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Characterization of Methicillin-Resistant *Staphylococcus aureus* Isolates from Food and Food Products of Poultry Origin in Germany▽

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During a survey of fresh chicken and turkey meat as well as chicken and turkey meat products for the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Germany, 32 (37.2%) of 86 samples were MRSA positive. Twenty-eight of these MRSA isolates belonged to clonal complex 398 (CC398), which is widespread among food-producing animals. These CC398 isolates carried SCC*mec* elements of type IV or V and exhibited *spa* type t011, t034, t899, t2346 or t6574 and either the known *dru* types dt2b, dt6j, dt10a, dt10q, dt11a, dt11v, and dt11ab or the novel *dru* types dt6m, dt10as, and dt10at. In addition, two MRSA sequence type 9 (ST9) isolates with a type IV SCC*mec* cassette, *spa* type t1430, and *dru* type dt10a as well as single MRSA ST5 and ST1791 isolates with a type III SCC*mec* cassette, *spa* type t002, and *dru* type dt9v were identified. All but two isolates were classified as multiresistant. A wide variety of resistance phenotypes and genotypes were detected. All isolates were negative for the major virulence factors, such as Panton-Valentine leukocidin, toxic shock syndrome toxin 1, or exfoliative toxins. In contrast to the MRSA CC398 isolates, the four ST9, ST5, or ST1791 isolates harbored the *egc* gene cluster for enterotoxin G, I, M, N, O, and U genes. Although the relevance of contamination of fresh poultry meat or poultry products with MRSA is currently unclear, the presence of multiresistant and, in part, enterotoxigenic MRSA emphasizes the need for further studies to elucidate possible health hazards for consumers.

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in livestock have gained particular attention during recent years (38). The identification of livestock-associated MRSA in food-producing animals has raised questions regarding the presence of MRSA in food of animal origin. Several studies were conducted in different parts of the world (i) to screen food of animal origin intended for human consumption for the presence of MRSA and also (ii) to identify the MRSA types present. A study from The Netherlands identified MRSA isolates in 11.9% of 2,217 samples tested (3). Differences in the prevalence of MRSA were detected with respect to the animal origin of the meat samples. MRSA was most prevalent in turkey (35.3%), followed by chicken (16.0%), veal (15.2%) pork (10.7%), and beef (10.6%). About 85% of the MRSA isolates were assigned to multilocus sequence type 398 (ST398) (3). Another study from The Netherlands focused on the detection of MRSA in pork and beef raw meat products sampled from retail stores (34). Among 79 samples, only two MRSA isolates were found, one (1.3%) of which was identified as an ST398 isolate. MRSA was not identified in a study conducted in Switzerland examining 100 pooled neck skin swabs from chicken carcasses and 460 food samples of animal origin. In Spain, Lozano et al. (24) identified only five MRSA isolates in 318 raw food samples. Of these, ST398 isolates were found in single samples of veal and pork, ST125 isolates in single chicken and rabbit samples, and an ST217 isolate in a sample from a wild boar. During a prevalence study of MRSA contamination of retail pork in Canada, Weese et al. detected MRSA in 31 (7.7%) of 402 samples. Ten of the 31 isolates were classified as ST398 by their nontypeability in SmaI pulsed-field gel electrophoresis and their *spa* type t034 (37).

In addition to these livestock-associated MRSA isolates, MRSA isolates that corresponded to types commonly found in humans were also detected in food of animal origin. This observation suggested that there is potential for contamination of food either at the slaughterhouse or during food processing, with humans being a likely source of contamination. Pu et al. (30) identified only two MRSA isolates in 120 retail meat samples sourced from 30 grocery stores in Baton Rouge, LA. One of them, however, was a Panton-Valentine leukocidin (PVL)-positive USA300 isolate known to be associated with community-associated MRSA infections, while the other was a PVL-negative USA100 isolate commonly found in health care-associated MRSA infections in the United States (30). In a Canadian study, Weese et al. (36) found 32 MRSA isolates in 678 food samples (pork, ground beef, and chicken) purchased at retail outlets. All 32 isolates were classified as Canadian epidemic MRSA-2 (= ST5-MRSA-II [26]), a human MRSA strain recognized as the most common cause of health care-associated infections in Canada but which has also been identified in pigs and pig farm personnel in that country (36). Two MRSA isolates, which displayed characteristics of community-associated MRSA isolates, were also detected among 444 retail raw chicken meat samples in Japan (19). MRSA ST72 from beef and pork as well as MRSA ST692 was

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identified at low frequencies (0.3 to 1.0%) in Korea (23). In addition, MRSA ST5 has also been identified from retail chicken in Korea (22).

These data show that different types of MRSA are present in food of animal origin in different countries. For Germany, very limited data about MRSA in food of animal origin are currently available. This applies in particular to MRSA in poultry and food of poultry origin (5). To gain insight into the MRSA types present in raw poultry meat and poultry meat products available from retail stores in the federal state Rhineland-Palatinate in Germany, a small-scale study focusing on chicken and turkey meat as well as the corresponding products was performed. The MRSA isolates obtained during this study were subjected to molecular analysis with particular reference to their genotypic characteristics, their virulence, and their antimicrobial resistance patterns.

