

Mosaic Staphylococcal Cassette Chromosome *mec* Containing Two Recombinase Loci and a New *mec* Complex, B2[∇]

R. Heusser, M. Ender, B. Berger-Bächli, and N. McCallum*

Institute of Medical Microbiology, University of Zurich, Gloriastr. 32, 8006 Zurich, Switzerland

Received 17 July 2006/Returned for modification 12 October 2006/Accepted 27 October 2006

A novel staphylococcal cassette chromosome (SCC) *mec* from a clinical methicillin-resistant *Staphylococcus aureus* isolate (ST100/CC5) had a mosaic structure, composed of SCC DNA from several different backgrounds. It harbored two complete *ccr* loci and a new variant of *mec* complex B, with Δ *mecRI* interrupted by the aminoglycoside resistance transposon Tn4001.

Methicillin resistance in *Staphylococcus aureus* (MRSA) is facilitated by the acquisition of the staphylococcal cassette chromosome *mec* (SCC*mec*), which integrates site specifically into the staphylococcal genome and carries *mecA*, encoding the alternative penicillin-binding protein PBP2a, a β -lactam-insensitive transpeptidase (6, 11, 13, 22, 26). The precise excision and site- as well as orientation-specific integration of this element depend on the action of cassette chromosome recombinase genes (*ccr*'s) located within the element (13).

Five main types of SCC*mec* have been described so far, each differing in size and composition and characterized according to its type of *ccr* locus and *mec* complex (3, 7, 25). *mec* complexes differ in the extents of insertion sequence (IS)-mediated deletions in the *mecA* regulatory genes *mecRI* and *mecI* and the presence and location of insertion sequence IS431, IS1182, or IS1272 (23). Apart from the *ccr* and *mec* complexes, and some common mobile resistance elements, SCC*mec* subtypes harbor variable J (junkyard) regions containing truncated and nonessential genes and genes of unknown functions (8). In addition to the major types, a number of new SCC elements, including non-*mecA*-carrying cassettes, have recently been discovered (4, 5, 9, 14, 15, 17, 19).

An epidemiological study of methicillin-resistant staphylococci from Zurich in 2003 identified several strains which contained multiple *ccr* loci (21). Here, we describe the SCC*mec* of one of these isolates, MRSA_{ZH47}.

MRSA_{ZH47} is of multilocus sequence type 100 and belongs to clonal complex 5, a genotype previously identified in Argentina (1, 24). In addition to its β -lactam resistance, it was resistant to aminoglycosides and carried a *blaZ*-encoded penicillinase. Its SCC*mec* type could not be determined by standard multiplex PCR (20), and additional *ccr* typing indicated that it contained both *ccr2* and *ccrC* loci (21).

Southern hybridization of SmaI-digested chromosomal DNA, separated by pulsed-field gel electrophoresis, showed that *mecA*- and *ccr2*-hybridizing sequences were collocated on a separate SmaI fragment from the *ccrC*-hybridizing sequence (data not shown).

Transient overexpression of *ccrAB2*, facilitating the precise excision of all major SCC*mec* types (10, 12, 13, 18), was used to cure MRSA_{ZH47}. Southern hybridization showed that the resulting oxacillin-susceptible clone MRSA_{ZH47c} had lost *mecA* and both *ccr* loci, indicating that all three were present on a single excisable SCC element containing an internal SmaI restriction site (data not shown). The susceptibility profile of MRSA_{ZH47c} showed that aminoglycoside resistance had also been lost.

A cosmid library of MRSA_{ZH47} DNA, consisting of over 600 clones with estimated inserts of about 45 kb, was constructed using a SuperCos1 cosmid vector kit (Stratagene, La Jolla, CA). Screening of the library by colony blot analysis using *ccr2*- or *ccrC*-specific probes identified 11 clones that hybridized to *ccr2*, 7 that hybridized to *ccrC*, and 2 that hybridized to both probes. Cosmids were end sequenced and the sequences compared to the genome sequence of *S. aureus* Mu50, revealing that the two cosmids hybridizing with both probes each contained one end of the SCC*mec* element and together completely covered it (data not shown).

