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Characterization of methicillin-resistant *Staphylococcus aureus* ST398 from cases of bovine mastitis

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Objectives: Twenty-five MRSA ST398 isolates from cases of bovine clinical mastitis and two isolates from farm personnel collected from 17 dairy farms in Germany were investigated for genetic relatedness, antimicrobial resistance and virulence properties.

Methods: Genomic relationships were determined by ApaI PFGE, *spa* typing, SCC*mec* typing and *dru* typing. Antimicrobial resistance phenotypes were determined by broth microdilution. Resistance and virulence genes were detected via a diagnostic DNA microarray and specific PCRs.

Results: Nine major ApaI PFGE patterns were detected. Three *spa* types (t011, t034 and t2576) and two SCC*mec* types (IV and V) were identified. Five different *dru* types were seen with dt11a being predominant. All isolates were negative for Panton–Valentine leucocidin, enterotoxin and exfoliative toxin genes. Ten resistance patterns were observed with 11 (40.7%) isolates being resistant to only β-lactam antibiotics and tetracyclines. Several resistance genes were detected: *blaZ* (penicillin resistance); *tet*(M), *tet*(K) and *tet*(L) (tetracycline resistance); *erm*(A), *erm*(B), *erm*(C) and *erm*(T) (macrolide/lincosamide/streptogramin B resistance); *aacA–aphD*, *aphA3*, *aadD* and *spc* (aminoglycoside or aminocyclitol resistance); *fexA* (phenicol resistance); *dfrK* (trimethoprim resistance); and *vga*(A) and *vga*(C) (pleuromutilin/lincosamide/streptogramin A resistance). The two human isolates were indistinguishable in their genotypic and phenotypic characteristics from the mastitis isolates of the same farm.

Conclusions: As previously described for ST398 from swine, isolates of this sequence type from cases of bovine mastitis also demonstrated a high degree of variability when ApaI PFGE profiles and other genotypic and phenotypic characteristics were compared. A uniform virulence gene pattern appeared to be conserved between ST398 isolates from both animal species.

Keywords: zoonosis, antimicrobial resistance, staphylococci, molecular epidemiology

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen in human medicine, but can also colonize and cause infections in a variety of animal species.[1] During recent years, MRSA isolates of the clonal lineage ST398 have gained particular attention as colonizers and—more rarely—causative agents of infections in pigs.[2,3] Previous studies have shown that ST398 isolates are not restricted to pigs, but can also be isolated from humans, dogs and horses.[4–6] Due to the low host specificity of MRSA ST398, transfer of such isolates between different animal species, but also between humans and animals, might occur in either direction. People with close contact with swine, but also with other MRSA ST398-carrying and -shedding animals, are at risk of being colonized by these isolates.[5–7] Colonized people may play a role in the further spread of MRSA ST398 between different farms and different animal species.[5,8] A first outbreak of MRSA ST398 in a Dutch hospital was reported in June 2007[9] and underlines its zoonotic potential. To follow the dissemination of MRSA ST398 isolates within and beyond different animal and human populations, tools for detailed characterization are necessary. In a previous study we showed that ApaI PFGE and *spa* typing supplemented by genotyping using a diagnostic DNA microarray are suitable tools to gain insight not only into the genetic diversity, but also into the virulence and antimicrobial resistance genes, of porcine MRSA ST398 isolates.[10]

In contrast to the wealth of data on MRSA isolates from companion animals, horses and pigs, comparatively little is known about MRSA isolates in cattle. The aim of this study was to characterize MRSA isolates from cases of bovine mastitis for their genomic relationships and for their resistance and virulence properties. For this, the diagnostic microarray and classic typing techniques have been applied and were supplemented by direct repeat unit (*dru*) typing, a new sequence-based typing technique that targets the variable-number tandem repeat (VNTR) region adjacent to IS*431* in SCC*mec* cassettes.[11]

Materials and methods

MRSA isolates

A total of 25 MRSA isolates from cases of bovine clinical mastitis and two isolates from farm personnel were included in this study. These isolates were obtained during 2008–09 and they originated from 17 dairy farms in four different regions in Germany, namely the south (Bavaria), the south-west (Baden-Württemberg), the north-west (North Rhine-Westphalia) and the north (Lower Saxony) of Germany (see Table 1). Depending on the availability of isolates as well as on the structure and size of the dairy farms, one to four bovine isolates per farm were included. The two human isolates were provided on a voluntary basis. Initial identification of the isolates as MRSA was conducted by the local diagnostic laboratories that provided the isolates [TGD Poing (Bavaria), CVUA Stuttgart/Fellbach (Baden-Württemberg), MBFG Wunstorf (Lower Saxony) and LUFA Nord-West Oldenburg (Lower Saxony)].