MATERIALS AND METHODS

Bacterial isolates. Eighty-six samples from food and food products of poultry origin were obtained from individual retail stores in the federal state Rhineland-Palatinate in the western part of Germany between May 2009 and December 2009. These included 22 samples from fresh turkey meat (15 from Germany, three from Italy, two from France, one from Austria, and one of unknown origin), 21 samples from turkey meat products (18 from Germany and three from Austria), 24 samples from fresh chicken meat (21 from Germany, two from Austria, and one from Hungary), and 19 samples from chicken meat products (17 from Germany, 1 from The Netherlands, and 1 of unknown origin). From each sample, 25 g of meat or meat product was minced, added to 225 ml Mueller-Hinton broth (supplemented with 6.5% [wt/vol] sodium chloride), homogenized for 1 min, and incubated at 35 to 37°C for 16 to 20 h. For selective enrichment of MRSA, 1 ml of this mixture was added to 9 ml of tryptone soya broth (Oxoid, Wesel, Germany) supplemented with 3.5 μg/ml cefoxitin and 75 μg/ml aztreonam. After another incubation at 35 to 37°C for 16 to 20 h, an aliquot of 10 μl was streaked on chromogenic MRSA selective agar (Brilliance MRSA agar; Oxoid) and incubated at 35 to 37°C for 18 to 24 h. Suspect denim blue colonies were subcultured on Columbia blood agar (supplemented with 7.5% [vol/vol] sheep blood). Further confirmatory tests for *S. aureus* included a coagulase test (BBL coagulase plasma rabbit plus EDTA; Becton Dickinson, Heidelberg, Germany) and a latex slide agglutination test (Staphytect Plus; Oxoid) for the detection of clumping factor, protein A, and capsular polysaccharides. For confirmation as MRSA, all isolates were subjected to a *mecA*-specific PCR as previously described (33). One MRSA isolate per positive sample was subjected to further analysis.

Antimicrobial susceptibility testing. All MRSA isolates were tested for their antimicrobial susceptibility by broth microdilution according to the recommendations given in document M31-A3 of the Clinical and Laboratory Standards Institute (1). For this, custom-made microtiter plate panels were used (MCS Diagnostics, Swalmen, The Netherlands), which included 10 to 12 concentrations of 30 antimicrobial agents in 2-fold dilution series. The tested compounds included penicillins (penicillin G, ampicillin, amoxicillin-clavulanic acid [2:1], and oxacillin), cephalosporins (cephalothin, cefotaxime, cefoperazone, cefquinome, and ceftiofur), tetracyclines (tetracycline and doxycycline), macrolides (erythromycin, spiramycin, tilmicosin, tulathromycin, and tylosin), lincosamides (clindamycin and pirlimycin), folate pathway inhibitors (trimethoprim and sulfamethoxazole-trimethoprim [19:1]), (fluoro)quinolones (nalidixic acid and enrofloxacin), phenicols (chloramphenicol and florfenicol), aminocyclitols (apramycin and spectinomycin), an aminoglycoside (gentamicin), a streptogramin (quinupristin-dalfopristin), a glycopeptide (vancomycin), and a pleuromutilin (tiamulin). For selected isolates which proved to be positive for the aminoglycoside resistance genes *aacA-aphD* and/or *aadD* in the microarray analyses, kanamycin MICs were determined by broth macrodilution (1). The reference strain *S. aureus* ATCC 29213 served as quality control strain in the MIC determinations.

DNA microarray analysis. A previously described diagnostic DNA microarray (StaphyType; Alere Technologies, Jena, Germany) was used to characterize the MRSA isolates (25, 26). This microarray-based assay is able to detect a total of 330 different sequences (~180 genes and alleles thereof), including *S. aureus*-specific genes, accessory gene regulator (*agr*) alleles, genes coding for virulence factors (toxins, enterotoxins, putative toxins, hemolysins, proteases, and biofilm formation molecules) and microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), capsule type-specific genes, and numerous antimicrobial resistance genes. Performance of the microarray analyses followed the recommendations of the manufacturer. Analysis of the recorded hybridization patterns was conducted using a designated reader and software (ArrayMate and IconoClust, both by Alere Technologies). Microarray analysis was supplemented by specific PCRs for recently identified antimicrobial resistance genes which have not yet been included in the microarray, such as *dfrK* (15), *vga*(C) (16), *erm*(T) (17), and *apmA* (7). Moreover, specific PCRs to confirm the linkage of the genes *tet*(L)-*dfrK* and *erm*(A)-*spc* were applied (8).

