JCM
Journals.ASM.org

Population Dynamics among Methicillin-Resistant *Staphylococcus aureus* Isolates in Germany during a 6-Year Period

Frieder Schaumburg,[a] Robin Köck,[b] Alexander Mellmann,[b] Laura Richter,[a] Felicitas Hasenberg,[a] André Kriegeskorte,[a] Alexander W. Friedrich,[c] Sören Gatermann,[d] Georg Peters,[a] Christof von Eiff,[a]* Karsten Becker,[a] and study group

Institute of Medical Microbiology, University Hospital Münster, Münster, Germany[a]; Institute of Hygiene, University Hospital Münster, Münster, Germany[b]; Department for Medical Microbiology and Infection Control, University Hospital Groningen, Groningen, The Netherlands[c]; and Department of Medical Microbiology, Institute for Hygiene and Microbiology, Ruhr University, Bochum, Germany[d]

Methicillin-resistant *Staphylococcus aureus* (MRSA) originated from the health care setting but is now emerging in communities without health care contact (CA-MRSA) or in livestock (LA-MRSA). The impact on the whole MRSA population was assessed in a German prospective multicenter study. Thirty-three laboratories consecutively collected up to 50 MRSA isolates from infection or carriage during two sampling periods in 2004 to 2005 and 2010 to 2011. Patient-related data were collected using a standardized questionnaire. Methicillin resistance was confirmed by the detection of *mecA* or its homologue $mecA_{LGA251}$. The *spa* type and major virulence factors were analyzed for each isolate. In total, 1,604 (2004 to 2005) and 1,603 (2010 to 2011) MRSA isolates were analyzed; one isolate from each sampling period harbored $mecA_{LGA251}$. LA-MRSA increased significantly (odds ratio [OR] = 22.67, 95% confidence interval [CI] = 8.51 to 85.49, $P < 0.0005$) and spread over Germany, originating from northwestern regions. Panton-Valentine leukocidin-positive CA-MRSA rose significantly, particularly in southern Germany, but the proportion in 2010 to 2011 remained low (2.7%, OR = 2.80, 95% CI = 1.54 to 5.34, $P < 0.0005$). The emerging MRSA clones changed the MRSA population in Germany during a 6-year period significantly. The ongoing epidemiological shift and changes of MRSA sources create a need for revision of guidelines for MRSA infection control and treatment.

Staphylococcus aureus is a bacterium causing skin and soft tissue infection, pneumonia, meningitis, endocarditis, and osteomyelitis. The first antistaphylococcal agent was penicillin, but soon after its introduction penicillin-resistant *S. aureus* spread globally in the 1950s and 1960s (9). Subsequently, members of the penicillinase-stable penicillins (i.e., methicillin) became the most important antibiotic drug for the treatment of *S. aureus* infection. However, the first methicillin-resistant *S. aureus* (MRSA) isolate was detected in England in 1961, and MRSA has spread throughout the world since then (14).

In Germany, MRSA isolates were reported for the first time in the 1970s and 1980s and comprised hospital-associated MRSA (HA-MRSA) (19, 27). German HA-MRSA isolates are clonal and mainly belong to *S. aureus* protein A locus (*spa*) types t002, t003, t008, and t032, which are associated with sequence types ST5, ST225, ST8, and ST22, as determined by multilocus sequence typing (MLST) (5). Typical risk factors for HA-MRSA carriage are history of hospitalization, residence in a nursing home, skin lesions, and hemodialysis, as well as indwelling catheters and inserted or implanted foreign bodies (15).

In the 1990s, new MRSA lineages emerged in communities without risk factors for HA-MRSA and spread in different countries to various extents (9). These community-associated MRSA (CA-MRSA) lineages are associated with skin and soft tissue infections and frequently harbor the Panton-Valentine leukocidin (PVL), a pore-forming toxin which lyses human neutrophils (20). While German CA-MRSA isolates are still rare and comprise different clonal lineages, isolates from the United States are dominated by the spread of MLSTs ST1 (USA400) and ST8 (USA300), suggesting a high transmissibility and/or virulence of these clones (8, 9).

In the early 2000s, so-called livestock-associated MRSA (LA-MRSA) emerged in Dutch pig farmers, who may have acquired this new subtype (ST398) from breeding animals (28). In regions with high density of livestock farming, LA-MRSA can be imported into hospitals, causing hospital-acquired infections (13, 15, 28).

The recent detection of a *mecA* homologue ($mecA_{LGA251}$), which is hitherto undetectable by routine molecular methods, raises the question of its prevalence (11, 18).

To elucidate if and to what extent the emergence of CA-MRSA and LA-MRSA may have changed the MRSA population in Germany, we performed a prospective longitudinal study and compared genotypes and virulence factors of human MRSA isolates from 33 laboratories throughout Germany collected in 2004 to 2005 with isolates obtained in the same study centers in 2010 to 2011.

