J Antimicrob Chemother 2015; **70**: 1298–1302
doi:10.1093/jac/dku531 Advance Access publication 28 January 2015

Journal of Antimicrobial Chemotherapy

Genome-wide analysis reveals two novel mosaic regions containing an ACME with an identical DNA sequence in the MRSA ST398-t011 and MSSA ST8-t008 isolates

Artur J. Sabat[1], Weronika M. Ilczyszyn[2], Miranda van Rijen[3], Viktoria Akkerboom[1], Bhanu Sinha[1], Jan Kluytmans[3], Jacek Miedzobrodzki[2], Hajo Grundmann[1] and Alexander W. Friedrich[1]*

[1]*Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands;* [2]*Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland;* [3]*Laboratory for Microbiology and Infection Control, Amphia Hospital, Breda, The Netherlands*

*Corresponding author. Department of Medical Microbiology, University Medical Center Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands. Tel: +31-50-361-3480; Fax: +31-50-361-9105; E-mail: alex.friedrich@umcg.nl

Received 4 September 2014; returned 6 October 2014; revised 14 November 2014; accepted 1 December 2014

Objectives: The presence of the arginine catabolic mobile element (ACME) in *Staphylococcus aureus* has been reported to enhance the colonization of the human host. The aim of this study was to determine the genetic organization of composite islands harbouring ACME.

Methods: Two ACME-positive *S. aureus* isolates obtained during two different surveys conducted in the Netherlands and Poland were characterized in this study. The isolates were analysed by *spa* typing, DNA microarrays and whole-genome sequencing.

Results: The two isolates harboured a truncated yet fully functional ACME type II with an identical nucleotide sequence, but differed in their adjacent mobile genetic elements. The first strain was a livestock-associated ST398-t011 MRSA, which had a staphylococcal cassette chromosome *mec* (SCC*mec*) composite island composed of SCC*pls* adjacent to *orfX* followed by ACME type II and SCC*mec* type IVa. The second ACME-positive isolate was an ST8-t008 MSSA. Its composite island showed an SCC-like element carrying the *ccrC* gene followed by ACME II.

Conclusions: This is the first report of an ACME in a livestock-associated MRSA ST398. It is also the first presentation of an ACME composite island structure in an MSSA isolate. Our findings indicate an extensive mosaicism of composite islands in *S. aureus*, which has implications for the transmissibility among humans and thus for public health.

Keywords: horizontal transfer, whole-genome sequencing, next-generation sequencing

Introduction

The success of the USA300 community-associated (CA) MRSA clone could partly be attributed to the presence of the arginine catabolic mobile element (ACME).[1] In USA300, the ACME is located downstream of the staphylococcal cassette chromosome *mec* (SCC*mec*) type IVa, creating the ACME-SCC*mec* composite island.[2] ACME enhances colonization of the skin and mucous membranes by *Staphylococcus aureus* by neutralizing the acidic pH from fatty acids, and improves the survival and growth of *S. aureus* by depleting L-arginine, which is the only substrate for the production of nitric oxide by macrophages.[1] A variant of USA300 ACME, called truncated ACME type II, has been detected in a small number of other MRSA lineages such as ST1-IVa, ST5-II, ST5-IV, ST5-V, ST22-IVa, ST22-IVh, ST59-IVa, ST97-V and ST239-III.[3–9] Recently, seven novel types of ACME-SCC*mec* composite island have been reported from MRSA strains with distinct genotypes.[3–7,10] In all isolates, the ACME-SCC*mec* elements were flanked by short direct repeat (DR) sequences, which differed mainly by point mutations.

The objective of this study was to determine the genetic organization of composite islands containing ACME using whole-genome sequencing (WGS).

