Characterization of Staphylococcal Cassette Chromosome mec Elements from Methicillin-Resistant Staphylococcus pseudintermedius Infections in Australian Animals

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ABSTRACT  We examined the oxacillin resistance phenotype and genomic structure of staphylococcal cassette chromosome mec (SCCmec) elements from 77 veterinary methicillin-resistant Staphylococcus pseudintermedius (MRSP) isolates. Isolates were characterized by oxacillin broth microdilution, whole-genome sequencing, and bioinformatics analysis. Five previously described SCCmec elements, and a sixth novel element, were identified: SCCmec III (also known as II-III), \( \Psi \)SCCmec\(_{57395} \), and SCCmec\(_{NA45} \) (a SCCmec VII variant), all previously described in MRSP, and SCCmec IVg and SCCmec V\(_{VT} \), previously described in both methicillin-resistant Staphylococcus aureus (MRSA) and MRSP. The sixth element was novel and found among nine geographically clustered isolates. This novel pseudostaphylococcal cassette chromosome (\( \Psi \)SCCmec\(_{KW21} \)) contained a class A mec gene complex but lacked ccr genes. It also harbored heavy metal (cadmium) resistance determinants. The median oxacillin MIC values among \( \Psi \)SCCmec\(_{KW21} \), SCCmec III, and SCCmec V\(_{VT} \) isolates were significantly higher than those determined for the SCCmec\(_{NA45} \) VII variant isolates and \( \Psi \)SCCmec\(_{57395} \) and SCCmec IVg isolates. \( \Psi \)SCCmec\(_{KW21} \) was found exclusively in sequence type 497 (ST497), an MRSP clone that is locally successful in Victoria, Australia. Future studies are necessary to determine if this clone has disseminated further afield and if \( \Psi \)SCCmec\(_{KW21} \) has moved into other MRSP lineages or staphylococcal species.

IMPORTANCE  Staphylococcus pseudintermedius is a significant veterinary pathogen and occasional cause of infections in humans. \( \beta \)-Lactams are an important group of antimicrobials used to treat staphylococcal infections in humans and animals. However, when staphylococci become methicillin resistant via the acquisition of a mobile genetic element called staphylococcal cassette chromosome mec (SCCmec), they become resistant to all \( \beta \)-lactams. This study detected a novel SCCmec element among a cluster of methicillin-resistant S. pseudintermedius isolates from animals in Australia. It also detected SCCmec elements in S. pseudintermedius that had high similarity to those identified in methicillin-resistant Staphylococcus aureus, demonstrating how human and animal pathogens can share the same resistance determinants.
KEYWORDS SCCmec, Staphylococcus pseudintermedius, antimicrobial resistance, methicillin resistance, veterinary, zoonotic infections

Methicillin-resistant Staphylococcus pseudintermedius (MRSP) has become an important opportunistic pathogen in veterinary small-animal practice (1) and is an occasional zoonotic pathogen (2). S. pseudintermedius is part of the canine skin microbiota but can cause a wide range of opportunistic clinical infections across many body systems. MRSP infections are more complicated than methicillin-susceptible S. pseudintermedius (MSSP) infections due to the lack of potential treatment options. In staphylococci, methicillin resistance is determined by the mecA gene and its homologues, mecB and mecC (3–5). mecA and mecC are harbored within a mobile genetic element called the staphylococcal cassette chromosome mec (SCCmec) element (4, 6), whereas mecB, typically found in Micrococcus caseolyticus (7), has recently been detected on a multidrug resistance plasmid in methicillin-resistant Staphylococcus aureus (MRSA) (5). SCCmec elements are composed of a methicillin resistance determinant (mecA, mecB, or mecC) contained within a mec gene complex and include site-specific recombinase genes (ccr), which are responsible for insertion of the SCCmec cassette into the core genome (6). SCCmec types were initially defined by their combination of the mec gene class complex and the ccr gene complex (8). However, assignment of nomenclature and classification of SCCmec elements have been hampered by the existence of composite cassettes (9, 10) and pseudo-SCCmec elements that do not harbor ccr genes (11). Eleven SCCmec cassettes are formally recognized in the database of the International Working Group on the Staphylococcal Cassette Chromosome (IWG-SCC) and were named I to XI according to the chronological order in which they were first reported. Several SCCmec elements are reported in MRSP, including SCCmec III (previously described as II-III [12]), which is found in the globally dominant sequence type 71 (ST71) lineage (13); SCCmec Vα variants (14); and a newly reported cassette, SCCmecNAA5, which harbors a class 1 mec gene complex and ccrC recombinase gene (15). Other SCCmec elements have been identified in S. pseudintermedius that are not recorded in the IWG-SCC database, including ψSCCmec57395 (11), which lacks ccr genes, and SCCmecKM241 (12) and SCCmecA116 (10) (Table 1). Despite the difficulties associated with characterizing some SCCmec elements, SCCmec typing can provide useful information about the phylogenetic and epidemiological origin of isolates. Echoing the epidemiological division of human-derived MRSA into health care-associated (HA) and community-associated (CA) lineages (16, 17), Kasai et al. found that veterinary MRSP isolates with SCCmec III tended to be HA-MRSP lineages whereas isolates with SCCmec V tended to be CA-MRSP (18). Recently, we described the molecular epidemiology of 77 MRSP isolates collected from clinical infections in animals in Australia during a national surveillance study and found that the population was phylogenetically diverse (19). The current study characterized the SCCmec elements in these isolates.