MATERIALS AND METHODS

Study design. In total, 36 microbiological laboratories throughout Germany associated with outpatient clinics and primary to tertiary care hospitals were invited to collect prospectively MRSA isolates from routine diagnostics. In two sampling periods (1 February 2004 to 31 January 2005 and 1 February 2010 to 31 January 2011), every laboratory collected the first 50 MRSA isolates detected irrespective of the type of specimen. Only one isolate per patient was included, and the laboratories were asked to indicate whether the sample was obtained from a case associated with

Received 10 May 2012 Returned for modification 12 June 2012
Accepted 10 July 2012
Published ahead of print 18 July 2012
Address correspondence to Karsten Becker, kbecker@uni-muenster.de.
* Present address: Christof von Eiff, Pfizer Pharma GmbH, Berlin, Germany.
Supplemental material for this article may be found at http://jcm.asm.org/.

doi:10.1128/JCM.01174-12

TABLE 1 Characteristics of MRSA patients in 2004 to 2005 and 2010 to 2011

Parameter	Result[a] for MRSA patients from: 2004–2005 (n = 1,604)	2010–2011 (n = 1,603)	OR (95% CI)	P
Mean age (yr) ± SD	64.9 ± 18.9	65.3 ± 20.4	NA	0.65
Proportion female	42.4 (680)	42.8 (671)	1.01 (0.88–1.17)	0.84
Risk factors for HA-MRSA				
History of MRSA infection/colonization	22.4 (219)	30.2 (219)	1.5 (1.2–1.88)	<0.0005
Hemo-/peritoneal dialysis in the past 12 mo	7.6 (63)	11.5 (59)	1.58 (1.06–2.33)	0.16
Surgery in the past 12 mo	52.6 (442)	54.9 (307)	1.1 (0.88–1.37)	0.40
Hospitalization in the past 12 mo	75.9 (635)	82.8 (512)	1.54 (1.17–2.02)	0.001
Residence in a day care or rehabilitation center	24.7 (198)	30 (137)	1.31 (1.00–1.71	0.04
MRSA infection				
Sepsis (foreign body associated)	3.2 (51)	2.3 (37)	0.72 (0.46–1.13)	0.13
Foreign body-associated infection	2.1 (33)	1.6 (26)	0.78 (0.45–1.36)	0.36
Pneumonia	7.5 (120)	3.6 (57)	0.46 (0.32–0.64)	<0.0005
Abscess	1.4 (22)	1.7 (27)	1.23 (0.67–2.28)	0.47
Meningitis	0.06 (1)	0 (0)	0 (0–39.02)	1
Surgical site infection	5.9 (94)	3.1 (49)	0.51 (0.35–0.73)	<0.0005
Skin and soft tissue infection	21.1 (339)	12.4 (199)	0.53 (0.43–0.64)	<0.0005
Urinary tract infection	3.2 (51)	2.1 (33)	0.64 (0.40–1.02)	0.05
Osteomyelitis	1.3 (21)	0.3 (4)	0.19 (0.05–0.56)	0.001
MRSA asymptomatic colonization	36.7 (589)	30.1 (483)	1.35 (1.16–1.56)	<0.0005
Care[b]				
Outpatient department	19.5 (312)	20.8 (333)	0.92 (0.77–1.10)	0.35
Intensive care unit	19.2 (308)	15.5 (249)	1.29 (1.07–1.56)	0.006
Dialysis department	0 (0)	0.6 (10)	0 (0–0.44)	<0.0005
Hemato-oncology department	2.1 (33)	1.2 (19)	1.75 (0.96–3.27)	0.05
General care wards	59.3 (951)	56.2 (901)	1.13 (0.98–1.31)	0.08

[a] Values are % (no.) except for ages.
[b] For 91 persons in 2010 to 2011, the type of care was not reported.

clinical signs of an *S. aureus* infection. A standardized questionnaire was completed for each isolate, including demographic data of the patient (age, gender) and sample-related data (date of sampling, sample material, in- or outpatient care, risk factors for MRSA colonization or infection, type of *S. aureus* infection). Samples and questionnaires were sent to the Institute of Medical Microbiology, University Hospital Münster, Münster, Germany, for molecular characterization, data entry, and analysis. Study centers which collected MRSA isolates during one sampling period only were excluded from the analysis.

Molecular characterization. Isolates were confirmed to be *S. aureus* by detection of the *S. aureus*-specific thermostable nuclease gene (*nuc*), and methicillin resistance was confirmed by *mecA* PCR (2). A PCR targeting the *mecA* homologue $mecA_{LGA251}$ was used for those isolates which were *mecA* negative but showed phenotypic methicillin resistance (18). Genes encoding the toxic shock syndrome toxin (*tst*), enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*), exfoliative toxins (*eta*, *etb*, *etd*), and the epidermal cell differentiation inhibitors (*edin-A*, *edin-B*, *edin-C*) were detected by multiplex PCRs (1, 3, 29, 30). Subtypes of the accessory gene regulator (*agr* I to IV) were detected by PCR (26). Sequence-based typing of the hypervariable region of the *S. aureus* protein A locus (*spa* typing) was performed for each isolate (21). Related *spa* types were clustered in *spa* clonal complexes (spa-CC) using the BURP (based upon repeat pattern) algorithm and applying the default parameters ($x = 5$, $y = 4$) as implemented in Ridom StaphType 2.2.1 (21). Multilocus sequence typing was performed for new *spa* types which were among the 10 most frequent *spa* types (10).

Statistical analyses. Data were analyzed with the software "R," version 2.13.1 (http://cran.r-project.org), and the package epicalc. Continuous variables were compared using Student's t test. Categorical variables were compared using the χ^2 test and the odds ratio (OR) and its 95% confidence interval (95% CI). The significance level was 0.05. Missing values were removed from the statistical analyses.

RESULTS

Study population. All 36 invited study centers participated in the first sampling period, 2004 to 2005, and 33 centers also took part in the second sampling period, 2010 to 2011. The three centers which did not contribute during the second period were excluded from the analysis. The majority of isolates were collected in northern, western, and southern Germany. While the mean ages and the proportions of females did not differ between the first and second sampling periods, there was a significant increase in patients with risk factors for MRSA colonization in 2010 to 2011 (Table 1).

