AAC Journals.ASM.org

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

B. A. Espedido,[a,b] J. A. Steen,[c,d] T. Barbagiannakos,[e] J. Mercer,[e] D. L. Paterson,[f] S. M. Grimmond,[c,d] M. A. Cooper,[d] I. B. Gosbell,[a,b,e] S. J. van Hal,[a,e] and S. O. Jensen[a,b]

Antibiotic Resistance & Mobile Elements Group, School of Medicine, University of Western Sydney, NSW, Australia[a]; Ingham Institute for Applied Medical Research, NSW, Australia[b]; Queensland Centre for Medical Genomics[c] and Institute for Molecular Bioscience,[d] University of Queensland, QLD, Australia; Department of Microbiology & Infectious Diseases, Sydney South West Pathology Service—Liverpool, NSW, Australia[e]; and Queensland Centre for Clinical Research, Royal Brisbane and Women's Hospital Campus, Brisbane, QLD, Australia[f]

Approximately 39% of methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type 239 (ST239)-like bloodstream isolates from Liverpool Hospital (obtained between 1997 and 2008) carry an arginine catabolic mobile element (ACME). Whole-genome sequencing revealed that an ACME II variant is located between *orfX* and SCC*mec* III, and based on pulsed-field gel electrophoresis patterns and temporal relationships of all ST239-like isolates ($n = 360$), ACME carriage may have contributed to subpulsotype strain replacement.

Strains of methicillin-resistant *Staphylococcus aureus* (MRSA) are a major cause of health care-associated infections around the world and are an emerging cause of infections in the wider community. In Australia, MRSA accounts for approximately 24% of *S. aureus* bloodstream infections and is associated with increased health care costs, morbidity, and mortality (16).

The arginine catabolic mobile element (ACME) is a putative virulence/colonization determinant that integrates into the chromosome at the same attachment site as staphylococcal chromosome cassette *mec* (SCC*mec*), presumably via the utilization of SCC*mec*-encoded cassette recombinases (2, 4, 5). ACME allotypes are defined by the presence or absence of the *arc* and *opp*-3 gene clusters (type I, *arc*$^+$ *opp*-3$^+$; type II, *arc*$^+$ *opp*-3$^-$; type III, *arc*$^-$ *opp*-3$^+$), and carriage of this element is thought to provide a competitive survival advantage. In this regard, the *arc* gene cluster (*arcCBDAR*) encodes a complete arginine deiminase pathway, which converts L-arginine to carbon dioxide, ATP, and ammonia, the last of which is important for survival at low pH (4). In general, ACME allotypes (and variants thereof) are broadly associated with certain coagulase-negative staphylococci (CoNS) (2, 10, 12). ACME carriage is uncommon in MRSA isolates, with the exception of USA300 (ST8-MRSA-IVa), for which evidence suggests that carriage has contributed to the successful spread of this community clone; a previous study showed that deletion of ACME I resulted in a fitness loss for a USA300 strain in a rabbit bacteremia model (5).

In this study, we examined the incidence of ACME carriage with respect to MRSA sequence type 239 (ST239)-like bloodstream isolates at Liverpool Hospital, Sydney, Australia, between 1997 and 2008. All 360 ST239-like isolates had previously been typed via pulsed-field gel electrophoresis (PFGE) using HARMONY criteria against known multilocus sequence-typed controls (15, 16); isolates were not subjected to multilocus sequence typing and are therefore referred to as ST239-like. Subsequently, we screened these isolates for the presence of an ACME allotype (I and II only) by directed *arc*A PCR (5). Whole-genome sequencing (WGS) was utilized to further characterize the ACME region in one *arcA*-positive isolate (Sa0059). A fragment library was generated and sequenced on an Ion Torrent PGM (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. A *de novo* assembly was generated using the CLC genomics workbench (CLC bio, Denmark), resulting in 1,437 contigs with an N_{50} of 2,501 bp and a maximum contig length of 16,659 bp. Contigs were ordered against JKD6008 (GenBank accession number CP002120), a recently described Australasian ST239-MRSA-III isolate (8), using Mauve contig mover (13). Gap closure for the *orfX*-ACME-SCC*mec* region was performed by sequencing PCR products using an ABI 3730*xl* capillary sequencer (Macrogen). A similar ACME allotype was inferred if the location of the ACME in other *arcA*-positive isolates was similar to that of Sa0059, as determined by directed PCR across the *orfX*-ACME junction using primers *orfX-SE_0098*-F (5′-GTTCCAGACGAAAAAGCACC-3′) and *orfX-SE_0098*-R (5′-ATTTTACTATCATATGGTTCAC-3′).

