Eur J Clin Microbiol Infect Dis (2009) 28:647–653
DOI 10.1007/s10096-008-0663-7

CONCISE ARTICLE

Variability of SCC*mec* in the Zurich area

M. Ender · S. Burger · A. Sokoli · R. Zbinden · B. Berger-Bächi · R. Heusser · M. M. Senn · N. McCallum

Received: 30 July 2008 / Accepted: 22 October 2008 / Published online: 26 November 2008

Abstract A periodic survey of methicillin-resistant *Staphylococcus aureus* (MRSA) in Zurich in 2004 and 2006 revealed a consistently low prevalence of MRSA. SCC*mec* and *ccr* typing showed fluctuations in the proportions of SCC*mec* types and in the carriage of mobile virulence determinants. Together with the presence of variant SCC*mec*s these findings suggest a high clonal diversity and level of SCC*mec* recombination. The prevalence of a local "drug clone", associated with low-level methicillin resistance and rapid growth, significantly decreased. This clone had spread among intraveneous drug users, steadily increasing from 1994 to 2001 and was dominant in 2001. Apparently, changes in the management of the Zurich drug scene have restricted the spread of this clone.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) were first reported only one year after introduction of the penicillinase-resistant antibiotic methicillin into clinical use and have since become an increasing worldwide problem [12]. Initially, MRSA were restricted to hospitals and sites of high antibiotic use, where they accumulated additional resistances and became multiresistant to all commonly used antibiotics. Recently, however, nonmultiresistant MRSA clones with lower oxacillin resistance levels, but apparently higher virulence, have emerged and begun spreading within the community and into hospitals [2, 5, 17, 18].

Methicillin resistance is based on the acquisition of an additional penicillin-binding protein (PBP), PBP2a, which has a lower affinity for β-lactams than the cells' own PBPs. Staphylococci differ in their ability to accept PBP2a for unknown reasons [13]. The restriction of MRSA to specific genetic backgrounds is also reflected by the observation that MRSA have generally evolved separately and sometimes even repeatedly into only five of the main 11 distinct epidemic susceptible *S. aureus* lineages [22]. Methicillin resistance thus depends on both the production of PBP2a and on the genetic composition of the core genome. Strain-specific resistance levels are therefore dictated by the host strain's genetic background and can vary extensively from extremely low (<1 μg/ml) to very high (>1000 μg/ml) oxacillin minimum inhibitory concentrations (MICs) [1].

The *mec*A gene, coding for PBP2a, is localized on the staphylococcal chromosomal cassette SCC*mec*, a unique genomic island, which integrates into the staphylococcal genome [8]. The *mec*A gene and its cognate regulatory elements *mecI-mecR1* form the *mec* complex. In some strains the regulatory genes *mecI-mecR1* may be truncated by insertion elements, giving rise to different *mec* complex loci. The other feature common to all SCC*mec* elements is the *ccr* locus, which encodes recombinases responsible for the site-specific integration and excision of SCC*mec* [9]. While the *ccr* genes from different SCC*mec* elements show sequence polymorphisms, the *mecA* gene is highly conserved. SCCmec elements vary to a great extent in size, due to additional resistance determinants, plasmids, transposons, IS elements, and genes unrelated to resistance. The

Electronic supplementary material The online version of this article (doi:10.1007/s10096-008-0663-7) contains supplementary material which is available to authorized users.

M. Ender · S. Burger · A. Sokoli · R. Zbinden · B. Berger-Bächi · R. Heusser · M. M. Senn · N. McCallum (✉)
Institute of Medical Microbiology, University of Zurich,
Gloriastrasse 32,
8006 Zurich, Switzerland
e-mail: mccallum@immv.uzh.ch

classification of SCC*mec* elements is based on the allotype of the *mec* and *ccr* complex as well as the presence of selected loci unrelated to methicillin resistance [19], which has lead to the identification of six major SCC*mec* types [10, 11, 14]. A new nomenclature has been proposed to accommodate novel and divergent SCC*mec* types [3]. The older, hospital-associated SCC*mec* types I, II, and III are generally larger due to the accumulation of unrelated resistance determinants and other extraneous DNA into the SCC*mec* element. The emerging community MRSA, with smaller SCC*mec* elements of type IV, V, or VI, are generally only oligoresistant, express lower methicillin resistance, and grow faster, which appears to give them a fitness advantage. Interestingly, the smaller SCC*mec* types have entered more diverse strain lineages than the larger types [21, 23], possibly due to increased mobility, or to a lower fitness burden associated with their typically lower/heterogeneous methicillin resistance.