MRSA typing. In total, 3,207 MRSA isolates were collected during the first (n = 1,604) and the second (n = 1,603) sampling periods. Of these, 1,236 isolates derived from infections and 1,072 from asymptomatic carriers. A clear attribution to "infection" or "asymptomatic carriage" was not possible for 899 isolates. All isolates were *nuc* positive and—with two exceptions—harbored the classical *mecA* gene. One isolate in each sampling period harbored $mecA_{LGA251}$ (*spa* type t843). The first isolate was recovered from sputum of a patient with *S. aureus* pneumonia, and the second isolate was isolated from a skin/mucosal swab for MRSA colonization of a patient who suffered from chronic respiratory tract disease.

Isolates of the first sampling period comprised 127 different

TABLE 2 Most frequent *spa* types of MRSA isolates from 2004 to 2005 and 2010 to 2011

	2004–2005				2010–2011			
Rank	*spa* type	MLST(s)[a]	Frequency [% (no.)]	Cumulative frequency (%)	*spa* type	MLST(s)[a]	Frequency, % (no.)	Cumulative frequency (%)
1	t003	ST5, ST225	40.15 (644)	40.15	t003	ST5, ST225	39.86 (639)	39.86
2	t032	ST22	15.59 (250)	55.74	t032	ST22	15.53 (249)	55.4
3	t008	ST8, ST247, ST250, ST254	7.48 (120)	63.22	t008	ST8, ST247, ST250, ST254	4.49 (72)	59.88
4	t002	ST5, ST231	6.86 (110)	70.08	t002	ST5, ST231	3.18 (51)	63.06
5	t001	ST5, ST222, ST228	5.93 (95)	76.01	t034	ST398[b]	2.56 (41)	65.62
6	t004	ST45	4.05 (65)	80.06	t011	ST398[b]	2.37 (38)	67.99
7	t041	ST111, ST228	1.50 (24)	81.56	t045	ST5, ST225	1.75 (28)	69.74
8	t038	ST45	1.00 (16)	82.56	t014	ST672, ST225[c]	1.31 (21)	71.05
9	t022	ST22	0.69 (11)	83.25	t8374	ST22[d]	1.19 (19)	72.24
10	t023	ST22	0.69 (11)	83.94	t022	ST22	1.19 (19)	73.43
>10	Others	—[e]	16.03 (258)	100	Others	—	26.58 (426)	100
Total			100 (1,604)				100 (1,603)	

[a] Associated multilocus sequence types as published on http://spaserver.ridom.de.
[b] Previously published (15).
[c] Previously published (23).
[d] This study.
[e] —, not determined.

spa types (two MRSA isolates [0.1%] were nontypeable), and isolates of the second period were associated with 236 different *spa* types (nine MRSA isolates [0.6%] were nontypeable) (see Table S1 in the supplemental material). The distributions of *spa* types stratified in spa-CCs in isolates from infection and carriage were similar. The proportion of classical HA-MRSA *spa* types (t003 and t032) did not change between 2004 to 2005 and 2010 to 2011 (Table 2). It is noteworthy that *spa* type t008 has mainly spread in southern Germany and decreased slightly in 2010 to 2011 (Table 2; Fig. 1). Isolates belonging to *spa* type t008 and t002 were predominant in southwest Germany in 2004 to 2005 (Fig. 1). Isolates of *spa* type t002 decreased in the whole country from 6.86% (n = 110) to 3.18% (n = 51) in 2010 to 2011. Isolates belonging to *spa* type t008 decreased from 7.48% (n = 120) in 2004 to 2005 to 4.49% (n = 72) in 2010 to 2011 (Table 2). Similarly, some of the most prevalent *spa* types in southeast Germany (t001), northern Germany (t004), Lower Saxony (t038), and central Germany (t041) were no longer predominant in 2010 to 2011 (Fig. 1). In 2010 to 2011, new MRSA isolates with *spa* types that are infrequent so far emerged in northern Germany (t4217, spa-CC 032, ST22; n = 12) and in southwest Germany (t4881, spa-CC 003, ST225; n = 6) (Fig. 1; see Table S1 in the supplemental material).

The proportion of PVL-positive isolates belonging to *spa* types associated with the classical PVL-positive European CA-MRSA clone (t002, t044) increased from 0.4% (n = 6) in 2004 to 2005 to 0.6% (n = 9) in 2010 to 2011. Similarly, *spa* types of PVL-positive