For isolates that carried *tet*(L) and *dfrK* but were negative for the PCR confirming linkage of the two genes, as well as for isolates that carried only *dfrK* without *tet*(L), a PCR assay was established to confirm the location of *dfrK* as part of transposon Tn*559* (18) in the chromosomal DNA. For this, the primers tnpC_fw (5′-TCGTTCGGAGGAAATCCTTA-3′) and radC_rv (5′-TCAAACC ACACTCCTTCAACC-3′) were used and an amplicon of 972 bp was expected. 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. One of the amplicons was sequenced for confirmation. For the detection of *vga*(B), the primers vgaB_fw (5′-GAATAA GGCGCAAGGAATGA-3′) and vgaB_rv (5′-TAGCTTGGCAAAAGCAACC T-3′) were used to generate a 601-bp amplicon. The PCR program was the same as described above, except that the annealing temperature was set at 54°C.

Molecular typing of MRSA isolates. All isolates were subjected to the two CC398-specific PCRs previously described by van Wamel et al. (35). Isolates which were negative in these PCRs were subjected to multilocus sequence typing (MLST) according to the protocol of Enright et al. (6). The sequences obtained for the seven housekeeping genes were compared to those deposited in the MLST database (http://saureus.mlst.net/). All MRSA isolates were subjected to *spa* sequence typing in accordance with the Ridom StaphyType standard protocol (http://spaserver.ridom.de). In addition, two PCR-directed typing methods applicable exclusively to methicillin-resistant staphylococci, *dru* typing and SCC*mec* typing, were applied. The *dru* amplicons were sequenced and compared with the *dru* sequences and *dru* types stored in the *dru* typing database (http://dru-typing.org) (8, 9). For SCC*mec* typing, the multiplex PCRs as described by Kondo et al. (21) were used. The discriminatory value for each typing method was calculated as an index of discrimination (*D*) as described by Hunter and Gaston (12).

RESULTS

MRSA detection and characterization. In total, 32 (37.2%) of the 86 samples were MRSA positive. These included 6 (25.0%) of the 24 samples from fresh chicken meat, 4 (21.1%) of the 19 samples from chicken meat products, 11 (50.0%) of the 22 samples from fresh turkey meat, and 11 (52.4%) of the 21 samples from turkey meat products. Among the MRSA-positive samples detected, only four, two from fresh turkey meat and two from turkey meat products, originated from the area where the samples had been taken (Table 1).

Twenty-eight of the 32 MRSA isolates reacted positively in both of the CC398-specific PCR assays described by van Wamel et al. (35) and were considered to belong to clonal complex 398 (CC398). Of the remaining four MRSA isolates, two exhibited ST9 (allelic profile 3-3-1-1-1-1-10), which belongs to CC9 (26). Another one was assigned to ST5 (allelic profile 1-4-1-4-12-1-10), whereas the last one was a single-locus variant of ST5, designated ST1791 (allelic profile 1-4-184-4-12-1-10). It should be noted that the *glpF* alleles 1 and 184 differ by a single nucleotide exchange (C at position 217 in allele 1 versus T in allele 184). Both ST5 and ST1791 belong to CC5 (26). The *spa* typing identified seven different *spa* types among the 32 isolates, each of them consisting of 5 to 10 repeats (Table 1; Fig. 1). Both ST9 isolates showed *spa* type t1430, while the ST5 and ST1791 isolates had *spa* type t002. The remaining five *spa* types were found in the CC398 isolates, with

TABLE 1. Molecular characteristics and virulence properties of the 32 MRSA isolates