The convergence of methicillin resistance with virulence is a dangerous development, especially if combined in rapidly growing strains. Examples include the acquisition of the arginine catabolic mobile element (ACME) associated with SCC*mec* [6], or the phage transmitted *pvl* genes [24] that are connected to many community-acquired MRSA (CA-MRSA). A particularly successful clone is strain USA300, which is spreading at a tremendous rate in the United States [15].

In recent years the prevalence of MRSA in the 920-bed University Hospital in Zurich had remained relatively low at approximately 3–6%. An epidemiological study performed in 2003 showed that about 30% of the MRSA isolates collected had low oxacillin MICs (43% had MIC ≤ 6 μg/ml) [20]. The majority belonged to a local MRSA clone spreading among intravenous drug users in the Zurich area. This particular "drug clone" appeared in 1994 and steadily proliferated, culminating in 2001 and remaining at quite a high level until 2004 when it constituted over 25% of all MRSA isolated [20]. The drug clone carries the novel SCC*mec*$_{N1}$ element, containing a *mecB* complex and the *dfrA* and *fusB1* genes coding for trimethoprim and fusidic acid resistance, respectively. It also contains two almost identical *ccrAB4*-like complexes that are similar to previously described *ccrAB4* complexes, and a core genome belonging to the successful Berlin CC45 clone [7, 20]. The very low level methicillin resistance of the drug clone is paired with extremely rapid growth; therefore, we were concerned that this clone may spread beyond its confined circle and acquire additional resistance determinants or virulence factors over time. To follow its fate 56 consecutive, non-duplicate MRSA isolates collected from April to December 2004 (referred to as 2004), mainly from the University Hospital in Zurich, and 55 isolates from June 2006 to February 2007 (referred to as 2006) were analysed.

Materials and methods

Polymerase chain reaction (PCR)

Specific primers were used to amplify *pvl* (PVL *lukS* fwd 5′-GTAAAATGTCTGGACATGATCCA-3′, PVL *lukF* rev 5′-CAACTGTATTGGATAGCAAAAGC-3′), *tst* (TSST up 5′-CCCTGTTCCCTTATTATCTA-3′, TSST down 5′-TTCCTTCGCTAGTATGTTGG-3′), and *arcA* (*arcA*+ 5′-GAGCCAGAAGTACGCGAG-3′, *arcA*- 5′-CACGTAA CTTGCTAGAACGAG-3′).

Susceptibility testing

Resistance levels for ciprofloxacin, chloramphenicol, trimethoprim, gentamicin, tetracycline, rifampin, fusidic acid, and the MLS antibiotics clindamycin and erythromycin were determined by disk diffusion according to CLSI [4], and oxacillin MICs were obtained by Etest (AB-Biodisk).

SCC*mec* typing

Isolates were typed according to Oliveira et al. [19]. *ccr* typing was performed as described previously [7, 20].

Pulsed-field gel electrophoresis

Strains were genotyped by pulsed-field gel electrophoresis of SmaI-digested chromosomal DNA, following the protocol given in [20].

Spa typing

spa sequence typing was performed using the Ridom StaphType *spa*-sequencing protocol (http://www.ridom.de/doc/Ridom_spa_sequencing.pdf) and the Ridom SpaServer to identify repeats and assign *spa* types (http://spaserver2.ridom.de/index.shtml).

Results and discussion

MRSA isolates were characterised and grouped according to their SCC*mec* typing profile and their *ccr* type. Antibiotic resistance levels to ciprofloxacin, chloramphenicol, trimethoprim, gentamicin, tetracycline, rifampin, fusidic acid, and MLS antibiotics were measured and oxacillin MICs were determined. Isolates were also screened for the presence of *pvl*, *tst*, and *arcA* by PCR. Unexpectedly, a rather large proportion of MRSA from the second collection period seemed to be unstable and 12 strains lost the *mecA* gene during storage at −80 °C. Eleven of these strains appeared to have lost their entire SCC*mec* elements, as they

Table 1 Characteristics of the analysed MRSA isolates grouped according to their SCC*mec* types