Molecular typing and DNA microarray analysis

All 27 MRSA isolates were subjected to *spa* sequence typing (http://spaserver.ridom.de), PFGE with ApaI as well as SmaI and PCR-directed SCC*mec* typing. For this, previously described protocols were applied.[10] The ApaI fragment patterns were analysed with the GelCompar software package (Applied Maths, Kortrijk, Belgium) and the similarities between the profiles were calculated using the Dice coefficient with a maximum position tolerance of 1.2%. The patterns were clustered by using the unweighted pair group method with arithmetic averages (UPGMA). For *dru* typing, primers dru1 (5′-GTTAGCATATTACCTCTCCTTGC-3′) and dru2 (5′-GCCGATTGTGCTTGATGAG-3′) were used.[11] The PCR program included an initial denaturation step at 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, 52°C for 1 min and 72°C for 1 min and a final extension at 72°C for 5 min. The amplicons were sequenced and compared with the *dru* sequences and *dru* types stored in the *dru* typing database available at http://dru-typing.org.

Detection of a variety of resistance, species, typing and virulence markers was conducted by using the previously described *S. aureus*-specific diagnostic DNA microarray (StaphyType; CLONDIAG, Jena, Germany) that recognizes 330 specific sequences (∼200 genes and alleles thereof).[10,12] Arrays were mounted in microtitre strips (ArrayStrip/ArrayMate/StaphyType system by CLONDIAG) and processed according to the manufacturer's protocols. An image of the array was recorded and automatically analysed using a designated reader and software (ArrayMate and IconoClust, both by CLONDIAG).

Susceptibility testing and detection of resistance genes

Antimicrobial susceptibility testing by broth microdilution followed the recommendations of the CLSI.[13] Custom-made microtitre plate panels were used. In total, susceptibilities to 27 antimicrobial agents were determined and for each of the antimicrobial agents, 10–12 concentrations in 2-fold dilution series were tested (see Table 2). In addition, isolates that were positive in the DNA microarray for the aminoglycoside resistance genes *aadD* and *aphA3* were tested by broth macrodilution[13] for their MICs of kanamycin. The reference strain *S. aureus* ATCC® 29213 served as a quality control strain in the MIC determinations.

The detection of resistance genes was performed mainly via the diagnostic microarray. Additional specific PCR assays served to detect resistance genes recently described to occur in MRSA ST398 isolates and/or not yet included in the microarray. These included the tetracycline resistance gene *tet*(L), the trimethoprim resistance gene *dfrK*, the pleuromutilin/lincosamide/streptogramin A resistance gene *vga*(C), the macrolide/lincosamide/streptogramin B (MLS_B) resistance gene *erm*(T) and the spectinomycin resistance gene *spc*.[14–17] For the detection of *tet*(L), a previously described PCR assay was used.[18] The following primer pairs were designed and used for the detection of the resistance genes: *dfrK* (dfrK_fw, 5′-GCTGCGATGGATAATGAACAG-3′; dfrK_rv, 5′-GGACGATTTCACAACCATTAAAGC-3′; amplicon size: 214 bp); *vga*(C) (vgaC_fw, 5′-CCGTATGCCCAGAGTGAGAT-3′; vgaC_rv, 5′-TGCTTGGGAACAAGTCCTTC-3′; amplicon size: 671 bp); *spc* (spc_fw, 5′-ACCAAATCAAGCGATTCAAA-3′; spc_rv, 5′-GTCACTGTTTGCCACATTCG-3′; amplicon size: 561 bp); and *erm*(T) (ermT_fw, 5′-ATTGGTTCAGGGAAAGGTCA-3′; ermT_rv, 5′-GCTTGATAAAATTGGTTTTTGGA-3′; amplicon size: 536 bp). For PCR-directed confirmation of the physical linkage between the genes *tet*(L) and *dfrK* the forward primer for *tet*(L) (TK1, 5′-CAAACTGGGTGAACACTG-3′) was combined with the above-mentioned reverse primer for *dfrK* to result in an amplicon of 1739 bp. For detection of the physical linkage between *erm*(A) and *spc*, the primer pair spc-erm(A)_1 (5′-AAGGCTATCGGGGAGAGTGT-3′) and spc-erm(A)_2 (5′-GCAACGAGCTTTGGGTTTAC-3′) was used to result in an amplicon of 548 bp. A standard PCR program consisting of initial denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 1 min, annealing for 1 min and primer extension at 72°C for 1 min and a final extension step at 72°C for 5 min was used. The annealing temperatures were 50°C (*dfrK*), 58°C [*vga*(C)], 52°C (*spc*), 45°C [*erm*(T) and *tet*(L)–*dfrK*] and 55°C [*spc*–*erm*(A)]. For the detection of *dfrK*, 35 cycles were used and all times during the cycles were reduced from 1 min to 30 s. For detection of the *tet*(L)–*dfrK* product, 35 cycles were also used and the time for primer extension during each cycle was 3 min.

