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Virulence and Resistance Determinants of German *Staphylococcus aureus* ST398 Isolates from Nonhuman Sources▽†

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A series of 100 *Staphylococcus aureus* isolates ascribed to sequence type 398 (ST398) and recovered from different sources (healthy carrier and diseased pigs, dust from pig farms, milk, and meat) in Germany were investigated for their virulence and antimicrobial resistance genetic background. Antimicrobial resistance was determined by the disk diffusion method. Virulence and resistance determinants (37 and 31 genes, respectively) were tested by PCR. Only two virulence profiles, including the accessory gene regulator *agrI* and three or four hemolysin-encoding genes, were detected. In contrast, 33 resistance profiles were distinguished (only 11 were shown by more than one isolate). Fifty-nine isolates were multiresistant (four or more antimicrobial classes), and 98 were methicillin resistant (*mecA* positive). All of the ST398 isolates showed resistance to tetracycline [encoded by *tet*(M) alone or together with *tet*(K) and/or *tet*(L)]. In addition, 98% were resistant to other antimicrobials, including macrolide-lincosamine-streptogramin B (70%, encoded by *ermA*, *ermB*, and *ermC*, alone or in combination), trimethoprim (65%, mostly due to *dfrK* and *dfrG*), kanamycin and gentamicin [29% and 14%, respectively, mainly related to *aac(6′)-Ie-aph(2″)-Ia* and/or *ant(4′)-Ia* but also to *aph(3′)-IIIa*], chloramphenicol (9%, *fexA* or *cfr*), quinupristin-dalfopristin (9%), ciprofloxacin (8%), and trimethoprim-sulfamethoxazole (4%). The heterogeneity of the resistance profiles underlines the ability of the ST398 clone to acquire multiple antimicrobial resistance genes. However, the virulence gene content of the tested isolates was low. Continuous surveillance is needed to clarify whether its pathogenicity potential for animals and humans will increase over time.

Methicillin-resistant *Staphylococcus aureus* (MRSA) of sequence type 398 (ST398) has gained particular attention during recent years because of its association with pigs and its ability to colonize pig farmers and other people in close contact with pigs (7, 12, 47). The MRSA isolates of ST398 usually lack important virulence determinants that are typical in other community and hospital MRSA isolates. The majority of the ST398 isolates analyzed so far carry only hemolysin-encoding genes (13, 21, 31, 32), although a small number of cases in which the isolates carried the bicomponent leukotoxin Panton-Valentine (*lukPV* genes) (43, 49) or staphylococcal enterotoxins (SEs, *se* genes) (21, 26) have also been reported. Genes for other toxins, like exfoliatins (ET, *et* genes), leukotoxins, and toxic shock syndrome toxin (TSST-1, *tst* gene) have not been found yet in ST398 isolates (13, 21, 31, 32, 44). The regulation of the expression of most extracellular virulence factors in *S. aureus* is under the control of a two-component signaling system called the accessory gene regulator (*agr*), which is polymorphic and divided into four distinct genetic groups (I to IV). A correlation exists between some *agr* groups and certain pathotypes and clonal complexes (CCs) (48), and CC398 seem to be associated with *agr* group I (*agrI*) (31, 32).

Typically, ST398 strains display a multiresistant phenotype. The majority of the isolates have been reported as MRSA, but in most of the epidemiological studies, the results were influenced by the use of selective isolation methods (8, 17). Methicillin-susceptible *S. aureus* (MSSA) strains have also been described (3, 17, 28, 43), and in the latest *S. aureus* surveillance studies of humans in Europe, all ST398 isolates detected were MSSA (16). Resistance to methicillin and other β-lactam antibiotics is mediated by *mecA* carrier elements called staphylococcal cassette chromosome *mec* (SCC*mec*). Together with methicillin resistance in ST398 isolates from swine, resistance to tetracycline, erythromycin, clindamycin, quinupristin-dalfopristin, ciprofloxacin, sulfonamides, trimethoprim, sulfamethoxazole-trimethoprim, and aminoglycosides has been reported (5, 9, 21, 38, 39).

The aim of the present work was to study the virulence and antimicrobial resistance genetic repertoire of a collection of 100 *S. aureus* strains previously ascribed to ST398 which were recovered from different sources (mainly livestock and food of animal origin) in Germany.

MATERIALS AND METHODS

Bacterial strains. A series of 100 ST398 *S. aureus* isolates, previously characterized by *spa* typing, SCC*mec* typing, pulsed-field gel electrophoresis (PFGE) analysis with SmaI and its neoschizomer Cfr9I, and multilocus sequence typing (MLST) (3), were included in this study. The isolates were selected from the

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strain collection of the National Reference Laboratory for Coagulase-Positive Staphylococci (NRL-Staph) at the Federal Institute for Risk Assessment (BfR). They had been isolated between 2004 and 2008 at different German regional laboratories or the NRL-Staph as part of various monitoring surveys and research projects. The selection of the isolates was done to cover a variety of animal species and tissues and different stages of the food chain. When several isolates with the same source were available, isolates from different farms, slaughter batches, and/or *spa*/SCC*mec* types were selected in order to cover a broad range of ST398 subtypes. The ST398 isolates had been collected from healthy asymptomatic carrier pigs at slaughter (42 isolates), clinical samples from pigs (29 isolates; information is available for 19 that were collected at postmortem from different altered tissues where no other *S. aureus* isolate was present), dust from pig farms (10 isolates), cow's milk (5 isolates from individual milk samples from quarters with subclinical mastitis), and meat from food-producing animals (14 isolates, including 8 from pigs, 4 from turkeys, 1 from a broiler chicken, and 1 from a cow).

