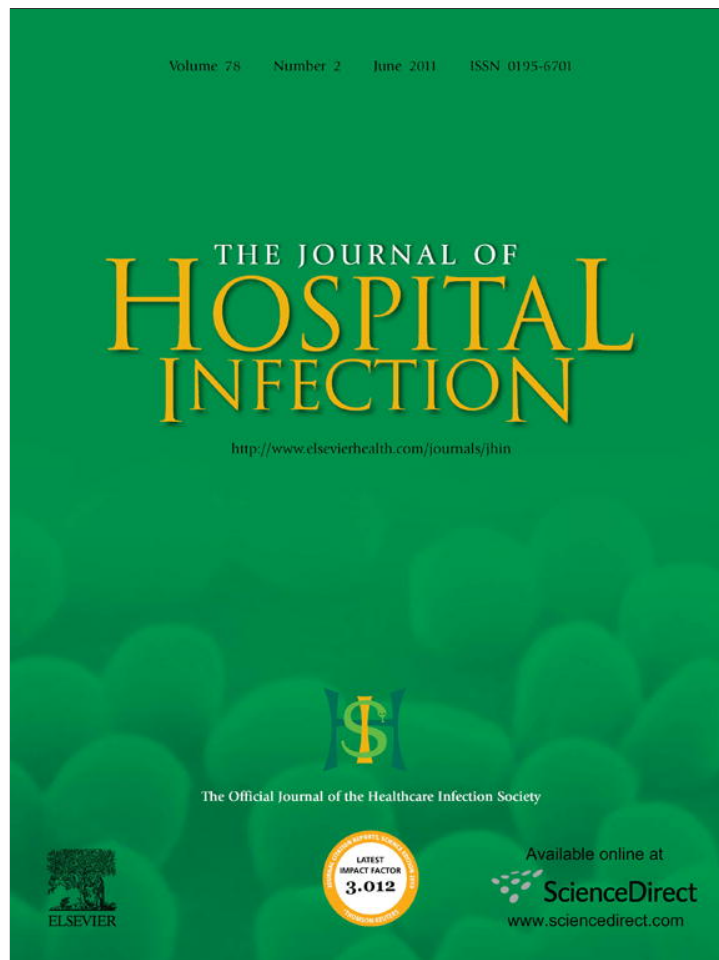


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## Prevalence and molecular epidemiology of meticillin-resistant *Staphylococcus aureus* in nursing home residents in northern Germany

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### SUMMARY

Nursing home residents are a population at risk for carrying meticillin-resistant *Staphylococcus aureus* (MRSA). To better guide infection control and healthcare network initiatives, we investigated the point prevalence and molecular epidemiology of MRSA colonisation among nursing home residents in Brunswick, northern Germany. Among the 32 participating nursing homes of the available 34 in the region, 68% of residents (1827 of 2688) were screened for nasal and/or wound colonisation. A total of 139 residents (7.6%; 95% confidence interval: 6.4–8.8%) were identified as MRSA positive, almost six-fold more than the 24 MRSA carriers (0.9%) expected according to the nursing homes' pre-test information. Although known risk factors including urinary tract catheters, wounds, preceding hospital admission, and high grade resident care were confirmed, none was sensitive enough to be considered as the sole determinant of MRSA carriage. *spa* typing revealed that more than 70% of isolates belonged to the Barnim strain (ST-22, EMRSA-15, CC22) typical for hospital-acquired MRSA in northern Germany. There was no evidence for the presence of community-acquired or livestock-associated *S. aureus* strains. These data show that in northern Germany MRSA has spread from the hospital environment to other healthcare institutions, which must now be regarded as important reservoirs for MRSA transmission.

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### Introduction

Prevalence of meticillin-resistant *Staphylococcus aureus* (MRSA) in Germany has increased markedly in both hospital- and community-associated settings in recent years, in common with the general worldwide trend.<sup>1,2</sup> Recent studies in different countries suggest that the epidemiology of MRSA has changed and that community- and healthcare-associated reservoirs of MRSA have expanded.<sup>3</sup> Nursing home residents represent a potential reservoir for MRSA. This is due to the prevalence of risk factors for MRSA colonisation in home residents including advancing age, age-associated morbidities such as diabetes and

renal insufficiency, high rate of urinary or other devices, the presence of chronic wounds, and recent healthcare or hospital contact.<sup>4,5</sup>

An understanding of the molecular epidemiology and prevalence of nursing home resident care-associated MRSA is essential for guiding infection control and healthcare network initiatives outside the hospital setting to restrict further spread of MRSA. Thus, between September and October 2009 we studied the point prevalence and molecular epidemiology of MRSA, as well as potential risk factors for MRSA colonisation of nursing home residents in the local health authority district of Brunswick, northern Germany. Brunswick is the second largest town in Lower Saxony, Germany, with about 245 000 inhabitants. It contains a number of healthcare facilities including a large academic teaching hospital of 1440 beds with more than 57 000 inpatients and almost 120 000 outpatients per year, three smaller hospitals with a combined total of about 500 beds, and 34 nursing homes with almost 3600 home residents.

