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Increase in the prevalence of Panton–Valentine leukocidin and clonal shift in community-onset methicillin-resistant *Staphylococcus aureus* causing skin and soft-tissue infections in the Rhine-Neckar Region, Germany, 2012–2016

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major challenge for patient care. Community-associated (CA)-MRSA often have a fitness and virulence advantage compared with their nosocomial counterparts. Increased mobility, travel activities and migration accelerate the intercontinental spread of virulent CA-MRSA strains. Outpatient clinics are the most important route of entry for CA-MRSA into hospitals. However, systematic data on CA-MRSA in Germany are limited. In this study, community-onset (CO)-MRSA skin and soft-tissue infection (SSTI) isolates in the Rhine-Neckar Region from 2012–2016 were characterised to gain an insight into their molecular epidemiology and to monitor potential introduction of virulent and dominant MRSA strains into our hospital. A total of 2475 patients with *S. aureus* SSTI were identified in the outpatient departments of our hospital, of which 94 (3.8%) were MRSA. In addition, 40.4% of the CO-MRSA harboured the virulence factor Panton–Valentine leukocidin (PVL). ST8-t008-MRSA-IVa/c (23.7%; 9/39) and ST80-t044-MRSA-IVc (15.8%; 6/38) were the predominant PVL-positive MRSA. Molecular typing and epidemiological data revealed that 42.6% (40/94) of strains could be traced back to a local origin and 44.7% (42/94) were endemic outside of Europe. Resistance to quinolones, clindamycin and macrolides was common, whilst resistance to trimethoprim/sulfamethoxazole, tetracycline, mupirocin, chlorhexidine and fusidic acid was low. No resistance to rifampicin, fosfomycin or linezolid was observed. This study provides insight into the clonal composition of CO-MRSA in the Rhine-Neckar Region. The increase of PVL-positive MRSA and the introduction of imported strains may affect the local MRSA landscape in the near future and should be monitored closely.

1. Introduction

The importance of methicillin-resistant *Staphylococcus aureus* (MRSA) as a nosocomial pathogen is indisputable [1,2]. Infection with MRSA is a major clinical burden and has been associated with high morbidity [2]. Current treatment options are limited and often suboptimal. Of particular importance is the acquisition of resistance to second-line agents, which restricts antimicrobial therapy and jeopardises treatment success. In addition, MRSA is causing great public concern owing to its transmissibility and asymptomatic carriers in the community [3]. In the general population, the spread and transmission of MRSA are not restricted by continental borders owing to increasing travel and migration activities [4–7].

In Germany, for yet unclear reasons, the percentage of nosocomial MRSA infection has dropped continually over the last years [8]. According to the current surveillance guidelines in Germany, MRSA isolated from blood cultures and cerebrospinal fluid must be reported to the healthcare authorities and may then be sent to a reference laboratory for further analysis, if required. Thus, most studies focus on the surveillance and clinical characteristics of healthcare-associated MRSA infections [9,10], whilst systematic data on community-onset (CO)-MRSA are scarce, rendering inferences about a similar trend in the community difficult. Although the genetic make-up of nosocomial MRSA and community-associated (CA)-MRSA is quite distinctive, some dominant CA-MRSA were able to penetrate into the healthcare setting and replace nosocomial clones, blurring the distinction between

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nosocomial and CA-MRSA. An example of this is the ST8 "USA300" MRSA strain, which dominates the MRSA landscape of the American continent both in the community and healthcare settings [11,12]. To date, it is unclear whether the overall decline in nosocomial MRSA in Europe is paralleled by an increased prevalence of CA-MRSA in the community or even partially compensated by an influx of these clones into hospitals. However, recent outbreaks and nosocomial transmission of virulent USA300 clones in Europe highlight the possibility of autochthonous spread of dominant MRSA clones and emphasise the importance of further data on CA-MRSA circulating in the community [13,14].

Although skin and soft-tissue infections (SSTIs) may be treated by local antimicrobial therapy or, in the case of skin abscesses, by incision and drainage [15], the importance of concomitant systemic antimicrobial therapy has recently been demonstrated by Daum et al. in a placebo-controlled trial [16]. Thus, resistance of CA- MRSA to non-β-lactam antibiotics is of relevance [17].