Sample origin	Isolate	Location where produced[a]	MLST type or clonal complex	*spa* type	*dru* type	SCC*mec* type	Presence of virulence and MSCRAMM genes					
							Enterotoxin genes	*lukD* + *lukE*	*hlb*	*cna*	*fib*	*fib* (MRSA 252)
Chicken meat	Chi-1	Germany, LS	CC398	t011	dt10a	V	−	−	−	+	−	+
	Chi-2	Germany, LS	CC398	t011	dt11a	V	−	−	−	+	−	+
	Chi-3	Austria	CC398	t034	dt6j	V	−	−	−	+	−	+
	Chi-4	Germany, LS	CC398	t011	dt11a	V	−	−	−	+	−	+
	Chi-5	Germany, LS	CC398	t011	dt2b	V	−	−	−	+	−	+
	Chi-10	Germany, LS	CC398	t011	dt11a	V	−	−	−	+	−	+
Chicken product	Chi-6	Germany, LS	ST9	t1430	dt10a	IV	G, I, M, N, O, U	−	+	−	+	−
	Chi-7	Germany, LS	CC398	t011	dt11a	V	−	−	−	+	−	+
	Chi-8	Unknown	CC398	t034	dt6j	V	−	−	−	+	−	+
	Chi-9	The Netherlands	ST9	t1430	dt10a	IV	G, I, M, N, O, U	−	+	−	+	−
Turkey meat	Tur-2	Germany, LS	ST5	t002	dt9v	III	G, I, M, N, O, U	+	+	−	+	−
	Tur-10	Germany, LS	CC398	t034	dt6j	V	−	−	−	+	−	+
	Tur-11	Germany, LS	CC398	t034	dt11a	V	−	−	−	+	−	+
	Tur-13	Unknown	CC398	t011	dt10q	IV	−	−	−	+	−	+
	Tur-14	Germany, RP	ST1791	t002	dt9v	III	G, I, M, N, O, U	+	+	−	+	−
	Tur-16	France	CC398	t899	dt10as	IV	−	−	−	+	−	+
	Tur-18	Italy	CC398	t6574	dt11ab	V	−	−	−	+	−	+
	Tur-19	Germany, LS	CC398	t011	dt10q	IV	−	−	−	+	−	+
	Tur-20	Germany, LS	CC398	t034	dt11v	V	−	−	−	+	−	+
	Tur-21	Germany, RP	CC398	t011	dt10q	IV	−	−	−	+	−	+
	Tur-22	Germany, BW	CC398	t034	dt6m	V	−	−	−	+	−	+
Turkey product	Tur-1	Germany, BW	CC398	t011	dt10q	IV	−	−	−	+	−	+
	Tur-3	Germany, BW	CC398	t011	dt11a	V	−	−	−	+	−	+
	Tur-4	Germany, BW	CC398	t011	dt10q	IV	−	−	−	+	−	+
	Tur-5	Germany, LS	CC398	t011	dt10q	IV	−	−	−	+	−	+
	Tur-6	Germany, RP	CC398	t034	dt6j	V	−	−	−	+	−	+
	Tur-7	Austria	CC398	t011	dt10q	IV	−	−	−	+	−	+
	Tur-8	Germany, RP	CC398	t034	dt6j	V	−	−	−	+	−	+
	Tur-9	Germany, BA	CC398	t2346	dt11a	V	−	−	−	+	−	+
	Tur-12	Germany, BW	CC398	t011	dt11a	V	−	−	−	+	−	+
	Tur-15	Germany, LS	CC398	t034	dt6j	V	−	−	−	+	−	+
	Tur-17	Austria	CC398	t011	dt10at	IV	−	−	−	+	−	+

[a] Regions in Germany: BA, Bavaria; BW, Baden-Württemberg; LS, Lower Saxony; RP, Rhineland-Palatinate.

t011 ($n = 16$) and t034 ($n = 9$) seen most frequently. SCC*mec* typing revealed the presence of SCC*mec* types III ($n = 2$), IV ($n = 11$), and V ($n = 19$). The *dru* typing identified 11 different *dru* types composed of 2 to 11 repeats (Table 1; Fig. 1). Three novel *dru* types, dt6m, dt10as, and dt10at, were identified for the first time during this study. Calculation of the discriminatory indices showed that *dru* typing had the highest discriminatory power ($D = 0.863$), followed by *spa* typing ($D = 0.681$), SCC*mec* typing ($D = 0.542$), and MLST ($D = 0.236$).

Virulence properties. Microarray analysis revealed a rather uniform virulence gene pattern among the MRSA isolates included in this study. All isolates were positive for the genes *hla* and *hld*, coding for α and δ hemolysins, for genes indicative of capsule type 5, and for the *icaACD* genes, whose products are involved in biofilm formation. Moreover, all MRSA isolates carried a similar set of genes for microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), including *clfA* and *clfB* (encoding clumping factors A and B), *fnbA* and *fnbB* (encoding fibronectin binding proteins A and B), and *ebpS* (encoding elastin binding protein) among others. All isolates were negative for the PVL genes *lukF-PV* and *lukS-PV*, the toxic shock syndrome toxin 1 *tst* alleles, the exfoliative toxin genes *eta*, *etb*, and *etd*, the arginine catabolic mobile element (ACME), and the genes *edin-A*, *edin-B*, and *edin-C*, encoding epidermal cell differentiation inhibitors.

Differences in the carriage of enterotoxin genes were seen between the ST9, ST5, and ST1791 isolates on one hand and the CC398 isolates on the other hand. The enterotoxin gene cluster *egc*, which comprises genes for the staphylococcal enterotoxins G, I, M, N, O, and U, was detected in the two ST9 isolates, the single ST5 isolate, and the single ST1791 isolate, whereas the CC398 isolates were negative for all enterotoxin genes tested. In addition, the single ST5 and ST1791 isolates were positive for the leukotoxin genes *lukD* and *lukE*. The ST9, ST5, and ST1791 isolates also differed in the carriage of specific MSCRAMM genes. These non-CC398 isolates were negative for *cna* (encoding collagen binding protein) but positive for the regular *fib* (encoding fibrinogen binding protein) allele present in *S. aureus* Newman (accession no. AP009351) and other *S. aureus* strains. The CC398 isolates were instead positive for the *fib* allele detected in MRSA 252 (accession no. BX571856) (Table 1). Moreover, these four isolates were also positive for the gene *hlb*, coding for the β hemolysin.