Materials and methods

Bacterial isolates

Two *S. aureus* isolates, which were ACME-positive based on DNA microarray (Alere Technologies) profiles, were obtained during two different surveys conducted in the Netherlands and Poland. Isolate RI46 was recovered

Table 1. DNA microarray hybridization profiles of isolates assigned to *spa* t008/MLST CC8:ST8 (isolate PL69) and *spa* t011/MLST CC8:ST398 (isolate RI46)

Genetic location or gene class	Gene(s)	PL69 result	RI46 result
SCC/SCC*mec*	*mecA*, *mecR*-truncated, *ccrA2*, *ccrB2*, *ugpQ*, *dcs*	negative	positive
	ccrC, *xylR*	positive	negative
	mecR, *mecI*, *ccrA1*, *ccrA3*, *ccrAA*-MRSAZH47, *ccrA4*, *ccrB1*, *ccrB3*, *ccrB4*, *merA*, *merB*, *kdpA*, *kdpB*, *kdpC*, *kdpD*, *kdpE*	negative	negative
ACME	*arcA*, *arcB*, *arcC*, *arcD*	positive	positive
Resistance	*blaZ*, *blaI*, *blaR*	positive	positive
	mecA, *aacA-aphD*, *tetM*	negative	positive
	ermC, *fosB*	positive	negative
	aadD, *aphA*, *cat*, *ermA*, *ermB*, *cfr*, *dfrA*, *far*, *fexA*, *linA*, *msrA*, *mefA*, mercury resistance locus, *mpbBM*, *mupR*, Q6GD50, *qacA*, *qacC*, *sat*, *tetK*, *vanA*, *vanB*, *vanZ*, *vatA*, *vatB*, *vga*, *vgb*	negative	negative
Virulence—enterotoxins	*entD*, *entJ*, *entR*	positive	negative
	entA, *entB*, *entC*, *entE*, *entG*, *entH*, *entI*, *entK*, *entL*, *entM*, *entN*, *entO*, *entQ*, *entU*, *tst1*	negative	negative
Virulence—leucocidins	*lukD*, *lukE*	positive	negative
	lukF-PV, *lukS-PV*	negative	negative
Virulence—haemolysins	*hla*, *hld*	positive	positive
	undisrupted *hlb*	negative	positive
Virulence—immune evasion	*sak*, *scn*	positive	negative
	chp	negative	negative
Virulence—exfoliative toxins	*etA*, *etb*, *etD*	negative	negative
Virulence—epidermal cell differentiation inhibitors	*edinA*, *edinB*, *edinC*	negative	negative
Virulence—proteases	*aur*, *sspA*, *sspB*, *sspP*	positive	positive
	splA, *splB*, *splE*	positive	negative
MSCRAMMs/adhesion factors	*bbp*, *clfA*, *clfB*, *ebh*, *eno*, *fib*, *ebpS*, *fnbA*, *fnbB*, *map*, *sdrC*, *sdrD*, *vwb*	positive	positive
	cna	negative	positive
	sasG	positive	negative

from the wound of a patient in the Netherlands in 2011 while isolate PL69 was obtained from the nose of an asymptomatic carrier in Poland in 2005.

Extraction of genomic DNA

Total DNA was extracted by enzymatic lysis using the buffers and solutions provided with the StaphyType DNA microarray kit (Alere Technologies GmbH, Jena, Germany) and the Qiagen DNeasy blood and tissue kit according to the manufacturers' instructions. To obtain an accurate quantification of the extracted genomic DNA for WGS, a fluorometric method specific for duplex DNA, a Qubit dsDNA BR assay kit and a Qubit fluorimeter (Life Technologies, The Netherlands) were used according to the manufacturer's instructions.

spa typing

The procedure was conducted as previously described.[11] The *spa* types were assigned using Ridom StaphType software version 1.4.6 (Ridom GmbH, Würzburg, Germany) and the Ridom SpaServer (http://www.spaserver.ridom.de).[12]

DNA microarray typing

DNA microarray analysis was performed using the Arraymate system (Alere Technologies) and the StaphyType kit (Alere Technologies) according to the manufacturer's instructions. The Alere StaphyType DNA microarray kit for *S. aureus* contains probes allowing the detection of ~170 distinct genes, including the ACME-*arcABCD* genes and SCC*mec* and SCC genes.