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## Methods

### Data and sample collection

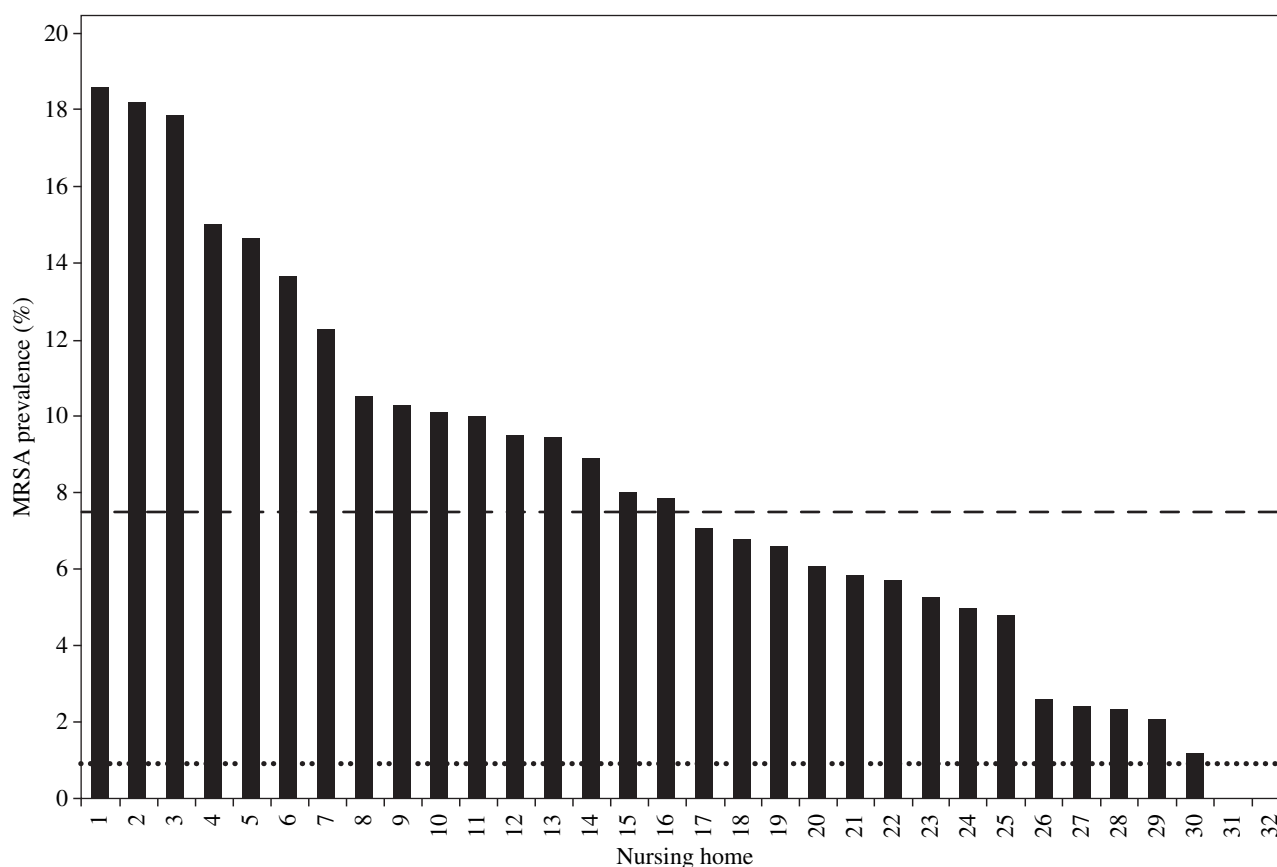
Swabs of the anterior nares and any superficial wounds were taken with sterile cotton-top swabs (Amies Transport swabs; Oxoid Deutschland GmbH, Wesel, Germany) by the nursing home staff after personal instruction by a member of the Health Authority or City Hospital Hygiene team at the beginning of each collection period. Each home was allowed 29 h for specimen collection from all participating residents and transport to the microbiology laboratory of the City Hospital Brunswick. All nursing homes were successively screened within eight weeks starting in September 2009. Each of the 1827 participants across the 32 participating nursing homes was surveyed for additional risk factor information in an accompanying questionnaire relating to diabetes, urinary catheters, hospital admission during the preceding six months, and grade 0–4 of resident care (grade 0: independent; grade 1: dependent on minor help with personal hygiene, nutrition, mobility or household at least once a day for a maximum of 90 min; grade 2: dependent for the parameters as grade 1 but for up to three times a day for a maximum of 3 h; grade 3: dependent for day and night for  $\geq 5$  h; grade 4: exceeding grade 3). Antibiotic usage was not included, since answers were deemed too ambiguous and thus error-prone. The total number of known MRSA-positive residents in each nursing home on the day of study (irrespective of their participation in this prevalence study) was obtained from the home management. Because of the blinded design of the study, we did not know whether a participant was known to be MRSA