Five previously characterized ST398 isolates collected from fecal samples of pigs in the Netherlands (3) were included as ST398 controls. Six non-ST398 *S. aureus* isolates from meat (turkey and chicken), pig, and human samples, as well as *S. aureus* NCTC 8325 and *S. aureus* ATCC 29213, were used as outgroup controls. The eight outgroup isolates had been previously characterized by *spa* typing, SCC*mec* typing, and SmaI PFGE (3, 4). MLST (10) of these isolates was performed for the present study.

Positive controls for PCR amplification analyses were taken from previously analyzed collections (2) or were provided by the European Community Reference Laboratory for Antimicrobial Resistance (EURL-AR, Food-DTU, Copenhagen, Denmark) and the German National Reference Centre for *Staphylococcus* (NRZ-Staph, Robert Koch Institute [RKI], Wernigerode, Germany).

Antimicrobial susceptibility testing. All isolates were tested for antimicrobial susceptibility by the disk diffusion method, using Mueller-Hinton agar and commercially available discs (Oxoid, Wesel, Germany). The antimicrobial agents tested were methicillin, oxacillin, tetracycline, gentamicin, kanamycin, rifampin, erythromycin, clindamycin, fusidic acid, quinupristin-dalfopristin, teicoplanin, chloramphenicol, ciprofloxacin, linezolid, trimethoprim, and trimethoprim-sulfamethoxazole. The methicillin-oxacillin-susceptible isolates were also tested for ampicillin and penicillin. The results were interpreted by following the CLSI breakpoints (6). For vancomycin susceptibility, the isolates were tested by agar broth dilution using a concentration range of 4 to 1 μl/ml (susceptible MICs are ≤2 μg/ml [6]). For selected isolates, the MICs for kanamycin, gentamicin and quinupristin-dalfopristin were also determined by broth microdilution (6). The MICs for these antimicrobials were interpreted using both CLSI (6) and EUCAST epidemiological cutoff values (www.eucast.org) (for kanamycin, susceptible MICs are ≤8 mg/ml; for gentamicin, ≤2 mg/ml; and for quinupristin-dalfopristin, ≤1 mg/ml). In all experiments, the strain *S. aureus* ATCC 29213 was used for quality assurance.

Virulence and resistance gene typing. All isolates were screened for virulence and resistance determinants by PCR amplification using primers previously described or designed for this study (see Table S1 in the supplemental material). The resistance determinants tested conferred resistance to ampicillin-penicillin (*blaZ*), methicillin-oxacillin (*mecA* and SCC*mec* type), macrolides (*msrA* and *msrB*), lincosamides (*linA*/*linA′*), streptogramins A (*vatA*, *vatB*, *vatC*, *vgaA*, *vgaB*, and *vgaC*), streptogramins B (*vgbA* and *vgbB*), macrolides-lincosamides-streptogramins B (MLS_B [*ermA*, *ermB*, and *ermC*]), tetracyclines [*tet*(K), *tet*(L), *tet*(M), and *tet*(O)], aminoglycosides (*aac(6′)-Ie-aph(2″)-Ia*, *ant(4′)-Ia*, and *aph(3′)-IIIa*), phenicols (*cat*::pC194, *cat*::pC221, *cat*::pC223, and *fexA*), trimethoprim (*dfrD*, *dfrK*, *dfrG*, and *dfrS1*), and phenicols-lincosamides-oxazolidinones-pleuromutilin-streptogramins A (*cfr*). The virulence determinants tested encoded hemolysins (*hla*, *hlb*, *hld*, *hlg*, and *hlg* variant), leukotoxins (*lukED*, *lukM*, and *lukPV*), exfoliatins (*eta*, *etb*, and *etd*), toxic shock syndrome toxin (*tst*), and SEs (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser*, and *seu*) or were markers of pathogenicity islands (*ear*, *splF*, and *bsaB*) or identified the type of the regulatory system *agr* (*agrI*, *agrII*, *agrIII*, and *agrIV*).

Dendrograms of similarity showing the clustering of the isolates according to virulence or the resistance gene profiles were generated with Bionumerics (BioNumerics version 5.1; Applied Maths, St.-Martens-Latem, Belgium) using the Jaccard's coefficient of similarity.

RESULTS

Virulence genes. The 100 isolates assigned to ST398 were negative for leukotoxins, exfoliatins, and superantigen toxins. They displayed two different virulence profiles: *hla hlb hld hlg agrI* (98 isolates) and *hla hlb hld agrI* (2 isolates) (Table 1). The ST398 isolates from the Netherlands displayed the *hla hlb hld hlg agrI* (3 isolates) and *hla hlb hld hlg seb agrI* (2 isolates) profiles, while the outgroup isolates showed profiles with a higher number of virulence genes (Table 1). A dendrogram of similarity built on the basis of the presence or absence of the screened virulence genes clearly separated the outgroup controls from the ST398 isolates, which clustered together in subcluster A1 (Fig. 1).

Phenotypic antimicrobial resistance. The ST398 isolates showed resistance to oxacillin (95%), tetracycline (100%), erythromycin-clindamycin (70%), trimethoprim (65%), kanamycin (29%), gentamicin (14%), quinupristin-dalfopristin, chloramphenicol (9% each), ciprofloxacin (8%), and trimethoprim-sulfamethoxazole (4%), arranged in 33 phenotypic resistance patterns (denoted by "R" and a number or alphanumeric) (Table 2; also see Table S2 in the supplemental material). Most of these included resistance to β-lactams and tetracyclines, together with trimethoprim and/or macrolide/lincosamide resistance. Only 13 of the resistance patterns were represented by more than one isolate (Table 2; also see Table S2 in the supplemental material), the most frequent being R21 (21 isolates), R8 (13 isolates), R11 (11 isolates), R28 (10 isolates), R25 and R29 (6 isolates each), and R15 (5 isolates). Only 14% of the series showed resistance to less than three classes of antimicrobials, whereas 21% were resistant to three classes and 30%, 33%, and 4% to four, five, and more than five classes, respectively. Within the outgroup isolates, eight additional resistance patterns (R7, R16, R17, R19, R22, R36, R37, and R38a) were identified (Table 2; also see Table S2 in the supplemental material).