Another important factor is the presence of the virulence marker Panton–Valentine leukocidin (PVL or *lukS*/*F*), a bicomponent pore-forming toxin causing tissue necrosis and lysis of phagocytic leukocytes [18]. For SSTI, the presence of PVL has major clinical therapeutic implications as clinical and epidemiological data suggest that PVL is associated with deep-seated abscesses, multiple lesions, recurring SSTI episodes, multiple antimicrobial resistances and outbreaks of SSTI [4,5,19]. Acquisition of PVL in *S. aureus* and MRSA is associated with community-acquired infections and is often found in returning travellers suffering from SSTI. Systematic data on the prevalence of PVL in Central Europe are scarce but it is estimated to be very low [6,20].

To monitor the introduction of dominant and virulent strains into the hospital setting, in this study CO-MRSA isolates from SSTI patients in outpatient departments of Heidelberg University Hospital (Heidelberg, Germany) between 2012 and 2016 were characterised by staphylococcal protein A (*spa*) type, staphylococcal cassette chromosome *mec* (SCC*mec*) type and detection of PVL. The main objective was to gain insights into the clonal composition and diversity of CO-MRSA as well as possible changes over time.

2. Materials and methods

2.1. Inclusion criteria and clinical information

A database search was performed of our laboratory information system and patient data management for methicillin-susceptible *S. aureus* and MRSA isolates causing SSTI from patients treated in outpatient departments of Heidelberg University Hospital between 2012 and 2016. CO-MRSA SSTIs were defined as SSTIs that were acquired outside of the hospital setting. Patients with a history of inpatient treatment 3 months prior to outpatient treatment were excluded. In case of chronic or multiple episodes of outpatient treatment for SSTI, only the primary episode was included in the analysis, i.e. each patient could only be included in the study once. Clinical information was obtained retrospectively from the patient's record. Information on chronic wounds, diabetes, recurrent infection(s) and history of travel or migration were collected. The main objective of the study was to characterise the population structure of CO-MRSA. Isolates were classified into CA-MRSA and hospital-associated (HA)-MRSA according to available epidemiological and genetic data.

2.2. Identification and antimicrobial resistance

Staphylococcus aureus strains were identified in routine microbiology by typical morphology, positive catalase reaction and slide agglutination test (Pastorex™ StaphPlus; Bio-Rad Laboratories GmbH, München, Germany). Antimicrobial resistance was determined using a VITEK®2 system (bioMérieux, Marcy-l'Étoile, France) and the results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints that were valid in the respective year. Presence of the *nuc* and *mecA* genes was confirmed by real-time PCR using the primers nuc-fw (5′-GCGATTGATGGTGATACGGTT-3′), nuc-rv (5′-AGCCAAGCCTTGACGAACTAAAGC-3′), mecA-fw (5′-GTAGAAATGACTGAACGTCCGATA A-3′) and mecA-rv (5′-CCAATTCCACATTGTTTCGGTCTAA-3′) under standard cycling conditions (60°C annealing temperature). All isolates were cryopreserved for further analyses.

2.3. spa typing, SCCmec typing, and detection of lukF/S and arginine catabolic mobile element (ACME)

All strains were tested for the presence of the PVL-encoding genes *lukF*/*S* by real-time PCR. *SCC*mec (types I–V) of all MRSA isolates were determined by multiplex PCR as described elsewhere [21]. All *S. aureus* isolates were subjected to *spa* typing [22]. *spa* types were clustered into *spa* clonal complexes (*spa*-CC) using the Based Upon Repeat Pattern (BURP) algorithm with parameters set to exclude if repeats were <5 and to cluster if cost ≤4 [23]. All MRSA with *spa* type t008 were subjected to ACME detection using the primers arcA-fw (5′-GCAGCAGAATCTATTACTGAGCC-3′) and arcA-rv (5′-TGCTAACTTTTCTATTGCTTGAGC-3′) as published previously [24]. Using *spa* type, SCC*mec* type, PVL and the presence of ACME, all isolates were further characterised according to the most probable country of origin by published epidemiological data (Table 1; Fig. 1B).

2.4. Mupirocin and chlorhexidine resistance

Due to the unavailability of clinical breakpoints and lack of standardised testing for chlorhexidine tolerance [25], susceptibility to this substance was determined genotypically with real-time PCR using the primers smr-fw (5′-GCCATAAGTACTGAAGTTATTGGA-3′), smr-rv (5′-GACTACGGTTGTTAAGACTAAACCT-3′), qacA/B-fw (5′-GCAGAAAGTGCAGAGTTCG-3′) and qacA/B-rv (5′-CCAGTCCAATCATGCCTG-3′). All isolates with phenotypic resistance to mupirocin were confirmed by PCR using the primers mupA-fw (5′-TATATTATGCGATGGAAGGTGTTG-3′) and mupA-rv (5′-AATAAAATCAGCTGGAAAAGTGTTG-3′).

