
Characterization of a novel staphylococcal cassette chromosome composite island from community-associated MRSA isolated in aged care facilities in Western Australia

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Background

In Western Australia (WA), clonal complex 5, ST835, community-associated (CA) MRSA is isolated almost exclusively from aged care facilities. In WA four different staphylococcal cassette chromosome (SCC) mec (SCCmec) elements have been identified in this ST, indicating high genetic activity in the SCCmec region.

Objectives

To investigate the SCC region of ST835 CA-MRSA WA MRSA-40 and determine the distribution of an SCCsorbitol element found within the region.

Results

The SCC region contained a composite island, SCC\textsubscript{mec\_WA\_MRSA-40-CI}, that was composed of three elements, ΨSCC\textsubscript{pls}, SCCsorbitol and SCC\textsubscript{mec\_VT (5C2&5)}. This is the first time that a sorbitol operon has been reported in an SCC element.

Conclusions

Generation of SCC\textsubscript{mec\_WA\_MRSA-40-CI} has involved multiple genetic events and recombination with CoNS has occurred during evolution of the SCC elements. While \textit{Staphylococcus aureus} is renowned for its ability to utilize mobile genetic elements to disseminate antimicrobial resistance, the SCC region of WA MRSA-40 shows that this clone has also utilized SCC elements to acquire extra virulence and possibly adapt to a niche environment.

Introduction

Antibiotic resistance places burdens on healthcare systems, and in Western Australia (WA) community-acquired (CA) MRSA is a major public health concern.\textsuperscript{1} Methicillin resistance occurs with the acquisition of a site-specific mobile resistance island called the staphylococcal cassette chromosome (SCC) mec (SCCmec). SCC elements that do not carry mec have also been
described. SCCs carry a ccr complex and adaptive genes, while pseudo (Ψ) SCCs carry adaptive genes only. SCC elements integrate sequentially into the rlm gene, to form a composite island (CI).

Between 2005 and 2012, 209 ST835 CA-MRSA (0.5% of the total) were isolated and characterized in WA as previously published.1 Twenty-eight (0.06% of the total) were designated WA MRSA-40 [SCCmec Vt (5C2&5)+2], with the others being WA MRSA-48 [115 isolates, SCCmec IVa (2B) and a variant sub-type of IV (2B)], WA MRSA-87 [5 isolates, SCCmec Vt (5C2&5)], WA MRSA-99 (1 isolate, novel SCCmec) and WA MRSA-103 (3 isolates, novel SCCmec). Considering that WA MRSA-87 may have acquired SCCsorbitol, generating WA MRSA-40, this demonstrates at least three SCCmec acquisitions.

WA MRSA-40 has been isolated almost exclusively from elderly patients and 79% of cases can be directly linked to carriage and infections in aged care facilities (G. W. Coombs, unpublished data). In the current investigation the WA MRSA-40 SCC region was found to consist of ΨSCCpls, SCCsorbitol and SCCmec inserted sequentially to form a CI, designated SCCmecWA MRSA-40-CI.

Materials and methods

Isolates were recovered between 2005 and 2012. The index WA MRSA-40 was isolated in 2005 from an elderly patient in an aged care facility.

DNA was extracted using the Invitrogen PureLink™ Genomic DNA Purification Kit (Invitrogen™ Pty Ltd, Mount Waverley, VIC, Australia) with the addition of lysostaphin for cell wall lysis.

Primers were designed to cover the region using the Expand Long Range dNTPack (Roche Diagnostics Ltd, Castle Hill, NSW, Australia). Amplicons were purified using the Ultra Clean DNA PCR Clean Up Kit (Mo Bio Laboratories, GeneWorks, Thebarton, SA, Australia) or the GENECLEAN® Turbo Kit (QBIogene Inc., 2008 Q St., Washington, DC, USA).

Sequences were assembled using MacVector 7.2 AssemblyLIGN™ 1.0.9 (Accelrys, Cambridge, UK) and BioEdit Sequence Alignment Editor 7.0.9 (www.mbio.ncsu.edu/bioedit/bioedit.html) and
analysed using MacVector 7.2. Homology searches were performed using BLAST at the National Centre for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov.

Forty-one ST835 MRSA isolates were investigated for presence of the sorbitol operon using primers srlDF (5′-CCAGTTTCTTCAATAATACCAGGTG-3′) and srlDR (5′-TTACTGGAGGTTTAGTCGGG-3′).

Results

The nucleotide sequence of SCCmec\(_{\text{WA MRSA-40-CI}}\) has been deposited under NCBI accession number JQ746621.