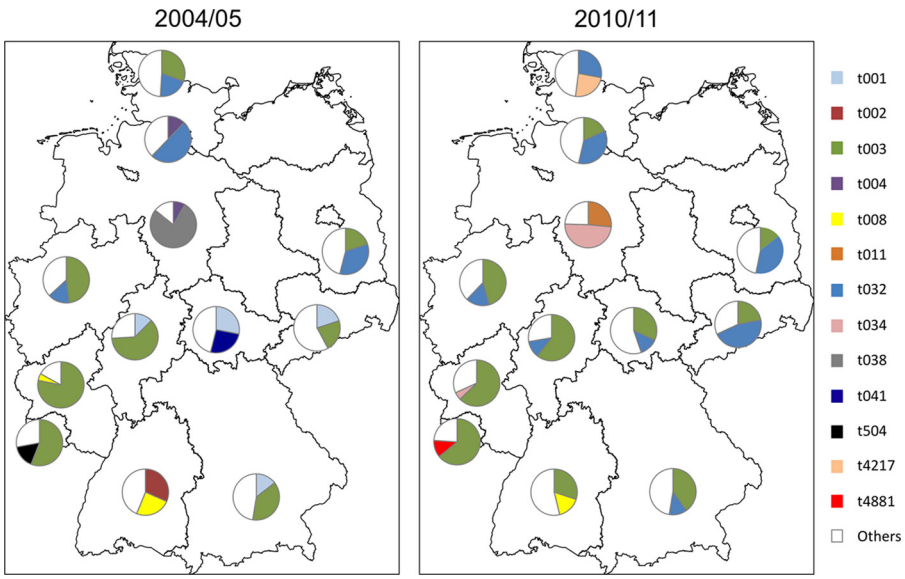

FIG 1 Distribution of the two most prevalent *spa* types of methicillin-resistant *Staphylococcus aureus* in Germany. Pie charts show the average prevalence in each federal state.

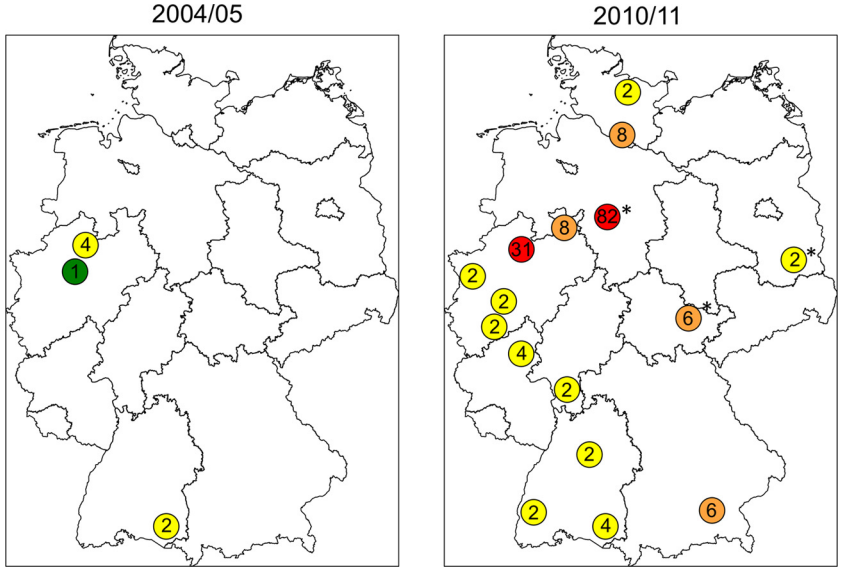

FIG 2 Distribution and spread of livestock-associated methicillin-resistant *Staphylococcus aureus* in Germany in 2004 to 2005 and 2010 to 2011. The numbers indicate the prevalence (%) of LA-MRSA isolates in the respective study centers (green, 1%; yellow, 2 to 5%; orange, 6 to 10%; red, >10%). Asterisks indicate that only one study center reported from the region.

isolates associated with the USA300 clone (t008) increased from 0.1% ($n = 2$) in 2004 to 2005 to 0.6% ($n = 10$) in 2010 to 2011. In total, CA-MRSA isolates rose significantly, particularly in southern Germany (OR = 2.80, 95% CI = 1.54 to 5.34, $P < 0.0005$).

The MRSA isolates associated with spa-CC011 (CC011 is indicative of ST398) belong to t034 and t011 and related *spa* types according to the BURP analysis (t108, t1451, t2576, t4395t, t8377). Isolates belonging to these *spa* types increased significantly from 0.3% ($n = 4$) in 2004 to 2005 to 5.4% ($n = 86$) in 2010 to 2011 (OR = 22.67, 95% CI = 8.51 to 85.49, $P < 0.0005$). In 2004 to 2005 these *spa* types, which are indicative of the LA-MRSA clonal lineage ST398, were—with one exception (Ravensburg, southwestern Germany)—found only in western Germany. These isolates were restricted to *spa* types t011 (Münster and Ravensburg) and t108 (Bochum). In 2010 to 2011 spa-CC011-associated *spa* types were detected all over Germany, but still with highest prevalence in northwestern Germany (Fig. 2). Association of these MRSA isolates with infection was reported only for isolates from 2010 to 2011; cases included skin and soft tissue infection ($n = 4$), foreign body infection ($n = 1$), sepsis ($n = 1$), pneumonia ($n = 1$), surgical site infection ($n = 1$), and endocarditis ($n = 1$). No or unknown association with infection was reported for 77 MRSA spa-CC011 isolates, which were isolated from urine ($n = 2$), sputum ($n = 3$), or skin/mucosal swabs ($n = 72$).

In 2004 to 2005 the *agr* II subtype was predominant (62.2%, $n = 998$) followed by *agr* I (34.5%, $n = 553$), *agr* III (1.8%, $n = 28$), and *agr* IV (1%, $n = 16$). Similarly, *agr* II was the most frequent subtype in 2010 to 2011 (54.3%, $n = 870$), followed by *agr* I (43.4%, $n = 696$) and *agr* III (2.2%, $n = 35$). In 2010 to 2011, *agr* IV was not detected.