2.5. Data and statistical analyses

All data were collected retrospectively with personal identifying information being pseudonymised before transfer into databases. Statistical procedures were performed using Stata Statistical Software: Release 14 (StataCorp LP, College Station, TX, USA). Contingency tables were constructed and χ^2 tests were used to calculate *P*-values for describing the association of PVL and clinical information. Logistic regression was used to calculate the odds ratio (OR), 95% confidence interval (CI) and *P*-value to analyse the association of PVL prevalence and year. *P*-values of <0.05 were considered statistically significant.

2.6. Ethical considerations

No additional procedure or additional data acquisition apart from routine clinical diagnostics were performed on patients included in the study. Surveillance and molecular characterisation were performed on the MRSA isolates as mandated by the infection control measures of the Department of Hospital Hygiene of Heidelberg University Hospital in accordance with the German Infection Protection Act. The Ethical review board was consulted with the study protocol to ensure conformity to current laws and regulations.

Table 1
Characteristics of community-onset methicillin-resistant *Staphylococcus aureus* (MRSA) skin and soft-tissue infection isolates in the Rhine-Neckar Region, Germany, 2012–2016[a].

spa type (*n*)	SCC*mec* (*n*)	*spa*-CC	ACME[b] (*n*)	PVL [*n* (%)]	Patients with history of travel or migration[c] (country; *n*)	Endemic area [Ref. PMID]
t003 (28)	II (28)	45	N/A	0	Egypt; 1	Germany (Rhine-Hesse epidemic clone)
t008 (12)[d]	IVa (7)	24	5	6 (85.7)	USA; 1	USA (USA300 epidemic clone) [22448902]
	IVc (5)		0	4 (80.0)		Latin America [22104084]
t002 (6)	II (1)	45	N/A	0	N/A	Germany
	IVa (1)		N/A	0		Africa [25983721]
	IVc (4)		N/A	2 (50.0)		South Asia [25982914]
t044 (6)	IVc (6)	Singleton	N/A	6 (100)	N/A	Middle East [22104084]
t437 (4)	V (4)	Singleton	N/A	2 (50.0)	Vietnam; 1	Asia (Taiwan) [21789606]
t019 (3)	IVc (3)	Singleton	N/A	2 (66.7)	N/A	Southwest Pacific [22104084]
t024 (2)	IVc (2)	24	N/A	0	N/A	Latin America [22745670], Africa [27873646]
t027 (2)	II (2)	Singleton	N/A	0	N/A	Unknown
t127 (2)	IVa (2)	Singleton	N/A	1 (50.0)	Syria; 1	Europe [26187827; 21447518]
t535 (2)	II (2)	Singleton	N/A	0	N/A	Germany [25704447][e]
t852 (2)	IVc (2)	5	N/A	2 (100)	N/A	Europe (Austria) [24391878], Oman [25356354]
t005 (1)	IVc	5	N/A	1 (100)	N/A	South Asia [20339016]
t012 (1)	NT	Singleton	N/A	0	N/A	Unknown
t032 (1)	IV	5	N/A	0	N/A	Germany [25704447][e]
t034 (1)	V	Singleton	N/A	1 (100)	N/A	Asia [24763740]
t035 (1)	II	45	N/A	0	N/A	Unknown
t045 (1)	II	45	N/A	0	N/A	Worldwide [18772392][e]
t11977 (1)	II	45	N/A	0	N/A	Unknown
t1250 (1)	V	Singleton	N/A	0	N/A	Unknown
t1339 (1)	IVa	Singleton	N/A	1 (100)	N/A	Africa [20298267]
t2051 (1)	IVc	45	N/A	1 (100)	N/A	Kuwait [28640870]
t223 (1)	IV	5	N/A	0	N/A	Jordan [25002017]
t304 (1)	IVa	24	N/A	0	N/A	South Asia [27203527]
t311 (1)	V	45	N/A	0	N/A	Europe [17553275][e]
t314 (1)	V	Singleton	N/A	1 (100)	N/A	Ghana [24586981][e]
t316 (1)	IVa	Singleton	N/A	1 (100)	N/A	Unknown
t334 (1)	IV	24	N/A	0	N/A	Unknown
t355 (1)	IV	Singleton	N/A	1 (100)	N/A	Europe [17553275][e]
t4103 (1)	IVa	NF	N/A	1 (100)	N/A	Unknown
t4690 (1)	V	Singleton	N/A	1 (100)	N/A	Unknown
t509 (1)	IVc	45	N/A	0	Sri Lanka; 1	Europe [26992009][e]
t6089 (1)	IV	Singleton	N/A	0	N/A	Unknown
t6380 (1)	IVa	24	N/A	1 (100)	N/A	Unknown
t657 (1)	V	Singleton	N/A	1 (100)	Nepal, India; 1	Asia (Bengal Bay clone) [18614654]
t665 (1)	IVa	Singleton	N/A	0	N/A	Caribbean [21789605]
t692 (1)	IVa	NF	N/A	1 (100)	Dominican Republic; 1	Middle America [23698534]