WGS

Next-generation sequencing was carried out using the Illumina MiSeq system and Nextera XT kit for the preparation of DNA libraries. SeqMan NGen software (version 11.2.1; DNASTAR, Madison, WI, USA) was used for the *de novo* assembly of the reads. The resulting contigs were ordered by Mauve Contig Mover[13] relative to the reference genomes of strains LA-ST398 (S0385) (GenBank accession number NC_017333) and M1 (GenBank accession number HM030720) for isolates RI46 and PL69, respectively. End-to-end alignment of contigs to find any overlap between adjoining contigs was carried out using SeqMan Pro software (version 10.1.2, DNASTAR). Contigs containing ACME or SCC*mec*-related DNA sequences were identified by BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The remaining gaps between ACME- and SCC*mec*-related contigs were closed by PCR amplification and Sanger sequencing.

Bioinformatics

Automated genome annotation was achieved using the SeqMan NGen and BLASTn software followed by manual sequence annotation and

editing using SeqBuilder software (version 11.2.1, DNASTAR). The DNA sequences were aligned using BLASTn software. The MLST STs were assigned through the publicly available MLST server (www.cbs.dtu.dk/services/MLST) on the basis of WGS data.[14]

Nucleotide sequence accession numbers

The nucleotide sequences of the composite islands containing ACME for PL69 and RI46 have been deposited in GenBank under the accession numbers KM252872 and KM252873, respectively.