Genotypic basis of resistance. The results for the genotypic bases of resistance of ST398 and outgroup isolates are summarized in Table 3. Among the German ST398 isolates, the following results were found. The ampicillin-penicillin resistance gene *blaZ* was detected in 94% of the isolates. For aminoglycoside resistance determinants, the gentamicin-kanamycin resistance gene *aac(6′)-Ie-aph(2″)-Ia* was found in 12 gentamicin-kanamycin-resistant isolates (12% of the total and 92% of those resistant to gentamicin-kanamycin) and in one gentamicin-resistant isolate (1% of the total and 7% of the gentamicin-resistant isolates), while the kanamycin resistance genes *ant(4′)-Ia* and *aph(3′)-IIIa* were detected in 37 (37% of the total and 76% of kanamycin-resistant isolates) and 2 (2% of the total and 7% of kanamycin-resistant isolates) isolates, respectively. Some of the isolates (with resistance profiles R4, R8a, R15c, R21h-R21n, R29b, R31a, and R33) were positive for the presence of genes conferring aminoglycoside resistance but did not express this resistance (MICs between 32 and 8 μg/ml) in assays using the CLSI clinical breakpoints. In one kanamycin-resistant isolate, none of the tested genes was present. For tetracycline resistance, the *tet*(M) gene was present in all ST398 isolates, alone (9%) or together with *tet*(K) (51%), *tet*(L) (22%), or both genes (18%). The genes encoding resistance to MLS_B, *ermA*, *ermB*, and *ermC*, were present in 35 (35%), 32 (32%), and 41 (41%) isolates, respectively, each gene being found alone or in combination in the following percentages of isolates: *ermA* (7%), *ermB* (9%), *ermC* (26%), *ermA ermB* (14%), *ermA ermC* (6%), *ermB ermC* (1%), and *ermA ermB ermC* (8%). No isolate was

TABLE 1. Virulence profiles of *S. aureus* ST398 isolates and non-ST398 outgroup isolates and their relationships with *spa* types and PFGE profiles[a]

Virulence profile	Virulence genotype[b]	Strain group	Source(s)[c]	*spa* type(s)	PFGE profile(s)[d]
V1 (101)	*hla hlb hld hlg agrI*	ST398	AP (42), CP (29), D (10), FP[e] (3), M (5), MC, MB, MP (7), MT (3)	t011 (37), t034 (31), t108 (10), t571 (2), t779, t1250, t1255, t1451 (4), t1457, t1580, t1793, t1928, t1985, t2011, t2346 (2), t2510, t2576 (4), t2970	C1 (11), C2 (2), C3 (3), C4, C5 (8), C6 (16), C7, C8 (2), C9 (6), C10 (2), C11 (3), C12 (8), C13, C14 (6), C15 (3), C16 (4), C17, C18 (6), C19, C20 (2), C21–C29, C30 (2), C31–C33
V2 (2)	*hla hlb hld agrI*	ST398	MT, MP	t108, t5210	C12, C24
V3 (2)	*hla hlb hld hlg seb agrI*	ST398	FP[e] (2)	t011 (2)	C34, C35
V4	*hla hlb hld hlg lukM egc*-like *splF agrIII*	ST433	CP	t318	S1
V5 (2)	*hla hlb hld hlg-v egc agrII*	ST9	CP, MB	t337, t1430	S2, S3
V6	*hla hld hlg-v lukED egc splF agrII*	ST5	MT	t002	S4
V7	*hla hlb hld hlg sec egc agrI*	ST217	CH	t032	S5
V8	*hla hlb hld hlg-v lukED egc sed sej ser splF agrII*	ST225	CH	t003	S6
V9	*hla hlb hld hlg-v lukED seb ear agrIV*	ST8	NCTC 8325	t211	Control PFGE 1
V10	*hla hlb hld hlg-v lukED sea egc ear splF agrII*	ST5	ATCC 29213	t002	Control PFGE 2

[a] The number of isolates is shown in parentheses when $n > 1$. In total, 113 isolates were analyzed.
[b] The *egc* and *egc*-like clusters include the genes *seg*, *sei*, *sem*, *sen*, and *seo*. The *egc*-like cluster includes also the gene *seu*.
[c] ATCC, American Type Culture Collection; CH, human clinical sample; AP, asymptomatic carrier pig; CP, pig clinical sample; D, dust from pig farms; FP, Dutch pig fecal sample (isolates included as ST398 controls); M, milk; MC, meat from cattle; MB, meat from broiler; MP, meat from pig; MT, meat from turkey; NCTC, National Collection of Type Cultures.
[d] C, Cfr9I PFGE profile; S, SmaI PFGE profile.
[e] ST398 isolates kindly provided by D. Mevius (Central Veterinary Institute of Waningen, Lelystad, Netherlands), used as control strains.

positive for the erythromycin resistance genes *msrA* and *msrB*. Three isolates (3%) carried the clindamycin resistance *linA*/*linA′* gene, but two of them were phenotypically susceptible (using the CLSI breakpoints). The remaining isolate was erythromycin and clindamycin resistant and also carried *ermB*. All quinupristin-dalfopristin-resistant isolates were negative for the tested genes conferring resistance to streptogramins of type A (*vatA*, *vatB*, *vatC*, *vgaA*, *vgaB*, and *vgaC*) and B (*vgbA* and *vgbB*). No isolate carried the tested chloramphenicol acetyltransferase genes (*cat*::pC194, *cat*::pC221, and *cat*::pC223), whereas two (2%) isolates were positive for *fexA*. One of these was also positive for the multidrug resistance gene *cfr*. The following four genes conferring trimethoprim resistance were detected, alone or in combination: *dfrS1 dfrD* (1%), *dfrK dfrG* (2%), *dfrG* (21%), and *dfrK* (39%).