spa, staphylococcal protein A; SCC*mec*, staphylococcal cassette chromosome *mec; spa*-CC, *spa* clonal complex; ACME, arginine catabolic mobile element; PVL, Panton–Valentine leukocidin; Ref., reference; PMID, PubMed ID; N/A, not available; NT, non-typeable; NF, no founder.
[a] All isolates were *mecA*-positive, sorted by the most common *spa* types in decreasing order.
[b] Detection of ACME was only performed for MRSA with the *spa* type t008
[c] Travel history was only recorded in the cases indicated.
[d] USA300 clusters were defined as ACME+PVL+ST8-t008-IVa MRSA for the North American variant and ACME–PVL+ST8-t008-IVc MRSA for the Latin American variant.
[e] No information on *SCC*mec type available.

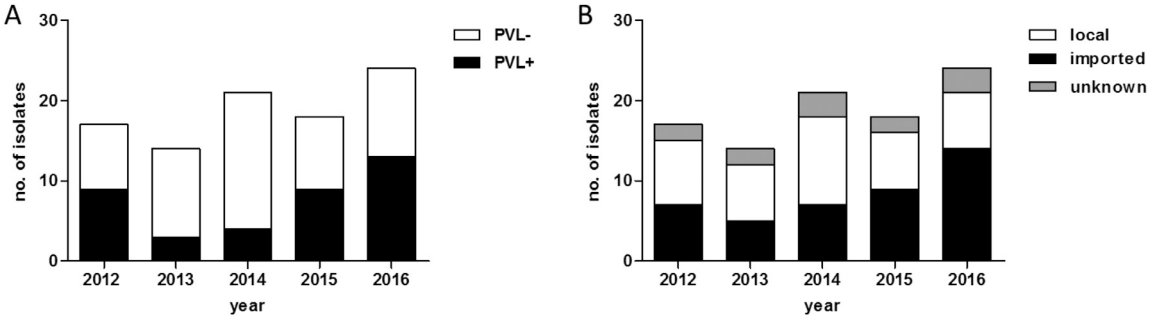

Fig. 1. (A) Percentage of Panton–Valentine leukocidin-positive (PVL+) (*lukF/S*-positive) and PVL-negative (PVL–) (*lukF/S*-negative) community-onset methicillin-resistant *Staphylococcus aureus* skin and soft-tissue infection isolates in Rhine-Neckar Region, Germany, 2012–216. (B) Percentage of isolates with local, imported or unknown origin. Absolute numbers: 2012, $n = 17$; 2013, $n = 14$; 2014, $n = 21$; 2015, $n = 18$; and 2016, $n = 24$.

Table 2
Frequency [*n* (%)] of Panton–Valentin leukocidin (PVL)-positive strains and clinical characteristics of community-onset methicillin-resistant *Staphylococcus aureus* (MRSA) skin and soft-tissue infection (SSTI) isolates in Rhine-Neckar Region, Germany, 2012–2026, according to the probable origin.