Results

Molecular typing and microarray analysis

All 27 isolates proved to be non-typeable by SmaI PFGE, but produced nine different major ApaI patterns with up to three subpatterns (Table 1). A comparison with the geographical origin of the isolates showed that pattern A was found among isolates from farms of all four geographical regions and pattern D was detected among isolates from two different farms each in the north-west and south of Germany. On the other hand, multiple isolates from the same farm often showed indistinguishable or very closely related ApaI patterns such as patterns B, F, E1 and E2 among the isolates from farms 1, 2, 4 and 5 in Baden-Württemberg (Table 1).

Three different *spa* types were identified with t011 ($n=23$) representing the dominant type. Three MRSA isolates showed t034 and a single isolate t2576 (Table 1). A closer look at the repeat units revealed that all three *spa* types are closely related; t034 (08-16-02-25-02-25-34-24-25) differed from t011 (08-16-02-25-34-24-25) by the duplication of the repeats 02-25 and t2576 (08-12-16-02-25-34-24-25) had an additional repeat 12 in comparison with t011.

SCC*mec* typing revealed the presence of a type IV SCC*mec* cassette and a non-typeable cassette in single isolates and type V cassettes in the remaining 25 isolates. The *dru* typing approach targets a non-coding VNTR region adjacent to IS*431*

Table 1. Comparison of the characteristics of the 27 MRSA ST398 isolates

Isolate[a]	Region-farm[b]	*spa* type	PFGE type	SCC*mec* type	*dru* type	Resistance pattern[c,d]	Resistance genes															
							blaZ-I-R	*tet*(M)	*tet*(K)	*tet*(L)	*erm*(A)	*erm*(B)	*erm*(C)	*erm*(T)	*vga*(A)	*vga*(C)	*dfrK*	*spc*	*aphA3*	*aacA–aphD*	*aadD*	*fexA*
3	BA-4	t011	I	IV	dt10q	BLA, TET, TMP	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−
40, 41	BA-3	t011	A1	V	dt11a	BLA, TET	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
9	BW-3	t011	A3	V	dt11a	BLA, TET	+	+	+	−												
58	BW-4	t011	A1	V	dt10a	BLA, TET	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
55, 56, **60**, hu15, hu17	BW-2	t011	B	V	dt11a	BLA, TET	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
1, 4	NW-1, NW-3	t011	D	V	dt11a	BLA, TET	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
48, **50**	BW-1	t011	E2	V	dt11a	BLA, TET, TIA	+	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−
6	LS-5	t034	H	non-typeable	dt11a	BLA, TET, MLS_B, TMP, KAN, SPT, TIA	+	+	−	+	+	−	+	−	−	+	+	+	+	−	+	−
5	LS-4	t011	G	V	dt11a	BLA, TET, MLS_B, TMP, GEN, KAN	+	+	+	−	−	−	+	−	−	−	−	−	−	+	−	−
7, 8	BA-1, BA-2	t011	D	V	dt11a	BLA, TET, MLS_B, TMP, SUL, GEN, KAN	+	+	+	+	−	−	−	+	−	−	+	−	−	+	+	−
53	BW-5	t034	E1	V	dt6j	BLA, TET, MLS_B, TMP, SPT, TIA	+	+	+	−	+	−	−	−	−	−	−	+	−	−	−	−
51	BW-5	t034	E1	V	dt6j	BLA, TET, MLS_B, TMP, SPT, TIA	+	+	+	+	+	+	−	−	−	−	+	+	−	−	−	−
2	NW-2	t011	A2	V	dt11a	BLA, TET, MLS_B, TMP, KAN, APR	+	+	+	+	−	+	−	−	−	−	+	−	−	−	+	−
11	LS-2	t2576	A3	V	dt11a	BLA, TET, MLS_B, TMP, KAN, APR	+	+	+	+	−	+	−	−	−	−	+	−	−	−	+	−
44, 46, 47	BW-4	t011	F	V	dt11ab	BLA, TET, MLS_B, TMP, SUL, GEN, KAN, TIA	+	+	+	+	−	+	−	−	+	−	+	−	−	+	+	−
10R, **12**	LS-1, LS-3	t011	C	V	dt11a	BLA, TET, MLS_B, TMP, CHL/FFC, KAN	+	+	+	+	−	−	−	+	−	−	+	−	−	−	+	+