A dendrogram of similarity was established on the basis of the presence or absence of the screened resistance genes (Fig. 2). The dendrogram separated susceptible isolates from the resistant isolates, which grouped in a large cluster (B) com-

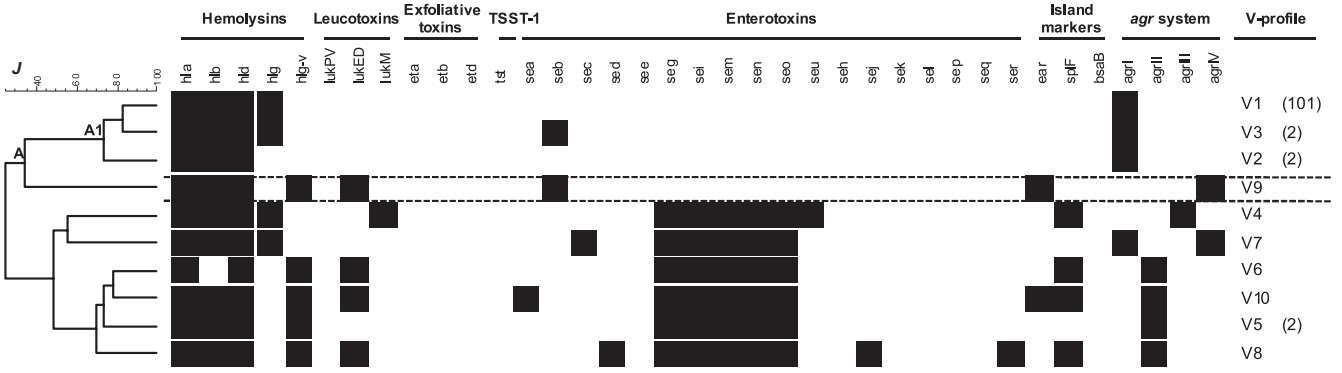

FIG. 1. Dendrogram showing the relatedness between virulence gene profiles (V-profile) for 30 exotoxin genes, 3 island markers, and the *agr* group of the ST398 and outgroup *S. aureus* isolates tested. The number of isolates is shown in parentheses when $n > 1$. Clusters are described in the text. The scale shows genetic similarity using Jaccard's coefficient (*J*). Dashed lines separate clusters/subclusters grouping ST398 isolates.

TABLE 2. Resistance profiles of ST398 and outgroup *S. aureus* isolates[a]

Genotypic resistance pattern[b]	Phenotypic resistance pattern[c]	Strain group(s)	Source(s)[d]
R0 (2)	Susceptible	ST8, ST433	NCTC, CP
R1	Amp-Pen	ST5	ATCC
R2[e]	Tet	ST398	M
R3[e]	Amp-Pen Tet	ST398	CP
R4	Amp-Pen Tet Ery-Cli	ST398	CP
R5	Amp-Pen Tet Ery-Cli-Syn	ST398	CP
R6[e]	Amp-Pen Tet Ery-Cli Tmp	ST398	CP
R7	Amp-Pen Gen Tet Ery-Cli	ST9	CP
R8 (13)	Met-Oxa Tet	ST398	AP (6), CP (2), FP[f], M (4)
R9	Met-Oxa Kan Tet	ST398	D
R10	Met-Oxa Gen-Kan Tet	ST398	D
R11 (11)	Met-Oxa Tet Ery-Cli	ST398	AP (4), CP (2), MC, MP (3), FP[f]
R12	Met-Oxa Kan Tet	ST398	CP
R13	Met-Oxa Tet Chl	ST398	AP
R14	Met-Oxa Tet Cip	ST398	AP
R15 (5)	Met-Oxa Tet Tmp	ST398	AP (2), D (2), MP
R16	Met-Oxa Syn Cip	ST217	CH
R17	Met-Oxa Gen Tet Ery-Cli	ST398	FP[f]
R18 (2)	Met-Oxa Kan Tet Ery-Cli	ST398	D, MB
R19	Met-Oxa Kan Tet Tmp	ST398	FP[f]
R20 (2)	Met-Oxa Gen-Kan Tet Tmp	ST398	AP (2)
R21 (21)	Met-Oxa Tet Ery-Cli Tmp	ST398	AP (9), CP (7), D (2), MP (3)
R22	Met-Oxa Tet Cip Tmp	ST398	FP[f]
R23	Met-Oxa Tet Sxt Tmp	ST398	CP
R24	Met-Oxa Gen Tet Ery-Cli Tmp	ST398	AP
R25 (6)	Met-Oxa Kan Tet Ery-Cli Tmp	ST398	AP (4), D, CP
R26	Met-Oxa Kan Tet Cip Tmp	ST398	AP
R27	Met-Oxa Kan Tet Ery-Cli Chl	ST398	CP
R28 (10)	Met-Oxa Gen-Kan Tet Ery-Cli Tmp	ST398	AP (5), D, CP (2), MT (2)
R29 (6)	Met-Oxa Tet Ery-Cli Syn Tmp	ST398	AP, D, CP, MP, MT (2)
R30	Met-Oxa Tet Ery-Cli Sxt-Tmp	ST398	AP
R31 (2)	Met-Oxa Tet Ery-Cli Cip Tmp	ST398	AP, CP
R32	Met-Oxa Tet Ery-Cli Chl Cip	ST398	AP
R33	Met-Oxa Tet Ery-Cli Chl Tmp	ST398	CP
R34	Met-Oxa Tet Ery-Cli-Syn Sxt-Tmp	ST398	AP
R35 (2)	Met-Oxa Tet Chl Cip Tmp	ST398	CP (2)
R36	Met-Oxa Kan Tet Ery-Cli Cip	ST5	MT
R37	Met-Oxa Kan Ery-Cli Cip Mup	ST225	CH
R38 (2)	Met-Oxa Kan Tet Ery-Cli Cip Tmp	ST9, ST398	CP, MB
R39	Met-Oxa Kan Tet Ery-Cli Chl Tmp	ST398	CP
R40	Met-Oxa Kan Tet Ery-Cli-Syn Chl Tmp	ST398	AP
R41	Met-Oxa Kan Tet Ery-Cli Chl Sxt-Tmp	ST398	CP