Characteristic	All strains ($n=94$)	Geographic origin[a]		
		Local ($n=40$)	Imported ($n=42$)	Unknown ($n=12$)
PVL-positive	38 (40.4)	4 (10.0)	30 (71.4)	4 (33.3)
Recurrent SSTI	19 (20.2)	7 (17.5)	8 (19.0)	4 (33.3)
Travel history	4 (4.3)	1 (2.5)	3 (7.1)	0 (0)
Migration	11 (11.7)	4 (10.0)	7 (16.7)	0 (0)
Chronic wounds [b]	24/87 (27.6)	15/39 (38.5)	6/39 (15.4)	3/9 (33.3)
Diabetes [b]	14/87 (16.1)	9/39 (23.1)	4/39 (10.3)	1/9 (11.1)

[a] Assignment based on PubMed data (see Table 1).
[b] Incomplete clinical data for 7 patients (1 local, 3 imported and 3 unknown).

3. Results

During the study period (2012–2016), 2475 patients with SSTI due to *S. aureus* were treated in outpatient departments of our hospital, of which 94 (3.8%; 95% CI 3.1–4.6%) had an infection with MRSA and were included in the study.

3.1. Patient characteristics

The mean ± standard deviation age of the study population was 53 ± 24.7 years (range 14–95 years). Four adolescents were included in the study (aged 14, 15, 16 and 16 years). Of the 94 patients, 52 (55.3%) were treated in the surgical outpatient department, 41 (43.6%) in the dermatology department and 1 (1.1%) in the travel clinic. Moreover, 56 patients (59.6%) were male, 19 (20.2%) had recurrent episodes of SSTI, 24 (27.6%) had chronic wounds and 14 (16.1%) had diabetes. In addition, 4 patients (4.3%) had a history of travel and 11 (11.7%) of migration (Table 2).

3.2. Molecular epidemiology and presence of Panton–Valentine leukocidin

Four *spa* clusters were identified: *spa*-CC 045 (42.6%; 40/94) with t002, t003, t035, t045, t311, t509, t2051 and t11977; *spa*-CC 024 (18.1%; 17/94) with t008, t024, t304, t334 and t6380; *spa*-CC 005 (5.3%; 5/94) with t005, t032, t223 and t852; and no-founder cluster (2.1%; 2/94) with t692 and t4103. In addition, there were 30 singletons (31.9%) including t012, t019, t027, t034, t044, t127, t314, t316, t355, t437, t535, t657, t665, t1250, t1339, t4690 and t6089. The most commonly isolated *spa* type was t003 (28/94; 29.8%) (Table 1).

Of the 94 isolates, 40.4% (38/94) were positive for PVL (PVL+) (Table 2). The presence of PVL was significantly associated with travel history (3% vs. 1%; $P < 0.05$) or migration (20% vs. 2%; $P=0.001$) and there was a trend towards an association with recurrent infection (4% vs. 1%; $P=0.08$). Diabetes or chronic wounds were negatively associated with the presence of PVL [2% vs. 32.9% ($P < 0.05$) and 4% vs. 30.8% ($P=0.01$), respectively]. An increasing proportion of PVL+ MRSA was observed from 2013–2016 ($P=0.01$; OR = 1.85, 95% CI 1.16–2.94) (Fig. 1A). However, due to the high proportion of PVL+ MRSA in 2012, this trend was not significant if 2012 was included in the analysis.

As a high percentage of strains were PVL+ and this was significantly associated with prior history of travel or migration, we aimed to identify clones that are known to circulate outside of Europe and may be imported into Germany. ST8-t008-MRSA-IVa/c ($n=9$) and ST80-t044-MRSA-IVc ($n=6$) were the predominant PVL+ MRSA strains circulating in our hospital/region.

The *spa* types, *SCC*mec types, information on travel or migration, presence of PVL (*lukF/S*) and endemic area are shown in Table 1. The analysis revealed that 44.7% of strains (42/94) were imported and 42.6% (40/94) were of local origin. The endemic area has not been described so far for 12.8% of the strains (12/94). An apparent clonal shift in favour of imported CA-MRSA clones was observed (Fig. 1B; Fig. 2).