Primers specific for known *orfX*, *ccrC2*, IS431, and *mecA* nucleotide sequences were used to synthesize long-range PCR products that were subcloned into either pUC19 or pBluescript SK(+). Inserts were end sequenced and the obtained sequences assembled. The double-stranded nucleotide sequence of the 33.7-kb element was completed by primer walking.

This SCC*mec* proved to be unique, containing elements and properties not previously described. GeneMark.hmm (16) and BLASTX (2) identified 33 open reading frames (ORFs), all of which were identical or highly similar in sequence to previously annotated staphylococcal genes (Table 1 and Fig. 1).

The *orfX* insertion site and the characteristic terminal inverted and direct repeats, generated upon insertion, were almost identical to those of other, previously described SCC*mec* elements (12). However, the left-end (proximal) direct repeat sequence contained a nucleotide transition of an adenine to guanine (Table 1), which has not been found elsewhere and increased the identity between the junctional direct repeats. This mutation did not impede the excision of the element, as demonstrated by the precise curing of SCC*mec*_{ZH47} from the chromosome of MRSA_{ZH47}. It could, however, possibly influence the stability or transfer frequency of the element.

SCC*mec*_{ZH47} contained a new *mec* complex that we have

* Corresponding author. Mailing address: Institute of Medical Microbiology, University of Zurich, 8006 Zurich, Switzerland. Phone: 41 44 634 2694. Fax: 41 44 634 4906. E-mail: mcallum@immv.unizh.ch.

[∇] Published ahead of print on 6 November 2006.