[a] The number of isolates is shown in parentheses when $n > 1$. In total, 113 isolates were analyzed.
[b] The subtyping of the resistance pattern according to the presence of a genetic background is shown in Fig. 2.
[c] Met, methicillin; Oxa, oxacillin; Tet, tetracycline; Gen, gentamicin; Kan, kanamycin; Ery, erythromycin; Cli, clindamycin; Mup, mupirocin; Syn, quinupristin-dalfopristin; Chl, chloramphenicol; Cip, ciprofloxacin; Tmp, trimethoprim; Sxt, trimethoprim-sulfamethoxazole. Intermediate susceptibility was considered resistance in this table (see Table S2 in the supplemental material for details). Isolates susceptible to methicillin and oxacillin were also tested for resistance to ampicillin and penicillin (Amp-Pen).
[d] ATCC, American Type Culture Collection; CH, human clinical sample; AP, asymptomatic carrier pig; CP, pig clinical sample; D, dust (from pig farms); FP, Dutch pig fecal sample; M, milk; MC, meat from cattle; MB, meat from broiler; MP, meat from pig; MT, meat from turkey; NCTC, National Collection of Type Cultures.
[e] *mecA*-positive isolates (MRSA).
[f] ST398 isolates kindly provided by D. Mevius (Central Veterinary Institute of Waningen, Lelystad, Netherlands), used as control strains.

prising two subclusters (B1 and B2). The resistant Dutch ST398 isolates and the outgroup isolates clustered together with the resistant ST398 isolates from Germany.

DISCUSSION

The emerging MRSA ST398 clonal group is widespread in the domestic pig population and can also be identified in other livestock species, such as veal calves, dairy cattle, and poultry (1, 12). Farmers and other livestock professionals handling carrier animals are at high risk of being colonized (7). Although infrequently, clinical infections with MRSA ST398 have been described, being in most cases related to these high-risk groups (12, 27, 45, 47). Human infections with MSSA ST398 have also been recorded, and they seem to be more frequent than those caused by MRSA ST398 (16). While the prevalence of the pathogen in farm animals and food has been studied extensively during the last few years, there is not much information on the molecular epidemiology of virulence and resistance determinants in this clonal group. The present work has investigated the virulence and resistance gene contents in a collection of 100 ST398 strains of nonhuman origin, representative of the German NRL-Staph collection (years 2004 to 2008), which was well characterized by several molecular methods previously (3).

The tested isolates of *spa* types assigned to ST398 lack sev-

TABLE 3. Genetic determinants conferring resistance to different classes of antimicrobials in ST398 and outgroup isolates of *S. aureus*[a]

Class of antimicrobial agent	Phenotypic resistance vs susceptibility[b]	Resistance genotype[c]	Strain group(s)	Source(s)[d]
β-Lactams	Amp-Pen-Met-Oxa^r (104)	*blaZ* SCC*mec* II	ST225	CH
		blaZ SCC*mec* IVa (5)	ST9, ST398 (4)	AP (3), MB, MT
		blaZ SCC*mec* V (73)	ST398 (73)	AP (25), CP (21), D (9), FP[e] (5), M (3), MB, MC, MP (6), MT (2)
		blaZ SCC*mec* V* (10)	ST398 (10)	AP (4), CP (4), D, MP
		blaZ SCC*mec* nt (9)	ST5, ST217, ST398 (7)	AP (6), CH, MP, MT
		SCC*mec* IVa	ST398	MT
		SCC*mec* V (4)	ST398 (4)	AP (3), M
		SCC*mec* V*	ST398	AP
	Amp-Pen^r Met-Oxa^s (3)	*blaZ* SCC*mec* V (2)	ST398 (2)	CP (2)
	Amp-Pen^r (2)	*blaZ* (4)	ST5, ST9, ST398 (2)	ATCC, CP (3)
	Amp-Pen-Met-Oxa^s (3)	*blaZ* SCC*mec* V	ST398	M
		None (2)	ST8, ST433	CP, NCTC
Tetracycline	Tet^r (108)	*tet*(M) (10)	ST9, ST398 (9)	AP (5), CP (3), MP (2)
		tet(L)	ST9	MB
		tet(M) *tet*(K) (56)	ST5, ST398 (55)	AP (16), CP (15), D (6), FP[e] (4), M (5), MB, MC, MP (5), MT (3)
		tet(M) *tet*(L) (22)	ST398 (22)	AP (13), CP (5), D, MP, MT (2)
		tet(M) *tet*(K) *tet*(L) (19)	ST398 (19)	AP (8), CP (7), D (3), FP[e]
	Tet^s (5)	*tet*(M) *tet*(K)	ST225	CH
		None (4)	ST5, ST8, ST217, ST433	ATCC, CH, CP, NCTC
Aminoglycosides	Gen^r (3)	*aac(6′)-Ie-aph(2″)-Ia ant(4′)-Ia*	ST398	AP
		ND (2)	ST9, ST398	CP, FP[e]
	Kan^r (20)	*ant(4′)-Ia* (15)	ST9, ST398 (14)	AP (4), CP (5), D (3), FP[e], MB (2)
		aph(3′)-IIIa (3)	ST5, ST398 (2)	AP (2), MT
		ND (2)	ST225, ST398	CH, CP
	Gen-Kan^r (13)	*aac(6′)-Ie-aph(2″)-Ia* (5)	ST398 (5)	AP (3), CP, D
		aac(6′)-Ie-aph(2″)-Ia ant(4′)-Ia (7)	ST398 (7)	AP (3), CP, D, MT (2)
		ant(4′)-Ia	ST398	AP
	Gen-Kan^s (77)	*ant(4′)-Ia* (15)	ST398 (15)	AP (6), CP (6), D, M, MP
		None (62)	ST 5, ST8, ST217, ST398 (58), ST433	AP (22), ATCC, CH, CP (16), D (4), FP[e] (3), M (4), MC, MP (7), MT (2), NTCT
MLS_B group[f]	Ery-Cli^r (74)	*erm*(A) (9)	ST5, ST398 (8)	AP (2), CP (3), FP[e], MP (2), MT
		erm(B) (8)	ST9, ST398 (7)	AP (5), D, MB, MT
		erm(C) (28)	ST9, ST225, ST398 (26)	AP (12), CH, CP (9), D (2), FP[e], MB, MP (2)
		erm(A) *erm*(B) (14)	ST398 (14)	AP (6), CP (3), D, MP (2), MT (2)
		erm(A) *erm*(C) (6)	ST398 (6)	AP, CP (3), D, MC
		erm(B) *erm*(C)	ST398	CP
		erm(B) *linA*/*linA′*	ST398	CP
		erm(A) *erm*(B) *erm*(C) (6)	ST398 (6)	AP (3), CP, D, MP
		ND (3)	ST398 (3)	CP (2), MT
	Ery-Cli^s (37)	*erm*(A)	ST217	CH
		erm(B)	ST398	D
		erm(C)	ST398	D
		linA/*linA′* (3)	ST398 (3)	CP (2), FP[e]
		erm(A) *erm*(B) *erm*(C) (2)	ST398 (2)	AP (2)
		None (29)	ST5, ST8, ST398 (26), ST433	AP (11), ATCC, CP (6), D (2), NCTC, M (5), MP, FP[e] (2)
Chloramphenicol	Chl^r (9)	*fexA*	ST398	CP
		fexA cfr	ST398	CP
		ND (7)	ST398 (7)	AP (3), CP (4)

