Identification of Multiresistance Gene cfr in Methicillin-Resistant *Staphylococcus aureus* from Pigs: Plasmid Location and Integration into a Staphylococcal Cassette Chromosome mec Complex

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The multiresistance gene *cfr* was found in 8/231 porcine methicillin-resistant *Staphylococcus aureus* isolates. They were characterized by multilocus sequence typing, *spa* typing, *dru* typing, and staphylococcal cassette chromosome mec (*SCCmec*) typing as ST627-t002-dt12w-IVb, ST6-t304-dt12w-IVb, ST9-t899-dt12w-IVb, ST9-t899-dt12ae-IVb, or ST63-t899-dt12v-IVb. Different *cfr* gene regions were detected on plasmids of ca. 35 kb in seven isolates. For the first time, an IS*Enfad-cfr-Is256* fragment was found to be inserted upstream of the *cfr* genes in a chromosomal *SCCmec IVb* element of the remaining isolate.

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) strains mainly play a role as colonizers of food-producing animals and humans who have occupational and otherwise close contact with these animals (1–5). However, they can also cause infections in humans (6) and animals (7, 8). The important LA-MRSA clones are usually of the multilocus sequence type 398 (ST398) in the United States (1, 2) and European countries (9) and/or of ST9 in Asian countries (10), including China. LA-MRSA strains have acquired a number of novel and unusual antimicrobial resistance genes (11–15), including multiresistance genes that confer resistance to critically and highly important antimicrobial agents in human medicine (16). One such gene is the gene *cfr*, which confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A (17). Oxazolidinones are last resort antimicrobial agents for the control of serious infections caused by MRSA and vancomycin-resistant enterococci in humans. Although *cfr*-carrying MRSA strains have been reported in human medicine (18, 19), *cfr*-carrying LA-MRSA strains of animal origin have been reported rarely (20, 21).

The 231 porcine MRSA isolates included in this study were collected in August 2012 and August 2013 from nasal swabs of healthy pigs at farms (*n* = 7) and slaughterhouses (*n* = 3) in the provinces of Henan (*n* = 119) and Shandong (*n* = 45) and in Shanghai (*n* = 15) and from the lungs of diseased pigs at an animal hospital in the Guangdong province (*n* = 52). All isolates were screened for the presence of *cfr* using previously described primers (22). Only eight (3.5%) of the MRSA isolates were positive for *cfr*. All *cfr*-carrying MRSA isolates had linezolid MICs of 4 mg/liter, which classifies them as borderline susceptible based on the current CLSI-approved breakpoints (23). *cfr*-positive staphylococci and enterococci with linezolid MICs of 4 mg/liter have been reported before (24, 25). The carriage rates for *cfr* differed geographically, with 5/15 (33.3%) *cfr*-positive isolates observed in Shanghai, followed by 2/52 (3.84%) in the Guangdong Province and 1/119 (0.84%) in the Henan Province. None of the isolates from Shandong contained *cfr*.

The eight *cfr*-carrying isolates were subjected to staphylococcal chromosomal cassette mec (*SCCmec*) typing, multilocus sequence typing (MLST) (http://saureus.mlst.net), *spa* typing (http://spaserver.ridom.de), *dru* typing (http://dru-typing.org), and Smal pulsed-field gel electrophoresis (PFGE) (4). All isolates shared *SCCmec* type IVb, while three different PFGE-MLST-*spa* gene profiles were observed: A-ST6-t304, B-ST6-720-1002, and C-ST63-t899 (Table 1). ST63 is a variant of ST9, differing by three single base pair exchanges in the *arcC* locus. The *dru* type dt12w was observed in six strains, while dt12v and the novel *dru* type dt12ae were observed in individual lung isolates collected in Guangdong.

All eight MRSA isolates were examined for their antimicrobial resistance phenotypes and genotypes. Resistance genes were detected by specific PCR assays (22, 26, 27) and sequence analyses. Antimicrobial susceptibility testing was performed by broth microdilution (28, 29), and 16 antimicrobial agents, including amoxicillin, penicillin G, ceftoxin, oxacillin, chloramphenicol, florfenicol, erythromycin, tiamulin, clindamycin, tetracycline, streptomycin, virginiamycin M1, linezolid, trimethoprim-sulfamethoxazole, spectinomycin, and vancomycin, were used. Besides *cfr*, all isolates harbored the *mecA* and *fexA* genes for resistance to methicillin and chloramphenicol-florfenicol, respectively, and all isolates, except HP15 and HP11, carried the tetracycline resistance gene *tet(L)*. Three isolates (HP29, HP30, and HP32) contained the gene *vga*(A)*v*, while another three isolates (SH150, 1518, and 1530) carried the gene *Isu(E)*, each of which code for ABC transporters that confer resistance to lincosamides, pleuromutilins, and streptogramin A. Isolates SH50, 1518, and 1530 also harbored a chromosomal 12,120-bp segment carrying a resistance gene cluster.
TABLE 1 Characteristics of the cfr-carrying MRSA isolates from pigs at slaughter and from the lungs of diseased pigs

