ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2005, p. 4263–4271 Vol. 49, No. 10
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.10.4263–4271.2005

Different Clonal Complexes of Methicillin-Resistant *Staphylococcus aureus* Are Disseminated in the Euregio Meuse-Rhine Region

Ruud H. Deurenberg,[1] Cornelis Vink,[1] Guy J. Oudhuis,[1] Jascha E. Mooij,[1] Christel Driessen,[1] Guy Coppens,[2] Jos Craeghs,[3] Els De Brauwer,[4] Sebastian Lemmen,[5] Hans Wagenvoort,[4] Alexander W. Friedrich,[6] Jacques Scheres,[1] and Ellen E. Stobberingh[1]*

Department of Medical Microbiology, Maastricht Infection Center (MINC), University Hospital Maastricht, Maastricht, The Netherlands[1]; Hospital East-Limbourg, Genk, Belgium[2]; General Hospital Vesalius, Tongeren, Belgium[3]; Atrium Medical Centre, Heerlen, The Netherlands[4]; Universitätsklinikum Aachen, Aachen, Germany[5]; and Institute of Hygiene, University of Münster, Münster, Germany[6]

Received 13 January 2005/Returned for modification 8 April 2005/Accepted 29 July 2005

The Euregio Meuse-Rhine (EMR) is formed by the border regions of Belgium, Germany, and The Netherlands. Cross-border health care requires infection control measures, in particular since the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) differs among the three countries. To investigate the dissemination of MRSA in the EMR, 152 MRSA isolates were characterized by pulsed-field gel electrophoresis (PFGE), SCC*mec* typing, and multilocus sequence typing. PFGE revealed major clonal groups A, G, L, and Q, suggesting dissemination of MRSA in the EMR. Group A harbored mainly SCC*mec* type III and sequence types (STs) 239 and 241. The majority of the strains from group G harbored SCC*mec* type I and ST8 and ST247, whereas most strains from group L carried either SCC*mec* type IV or type I. Within group L, ST8 and ST228 were found, belonging to clonal complexes 8 and 5, respectively. Most strains from group Q included SCC*mec* type II and were sequence typed as ST225. Both ST225-MRSA-II and ST241-MRSA-III were novel findings in Germany. In addition, the SCC*mec* type of two isolates has not been described previously. One strain was classified as SCC*mec* type III but harbored the *pls* gene and the *dcs* region. Another strain was characterized as SCC*mec* type IV but lacked the *dcs* region. In addition, one isolate harbored both SCC*mec* type V and Panton-Valentine leukocidin. Finally, the SCC*mec* type of the strains was found to be correlated with the antibiotic susceptibility pattern.

Staphylococcus aureus is a potentially pathogenic bacterium that can cause various diseases such as postoperative wound infections and necrotizing pneumonia (20). *S. aureus* has a strong adaptive power to antibiotics. Since the introduction of methicillin in 1959, methicillin-resistant *S. aureus* (MRSA) strains have been isolated, first in the United Kingdom in 1961 and subsequently in other parts of the world. Although most of the MRSA strains are hospital acquired, community-acquired strains (CA-MRSA) have also recently been reported (32).

Resistance of MRSA strains to methicillin is determined by the presence of the *mecA* gene, which encodes the penicillin binding protein 2a. The *mecA* gene is localized on a mobile genetic element, which is designated the *s*taphylococcal *c*hromosomal *c*assette *mec* (SCC*mec*) (3, 14, 21). Currently, five main types of SCC*mec* (types I to V) are distinguished. SCC*mec* types I, II, and III are associated with hospital-acquired MRSA, whereas types IV and V are associated with CA-MRSA (12, 15). SCC*mec* types I, IV, and V exclusively encode resistance to β-lactam antibiotics. By contrast, SCC*mec* types II and III determine multiresistance, as these cassettes carry both integrated plasmid sequences (e.g., pT181 and pUB110) and transposons (e.g., Tn*554*) containing drug resistance genes. Besides the resistance genes on SCC*mec*, *S. aureus* can also carry drug resistance genes on other sites of its chromosome and on plasmids. Also situated on SCC*mec* are genes responsible for the regulation of the transcription of *mecA*: Δ*mecR1* (on SCC*mec* types I, IV, and V) and *mecR1* and *mecI* (on SCC*mec* types II and III) (13, 14, 15). For integration into and excision from the chromosome at a specific site (*attBscc*), genes encoding chromosomal cassette recombinases (*ccr*) are located within the SCC*mec* elements. These genes are designated *ccrA1* and *ccrB1* (in SCC*mec* type I), *ccrA2* and *ccrB2* (in SCC*mec* types II and IV), *ccrA3* and *ccrB3* (in SCC*mec* type III), and *ccrC* (in SCC*mec* type V) (6, 12, 15).

The Euregio Meuse-Rhine (EMR) is a region consisting of the Belgian provinces of Limbourg and Liège, the German-speaking region of Belgium, the region Aachen in Germany, and the southern part of the Dutch province of Limbourg, with an area of 10.478 km^2. Cross-border patient mobility and free access to health care facilities within the European Union in general, and the EMR in particular, are important issues for patients, doctors, hospitals, sickness funds, and health care insurance companies. Each year, many thousands of the 3.7 million inhabitants of the EMR cross the border to visit a medical specialist or a hospital on the other side of the border. In an official publication of the European Commission (D. Byrne, Maastricht Conference on Cross-Border Health Care, Maastricht, The Netherlands, 8 June 2004), the EMR was therefore recently mentioned as a model region for the European Union in the field of cross-border health care and cross-border cooperation of hospitals and sickness funds. Nevertheless, an

* *Corresponding author. Mailing address: Department of Medical Microbiology, University Hospital Maastricht, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands. Phone: 31-43-3874644. Fax: 31-43-3876643. E-mail: est@lmib.azm.nl.