During the study period, ST239-like isolates accounted for 78.6% (360/458) of all MRSA isolates and displayed a high degree of diversity with 61 PFGE subpulsotypes. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram analysis revealed that these subpulsotypes clustered into five distinct clades (Fig. 1A), with clades 1 and 4 collectively accounting for 87.5% (315/360) of all ST239-like isolates. Interestingly, analysis of temporal relationships revealed that a subpulsotype strain replacement event had occurred; isolates from clade 4 (CL4) peaked in 2002 and were completely replaced by those from clade 1 (CL1) in 2008 (Fig. 1B). Screening of isolates for an ACME allotype revealed that 88% of CL1 isolates tested were positive for *arcA*, com-

Received 3 January 2012 Returned for modification 8 February 2012
Accepted 28 February 2012
Published ahead of print 5 March 2012
Address correspondence to S. O. Jensen, s.jensen@uws.edu.au, or S. J. van Hal, Sebastian.vanHal@sswahs.nsw.gov.au.
B.A.E. and J.A.S. contributed equally to this article.

doi:10.1128/AAC.00013-12

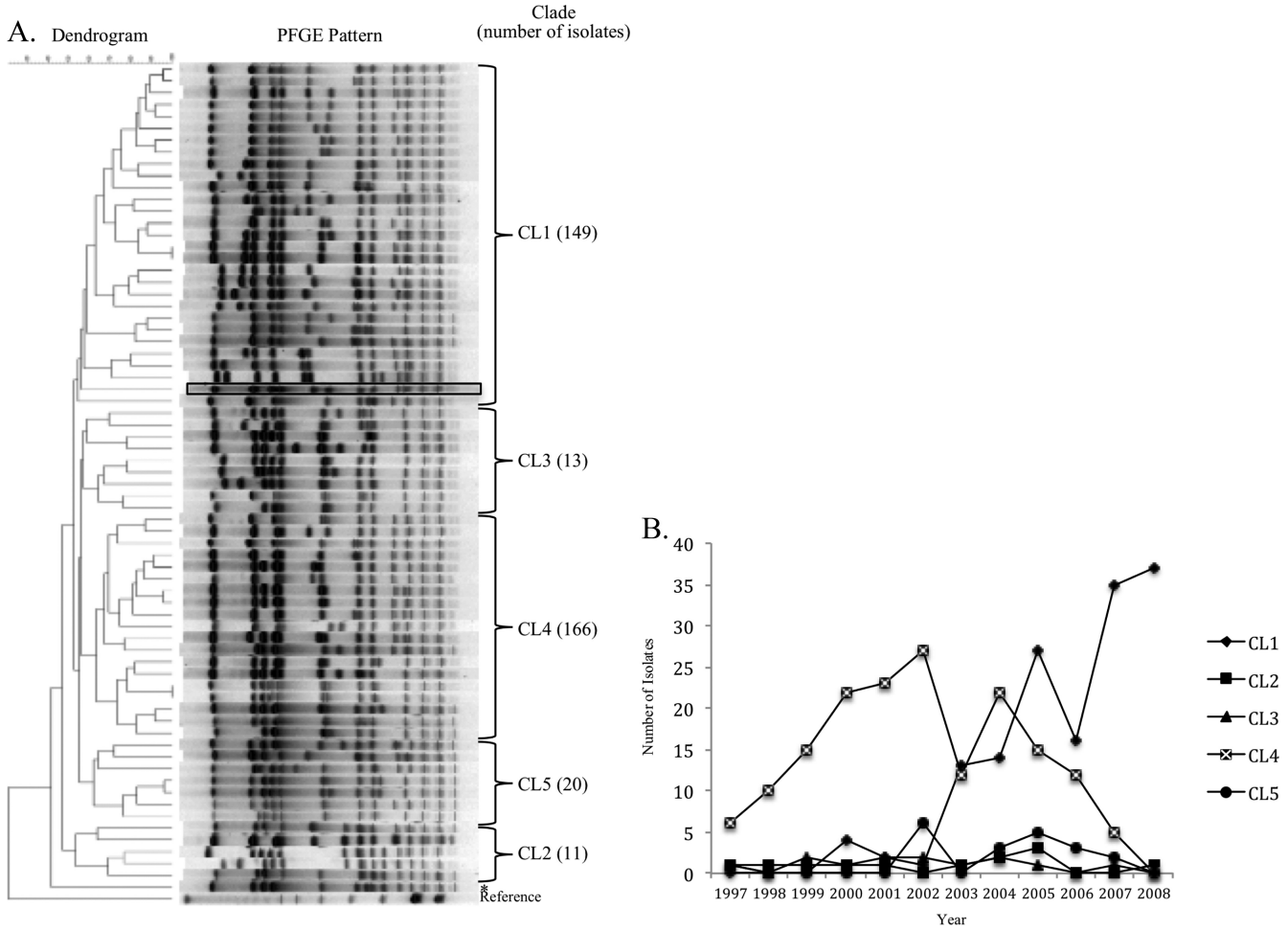

FIG 1 (A) UPGMA dendrogram showing the five major clades of representative ST239-like PFGE patterns (Liverpool Hospital; 1997 to 2008) that was generated using BioNumerics (Applied Maths, Kortrijk, Belgium). The asterisk denotes a single PFGE pattern that does not cluster within the major clades (1 to 5), and the Sa0059 (clade 1 isolate) pulsotype is boxed. (B) Distribution of ST239-like isolates per clade during the study period.

pared to only 5.5% of CL4 isolates (Table 1). A detailed analysis of contigs obtained from sequencing the CL1 isolate Sa0059 that did not order against JKD6008 (44/1,437 contigs) revealed a genomic region located between *orfX* and SCC*mec*III (Fig. 2B) that was identical to ΔACME II, a novel ACME II variant recently identified in ST22 isolates from Ireland (14) (Fig. 2A); 9 contigs were