Methicillin-Resistant and -Susceptible *Staphylococcus aureus* Strains of Clonal Lineages ST398 and ST9 from Swine Carry the Multidrug Resistance Gene *cfr*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen that causes health care- and community-associated infections in humans worldwide (2, 7, 18). Recent reports indicated that MRSA isolates of multilocus sequence type ST398 have their main reservoir in swine (4, 9, 25). MRSA strains of clonal lineage ST398 but also methicillin-susceptible *S. aureus* (MSSA) strains of other clonal lineages are able to colonize and cause infections in various other animal species and humans (14, 20, 24, 28–30). Currently, the colonization of persons with exposure to swine farming and subsequent infections in humans are a matter of worldwide concern (9, 27, 30, 31).

Due to the common multiresistance phenotype displayed by MRSA ST398 strains, therapeutic options are limited. Among the antimicrobial agents considered the most-promising therapeutics, oxazolidinones play a dominant role in the treatment of MRSA infections (5). In addition, pleuromutilins, represented by the recently FDA-approved retapamulin, are important drugs for the therapy of *S. aureus* skin infections (10). However, transferable oxazolidinone and pleuromutilin resistance mediated by the gene *cfr* has been reported recently in staphylococci from animals in Germany and Denmark (11, 12) and from humans in Colombia and the United States (17, 22).

The gene *cfr* codes for a methyltransferase that targets 23S rRNA. The Cfr-mediated resistance phenotype includes besides oxazolidinones and pleuromutilins—phenicols, lincosamides, and streptogramin A antibiotics, all of which share overlapping binding sites in close proximity to 23S rRNA (15).

To date, very little is known about the dissemination of the gene *cfr* among *S. aureus* strains from animals. The aim of the present study was to investigate *S. aureus* strains of porcine origin for the presence of this and other antibiotic resistance genes and to further characterize *cfr*-positive strains. For this, nasal swabs were taken from 846 swine from 367 farms all over Germany during 2007 and investigated at the National Reference Laboratory for Staphylococci in Germany at the Robert Koch Institute (RKI). A total of 110 porcine *S. aureus* isolates were identified. Studies at the Institute of Farm Animal Genetics of the Friedrich-Loeffler-Institute (FLI) included the screening of 90 porcine coagulase-positive and coagulase-variable staphylococci collected all over Germany from diseased swine in the BIT-GermVet study 2004-2006 (19) and 56 non-related porcine *S. aureus* strains provided by veterinary diagnostic laboratories from all over Germany and collected mainly in 2008. In total, two staphylococcal strains of porcine origin, one from the RKI study and the other from the FLI study, displayed a resistance phenotype indicative of the presence of *cfr* (Table 1). Both strains originated from swine farms in different geographic areas of northern Germany, were isolated in 2004 and 2007, respectively, and carried the gene *cfr*, as confirmed by PCR analysis and sequencing of the amplicons. One of these strains showed an oxacillin MIC of ≥32 μg/ml and was classified as a MRSA strain. The mecA gene and staphylococcal cassette chromosome mec type V were detected by multiplex PCR assays (13). The species identification of both strains as *S. aureus* was confirmed biochemically with the ID32Staph system (bioMérieux, Nürtingen, Germany). The further characterization of these strains included multilocus sequence typing (MLST) (6) and *spa* typing (http://spaserver2.ridom.de/index.shtml). The *cfr*-carrying MRSA strain belonged to the clonal lineage ST398 with the MLST allelic profile 3-35-2-20-26-39. This isolate was characterized by *spa* typing as t034. In contrast, the *cfr*-carrying MSSA strain exhibited *spa* type t3198 and was assigned to the MLST type ST9 based on its allelic profile, 3-3-1-1-1-1-10.

