Characterization and Distribution of the Arginine Catabolic Mobile Element in Methicillin-resistant *Staphylococcus aureus* ST239 Isolates from Liverpool Hospital, NSW, Australia

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Abstract

Background: The arginine catabolic mobile element (ACME) is widely distributed amongst certain coagulase negative staphylococcal species. However, with respect to methicillin-resistant *Staphylococcus aureus* (MRSA), ACME is mainly found in association with ST8-MRSA-IVa and evidence suggests that it has contributed to the successful spread of this community clone. The aim of this study was to determine the distribution of ACME amongst ST239-MRSA-III isolates from Liverpool Hospital, NSW, Australia, and characterize its genetic context.

Methods: All MRSA blood stream infection isolates from Liverpool Hospital (between 1997 and 2008) have been typed by pulsed-field gel electrophoresis (PFGE) using HARMONY criteria against known multilocus sequence typed controls. A selection of isolates identified as ST239 (360 in total) was screened for the presence of ACME and one isolate was chosen for whole genome sequencing (WGS).

Results: A UPGMA dendrogram based on the ST239 PFGE patterns revealed large diversity, with 61 sub-pulsotypes clustering into several distinct clades. Two of these clades (1 and 4) predominated and based on temporal relationships were responsible for sub-pulsotype replacement events. At present, ACME has been exclusively identified in clade 1 sub-pulsotypes and WGS of one isolate has revealed that an ACME II variant is located between orfX and SCC*mec* III.

Conclusions: These results suggest that ACME carriage is responsible, at least in part, for the current sub-pulsotype replacement event (*i.e.*, clade 1 isolates replacing clade 4 isolates) and represent the first genetic characterization of ACME in ST239-MRSA-III.

Introduction

Methicillin-resistant *S. aureus* (MRSA) is an important pathogen causing infections in both the community and hospital setting. In Australia, MRSA accounts for ~24% of *S. aureus* bloodstream infections and is associated with increased health-care costs, morbidity and mortality[8].

The arginine catabolic mobile element (ACME) is a putative virulence determinant that can be classified based on the absence/presence of the *arc* and *opp*-3 gene clusters (Type I, *arc* $^+$ *opp*-3 $^+$; Type II, *arc* $^+$ *opp*-3 $^-$; Type III, *arc* $^-$ *opp*-3 $^+$). ACMEs are suggested to aid in bacterial growth and provide a competitive survival advantage as a previous study showed that deletion of ACME I resulted in a fitness loss for a USA300 strain (ST8-MRSA-IVa) in a rabbit bacteremia model[3].

Liverpool Hospital is a 600-bed teaching hospital that provides medical and surgical care to the surrounding community of approximately 850,000 people. From 1997-2008, ST239-MRSA-III was the predominant sequence type (ST) among hospital-acquired bloodstream isolates. This study characterized molecular epidemiological trends of ST239 bloodstream isolates in our hospital over the 12-year period. Furthermore, the presence of an ACME in the Liverpool ST239 population was examined to determine its possible role in the success of this ST in our hospital.

Methods

MRSA isolates: Between 1996 and 2008, inclusive, MRSA was identified by conventional techniques in 458 positive blood cultures (BacT/ALERT 3D; bio-Mérieux, Marcy l'Etoile FRA) obtained from 409 patient episodes at Liverpool Hospital, Sydney, Australia and were included in this study[8].

Pulsed-field Gel Electrophoresis (PFGE): PFGE with Sma I restriction was performed as previously described[9]. Isolates were classified based on band profile comparisons to known multi-locus sequence types.

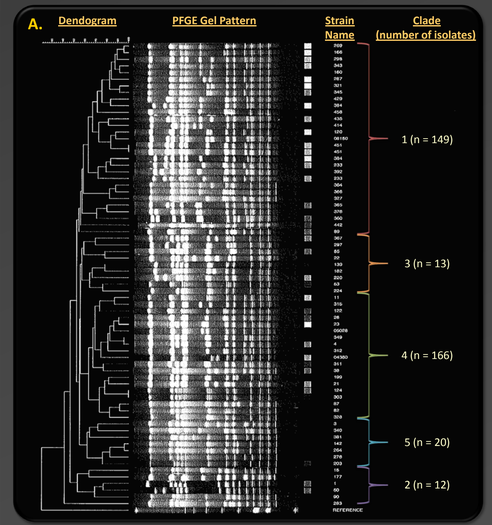
Whole Genome Sequencing (WGS): A fragment library suitable for sequencing on the Ion Torrent platform (Life Technologies; Carlsbad CA) was generated as per the manufacturers instructions. Sequence data was mapped to *S. aureus* JKD6008 (GenBank CP002120) using Mauve contig mover[6], and contigs that could not be placed were further interrogated as novel genomic content. For the identification of novel genomic content, a *de novo* assembly was generated using CLC genomics workbench (CLC bio, Denmark).