SCCmec\(_{\text{WA MRSA-40-CI}}\) was 72,521 bp and comprised three elements, \(\Psi\)SCC\(_{\text{pls}}\), SCCsorbitol and SCCmec\(_{\text{VT (5C2&5)}}\), inserted in the attB site of rlm\(_H\). Repeats are presented in Figure 1 and the ORFs of \(\Psi\)SCC\(_{\text{pls}}\) and SCCsorbitol are presented in Table S1 (available as Supplementary data at JAC Online).

SCCmec\(_{\text{WA MRSA-40-CI}}\) was flanked by 15 bp imperfect direct repeats (DRs), DR-1 and DR-4. Similar DRs were found at the junctions of \(\Psi\)SCC\(_{\text{pls}}\) and SCCsorbitol (DR-2) and SCCsorbitol and the left-hand end of SCCmec (DR-3). DR-4 was 100% homologous with the DR at attB of \(Staphylococcus aureus\) NCTC8325 while DR-3 had 2 bp dissimilar. DR-2 and DR-1 had 2 and 3 bp dissimilar with NCTC8325, respectively. Unique 39 bp imperfect DRs flanking SCCsorbitol (DR-5 and DR-6) were found. Imperfect inverted repeats (IRs) of 7 bp were identified within DR-1 (IR-1), and immediately preceding DR-2 (IR-2). Imperfect 24 bp IRs were identified from within DR-2 (IR-3) and immediately preceding DR-3 (IR-4), and from within DR-3 (IR-5) and immediately preceding DR-4 (IR-6). Flanking the junction between \(\Psi\)SCC\(_{\text{pls}}\) and SCCsorbitol, a large IR was found as previously described in the SCC-CI of \(Staphylococcus epidermidis\) ATCC 12228 with a 13 bp perfect IR separated by 29 nt (IR-7 and IR-8). The left and right extremities of the SCCmec were flanked by
DR-3 and IR-5 and DR-4 and IR-6, respectively. Apart from a 1 bp difference in DR-4 these repeats were identical to those of the SCCmec V\(_1\) (5C2&5) of PM1 (GenBank: AB462393).

Homologies between \(\Psi^{\text{SCC}}\)plS and SCCsorbitol and other elements in the NCBI database are presented in Figure 2. \(\Psi^{\text{SCC}}\)plS is 11 736 bp and comprises eight ORFs, including a plS gene which encodes a plasmin-sensitive protein, genes encoding a poly (glycerol-phosphate) alpha-glucosyl transferase, a glucosyl transferase group 1 family protein, a truncated MurF-like protein and five hypothetical proteins. There were high homologies with \(\Delta\)J1 SCCmec I (nt 13 026–14 313, GenBank: FR753166) and a genomic region of \(S.\) epidermidis BCM-HMP0060 (nt 20 4545–20 5591, GenBank: NZ_GG696727.1).

SCCsorbitol is a 19 497 bp mosaic structure comprising 23 ORFs including a truncated type 1 restriction modification system, a speG-like gene, a ccrA2B2 complex and a sorbitol phosphotransferase operon. There were nucleotide homologies of 84% to 100% with \(S.\) epidermidis ATCC 12228 SCC-CI (nt 51 481–55 510 and nt 35 550–39 459, GenBank: AE015929), the ACME II and SCCmec of \(S.\) aureus M1 type IV SCCmec (nt 1–5024, GenBank: HM030720), the ACME II-CI of \(S.\) aureus M08/0126 (nt 40 175–47 028, GenBank: FR753166) and chromosomes of \(Staphylococcus\) carnosus TM300 (nt 23 85782–239 0926, GenBank: AM295250), \(Staphylococcus\) hominis C80 (Bioproject PRJNA38759) and \(S.\) hominis SK119 (Bioproject PRJNA34087).2–4 The most extensive homology in the ccrA2B2 downstream region was with ATCC 12228 SCC-CI ACME II, with the exception of an IS\textit{Sep1} element and an adjacent ORF that were present in ATCC12228. Otherwise, there was 100% nucleotide identity between SCCsorbitol nt 12 198–15 955 and ATCC 12228 ACME II.

Upstream of the ccrA2B2 complex of SCCsorbitol is a 5120 bp sorbitol operon flanked by 6 bp imperfect IRs (not shown), indicating an integration event. This operon has 99.7% homology with an operon found in the J1 region of type V (5C2&5) SCCmec of \(S.\) aureus JCSC4610 (nt 38 687–43 806, GenBank: AB773816)5 and 71%–73% homology with the chromosomal sorbitol operon of \(S.\) carnosus TM300 (nt 2 390 896–2 387 006, GenBank: AM295250). The sorbitol operon has a G+C
content of 27.7% while the G+C content of the whole SCC region is 30.9% suggesting possible interspecies transfer.\(^6\)