Antimicrobial resistance phenotypes and genotypes. As MRSA isolates, all carried the *mecA* gene and exhibited oxacillin MICs of 4 to ≥32 μg/ml. Thirty isolates exhibited a

(a) *spa* types

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t002:        26-23-17-34-17-20-17-12-17-16
t011:        08-16-  -02-25-  -  -  -  -34-24-  -25
t034:        08-16-  -02-25-  -  -02-25-34-24-  -25
t2346:       08-16-  -02-25-  -  -  -  -34-24-24-25
t6574:      385-16-  -02-25-  -  -  -  -34-24-  -25
t899:        07-16-23-02-  -  -  -  -  -34
t1430:       07-16-23-02-  -12-23-02-  -34
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(b) *dru* types

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dt2b:       5a-  -  - -  -  -  -  -  -  -3e
dt6j:       5a-2d-4a-0-2d-  -  -  -  -  -3e
dt6m:       5a-2d-3f-0-2d-  -  -  -  -  -3e
dt9v:       5a-2d-4a-0-2d-  -  -2g-3b-4e-3e
dt10a:      5a-2d-4a-0-2d-5b-3a-2g-3b-4e
dt10q:      5a-2d-4a-0-2d-5b-3a-2g-2c-4e
dt10as:     5a-2d-4a-0-2d-5b-2a-2g-4b-4e
dt10at:     5a-2d-4a-0-3c-5b-3a-2g-2c-4e
dt11a:      5a-2d-4a-0-2d-5b-3a-2g-3b-4e-3e
dt11v:      5a-2d-4a-0-3c-5b-3a-2g-3b-4e-3e
dt11ab:     5a-2d-4a-0-2d-5b-3a-2g-3b-4e-2f
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(C) *dru* repeats

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4a:  ATAAGAGGTTTGTTAAAAGCAGTTCTCAGTAAAATTACAG
3f:  ATAAGAGGTAAGTTAAAAGCAGTTCTCAGTAAAATTACAG

2d:  ATAAGAGGTTTGTTAAAAGCAGTTCTAAGTAAAATTGCAG
3c:  ATAAGAGGTTTGTTAAAAGCAGTTCTCAGTAAAATTGCAG

2a:  ATAAGGGGTAAGTTAAAAGCAGTTCTAAGTAAAATTGCAG
3a:  ATAAGGGGTAAGTTAAAAGCAGTTCTCAGTAAAATTGCAG

3b:  ATAAGAGGTGCGTTAAAAGCAGTTCTCAGTAAAATTGCTG
2c:  ATAAGAGGTGCGTTAAAAGCAGTTCTCAGTAAAATTGCAG
4b:  ATAAGAGGTTTGTTAAAAGCAGTTCTCAGTAAAATTGCTG
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FIG. 1. Comparison of *spa* and *dru* types and selected *dru* repeats found among the MRSA isolates from fresh poultry meat and poultry products. The variable nucleotides in the *dru* repeats are shaded.

multidrug resistance phenotype with resistance to ≥3 classes of antimicrobial agents (Table 2). Among the most common additional resistance properties, tetracycline resistance was found in 31 (96.9%), combined resistance to macrolides, lincosamides, and streptogramin B (MLS_B) antibiotics in 28 (87.5%), and trimethoprim resistance in 24 (75.0%) isolates. In contrast, resistance to enrofloxacin or apramycin was detected in only five (15.6%) isolates or one (3.1%) isolate, respectively. One of the two non-multidrug-resistant isolates was from a chicken product and exhibited resistance to only β-lactam antibiotics and enrofloxacin. The other isolate was from a turkey product and exhibited resistance to only β-lactam antibiotics and tetracyclines (Table 2).