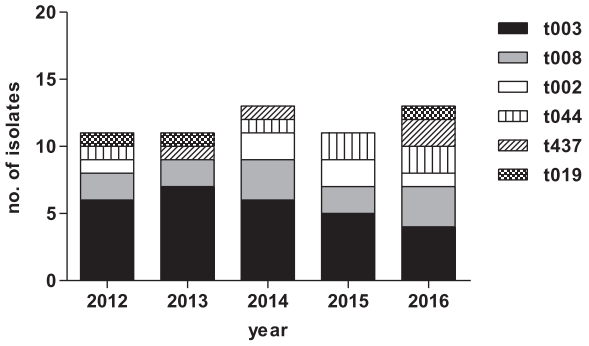

Fig. 2. Percentage of the six most frequent staphylococcal protein A (*spa*) types among community-onset methicillin-resistant *Staphylococcus aureus* (MRSA) skin and soft-tissue infection isolates in Rhine-Neckar Region, Germany, 2012–2016. The proportion of the predominant ST5-t003 Rhine-Hesse MRSA is declining over time (black bars), whereas the proportion of imported MRSA strains is increasing. Absolute numbers: 2012, t003 ($n=6$), t008 ($n=2$), t002 ($n=1$), t044 ($n=1$) and t019 ($n=1$); 2013, t003 ($n=7$), t008 ($n=2$), t437 ($n=1$) and t019 ($n=1$); 2014, t003 ($n=6$), t008 ($n=3$), t002 ($n=2$), t044 ($n=1$) and t437 ($n=1$); 2015, t003 ($n=5$), t008 ($n=2$), t002 ($n=2$) and t044 ($n=2$); and 2016, t003 ($n=4$), t008 ($n=3$), t002 ($n=1$), t044 ($n=2$), t0437 ($n=2$) and t019 ($n=1$). Underlined *spa* types are locally endemic.

3.3. Major epidemic MRSA clones

Several MRSA belonging to major circulating epidemic MRSA clones were identified. Twenty-eight belonged to the endemic CC5-t003-II Rhine-Hessen clone, five to ACME+ST8-t008-IVa 'USA300', four to ACME–ST8-t008-IVc 'USA300-Latin American variant', six to ST80-t044-IVc, three to ST30-t019-IVc 'Southwest Pacific MRSA clone' and one to ST772-t657-V 'Bengal Bay MRSA clone'.

3.4. Antimicrobial resistance

A high level of resistance to fluoroquinolones (57.4%), clindamycin (56.4%) and macrolides (68.1%) was observed, whilst resistance to tetracycline (17.0%), trimethoprim/sulfamethoxazole (SXT) (7.5%) and fusidic acid (11.7%) was low. No resistance to rifampicin, fosfomycin and linezolid was detected. In addition, 2.1% and 4.3% of strains were resistant to the decolonising agents mupirocin and chlorhexidine, respectively (Table 3).

Moreover, the distribution of antimicrobial resistance among local and imported clones differed substantially: 85.0% of locally acquired versus 33.3% of imported isolates were resistant to

Table 3
Frequency of resistance [*n* (%)] to orally administered antibiotics and decolonising agents of community-onset methicillin-resistant *Staphylococcus aureus* skin and soft-tissue infection isolates in Rhine-Neckar Region, Germany, 2012–2016.

Antimicrobial agent	All strains ($n=94$)	Geographic origin		
		Local ($n=40$)	Imported ($n=42$)	Unknown ($n=12$)[a]
Fluoroquinolones	54 (57.4)	34 (85.0)	14 (33.3)	6 (50.0)
SXT[b]	7/93 (7.5)	2/39 (5.1)	3 (7.1)	2 (16.7)
Clindamycin	53 (56.4)	34 (85.0)	12 (28.6)	7 (58.3)
Macrolides	64 (68.1)	34 (85.0)	21 (50.0)	9 (75.0)
Tetracycline	16 (17.0)	3 (7.5)	10 (23.8)	3 (25.0)
Rifampicin	0	0	0	0
Fosfomycin	0	0	0	0
Fusidic acid	11 (11.7)	4 (10.0)	6 (14.3)	1 (8.3)
Linezolid	0	0	0	0
Mupirocin	2 (2.1)	1 (2.5)	1 (2.4)	0
qacA/B[c]	4 (4.3)	1 (2.5)	3 (7.1)	0

SXT, trimethoprim/sulfamethoxazole.
[a] Travel history was not apparent from or stated explicitly in the patients' records.
[b] One missing data for SXT resistance (local origin).
[c] *qacA/B* is associated with tolerance to cationic antiseptics (e.g. chlorhexidine digluconate).

fluoroquinolones ($P < 0.001$), 85.0% versus 28.6% to clindamycin ($P < 0.001$) and 85.0% versus 50.0% to macrolides ($P=0.001$), respectively. Resistance to tetracycline was higher among imported clones (23.8% vs. 7.5%; $P=0.1$). The percentage of strains resistant to SXT and fusidic acid was similar [7.1% vs. 5.0% ($P=0.5$) and 14.3% vs. 10.0% ($P=0.7$), respectively] (Table 3). Over 72% of MRSA (68/94) were resistant to two or more of the following non-β-lactam antibiotics: clindamycin; fluoroquinolones; SXT; tetracycline; macrolides and rifampicin.

