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Emergence of SCC*mec* Type IV as the Most Common Type of Methicillin-Resistant *Staphylococcus aureus* in a University Hospital

A.M. Strandén, R. Frei, H. Adler, U. Flückiger, A.F. Widmer

Abstract

Background: The epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) has dramatically changed over the last decade by the emergence of community-associated MRSA (CA-MRSA). Recent studies indicate that these strains have already spread to hospitals. To evaluate if SCC*mec* type IV and Panton–Valentine leukocidin (PVL) are unambiguous markers of CA-MRSA, we analyzed 77 sporadic MRSA strains isolated, in our low MRSA incidence university hospital, from inpatients between 2000 and 2004.

Methods: MRSA strains were analyzed by staphylococcal cassette chromosome *mec* (SCC*mec*) typing, PCR for PVL genes and pulsed-field gel electrophoresis (PFGE). MRSA was classified in HA-MRSA or CA-MRSA according to Centers for Disease Control and Prevention (CDC) criteria. Antimicrobial susceptibility testing was performed using microbroth dilution method following CLSI recommendations.

Results: Among 77 sporadic single-patient strains, SCC*mec* types I–IV and four subtypes were identified. Type IV/IVA was most common (42.9%).The distribution of SCC*mec* types changed over the years. Type IV/IVA strains increased from 33.3% in 2000 to 57.9% in 2004. Type IV strains were resistant to ciprofloxacin in 81.8%, and in 9.1% to tobramycin while type IVA strains were 100% resistant to both antimicrobials. In contrast, non-type IV/IVA strains were resistant to ciprofloxacin in 86.4%, and in 75.0% to tobramycin. Only one strain was PVL positive and harbored SCC*mec* type III variant. By PFGE analysis, the 33 SCC*mec* type IV/IVA strains comprised 12 distinct genotypes. 36.4% of 11 CA-MRSA and 43.9% of 66 HA-MRSA harbored SCC*mec* type IV/IVA.

Conclusion: Type IV/IVA has become the most common SCC*mec* type in inpatients of our university hospital. The SCC*mec* type IV/IVA is present in both CA-MRSA and HA-MRSA limiting its use as a marker for CA-MRSA.

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become the most important emerging pathogen worldwide. Methicillin resistance in staphylococci is caused by the expression of penicillin-binding protein 2a (PBP2a) encoded by the *mecA* gene that is located on a genetic element called the staphylococcal cassette chromosome (SCC). SCC*mec* is a mobile DNA element of 21–67 kb and is integrated into the chromosome of MRSA [1, 2]. Hospital-associated MRSA (HA-MRSA) isolates are typically resistant to multiple antibiotics, negative for Panton–Valentine leukocidin (PVL) encoding genes and primarily harbor SCC*mec* types I, II, III, and rarely IV [1, 3, 4]. Up to six main types of SCC*mec* and many variants, especially of type IV are known [1, 5, 6]. Type IV is present in many more genetic backgrounds than other types suggesting an enhanced mobility [7–9]. In contrast to HA-MRSA, community-associated MRSA (CA-MRSA) strains are commonly susceptible to the majority of non-β-lactam antibiotics, frequently expressing genes encoding for PVL and are predominantly of the SCC*mec* types IV and V, and present multiple patterns by pulsed-field gel electrophoresis [10, 11].

Recently, the emergence of CA-MRSA dramatically increased the importance and awareness of this multiresistant pathogen [12]. In contrast to HA-MRSA, CA-MRSA infections typically occur in young, athletic and healthy individuals without exposure to healthcare institutions [13].

The University Hospital Basel (UHB), Basel, Switzerland, has a low overall incidence of MRSA, between 0.104 and 0.179 per 1,000 patient days with very rare clusters. On average, less than two bloodstream infections with MRSA per year are observed in a setting with more than 700 episodes of bloodstream infections per year. This environment allows testing the hypothesis, if SCC*mec* type IV and PVL are markers of CA-MRSA among strains found in hospitalized patients. We, therefore, compared the presence

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of these molecular traits with epidemiological data obtained from inpatients between 2000 and 2004.

Patients and Methods

Epidemiological Data and Setting

Since 1996, demographic data of all patients admitted to the UHB and colonized or infected with MRSA were prospectively collected in a case report form, and data transferred to a database (MS Access, USA). MRSA was classified as CA-MRSA or HA-MRSA according to Centers for Disease Control and Prevention (CDC) criteria [14]. Since 1992, the hospital follows a ''search and destroy'' policy, adapted from guidelines issued by the Dutch Infection Control Society [15]. Our approach was successfully validated in a foreign hospital [16]. Patients with risk factors or those previously colonized or infected with MRSA are routinely screened on admission by swabs of nose, throat, and any wounds present [21]. Since 1992, MRSA strains are saved at –70 °C. All consecutive single patient isolates of hospitalized patients between 2000 and 2004 were included in this study. Strains from outpatients and from epidemics or clusters were excluded. Only one isolate per patient was analyzed.