TABLE 1. ORFs encoded on SCCmec_{ZH47}

Coding sequence no.	Name ^a	Position (bp)	Identity (%) ^b	Homolog(s) ^c	Information ^d
	DR-L	754–771			Integration site sequence of SCCmec, A→G substitution at position 16 of left direct repeat
1	IR-L ZH02	757–764 1017–1322	98	CZ078 (85/2082)	Integration site sequence of SCCmec; left inverted repeat Hypothetical protein, predicted restriction endonuclease domain (COG 3183)
2	ZH03	1887–2384	100	CZ077 (85/2082)	Conserved hypothetical protein (COG 3680)
3	ZH04	2468–3967	100	CZ076 (85/2082)	Hypothetical protein
4	ZH05	4193–5293	100	CZ075 (85/2082)	Hypothetical protein; DNA polymerase A family domain (Pfam 00476.12)
5	ZH06	5286–5657	94	CZ074 (85/2082)	Hypothetical protein (DUF 1092)
6	ZH07	5654–7273	100	3 half: CG008 (85/3907) 100 5 half: unnamed ORF (TSGH17)	Hypothetical protein; POX_D5 domain associated with viral DNA replication (Pfam 03288.11)
7	<i>ccrC</i>	7498–9174	94	<i>ccrC2</i> (TSGH17) 89 <i>ccrC3</i> (85/2082)	Cassette chromosome recombinase C
8	ZH09	9280–9618	98	SSP0034 (ATCC 15305)	Hypothetical protein
9	ZH10	9714–10025	90	SSP0032 (ATCC 15305)	Hypothetical protein, contains SmaI restriction site
10	ZH11	10041–10547	90	CZ068 (85/2082)	Conserved in gram-positive and -negative bacteria but of unknown function (COG 4333)
11	IS431	10696–11370	99	IS431 (SCCmec types I–IV)	Insertion sequence IS431
12	ZH13	11628–11795	100	ORF CN041 (N315) and in SCCmec types I–V	Putative HMG-coenzyme A-synthase (cholesterol biosynthesis)
13	<i>ugpQ</i>	12712–13455	100	<i>ugpQ</i> (all SCCmec types)	Glycerophosphoryl diester phosphodiesterase
14	ZH15	13552–13980	100	SA0037 (N315) and in SCCmec types I–V	Hypothetical protein, MaoC-like domain (Pfam 01575.11)
15	<i>mecA</i>	14026–16032	100	WIS (SCCmec type V) and TSGH17 (SCCmec type V _T)	Penicillin-binding protein PBP2a
16	Δ <i>mecRI</i> '	16132–16958	100	Δ <i>mecRI</i> from <i>mec</i> complex B	First 826 bp of Δ <i>mecRI</i> , truncated signal transducer MecR1 from <i>mec</i> complex B
	DR _{Tn4001}	16951–16958			Repeated region generated by transposon integration
	IR _{Tn4001}	16959–17059			Repeated region generated by transposon integration
17	IS256L	17060–18232	100	Tn4001	IS of Tn4001
18	<i>aac</i>	18235–18681	100	Tn4001	Putative <i>N</i> -acyltransferase, GNAT family
19	<i>aac</i> (2')– <i>aph</i> (6'')	18682–20121	100	Tn4001	Aminoglycoside-(2')-acetyltransferase-aminoglycoside-(6'')-phosphotransferase
20	IS256R	20251–21423	100	Tn4001	IS of Tn4001
	IR _{Tn4001}	21424–21524			Repeated region generated by transposon integration
	DR _{Tn4001}	21525–21532			Repeated region generated by transposon integration
21	Δ <i>mecRI</i> '	21533–21692	100	Δ <i>mecRI</i> from <i>mec</i> complex B	Remainder of Δ <i>mecRI</i> , interrupted by Tn4001 insertion
22	ZH22	21595–21924	100	Δ <i>hsdR</i> (MW2)	Truncated hypothetical protein, similar to type I restriction endonuclease
23	ZH23	21915–23438	100	IS1272 transposase from <i>mec</i> complex B	aa 1–102: transposase and inactivated derivatives (COG 3666) aa 185–459: transposase DDE domain
24	ZH24	23574–24083	100	Various type IV SCCmecs	Hypothetical protein
25	ZH25	24095–24406	100	Various type IV SCCmecs	Hypothetical protein
26	ZH26	24493–24843	100	Various type IV SCCmecs	Hypothetical protein
27	<i>ccr2B</i>	25365–26993	100	<i>ccrB</i> SCCmec type IVd (JCSC4469)	Cassette chromosome recombinase B
28	<i>ccr2A</i>	27015–28364	100	<i>ccrA</i> SCCmec type IVe (AR43)	Cassette chromosome recombinase A
29	ZH29	28598–30391	100	R004 (MR108)	aa 1–382: superfamily II helicase and inactivated derivatives (COG 5519)
30	ZH30	30391–30687	100	Different type IV SCCmecs	Hypothetical protein
31	ZH31	30880–31926	100	Different type IV SCCmecs (ATCC 12228)	Hypothetical protein
32	ZH32	32381–33535	100	CR008 (MR108)	aa 1–122: <i>abi</i> alpha protein, predicted transcriptional regulator (COG 2865)
33	ZH33	33565–34365	100	Different type IV SCCmecs and SE0042 (ATCC 12228)	Hypothetical protein, abortive phage resistance protein
	IR-R	34423–34430			Integration site sequence of SCCmec; right inverted repeat
	DR-R	34432–34449			Integration site sequence of SCCmec; right direct repeat

^a ORFs of known functions are named accordingly; putative and hypothetical ORFs have been assigned ZH numbers.

^b Amino acid sequence identity.

^c The name of the homologous gene is indicated, and the strain in which it was found is indicated in parentheses. If homologous ORFs were unnamed, the SCCmec type in which they were found is indicated.

^d Information about the element, e.g., about the encoded protein. Positions and reference numbers of known protein domains are indicated. aa, amino acids; HMG, 3-hydroxy-3-methylglutaryl; COG, cluster of orthologous groups of proteins.