Characterization of SCCmec elements. Isolates originated from a larger collection of 669 S. pseudintermedius isolates collected during the first Australian survey into antimicrobial resistance in veterinary pathogens (20, 21). All isolates were identified using a BD Bruker MALDI Biotyper and were screened for methicillin resistance using manual oxacillin broth microdilution according to CLSI guidelines (22). The end dilution for MIC testing was 64 mg/liter oxacillin. Oxacillin resistance was confirmed by detection of an oxacillin MIC of ≥0.5 mg/liter using Vitek 2, in accordance with the manufacturer’s instructions, and detection of the mecA gene by whole-genome sequencing as previously described (19). DNA extraction, library preparation, and initial de novo assembly were undertaken as previously described (19). SCCmec typing was undertaken by downloading the sequences of the mec gene complex and ccr elements of the SCCmec elements described by the IWG-SCC (8) from the NCBI online database (https://www.ncbi.nlm.nih.gov/). SCCmec elements previously identified in S. pseudintermedius but not included in the SCCmec working group website were also downloaded (Table 1). Downloaded SCCmec element sequences underwent BLASTn searches.
against de novo contigs using CLC Genomics Workbench. BLASTn results required more than 85% sequence homology to be assigned a particular ccr gene. mec gene complexes were assigned based on the gene structure of mecA, its regulators, and associated insertion sequences (Is). If a SCCmec type could not be assigned, contigs were mapped against a scaffold of reference SCCmec types (B) and the reference methicillin-susceptible S. pseudintermedius genome, ED99 (accession no. CP002478.1). The Kruskal-Wallis test was used to determine whether the median oxacillin MICs differed across SCCmec types. The Mann-Whitney U test was used to assess differences between SCCmec types in the median oxacillin MIC. SCCmec types with more than eight isolates were compared as separate entities in analyses; other isolates were grouped together.

Diversity of SCCmec types. Six SCCmec types were identified among 74 of the 77 MRSP from Australia (Table 2). The SCCmec type of the remaining three isolates (two ST497 isolates and one ST71 isolate) could not be determined because of poor sequencing quality (low read coverage and contig breaks) in the region around the mecA gene. Isolates from the same multilocus sequence type (MLST) tended to harbor the same SCCmec type. Four of the SCCmec types have previously been characterized in MRSP or MRSA as follows: SCCmec III (n = 34) and SCCmec57395 (n = 7), previously described in MRSP; and SCCmec V (n = 10) and SCCmec IVg (n = 5), previously described in MRSP and MRSA (11, 12, 23, 24). The 34 SCCmec III isolates, which were mostly ST71 and ST316, demonstrated 98% to 100% sequence homology to the mec and ccr gene complexes of the III element from MRSP KM1381 (12). SCCmec elements had no ccr genes but had 99 to 100% sequence homology to the region spanning from orfx to IS256 from MRSP 57395 (11). The V isolates displayed 96% to 100% homology to the ccrC1 gene from MRSP 06-3228 (23) and 100% homology to its mecA and IS431 genes, but the mecR1 gene was variably truncated from 23 bp to 93 bp. SCCmec IVg isolates had 97% to 100% sequence homology to the entire cassette from MRSA isolated from bovine milk described previously by Kwon et al. (24).

The fifth SCCmec element has 99% sequence homology to the novel element recently reported in MRSP NA45 (15). In our study, this element was identified in nine geographically dispersed isolates from eight different STs (Table 2). These isolates harbored a class C1 mec complex with 99% sequence homology to SCCmec X from MRSA JCSC6945 but did not harbor the same ccr genes as this element. Instead, the isolates harbored a recombinase gene with 99% homology to ccrC6, recently identified in a 43,922-bp SCCmec element in ST84 MRSP (15) and methicillin-resistant S. schleiferi

| SCCmec name | Reference isolate | Accession number | mec complex | ccr complex |
|-------------|-------------------|-----------------|-------------|-------------|
| SCCmec57395 | MRSP 57395        | HE984157.2      | C1          | No ccr      |
| SCCmec150241| MRSP KM241        | AM904731.1      | A           | A5B3        |
| SCCmecA16   | MRSP A16          | LN864705.1      | A           | A1B3        |
| SCCmecNA45  | MRSP NA45         | CP016072.1      | C1          | C6          |