4. Discussion

The present study analysed CA-MRSA clones causing SSTI circulating in the Rhine-Neckar Region, Germany. Of particular interest is the presence of the virulence marker PVL, which is associated with higher clinical severity and intercontinental travel [5,17]. Data on PVL prevalence in CO-MRSA in Germany and Europe are scarce. Most studies focus on the characterisation of a collection of HA-MRSA and CA-MRSA. For this reason, it is difficult to compare the current study with published reports. There are, however, similar studies despite different study premises, which may assist in the interpretation of the current findings. In Germany, the prevalence of PVL in circulating CA-MRSA was considered to be very low. In 2011, Schaumburg et al. reported 2.7% PVL-positive MRSA isolates collected in outpatient clinics as well as hospitals. However, that study included both asymptomatic carrier and clinical MRSA isolates [10]. In Central Europe, the PVL prevalence is considered to be low [6,20,26]. In a recent European multicentre study also conducted in tertiary-care hospitals, Bouchiat et al. detected 9% and 8% PVL+ MRSA clones overall and in the German study centre, respectively [20]. These findings are in sharp contrast to the current observations of a much higher PVL prevalence in CA-MRSA from SSTI in the Rhine-Neckar Region. We found that overall from 2012–2016, on average 40.4% of CA-MRSA was PVL+, with proportions ranging from 19% to 54% per year of the study period. Acquisition PVL+ *S. aureus* was commonly found to be travel-associated, thus importation through travel and migration is the most probable source of these MRSA [4,5]. There was a slight increase in the number of patients with a documented history of migration from 2012 to 2016. In 2012 there were no patients with documented migration, one in 2013, one in 2014, three in 2015 and six in 2016.

To some extent the differences between the current findings and other groups' reports may be explained by selection bias and/or study parameters. The presence of PVL is associated with a more severe clinical presentation [4,5]. Patients with uncomplicated SSTI are less likely to seek immediate medical advice. Patients with more severe SSTI will usually consult a general practitioner before being referred to a specialist and would not be considered as an emergency. Hence, these cases rarely end up in the emergency room in Germany, which was the premise of a study conducted by Bouchiat et al. [20]. The premise of the current study was outpatient departments, such as the ambulatory surgical unit and the dermatology unit. However, whilst this may explain a higher proportion of PVL+ CA-MRSA isolated in this study compared with that reported for emergency departments in Europe and Germany by Bouchiat et al. [20], this does not explain the increase in PVL+ CA-MRSA over time presented here.

In their recent publication, the German National Reference Laboratory for Staphylococci reported an abundance of PVL+ MRSA from children admitted to hospitals in Frankfurt-Höchst in the years 2012–2015. Of 42 MRSA isolates identified, 40 (95%) were classified as CA-MRSA owing to their molecular characteristics, 80% of which harboured the PVL gene [27]. The majority of children (39/40) suffered from SSTI and 90% were immigrants. Layer et al. also analysed a number of CA-MRSA strains sent randomly from all regions of Germany [27]; 63.6% (464/729) of these isolates were PVL+. For some of the strains, information on prior history of travel or migration was available. As submission to a reference centre is likely to select for isolates from more complicated cases, this proportion is likely an overestimation of the PVL prevalence in CA-MRSA circulating in Germany.

4.1. Antimicrobial resistance

Although the role of systemic antibiotic therapy in the treatment of SSTI has been disputed and highly debated over the past years, recent studies by Daum et al. [16] and Hogan et al. [28] demonstrated that adjunctive antibiotic therapy was more favourable than incision and surgical drainage only to prevent recurrent episodes. Moreover, not only complicated SSTI but also smaller skin abscesses benefit from systemic antimicrobial therapy. Thus, particularly for MRSA, resistance to non-β-lactams antibiotics is relevant [17], as the current data suggest that >72% of locally circulating MRSA were resistant to two or more non-β-lactam antibiotics. A recent report suggests that resistance genes may be integrated into the SCC*mec* cassette and no additional extrinsic selection pressure might be needed for their spread, rather they are transferred along with SCC*mec* [29]. Therefore, the emergence of resistance/multiresistance to second-line agents in MRSA should be monitored closely. Only a small percentage of CO-MRSA

was resistant to mupirocin and harboured *qacA*/*B*, which is associated with chlorhexidine tolerance [30]. Data in the current study suggest that the use of mupirocin is justified to eradicate CO-MRSA colonisation.