positive, or if known MRSA-positive residents participated in the study and whether they were correctly identified. Informed consent was obtained prior to analysis from the management of each nursing home as well as from each individual resident or his or her custodian, respectively. The study protocol was approved by the head of the medical ethics committee of Hanover Medical School and the commissioner for data protection of the City Hospital Brunswick.

Swabs were inoculated on to cefoxitin-containing chromogenic agar plates (CHROMagar, MD; MAST Diagnostics, Reinfeld, Germany) and incubated for 48 h at 30 °C in ambient air. Suspicious colonies were confirmed as *S. aureus* by Gram staining, catalase and coagulase testing (Slidex Staph kit, bioMérieux, Nürtingen, Germany) followed by antibiotic susceptibility testing using Vitek II system (P-592 card, bioMérieux). Any discrepancies were resolved using polymerase chain reaction detecting *mecA* and *orfX* as described elsewhere.<sup>6</sup>

### Statistical analysis

Fisher's exact test and  $\chi^2$ -test were performed using Prism 4 for Windows (GraphPad Prism). Where a risk factor criterion had only two possible categories (e.g. sex; wound swabs, diabetes, urinary tract catheter and hospital stay within the previous 6 months – all yes/no), then Fisher's exact test [two-tailed, 95% confidence intervals (CI) and relative risk as the test statistic] was used, the relative risk test statistic being the ratio of proportions. Where a risk factor criterion had several possible categories (e.g. resident care



**Figure 1.** Meticillin-resistant *Staphylococcus aureus* (MRSA) prevalence in 32 nursing homes in Brunswick, northern Germany. Prevalence is given in percentage of total residents screened and homes (represented by a number) are sorted by prevalence. The dotted line represents the expected rate (0.9%) of MRSA prevalence according to the nursing homes' pre-study information; the dashed line the mean MRSA rate detected (7.6%).

dependency), then  $\chi^2$ -test (95% CI) was used, and  $\alpha < 5\%$  ( $P < 0.05$ ) considered significant.

### spa typing

For molecular typing of isolated strains, the repeat region (X region) of the protein A gene (*spa*) was analysed. Colonies grown on sheep blood agar plates were suspended in 0.5 mL of Tris/HCl (10 mM, pH 7.5 supplemented with 0.1 mM EDTA). Cells were disrupted in a Fast Prep-24 instrument for 40 s, intensity 5.5 (MP Biomedicals, Inc., Solon, OH, USA). The repeat region was amplified using primers *spa*-1113fn (5'-TAAAGACGATCCWTCGGTGAGC-3') and *spa*-1514rn (5'-SAGCAGTAGTCCGCTTTGCT-3'), and sequenced using an ABI PRISM BigDye Terminator v1.1 Ready Reaction cycle sequencing kit (Applied Biosystems, Darmstadt, Germany) on an ABI PRISM 3100 genetic analyser (Applied Biosystems). The *spa* types were assigned through the Ridom web server. Sequences of new *spa* types were deposited in the GenBank database under accession numbers HM209052 and HM209053.