TABLE 1. Distribution of SCC*mec* types within MRSA isolates in the EMR, 1999–2004

Country	No. (%) of:					Total (no. [%])
	SCC*mec* I	SCC*mec* II	SCC*mec* III	SCC*mec* IV	NT[a]	
Belgium	24 (60)	2 (5)	1 (2.5)	13 (32.5)	0 (0)	40 (100)
Germany	8 (16)	14 (29)	18 (37)	7 (14)	2 (4)	49 (100)
The Netherlands	12 (19)	2 (3)	16 (25)	31 (49)	2 (3)	63 (100)
Total	44 (29)	18 (12)	35 (23)	51 (34)	4 (3)	152 (100)

[a] NT, not typeable.

important issue of concern that is related to cross-border health care is the dissemination of multiresistant bacteria. In this regard, it is interesting to note that the three countries forming the EMR differ considerably in prevalence of MRSA isolated in hospitals (23.6%, 13.8%, and 0.6% in Belgium, Germany, and The Netherlands, respectively) (31). Consequently, the cross-border transfer of patients may have an important impact on the dissemination and prevalence of MRSA, in particular in cases where patients are transferred from countries with a relatively high prevalence to a country with a low prevalence. Therefore, we investigated the dissemination of MRSA isolates between hospitals from the EMR during the last 5 years. The MRSA isolates were subjected to pulsed-field gel electrophoresis (PFGE), SCC*mec* typing, and multilocus sequence typing (MLST) (2). As the presence of Panton-Valentine leukocidin (PVL) genes was suggested to be an important characteristic of CA-MRSA (33), the isolates were also subjected to a PVL-specific real-time PCR.

MATERIALS AND METHODS

Clinical isolates. One hundred fifty-two isolates of MRSA from individual patients, isolated between December 1999 and February 2004 from five geographically closely related hospitals in the EMR, were included in the study (Table 1). These included two Belgian hospitals (Hospital East-Limbourg, Genk, an 822-bed general hospital, and General Hospital Vesalius, Tongeren, a 355-bed general hospital), one German hospital (Universitätsklinikum Aachen, a tertiary 1,500-bed university hospital), and two Dutch hospitals (Atrium Medical Centre, Heerlen, an 811-bed general hospital, and University Hospital Maastricht, a tertiary 680-bed university hospital). The 152 MRSA isolates included 40 isolates from Belgium, 49 from Germany, and 63 from The Netherlands (Table 1). The isolates from the Belgian and German hospitals represented a random selection of 50% of the clinical isolates. The Dutch isolates were derived from surveillance cultures from patients at risk for MRSA carriage who were admitted to the two Dutch hospitals during the study period. During this period, infections with MRSA were not found in the two Dutch hospitals. All strains were identified as *S. aureus* by catalase and coagulase testing. Methicillin resistance was determined by the disk diffusion test with oxacillin concentration disks, according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (22), and by PCR amplification of the *mecA* gene (26).

Reference strains. MRSA strains COL, BK2464, ANS46, HDE288, and WIS were used as reference strains for SCC*mec* types I, II, III, IV, and V, respectively (15, 23). *S. aureus* strain 1206 was used as a positive control for the Tn*554* PCR (35). For PFGE, reference strain *S. aureus* Ps 47 was used as a molecular weight marker, whereas MRSA strains BK2464, COL, HDE288, HU25, and PER34 were used as reference strains for the New York/Japan, archaic, pediatric, Brazilian, and Iberian clonal types, respectively (24).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed by microbroth dilution according to the NCCLS guidelines (22) for the following antibiotics: amikacin, amoxicillin, cefazolin, ciprofloxacin, clindamycin, co-trimoxazole, doxycycline, erythromycin, flucloxacillin, gentamicin, penicillin, rifampin, and vancomycin.

SCC*mec* typing. SCC*mec* typing was essentially carried out as described by Oliveira and de Lencastre (23), in which *mecA* and six different loci on SCC*mec* (Fig. 1) were amplified by PCR with the following modifications. PCR amplification of *mecA* sequences was carried out with primers *mec*A1 and *mec*A2 (Sigma Genosys, The Netherlands), resulting in a PCR product of 527 bp instead of 162 bp (26). PCR was performed in a volume of 50 μl containing 10 μl of a 0.5 McFarland suspension (1.5×10^8 CFU/ml) of the MRSA strain, 0.2 mM of each deoxynucleoside triphosphate (Amersham Biosciences, The Netherlands), 1× PCR buffer (QIAGEN, The Netherlands), 1.25 U HotStar*Taq* (QIAGEN, The Netherlands), and primers. The primer concentrations used were similar to those previously described (23), except for those of the *mecA* primers, which were 0.6 μM for both *mec*A1 and *mec*A2. The amplifications were performed on a GeneAmp PCR system, model 9600 (Applied Biosystems, The Netherlands), with the following program: 15 min at 94°C, followed by 30 cycles of 30 s at 94°C,

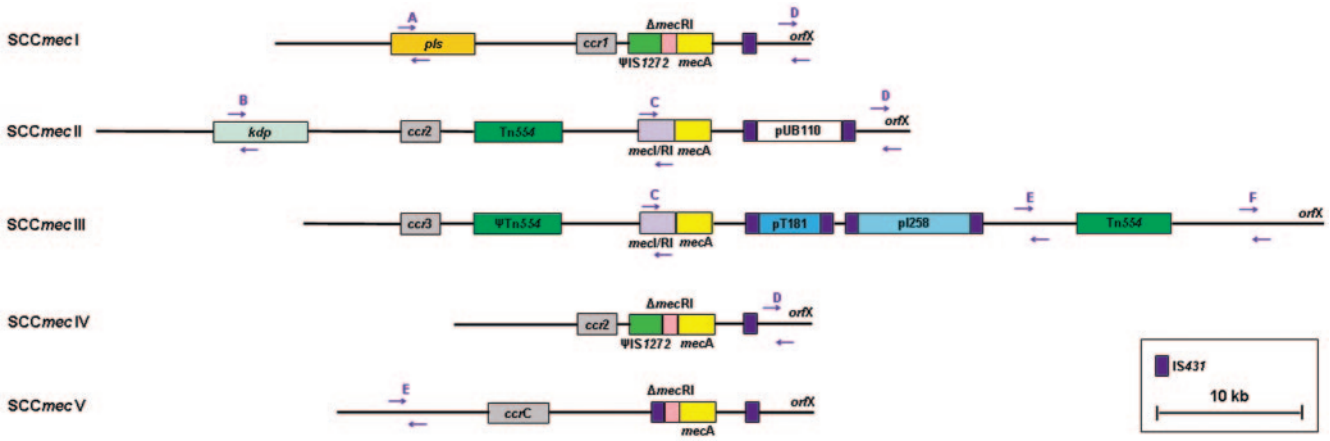

FIG. 1. Schematic drawing of SCC*mec* types I to V. The major elements (*ccr* genes, IS*431*, IS*1272*, *mecA*, *mecI*, *mecRI*, *orfX*, pI258, pT181, pUB110, and Tn*554*) of the five SCC*mec* types are given, as are the six different loci (A to F) used for the typing of SCC*mec* according to the method of Oliveira and de Lencastre (23). The primers of the PCR for the six different SCC*mec* loci are indicated by arrows.