Strain	SCC*mec*	*ccr*	MIC[a] [μg/ml]	Resistance phenotype									Virulence determinants[b]			Year
			Ox	Ci	Cm	Gm	Tc	Ra	Tr	Fa	Cl	Er	*tst*	*pvl*	*arcA*	
ZH 123	**I**	*AB1*	12	S	S	S	S	S	S	**R**	S	**R**	+			2004
ZH 129	**I**	*AB1*	12	S	S	S	S	S	S	**R**	S	**R**	+			
ZH 138	**I**	*AB1*	>256	**R**	S	**R**	S	S	S	S	**R**	**R**				
ZH 141	**I**	*AB1*	>256	**R**	I	**R**	S	S	S	S	**R**	**R**				
ZH 261	**I**	*AB1*	>256	**R**	S	**R**	S	S	S	S	**R**	**R**				2006
ZH 276	**I**	*AB1*	>256	**R**	**R**	**R**	S	S	S	S	**R**	**R**				
ZH 317	**I**	*AB1*	>256	**R**	I	**R**	I	S	S	S	**R**	**R**				
ZH 150	**II**	*AB2*	192	**R**	S	S	S	S	S	S	**R**	**R**				2004
ZH 104	**II**	*AB2*	256	**R**	S	S	S	S	S	S	S	**R**				
ZH 111	**II**	*AB2*	256	**R**	I	S	S	S	S	S	**R**	**R**				
ZH 149	**II**	*AB2*	256	**R**	S	S	S	S	S	S	**R**	**R**				
ZH 151	**II**	*AB2*	256	**R**	S	S	S	S	S	S	S	**R**				
ZH 281	**II**	*AB2*	0.25	S	S	S	**R**	S	**R**	S	S	S				2006
ZH 286	**II**	*AB2*	1	**R**	I	S	S	S	S	S	**R**	**R**				
ZH 297	**II**	*AB2*	24	**R**	S	S	S	S	S	S	S	S				
ZH 309	**II**	*AB2*	192	**R**	S	S	S	S	S	S	**R**	**R**				
ZH 270	**II**	*AB2*	256	**R**	S	S	S	S	S	S	**R**	**R**				
ZH 278	**II**	*AB2*	256	**R**	S	S	S	S	S	S	**R**	**R**				
ZH 284	**II**	*AB2*	256	**R**	I	S	I	S	S	S	**R**	**R**				
ZH 295	**II**	*AB2*	256	**R**	S	S	S	S	S	S	**R**	**R**				
ZH 316	**II**	*AB2*	256	**R**	I	I	R	S	S	S	**R**	**R**				
ZH 135	**III**	*AB3 & C*	>256	**R**	S	**R**	**R**	S	**R**	S	S	**R**				2004
ZH 139	**III**	*AB3 & C*	>256	**R**	**R**	**R**	**R**	**R**	**R**	**R**	S	**R**				
ZH 146	**III**	*AB3 & C*	>256	**R**	**R**	**R**	**R**	**I**	**R**	**R**	S	**R**				
ZH 148	**III**	*AB3 & C*	>256	**R**	**R**	**R**	**R**	**R**	**R**	**R**	S	**R**				
ZH 153	**III**	*AB3 & C*	>256	**R**	**R**	**R**	**R**	I	**R**	**R**	S	**R**				
ZH 162	**III**	*AB3 & C*	>256	**R**	**R**	**R**	**R**	**R**	**R**	**R**	S	**R**				
ZH 163	**III**	*AB3 & C*	>256	**R**	**R**	**R**	**R**	I	**R**	**R**	S	**R**				
ZH 264	**III**	*AB3 & C*	>256	**R**	S	**R**	**R**	**R**	**R**	I	**R**	**R**				2006
ZH 265	**III**	*AB3 & C*	>256	**R**	S	**R**	**R**	**R**	**R**	S	**R**	**R**				
ZH 115	**IV**	*AB2*	1	S	S	S	I	S	S	S	S	**R**				2004
ZH 116	**IV**	*AB2*	1	S	S	S	I	S	S	S	S	**R**				
ZH 118	**IV**	*AB2*	1	S	S	S	**R**	S	S	S	S	**R**				
ZH 113	**IV**	*AB2*	1.5	S	I	S	**R**	S	S	S	S	**R**				
ZH 117	**IV**	*AB2*	1.5	S	S	S	**R**	S	S	S	S	**R**				
ZH 120	**IV**	*AB2*	2	S	S	S	S	S	S	**R**	I	**R**				
ZH 108	**IV**	*AB2*	3	S	S	S	**R**	S	S	S	S	**R**				
ZH 131	**IV**	*AB2*	4	I	S	S	**R**	S	S	**R**	S	S				
ZH 155	**IV**	*AB2*	4	I	S	S	S	S	S	S	S	S				
ZH 130	**IV**	*AB2*	16	I	S	S	S	S	S	S	S	S		+		
ZH 158	**IV**	*AB2*	48	S	S	S	S	S	S	**R**	I	**R**				
ZH 110	**IV**	*AB2*	64	S	S	S	S	S	S	S	S	S		+		
ZH 119	**IV**	*AB2*	96	S	S	S	**R**	S	S	S	S	**R**				
ZH 114	**IV**	*AB2*	128	S	S	S	**R**	S	S	S	S	I			+	
ZH 124	**IV**	*AB2*	256	**R**	S	S	**R**	S	S	S	**R**	**R**		+	+	
ZH 121	**IV**	*AB2*	>256	**R**	S	S	S	S	S	S	S	S				
ZH 136	**IV**	*AB2*	>256	**R**	S	S	S	S	S	S	**R**	**R**				
ZH 154	**IV**	*AB2*	>256	**R**	S	S	I	S	S	S	S	S				