Besides the *mecA* gene, all isolates except the ST1791 isolate carried the *blaZ/I/R* gene cluster for a staphylococcal narrow-spectrum β-lactamase. All but one of the tetracycline-resistant isolates carried the tetracycline resistance gene *tet*(M), often in combination with the genes *tet*(K), *tet*(L), or *tet*(K) plus *tet*(L) (Table 2). The remaining tetracycline-resistant isolate, of ST9, carried solely the *tet*(L) gene, whereas the other ST9 isolate was the only one that was tetracycline susceptible. The dihydrofolate reductase gene *dfrK* was detected in all but one of the trimethoprim-resistant isolates which harbored *dfrS1* (also known as *dfrA*). Specific PCR assays confirmed that the *dfrK* gene was linked to the *tet*(L) gene in eight isolates, whereas it was part of the chromosomally located transposon Tn*559* in seven cases. The location of *dfrK* and its potential linkage to other resistance genes in the remaining eight *dfrK*-positive isolates are unknown. The four rRNA methylase genes *erm*(A), *erm*(B), *erm*(C), and *erm*(T) were detected alone or in various combinations (Table 2). The simultaneous occurrence of *erm*(T) together with *erm*(C) was a novel observation. The spectinomycin resistance gene *spc* was detected in 15 of the 19 isolates with spectinomycin MICs of ≥512 μg/ml. It proved to be linked to the MLS_B resistance gene *erm*(A) in all 15 isolates. The genetic basis of spectinomycin resistance in the other four isolates remains to be clarified. Apramycin resistance was recorded in only a single isolate and was shown to be due to the presence of the gene *apmA*. Two aminoglycoside resistance genes, *aacA-aphD*, known to mediate resistance to gentamicin and kanamycin, and *aadD*, known to confer resistance to kanamycin and neomycin, were detected either alone or in combination in isolates that displayed resistance to gentamicin and/or kanamycin. In contrast, the isolate Chi-1 from chicken meat, with a gentamicin MIC of 16 μg/ml, did not exhibit the presence of a gene for gentamicin resistance. Ten of the 16 isolates with tiamulin MICs of ≥16 μg/ml carried any of the genes *vga*(A) ($n = 5$) or *vga*(C) ($n = 5$), both of which code for ABC transporters that confer combined resistance to streptogramin A antibiotics, lincosamides, and pleuromutilins. Since none of the remaining six isolates carried the *vga*(B) gene, the genetic basis of tiamulin resistance in these cases remains to be determined. All isolates were negative in the microarray for the lincosamide resistance gene *lnu*(A), the macrolide resistance genes *msr*(A), *mph*(C), and *mef*(A), the streptogramin A resistance genes *vat*(A), and *vat*(B), the streptogramin B resistance gene *vgb*(A), and the kanamycin/neomycin resistance gene *aphA3*.

DISCUSSION

The occurrence of MRSA not only in food-producing animals but also in food of animal origin might represent a relevant issue with regard to food safety and consumer protection. For eradication, it is important to identify the origin of the isolates and their dissemination on the farm and along the food chain, including potential ways of transmission and the vectors involved in the spread. To evaluate potential health hazards, it is necessary to know the pathogenic potential of the MRSA isolates. Thus, molecular typing of the isolates and a comprehensive analysis of their virulence and antimicrobial resistance properties will provide relevant information for epidemiological studies and for risk analysis. The application of four different typing techniques, two applicable to *S. aureus* in general and two applicable only to methicillin-resistant staphylococci, identified a hierarchy in these methods with regard to their discriminatory power. The highest discriminatory value of 0.863 was calculated for *dru* typing. This means that two isolates randomly selected from the test population can be assigned to different *dru* types with a probability of 86.3%. When *dru* typing was combined with *spa* typing, the discriminatory power was increased slightly to 0.893.

The molecular characterization of the MRSA isolates from

TABLE 2. Antimicrobial resistance phenotypes and genotypes of the 32 MRSA isolates