associated with this region. The remaining 35 contigs that did not order against JKD6008 displayed homology to the core genome of various staphylococcal bacteriophages and the genomic island vSaβ (type II) (1). While the latter encodes putative virulence factors, this genomic island is commonly present in MRSA (including other sequenced ST239 strains) (7, 9), and its distribution among our ST239-like isolates was not further examined. Note that all *arcA*-positive ST239-like isolates most likely carried ΔACME II, as they were also positive for *orfX-SE_0098*.

TABLE 1 PCR screening for the presence of *arcA* in ST239 MRSA isolates

Clade	No. of isolates	No. (%) of isolates with each *arcA* status[a]		
		Positive	Negative	Not tested[b]
1	149	125 (88)	17 (12)	7
2	11	0 (0)	10 (100)	1
3	13	1 (7.7)	12 (92.3)	
4	166	9 (5.5)	154 (94.5)	3
5	20	0 (0)	20 (100)	
All clades[c]	360	135 (38.7)	214 (61.3)	11

[a] Percentage of total isolates tested.
[b] Isolates could no longer be cultured from the original glycerol stock.
[c] Includes the single isolate represented by the PFGE pattern that does not cluster within the major clades (see Fig. 1).

This is the first genetic characterization of an ACME allotype in MRSA ST239-like isolates and the first report of ΔACME II in Australia. In addition, our data suggest that carriage of this ACME variant may have been, at least in part, responsible for the observed subpulsotype replacement event. Thus, ΔACME II may be contributing to the competitive fitness of CL1 isolates and their ability to colonize the skin and mucosal surfaces, as proposed for ACME I with respect to the successful spread of USA300 (4, 5). In this regard, it is interesting to note that the *arcA*-positive CL4 isolates (although limited in number) are temporally spread throughout the study period, similar to those of CL1, implying that the genetic background of CL1 isolates is more amenable to the carriage of ΔACME II.