Bacterial Strains and Susceptibility Testing

Methicillin-resistant *Staphylococcus aureus* isolates were detected by standard culture methods. Colonies suspected to be *Staphylococcus aureus* were screened with the latex agglutination test PASTOREX STAPH PLUS (BIO-RAD, France) and confirmed as such with Gram stain, catalase, and aurease (Rapidec staph, bioMérieux, France). Resistance to oxacillin was determined according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), formerly NCCLS (http://www.clsi.org), using an oxacillin disk and/or screen agar (MRSA Screen Agar, bioMérieux) and confirmed by the detection of penicillin-binding protein 2a (MRSA-Screen, Denka Seiken, Japan). Any discrepant results were resolved by PCR testing using primers targeting *femA* and *mecA* gene. The susceptibility against 12 antimicrobial compounds was tested using microbroth dilution method (Micronaut^{TM} system, Merlin, Germany) following CLSI recommendations.

SCC*mec* Typing

The SCC*mec* multiplex PCR assay was performed according to the method of *Oliveira* and *de Lancastre* [17] with modifications in the primer concentrations. Concentrations for primers were as follows: 0.25 μM for CIF2 F2, CIF2 R2, MECI P2, MECI P3, RIF5 F10, RIF5 R13; 0.3 μM for IS431 P4, pUB110 R1; 0.5 μM for pT181 R1; 1.0 μM for DCS F2, DCS R1, RIF4 F3, RIF4 R9, MECA P4, MECA P7 and 1.7 μM for KDP F1, KDP R1. The multiplex PCR included eight loci (A–H) [17]. MRSA strains NCTC10442, N315, 85/2082, and WSPP A were included as controls for SCC*mec* types I, II, III, and IV, respectively. PCR products were resolved by electrophoresis and visualized with ethidium bromide on 2% agarose gels.

PVL

The PVL genes (*lukS-PV*, *lukF-PV*) were detected by PCR according to the method of *Lina* et al. [18]. The PCR products were visualized with ethidium bromide on 1.5% agarose gels.

Pulsed-Field Gel Electrophoresis

All isolates were analyzed by pulsed-field gel electrophoresis (PFGE) as described previously [19]. After *Sma*I digestion the DNA restriction fragments were separated by PFGE and dendrograms were drawn using the software GelCompar 4.5 (Applied Maths, Belgium). The fragment patterns were interpreted according to the criteria of *Tenover* et al. [20].

Results

A total of 187 single patient isolates were recorded in the database from inpatients, outpatients, and healthcare workers from 2000 to 2004. Among 104 MRSA strains isolated from inpatients, 93 were available for the analysis by SCC*mec* typing, by PVL and PFGE; 11 strains were accidentally disposed or were not viable anymore; 17.2% (16/93) of the strains originated from an MRSA epidemic and were therefore excluded. Fourteen of these isolates possessed the SCC*mec* type I and two isolates type IVA. Among the remaining 77 sporadic cases, the SCC*mec* type IV/IVA was the most common representing 42.9% of cases (Figure 1). The rate of SCC*mec* type IV/IVA strains increased from 33.3% in 2000 to 57.9% in 2004 (Figure 2). The incidence density of MRSA by inpatients increased in the same period from 0.104 to 0.165 per 1,000 patient days (Figure 2).

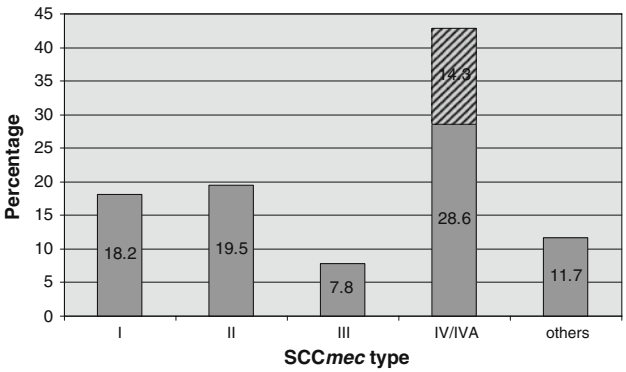

Figure 1. Prevalence of SCC*mec* types over a 5-year period in percentage. The bar labeled with IV/IVA comprises SCC*mec* type IV (gray) and type IVA (hatched).