Origin	Isolate	Resistance pattern[a]	Presence of resistance genes[b]										
			blaZ/I/R	*tet*	*dfr*	*tet*(L)-*dfrK*	*tnpC-dfrK*	*erm*	*erm*(A)-*spc*	*spc*	*aacA-aphD*	*aadD*	*apmA*	*vga*
Chicken meat	Chi-1	BLA, TET, TMP, MLS_B, GEN, KAN, APR	+	(K), (L), (M)	*K*	+	−	(B)				+	+	
	Chi-2	BLA, TET, TMP, MLS_B, SPC	+	(K), (L), (M)	*K*	+	−	(A), (C)	+	+				
	Chi-3	BLA, TET, TMP, MLS_B, SPC, TIA, (Q/D)	+	(K), (M)	*K*		−	(A)	+	+				−
	Chi-4	BLA, TET, TMP, MLS_B, KAN, TIA, (Q/D)	+	(K), (L), (M)	*K*	+	−	(C)				+		(A)
	Chi-5	BLA, TET, MLS_B, TIA, Q/D	+	(K), (M)				(C)						(A)
	Chi-10	BLA, TET, TMP, MLS_B, SPC	+	(K), (L), (M)	*K*	+	−	(A), (C)	+	+				
Chicken product	Chi-6	BLA, TET, TMP, MLS_B, (KAN), ENR	+	(L)	*K*	+	−	(B)				+		
	Chi-7	BLA, TET, TMP, MLS_B, SPC, TIA, Q/D	+	(K), (L), (M)	*K*	+	−	(A), (C)	+	+				(C)
	Chi-8	BLA, TET, TMP, MLS_B, SPC, TIA, (Q/D)	+	(K), (M)	*K*		−	(A), (B)	+	+				(C)
	Chi-9	BLA, ENR	+											
Turkey meat	Tur-2	BLA, TET, MLS_B, SPC, ENR	+	(M)				(A)	+	+				
	Tur-10	BLA, TET, TMP, MLS_B, SPC, TIA, (Q/D)	+	(K), (M)	*K*		−	(A), (B)	+	+				−
	Tur-11	BLA, TET, TMP, MLS_B, SPC, KAN, TIA, (Q/D)	+	(L), (M)	*K*	+	−	(A)	+	+		+		(C)
	Tur-13	BLA, TET, TMP, MLS_B, GEN, KAN	+	(L), (M)	*K*	−	+	(T)			+	+		
	Tur-14	BLA, TET, MLS_B, SPC, ENR	−	(M)				(A)	+	+				
	Tur-16	BLA, TET, TMP, MLS_B, TIA, (Q/D)	+	(L), (M)	*S1*			(B)						(A)
	Tur-18	BLA, TET, GEN, KAN, TIA	+	(M)							+			(A)
	Tur-19	BLA, TET, TMP, MLS_B, SPC, TIA, Q/D	+	(K), (M)	*S1+K*		−	(A), (C)	+	+				−
	Tur-20	BLA, TET, TMP, MLS_B, SPC, KAN, TIA, (Q/D)	+	(L), (M)	*K*	+	−	(A)	+	+		+		(C)
	Tur-21	BLA, TET, TMP, MLS_B, SPC, (GEN), KAN	+	(L), (M)	*K*	−	+	(C)		−	+			
	Tur-22	BLA, TET, TMP, MLS_B, SPC, TIA, Q/D	+	(M)	*K*		−	(A), (C)	+	+				−
Turkey product	Tur-1	BLA, TET, TMP, MLS_B, SPC, GEN, KAN	+	(L), (M)	*K*	−	+	(T)		−	+	+		
	Tur-3	BLA, TET	+	(K), (M)										
	Tur-4	BLA, TET, TMP, MLS_B, GEN, KAN	+	(L), (M)	*K*	−	+	(T)			+			
	Tur-5	BLA, TET, TMP, MLS_B, SPC, (GEN), KAN, ENR	+	(L), (M)	*K*	−	+	(C), (T)		−	+	+		
	Tur-6	BLA, TET, TMP, MLS_B, SPC, TIA, (Q/D)	+	(K), (M)	*K*		−	(A)	+	+				−
	Tur-7	BLA, TET, TMP, MLS_B, SPC, GEN, KAN	+	(L), (M)	*K*	−	+	(C)		−	+			
	Tur-8	BLA, TET, TMP, MLS_B, SPC, TIA, (Q/D)	+	(K), (M)	*K*		−	(A), (B)	+	+				−
	Tur-9	BLA, TET, MLS_B, TIA, (Q/D)	+	(K), (M)				(C)						(A)
	Tur-12	BLA, TET, MLS_B	+	(K), (M)				(C)						
	Tur-15	BLA, TET, TMP, MLS_B, SPC, TIA, (Q/D)	+	(K), (M)	*K*		−	(A)	+	+				(C)
	Tur-17	BLA, TET, TMP, GEN, KAN	+	(L), (M)	*K*	−	+				+			

[a] Abbreviations of antimicrobial agents: APR, apramycin; BLA, β-lactam antibiotics; ENR, enrofloxacin; GEN, gentamicin; KAN, kanamycin; MLS_B, macrolides-lincosamides-streptogramin B; Q/D, quinupristin-dalfopristin; SPC, spectinomycin; TET, tetracyclines; TIA, tiamulin; TMP, trimethoprim. Parentheses indicate that based on the MICs, these isolates proved to be intermediate or borderline susceptible to the antimicrobial agent. Despite the lack of CLSI-approved breakpoints, isolates that showed high MICs of TMP (≥256 μg/ml), SPC (≥512 μg/ml), TIA (≥16 μg/ml), or APR (≥64 μg/ml) were considered resistant.

[b] The different subtypes or combinations of subtypes of *tet*, *dfr*, *erm*, and *vga* genes present are indicated by the corresponding letters. It should be noted that the Tn*4003*-associated *dfrS1* gene is also referred to as *dfrA* in the literature. Shading indicates that based on the resistance phenotype, the presence of a corresponding resistance gene or amplicons was not expected.

fresh poultry meat and poultry products identified the vast majority as livestock-associated MRSA CC398. The *spa* types detected among the isolates of the present study were mainly those previously seen in MRSA ST398 from swine (10, 13), cattle (8), and poultry (5, 27, 29). Only *spa* type t6574, identified in an MRSA isolate from turkey meat imported from Italy, appeared as a *spa* type that has so far not been described in MRSA CC398. Comparison of the *dru* types detected among the isolates in the present study with *dru* types determined in previous studies revealed that certain *dru* types may be present in different SCC*mec* cassettes, such as dt10a in a type V cassette of an ST398 isolate (8) or dt11a in a type IV cassette of a human MRSA ST22 isolate (32). Among the four non-CC398 isolates in the present study were two ST9 isolates with *spa* type t1430 and *dru* type dt10a. In a recent screening of professional food handlers and food of animal origin in The Netherlands, one MRSA isolate from a chicken meat sample belonged to ST9/t1430 (4). One of the two ST9 isolates in the present study was also from a chicken product imported from The Netherlands.

