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

Corinna Kehrenberg,[1]* Christiane Cuny,[2] Birgit Strommenger,[2] Stefan Schwarz,[1]* and Wolfgang Witte[2]

Institute of Farm Animal Genetics, Friedrich-Loeffler-Institute, Neustadt-Mariensee, Germany,[1] and Robert Koch Institute, Wernigerode Branch, 38855 Wernigerode, Germany[2]

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Methicillin-resistant *Staphylococcus aureus* clonal lineage ST398 and methicillin-susceptible lineage ST9 strains have their main reservoir in swine but can colonize and cause infections in humans. The phenicol/lincosamide/oxazolidinone/pleuromutilin/streptogramin A multidrug resistance gene *cfr* was detected in isolates of both clonal lineages, rendering a spread to humans with exposure to swine farming possible.

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 A2503 in 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 A2503 (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 BfT-GermVet study 2004-2006 (19) and 56 nonrelated 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 3-35-19-2-20-26-39. This isolate was characterized by *spa* typing as t034. In contrast, the *cfr*-carrying MSSA strain exhibited s*pa* 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 plas-

* Corresponding author. Mailing address: Institute of Farm Animal Genetics, Friedrich-Loeffler-Institute (FLI), Höltystr. 10, 31535 Neustadt-Mariensee, Germany. Phone for Corinna Kehrenberg: 49-5034-871242. Fax: 49-5034-871246. E-mail: corinna.kehrenberg@fli.bund.de. Phone for Stefan Schwarz: 49-5034-871241. Fax: 49-5034-871246. E-mail: stefan.schwarz@fli.bund.de.

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TABLE 1. MICs of the *cfr*-carrying MRSA ST398 and MSSA ST9 strains and their pSCFS3-carrying *S. aureus* RN4220 transformants

Bacterial strain	Resistance genes	MIC (μg/ml)[a]									
		TIA	VIR M_1	LZD	CLI	CHL	FFC	ERY	Q/D	TET	OXA
MRSA ST398	*cfr*, *fexA*, *erm*(A), *tet*(M), *mecA*	≥128	128	4	≥64	≥128	≥128	≥64	4	64	≥32
MSSA ST9	*cfr*, *fexA*, *erm*(C)	≥128	128	8	≥64	≥128	≥128	≥64	4	0.12	0.06
S. aureus RN4220		0.063	4	0.5	0.125	4	2	0.25	0.25	0.25	0.06
S. aureus RN4220/pSCFS3 (ST398 transformant)	*cfr*, *fexA*	≥128	128	4	≥64	≥128	128	0.25	1	0.25	0.06
S. aureus RN4220/pSCFS3 (ST9 transformant)	*cfr*, *fexA*	≥128	128	4	≥64	≥128	128	0.25	1	0.25	0.06

[a] The antimicrobial agents are abbreviated as follows: TIA (tiamulin), VIR M_1 (virginamycin M_1), LZD (linezolid), CLI (clindamycin), CHL (chloramphenicol), FFC (florfenicol), ERY (erythromycin), Q/D (quinupristin-dalfopristin), TET (tetracycline), and OXA (oxacillin).

mid pSCFS3 (12). The *cfr*-containing BglII fragments obtained from the *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 *cfr* as well as the phenicol exporter gene *fexA*. The latter is usually part of transposon Tn*558*, and the *fexA* reading frame is completely retained on plasmid pSCFS3. In contrast, the Tn*558*-associated transposase genes *tnpA* and *tnpB* are deleted in plasmid pSCFS3 by the insertion of a 4,674-bp segment. This segment carries the *cfr* gene and a copy of the insertion sequence IS*21-558*, which is supposed to be involved in the mobility of *cfr* (11).

Although plasmid pSCFS3 is not conjugative, it was shown to be transferable into another staphylococcal recipient strain which then expressed the *cfr*-associated resistance phenotype. When entering a new host cell, plasmid pSCFS3 may undergo recombinational events with plasmids already residing in new host cells. In this regard, it should be noted that a pSCFS3-analogous *cfr* gene region was recently found on a 55-kb plasmid in a *cfr*-carrying MSSA isolate detected during the 2007 LEADER program in the United States (17). Although our results indicated that the *cfr*-carrying plasmids in the two porcine strains harbored only the resistance genes *cfr* and *fexA*, PCR analysis (12, 21) revealed that both strains also carried a macrolide, lincosamide, and streptogramin B resistance gene, *erm*(A) in MRSA ST398 and *erm*(C) in MSSA ST9, and that the MRSA isolate was also resistant to tetracyclines via a *tet*(M) gene. These observations point toward further limited therapeutic options in the control of these *cfr*-carrying strains.

Previous studies suggested that there is a high risk for swine farmers, veterinarians, or people with exposure to swine farming to be colonized with *S. aureus* strains of sequence types ST398 or ST9 (1, 23). 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 *cfr*. In addition, *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 *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 *cfr* in *S. aureus* strains from swine, we report here the first two porcine *S. aureus* isolates of sequence types ST398 and ST9 that carry the multidrug resistance gene *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 *S. aureus* ST398 or ST9 from swine to humans and a further spread of human-adapted *S. aureus* strains cannot be excluded.

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