WA MRSA-40 SCC\textit{mec} was 41 289 bp and 99% homologous with SCC\textit{mec} V\(_1\) (5C2&5) of PM1. There were 16 nt differences, with the majority occurring in the IS\textit{431} transposases. IS\textit{431}s left (L) and right (R) of WA MRSA-40 were identical and encoded three premature stop codons. When compared with IS\textit{431}L and R transposase sequences of PM1, WA MRSA-40 possessed 12 and 0 bp differences, respectively. When compared with the intact transposase sequences of IS\textit{431}s L and R of the prototype type V strain W1S (GenBank: AB121219) WA MRSA-40 had 15 and 10 bp differences, respectively. There was also polymorphism in the J2 region between ORF M23 and the equivalent locus, ORF No. 25 of PM1, which encodes a putative DNA polymerase A motif protein.

Seven of the 41 ST835 isolates carried the sorbitol operon, implying they carried SCC\textit{sorbitol}; all of these belonged to the WA MRSA-40 group. WA MRSA-40 was originally recovered in 2005; three further strains were then recovered in 2006, two in 2009 and one in 2011. Six were from infections and one was a colonizer. All were from the metropolitan area with three from one aged care facility. No epidemiological linkage was made.

**Discussion**

The structural genetics of SCC\textit{mec}\textsubscript{WA MRSA-40-CI} from a genotype of CA-MRSA found in aged care facilities in WA have been characterized.

Ψ\textit{SCCplls} has been reported in other staphylococci where it is known as ΔJ1 SCC\textit{mec} I. The \textit{plls} gene has been associated with virulence and found on other mobile elements including the SCC\textit{mec} and plasmids of animal and human origin (GenBank: GQ900485 and GQ900474, respectively) in different lineages of \textit{S. aureus}. High homology with a genomic region of \textit{S. epidermidis} BCM-HMP0060 implicates \textit{S. epidermidis} in the generation of Ψ\textit{SCCplls}.
This is the first report of a sorbitol phosphotransferase operon on an SCC element. Such an operon has previously been reported in the J1 region of a type V (5C2) SCCmec and on the chromosome of S. carnosus.\textsuperscript{4,5} Frequent use of dietary sorbitol, used 3.7 times more frequently by elderly individuals, has been shown to increase the number of sorbitol-utilizing bacteria.\textsuperscript{7,8} It is therefore possible that the elderly index patient could have been exposed to dietary sorbitol in the care environment and SCCsorbitol mediated an advantage to the bacterial cell by enabling it to utilize sorbitol as an energy source. The failure to demonstrate the presence of SCCsorbitol beyond 2012 could indicate that the metabolic burden of carriage outweighed the environmental advantage conveyed.

WA MRSA-40 encoded a Taiwanese type V\textsubscript{T} (5C2&5) SCCmec that has been shown to be mobile amongst CA-MRSA in WA.\textsuperscript{1} It is interesting that the majority of polymorphisms in the SCCmec region occurred in the IS\textsubscript{431}L and R transposases. Thus far, the IS\textsubscript{431}L and R transposase sequences of the prototype SCCmec type V (5C2) strain W1S,\textsuperscript{9} and IS\textsubscript{431}L of PM1, are the only SCCmec type V-associated IS\textsubscript{431} transposases that have been intact and presumably active. As previously reported, some mec-associated IS\textsubscript{431} polymorphisms have resulted in premature stop codons, which would inactivate the transposases.\textsuperscript{10} This could represent an evolutionary adaptation that has stabilized type V SCCmecs.

In conclusion, it is evident that generation of SCCmec\textsubscript{WA MRSA-40-CI} has involved numerous genetic events and recombination with CoNS has occurred during evolution of ΨSCC\textsubscript{pls} and SCCsorbitol. Furthermore, this study has demonstrated how a virulent, antibiotic resistant lineage of CA-MRSA may have utilized an SCC element to increase competitiveness in a niche environment.

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**Transparency declarations**

None to declare.
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References


Figure 1. SCCmec composite island of WA MRSA-40. SCC elements are separated by vertical lines and are indicated above the figure. Genes and ORFs are shown as blocked arrows indicating their direction of transcription. Genes with relevance to the text are labelled. Positions, directions and sequences of repeats are shown below the figure (not drawn to scale). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.
Figure 2. Comparison of ΨSCCpls and SCCsorbitol of WA MRSA-40 with staphylococci encoding homologous regions. The ΨSCCpls and SCCsorbitol region of WA MRSA-40 is presented in the centre of the figure and homologous regions from other staphylococci are presented above or below. The source strain of each region is indicated. Genes and ORFs are shown as blocked arrows indicating their direction of transcription. Genes with relevance to the text are labelled. Regions of homology are indicated with grey shading. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.