30 s at 53°C, and 60 s at 72°C, followed by a postextension step of 10 min at 72°C. The PCR products were separated on 2% agarose gels in Tris-acetate-EDTA buffer, stained with ethidium bromide, and visualized with UV light by using a FluorChem imaging system (Alpha Innotech Corporation, The Netherlands).

PCR for *ccrAB* and *ccrC*. Most of the primers used for amplification of *ccrAB* and *ccrC* were used as described previously (11, 15). Primer β2, however, was replaced by a primer with the following sequence: 5′-ATTGCCTTGATAATA GCCTCT-3′ (primer β2a). The following reaction conditions were used: either 1.2 μM of forward primer β2a for *ccrAB* or 0.4 μM of forward primer γF for *ccrC*; 0.4 μM of reverse primer α2, α3, α4, or γR; 0.2 mM of each deoxynucleoside triphosphate; 1× PCR buffer; 2.5 U of HotStar*Taq* DNA polymerase; and 10 μl of a 0.5 to 1 McFarland suspension (1.5×10^8 to 3×10^8 CFU/ml) in a total volume of 50 μl. Amplification was performed on a GeneAmp PCR system, model 9600, by using the following program: 15 min at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C, followed by an extension step of 10 min at 72°C. PCR products were analyzed by electrophoresis through 1% agarose gels as described above.

PCR for Tn*554*. The primers for Tn*554* were used as described previously (16). The reaction conditions used were similar to those for the *ccr* PCR. Amplification was performed on a GeneAmp PCR system, model 9600, by using the following program: 15 min at 94°C, followed by 34 cycles of 1 min at 94°C, 1 min at 63°C, and 1 min at 72°C, followed by an extension step of 10 min at 72°C. PCR products were analyzed by electrophoresis through 1% agarose gels as described above.

PFGE. PFGE was carried out essentially as described previously (11). The banding patterns were visualized with UV light by using a FluorChem imaging system. Subsequently, the patterns were analyzed with Dice comparison and unweighted-pair group matching analysis settings with GelCompar II 3.5 (Applied Maths, Sint-Martens-Latem, Belgium) according to the scheme of Tenover et al. (30). The position tolerance was set at 2.0%, and isolates with a similarity index of 0.80 or more were classified as a clonal group (5, 25).

MLST. It has previously been shown that MRSA strains from one major clonal group, as demonstrated by PFGE, have either the same sequence type (ST) or STs that are related to a single clonal complex (5, 7, 25, 29). Therefore, two representative strains from each of the major clonal groups as obtained through PFGE (5, 7, 25, 29) were used for MLST (9). The primers used for MLST were identical to those described previously (9), with the exception of primers *glpF*-Dn and *gmk*-Up, which were replaced by primers *glpF*-Dna (5′-TGGTAAAATCG CATGTGCAATTC-3′) and *gmk*-Upa (5′-ATCGTTTTATCAGGACCATC-3′), respectively. The PCR products were sequenced using an ALFexpress II automatic sequencer (Amersham Biosciences, The Netherlands). Finally, the STs were determined by using the MLST database (http://www.mlst.net).

Real-time PCR for PVL. PVL was detected with a real-time PCR method as described previously (8).

Statistical analysis. The correlation between SCC*mec* type and antibiotic susceptibility pattern was determined by canonical discriminant analyses with the software package SPSS 11.0.1 (SPSS, Inc., The Netherlands). Canonical discriminant analyses are used for the investigation of one or more normally distributed interval independent variables (SCC*mec* type) and a categorical dependent variable (susceptibility pattern). This is a multivariate technique that considers the latent dimensions in the independent variables for predicting group membership in the categorical dependent variable.

RESULTS

Distribution of SCC*mec* types. The SCC*mec* types (Fig. 1) could be determined for 148 of the 152 (97.4%) clinical isolates of MRSA. Only four of the five different types of SCC*mec* were found (I to IV [Table 1]) with the method described by Oliveira and de Lencastre (23). SCC*mec* types I and IV were predominant, with 29% and 34%, respectively, and types II and III were less common, with 12% and 23%, respectively. The different SCC*mec* types were not distributed similarly among the three countries from the EMR. In Belgium, SCC*mec* types I and IV predominated, whereas in Germany, the most common types were types II and III. In The Netherlands, the most frequently found types were types I, III, and IV (Table 1).

The SCC*mec* types of 4 (DOM068, DOM083, DOM114, and DOM012) of the 152 MRSA isolates (3%) could not be determined according to the method described by Oliveira and de Lencastre (23). DOM068 was found to contain loci A, C, D, and E, as well as F (Table 2), which is uncommon to the prototypes of the five known SCC*mec* types, as shown in Fig. 1. Another isolate, DOM083, was also found to have a unique SCC*mec* organization, possessing loci C, D, E, and F (Table 2). The SCC*mec* structure of isolates DOM068 and DOM083 was further characterized by determination of the presence of two other loci from the SCC*mec* cassette, i.e., *ccr* and Tn*554*. Both strains were found to possess Tn*554* as well as *ccrAB3*, indicating that their SCC*mec* cassettes most strongly resemble the type III cassette (Table 2).

TABLE 2. Results of non-typeable[b] SCC*mec* within MRSA isolates

Code[a]	Country	Locus (loci)[c]	*ccr*	Tn*554*	SCC*mec* type
DOM012	The Netherlands	—	*ccrA2*	−	IV
DOM068	Germany	A, C to F	*ccrA3*	+	III
DOM083	Germany	C to F	*ccrA3*	+	III
DOM114	The Netherlands	E	*ccrC*	−	V

[a] All strains were positive for *mecA*.
[b] According to the classification scheme of Oliveira and de Lencastre (23).
[c] The distributions of the six different loci among the SCC*mec* types are given in Fig. 1. —, isolate lacked all six loci.

The cassette of another strain, DOM114, was found to contain only a single locus from the six loci that were defined by Oliveira and de Lencastre (23), i.e., locus E (Table 2). Since DOM114 was also found to possess the *ccrC* gene, its SCC*mec* cassette can be classified as type V (Table 2).

The fourth "nontypeable" isolate, DOM012, lacked all six loci as defined by Oliveira and de Lencastre (23). However, this strain was found to contain *ccrAB2*, which is characteristic for both type II and type IV cassettes. Since the DOM012 cassette did not contain Tn*554*, it was classified as type IV (Table 2).