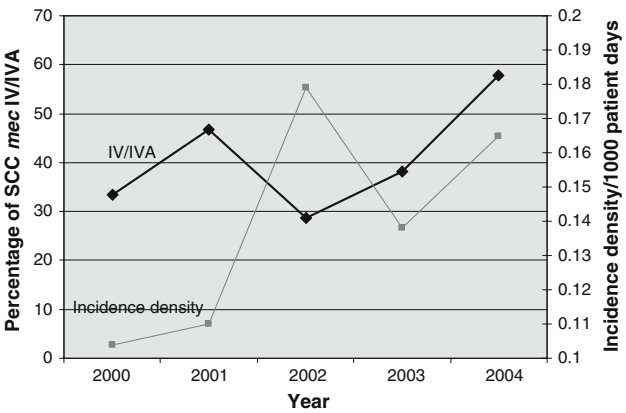

Figure 2. Percentage of SCC*mec* type IV/IVA strains and the incidence density of MRSA in inpatients per 1,000 patient days.

Table 1
Definitions of SCC*mec* types and subtypes.

SCC*mec* type	I	IA	II	III	IV	IVA	Type III variant	New variant	New variant	Only mec
Locus	A, D	A, G, D	D, B, C	F, H, E, C	D	G, D	F, E	A, F, E	F, D	
No. of isolates	14	1	15	6	22	11	5	1	1	1
No. of PVL positive isolates	0	0	0	0	0	0	1	0	0	0

Nine different SCC*mec* types and subtypes were detected (Figure 1; Table 1).

From the 77 analyzed strains, 11 (14.3%) were classified as CA-MRSA, 66 (85.7%) as HA-MRSA. Of CA-MRSA and of HA-MRSA, 36.4% and 43.9% harbored SCC*mec* type IV/IVA, respectively. The rate of type IV/IVA strains by HA-MRSA increased from 33.3% to 66.7%. Surprizingly, only one strain classified as HA-MRSA was positive for PVL (Table 1).

By PFGE analysis, the 33 SCC*mec* type IV/IVA strains comprised 12 distinct genotypes (Figure 3). 24.2% of these strains originated from the infected and 75.8% from colonized patients.

The most discriminating antimicrobial resistance rates among SCC*mec* type IV, IVA and non-type-IV are given in table 2.

Discussion

In our setting, the low incidence of MRSA coupled with a prospective active screening program using a standardized case report form provided a unique opportunity to classify MRSA patients with epidemiological data and results from molecular typing [21].

From the 77 sporadic cases, 11 (14.3%) were CA-MRSA and 66 (85.7%) HA-MRSA. SCC*mec* type IV/IVA was already common in the year 2000 and has become the most common type with 57.9% prevalence in 2004. PFGE analysis of the 33 SCC*mec* types IV/IVA isolates comprised 12 distinct genotypes. Several strains had similar PFGE patterns, but were epidemiologically unrelated. They were isolated from different years and/or different locations, suggesting an epidemic clone in the community. Similar to a recent observation in Chicago, it