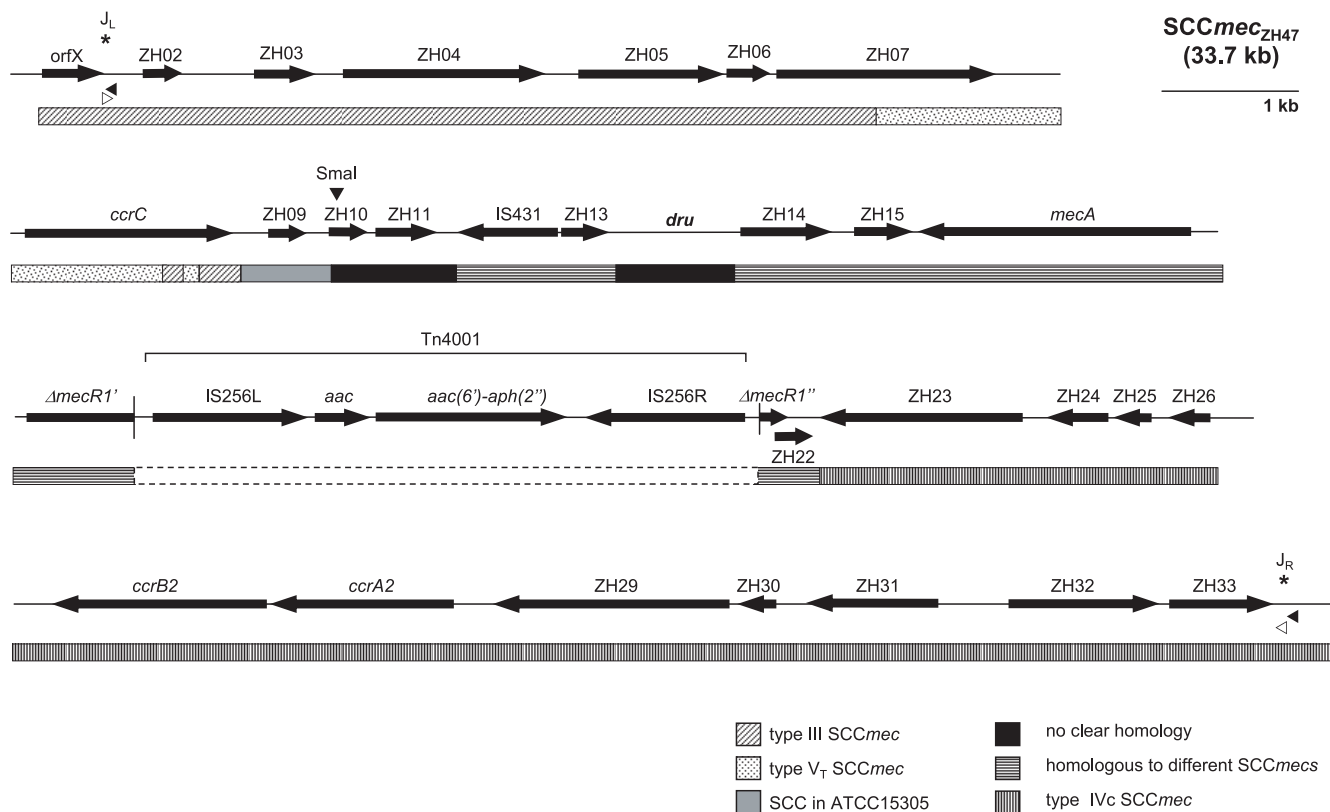


FIG. 1. Genetic map of the mosaic SCCmec element from MRSA_{ZH47}. Open reading frames are symbolized by arrows, and the bars below them indicate homologies to previously described SCC elements. Integration site sequences for SCCmec (*), direct repeats of chromosomal junctions (◄), and inverted repeats (▷) are indicated. J_L denotes the junction proximal and J_R the junction distal to the origin of replication.

named B2 because of its similarity to *mec* complex B. The new B2 complex differed in that the 987-bp Δ *mecRI* fragment was interrupted by insertion of the aminoglycoside resistance transposon Tn4001 at base pair position 820 (Fig. 1), and while the *mecA* promoter region was identical to that of *mec* complex B, the *mecA* gene sequence was identical to that of SCCmec types V and V_T.

In addition to a *ccrAB2* locus at the usual position downstream of *mecA*, the element possessed a *ccrC* locus between *orfX* and the *dru* element (Fig. 1). The *ccrA2* sequence was identical to that of SCCmec type IVe, while the *ccrB2* sequence was identical to that of SCCmec type IVd. Comparison of the *ccrC* sequence to published variants revealed high levels of similarity to the *ccrC2* and *ccrC3* sequences of SCCmec types V_T and III, respectively (Table 1).

