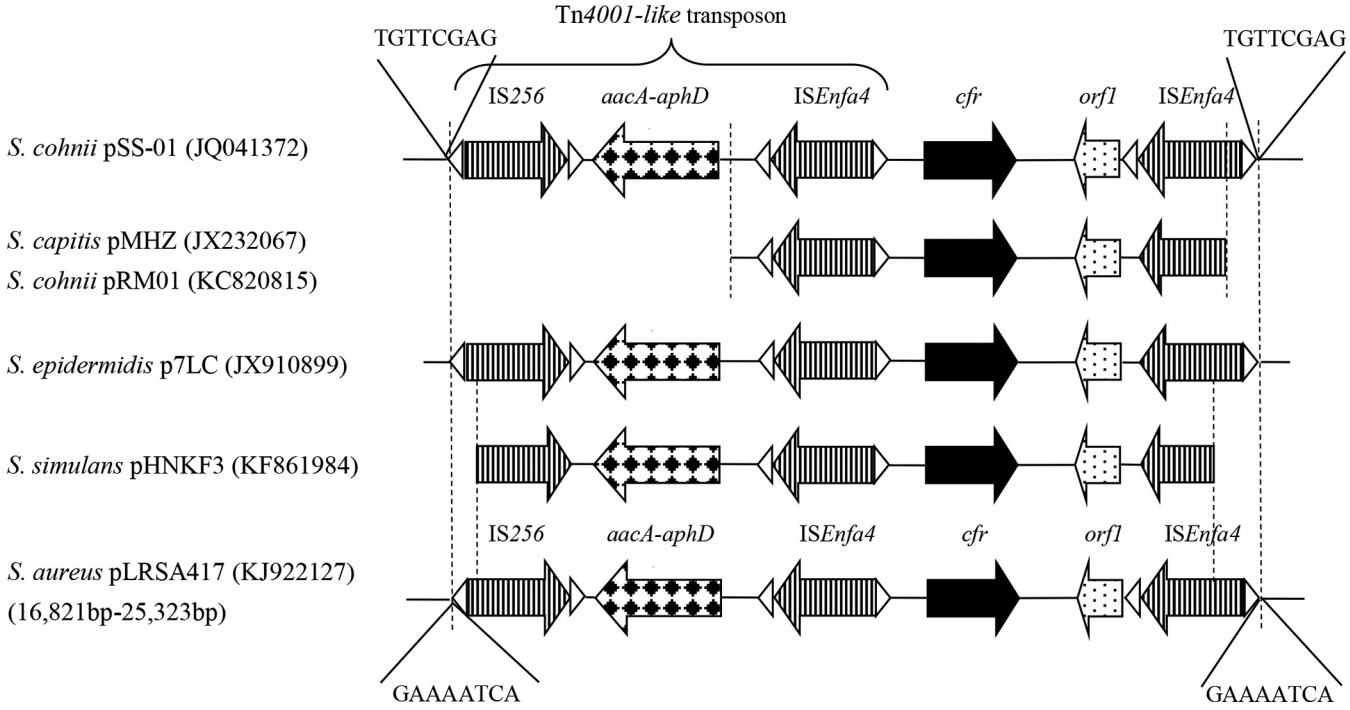

FIG 2 Schematic representation and comparison of the *cfr* genetic environment. Linear genetic maps of plasmids are presented, with the accession numbers given in parentheses. Genes and their corresponding transcription orientations are indicated by arrows with various shading patterns. The region within the two dotted lines between plasmid maps illustrates that they share high homology (>95% nucleotide identity). The structure of a Tn*4001-like* transposon is indicated. The triangles represent the inverted repeats of the respective mobile elements, and target site duplications are presented as 8-bp sequences.

An 8,487-bp fragment containing a Tn*4001*-like transposon, *cfr*, *orf1*, and IS*Enfa4* was flanked by two 8-bp target site duplications (TSDs), the signature of a transposition event. This DNA fragment, flanked by different TSDs, showed a sequence identical to that of pSS-01 from *S. cohnii* of swine origin in China (18) except for three single-nucleotide polymorphisms and two deletions. A similar genetic environment surrounding the *cfr* gene can be observed in several plasmids from clinical CoNS, including pMHZ in *S. capitis* from Hangzhou (9), pRM01 and pRA01 in *S. cohnii* from Beijing and Rui'an (10), p7LC in *S. epidermidis* from the United States (19), and pHNKF3 from a pig in Guangzhou (Fig. 2). It seemed that the genetic structures of the Tn*4001*-like transposon, *cfr*, and IS*Enfa4* play an important role in the mobility of *cfr* in different staphylococcal plasmids of different origins. In addition to IS*Enfa4*, another insertion sequence, IS*21-558*, was found in staphylococci from both humans and swine in China (18). These observations further proved that the genetic environment of the *cfr* gene in clinical staphylococci in China was closely related to that in livestock isolates, and the transmission of *cfr*-carrying fragments and/or plasmids between staphylococci from animals and humans appears to be likely.

Two outbreaks of *cfr*-carrying MRSA and *S. epidermidis* in Madrid, Spain, and Ohio were described in 2010 (20–22). Both outbreaks were associated with nosocomial transmission and prior linezolid exposure. In the current study, however, linezolid therapy seemed not to contribute to the emergence of resistant strains, according to records regarding previous antimicrobial therapy. Notably, 16 MRCoNS with the same *cfr*-carrying plasmid isolated from ICUs were identified in the previous 3 years. CoNS, which are usually ignored, might become the reservoir of the *cfr* gene and result in the dissemination of the resistance gene.

In conclusion, this is the first report of clonal spread of *cfr*-harboring MRSA in China. The same *cfr*-carrying plasmid disseminated among multiple staphylococcal species in ICUs of our hospital. The *cfr* gene, carried by a similar mobile genetic organization, appears to easily transfer horizontally among bacteria of both human and animal origins in China.

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