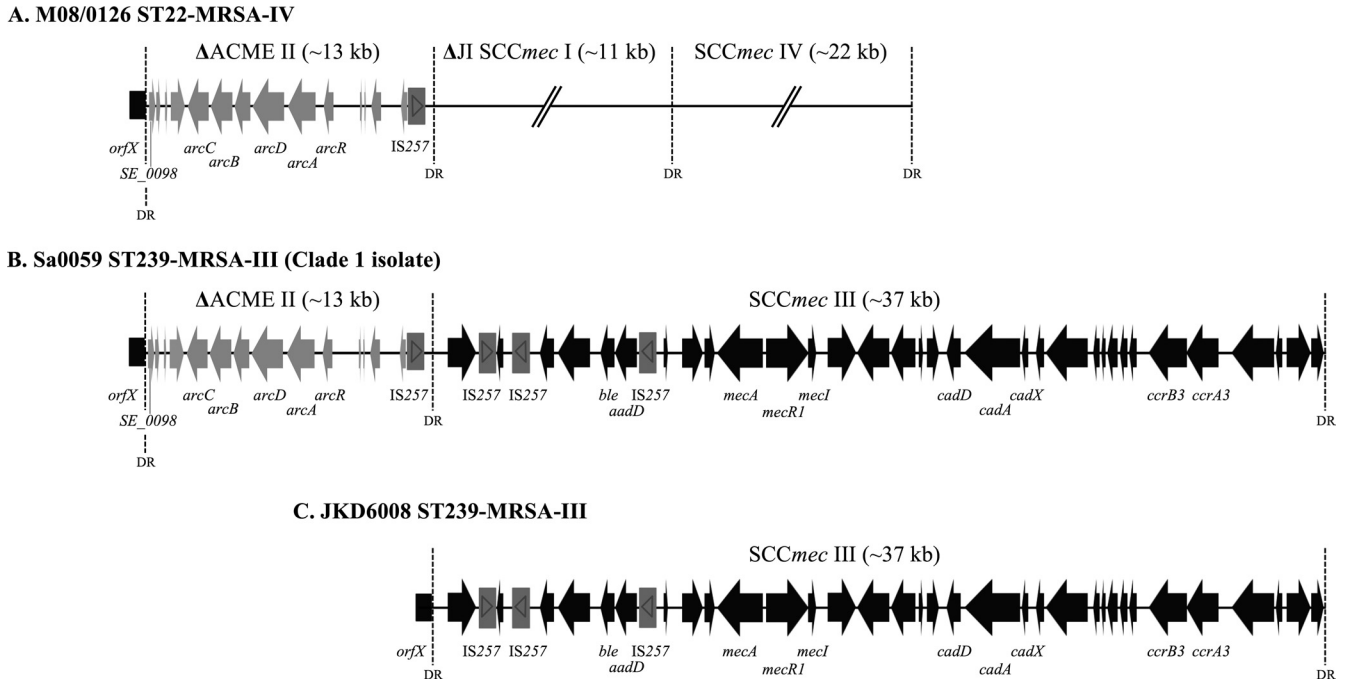

FIG 2 Schematic representation of the *orfX*-ACME-SCC*mec* structure from M08/0126 ST22-MRSA-IV (GenBank accession number FR753166) (A), Sa0059 ST239-MRSA-III (clade 1 isolate; GenBank accession number JQ412578) (B), and JKD6008 ST239-MRSA-III (GenBank accession number CP002120) (C). Only the following selected genes are annotated: the arginine deiminase pathway (*arcCBDAR*), the SCC*mec* III cassette recombinases (*ccrB3*, *ccrA3*), and the determinants encoding resistance to bleomycin (*ble*), aminoglycosides (*aadD*), methicillin (*mecA*), and cadmium (*cadDAX*). Arrowheads within gray rectangles denote the transposase (and the direction of its transcription) of IS*257* (also referred to as IS*431*). DR, direct repeat. Black rectangles denote *orfX*.