[a]The 16 isolates printed in bold type were selected as representative of the respective type and MIC values for them are included in Table 2.
[b]BA, Bavaria; BW, Baden-Württemberg; LS, Lower Saxony; NW, North Rhine-Westphalia.
[c]APR, apramycin; BLA, β-lactam antibiotics; CHL/FFN, chloramphenicol/florfenicol; GEN, gentamicin; KAN, kanamycin; MLS_B, macrolides/lincosamides/streptogramin B; SPT, spectinomycin; SUL, sulphonamides; TET, tetracyclines; TIA, tiamulin; TMP, trimethoprim.
[d]Despite the lack of CLSI-approved breakpoints, isolates that showed high MIC values of TMP (≥256 mg/L), KAN (≥64 mg/L), SPT (≥512 mg/L), TIA (≥16 mg/L), APR (≥64 mg/L) and FFN (≥32 mg/L) were considered resistant.

Table 2. Distribution of MIC values for the 16 different MRSA ST398 types identified in this study

	No. of isolates with MIC (mg/L)															Susceptible		Intermediate		Resistant[a]	
Antimicrobial agent(s)	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	no.	%	no.	%	no.	%
Oxacillin + 2% NaCl	—	—	—	—	—	—	—	—	1	9	6					—	—			16	100
Penicillin	—	—	—	—	—	—	—	—	2	6	5	3				—	—			16	100
Ampicillin	—	—	—	—	—	—	—	—	4	6	4	2	—			—	—			16	100
Amoxicillin/clavulanic acid[b]	—	—	—	—	—	—	—	11	3	2	—	—	—			11	68.7			5	31.3
Ceftiofur	—	—	—	—	—	—	—	1	10	3	—	2	—			—	—	1	6.3	15	93.7
Cefquinome	—	—	—	—	—	—	—	12	2	2	—	—									
Cefalotin		—	—	—	—	—	9	2	2	2	1	—	—	—		13	81.2	2	12.5	1	6.3
Cefotaxime	—	—	—	—	—	—	—	—	1	11	2	2				1	6.3	13	81.2	2	12.5
Cefoperazone		—	—	—	—	—	—	—	2	8	2	4				10	62.5	2	12.5	4	25
Erythromycin	—	—	—	—	7	—	—	—	—	—	—	9				7	43.8	—	—	9	56.2
Spiramycin		—	—	—	—	—	—	—	7	—	1	—	—	8							
Tylosin tartrate	—	—	—	—	—	—	6	2	—	—	—	—	8								
Clindamycin	—	—	—	3	3	1	—	—	1	—	—	—	8			6	37.5	1	6.3	9	56.2
Pirlimycin	—	—	—	—	—	6	1	—	—	—	1	—	8			7	43.8			9	56.2
Tetracycline			—	—	—	—	—	—	—	—	—	12	4	—	—	—	—	—	—	16	100
Chloramphenicol					—	—	—	—	9	6	—	—	1	—	—	9	56.2	6	37.5	1	6.3
Florfenicol			—	—	—	—	1	8	6	—	—	1	—	—	—						
Gentamicin			—	7	3	1	—	—	2	—	2	1	—	—	—	11	68.7	2	12.5	3	18.8
Spectinomycin			—	—	—	—	—	—	—	—	—	6	7	—	3						
Apramycin	—	—	—	—	—	—	7	5	1	1	—	—	2								
Nalidixic acid		—	—	—	—	—	—	—	—	—	5	8	3	—							
Enrofloxacin	—	—	4	11	1	—	—	—	—	—	—										
Trimethoprim		—	—	3	3	—	—	—	—	—	—	—	—	10							
Sulfamethoxazole/trimethoprim[b]	4	2	—	3	3	1	1	—	—	—	—	2				14	87.5			2	12.5
Tiamulin	—	—	—	1	—	10	—	—	—	2	—	—	3								
Quinupristin/dalfopristin	—	—	—	—	8	3	4	1	—	—	—					11	68.7	4	25	1	6.3
Vancomycin	—	—	—	—	9	7	—	—	—	—	—					16	100	—	—	—	—