PFGE analyses. Each of the MRSA strains was subjected to analysis by PFGE. On the basis of the PFGE patterns, a dendrogram was constructed (Fig. 2). One of the isolates (DOM152) could not be typed due to repeated difficulties with the isolation of DNA from this strain. A total of 32 clonal groups (A to AF) were distinguished, of which four were major clonal groups (A, G, L, and Q). Major clonal group A was closely related to the clonal groups B, C, D, and E. Taken together, these groups included 34 of the 152 MRSA isolates (22%). A large majority of these 34 strains (88%), isolated from both Germany and The Netherlands, harbored SCC*mec* type III.

The second major clonal group, group G, contained 26 of the 152 MRSA isolates (17%), of which 21 (81%) harbored SCC*mec* type I and 5 (19%) harbored SCC*mec* type IV. Most isolates from this group originated from Belgium and The Netherlands. Group G was also found to include reference strain COL, representative for the archaic clone, which is one of the six major MRSA clones spread worldwide (2). Clonal group F, which was related to group G, comprised only four isolates (3%), three of which harbored SCC*mec* type IV and one of which harbored SCC*mec* type V. The reference strain for the Iberian clone (2), PER34, was linked to clonal group F.

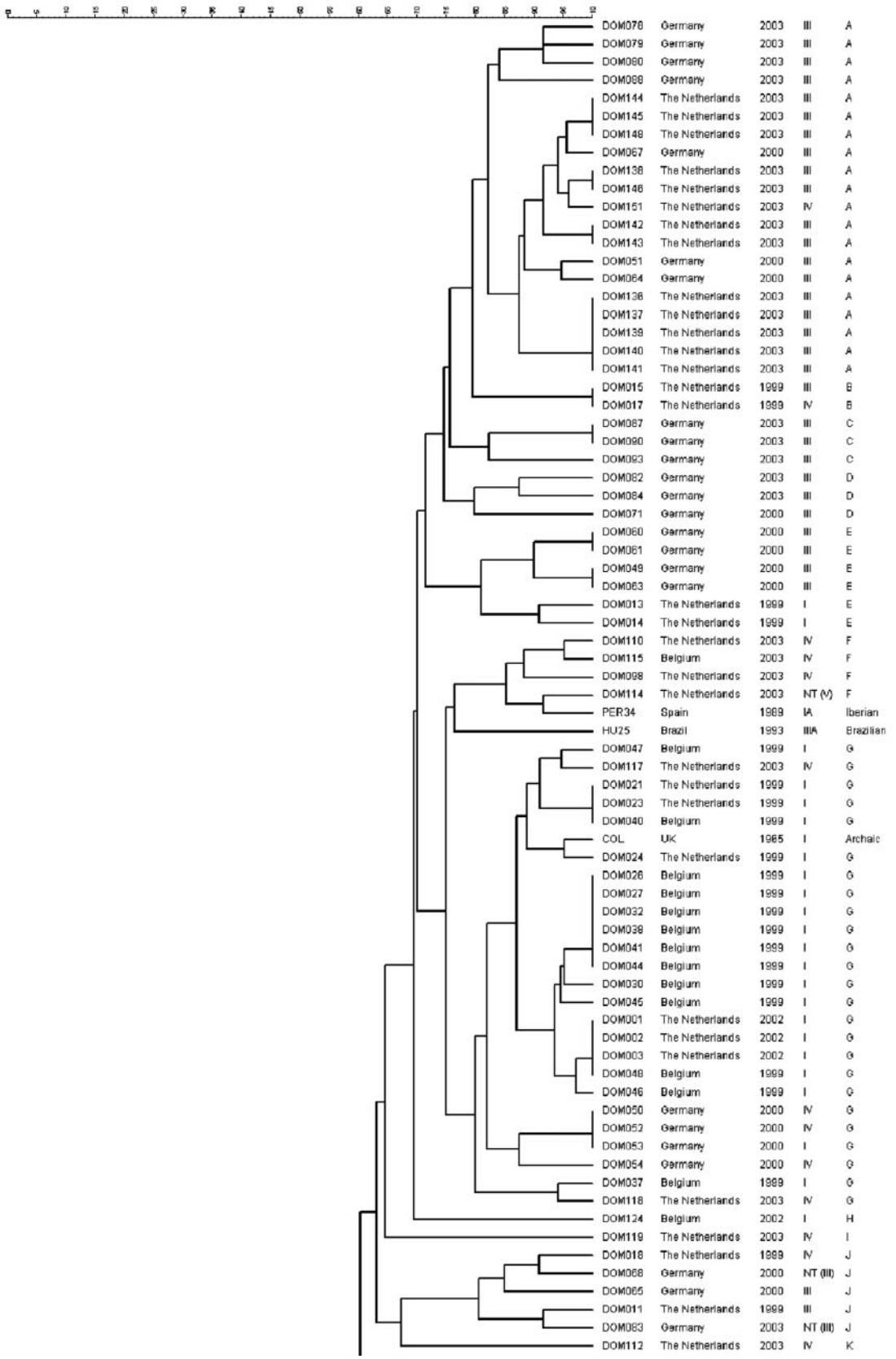

FIG. 2. Dendrogram of the 152 clinical MRSA isolates and five reference clones. The five columns on the right represent MRSA isolate code, country of origin, year of isolation, SCC*mec* type, and clonal group, respectively. NT, not typeable.