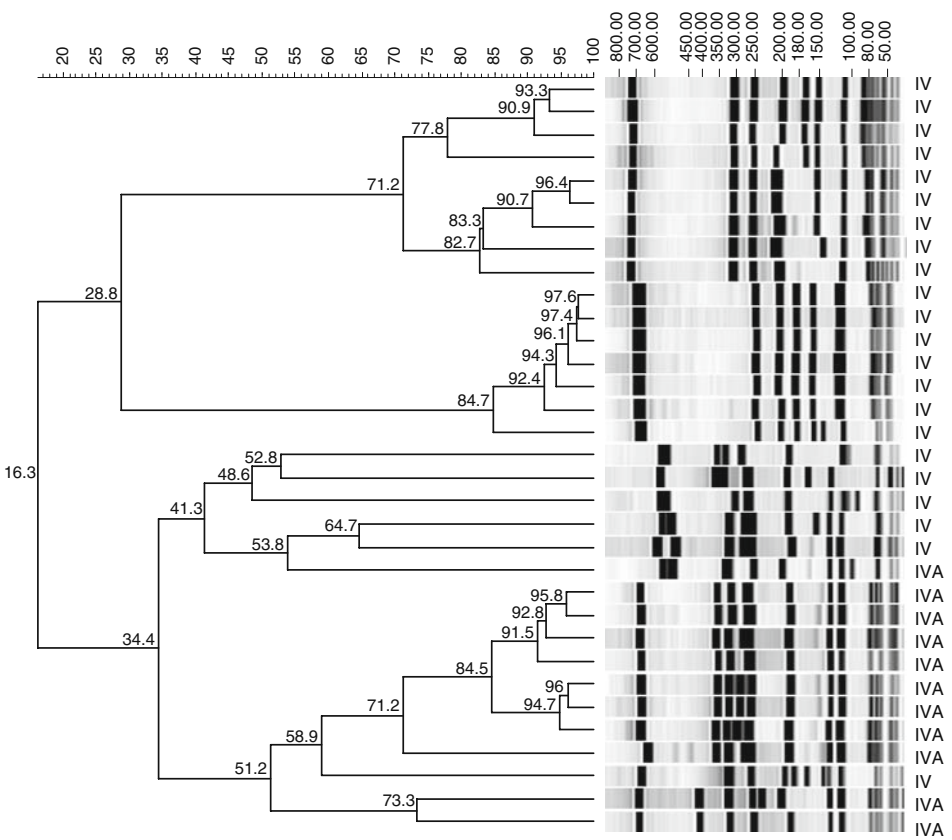

Figure 3. PFGE analysis with dendrogram of SCC*mec* type IV/IVA isolates.

Table 2
The antimicrobial resistance of SCC*mec* type IV, IVA and non-type IV/IVA strains

SCC*mec* type	Resistance (%)		
	Ciprofloxacin	Clarithromycin	Tobramycin
IV	82	50	9
IVA	100	55	100
Non-IV/IVA	86	73	75

appears that SCC*mec* type IV/IVA has spread in the hospital without evidence for clusters or epidemics [22].

In contrast to the study of *Popovich* [22], who exclusively collected strains from invasive bloodstream infections, only 24% of patients in our study were infected with MRSA rather than colonized. About 43.9% of HA-MRSA and 36.4% of CA-MRSA were of SCC*mec* type IV/IVA. In USA, HA-MRSA primarily harbors SCC*mec* types I, II, III, and occasionally IV and CA-MRSA are predominantly of the SCC*mec* type IV [11]. In Geneva, another Swiss town, the most frequent MRSA strains were of SCC*mec* type I (86%) followed by type IV (11%), whereas other types were detected only on rare occasions [25]. However, similar to our observation, the number of type IV isolates has considerably increased during the last years [23]. The latter study analyzed a highly selective strain collection of non-multidrug-resistant and community-acquired MRSA, including those from epidemics. In contrast, our study used all consecutive strains from hospitalized patients. Other authors also reported an increase of SCC*mec* type IV in the hospital setting [24]. To summarize, MRSA harboring genetic markers of CA-MRSA have increased over the past years at our hospital and appear to replace traditional hospital MRSA strains. Bootsma et al. [26] already predicted in their model that CA-MRSA strains would replace, not add to, traditional HA-MRSA strains. Data from our study and those by *Popovich* support this model [22].

The strength of our study is the unique environment of the hospital: less than 0.5% of all patients admitted to the hospital are colonized with MRSA, as reported by *Mertz* et al. [21] and confirmed by a recent screening of 1,000 patients on admission (data not shown). In addition, the surveillance program identifies only between 2 and 10 transmissions per year in our 750-bed tertiary care hospital. Therefore, patients are very unlikely to be misclassified as sporadic when they were in fact part of a cluster. Replacement of traditional hospital-associated MRSA strains with CA-MRSA strains would have serious infection-control implications in a low-prevalence setting; it may render infection control activities futile if multiple clusters of CA-MRSA spread in the hospital with unknown modes of transmission. Cross-transmission of CA-MRSA and familial transmissions have been documented earlier [27]. The MRSA low incidence countries could use another strategy for the control of MRSA, if the prevalence of MRSA is growing despite the fact that hospital-associated cases are under control.

The search and destroy policy of our institution may have successfully controlled HA-MRSA, likely overrepresenting type IV by eliminating type I and type II strains [14].

Similar to *Qi* et al., our isolates of SCC*mec* type IV were mostly resistant to ciprofloxacin and clarithromycin [28]. The SCC*mec* type IVA strains were more resistant than type IV in our setting. In contrast to the study results of USA, we had only one PVL positive strain [11]. This may be due to the predominance of colonized patients in our data set, exclusion of outpatients or differences between the rates of different countries or the time frame of data collection [29]. In fact, few PVL positive strains by SCC*mec* type IV (12%) have also been found in Zurich, Switzerland [28]. In our setting, PVL did not prove to be a reliable marker for CA-MRSA.

In conclusion, we have demonstrated that SCC*mec* type IV/IVA is present in both CA-MRSA and HA-MRSA, limiting its use as a marker for CA-MRSA. The epidemiology of MRSA in hospitals is rapidly changing, and MRSA SCC*mec* type IV may become the most common type in hospitals in near future.

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