MRSA exotoxin characterization. Overall, 16 (1%) and 44 (2.7%) PVL-positive MRSA isolates were found in 2004 to 2005 and 2010 to 2011, respectively (Table 3). Taking into account the results of both sampling periods, they were isolated from skin and soft tissue infections (26.7%, $n = 16$), abscesses (15%, $n = 9$), pneumonia and postsurgical wound infections (3.3%, $n = 2$, each), and urinary tract infections (1.7%, $n = 1$). For 16.7% ($n = 10$), no association with infection was reported, and for 28.3% ($n = 17$) PVL-positive isolates, the association with an infection was unclear. These isolates were derived from skin/mucosal swabs (52.9%, $n = 9$), aspirates/secretions (23.4%, $n = 4$), wounds (17.7%, $n = 3$), and other specimens (5.9%, $n = 1$). In 2004 to 2005, PVL-positive MRSA isolates were more frequently isolated from skin/mucosal swabs than in 2010 to 2011 (75.0% versus 36.7%).

The risk factors history of hospitalization and MRSA detection

TABLE 3 Virulence factors of German MRSA isolates from 2004 to 2005 and 2010 to 2011

Virulence factor	% (no.) of MRSA isolates from[a]: 2004–2005	2010–2011	OR (95% CI)	*P*
lukS-PV/*lukF*-PV	1.0 (16)	2.7 (44)	2.80 (1.54–5.34)	<0.0005
tst	9.6 (154)	0.9 (14)	0.08 (0.04–0.14)	<0.0005
sea	13.6 (218)	6.6 (105)	0.45 (0.35–0.57)	<0.0005
seb	0.5 (8)	0.4 (7)	0.88 (0.27–2.77)	0.8
sec	13.3 (213)	20.1 (322)	1.64 (1.35–1.99)	<0.0005
sed	56.2 (901)	49.9 (800)	0.78 (0.67–0.9)	<0.0005
seg	88.5 (1,419)	85.0 (1,363)	0.74 (0.60–0.91)	0.004
seh	0.3 (5)	0.7 (11)	2.21 (0.71–8.13)	0.13
sei	90.2 (1,447)	85.2 (1,366)	0.63 (0.50–0.78)	<0.0005
sej	45.0 (721)	47.5 (762)	1.11 (0.96–1.28)	0.14
eta	0.4 (6)	0 (0)	0 (0–0.85)	0.03
etb	0.1 (2)	0 (0)	0 (0–5.33)	0.5
etd	1.0 (16)	0.5 (8)	0.5 (0.18–1.24)	0.1
edin-A	0 (0)	0.06 (1)	Inf[b] (0.03–Inf)	0.5
edin-B	0.9 (14)	0.5 (8)	0.57 (0.21–1.46)	0.2
hlg	99.8 (1,600)	100 (1,603)	Inf (0.66–Inf)	0.13

[a] Percentages are given as relation to the respective evaluable questionnaire data.
[b] Inf, infinity.

TABLE 4 Risk factors for HA-MRSA colonization in PVL-positive isolates

Risk factor	% (no.) of patients with:		OR (95% CI)	*P*
	PVL-positive MRSA	PVL-negative MRSA		
History of MRSA colonization	20 (6)	25.9 (432)	0.72 (0.24–1.82)	0.47
MRSA detection >48 h after admission	27.6 (8)	52.1 (875)	0.35 (0.13–0.83)	0.01
Dialysis in the past 12 mo	13 (3)	9.0 (119)	1.51 (0.28–5.21)	0.46
Surgery in the past 12 mo	42.9 (9)	53.7 (740)	0.65 (0.24–1.68)	0.32
Hospitalization in the past 12 mo	50 (11)	79.3 (1,136)	0.26 (0.1–0.67)	0.001
Residence in a nursing home or rehabilitation center in the past 12 mo	14.3 (3)	26.8 (332)	0.45 (0.09–1.57)	0.32

in specimens obtained more than 48 h after admission were significantly less frequently reported for PVL-positive MRSA isolates than for PVL-negative isolates (Table 4). Furthermore, the mean number of risk factors for health care-associated MRSA (HA-MRSA) colonization in persons with PVL-positive isolates was 0.7 (range, 0 to 4) compared to 1.2 (range, 0 to 6) for persons with PVL-negative isolates ($P = 0.001$). Only the risk factor proportion of persons who had hemodialysis or peritoneal dialysis in the past 12 months was more prevalent in the PVL-positive group (Table 4).

PVL-positive isolates from 2004 to 2005 belonged to *spa* types/STs t044/ST80 ($n = 5$), t003/ST5 ($n = 2$), and t008/ST8 ($n = 2$), followed by t001, t002, t019, t032, t041, t808, and t322 ($n = 1$ each) (Fig. 3). In 2010 to 2011, PVL-positive isolates belonging to *spa* types/STs t008/ST8 ($n = 10$), t044/ST80 ($n = 5$), t002/ST5 ($n = 4$), and t318/ST30 ($n = 4$) became the most prevalent, followed by t437 ($n = 3$); t003, t019, t203, and t4741 ($n = 2$ each); and t005, t016, t045, t657, t692, t791, t967, t2518, t7656, and t8376 ($n = 1$ each). PVL-positive MRSA isolates were present in four major spa-CCs (spa-CC003, -008, -012, and -032).

DISCUSSION

This is the first systematic prospective and nationwide multicenter study analyzing the molecular background and exotoxin equipment of MRSA isolates in Germany. Main findings are an increasing proportion of LA-MRSA and PVL-positive MRSA isolates between 2004 to 2005 and 2010 to 2011.