	Chl^s (104)	None (104)	ST5 (2), ST8, ST9 (2), ST217, ST225, ST398 (97), ST433	AP (39), ATCC, CH (2), CP (25), D (10), FP^e (5), M (5), MB (2), MC, MP (8), MT (5), NTCT
	Trimethoprim	Tmp^r (65)	*dfrK* (40)	ST9, ST398 (39)	AP (22), CP (11), D (2), FP^e, MB, MP, MT (2)
			dfrG (22)	ST398 (22)	AP (5), CP (9), D (2), FP^e, MP (3), MT (2)
			dfrK dfrG (2)	ST398 (2)	D, MP
			dfrD dfrSI	ST398	AP
		Tmp^s (48)	*dfrK* (2)	ST398 (2)	D (2)
			ND (3)	ST398 (3)	AP, D (2)
			None (43)	ST5 (2), ST8, ST9, ST217, ST225, ST398 (33), ST433,	AP (13), ATCC, CH (2), CP (11), D, FP^e (3), M (5), MB, MC, MP (3), MT, NCTC

[a] The number of isolates is shown in parentheses when $n > 1$. In total, 113 isolates were analyzed.

[b] Amp, ampicillin; Pen, penicillin; Met, methicillin; Oxa, oxacillin; Tet, tetracycline; Gen, gentamicin; Kan, kanamycin; Ery, erythromycin; Cli, clindamycin; Chl, chloramphenicol; Tmp, trimethoprim. Superscript "r" and "s" denote resistance and susceptibility, respectively. Intermediate susceptibility was considered resistance in this table. Met-Oxa-resistant isolates were considered Amp-Pen resistant.

[c] Gene or genes related to a determined resistance pattern. SCC*mec* V* corresponds to isolates that were SCC*mec* III by the Zhang et al. (50) typing protocol but amplified the *ccrC* of SCC*mec* type V (3). ND, not determined; nt, not typeable.

[d] ATCC, American Type Culture Collection; CH, human clinical sample; AP, asymptomatic carrier pig; CP, pig clinical sample; D, dust (from pig farms); FP, Dutch pig fecal sample; M, milk; MC, meat from cattle; MB, meat from broiler; MP, meat from pig; MT, meat from turkey; NCTC, National Collection of Type Cultures.

[e] ST398 isolates kindly provided by D. Mevius (Central Veterinary Institute of Waningen, Lelystad, Netherlands), used as control strains.

[f] MLS, macrolide, lincosamide, and streptogramin B group.

eral clinically important *S. aureus*-associated virulence factors regardless of their source. This is in line with the results of other studies (13, 21, 31, 32) and with the fact that clinical disease in swine and humans colonized by ST398 MRSA is rarely observed (16, 45, 47). However, cases of clinical and subclinical mastitis associated with this type of MRSA in dairy herds have been reported (13, 40), as have pathological lesions of pigs (28). From the 37 genes tested in this study that are related to virulence, the presence of pathogenicity islands, and regulators, we could only detect hemolysin-encoding genes (*hla*, *hlb*, *hld*, and *hlg*) and the *agr* group. None of the German isolates carried enterotoxins, but *seb* was present in two of the Dutch ST398 isolates. Low levels of occurrence of either *seb sek* and *seq*, or *sed* and *seg* were also reported for MRSA ST398 isolates from Germany (21) and France (26). It has been speculated that the environment contributes to the low presence of virulence determinants in this clone. However, some studies focused on the pig population have shown how in other *S. aureus* isolates (i.e., MSSA isolates from undetermined CCs), the presence of enterotoxins can be high (33). The Panton-Valentine leukocidin (PVL) was absent in our isolates. Genes encoding this toxin, which is frequently present in community-acquired *S. aureus* isolates, have also been detected in ST398 isolates from hospitalized patients (49) and, at a low frequency, in pig isolates (43). The majority of PVL-positive ST398 isolates described so far has been obtained from humans without exposure to animal husbandry (46, 49).