Both strains, MRSA ST398 and MSSA ST9, carried the *cfr* gene on ca. 36-kb plasmids, as confirmed by protoplast transformation into *S. aureus* RN4220 (12). The transformants exhibited the elevated MICs of phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics, which confirmed the functional activity of the gene *cfr* (Table 1). Both plasmids were indistinguishable by their BglII restriction patterns from the previously described *cfr*-carrying plasm-
mid pSCFS3 (12). The cfr-containing BglII fragments obtained from the \textit{S. aureus} RN4220 transformants were cloned into the BamHI site of vector pBluescript II SK+ (Stratagene, Amsterdam, The Netherlands). A sequence analysis of the cfr gene regions confirmed the assignment of both plasmids to the pSCFS3 type (12). This plasmid type carries the multidrug resistance gene \textit{cfr} as well as the phenicol exporter gene \textit{fexA}. The latter is usually part of transposon Tn558, and the \textit{fexA} reading frame is completely retained on plasmid pSCFS3. In contrast, the Tn558-associated transposable genes \textit{tnpA} and \textit{tnpB} are deleted in plasmid pSCFS3 by the insertion of a \textit{tnpB}-associated transposase genes reading frame is completely retained on plasmid pSCFS3. In

Previous studies suggested that there is a high risk for swine farmers, veterinarians, or people with exposure to swine farming to be colonized with \textit{S. aureus} strains of sequence types ST398 or ST9 (1). As in other European countries, MRSA ST398 is widely disseminated as a nasal colonizer among pigs in Germany (16) and represented 0.22% of all MRSA strains from human infections in Germany in 2006/2007 (3). MRSA ST398 was also detected in outpatients or inpatients with ventilator-associated pneumonia in central Europe (30) and was assumed to have already entered the food chain (26). Among the 655 MRSA ST398 strains from pigs as well as from colonization and infections in humans that have been tested to date at RKI and FLI, only the single strain described in this study carried the gene \textit{cfr}. In addition, \textit{S. aureus} ST9 strains have

been shown to be frequently disseminated among swine in France and have not been reported so far from other animal species (1). However, MSSA ST9 strains have occasionally been isolated from healthy human carriers (8). This sequence type seems to be rare among \textit{S. aureus} isolates from infections in humans in Germany: in a study conducted by RKI and comprising 2,353 strains collected from various infections in humans all over Germany in 2007, only a single ST9 strain was identified.

Even if our initial studies do not allow us to estimate the prevalence of the gene \textit{cfr} in \textit{S. aureus} strains from swine, we report here the first two porcine \textit{S. aureus} isolates of sequence types ST398 and ST9 that carry the multidrug resistance gene \textit{cfr} on a plasmid. As there is obviously no pronounced host specificity with respect to the colonization of pigs and humans, the transfer of cfr-carrying \textit{S. aureus} ST398 or ST9 from swine to humans and a further spread of human-adapted \textit{S. aureus} strains cannot be excluded.

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\textit{REFERENCES}


\begin{table}
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\textbf{Bacterial strain} & \textbf{Resistance genes} & \textbf{TIA} & \textbf{VIR M$_1$} & \textbf{LZD} & \textbf{CLI} & \textbf{CHL} & \textbf{FFC} & \textbf{ERY} & \textbf{Q/D} & \textbf{TET} & \textbf{OXA} \\
\hline
\textit{S. aureus} ST398 & \textit{cfr}, \textit{fexA}, \textit{erm}(A), \textit{tet}(M), \textit{meC} & 128 & 128 & 4 & 64 & 128 & 128 & 25 & 0.25 & 0.25 & 0.25 \\
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\textit{S. aureus} RN4220 & \textit{cfr}, \textit{fexA}, \textit{erm}(C) & 128 & 128 & 4 & 64 & 128 & 128 & 25 & 0.25 & 0.25 & 0.25 \\
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\textit{S. aureus} RN4220/pSCFS3 (ST398 transformant) & \textit{cfr}, \textit{fexA} & 128 & 128 & 4 & 64 & 128 & 128 & 25 & 0.25 & 0.25 & 0.25 \\
\hline
\textit{S. aureus} RN4220/pSCFS3 (ST9 transformant) & \textit{cfr}, \textit{fexA} & 128 & 128 & 4 & 64 & 128 & 128 & 25 & 0.25 & 0.25 & 0.25 \\
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\caption{MICs of the \textit{cfr}-carrying MRSA ST398 and MSSA ST9 strains and their pSCFS3-carrying \textit{S. aureus} RN4220 transformants.}
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