TABLE 2 SCCmec types and multilocus sequence types (MLST) of methicillin-resistant Staphylococcus pseudintermedius isolates from clinical infections in animals in Australia, 2013 to 2014

| Element | MLST* |
|---------|-------|
| SCCmec III (n = 34) | ST71 (n = 25), ST316 (n = 8), ST25 (n = 1) |
| SCCmec57395 (n = 7) | ST45 (n = 6), ST544 (n = 1) |
| SCCmec V (n = 10) | ST496 (n = 8), ST16 (n = 1), ST51 (n = 1) |
| SCCmec IVg (n = 5) | ST498 (n = 3), ST25 (n = 1), ST539 (n = 1) |
| SCCmec57395 (VII variant) | ST64 (n = 2), ST84 (n = 1), ST283 (n = 1), ST499 (n = 1), ST500 (n = 1), ST501 (n = 1), ST525 (n = 1), ST547 (n = 1) |
| SCCmec57395 (VII variant) | ST497 (n = 9) |
| Not determined* | ST497 (n = 2), ST71 (n = 1) |

*MLST, multilocus sequence types.

*The SCCmec57395 VII variant element harbors a C1 mec complex and a ccrC6 element (15). SCCmec57395 is a novel element that is described in this paper.

*The SCCmec elements of three isolates could not be determined due to poor sequence and assembly quality (contig breaks around the mecA gene).
This element is also present in ST398 MRSA RIVM3897 (26), but the RIVM3987 element lacks the final 8,164 bp at the 3' end of the SCCmec cassette in ST84 MRSP and MRSS. The nine isolates in this study showed 99% homology to the entire SCCmec cassette from MRSP NA45, which contained heavy metal resistance genes \( \text{arsB}, \text{arsC}, \text{arsR} \) (arsenic resistance) and \( \text{copA} \) (copper resistance) but no antimicrobial resistance genes. On the basis of typing recommendations of the IWG-SCC (8), the element in these nine isolates and in MRSP NA45 could be described as SCCmec VII because it harbors a class C1 mec gene complex and \( \text{ccrC} \) recombinase gene (Fig. S1). However, as the mec gene complex is positioned in reverse orientation in comparison to SCCmec VII, we feel that it is more appropriate to refer to this cassette as "SCCmec NA45," a SCCmec VII variant. Most of the isolates harboring the SCCmec VII variant SCCmec NA45 did not harbor the variable repeat region of the \( \text{spa} \) gene and therefore could not be assigned a \( \text{spa} \) type (19).

The final nine MRSP isolates, all ST497, could not be mapped to previously described SCCmec elements. All ST497 isolates were from a geographic cluster in Melbourne, Victoria (19). To characterize the novel element found in the remaining nine isolates, a representative ST497 isolate (KW21) underwent further sequencing by Illumina HiSeq and Nanopore long-read technology using MinIon. MinIon long reads were used to verify the structure of the \textit{de novo} assembly. \textit{De novo} assembly of Illumina HiSeq reads using Geneious yielded a 319,216-bp contig that contained the entire putative SCCmec element. This contig was subjected to a blast search against the NCBI database to determine putative components of the element. The element was annotated using PROKKA (27) and manually verified using the BLASTn algorithm in CLC Genomics Workbench.

\textbf{ΨSCCmec\textsubscript{KW21} element.} The novel SCCmec element, designated "ΨSCCmec\textsubscript{KW21}," was integrated at the 3' end of the \( \text{orfX} \) gene (\textit{rmlH}) (Fig. 1). The characteristic direct repeat and insertion sequences (\textit{I\textsubscript{SS}}) that typically flank SCCmec elements were absent at the right side (10, 28). The element contained a class A mec gene complex that had 99% BLASTn similarity to the class A mec gene complex SCCmec cassettes from MRSA JKD6008 (29), MRSP KM241 (12) and MRSP AI16 (10) (accession numbers \textit{CP002120.1}, \textit{CP017155.1}, and \textit{CP002121.1} respectively).
AM904731.1, and LN864705 respectively). The element did not contain any ccr genes. The 16,711-bp region of \(\Psi^+\text{SCCmec}_{\text{KW21}}\) that spanned from orfX to the cadmium resistance operon, cadCAD, had 99% sequence homology to the same region of SCCmec III from ST239 MRSA isolate JKD6008 (Fig. 1). We therefore propose that \(\Psi^+\text{SCCmec}_{\text{KW21}}\) is a truncated version of SCCmec III that lost the segment containing ccr genes after the cassette was inserted into the genome. Unlike SCCmec III from ST239 MRSA, \(\Psi^+\text{SCCmec}_{\text{KW21}}\) does not harbor ccr genes; instead, cadA and cadC are bordered by a truncated IS30 family transposase. To the right of this transposase is a 10,116-bp region that had 97% BLASTn similarity to a chromosomal region of Macrococcus sp. IME1152 (accession number CP017156.1) that includes the copY gene, encoding a putative copper transport repressor. BLASTn analysis of the de novo contigs of ST497 isolates against the SCCmec element from KW21 revealed that all nine isolates had the same mec gene complex, the genomic region with Macrococcus sequence homology, and no ccr genes. Consequently, they were considered to have the same pseudoelement as KW21. ST239 is an important health care–associated MRSA lineage in humans, so screening of healthy and diseased animals across Australia is now indicated to determine if ST497 and/or other lineages harboring \(\Psi^+\text{SCCmec}_{\text{KW21}}\) continue to exist within a geographic cluster or whether this lineage has disseminated across Australia or overseas, as has occurred with ST239 MRSA in humans (30).