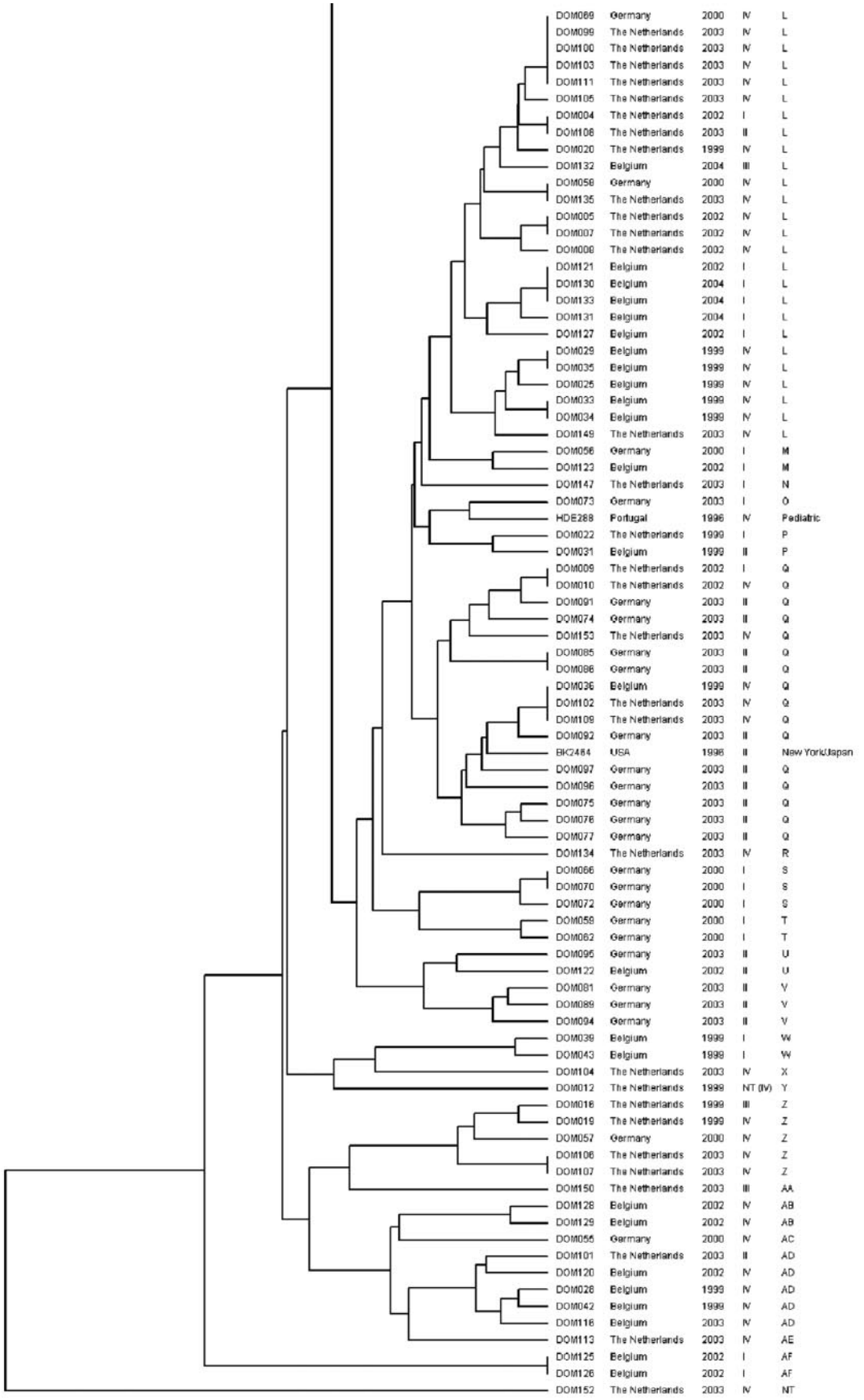

FIG. 2—*Continued.*

TABLE 3. Typing results of the four major clonal groups

Code	Country	Major clonal group	SCC*mec* type	MLST	ST	CC[a]
DOM078	Germany	A	III	2-3-1-1-4-4-30	241	8
DOM141	The Netherlands	A	III	2-3-1-1-4-4-3	239	8
DOM038	Belgium	G	I	3-3-1-12-4-4-16	247	8
DOM053	Germany	G	I	3-3-1-1-4-4-3	8	8
DOM111	The Netherlands	L	IV	3-3-1-1-4-4-3	8	8
DOM131	Belgium	L	I	1-4-1-4-12-24-29	228	5
DOM077	Germany	Q	II	1-4-1-4-12-25-10	225	5
DOM092	Germany	Q	II	1-4-1-4-12-25-10	225	5

[a] CC, clonal complex.

The third major clonal group, group L, included 26 of the 152 MRSA isolates (17%). Of these, 18 (69%) contained SCC*mec* type IV, 6 (23%) contained SCC*mec* type I, 1 (4%) contained SCC*mec* type II, and 1 (4%) contained SCC*mec* type III. Most of the strains were isolated in Belgium and The Netherlands. None of the reference strains were linked to this clonal group.

The fourth major group, group Q, contained 16 of the 152 MRSA isolates (11%). Ten (63%) of these carried SCC*mec* type II, whereas five (31%) contained SCC*mec* IV and one (6%) contained SCC*mec* type I. All except one of the strains from this group were isolated in either Germany or The Netherlands. Group Q also included reference strain BK2464, which is a representative of the New York/Japan clone (2).

MLST analyses. To investigate which major MRSA clones from which clonal complexes are disseminated in the EMR, two representative strains from each major clonal PFGE group were subjected to MLST. As shown in Table 3, MLST identified six different sequence types (ST8, -225, -228, -239, -241, and -247) that belonged to two clonal complexes (CC5 and CC8). In major clonal group A, two different STs, which both belong to CC5, were found: (i) ST239-MRSA-III, a single-locus variant (SLV) of ST8 and representative for the Brazilian clone, and (ii) ST241-MRSA-II, an SLV of ST239-MRSA-III (at locus *yqiL*). The two STs that were found in major clonal group G are different SLVs of a single ST, i.e., ST250; ST247-MRSA-I is an SLV at the *gmk* locus, and ST8-MRSA-I is an SLV at the *yqiL* locus of ST250 (10). Interestingly, the strains from major clonal group L (DOM111 and DOM131) were typed as ST8-MRSA-IV and ST228-MRSA-I, respectively, which belong to different clonal complexes (CC8 and CC5, respectively). Both strains from major clonal group Q were typed as ST225-MRSA-II, an SLV at the *tpi* locus of ST5 (10).

Prevalence of PVL. Only 2 (DOM103 and DOM114) of the 152 MRSA isolates (1.3%) contained PVL. Although both strains were isolated in The Netherlands in 2003, they differed in both SCC*mec* type and PFGE type. Strain DOM103 was classified within major clonal group L, harboring SCC*mec* type IV, whereas strain DOM114 was classified within group F, carrying SCC*mec* type V (Fig. 2).