Table 1 (continued)

Strain	SCC*mec*	*ccr*	MIC[a] [μg/ml]	Resistance phenotype									Virulence determinants[b]			Year
			Ox	Ci	Cm	Gm	Tc	Ra	Tr	Fa	Cl	Er	*tst*	*pvl*	*arcA*	
ZH 313	**IV**	*AB2*	1.5	S	S	S	S	S	S	S	S	S		+		2006
ZH 282	**IV**	*AB2*	3	S	S	S	S	S	S	S	S	S				
ZH 268	**IV**	*AB2*	6	S	I	S	S	S	S	S	S	S				
ZH 314	**IV**	*AB2*	24	I	I	S	**R**	S	I	**R**	S	S		+		
ZH 304	**IV**	*AB2*	64	S	I	I	S	S	S	S	S	I				
ZH 285	**IV**	*AB2*	128	S	S	S	S	S	S	S	S	S				
ZH 263	**IV**	*AB2*	256	**R**	S	S	S	S	S	S	S	**R**				
ZH 271	**IV**	*AB2*	256	**R**	S	S	S	S	S	S	S	**R**		+	+	
ZH 312	**IV**	*AB2*	256	**R**	S	S	**R**	S	S	S	I	**R**		+		
ZH 262	**IV**	*AB2*	>256	**R**	S	S	**R**	S	S	I	S	**R**				
ZH 266	**IV**	*AB2*	>256	S	S	S	S	S	**R**	S	S	**R**				
ZH 272	**IV**	*AB2*	>256	**R**	S	S	S	S	S	S	S	**R**				
ZH 274	**IV**	*AB2*	>256	I	S	S	S	S	S	S	S	**R**				
ZH 275	**IV**	*AB2*	>256	S	S	S	S	S	S	S	S	S				
ZH 279	**IV**	*AB2*	>256	**R**	S	S	S	S	S	S	S	**R**				
ZH 283	**IV**	*AB2*	>256	**R**	S	S	S	S	S	S	S	**R**				
ZH 291	**IV**	*AB2*	>256	**R**	S	S	S	S	S	**R**	S	**R**		+		
ZH 294	**IV**	*AB2*	>256	**R**	S	S	S	S	S	I	S	**R**				
ZH 310	**IV**	*AB2*	>256	**R**	S	S	S	S	S	I	S	S				
ZH 137	**N1**	*AB4*	1.5	**R**	S	S	S	S	**R**	**R**	S	S				2004
ZH 144	**N1**	*AB4*	1.5	**R**	I	S	I	S	**R**	**R**	S	S				
ZH 145	**N1**	*AB4*	1.5	**R**	I	I	S	S	**R**	**R**	S	S				
ZH 161	**N1**	*AB4*	1.5	**R**	S	I	S	S	**R**	**R**	S	S				
ZH 122	**N1**	*AB4*	2	**R**	S	S	S	S	**R**	**R**	S	S				
ZH 126	**N1**	*AB4*	2	**R**	S	S	S	S	**R**	**R**	S	S				
ZH 127	**N1**	*AB4*	2	**R**	S	S	S	S	**R**	**R**	S	S				
ZH 103	**N1**	*AB4*	3	**R**	S	S	S	S	**R**	**R**	S	S				
ZH 105	**N1**	*AB4*	3	**R**	S	S	S	S	**R**	**R**	S	S				
ZH 109	**N1**	*AB4*	3	S	S	S	S	S	**R**	**R**	S	S				
ZH 125	**N1**	*AB4*	3	**R**	S	S	S	S	**R**	**R**	S	S				
ZH 147	**N1**	*AB4*	3	**R**	S	S	S	S	**R**	**R**	S	S				
ZH 107	**N1**	*AB4*	4	S	S	S	S	S	**R**	**R**	S	S				
ZH 128	**N1**	*AB4*	4	**R**	S	S	S	S	**R**	**R**	S	S				
ZH 132	**N1**	*AB4*	4	S	S	S	S	S	**R**	**R**	S	S				
ZH 106	**N1**	*AB4*	6	S	S	S	S	S	**R**	**R**	S	S				
ZH 112	**N1**	*AB4*	6	**R**	S	S	S	S	**R**	**R**	S	S				
ZH 277	**N1**	*AB4*	1.5	**R**	I	S	S	S	**R**	**R**	S	S				2006
ZH 287	**N1**	*AB4*	1.5	**R**	S	S	S	S	**R**	**R**	S	S				
ZH 292	**N1**	*AB4*	2	S	S	S	S	S	**R**	**R**	S	S				
ZH 315	**N1**	*AB4*	3	S	S	S	S	S	**R**	**R**	S	S				
ZH 273	**VI**	*AB4*	>256	**R**	S	S	**R**	**R**	**R**	**R**	**R**	**R**				2006
SCC*mec* variants																
ZH 159	**I**	*AB2*	192	S	**R**	S	**R**	S	S	S	S	S				2004
ZH 152	**II-VAR**	*AB1 & AB4*	1	**R**	S	S	S	S	S	**R**	S	S				2004
ZH 134	**II**	*AB1*	48	**R**	S	**R**	**R**	**R**	**R**	S	**R**	**R**	+			
ZH 133	**I/II-VAR**	*AB1*	>256	**R**	S	**R**	**R**	**R**	S	S	**R**	**R**				
ZH 299	**II**	*AB2 & AB1*	0.75	S	S	S	S	S	S	S	S	S		+		2006
ZH 293	**II-VAR**	*AB2 & AB4*	6	I	S	S	S	S	S	S	S	S	+			