The element as a whole appeared mosaic in structure. The presence of both a *ccrAB2* locus and a variant *ccrC* locus and of regions with strong similarity to several different SCC elements, including the typical hospital-acquired MRSA type III SCCmec, the community-associated MRSA SCCmec types IV and V_T, and SCCmec from the non-*S. aureus* species *Staphylococcus saprophyticus*, suggests that SCCmec_{ZH47} had been assembled via several recombination events (Fig. 1).

Most of the new SCC and SCCmec elements recently discovered, including the SCCmec_{ZH47} described here, appear to have acquired regions from other SCC elements, suggesting

that significant intra- and interspecies exchange and recombination of SCC DNA occurs.

Nucleotide sequence accession number. The nucleotide sequence newly determined in this study was deposited in the EMBL database under accession number AM292304.

This study was supported by Swiss National Science Foundation grant NF31-105390/1.

REFERENCES

- Aires de Sousa, M., and H. de Lencastre. 2003. Evolution of sporadic isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals and their similarities to isolates of community-acquired MRSA. *J. Clin. Microbiol.* **41**:3806–3815.
- Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**:3389–3402.
- Barberis-Maino, L., B. Berger-Bächli, H. Weber, W. D. Beck, and F. H. Kayser. 1987. IS431, a staphylococcal insertion sequence-like element related to IS26 from *Proteus vulgaris*. *Gene* **59**:107–113.
- Boyle-Vavra, S., B. Ereshefsky, C. C. Wang, and R. S. Daum. 2005. Successful multiresistant community-associated methicillin-resistant *Staphylococcus aureus* lineage from Taipei, Taiwan, that carries either the novel staphylococcal chromosome cassette *mec* (SCCmec) type V_T or SCCmec type IV. *J. Clin. Microbiol.* **43**:4719–4730.
- Chongtrakool, P., T. Ito, X. X. Ma, Y. Kondo, S. Trakulsomboon, C. Tiensasitorn, M. Jamklang, T. Chavalit, J. H. Song, and K. Hiramatsu. 2006. Staphylococcal cassette chromosome *mec* (SCCmec) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCCmec elements. *Antimicrob. Agents Chemother.* **50**:1001–1012.
- Hartmann, B. J., and A. Tomasz. 1984. Low-affinity penicillin-binding pro-