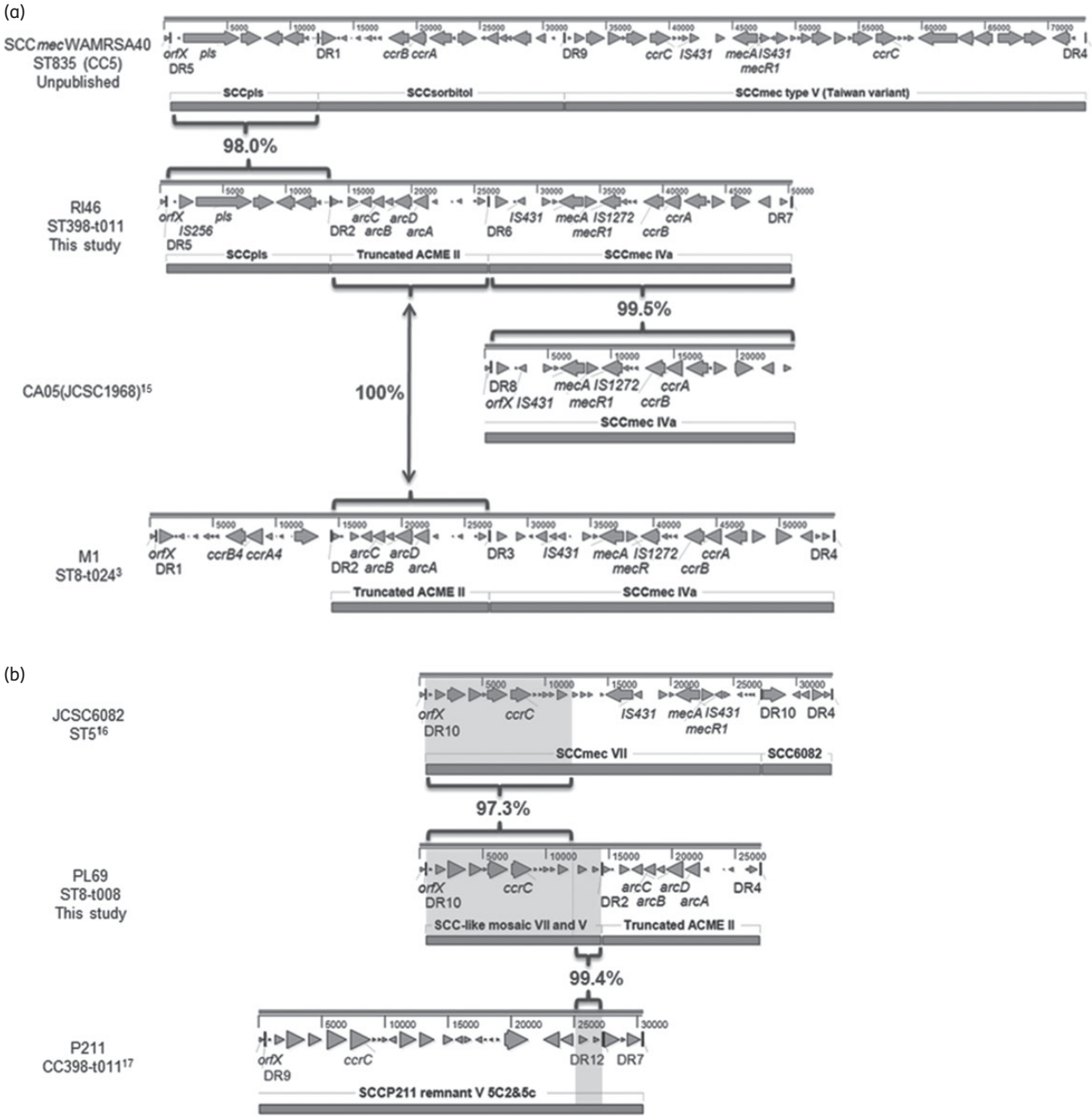

Figure 1. Structure comparison of the composite islands from *S. aureus* isolates: (a) SCC*mec*WAMRSA40 (GenBank accession number JQ746621), RI46 (GenBank accession number KM252873), CA05(JCSC1968) (GenBank accession number AB063172) and M1 (GenBank accession number HM030720); (b) JCSC6082 (GenBank accession number AB373032), PL69 (GenBank accession number KM252872) and P211 (GenBank accession number KF593810); and (c) CMFT3119 (GenBank accession number HF569105) and PL69. The numbers in the ruler are designated relative to the position of A in the ATG start codon of the *orfX* gene. The arrows indicate the genes. Only the following selected genes are annotated: rRNA methyltransferase (*orfX*) containing the SCC*mec* insertion site, the arginine deiminase pathway (*arcCBDA*), the SCC*mec* cassette recombinases (*ccrA*, *ccrB* and *ccrC*), the determinant encoding resistance to methicillin (*mecA*) and its regulatory gene (*mecR1*), the transposases of IS*256*, IS*431* and IS*1272*, and plasmin-sensitive surface (*pls*) protein. The vertical bars indicate DRs. Sequences of DRs: DR1, GAAGCATATCATAAGTGA; DR2, GAAGCGTATCATAAGTGA; DR3, GAAGCGTATCACAAATAA; DR4, GAGGCGTATCATAAGTAA; DR5, GAAGCGTACCACAAATAA; DR6, GAAGCATATCATAAATGA; DR7, GAGGCTTATCATAAATGA; DR8, GAAGCTTATCATAAGTAA; DR9, GAGGCTTATCATAAATAA; DR10, GAAGCGTATCATAAATAA; DR11, GAGGCGTATCATAAGTAG; and DR12, GAAGCATATCATAAATAA.