We detected an increased proportion of patients with risk factors for HA-MRSA colonization. The higher proportion of patients who had been hospitalized in the past 12 months in 2010 to 2011 may reflect the 8.3% increase of inpatient cases in German hospitals from 2004 (20,365 cases per 100,000 inhabitants) to 2010 (22,057 cases per 100,000 inhabitants) (24). In contrast, the increased proportion of patients with a history of MRSA infection/colonization in 2010 to 2011 does not reflect the stable or decreasing proportion of MRSA infection/colonization in recent years (19.5 to 21.2% between 2004 and 2009) (17, 22).

The vast majority of MRSA isolates harbored the classical *mecA* gene; only two isolates (0.06%) with the *mecA* homologue $mecA_{LGA251}$ were collected in 2004 to 2005 and 2010 to 2011. Similarly low rates of $mecA_{LGA251}$-positive MRSA isolates were also reported in retrospective analyses of German strain collections (6, 18). The two isolates from our study belonged to *spa* type t843, which is the most frequent *spa* type in $mecA_{LGA251}$-positive isolates from cows and humans in Great Britain and Denmark (11). While the proportion of $mecA_{LGA251}$ MRSA isolates seemed to increase in Denmark from 2008 (0.12%) to 2010 (0.67%), we observed a con-

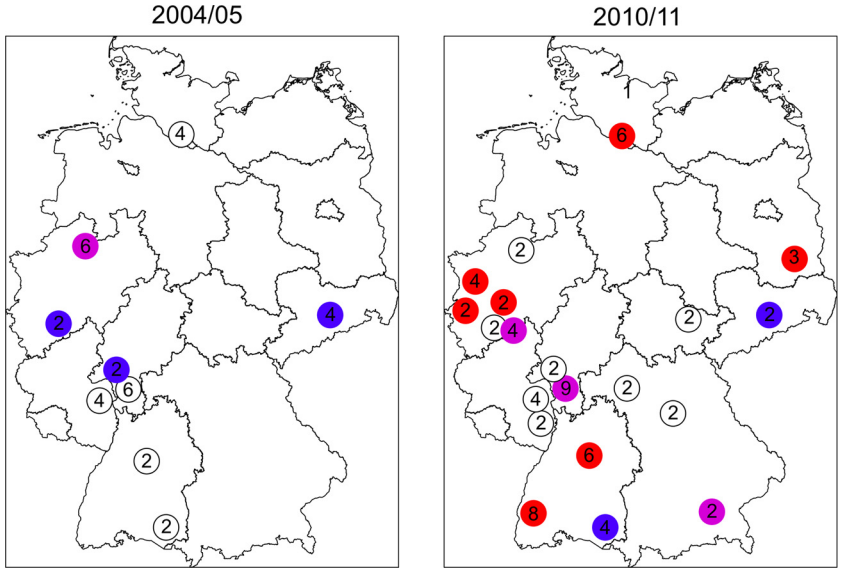

FIG 3 Distribution and spread of PVL-positive methicillin-resistant *Staphylococcus aureus* in Germany in 2004 to 2005 and 2010 to 2011. The numbers indicate the proportions (%) of PVL-positive MRSA isolates in the respective study centers. Colors indicate detection of ST8 (red), ST80 (blue), ST8 and ST80 (violet), and non-ST8/non-ST80 (white).

tinuously low prevalence (0.06%) (11). There is evidence, at least for one isolate, which was isolated from a pneumonia patient, that MRSA harboring $mecA_{LGA251}$ can cause clinical infection.

The major MRSA lineages in Germany, which are traditionally named after the region of their first occurrence, comprise the Rhine-Hessen MRSA (t002/ST5 and t003/ST225), the Barnim MRSA (t032/ST22), and the northern German MRSA clone (t008/ST8). The proportion of the classical HA-MRSA lineages (t003/ST225 and t032/ST22) was stable during the study period (Table 2), which is consistent with recent reports (5). The origin of the emerging MRSA *spa* type t4217/ST22 in northern Germany in 2010 to 2011 is unclear as this *spa* type is not associated with published outbreaks in this area and as its occurrence cannot be explained by a hypothetical cross-border transfer from Denmark. Similarly, the source of MRSA t4881/ST225 in southwest Germany (Fig. 1) is unclear. A cross-border import of these isolates from France is unlikely, as the major French MRSA clones belong to t008/ST8, t024/ST8, and t777/ST5 (12). The t4881 clone might have evolved from t504, which was the second most common *spa* type in this region in 2004 to 2005. Since MRSA t504 (26-17-20-17-12) and t4881 (26-17-20-17-13-17-17-16) have similar repeat patterns, a microevolution from t504 to t4881 is possible, although deletions of *spa* repeats are more frequent than duplications in *spa* type alterations (4).

Importantly, *spa* types associated with spa-CC011 (t011, t034) are presumptively LA-MRSA within the MLST clonal complex 398. While LA-MRSA isolates were initially detected in commercially raised pigs, their spread to other animals (cattle, chicken, horses, and pets) as well as farmers, veterinarians, and other exposed persons has been reported (7). Here, we show a significantly increasing prevalence of LA-MRSA-associated *spa* types from 0.3% (2004 to 2005) to 5.4% (2010 to 2011) (28). However, the prevalence of LA-MRSA was particularly high in northwestern Germany, which is characterized by a high density of livestock production (Lower Saxony, North Rhine-Westphalia). Proportions of up to 20% of all MRSA isolates have been reported from "pig-dense" regions, consistent with our results (16). However, the high prevalence in Lower Saxony (82%) (Fig. 2) might be questionable, as only one laboratory reported results from this region.