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Origin</th>
<th>Sample site</th>
<th>Orientation</th>
<th>Resistance phenotype</th>
<th>dru</th>
<th>pfge type</th>
<th>SCCmec type</th>
<th>gpt</th>
<th>art</th>
<th>eae</th>
<th>hp1</th>
<th>IS256</th>
<th>mecr1</th>
<th>mecR1</th>
<th>J1 region</th>
<th>J2 region</th>
<th>J3 region</th>
<th>Location of resistance genes (kb) on the chromosomal DNA of MRSA isolate JCSC6690</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP15</td>
<td>SH</td>
<td>Nose</td>
<td>N/A</td>
<td>1-12v (N)</td>
<td></td>
<td>A1 -H11011</td>
<td>SS, HC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>dmeR1 and eaeA located at the left border (1,157 bp) of SCCmec, with the genes aadE (streptomycin resistance), spa (speci-</td>
</tr>
<tr>
<td>HP11</td>
<td>SH</td>
<td>Nose</td>
<td>N/A</td>
<td>1-12v (N)</td>
<td></td>
<td>B1 -H11011</td>
<td>SS, HC</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mecr1 and spa (speci-</td>
</tr>
<tr>
<td>HP10</td>
<td>SH</td>
<td>Nose</td>
<td>N/A</td>
<td>1-12v (N)</td>
<td></td>
<td>B1 -H11011</td>
<td>SS, HC</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>mecr1 and spa (speci-</td>
</tr>
<tr>
<td>HP30</td>
<td>SH</td>
<td>Nose</td>
<td>N/A</td>
<td>1-12v (N)</td>
<td></td>
<td>B1 -H11011</td>
<td>SS, HC</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>mecr1 and spa (speci-</td>
</tr>
<tr>
<td>HP29</td>
<td>SH</td>
<td>Nose</td>
<td>N/A</td>
<td>1-12v (N)</td>
<td></td>
<td>B1 -H11011</td>
<td>SS, HC</td>
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<td>mecr1 and spa (speci-</td>
</tr>
<tr>
<td>HP30</td>
<td>SH</td>
<td>Nose</td>
<td>N/A</td>
<td>1-12v (N)</td>
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<td>B1 -H11011</td>
<td>SS, HC</td>
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<td>mecr1 and spa (speci-</td>
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<tr>
<td>HP30</td>
<td>SH</td>
<td>Nose</td>
<td>N/A</td>
<td>1-12v (N)</td>
<td></td>
<td>B1 -H11011</td>
<td>SS, HC</td>
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<td>mecr1 and spa (speci-</td>
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<tr>
<td>SH50</td>
<td>HN</td>
<td>Nose C1</td>
<td>N/A</td>
<td>1-12v (N)</td>
<td></td>
<td>C1 -H11011</td>
<td>SS, HC</td>
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<td>mecr1 and spa (speci-</td>
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<tr>
<td>1518</td>
<td>GD</td>
<td>Lung C2</td>
<td>C3</td>
<td>1-12v (N)</td>
<td></td>
<td>C3 -H11011</td>
<td>SS, HC</td>
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<td>mecr1 and spa (speci-</td>
</tr>
<tr>
<td>1530</td>
<td>GD</td>
<td>Lung C3</td>
<td>C3</td>
<td>1-12v (N)</td>
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<td>C3 -H11011</td>
<td>SS, HC</td>
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<td>mecr1 and spa (speci-</td>
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With the genes aadE (streptomycin resistance), spa (speci- |

In conclusion, the best of our knowledge, this is the first time that cfr was detected in the J region of an SCCmec element. The finding that cfr-carrying MRSA isolates from pigs carry additional resistance genes warrants continuous surveillance, as it underlines the potential for coselection and persistence of these isolates under the selective pressure by various other antimicrobial agents.
Nucleotide sequence accession number. The DNA sequence of the cfr-carrying segment in MRSA isolate 1518 was assigned GenBank accession no. KP777553.

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