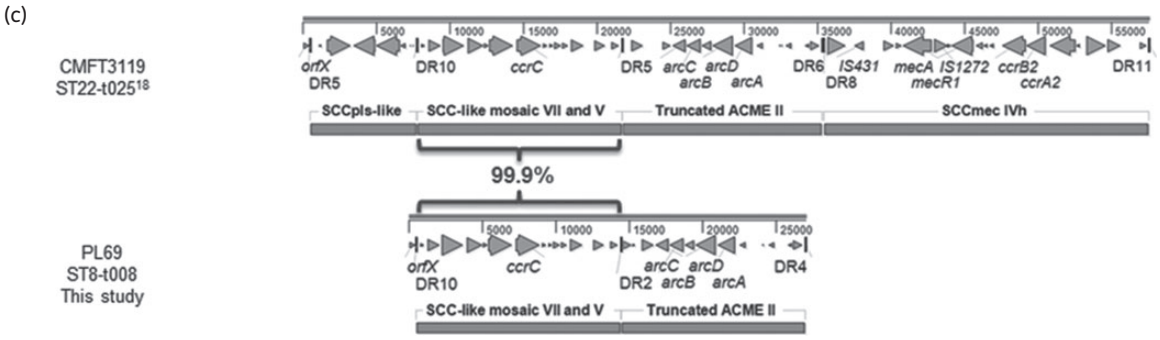

Figure 1. *Continued*

Results

ACME-SCCmec composite island in RI46

The ACME-positive isolate RI46 (Table 1) belonged to MLST ST398 and *spa* type t011, consistent with the most frequent lineage of livestock-associated MRSA in the Netherlands. To our knowledge, this is the first report of an ACME in MRSA ST398. Sequence analysis revealed a new organization of the ACME-SCC*mec* composite island in this isolate (Figure 1a). The ACME-SCC*mec* composite island was 49 796 bp in size. It was composed of the SCC*pls* region adjacent to *orfX*, followed by the truncated form of ACME type II and SCC*mec* type IVa. The SCC*pls* region corresponded to a truncated J1 region of SCC*mec* I, which has previously been found in the ACME-SCC*mec* composite island of MLST ST22[5] and in clonal complex (CC) 5.[6,7,10] SCC*pls* (truncated J1 SCC*mec* type I) had already been identified together with SCC*mec* type IVa in a composite island,[10] but has never been shown to be located upstream and separated by truncated ACME II as was determined for this isolate (Figure 1a). The SCC*pls* region in this isolate was 13 059 bp long and, among all the sequences deposited in the GenBank database, showed the highest similarity to a corresponding region in a CA MRSA isolate (of MLST ST835/CC5) from Western Australia (Figure 1a). The SCC*pls* regions in both isolates were highly conserved (98% identity) with the exception of the IS*256* element, which was present only in the ST398 isolate (Figure 1a). IS*256* was inserted 1023 bp downstream of the *orfX* gene. BLAST searches in the GenBank database showed that the truncated ACME II sequence of 12 545 bp in the ST398 isolate was identical to those in the MRSA isolates M1 (CC8:ST8-t024-IVa, GenBank accession number HM030720),[3] Sa0059 (CC8:ST239-III, GenBank accession number JQ412578),[4] R15 (CC8:ST8-t064-IVa, GenBank accession number KF184643),[10] R17 (CC8:ST8-t064-IVa, GenBank accession number KF184644),[10] R95 (CC5:ST5-t002-IVa, GenBank accession number KF184645)[10] and R99 (CC5:ST5-t002-IVa, GenBank accession number KF234240).[10] SCC*mec* IVa (24 120 bp) in the RI46 isolate showed the highest homology (99.5% identity) with MRSA isolate CA05(JCSC1968) (GenBank accession number AB063172)[15] (Figure 1a).