Table 1 (continued)

Strain	SCC*mec*	*ccr*	MIC[a] [μg/ml]	Resistance phenotype									Virulence determinants[b]			Year
			Ox	Ci	Cm	Gm	Tc	Ra	Tr	Fa	Cl	Er	*tst*	*pvl*	*arcA*	
ZH 143	**III-VAR**	*C*	3	S	S	**R**	S	S	S	S	S	S		+		2004
ZH 269	**III-VAR**	*C*	6	S	S	**R**	S	S	**R**	S	S	S				2006
ZH 298	**IV**	*AB1*	0.38	S	S	S	S	S	S	**R**	S	S				2006
ZH 305	**IV**	*AB2 & AB4*	3	S	**R**	S	**R**	S	**R**	**R**	S	I				

MRSA methicillin-resistant *Staphylococcus aureus*, Ci ciprofloxacin, Cm chloramphenicol, Gm gentamicin, Tc tetracycline, Ra rifampin, Tr trimethoprim, Fa fusidic acid, Cl clindamycin, Er erythromycin

[a] *MIC* minimal inhibitory concentration determined for Ox (oxacillin)

[b] *tst* encodes toxic shock syndrome toxin, *pvl* encodes Panton-Valentine leukocidin, *ArcA* belongs to the arginine deiminase pathway gene cluster located on the ACME element

gave no amplification products from SCC*mec* typing PCR. The remaining strain amplified a single band containing the *dcs* region present in SCC*mec* types I, II, and IV, but appeared to have lost the region(s) that may have encoded other SCC*mec* typing loci, including the *mecA* gene. Pulsed-field gel electrophoresis (PFGE) analysis of these 12 strains identified ten distinct SmaI restriction profiles (Supplementary Fig. 1), indicating that the majority of the isolates were not closely related and hence loss of *mecA* was not associated with a specific clonal background(s). Susceptibility profiles of these strains also showed that there was no clear relationship between these strains (Supplementary Table 1). These strains were subsequently removed from the study. The remaining 99 isolates included 56 from 2004 and 43 from 2006 (Table 1).