Correlation between SCC*mec* type and antibiotic susceptibility pattern. The antibiotic resistance patterns for the SCC*mec* types are presented in Table 4. Only the non-β-lactam antibiotics are presented in this table, since all MRSA strains were resistant to the four β-lactam antibiotics tested, i.e., amoxicillin, cefazolin, flucloxacillin, and penicillin. To investigate if a correlation exists between the SCC*mec* type and the antibiotic susceptibility pattern of MRSA isolates, canonical discriminant analyses were performed. As shown in Fig. 3, the SCC*mec* types were centered around the four group centroids, indicating that SCC*mec* type and susceptibility pattern were indeed correlated. More specifically, the antibiotic susceptibility pattern had a predictive value of 84.1% for SCC*mec* type I, 83.3% for SCC*mec* type II, 85.7% for SCC*mec* type III, and 86.3% for SCC*mec* type IV (Table 5). As shown in Table 4 and Fig. 3, the correlation is more pronounced for SCC*mec* types II, III, and IV than for SCC*mec* type I.

DISCUSSION

Monitoring the dissemination of MRSA in the EMR is important, since known and novel MRSA clones may spread from

TABLE 4. Distribution of the non-β-lactam antibiotic resistance patterns among the 152 MRSA strains and their corresponding SCC*mec* types

SCC*mec* type	No. of strains	No. (%) of resistant MRSA strains[a]								
		AMK	CIP	CLI	COT	DOX	ERY	GEN	RIF	VAN
I	44	43 (98)	42 (96)	32 (73)	42 (96)	17 (39)	38 (86)	42 (96)	7 (16)	0 (0)
II	18	17 (94)	17 (94)	17 (94)	3 (17)	1 (6)	17 (94)	1 (6)	1 (6)	0 (0)
III	35	35 (100)	33 (94)	30 (86)	34 (97)	32 (91)	34 (97)	32 (91)	6 (17)	0 (0)
IV	51	34 (67)	40 (78)	11 (22)	10 (20)	0 (0)	28 (55)	22 (43)	0 (0)	0 (0)
NT[b]	4	3 (75)	2 (50)	1 (25)	2 (50)	2 (50)	1 (25)	3 (75)	2 (50)	0 (0)

[a] AMK, amikacin; CIP, ciprofloxacin; CLI, clindamycin; COT, co-trimoxazole; DOX, doxycycline; ERY, erythromycin; GEN, gentamicin; RIF, rifampin; VAN, vancomycin.
[b] NT, not typeable.

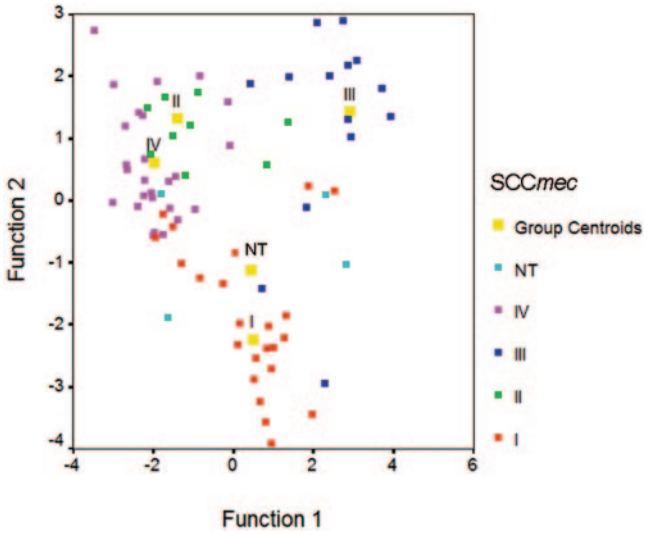

FIG. 3. Statistical analyses of SCC*mec* types and antibiotic susceptibility patterns. The discriminant function 1 and function 2 are latent variables that are created as a linear combination of discriminating variables. NT, not typeable.

TABLE 5. Correlation between the SCC*mec* types and the antibiotic susceptibility patterns of the 152 MRSA strains

SCC*mec* type	Predicted SCC*mec* type (no. [%])[a]					Total (no. [%])
	I	II	III	IV	NT[b]	
I	37 (84.1)	0 (0)	2 (4.5)	5 (11.4)	0 (0)	44 (100)
II	0 (0)	15 (83.3)	1 (5.6)	1 (5.6)	1 (5.6)	18 (100)
III	3 (8.6)	1 (2.9)	30 (85.7)	1 (2.9)	0 (0)	35 (100)
IV	0 (0)	7 (13.7)	0 (0)	44 (86.3)	0 (0)	51 (100)
NT[b]	0 (0)	0 (0)	1 (25)	1 (25)	2 (50)	4 (100)

[a] Predicted from susceptibility pattern.
[b] NT, not typeable.

country to country through cross-border patient care. In particular, the spread of MRSA harboring either SCC*mec* type II or type III, which encodes multiresistance, could pose a serious threat to health care facilities.

In this study, 152 MRSA strains were characterized, isolated in hospitals from the EMR between 1999 and 2004. Typing of the strains by PFGE revealed four major clonal groups, suggesting dissemination of MRSA in the EMR. The strains that were classified within major clonal group A and within the closely related, minor clonal groups B to E comprised 22% of all isolates. Two representative strains from group A were also typed by MLST and were classified as ST239-MRSA-III and ST241-MRSA-III. Both STs form part of CC8 (10). Although strains with the signature of ST239-MRSA-III were previously found in both Germany and The Netherlands, this study is the first to report the presence of ST241-MRSA-III in Germany (10).

The second major clonal group, group G, included MRSA strains harboring mainly SCC*mec* type I (Fig. 2). MLST of two representative clones from group G revealed two different STs, which were classified within the same clonal complex (CC8), i.e., ST8-MRSA-I and ST247-MRSA-I. Both STs have previously been found in the countries from the EMR: ST8-MRSA-I in Belgium and The Netherlands and ST247-MRSA-I in Belgium and Germany (7, 10, 36).

MRSA strains from major clonal group L harbored mainly SCC*mec* types IV and I. From this group, two strains with different SCC*mec* types (one strain of type IV and one of type I) were selected for analysis by MLST. Thus, these strains were classified as ST8-MRSA-IV and ST228-MRSA-I, respectively. Interestingly, these STs belong to different clonal complexes, i.e., CC8 and CC5, respectively. Although these clonal complexes were more closely related to each other than they are to other clonal complexes (19), the finding of strains from different MLST clonal complexes within a single PFGE clonal group was novel for *S. aureus*. Nevertheless, this finding was in line with previous reports that demonstrated the higher discriminatory power of MLST over PFGE for bacterial species other than *S. aureus*, such as *Vibrio cholerae*, *Salmonella*, and *Listeria monocytogenes* (17, 18, 28). Both ST8-MRSA-IV and ST228-MRSA-I were found previously in the EMR countries: ST8-MRSA-IV in Germany and The Netherlands and ST228-MRSA-I in Belgium and Germany (10, 36).