ACME-SCC composite island in PL69

The ACME-positive isolate PL69 (Table 1) belonged to ST8 and *spa* type t008. The microarray results showed that isolate PL69 was ACME-positive and SCC*mec*-negative, which is a rare finding in *S. aureus*. Moreover, the microarray hybridization profile showed a signal from the *ccrC* probe indicating the potential presence of an SCC*mec* remnant DNA in this MSSA isolate. The overall structure of the 26 539 bp region encompassing the ACME composite island from PL69 is illustrated in Figure 1(b and c). Sequence analysis revealed that the ACME in isolate PL69 was 12 545 bp in length and identical to that of RI46. It was bounded by DR2 and DR4 (Figure 1b and c). Between *orfX* and the ACME was a region of 13 940 bp flanked by DR10 and DR2 that carried a type 5 *ccr* gene complex, but lacked the *mec* complex, including the *mecA* gene (Figure 1b and c). The region was made up of two distinct parts (Figure 1b). Immediately downstream of the *orfX* locus was an SCC-like element composed of 11 882 bp carrying the *ccrC* gene. It showed the highest homology to the SCC*mec* type VII of MRSA isolate JCSC6082 (ST5, GenBank accession number AB373032)[16] (97.3% identity; Figure 1b). The consecutive region consisted of 2058 bp and had 99.4% homology with a corresponding region of the SCC*mec* remnant type V(5C2&5)c of the MSSA P211 isolate (CC398-t011, GenBank accession number KF593810)[17] (Figure 1b). Interestingly, the whole nucleotide sequence located between DR10 and DR2 was found to be almost intact (99.9% identity) in the corresponding region of the MRSA isolate CMFT3119 (CC22:ST22-t025-IVh, GenBank accession number HF569105)[18] (Figure 1c). The ACME of the CMFT3119 isolate was longer (13 594 bp) than that of PL69. Moreover, the first 2366 bp of ACME in the CMFT3119 isolate did not share the same degree of homology with PL69, while the downstream end (11 228 bp) was highly similar to that of PL69 (99.6% identity).

Discussion

This is the first report of an ACME and the *pls* gene in MRSA ST398. The *pls* gene encoding the plasmin-sensitive protein has been implicated as a virulence factor in a murine septic arthritis model.[19] These elements may increase the transmissibility, the ability to colonize humans and the increased of virulence of ST398 isolates commonly associated with livestock in the Netherlands.

The identical nucleotide sequence of truncated ACME type II in isolates of distinct genotypes indicates its recent horizontal acquisition. We have demonstrated complete identity of the entire ACME-SCC*mec* composite island in two distinct MRSA lineages.[10] In this study we identified the ACME element in combination

with different mobile genetic elements that have previously been reported.[3–7,10] This indicates that composite islands containing ACME have evolved by acquiring different genetic elements in several independent steps. Our study raises the question of whether the MSSA isolate PL69 was derived from an MRSA ancestor. We suggest that ACME is transferred independently of SCC*mec* elements and that Ccr recombinases from SCC-like elements may be involved in the transfer of ACME and other genetic elements that do not possess the *mec* complex. Our findings also show that despite the syntenic and largely conserved nature of the core genome in *S. aureus*, composite islands display an astonishing degree of mosaicism as a result of horizontal gene transfer during multiple and frequent recombination events.

Acknowledgements

Some of the data included in this manuscript were presented at the Third ASM-ESCMID Conference on Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications, Copenhagen, 2013 (Poster 78B).

Funding

This work was supported by the Interreg IVa-funded projects EurSafety Health-net (III-1-02=73) and SafeGuard (III-2-03=025), part of a Dutch-German cross-border network supported by the European Commission, the German Federal States of Nordrhein-Westfalen and Niedersachsen, and the Dutch provinces of Overijssel, Gelderland and Limburg. This work was supported in part by a grant (N N401 017740) from the Polish National Center for Research NCN.

Transparency declarations

None to declare.

References

1 Thurlow LR, Joshi GS, Clark JR *et al*. Functional modularity of the arginine catabolic mobile element contributes to the success of USA300 methicillin-resistant *Staphylococcus aureus*. *Cell Host Microbe* 2013; **13**: 100–7.

2 Diep BA, Gill SR, Chang RF *et al*. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. *Lancet* 2006; **367**: 731–9.

3 Bartels MD, Hansen LH, Boye K *et al*. An unexpected location of the arginine catabolic mobile element (ACME) in a USA300-related MRSA strain. *PLoS One* 2011; **6**: e16193.