Previous studies by Ghaznavi-Rad et al. (6) and Monecke et al. (11) also detected the *arcA* gene in ST239 isolates (albeit at low levels), but in each case, the ACME allotype was not further characterized. As ΔACME II has been described only in ST22-MRSA-IVh isolates from Dublin, Ireland (14), ST22 may represent an MRSA reservoir of this ACME II variant; ST22-like isolates were also recovered from Liverpool Hospital during the study period (15, 16). However, as the isolates from Ireland represent a distinct ST22-MRSA-IV strain, it is also likely that the ΔACME II identified in our study was independently obtained (by ST239) from a coagulase-negative staphylococcal source due to the prevalence and exclusivity of ACME II in CoNS. In support of this, Bartels et al. (3) recently described a USA300-related strain that had acquired an ACME II variant (similar to ΔACME II) that was most likely from a *Staphylococcus epidermidis* background. In any case, the emergence of ΔACME II in MRSA ST239-like isolates suggests that unlike ACME I, this ACME II variant may provide a selective advantage to a number of different MRSA sequence types and thus more broadly enhance the dissemination of already successful MRSA clones that are resistant to multiple antimicrobial agents. Furthermore, our results emphasize the central role that mobile elements play in the acquisition of new properties and, thus, the "short-term" evolution of this important human pathogen. Additional studies are required to demonstrate the advantageous potential of ΔACME II carriage and to ascertain if the genetic background of CL1 ST239-like isolates (in comparison to that of CL4 isolates, for example) is more amenable to harboring an ACME allotype.

ACKNOWLEDGMENTS

M.A.C. acknowledges the support of an NHMRC Australia Fellowship (AF511105). S.M.G. is an NHMRC Senior Research Fellow.

REFERENCES

1. **Baba T, et al.** 2002. Genome and virulence determinants of high virulence community-acquired MRSA. Lancet **359**:1819–1827.
2. **Barbier F, et al.** 2011. High prevalence of the arginine catabolic mobile element in carriage isolates of methicillin-resistant *Staphylococcus epidermidis*. J. Antimicrob. Chemother. **66**:29–36.
3. **Bartels MD, Hansen LH, Boye K, Sorensen SJ, Westh H.** 2011. An unexpected location of the arginine catabolic mobile element (ACME) in a USA300-related MRSA strain. PLoS One **6**:e16193.
4. **Diep BA, et al.** 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. Lancet **367**:731–739.
5. **Diep BA, et al.** 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.
6. **Ghaznavi-Rad E, et al.** 2010. Predominance and emergence of clones of hospital-acquired methicillin-resistant *Staphylococcus aureus* in Malaysia. J. Clin. Microbiol. **48**:867–872.
7. **Holden MT, et al.** 2010. Genome sequence of a recently emerged, highly transmissible, multi-antibiotic- and antiseptic-resistant variant of methicillin-resistant *Staphylococcus aureus*, sequence type 239 (TW). J. Bacteriol. **192**:888–892.
8. **Howden BP, et al.** 2010. Complete genome sequence of *Staphylococcus aureus* strain JKD6008, an ST239 clone of methicillin-resistant *Staphylococcus aureus* with intermediate-level vancomycin resistance. J. Bacteriol. **192**:5848–5849.
9. **Li Y, et al.** 2011. Complete genome sequence of *Staphylococcus aureus* T0131, an ST239-MRSA-SCC*mec* type III clone isolated in China. J. Bacteriol. **193**:3411–3412.

10. **Miragaia M, et al.** 2009. Genetic diversity of arginine catabolic mobile element in *Staphylococcus epidermidis*. PLoS One **4**:e7722.
11. **Monecke S, et al.** 2011. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. PLoS One **6**:e17936.
12. **Pi B, Yu M, Chen Y, Yu Y, Li L.** 2009. Distribution of the ACME-*arcA* gene among meticillin-resistant *Staphylococcus haemolyticus* and identification of a novel *ccr* allotype in ACME-*arcA*-positive isolates. J. Med. Microbiol. **58**:731–736.
13. **Rissman AI, et al.** 2009. Reordering contigs of draft genomes using the Mauve Aligner. Bioinformatics **25**:2071–2073.
14. **Shore AC, et al.** 2011. 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. **55**:1896–1905.
15. **van Hal SJ, et al.** 2011. Methicillin-resistant *Staphylococcus aureus* vancomycin susceptibility testing: methodology correlations, temporal trends and clonal patterns. J. Antimicrob. Chemother. **66**:2284–2287.
16. **van Hal SJ, Jones M, Gosbell IB, Paterson DL.** 2011. Vancomycin heteroresistance is associated with reduced mortality in ST239 methicillin-resistant *Staphylococcus aureus* blood stream infections. PLoS One **6**:e21217.