**Variation in oxacillin MICs amongst different SCCmec types.** Fig. 2 shows the oxacillin MIC range for the major SCCmec types. The median oxacillin MICs differed significantly depending on SCCmec type (Fig. 2; \(P < 0.001\)). The median oxacillin MICs of \(\Psi^+\text{SCCmec}_{57395}\) and SCCmec IVg isolates (1 mg/liter) and of SCCmecNA45-VII variant isolates (4 mg/liter) were significantly lower than the median oxacillin MIC of SCCmec III, SCCmec V, and \(\Psi^+\text{SCCmec}_{\text{KW21}}\) isolates (64 mg/liter; \(P < 0.01\)). Recently, Kasai and colleagues (18) similarly reported differences in oxacillin MIC on the basis of MRSP SCCmec types. Specifically, they found that isolates with SCCmec III generally had higher oxacillin MICs and were more often associated with suspected hospital-acquired infections than isolates with SCCmec V. Analogous to analyses of MRSA in human medicine, they concluded that oxacillin MIC may give clues as to an isolate’s epidemiological origin, where a high oxacillin MIC may indicate that an MRSP isolate is from a successful “health care–associated” clone whereas isolates with lower MICs may represent “community–associated” clones (18). There were insufficient epidemiological data available in our study to draw similar conclusions, but our results do support the notion that the oxacillin MIC is significantly affected by the SCCmec type and that isolates of the same multilocus sequence type generally harbor the same SCCmec type. Thus, it follows that different MRSP lineages would demonstrate different oxacillin MICs. The mecA gene can be repressed by either mecl or blai, but MRSA and MRSP isolates with bla regulators typically demonstrate more rapid induction and higher expression of mecA than isolates with mec regulators alone (31–33). To determine whether the
oxacillin MIC was influenced by the presence of the \textit{blaI} and \textit{mecI} genes rather than simply by the SCC\textit{mec} type, we screened all isolates for these repressor genes using a BLAST-based command line script (screen\_assembly3.py: \url{https://github.com/shimbalama/screen\_assembly}). Most (45/77) isolates harbored \textit{blaI}, but only SCC\textit{mec} III and \textit{\textbackslash{}mec\textbackslash{}KW21} harbored both \textit{blaI} and \textit{mecI}. The high median oxacillin MIC (64 mg/liter) of III and \textit{\textbackslash{}mec\textbackslash{}KW21} isolates could have been due to the fact that \textit{blaI} attenuates the strong \textit{mecA} repression expected from the cognate \textit{mec} regulators (32).

In summary, we found six SCC\textit{mec} types among 77 MRSP isolates collected from clinical infections in Australian animals. The oxacillin MIC varied according to SCC\textit{mec} type. We also described \textit{\textbackslash{}mec\textbackslash{}KW21}, a novel pseudo-SCC\textit{mec} element that was found only in a geographic cluster of clinical isolates. This report highlights the utility of nation-wide surveillance studies in unearthing novel and emerging resistance determinants and demonstrates how genomic resistance elements found in significant human pathogens such as \textit{S. aureus} can also be found in veterinary pathogens such as \textit{S. pseudintemedius}.

\textbf{Accession number(s).} The genomic sequence of \textit{\textbackslash{}mec\textbackslash{}KW21} has been deposited in the National Center for Biotechnology Information (NCBI) database under GenBank accession number MH713898. The contig sequences of the MRSP isolates described in this study were also deposited under BioProject number PRJNA439160 and BioSample accession numbers SAMN08741522 to SAMN08741590.

\section*{SUPPLEMENTAL MATERIAL}
Supplemental material for this article may be found at \url{https://doi.org/10.1128/mSphere.00491-18}.

\section*{FIG S1, TIF file, 2.7 MB.}

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