Important virulence factors such as PVL and toxic shock syndrome toxin 1, as well as exfoliative toxins, were not identified in any of the MRSA isolates from poultry and poultry products. This is in accordance with the findings of ST398 isolates from pigs and cattle (8, 13). Although enterotoxin genes of types B, K, and Q have previously been detected in single MRSA ST398 isolates from pigs (13), none of the MRSA CC398 isolates from poultry or poultry products was positive in the microarray for enterotoxin genes. Based on the currently available data, the carriage of enterotoxin genes seems to be rare among CC398 isolates (8, 11, 13). However, the MRSA ST9, ST5, and ST1791 isolates (Table 1) carried the *egc* gene cluster for enterotoxin genes *seg-sei-sem-sen-seo-seu*. The presence of this gene cluster is a common feature of CC9 and CC5 isolates (26). MRSA ST1 and MRSA ST72 carrying the enterotoxin genes *seg-sei* or *sea-seg-sei*, respectively, were found in raw beef and fish samples in Korea (31).

Thirty of the 32 MRSA isolates in the present study were classified as multiresistant by resistance to at least three classes of antimicrobial agents. The simultaneous occurrence of more than one *tet* or *erm* gene has previously been observed in ST398 isolates of porcine and bovine origin (8, 13). The same is true for the presence of the *blaZ/I/R* gene cluster and the *mecA* gene (8, 13). The recently identified trimethoprim resistance gene *dfrK* (15) proved to be the dominant *dfr* gene in MRSA CC398 and was also found in one of the two ST9 isolates (Table 2). PCR assays confirmed that the *dfrK* gene was in most cases either linked to the *tet*(L) gene (15) or part of the transposon Tn*559* (18). In staphylococci, transposon Tn*559* has been identified in only a single methicillin-susceptible ST398 isolate (18). The finding that it is also present in MRSA isolates from poultry and poultry products is a novel observation. The linkage of the resistance genes *erm*(A) and *spc* strongly suggested the presence of a Tn*554*-like transposon (28). The isolate Chi-1 did not harbor a gene for gentamicin resistance but had a gentamicin MIC of 16 μg/ml, which classified this isolate as borderline resistant. The same isolate, however, carried the gene *apmA* for apramycin resistance. Although *apmA* does not confer gentamicin resistance, it has been shown to elevate the MIC of gentamicin from 0.25 to 8 μg/ml in *S. aureus* RN4220 transformants that carry a plasmid-borne *apmA* gene (7). Thus, a contribution of *apmA* to this borderline gentamicin resistance is possible. Four of the MRSA isolates were identified as resistant to the streptogramin A plus B combination quinupristin-dalfopristin by showing a MIC of 4 or 8 μg/ml, while another 11 isolates were classified as intermediate by showing a MIC of 2 μg/ml (2) (Table 2). Nine of these 15 isolates carried at least one *erm* gene, whose product also specifies resistance to streptogramin B antibiotics, together with a *vga*(A) or *vga*(C) gene, whose products have been shown to mediate resistance to streptogramin A antibiotics in addition to pleuromutilins and lincosamides (14, 16). Since all isolates tested negative for *vat*(A), *vat*(B), *vga*(B), and *vgb*(A), the classification of these nine isolates as intermediate or resistant to quinupristin-dalfopristin most likely resulted from the synergistic effect of an *erm* gene and a *vga* gene.

When comparing all characteristics listed in Tables 1 and 2, a wide variety of MRSA isolates were detected among the 32 isolates tested. Only three pairs of isolates, Chi-2 and Chi-10, Chi-3 and Tur-6, and Tur-8 and Tur-10, proved to be indistinguishable or closely related. The last two pairs of isolates differed from one another only by the presence or absence of an *erm*(B) gene (Table 2). In general, the MRSA CC398 isolates from poultry and poultry products showed characteristics similar to those of isolates from pigs and cattle (8, 13). In addition, the ST9, ST5, and ST1791 isolates corresponded closely in their microarray patterns to human strains of CC9 and CC5 (26). As previously stated by Weese et al. (36), the relevance of MRSA contamination of retail meat is unknown and is controversial (20). Different screening studies resulted in strikingly different MRSA prevalences. This may be due to differences in the sampling plans and the MRSA detection procedures applied, but it may also reflect country-specific or food-specific true differences in the MRSA prevalence. This study presented only a time-limited snapshot of the presence of MRSA in fresh chicken and turkey meat as well as in the corresponding products sold in Germany, and it is uncertain in how far the results found in the federal state Rhineland-Palatinate can be extrapolated to other federal states in Germany or to Germany in general. Nevertheless, the observation that 37.2% of the samples tested were MRSA positive is alarming and needs further investigation. Specifically, longitudinal studies with a farm-to-fork approach are needed to identify the sources of contamination and to clarify whether isolates found as commensals in poultry are indistinguishable in their genotypic characteristics from those found in fresh poultry meat and poultry products.

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