MRSA strains from the fourth major clonal group, group Q, harbored mainly SCC*mec* type II. Two representatives from this group were both typed as ST225-MRSA-II, an ST belonging to CC5. Although ST225-MRSA-II has previously been found in the United States (29), the finding of this ST in Germany is novel. Since ST225-MRSA-II is an SLV at the *tpi* locus of strain ST5-MRSA-II, which was previously found in Belgium (7), ST225-MRSA-II may be derived from ST5-MRSA-II. Recent studies reported the finding of ST22-MRSA-IV and ST45-MRSA-IV in Belgium and ST45-MRSA-I and ST45-MRSA-IV in The Netherlands (7, 34). These STs, however, were not found in this study.

For 4 of the 152 MRSA isolates (3%) the SCC*mec* type could not be determined using the method described by Oliveira and de Lencastre (23). The percentage of nontypeable SCC*mec* cassettes was low compared to that from other studies, in which 10 to 15% could not be typed (4, 11). Since two of the nontypeable MRSA strains (DOM068 and DOM083) were found to possess *ccrAB3*, they could be considered SCC*mec* type III strains. However, compared to the type III cassette prototype (Fig. 1), strain DOM068 contained two additional loci, locus A (*pls* gene) and locus D (*dcs* region). Strains with an organization of SCC*mec* loci similar to that for DOM068 have not been reported yet. In contrast, a strain with an additional locus D as opposed to the type III prototype, as seen in strain DOM083, has previously been described by Aires de Sousa and de Lencastre (1).

Another nontypeable strain, DOM012, was found to contain only a single SCC*mec* locus apart from *mecA*. This locus, *ccrAB2*, was present in the prototype cassettes of both type II and type IV. However, since strain DOM012 possessed neither *mecI* (locus C) nor Tn*554*, its gene cassette had a higher similarity with the cassette of type IV than with that of type II. Also, the resistance to β-lactam antibiotics found was in line with SCC*mec* type IV. We therefore concluded that strain DOM012 carries SCC*mec* type IV but lacks locus D.

The low prevalence of PVL was in accordance with previous studies (8, 27). Both strains (DOM103 and DOM114) were very likely not related, as they were classified within different

clonal groups and harbored different SCC*mec* types. DOM114 is to our knowledge the first reported PVL-positive MRSA strain carrying SCC*mec* type V.

Although antibiotic resistance in *S. aureus* can also be determined by sequences other than SCC*mec*, a correlation of approximately 85% was found between antibiotic susceptibility pattern and SCC*mec* type. Rapid identification of the SCC*mec* type of MRSA isolates by PCR could therefore be useful to predict the antibiotic susceptibility pattern of isolates and, consequently, guide the choice of antibiotics used for treatment. Hence, the identification of the SCC*mec* type of clinical isolates might contribute to prevent the unnecessary use of vancomycin, which is needed only in case of MRSA isolates harboring SCC*mec* type II or III.

In summary, MRSA strains belonging to clonal complexes 5 and 8 were disseminated in the EMR and several "new" types were found: both ST225-MRSA-II and ST241-MRSA-III were novel findings in the German part of the EMR. Furthermore, one strain was classified as SCC*mec* type III, but contained the *pls* gene and the *dcs* region, and another strain was characterized as SCC*mec* type IV, but lacked the *dcs* region. One isolate harboring both SCC*mec* type V and PVL was found.

ACKNOWLEDGMENTS

The study was partly performed within the framework of the Interreg-III project "Cross-Border Health Care in the Euregio Meuse Rhine."

We thank H. de Lencastre and D. C. Oliveira from The Rockefeller University, New York, N.Y., for providing the reference strains for SCC*mec* typing; T. Ito from the Juntendo University, Tokyo, Japan, for providing the reference strain (WIS) for SCC*mec* V; and W. Wannet from the National Institute of Public Health and Environment (RIVM) in Bilthoven, The Netherlands, for the MRSA cluster 28 strain. We are grateful to Monique Coomans and Alexandra Heinzmann for excellent technical assistance with the MIC determinations, Peter Terporten for the statistical analysis, and Erik Beuken for his help with DNA sequencing.

