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 (SCCmec II)-spa t311, were investigated in this study. Complete sequencing of a cfr-carrying plasmid, pLRSA417, revealed an 8,487-bp fragment containing a Tn4001-like transposon, cfr, orf1, and ISEnf. 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), Ru’ian (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.

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

* TZP, piperacillin-tazobactam; CMN, cefminox; MOX, moxifloxacin; MEM, meropenem; TEC, teicoplanin; AK, amikacin; PB, polymyxin B.
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 (SCCmec) 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)-SCCmec 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.

![Genetic map of pLRSA417](http://aac.asm.org)
An 8,487-bp fragment containing a Tn4001-like transposon, cfr, orf1, and ISEnfa4 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), pTLC in S. epidermidis from the United States (19), and pHNF3 from a pig in Guangzhou (Fig. 2). It seemed that the genetic structures of the Tn4001-like transposon, cfr, and ISEnfa4 play an important role in the mobility of cfr in different staphylococcal plasmids of different origins. In addition to ISEnfa4, another insertion sequence, IS21-558, is usually ignored, might become the reservoir of the cfr gene in clinical staphylococci in China. The same genetic environment surrounding the cfr gene and the presence of other mechanisms account for the very high linezolid resistance of Staphylococcus aureus from Hong Kong by phenotypic typing, pulsed-field gel electrophoresis, and fluorescent amplified-fragment length polymorphism analysis. J Clin Microbiol 41:4980–4985. http://dx.doi.org/10.1128/JCM.41.11.4980-4985.2003.