The low number of virulence factors in ST398 isolates is in clear contrast to the high number of resistance determinants. In this respect, type I and II restriction-modification systems detected in the genome of ST398 (36) may have interfered so far with the acquisition of virulence factors, which are mainly encoded by pathogenicity islands or phages (34). However, this does not explain the high number of resistance genes carried by the clone. In *S. aureus*, resistance genes are mainly located in plasmids and, depending on size and GC content, their chances to escape restriction might be high. Moreover, selection pressure on ST398 isolates induced by the use of antimicrobials in livestock production could have potentiated positive selection for resistance determinants, while infrequent contact with pathogenic bacteria, more common in hospitals, could explain why the clone still lacks many virulence determinants.

In different countries, different antimicrobial usage habits can influence the antimicrobial resistance patterns (regarding phenotypes and mobile genetic determinants) observed in the bacterial populations. The nonhuman German ST398 isolates analyzed in this study were highly heterogeneous with regard to antimicrobial resistance properties. This may be due to the livestock being raised with different types of antimicrobials, but data on the antimicrobial usage on the farms from which the isolates came are not available. A high percentage (59%) of multiresistance was present in the series. Almost 37% of the isolates, 43% of them recovered from healthy carrier pigs, showed resistance to five or more classes of antimicrobials, whereas only 28% did in a study of 55 German ST398 isolates from diseased swine (21). All 100 isolates were resistant to tetracycline, a property of the ST398 clone also found by other authors (9, 41). This resistance is mostly related to the presence of the *tet*(M) gene located in transposons Tn*5801* and Tn*916*. Only a few ST398 isolates lacking *tet*(M) have been

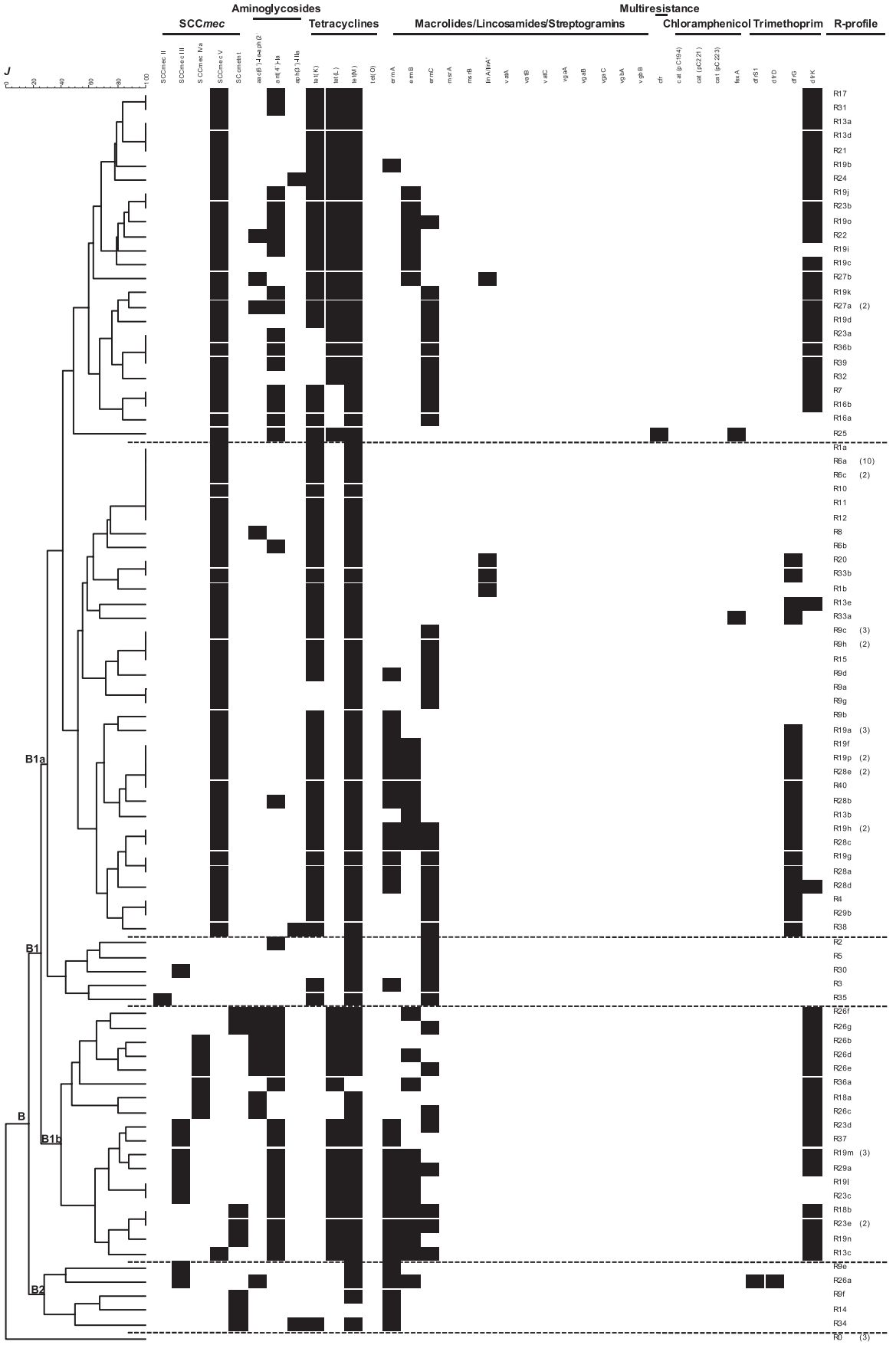

FIG. 2. Dendrogram showing the relatedness between resistance gene profiles (R-profile) for 31 resistance genes or cassettes of the ST398 and outgroup *S. aureus* isolates tested. The scale shows genetic similarity using Jaccard's coefficient (*J*). Dashed lines separate clusters/subclusters.