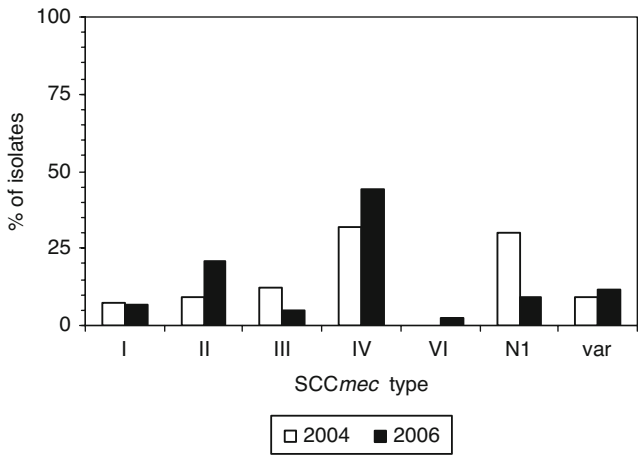

Fig. 1 Percentage of SCC*mec* types from isolates collected during 2004 and 2006

SCC*mec* type I

The smallest group, containing only seven isolates, was composed of SCC*mec* type I MRSA and remained stable over the observed period (7.1% in 2004; 6.9% in 2006) (Fig. 1). Five of these isolates were highly oxacillin resistant, with MICs >256 μg/ml, and were resistant to ciprofloxacin, gentamicin, clindamycin, and erythromycin. The remaining two strains carried only fusidic acid and erythromycin resistances, were heterogeneously oxacillin resistant, with MICs of 12 μg/ml, and contained the *tst* gene. One isolate from 2004 (ZH159) had a SCC*mec* type I multiplex PCR profile, but contained a *ccrAB2* complex and was therefore grouped among the variants.

SCC*mec* type II

A total of 14 isolates had typical SCC*mec* type II profiles, containing the *ccrAB2* complex. This group strongly increased from 2004 (8.9%) to 2006 (20.9%) (Fig. 1). Generally, they had high oxacillin MICs and were resistant to ciprofloxacin and erythromycin. Five other isolates amplified multiplex PCR profiles identical or similar to that of type II, but were classified as containing variant SCC*mecs* on the basis of *ccr* typing. Two of these contained only *ccrAB1* and were considerably more multiresistant than the normal SCC*mec* type II isolates, and one contained the *tst* gene. The other three had multiple *ccr* complexes: ZH299 contained both *ccrAB2* and *ccrAB1*, was PCR-positive for *pvl*, and had no additional antibiotic resistances; ZH293 had *ccrAB2* as well as *ccrAB4* and carried *tst*; the third strain (ZH152) contained both *ccrAB1* and *ccrAB4*.

SCC*mec* type III

SCC*mec* type III is a composite element containing both *ccrAB3* and *ccrC* complexes. Only a small proportion of isolates contained SCC*mec* type III, which further declined from 2004 (12.5%) to 2006 (4.6%) (Fig. 1). SCC*mec*-type III MRSA were by far the most multiresistant, with resistances to at least six of the nine antibiotic classes tested. Two isolates had SCC*mec* type III-like multiplex PCR profiles, except that one from 2004 (ZH143) lacked the SCC*mec* type III J3-specific band and another from 2006 (ZH269) lacked the SCC*mec* type III J1-specific band. Both isolates amplified only *ccrC* and one was a carrier of *pvl*. Unlike the classical SCC*mec* type III isolates, which had an oxacillin MIC >256 μg/ml, these two were low-level resistant strains with oxacillin MICs of 3 and 6 μg/ml, respectively. These two isolates were also much less multiresistant and have therefore been grouped among those carrying variant SCC*mec*s.

SCC*mec* type IV

The largest proportion of MRSA isolates contained SCC*mec* type IV, with 18 isolates in 2004 (32%) and 19 in 2006 (44%) (Fig. 1). The oxacillin MICs in this group of strains ranged from 1 to over 256 μg/ml. Eight out of the 37 type IV MRSA were *pvl* positive. Two of these isolates also carried an ACME and were closely related to strain USA300 as they had the same SmaI PFGE pattern. A third type IV SCC*mec* strain with a different PFGE pattern also carried ACME, but was *pvl* negative. Type IV MRSA generally had few additional resistances to other antibiotics. The most common resistances found, in decreasing order, were erythromycin in 22 (59.4%), tetracycline in 11 (29.7%), ciprofloxacin in 14 (37.8%), and fusidic acid in five (13.5%) strains. Three additional strains had SCC*mec* type IV multiplex PCR profiles, but were segregated from this group due to their *ccr* type. One isolate (ZH273) appeared to contain SCC*mec* type VI on the basis of amplifying a *ccrAB4* complex in addition to the *mecA* and *dcs* bands, common to both types IV and VI [16]. This strain was significantly more multiresistant than SCC*mec* type IV strains. Another isolate (ZH305) contained both *ccrAB2* and *ccrAB4* and was resistant to four of the ten antibiotics tested. The third (ZH298) contained a *ccrAB1* complex, had an exceptionally low oxacillin MIC of 0.38 μg/ml, and its only additional resistance was to fusidic acid. Both ZH305 and ZH298 were grouped with the variant SCC*mec* types.