[a]Isolates were classified as susceptible, intermediate or resistant, if applicable breakpoints were available in the CLSI documents M31-A3 or M100-S19.[13,24]
[b]The MIC values of amoxicillin/clavulanic acid (2:1) and sulfamethoxazole/trimethoprim (19:1) are given as amoxicillin and trimethoprim MIC values, respectively.
Black vertical lines indicate the CLSI breakpoints used to classify the isolates as susceptible, intermediate (if available) or resistant.
Concentrations not included in the test panel are shaded grey. The isolates displayed in the lowest not tested concentration showed growth in the highest test concentration and the MIC value is equal to or greater than the concentration following the highest test concentration.

in the SCC*mec* cassette, which consists of 40 bp direct-repeat units. Among the 27 MRSA isolates, five different *dru* types were detected with dt11a (repeat pattern 5a-2d-4a-0-2d-5b-3a-2g-3b-4e-3e) being present in 20 isolates. The *dru* types dt11ab (5a-2d-4a-0-2d-5b-3a-2g-3b-4e-2f) and dt6j (5a-2d-4a-0-2d-3e) were present in three and two isolates, respectively, while the types dt10a (5a-2d-4a-0-2d-5b-3a-2g-3b-4e) and dt10q (5a-2d-4a-0-2d-5b-3a-2g-2c-4e) were found in single isolates. It should be noted that dt11ab and dt6j are novel *dru* types that have been identified during the course of this study. Moreover, the comparison with SCC*mec* types showed that the single isolate with a type IV SCC*mec* cassette also had the unique *dru* type dt10q, whereas four different *dru* types were found among the 25 isolates carrying a type V SCC*mec* cassette.

Multilocus sequence typing (MLST) was conducted for four selected isolates, namely for bovine isolates 3 (*spa* type t011, SCC*mec* type IV, *dru* type dt10q), 11 (t2576, V, dt11a) and 53 (t034, V, dt6j) and the human isolate hu15 (t011, V, dt11a). All four isolates were assigned to the clonal lineage ST398 with the MLST allelic profile 3-35-19-2-20-26-39.

Microarray-based genotyping analysis revealed a uniform virulence gene pattern among the 27 MRSA isolates. All isolates were positive for α and δ haemolysin, *agr* group 1 and capsule type 5 and carried a similar set of MSCRAMM (microbial surface components recognizing adhesive matrix molecules) genes, including *clfA* and *clfB* (clumping factors A and B), *fnbA* and *fnbB* (fibronectin binding proteins A and B), *fib* (fibrinogen binding protein), *cna* (collagen binding protein) and *ebpS* (elastin binding protein) among others. All isolates were negative for the Panton–Valentine leucocidin genes *lukF-PV* and *lukS-PV*, the toxic shock syndrome toxin 1 (TSST-1) gene *tst*, the exfoliative toxin genes *etA*, *etB* and *etD* as well as for the staphylococcal enterotoxin genes.

Antimicrobial resistance phenotypes and genotypes

Susceptibility testing revealed the presence of 10 different susceptibility patterns (Table 1). The most frequently seen resistance pattern consisted of resistance to β-lactams and tetracyclines only and was detected in 11 isolates. Another three isolates showed elevated MICs of either trimethoprim or tiamulin in addition to β-lactam and tetracycline resistance (Table 1). The remaining 13 isolates, however, exhibited more expanded resistance patterns that included resistance to members of six to eight classes of antimicrobial agents (Table 1). All MRSA isolates were susceptible to vancomycin (MICs of 0.5–1 mg/L) and showed low enrofloxacin MICs of 0.12–0.5 mg/L. Although MIC values for all 27 isolates were determined, only those for the 16 isolates representing the different MRSA ST398 types, as identified on the basis of all genotypic and phenotypic characteristics, are included in Table 2 to avoid bias by multiple inclusion of isolates of the same type.