### Results

Thirty-two of 34 (91.4%) nursing homes in Brunswick agreed to participate in this study. Of a total of 2688 residents 1847 individually agreed to participate, of which 1827 were available for analysis and screened between September and October 2009, representing 68.5% of the nursing home population of Brunswick. The study population included 21% males with a mean age of 78 years, and 79% females with a mean age of 86.2 years. A total of 139 residents were identified as MRSA positive in their nose and/or wounds, yielding a prevalence of 7.6% (CI: 6.4–8.8) (assuming a random association of study participation and MRSA carrier status). The variation of prevalence rates (0–18.6%) observed among the different nursing homes indicates a normal distribution without obvious outliers that may account for the unexpectedly high prevalence (Figure 1). The rate of MRSA-positive residents identified was almost six-fold higher than the 24 already known MRSA-positive residents (0.9%) reported by the nursing home management at the beginning of the study. However, because of the blinded design of the study we do not know whether these previously known MRSA-positive residents participated in the study, or whether they were correctly identified. Eighteen of the 139 MRSA-positive residents had been treated as inpatients in hospital during the last two years and had already been identified as MRSA positive as documented in the microbiology laboratory information system. Thus, the vast majority of MRSA-positive residents were not known by the nursing home management or by the microbiology department.

Data regarding the resident cohort and risk factor analysis are summarised in Table I. Only 3% of the residents had open wounds, although almost 8% (7.91%, Table I) of MRSA-positive residents had open wounds. Urinary catheters, hospital stay within the preceding six months, and high grade care dependency (grade  $\geq 3$ ) were also significantly associated with MRSA colonisation. By contrast, gender, despite a slight tendency for higher MRSA prevalence among male residents, and diabetes were not significantly associated with MRSA colonisation in our analysis.

Among 137 MRSA isolates available for *spa* typing, 21 different types could be observed that clustered into five distinct groups (Table II). The largest group of *spa* types (121/137, 88.3%) belonged to the EMRSA-15 complex, with *spa* type t032 predominating (97/137, 70.7%). The other *spa* types of this complex differ from t032 by short sequence repeat (SSR) unit deletions (t020, t022, t025, t608, t379, t578, 1904, t1862), which is the major cause of genetic rearrangement within an SSR region,<sup>7</sup> or duplications or insertions

**Table I**

Characteristics and risk factor analysis of the resident study cohort

	MRSA <sup>+</sup>	MRSA <sup>-</sup>	MRSA prevalence			Sensitivity (%) <sup>a</sup>
			%	Test statistics <sup>b</sup>	P-value	
<b>Male</b>						
No. (years)	38	345	9.92	1.419	0.0648	27.34
Age (years)	80.26	77.01				
<b>Female</b>						
No. (years)	101	1343	6.99			
Age (years)	85.38	86.27				
<b>Wound swabs</b>						
Yes	11	43	20.37	2.822	0.0018	7.91
No	128	1645	7.22			
<b>Diabetes</b>						
Yes	42	428	8.94	1.245	0.2265	30.22
No	97	1254	7.18			
Not specified	0	6				
<b>Urinary catheter</b>						
Yes	25	117	17.61	2.596	<0.0001	17.99
No	114	1567	6.78			
Not specified	0	4				
<b>Hospital stay within previous 6 months</b>						
Yes	63	453	12.21	2.095	<0.0001	45.32
No	76	1228	5.83			
Not specified	0	7				
<b>Resident care grade</b>						
0	1	49	2	41.8 <sup>c</sup>	<0.0001	0.72
1	38	655	5.48			27.34
2	53	654	7.5			38.13
3	45	325	12.16			32.37
3+/4	2	0				1.44
Not specified	0	5				
<b>Total</b>	<b>139 (7.6)</b>	<b>1688 (92.4)</b>				

<sup>a</sup> The sensitivity lists the percentage of MRSA-positive residents that would have been identified using the indicated risk factor as sole screening criterion.

<sup>b</sup> Relative risk test-statistic.

<sup>c</sup>  $\chi^2$ -Test statistic with four degrees of freedom.

(t1032, t965, as well as a newly identified *spa* type termed tbs1) where a single genetic event could account for the sequence divergence between the types. Three further *spa* types differed from t032 by deletions and changes of single SSR units (t2113, t1499, as well as a second newly identified *spa* type termed tbs2). A second group of *spa* types (6/137, 4.4%) comprised t002, t003 and t105, observed in similar low abundance as t008 (5/137, 3.6%).

All *spa* types could be ascribed to known hospital-acquired MRSA clones prevalent in Germany.<sup>2,8</sup> The two novel *spa* types found in this study were clearly attributable to the CC22 complex.