Drug clones

Twenty one isolates that amplified only the *mecA* band by SCC*mec* typing were confirmed by drug clone specific *ccrAB* PCR [7] to carry the drug clone SCC*mec* and were shown to produce the characteristic SmaI pattern of the original type clone CH482 by PFGE [7, 20], which belongs to ST45 and has the *spa* type t065. All drug clones were also trimethoprim and fusidic acid resistant. All except six were ciprofloxacin resistant. Their oxacillin resistance levels were low, between 1.5 and 6 μg/ml, and none had acquired additional resistances, suggesting that the drug clone maintained all its characteristics and showed no further evolution to multiresistance or higher oxacillin resistance. The number of drug clone MRSA isolates detected was drastically reduced from 17 isolates in 2004 (30.3%) to only four in 2006 (9.3%) (Fig. 1).

Conclusion

The University Hospital of Zurich has a very low prevalence of MRSA compared to many other worldwide healthcare facilities. Periodic surveillance of MRSA, based on SCC*mec* and *ccr* typing, was performed to follow the evolution of MRSA diversity between 2003 and 2006. During this period, numbers of MRSA carrying typical hospital acquired SCC*mec* type I, II, and III elements appeared to have remained relatively stable (28.5% in 2004 versus 32.4% in 2006). However, large fluctuations within this group included an increase of more than twofold in SCC*mec* type II isolates and a drastic reduction of type III isolates. SCC*mec* type IV-containing isolates have remained the most prevalent, making up 32.1% and 44.2% of all isolates in 2004 and 2006, respectively. The drug clone, which was very prominent in 2003, seems to be on the decline and does not seem to have acquired any additional resistances, nor spread into the hospital. As a long-term consequence of the disruption of the drug scene in 1995, the locally restricted drug clone is dying out, or has possibly moved to other locations that are not in the catchment area of the University Hospital. The frequency of drug addicts hospitalized at the University Hospital has also declined in recent years.

Ten strains appeared to contain variant SCC*mec* types. Many of these were similar to defined SCC*mec*s but appeared to have undergone deletions, rearrangements, or recombinations, leading to losses of *ccr* loci or to the accumulation of multiple *ccr*'s. Generally, strains carrying these variant SCC*mec*s were also divergent in their resistance patterns, oxacillin MIC, and bearing mobile virulence factors such as *pvl*, *tst*, or *arcA*, compared to the corresponding SCC*mec* type they were most similar to. The observation that almost 10% of the analysed MRSA have undergone SCC*mec* rearrangements suggests that this chromosomal region is very active. PFGE analysis showed significant genetic diversity amongst the isolates with

variant SCC*mec* profiles (Supplementary Fig. 2), indicating that the recombination events leading to the evolution of new SCC*mec* structures had not occurred exclusively or preferentially in any specific clonal background.