- tein associated with β -lactam resistance in *Staphylococcus aureus*. *J. Bacteriol.* **158**:513–516.
7. Hiramatsu, K., K. Asada, E. Suzuki, K. Okonogi, and T. Yokota. 1992. Molecular cloning and nucleotide sequence determination of the regulator region of *mecA* gene in methicillin-resistant *Staphylococcus aureus* (MRSA). *FEBS Lett.* **298**:133–136.
 8. Hiramatsu, K., Y. Katayama, H. Yuzawa, and T. Ito. 2002. Molecular genetics of methicillin-resistant *Staphylococcus aureus*. *Int. J. Med. Microbiol.* **292**:67–74.
 9. Holden, M. T., E. J. Feil, J. A. Lindsay, S. J. Peacock, N. P. Day, M. C. Enright, T. J. Foster, C. E. Moore, L. Hurst, R. Atkin, A. Barron, N. Bason, S. D. Bentley, C. Chillingworth, T. Chillingworth, C. Churcher, L. Clark, C. Corton, A. Cronin, J. Doggett, L. Dowd, T. Feltwell, Z. Hance, B. Harris, H. Hauser, S. Holroyd, K. Jagels, K. D. James, N. Lennard, A. Line, R. Mayes, S. Moule, K. Mungall, D. Ormond, M. A. Quail, E. Rabinowitsch, K. Rutherford, M. Sanders, S. Sharp, M. Simmonds, K. Stevens, S. Whitehead, B. G. Barrell, B. G. Spratt, and J. Parkhill. 2004. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. *Proc. Natl. Acad. Sci. USA* **101**:9786–9791.
 10. Ito, T., Y. Katayama, K. Asada, N. Mori, K. Tsutsumimoto, C. Tiensasitorn, and K. Hiramatsu. 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.
 11. Ito, T., Y. Katayama, and K. Hiramatsu. 1999. Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. *Antimicrob. Agents Chemother.* **43**:1449–1458.
 12. Ito, T., X. X. Ma, F. Takeuchi, K. Okuma, H. Yuzawa, and K. Hiramatsu. 2004. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. *Antimicrob. Agents Chemother.* **48**:2637–2651.
 13. Katayama, Y., T. Ito, and K. Hiramatsu. 2000. A new class of genetic element, staphylococcal cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **44**:1549–1555.
 14. Katayama, Y., F. Takeuchi, T. Ito, X. X. Ma, Y. Ui-Mizutani, I. Kobayashi, and K. Hiramatsu. 2003. Identification in methicillin-susceptible *Staphylococcus hominis* of an active primordial mobile genetic element for the staphylococcal cassette chromosome *mec* of methicillin-resistant *Staphylococcus aureus*. *J. Bacteriol.* **185**:2711–2722.
 15. Kuroda, M., A. Yamashita, H. Hirakawa, M. Kumano, K. Morikawa, M. Higashide, A. Maruyama, Y. Inose, K. Matoba, H. Toh, S. Kuhara, M. Hattori, and T. Ohta. 2005. Whole genome sequence of *Staphylococcus saprophyticus* reveals the pathogenesis of uncomplicated urinary tract infection. *Proc. Natl. Acad. Sci. USA* **102**:13272–13277.
 16. Lukashin, A., and M. Borodovsky. 1998. GeneMark.hmm: new solutions for gene finding. *Nucleic Acids Res.* **26**:1107–1115.
 17. Luong, T. T., S. Ouyang, K. Bush, and C. Y. Lee. 2002. Type 1 capsule genes of *Staphylococcus aureus* are carried in a staphylococcal cassette chromosome genetic element. *J. Bacteriol.* **184**:3623–3629.
 18. Ma, X. X., T. Ito, C. Tiensasitorn, M. Jamklang, P. Chongtrakool, S. Boyle-Vavra, R. S. Daum, and K. Hiramatsu. 2002. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strain. *Antimicrob. Agents Chemother.* **46**:1147–1152.
 19. Mongkolrattanothai, K., S. Boyle-Vavra, T. V. Murphy, and R. S. Daum. 2004. Novel non-*mecA*-containing staphylococcal chromosomal cassette composite island containing *pbp4* and *tagF* genes in a commensal staphylococcal species: a possible reservoir for antibiotic resistance islands in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **48**:1823–1836.
 20. Oliveira, D. C., and H. de Lencastre. 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.
 21. Qi, W., M. Ender, F. G. O'Brien, A. Imhof, C. Ruef, N. McCallum, and B. Berger-Bächi. 2005. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Zürich, 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.
 22. Reynolds, P. E., and D. F. Brown. 1985. Penicillin-binding proteins of β -lactam-resistant strains of *Staphylococcus aureus*. Effect of growth conditions. *FEBS Lett.* **11**:28–32.
 23. Shore, A., A. S. Rossney, C. T. Keane, M. C. Enright, and D. C. Coleman. 2005. Seven novel variants of the staphylococcal chromosomal cassette *mec* in methicillin-resistant *Staphylococcus aureus* isolates from Ireland. *Antimicrob. Agents Chemother.* **49**:2070–2083.
 24. Sola, C., P. Cortes, H. A. Saka, Cordoba MRSA Collaborative Study Group, A. Vindel, and J. L. Bocco. 2006. Evolution and molecular characterization of methicillin-resistant *Staphylococcus aureus* epidemic and sporadic clones in Cordoba, Argentina. *J. Clin. Microbiol.* **44**:192–200.
 25. Tesch, W., C. Ryffel, A. Strässle, F. H. Kayser, and B. Berger-Bächi. 1990. Evidence of a novel staphylococcal *mec*-encoded element (*mecR*) controlling expression of penicillin-binding protein 2'. *Antimicrob. Agents Chemother.* **34**:1703–1706.
 26. Utsui, Y., and T. Yokota. 1985. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **28**:397–403.