### Discussion

Meticillin-resistant *S. aureus* (MRSA) represents a global problem with an increasing demand on healthcare resources in many countries including Germany. A wide geographical distribution of *spa* types was described recently in all European countries with considerable differences in genetic diversity of MRSA between countries.<sup>2</sup> An analysis of 967 MRSA isolates from patients with invasive *S. aureus* infections revealed that invasive MRSA clones in Europe display a typical epidemic behaviour and have a predominantly regional distribution with t032 predominating in the UK and Ireland but also in the Brandenburg area of East Germany, whereas central Germany has been dominated by t003.

Nursing home residents represent a population at risk for carrying MRSA because of greater age, age-associated morbidity, urinary devices, and high rate of hospital or healthcare contacts, thus representing a potential 'hot spot' for MRSA exchange between hospital, outpatient, healthcare and community. Understanding the epidemiology of community- and healthcare-associated MRSA is

**Table II**  
Frequency of individual *spa* types identified among the 137 isolates available for typing

<i>spa</i> type	Short sequence repeat profile														Predicted clonal complex (CC)	No. of isolates (%)		
t032	26	23	23	13	23	31	29	17	31	29	17	25	17	25	16	28	CC22	97 (70.7)
t020	26	23				31	29	17	31	29	17	25	17	25	16	28	CC22	6 (4.4)
t022	26	23		13	23	31	29	17	31	29	17	25	17	25	16	28	CC22	5 (3.6)
t025	26	23	23	13	23		29	17	31	29	17	25	17	25	16	28	CC22	3 (2.2)
t608	26	23							31	29	17	25	17	25	16	28	CC22	2 (1.5)
t379	26	23	23	13	23	31	29	17				25	17	25	16	28	CC22	1 (0.7)
t578	26	23	23	13	23	31	29	17	31	29	17	25	17	25		28	CC22	1 (0.7)
t904	26	23	23	13	23	31	29	17	31			17	25	16		28	CC22	1 (0.7)
t1865	26	23	23						17	31	29	17	25	16		28	CC22	1 (0.7)
t1032	26	23	23	13	23	31	29	17	31	29	17	25	16	16		28	CC22	1 (0.7)
t965	26	23	23	13	23	31	29	17	31	29	17	25	17	25	16	28	CC22	2 (1.5)
tbs1	26	23	23	13	23	31	29	17	31	29	17	25	17	25	16	28	CC22	1 (0.7)
t2113	26	23	23	13	23	31	29	17	31	29	17		24	25	16	28	CC22	1 (0.7)
t1499	26	23		16	23	31	29	17	31	29	17	25	17	25	16	28	CC22	1 (0.7)
tbs2	7	23		13	23	31	29	17	31	29	17	25	17	25	16	28	CC22	1 (0.7)
t003	26				17	20	17	12	17	17	16					CC5	3 (2.2)	
t002	26	23	17	34	17	20	17	12	17		16					CC5	2 (1.5)	
t105	26	23	17	34	17	20	17		17		16					CC5	1 (0.7)	
t008	11	19	12	21	17	34	24	34	22	25						CC8	5 (3.6)	
t040	9	2	16	13	17	34	16	34								CC45	1 (0.7)	
t091	7	23	21	17	34	12	23	2	12	23						CC7	1 (0.7)	

essential to guide new control initiatives to prevent these organisms from becoming endemic in geographical areas. Thus, control efforts aimed at interrupting the spread within and between healthcare institutions is imperative. Only few studies to date have addressed the MRSA prevalence in residents of nursing homes in Germany.

In the present study, we screened 1827 (68%) of 2688 residents from 32 nursing homes for nasal and/or wound colonisation with MRSA and identified 139 residents (7.6%; CI: 6.4–8.8%) as MRSA positive. Known risk factors for MRSA colonisation including

urinary tract catheters, wounds, preceding hospital admission, and high grade care dependency were confirmed, but none were sensitive enough to predict the majority of MRSA-positive residents. More than 70% of the MRSA isolates belonged to the Barnim clone typical for hospital-acquired MRSA in northern Germany.<sup>2,8</sup> There was no evidence of the presence of community-acquired or livestock-associated *S. aureus* strains, so testing for Panton–Valentine leucocidin (PVL) was not performed. *spa* type t008 (with five isolates in our study) commonly attributed to the community-acquired USA300 clone is typical for the