References

1. Berger-Bächi B, Rohrer S (2002) Factors influencing methicillin resistance in staphylococci. Arch Microbiol 178:165–171. doi:10.1007/s00203–002–0436–0
2. Charlebois ED, Perdreau-Remington F, Kreiswirth BN, Bangsberg DR, Ciccarone D, Diep BA, Ng VL, Chansky K, Edlin B, Chambers HF (2004) Origins of community strains of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 39:47–54. doi:10.1086/421090
3. Chongtrakool P, Ito T, Ma X, Kondo Y, Trakulsomboon S, Tiensasitorn C, Jamklang M, Chavalit T, Song J, Hiramatsu K (2006) Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: A proposal for a new nomenclature for SCC*mec* elements. Antimicrob Agents Chemother 50:1001–1012. doi:10.1128/AAC.50.3.1001–1012.2006
4. CLSI, Institute CaLS (2007) Performance standards for antimicrobial susceptibility testing. 17th informational supplement. Clinical Laboratory Standards Institute, Wayne, Pennsylvania, USA
5. Deresinski S (2005) Methicillin-resistant *Staphylococcus aureus*: an evolutionary, epidemiologic, and therapeutic odyssey. Clin Infect Dis 40:562–573. doi:10.1086/427701
6. Diep BA, Stone GG, Basuino L, Graber CJ, Miller A, des Etages S-A, Jones A, Palazzolo-Ballance AM, Perdreau-Remington F, Sensabaugh GF, DeLeo FR, Chambers HF (2008) The arginine catabolic mobile element and staphylococcal chromosomal cassette *mec* linkage: convergence of virulence and resistance in the USA300 clone of methicillin-resistant *Staphylococcus aureus*. J Infect Dis 197:1523–1530. doi:10.1086/587907
7. Ender M, Berger-Bächi B, McCallum N (2007) Variability in SCC*mec*$_{N1}$ spreading among injection drug users in Zurich, Switzerland. BMC Microbiol 7:62. doi:10.1186/1471–2180–7–62
8. Hiramatsu K, Katayama Y, Yuzawa H, Ito T (2002) Molecular genetics of methicillin-resistant *Staphylococcus aureus*. Int J Med Microbiol 292:67–74. doi:10.1078/1438–4221–00192
9. Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, Hiramatsu K (2001) Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 45:1323–1336. doi:10.1128/AAC.45.5.1323–1336.2001
10. Ito T, Kuwahara K, Hiramatsu K (2007) Staphylococcal cassette chromosome *mec*(SCC*mec*) analysis of MRSA. Methods Mol Biol 391:87–102
11. Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K (2003) Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. Drug Resist Updat 6:41–52. doi:10.1016/S1368–7646(03)00003–7
12. Jevons MP (1961) "Celbenein"-resistant staphylococci. BMJ 124:124–125
13. Katayama Y, Robinson DA, Enright MC, Chambers HF (2005) Genetic background affects stability of *mecA* in *Staphylococcus aureus*. J Clin Microbiol 43:2380–2383. doi:10.1128/JCM.43.5.2380–2383.2005
14. Kondo Y, Ito T, Ma X, Watanabe S, Kreiswirth B, Etienne J, Hiramatsu K (2006) Combination of multiplex PCRs for SCC*mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob Agents Chemother 51:264–274. doi:10.1128/AAC.00165–06
15. Liu C, Graber CJ, Karr M, Diep BA, Basuino L, Schwartz BS, Enright MC, O'Hanlon JC, Thomas SJ, Perdreau-Remington F, Gordon S, Gunthorpe H, Jacobs R, Jensen P, Leoung G, Rumack JS, Chambers HF (2008) A population-based study of the incidence and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* disease in San Francisco, 2004–2005. Clin Infect Dis 46:1637–1646. doi:10.1086/587893
16. Milheiriço C, Oliveira DC, de Lencastre H (2007) Update to the multiplex PCR strategy for assignment of mec element types in *Staphylococcus aureus*. Antimicrob Agents Chemother 51:3374–3377. doi:10.1128/AAC.00275–07
17. O'Brien FG, Pearman JW, Gracey M, Riley TV, Grubb WB (1999) Community strain of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. J Clin Microbiol 37:2858–2862
18. Okuma K, Iwakawa K, Turnidge JD, Grubb WB, Bell JM, O'Brien FG, Coombs GW, Pearman JW, Tenover FC, Kapi M, Tiensasitorn C, Ito T, Hiramatsu K (2002) Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J Clin Microbiol 40:4289–4294. doi:10.1128/JCM.40.11.4289–4294.2002
19. Oliveira D, de Lencastre H (2002) Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 46:2155–2161. doi:10.1128/AAC.46.7.2155–2161.2002
20. Qi W, Ender M, O'Brien F, Imhof A, Ruef C, McCallum N, Berger-Bächi B (2005) Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Zurich, Switzerland (2003): prevalence of type IV SCC*mec* and a new SCC*mec* element associated with isolates from intravenous drug users. J Clin Microbiol 43:5164–5170. doi:10.1128/JCM.43.10.5164–5170.2005
21. Robinson DA, Enright MC (2003) Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 47:3926–3934. doi:10.1128/AAC.47.12.3926–3934.2003
22. Robinson DA, Enright MC (2004) Multilocus sequence typing and the evolution of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 10:92–97. doi:10.1111/j.1469–0691.2004.00768.x
23. Saïd-Salim B, Mathema B, Kreiswirth BN (2003) Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging pathogen. Infect Control Hosp Epidemiol 24:451–455. doi:10.1086/502231
24. Wannet WJB, Spalburg E, Heck MEOC, Pluister GN, Tiemersma E, Willems RJL, Huijsdens XW, de Neeling AJ, Etienne J (2005) Emergence of virulent methicillin-resistant *Staphylococcus aureus* strains carrying Panton-Valentine leucocidin genes in The Netherlands. J Clin Microbiol 43:3341–3345. doi:10.1128/JCM.43.7.3341–3345.2005