CA-MRSA isolates frequently harbor PVL-encoding genes and are highly prevalent in the United States but are still rare in Germany. In our comparative analysis, we showed that the proportion of PVL-positive isolates among MRSA isolates has significantly increased between 2004 to 2005 (1.0%) and 2010 to 2011 but that the proportion remains low (2.7%) (Table 3). While the European CA-MRSA clone (t044, ST80 [17]) was the most prevalent PVL-positive MRSA clone in 2004 to 2005, the USA300 clone (t008, ST8 [17]) became predominant in 2010 to 2011. Interestingly, in both sampling periods increasing high proportions of PVL-positive MRSA isolates were found in the cities of Hamburg (2004 to 2005, 2.0%; 2010 to 2011, 6.1%) and Frankfurt am Main (2004 to 2005, 6.5%; 2010 to 2011, 8.8%). These metropolitan areas have a high population density and are international aviation hubs. Several studies have shown that PVL-positive MRSA and methicillin susceptible *S. aureus* can be imported and spread through international travel (25, 31).

The different proportions of genes encoding members of the pyrogenic toxin superantigen (PTSAg) family in 2004 to 2005 compared to 2010 to 2011 might be due to changes in dominating clonal lineages. In particular, the decrease of *tst* and enterotoxin gene-harboring strains might be explained by the advent of LA-MRSA CC398 isolates which only sporadically harbor these PTSAgs.

Our study comprises nationwide data from laboratories which perform microbiological diagnostics for outpatient care and for primary to tertiary care hospitals and includes isolates from carriage and infection. Since the same laboratories participated in both sampling periods, we controlled for the geographical bias (12). As we included only consecutively collected samples, we ruled out a reporting bias.

One limitation of our study is the underrepresentation of isolates from the eastern part of Germany, especially from the capital of Berlin, the inclusion of which might have added important information regarding the prevalence of PVL-positive isolates in urban areas. Second, we did not assess the contact to animals as a risk factor for MRSA carriage or infection in 2004 to 2005, because there was only a limited awareness of this MRSA reservoir in that time.

In conclusion, the emergence of novel MRSA clonal lineages highlights the possibility of substantial changes in MRSA epidemiology within relatively short time periods.

ACKNOWLEDGMENTS

The following persons (in alphabetical order) in Germany were participants of the study group: M. Abele-Horn, Würzburg; Aepfelbacher, Hamburg; F. Albert, Erlangen; A. Anders, Bochum; W. Bär and B. Beyreiß, Cottbus; G. Bierbaum, Bonn; U. Bührlen, Stuttgart; K. H.-U. Borg, Hamburg; Claußen, Hannover; C. Diaz, Cologne; A. Ditzen, Dresden; M. Dobonici, Ludwigshafen; U. Eigner, Heidelberg; H. Erichsen, Moers; A. Fahr, Heidelberg; P. Finzer, Moers; U. Frank, Freiburg; C. Freytag, Bad-Oeynhausen; M. Frosch, Würzburg; G. Funke, Ravensburg; S. Gatermann, Bochum; J. Geisen, Cologne; W. Hell, Koblenz; M. Herrmann, Homburg; U. Höffler, Ludwigshafen; E. Jacobs, Dresden; B. Jansen, Mainz; D. Jonas, Freiburg; M. Kaase, Bochum; M. Kaulfers and J. K. Knobloch, Hamburg; M. Kresken and B. Körber-Irrgang, Rheinbach; M. van der Linden, Aachen; A. Lommel and C. Lücking, Ludwigshafen; D. Mack, Hamburg; S. Müller, Würzburg; L. von Müller, Homburg; A. Müller-Chorus, Bochum; K. Noldt, Hamburg; W. Pfister, Jena; T. Regnath, W. Reiter, Stuttgart; J. Rissland, Koblenz; A. Roggenkamp, Münich; U. Rohr, Bochum; E. Rosenthal, Wiesbaden; S. Schade and S. Scherpe, Hamburg; T. Schmidt-Wieland, Ravensburg; C. Schoerner, Erlangen; S. Schubert, Kiel; R. Schwarz, Cologne; K. Schwegmann, Hannover; H. Seifert, Cologne; V. Simon, Münich; E. Straube, Jena; M. Trautmann, Stuttgart; U. Ullmann, Kiel; U. Vogel, Würzburg, M. Vogt, Koblenz; H. von Wulffen, Hamburg; T. Wichelhaus, Frankfurt; M. L. Wimmer-Dahmen, Mönchengladbach; J. Wüllenweber, Homburg; B. Würstl, Münich; W. Kalka-Moll, Mönchengladbach; S. Monecke, Dresden; R. R. Reinert, Aachen; B. Zöllner, Moers.

This work was supported and performed under the auspices of the Paul Ehrlich Gesellschaft für Chemotherapie (K.B. and C.V.E.), working group Staphylococcal infections, and in part supported by grants to R.K., A.W.F., G.P., and K.B. (01KI1014A) within the MedVet-Staph project by the Bundesministerium für Bildung und Forschung.