**Table III**  
MRSA prevalence and molecular typing in European nursing homes

Country	LTCF	No. screened	MRSA <sup>+</sup>	Molecular data	Reference
UK (Leeds)	39 homes	715	159 (22%)	All PVL <sup>-</sup> (of 74 tested); EMRSA-15 (78%); local nosocomial epidemic strain	15,24
UK (Leeds)	69 homes	1245	244 (20%)		15
UK (Leeds)	66 homes	1077	205 (19%)		15
UK (Northamptonshire)	17 homes	275	13 (4.7%)	EMRSA-15, EMRSA-16	17
UK (Birmingham)	10 homes	191	33 (17.3%)	Phage-type pattern of EMRSA-15 (22 of 31 isolates)	16
Ireland	45 homes	1111	267 (23.3%)	All isolates tested PVL <sup>-</sup> ;	21
Ireland	32 homes	792	135 (17%)	t032 (N = 14; ST-22), t022 (N = 3, ST-22), t190 (N = 5, ST-8), t379 (N = 1) <sup>a</sup>	18
Ireland (Dublin)	6 homes	754	65 (8.6%)	Intervention study <sup>b</sup>	25
		743	75 (10.1%)	Two analyses, 5–6 months apart	
Italy (Bergamo)	NS	88	17 (19.3%)	All PVL <sup>-</sup> ;	19
Spain (Catalonia and Balearic Islands)	9 homes	1377 nasal 82 decubitus	213 (15.5%) 49 (59%)	19 of 20 <i>spa</i> t008 (ST8, SCCmec IV) <sup>c</sup> 230 (16.7%) MSSA, 916 (66.5%) non-carriers; 18 residents only colonised in decubitus ulcer; CC5- and ST22-MRSA IV	20,26
Slovenia (Novo mesto)	1 home	107/91	10 (9.3%)/8 (8.8%)	Two different screens, 3 months apart	13,14
Germany (Höxter)	5 hospitals 5 homes 5 rehabilitation centres	1083	3.4% 2.3% 1.2%	All PVL <sup>-</sup> ; t003 (ST225, 'Rhine-Hessen' strain; 30%), t032 (ST22, Barnim strain; 22.2%)	12
Germany (Rhine-Neckar)	47 homes	3236	36 (1.1%)	30 of 36 were 'Rhine-Hessen' (endemic hospital strain in Germany)	11
Germany (Brunswick)	32 homes	1827	139 (7.6%)	See Table II	

MRSA, methicillin-resistant *Staphylococcus aureus*; LTCF, long term care facility; PVL, Pantone–Valentine leucocidin.

<sup>a</sup> *spa* typing was done on only 23 isolates; 7.8% staff members were MRSA carriers.

<sup>b</sup> Twelve of 333 staff members were MRSA carriers.

<sup>c</sup> Three of 51 (5.8%) staff members were MRSA carriers.

hospital-acquired 'northern German' subclone in Germany.<sup>9,10</sup> Thus, there has been transmission of hospital-acquired MRSA to other healthcare institutions.

Similar observations have been made by other investigators in urban care homes in Germany and Europe, where mainly hospital-acquired PVL-negative MRSA isolates had been identified.<sup>4</sup> However, significantly lower rates between 1.1% and 2.3% have been reported in earlier studies from Germany (Table III).<sup>11,12</sup> Considerably higher rates outside the hospital have been reported in other European countries, including 8.6% in Ireland and about 9% in Slovenia to about 20% in England (with 78% belonging to EMRSA-15) and Italy.<sup>13–19</sup> A recent long-term follow-up study from Spain revealed further that almost half of the MRSA carriers (47.8%) had persistent colonisation for six or more months, whereas only 19% had transient and about 4% intermittent MRSA colonisation. The annual incidence of MRSA acquisition in this study was reported to be around 20%.<sup>20</sup>

Nursing home staff were not included in our study. The few studies that tested for MRSA colonisation among nursing staff reported prevalence rates between 5% and 8%, slightly lower than in the respective cohorts analysed.<sup>18–21</sup> However, the impact of staff colonisation on MRSA transmission is far from clear and has therefore been excluded in our study. Similarly, transmission to household contacts has been reported to occur in 19–50% of an index case, the effect of which is most likely self-limited and, again, the impact on transmission remains to be defined.<sup>22,23</sup>

In summary, our results show that in northern Germany MRSA has successfully spread from the hospital environment to long term care facilities. Therefore, long term care residents must be regarded as an important reservoir for MRSA transmission.

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## Conflict of interest statement

None.

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None.

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