We demonstrated that the resistance profile of the local clones differed substantially from that of the imported clones. Although we would recommend obtaining a sample for microbiological analysis, especially in complicated or recurrent cases, initial empirical therapy may be guided by the individual patient history and local epidemiology. A history of travel or migration may lead to a different empirical therapy in those patients than in patients with chronic conditions such as diabetes or chronic wounds [5].

4.2. Abundance of epidemic CA-MRSA clones

The most frequent *spa* type, comprising 29.8% of analysed isolates, was t003 belonging to the CC5-t003-MRSA-II 'Rhine-Hesse epidemic clone'. This was expected as this is, and has been, the dominant clone circulating in the healthcare setting in our region for some years [31]. However, a shift towards CA-MRSA clones was observed, which are characterised by the smaller SCC*mec* cassettes of type IV and V. In 2012 and 2013, t003 was still the predominant clone, but then the proportion of t003 MRSA continuously declined until 2016. We hypothesised that these changes might be attributed to variations in patient demographics owing to increased travel activities and/or migration, rather than clonal replacement of local strains. Judging by the dynamic and data extrapolation, this clonal shift may be more apparent in the coming years and a clonal replacement may be anticipated as a consequence. Such a phenomenon has been described for the 'USA300' PVL+ ST8-t008-MRSA-IV on the American continent and for PVL+ CA-MRSA in Ireland [11,32].

The second most frequent *spa* type, comprising 12.8%, was ST8-t008 MRSA, of which 75% (9/12) belonged to the USA300 clonal lineage. Its proportion remained stable over the years. In recent years, outbreaks and transmission of these MRSA clones have been reported in Germany, France and Belgium [13,14,33]. Interestingly, ST8-t008 USA300 MRSA was not found in the study by Bouchiat et al. [20], although its presence has been documented previously in Germany [10,34]. Indeed, through the StaphTrav surveillance network (http://www.staphtrav.eu), our group identified USA300 MRSA as the most commonly imported MRSA clone [5]. Taken together, ST8 MRSA might be emerging in Germany and surveillance measures should be adjusted to anticipate further transmission and spread, as the current German Infection Protection Act requires notification for MRSA isolated from blood cultures and cerebrospinal fluid only.

Altogether, 44.7% of the MRSA isolates were epidemic outside of Europe and thus likely to have been imported to Germany. Especially the rise in 2015 and 2016 may be connected to increased migratory activities in this period.

This study has limitations. A monocentric study was conducted and the epidemiology and clinical course of MRSA SSTIs are heterogeneous and may differ between regions. We were only able to analyse 94 MRSA isolates over the study period owing to the low prevalence of MRSA in Germany. Selection bias caused by the various mechanisms that underlie patient referral to outpatient departments at a tertiary-care hospital undoubtedly affect the generality of our findings to the general population of our region. Microbiological diagnostic and antimicrobial susceptibility testing is not a standard procedure for SSTI in the outpatient setting. Hence, the abundance of CA-MRSA might be underestimated owing to undetected and undocumented cases.

The current surveillance regulations in Germany are not sufficient to detect clonal shifts and emerging highly pathogenic MRSA clones circulating in the community. Although there is supporting evidence that CA-MRSA clones are invading the healthcare setting, surveillance regulations have yet to be adapted. In light of the current findings, we recommend to expand the current MRSA reporting practice to include the notification of CO-MRSA SSTIs.

5. Conclusion

The data presented here and the limited systematic data available for Germany highlight the urgent need for continuous surveillance of CA-MRSA as the epidemiology may change rapidly. The findings of the current study suggest that the abundance of PVL+ CA-MRSA is alarming and, at the current rate, might invade hospitals and dominate the HA-MRSA landscape in the future. An increasing heterogeneity of resistances to non-β-lactam antibiotics among MRSA underlines the importance of antimicrobial susceptibility testing for *S. aureus* causing SSTI in guiding clinicians towards an appropriate antimicrobial therapy.

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Competing interests

None declared.

Ethical approval

The Ethical Review Board was consulted to verify conformity to the German Infection Protection Act; no specific ethical approval required [S-474/2018].

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