described so far (21, 22). Nearly all isolates (91%) in this study also carried additional tetracycline resistance genes, like *tet*(K) and/or *tet*(L), that are normally harbored on plasmids. The latter gene, which has rarely been described in non-ST398 MRSA isolates (14, 42), was relatively common in our series (40%), as well as in other isolates from the ST398 clone (24%) (13, 21). The prevalence of trimethoprim resistance was also high (65%), and the *dfrK* and *dfrG* genes, which have rarely been detected among other MRSA isolates, seem to be frequent in German ST398 isolates (13, 21). Colocalization of the *tet*(L) and *dfrK* genes on the pKKS2187 plasmid was described in a German MRSA ST398 isolate from a diseased pig (22). In 36% of our isolates, both genes appeared together, with 4% and 6% being positive for only *tet*(L) or only *dfrK*, respectively. Chloramphenicol resistance was expressed in nine isolates (9%), but only two of them were positive for *fexA*, a gene that has rarely been detected in staphylococci from animal sources (25). Among these *fexA*-positive isolates, one isolate also carried the multidrug resistance determinant *cfr*, which was previously found, associated with *fexA*, in individual ST398 and ST9 isolates (24). This indicates that, currently, both genes are infrequent in the German ST398 population.

In the present study, the percentage of resistance to gentamicin (14%) was similar to that reported for diseased swine by Kadlec et al. (21) but, in comparison to that report, the percentage of resistance to kanamycin was significantly higher (29% versus 7%; χ^2 test, $P = 0.003$) in our study. High percentages of gentamicin and/or kanamycin resistance (22% and 41%, respectively) were reported in ST398 isolates from cases of bovine mastitis (13). The genes implicated in the aminoglycoside resistance of the isolates from our series were *aac(6′)-Ie-aph(2″)-Ia* and/or *ant(4′)-Ia*, very frequently present in *S. aureus* isolates (14) and ST398 isolates from other series (13, 21). The *aph(3′)-IIIa* gene, infrequent in *S. aureus* isolates, was found in only 2% of our series. In 16 isolates (16%) that had repeatedly been shown to be susceptible to kanamycin in disc diffusion assays (corresponding to MICs of ≤16 μg/ml), *ant(4′)-Ia* could be detected. With their MICs having been tested by broth microdilution and considering the EUCAST cutoff values (susceptible range, ≤8 μg/ml), the same isolates showed either susceptibility or resistance (various MICs between 32 and 8 μg/ml). This phenomenon has also been noticed in other studies (11, 19, 30) and could be due to a requirement for the induction of transcription or the presence of an inactive *ant(4′)-Ia.* It has been demonstrated that a rearrangement between the *blaZ* gene and a Tn*4001*-IS*257* hybrid structure has developed an *aac(6′)-Ie-aph(2″)-Ia* gene inducible by β-lactams (20). In our series, all except one of the *aac(6′)-Ie-aph(2″)-Ia*-positive strains were also positive for *blaZ*, but in the present work, no further investigations into the presence of this hybrid structure were conducted. Regarding the MLS_B resistance, several *erm* genes were found (prevalences of 30 to 40%), appearing either alone or in all possible combinations. This is interesting because, in *S. aureus*, the *ermA* gene is the most frequent determinant that confers constitutive resistance to MLS_B, while *ermC* is more common in strains with an inducible phenotype (14, 15, 18). The *ermB* gene has been identified in multiple bacterial genera (35). It has been found more frequently in staphylococci from animal sources than from human sources (14, 15, 18, 37). None of the nine (9%) quinupristin-dalfopristin-resistant isolates carried the genes coding for resistance to type A and B streptogramins tested in the study. The novel gene *vgaC* found in the ST398 clone (23) was also absent. This suggests that the clone possesses other mechanisms responsible for this resistance.

When looking at the clustering analyses (Fig. 2), there was a correlation between the resistance profiles of the isolates, their Cfr9I PFGE profiles, and their SCC*mec* types previously described by Argudín et al. (3). Isolates with SCC*mec* type V usually carried *tet*(K) and clustered together in subcluster B1a. These isolates also clustered together at a similarity of 0.63 when analyzed by Cfr9I PFGE (3). Conversely, subcluster B1b grouped mainly isolates with SCC*mec* IVa or V* without *tet*(K), and their PFGE profiles were also grouped within a different cluster (similarity of 0.64) (3). Some of the SCC*mec* V carriers also have *dfrK*, like most of the SCC*mec* IVa or V* carriers. Despite the differences, all of the ST398 Cfr9I PFGE profiles clustered together and were separate from the outgroup strains of different CCs.

So far, the virulence gene content of the ST398 clone appears to be low. However, continuous surveillance is needed in order to clarify whether the pathogenicity potential of the clone will evolve in the coming years. Moreover, MRSA ST398 is a reservoir for multiple determinants (13, 21, 26, 29) conferring resistance to several antimicrobials of clinical relevance. For the same phenotypic resistance, one or more genes, frequently found on plasmids [*blaZ*, *ant(4′)-Ia*, *tet*(K), *tet*(L), *ermC*, *dfrD*, and *dfrK*] or transposons [*aac(6′)-Ie-aph(2″)-Ia*, *aph(3′)-IIIa tet*(M), *ermA*, *ermB*, and *dfrS1*], are present in these isolates. Many of them may be colocalized on the same genetic element (22, 34). The high prevalence of this type of *S. aureus* in livestock and food, together with the carriage of multiple resistance determinants with a high risk of spread, underlines the importance of controlling this emergent bacterium in the animal population and its spread to exposed humans.

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