All 27 MRSA isolates carried *mecA* and the β-lactamase operon *blaZ-blaI-blaR*. Tetracycline resistance, also present in all 27 isolates, was mediated by the genes *tet*(M)+*tet*(K) in 15 isolates, *tet*(M)+*tet*(K)+*tet*(L) in 10 isolates as well as *tet*(M)+*tet*(L) or *tet*(M) alone in single isolates. All 11 *tet*(L)-positive isolates also carried the trimethoprim resistance gene *dfrK* and exhibited high trimethoprim MICs of ≥256 mg/L. Moreover, in all 11 cases, a physical linkage between *tet*(L) and *dfrK* could be confirmed by PCR. A single isolate carried a *dfrK* gene without *tet*(L). In two trimethoprim-resistant isolates the trimethoprim resistance gene could not be identified (Table 1). MLS_B resistance, seen in 13 isolates, was mediated by either *erm*(A) ($n=1$), *erm*(B) ($n=5$), *erm*(C) ($n=1$), *erm*(A)+*erm*(B) ($n=1$) or *erm*(A)+*erm*(C) ($n=1$). In the remaining four isolates the MLS_B resistance gene *erm*(T) was detected. High spectinomycin MICs of ≥512 mg/L were detected in three isolates that carried the gene *spc* and were positive for *erm*(A). The physical linkage between *erm*(A) and *spc* could be confirmed by PCR in all three cases. All six gentamicin-resistant isolates were also kanamycin resistant and harboured the resistance gene *aacA–aphD* whose gene product confers combined resistance to gentamicin and kanamycin. Regardless of the presence of the gene *aacA–aphD*, all but one of the kanamycin-resistant isolates carried the *aadD* gene and one of the kanamycin-resistant but gentamicin-susceptible isolates harboured an *aphA3* gene in addition to *aadD* (Table 1). Two isolates that represented the same MRSA ST398 type were identified as chloramphenicol resistant and also showed high florfenicol MICs of 64 mg/L. The phenicol exporter gene *fexA* was identified in these two isolates. A trimodal distribution of tiamulin MICs was observed. The isolates with tiamulin MICs of 16 mg/L carried the gene *vga*(A), whereas one of the three isolates with tiamulin MICs of ≥128 mg/L carried the recently described gene *vga*(C). The genetic basis of the high tiamulin MICs for the remaining two MRSA isolates remains to be identified.

Discussion

Mastitis is economically the most relevant disease of dairy cattle in which staphylococci play an important role. Currently, MRSA isolates are found only rarely to be associated with bovine mastitis. Although the first MRSA isolates from bovine mastitis were described in 1975,[19] few studies have dealt with detailed strain characterization.[20–22] In most of these cases, MRSA isolates related to those found in human medicine have been identified.[20–22] In a study from Hungary, PVL-negative MRSA isolates of MLST type ST1/*spa* type t127 carrying a SCC*mec* type IVa cassette and displaying the same SmaI PFGE pattern were identified.[22] A recent study from Turkey identified different PVL-negative MRSA isolates from bovine milk samples; ST8/t190 carrying a type IV SCC*mec* cassette as well as ST239 (and a single allele variant of it)/t030 carrying a type III SCC*mec* cassette.[20] A study of *S. aureus* from cows in Germany and Switzerland identified two MRSA isolates among 128 *S. aureus*, one of which was a clonal complex 8 strain related to the epidemic MRSA strain Irish 01 while the other was an ST398/t034 strain.[21] Three MRSA ST398 isolates of *spa* type t034 have also been identified in the present study in two farms, one from the north and one from the south-west of Germany (Table 1).

Most of the MRSA isolates in the aforementioned studies closely resembled isolates found in human medicine. The presence of hospital-related MRSA isolates in either infected dairy cattle or mastitic milk samples may indicate transmission of these isolates between humans and animals. In the present study, we identified exclusively MRSA ST398 isolates of different *spa* and *dru* types. All these isolates were PVL negative and also negative for staphylococcal enterotoxins, which may play

an important role in food intoxication following the consumption of milk contaminated with *S. aureus*. In one farm from Baden-Württemberg (BW-2; Table 1), two farm workers provided a nasal swab on a voluntary basis and, in both cases, MRSA isolates were identified that were indistinguishable from the three bovine isolates obtained from the same farm. This observation also suggests transfer of MRSA isolates between animals and personnel of the same farm. Since MRSA ST398 has been identified rarely among cattle so far, inquiries at the farms revealed that some of the farms also kept pigs and in one case that a milker worked part-time in a swine production unit. Unfortunately, no samples of the respective swine or the persons have been made available to confirm the presence/absence of MRSA ST398.