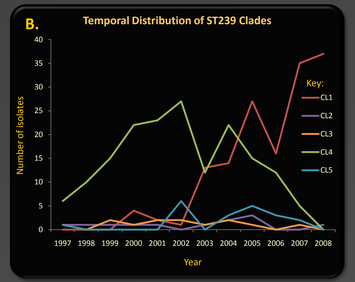
ACME screening: The *arc*A gene was detected by PCR as previously described[2]. To confirm the location of ΔACME II in relation to orfX, the following primers were used: orfX-ACME-F (5'-GTTCCAGACGAAAAAGCACC-3'); orfX-ACME-R (5'-ATTTTACTATCATATGGTTCAC-3').

Results

During the 12-year study period, ST239 was the prevailing MRSA ST among bloodstream isolates from Liverpool Hospital (360/458 isolates, 78.6%) as determined by PFGE, with five clades (CL1-5) comprising 61 PFGE sub-pulsotypes observed (Figure 1a). Although CL4 represents the dominant sub-pulsotypes, CL1 sub-pulsotypes had completely replaced these by the end of the study period (Figure 1b).

As previous studies had attributed the successful spread of USA300 to ACME carriage, we screened our isolates for *arc*A via PCR to determine if an ACME was involved with the CL1/4 replacement event. Of the isolates tested (n=349), 88% of CL1 isolates were positive for *arc*A compared to only 6% of CL4 isolates (Figure 1c), suggesting that the presence of an ACME in these strains may be contributing to their recent success. WGS was employed to further characterize the ACME region in one CL1 isolate (*S. aureus* Sa0059). 1,437 contigs were obtained with an N_{50} of 2501 bp, and a max contig length of 16,659 bp. Contigs were ordered against the genome of *S. aureus* JKD6008, a recently described Australian ST239-MRSA-III isolate, and contigs that could not be placed were further interrogated as novel genomic content. One such region of novel genomic content was found inserted between orfX and SCC*mec* III of Sa0059 (Figure 2) and was identical to ΔACME II, a novel ACME variant described earlier this year in an Irish ST22 isolate[7]. The position of ΔACME II in the other *arc*A-positive isolates in our study was confirmed by PCR (Figure 1c) using primers binding to orfX and SE_0098.





C.

PCR Screen Results

Clade	*arc*A Positive	*arc*A Negative	*arc*A Not tested	orfX-ACME Positive	orfX-ACME Negative	orfX-ACME Not tested
1	125	17	7	125	0	24
2	0	11	1	-	-	12
3	1	12	-	1	0	12
4	9	154	3	9	0	157
5	0	20	-	-	-	20

Figure 1. (A) Comparison of ST239 clades 1-5 from Liverpool Hospital (1997-2008) based on representative sub-pulsotype PFGE patterns using BioNumerics (Applied Maths; Kortrijik, BEL). (B) Distribution of ST239 isolates per clade from Liverpool Hospital over the study period. (C) Results for PCR screens targeting the *arc*A gene in ACMEs and the boundary between orfX and ΔACME II (SE_0098; see *Figure 2*).

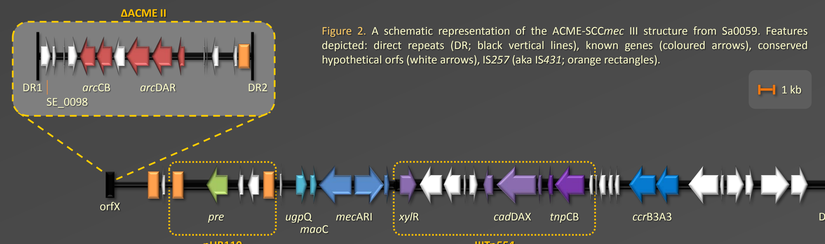


Figure 2. A schematic representation of the ACME-SCC*mec* III structure from Sa0059. Features depicted: direct repeats (DR; black vertical lines), known genes (coloured arrows), conserved hypothetical orfs (white arrows), IS*257* (aka IS*431*; orange rectangles).

Conclusion

To our knowledge, this is the first genetic characterization of an ACME allotype in ST239-MRSA-III and the first report of ΔACME II in Australia. Although previous studies detected the *arc*A gene in 3/360 Malaysian ST239 isolates[4] and 64/76 Australian USA300-like isolates[5], the ACME was not further characterized. Genetic comparisons between the *arc*A-positive isolates from those studies and our isolates would be beneficial in determining geo-temporal relationships among Australasian MRSA, particularly among ST239 isolates. Furthermore, ΔACME II was first described in 15 ST22-MRSA-IVh isolates from an Irish hospital[7]. Interestingly, ST239 and ST22 are the dominant STs in hospital-acquired MRSA in Australia (62.2% and 37.2% of MRSA, respectively)[1] and in our hospital (78.6% and 10.5%, respectively; unpublished data). While screening of ST22 isolates from our hospital for ΔACME II is needed, a single ST22 isolate could have been the source of ΔACME II found in our ST239 isolates.

The presence of ΔACME II in our ST239-MRSA-III coincides with the replacement of CL4 sub-pulsotype isolates by those of CL1. Additional studies are required to ascertain if the genetic background of CL1 isolates was more favorable to harboring an ACME and to demonstrate *in vivo* the advantageous potential of ΔACME II carriage.

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