4 Espedido BA, Steen JA, Barbagiannakos T *et al*. Carriage of an ACME II variant may have contributed to methicillin-resistant *Staphylococcus aureus* sequence type 239-like strain replacement in Liverpool Hospital, Sydney, Australia. *Antimicrob Agents Chemother* 2012; **56**: 3380–3.

5 Shore AC, Rossney AS, Brennan OM *et al*. Characterization of a novel arginine catabolic mobile element (ACME) and staphylococcal chromosomal cassette *mec* composite island with significant homology to *Staphylococcus epidermidis* ACME type II in methicillin-resistant *Staphylococcus aureus* genotype ST22-MRSA-IV. *Antimicrob Agents Chemother* 2011; **55**: 1896–905.

6 Urushibara N, Kawaguchiya M, Kobayashi N. Two novel arginine catabolic mobile elements and staphylococcal chromosome cassette *mec* composite islands in community-acquired methicillin-resistant *Staphylococcus aureus* genotypes ST5-MRSA-V and ST5-MRSA-II. *J Antimicrob Chemother* 2012; **67**: 1828–34.

7 Takano T, Hung WC, Shibuya M *et al*. A new local variant (ST764) of the globally disseminated ST5 lineage of hospital-associated methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the virulence determinants of community-associated MRSA. *Antimicrob Agents Chemother* 2013; **57**: 1589–95.

8 Ellington MJ, Yearwood L, Ganner M *et al*. Distribution of the ACME-*arcA* gene among methicillin-resistant *Staphylococcus aureus* from England and Wales. *J Antimicrob Chemother* 2008; **61**: 73–7.

9 Goering RV, McDougal LK, Fosheim GE *et al*. Epidemiologic distribution of the arginine catabolic mobile element among selected methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates. *J Clin Microbiol* 2007; **45**: 1981–4.

10 Sabat AJ, Köck R, Akkerboom V *et al*. Novel organization of the arginine catabolic mobile element and staphylococcal cassette chromosome *mec* composite island and its horizontal transfer between distinct *Staphylococcus aureus* genotypes. *Antimicrob Agents Chemother* 2013; **57**: 5774–7.

11 Aires-de-Sousa M, Boye K, de Lencastre H *et al*. High interlaboratory reproducibility of DNA sequence-based typing of bacteria in a multicenter study. *J Clin Microbiol* 2006; **44**: 619–21.

12 Harmsen D, Claus H, Witte W *et al*. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. *J Clin Microbiol* 2003; **41**: 5442–8.

13 Rissman AI, Mau B, Biehl BS *et al*. Reordering contigs of draft genomes using the Mauve aligner. *Bioinformatics* 2009; **25**: 2071–3.

14 Larsen MV, Cosentino S, Rasmussen S *et al*. Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Micobiol* 2012; **50**: 1355–61.

15 Ma XX, Ito T, Tiensasitorn C *et al*. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother* 2002; **46**: 1147–52.

16 Berglund C, Ito T, Ikeda M *et al*. Novel type of staphylococcal cassette chromosome *mec* in a methicillin-resistant *Staphylococcus aureus* strain isolated in Sweden. *Antimicrob Agents Chemother* 2008; **52**: 3512–6.

17 Vandendriessche S, Vanderhaeghen W, Larsen J *et al*. 2014. High genetic diversity of methicillin-susceptible *Staphylococcus aureus* (MSSA) from humans and animals on livestock farms and presence of SCC*mec* remnant DNA in MSSA CC398. *J Antimicrob Chemother* 2014; **69**: 355–62.

18 Hill-Cawthorne GA, Hudson LO, El Ghany MF *et al*. Recombinations in staphylococcal cassette chromosome *mec* elements compromise the molecular detection of methicillin resistance in *Staphylococcus aureus*. *PLoS One* 2014; **9**: e101419.

19 Josefsson E, Juuti K, Bokarewa M *et al*. The surface protein Pls of methicillin-resistant *Staphylococcus aureus* is a virulence factor in septic arthritis. *Infect Immun* 2005; **73**: 2812–7.