REFERENCES

1. **Aires de Sousa, M., and H. de Lencastre.** 2003. Evolution of sporadic isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals and their similarities to isolates of community-acquired MRSA. J. Clin. Microbiol. **41:**3806–3815.
2. **Aires de Sousa, M., and H. de Lencastre.** 2004. Bridges from hospitals to the laboratory: genetic portraits of methicillin-resistant *Staphylococcus aureus* clones. FEMS Immunol. Med. Microbiol. **40:**101–111.
3. **Berger-Bächi, B., and S. Rohrer.** 2002. Factors influencing methicillin resistance in staphylococci. Arch. Microbiol. **178:**165–171.
4. **Chung, M., G. Dickinson, H. De Lencastre, and A. Tomasz.** 2004. International clones of methicillin-resistant *Staphylococcus aureus* in two hospitals in Miami, Florida. J. Clin. Microbiol. **42:**542–547.
5. **Coombs, G. W., G. R. Nimmo, J. M. Bell, F. Huygens, F. G. O'Brien, M. J. Malkowski, J. C. Pearson, A. J. Stephens, P. M. Giffard, and Australian Group for Antimicrobial Resistance.** 2004. Genetic diversity among community methicillin-resistant *Staphylococcus aureus* strains causing outpatient infections in Australia. J. Clin. Microbiol. **42:**4735–4743.
6. **Daum, R. S., T. Ito, K. Hiramatsu, F. Hussain, K. Mongkolrattanothai, M. Jamklang, and S. Boyle-Vavra.** 2002. A novel methicillin-resistance cassette in community-acquired methicillin-resistant *Staphylococcus aureus* isolates of diverse genetic backgrounds. J. Infect. Dis. **186:**1344–1347.
7. **Denis, O., A. Deplano, C. Nonhoff, R. De Ryck, R. de Mendonca, S. Rottiers, R. Vanhoof, and M. J. Struelens.** 2004. National surveillance of methicillin-resistant *Staphylococcus aureus* in Belgian hospitals indicates rapid diversification of epidemic clones. Antimicrob. Agents Chemother. **48:**3625–3629.
8. **Deurenberg, R. H., C. Vink, C. Driessen, M. Bes, N. London, J. Etienne, and E. E. Stobberingh.** 2004. Rapid detection of Panton-Valentine leukocidin from clinical isolates of *Staphylococcus aureus* strains by real-time PCR. FEMS Microbiol. Lett. **240:**225–228.
9. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.
10. **Enright, M. C., D. A. Robinson, G. Randle, E. J. Feil, H. Grundmann, and B. G. Spratt.** 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. USA **99:**7687–7692.
11. **Hanssen, A.-M., G. Kjeldsen, and J. U. Ericson Sollid.** 2004. Local variants of staphylococcal cassette chromosome *mec* in sporadic methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci: evidence of horizontal gene transfer? Antimicrob. Agents Chemother. **48:**285–296.
12. **Hiramatsu, K., L. Cui, M. Kuroda, and T. Ito.** 2001. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol. **9:**486–493.
13. **Ito, T., Y. Katayama, K. Asada, N. Mori, K. Tsutsumimoto, C. Tiensasitorn, and K. Hiramatsu.** 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **45:**1323–1336.
14. **Ito, T., K. Okuma, X. X. Ma, H. Yuzawa, and K. Hiramatsu.** 2003. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. Drug Resist. Updates **6:**41–52.
15. **Ito, T., X. X. Ma, F. Takeuchi, K. Okuma, H. Yuzawa, and K. Hiramatsu.** 2004. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. Antimicrob. Agents Chemother. **48:**2637–2651.
16. **Jensen, L. B., N. Frimodt-Moller, and F. M. Aarestrup.** 1999. Presence of *erm* gene classes in Gram-positive bacteria of animal and human origin in Denmark. FEMS Microbiol. Lett. **170:**151–158.
17. **Kotetishvili, M, O. C. Stine, Y. Chen, A. Kreger, A. Sulakvelidze, S. Sozhamannan, and J. G. Morris, Jr.** 2003. Multilocus sequence typing has better discriminatory ability for typing *Vibrio cholerae* than does pulsed-field gel electrophoresis and provides a measure of phylogenetic relatedness. J. Clin. Microbiol. **41:**2191–2196.
18. **Kotetishvili, M., O. C. Stine, A. Kreger, J. G. Morris, Jr., and A. Sulakvelidze.** 2002. Multilocus sequence typing for characterization of clinical and environmental *Salmonella* strains. J. Clin. Microbiol. **40:**1626–1635.
19. **Lindsay, J. A., and M. T. Holden.** 2004. *Staphylococcus aureus*: superbug, super genome? Trends Microbiol. **12:**378–385.
20. **Lowy, F. D.** 1998. *Staphylococcus aureus* infections. N. Engl. J. Med. **339:** 520–532.
21. **Mongkolrattanothai, K., S. Boyle, M. D. Kahana, and R. S. Daum.** 2003. Severe *Staphylococcus aureus* infections caused by clonally related community-acquired methicillin-susceptible and methicillin-resistant isolates. Clin. Infect. Dis. **37:**1050–1058.
22. **National Committee for Clinical Laboratory Standards.** 1999. Performance standards for antimicrobial susceptibility testing; ninth informational supplement M100-S9. National Committee for Clinical Laboratory Standards, Wayne, Pa.
23. **Oliveira, D. C., and H. de Lencastre.** 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **46:** 2155–2161.
24. **Oliveira, D. C., A. Tomasz, and H. de Lencastre.** 2001. The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated *mec* elements. Microb. Drug Resist. **7:**349–361.
25. **Perez-Roth, E., F. Lorenzo-Diaz, N. Batista, A. Moreno, and S. Mendez-Alvarez.** 2004. Tracking methicillin-resistant *Staphylococcus aureus* clones during a 5-year period (1998 to 2002) in a Spanish hospital. J. Clin. Microbiol. **42:**4649–4656.
26. **Predari, S. C., M. Ligozzi, and R. Fontana.** 1991. Genotypic identification of methicillin-resistant coagulase-negative staphylococci by polymerase chain reaction. Antimicrob. Agents Chemother. **35:**2568–2573.
27. **Prevost, G., P. Couppie, P. Prevost, S. Gayet, P. Petiau, B. Cribier, H. Monteil, and Y. Piemont.** 1995. Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. J. Med. Microbiol. **42:**237–245.
28. **Revazishvili, T., M. Kotetishvili, O. C. Stine, A. S. Kreger, J. G. Morris, Jr., and A. Sulakvelidze.** 2004. Comparative analysis of multilocus sequence typing and pulsed-field gel electrophoresis for characterizing *Listeria monocytogenes* strains isolated from environmental and clinical sources. J. Clin. Microbiol. **42:**276–285.
29. **Shukla, S. K., M. E. Stemper, S. V. Ramaswamy, J. M. Conradt, R. Reich, E. A. Graviss, and K. D. Reed.** 2004. Molecular characteristics of nosocomial and Native American community-associated methicillin-resistant *Staphylococcus aureus* clones from rural Wisconsin. J. Clin. Microbiol. **42:**3752–3757.
30. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
31. **Tiemersma, E. W., S. L. A. M. Bronzwaer, O. Lyytikäinen, J. E. Degener, P. Schrijnemakers, N. Bruinsma, J. Monen, W. Witte, H. Grundmann, and**

European Antimicrobial Resistance Surveillance System participants. 2004. Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. Emerg. Infect. Dis. **10:**1627–1634.
32. **van den Broek, P. J.** 2003. *Staphylococcus aureus*, a successful pathogen. Ned. Tijdschr. Geneeskd. **147:**1045–1048. (In Dutch.)
33. **Vandenesch, F., T. Naimi, M. C. Enright, G. Lina, G. R. Nimmo, H. Heffernan, N. Liassine, M. Bes, T. Greenland, M. E. Reverdy, and J. Etienne.** 2003. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg. Infect. Dis. **9:**978–984.
34. **Wannet, W. J., E. Spalburg, M. E. Heck, G. N. Pluister, R. J. Willems, and A. J. De Neeling.** 2004. Widespread dissemination in The Netherlands of the epidemic Berlin methicillin-resistant *Staphylococcus aureus* clone with low-level resistance to oxacillin. J. Clin. Microbiol. **42:**3077–3082.
35. **Westh, H., D. M. Hougaard, J. Vuust, and V. T. Rosdahl.** 1995. Prevalence of *erm* gene classes in erythromycin-resistant *Staphylococcus aureus* strains isolated between 1959 and 1988. Antimicrob. Agents. Chemother. **39:** 369–373.
36. **Witte, W.** 2004. International dissemination of antibiotic resistant strains of bacterial pathogens. Infect. Genet. Evol. **4:**187–191.