REFERENCES

1. **Becker K, et al.** 2003. Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. J. Clin. Microbiol. **41**:1434–1439.
2. **Becker K, et al.** 2006. Does nasal cocolonization by methicillin-resistant coagulase-negative staphylococci and methicillin-susceptible *Staphylococcus aureus* strains occur frequently enough to represent a risk of false-

positive methicillin-resistant *S. aureus* determinations by molecular methods? J. Clin. Microbiol. **44**:229–231.
3. **Becker K, Roth R, Peters G.** 1998. Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. J. Clin. Microbiol. **36**:2548–2553.
4. **Boye K, Westh H.** 2011. Variations in *spa* types found in consecutive MRSA isolates from the same patients. FEMS Microbiol. Lett. **314**:101–105.
5. **Cuny C, Layer F.** 2011. Auftreten und Verbreitung von MRSA in Deutschland 2010. Epidemiologisches Bull. **26**:233–241.
6. **Cuny C, Witte W.** 2011. Auftreten von MRSA mit negativem Nachweis für *mecA* (PCR) und Penicillin-Bindeprotein PBP2a (Agglutinationstest). Epidemiologisches Bull. **38**:351–352.
7. **Cuny C, et al.** 2009. Nasal colonization of humans with methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 with and without exposure to pigs. PLoS One **4**:e6800. doi:10.1371/journal.pone.0006800.
8. **David MZ, Daum RS.** 2010. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin. Microbiol. Rev. **23**:616–687.
9. **Deleo FR, Otto M, Kreiswirth BN, Chambers HF.** 2010. Community-associated meticillin-resistant *Staphylococcus aureus.* Lancet **375**:1557–1568.
10. **Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus.* J. Clin. Microbiol. **38**:1008–1015.
11. **García-Álvarez L, et al.** 2011. Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect. Dis. **11**:595–603.
12. **Grundmann H, et al.** 2010. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. PLoS Med. **7**:e1000215. doi:10.1371/journal.pmed.1000215.
13. **Hartmeyer GN, Gahrn-Hansen B, Skov RL, Kolmos HJ.** 2010. Pig-associated methicillin-resistant Staphylococcus aureus: family transmission and severe pneumonia in a newborn. Scand. J. Infect. Dis. **42**:318–320.
14. **Jevons MP.** 1961. "Celbenin"-resistant Staphylococci. BMJ **1**:124–125.
15. **Köck R, et al.** 2009. Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) among pigs on German farms and import of livestock-related MRSA into hospitals. Eur. J. Clin. Microbiol. Infect. Dis. **28**:1375–1382.
16. **Köck R, et al.** 2011. Characteristics of hospital patients colonized with livestock-associated meticillin-resistant *Staphylococcus aureus* (MRSA) CC398 versus other MRSA clones. J. Hosp. Infect. **79**:292–296.
17. **Köck R, et al.** 2011. The epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in Germany. Dtsch. Arztebl. Int. **108**:761–767.
18. **Kriegeskorte A, et al.** 2012. Human MRSA isolates with novel genetic homolog, Germany. Emerg. Infect. Dis. **18**:1016–1018.
19. **Lenz W, Eilers E, Lehmacher U.** 1988. Characterization of methicillin-resistant *Staphylococcus aureus* strains isolated from 1974 to 1983 in West Germany with respect to the results of lysotyping. Zentralbl. Bakteriol. Mikrobiol. Hyg. **268**:277–293.
20. **Löffler B, et al.** 2010. *Staphylococcus aureus* Panton-Valentine leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS Pathog. **6**:e1000715. doi:10.1371/journal.ppat.1000715.
21. **Mellmann A, et al.** 2008. Characterization of clonal relatedness among the natural population of *Staphylococcus aureus* strains by using *spa* sequence typing and the BURP (based upon repeat patterns) algorithm. J. Clin. Microbiol. **46**:2805–2808.
22. **Meyer E, Schwab F, Schroeren-Boersch B, Gastmeier P.** 2011. Increasing consumption of MRSA-active drugs without increasing MRSA in German ICUs. Intensive Care Med. **37**:1628–1632.
23. **Nienhoff U, et al.** 2009. Transmission of methicillin-resistant *Staphylococcus aureus* strains between humans and dogs: two case reports. J. Antimicrob. Chemother. **64**:660–662.
24. **Statistisches Bundesamt.** 2012. Gesundheit. Grunddaten der Krankenhäuser. 2010. Fachserie 12 Reihe 6.1.1. Statistisches Bundesamt, Wiesbaden, Germany.
25. **Stenhem M, et al.** 2010. Imported methicillin-resistant *Staphylococcus aureus*, Sweden. Emerg. Infect. Dis. **16**:189–196.
26. **von Eiff C, Friedrich AW, Peters G, Becker K.** 2004. Prevalence of genes encoding for members of the staphylococcal leukotoxin family among clinical isolates of *Staphylococcus aureus.* Diagn. Microbiol. Infect. Dis. **49**:157–162.
27. **Witte W, Nguyen VD, Dunnhaupt K.** 1986. Methicillin-resistant *Staphylococcus aureus* (MRSA) in the German Democratic Republic. Incidence and strain-characteristics. J. Hyg. Epidemiol. Microbiol. Immunol. **30**:185–193.
28. **Wulf M, Voss A.** 2008. MRSA in livestock animals—an epidemic waiting to happen? Clin. Microbiol. Infect. **14**:519–521.
29. **Yamaguchi T, et al.** 2001. Complete nucleotide sequence of a *Staphylococcus aureus* exfoliative toxin B plasmid and identification of a novel ADP-ribosyltransferase, EDIN-C. Infect. Immun. **69**:7760–7771.
30. **Yamaguchi T, et al.** 2002. Identification of the *Staphylococcus aureus etd* pathogenicity island which encodes a novel exfoliative toxin, ETD, and EDIN-B. Infect. Immun. **70**:5835–5845.
31. **Zanger P, et al.** 2012. Import and spread of Panton-Valentine leukocidin-positive *Staphylococcus aureus* through nasal carriage and skin infections in travelers returning from the tropics and subtropics. Clin. Infect. Dis. **54**:483–492.