A comparison of the characteristics of the bovine MRSA ST398 isolates with those previously described for porcine MRSA ST398 isolates[10] revealed similarities with regard to the typing results, the virulence patterns and the resistance patterns. The most common SCC*mec* and *spa* types in both test populations were SCC*mec* V and t011 and t034. Bovine and porcine isolates showed largely similar virulence patterns except that few porcine isolates were positive for the staphylococcal enterotoxin genes *seb* or *sek* and *seq*.[10] When comparing the resistance genotypes and phenotypes detected, again striking similarities to the porcine isolates became apparent. All bovine isolates were resistant to β-lactams and tetracyclines. In addition to the *mecA* gene, all bovine MRSA ST398 carried the *blaZ-blaI-blaR* β-lactamase operon. For tetracycline and MLS_B resistance genes, the most common staphylococcal *tet* genes [*tet*(M), *tet*(K) and *tet*(L)] and *erm* genes [*erm*(A), *erm*(B) and *erm*(C)] were detected alone or in different combinations (Table 1). Novel resistance genes detected so far only among porcine ST398 isolates have also been identified among bovine isolates. These include the trimethoprim resistance gene *dfrK*, the pleuromutilin/lincosamide/streptogramin A resistance gene *vga*(C) and the MLS_B resistance gene *erm*(T).[14–16] All *tet*(L)-positive bovine isolates carried the *dfrK* gene downstream of *tet*(L) as previously described in a porcine MRSA ST398 isolate.[14] Moreover, all *erm*(A)-positive isolates exhibited high spectinomycin MICs and carried the *spc* gene downstream of *erm*(A). This linkage suggests that the respective isolates may have acquired a Tn*554*-like transposon in which these two genes are known to be present.[17] As previously seen in porcine MRSA ST398 isolates, all chloramphenicol-resistant bovine isolates also carried the phenicol exporter gene *fexA* rather than any of the plasmid-borne chloramphenicol acetyltransferase genes usually found in staphylococci. The aminoglycoside resistance genes *aadD* and *aacA–aphD* have also been described in porcine MRSA ST398 isolates.[3,10,15] It should be noted that the resistance genes *tet*(M), *erm*(A), *erm*(B) and *aacA–aphD* have also been identified recently in Turkish MRSA isolates from bovine milk.[20] The detection of the gene *vga*(A) in bovine MRSA ST398 isolates that exhibited tiamulin MICs of 16 mg/L was a novel observation. Variants of *vga*(A) have been described to mediate pleuromutilin resistance in addition to resistance to lincosamides and streptogramin A antibiotics.[23] However, the tiamulin MICs for these *vga*(A)-carrying isolates were, at ≥128 mg/L, distinctly higher than the ones observed in our study.[23]

Molecular typing as well as the resistance genotypes and phenotypes identified 16 different types among the 27 MRSA ST398 isolates included in this study (Table 1). If more than a single isolate per farm was available, these isolates usually exhibited very similar characteristics. Occasionally, isolates displaying indistinguishable characteristics were also found in different farms from the same geographical area, such as NW-1 and NW-3, BA-1 and BA-2, or LS-1 and LS-3. It was not possible to find out whether links between these farms existed that might help to explain the occurrence of indistinguishable MRSA ST398 isolates in these farms. In one case (farm BW-4), two MRSA ST398 types were isolated that differed in their ApaI PFGE pattern, *dru* type and resistance pattern (Table 1). Based on the findings of this study, it seems that there was no single MRSA ST398 type disseminated in dairy farms all across Germany. More likely, most of the dairy farms in which MRSA-mediated mastitis was detected, had a more or less farm-specific MRSA ST398 type. This can indicate either multiple importations of ST398 from external diverse sources or a very high rate of gene transfer leading to diversification within a rather short time. Detailed epidemiological studies are necessary to elucidate the ways by which the dairy cattle have acquired the MRSA ST398 isolates and what role was played by other animals on the farms or farm personnel in the dissemination of such isolates.

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Transparency declarations

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