Genetic organization of mecA and mecA-regulatory genes in epidemic methicillin-resistant Staphylococcus aureus from Australia and England

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The mecA gene that encodes methicillin resistance in Staphylococcus aureus may be regulated by the mecR1 and mecI genes, and this region has been referred to as the mec complex. An analysis of these regulatory genes in 35 epidemic methicillin-resistant S. aureus (MRSA) isolated in England and Australia has found that they contain three classes of mec complex. Firstly, the Class A mec complex with complete mecR1 and mecI genes. Secondly, a new variant of Class A, the Class A1 mec complex, with a 166 bp deletion in the membrane-spanning domain of mecR1 and a complete mecI gene. Thirdly, the Class B mec complex, in which the penicillin-binding domain of mecR1 and the whole mecI gene are deleted by the insertion of a partial sequence of IS1272. Seven MRSA isolated in England and Australia over different time periods had the Class A mec complex. However, the isolates did not have closely related pulsed-field gel electrophoresis (PFGE) patterns. The Class A1 mec complex was found in 12 Australian isolates and the English epidemic MRSA, EMRSA-1. All these organisms were isolated in the early 1980s and had closely related PFGE patterns. The Class B mec complex region was found in nine EMRSA and seven Australian MRSA isolated over the period from the 1970s to 2000. These isolates had related PFGE patterns. The mecA region was also compared in the isolates and all but two of the isolates had an XbaI restriction site. These results support the global spread of epidemic clones and confirm the close relationship between the Australian and English MRSA strains.

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) were first reported in 1961.¹ Although there were sporadic outbreaks of MRSA they did not become a major problem until the late 1970s and early 1980s when outbreaks were reported from many parts of the world.² The strains demonstrated an ability to spread within and between hospitals and are known as epidemic MRSA or EMRSA.² This raises the question of the relationship of the earlier and later MRSA. Initial evidence indicated that the early isolates were clonal and that the later isolates were different.² However, there is now conflicting evidence on the relationship of the different isolates. Basically there are two possibilities. First, S. aureus has acquired the genes for methicillin resistance and all subsequent isolates are clonal and differ due to changes brought about by mutation, phages, plasmids and transposons. The other possibility is that different strains of S. aureus have acquired the methicillin resistance gene at different times. Although it may not always be easy to distinguish between these two alternatives, a finding that the genes for methicillin resistance are different in different isolates is more likely to indicate that strains have independently acquired the gene for methicillin resistance. Many different approaches have now been used to compare the overall relatedness of MRSA isolates.³ Methods that have been particularly useful are contour-clamped homogeneous electric field electrophoresis (CHEF),³ and more recently spaA sequencing,⁴ multilocus sequence...
typing (MLST), DNA microarray and sequencing of the total MRSA genome. These techniques all confirm that MRSA have diverse genetic backgrounds.

The mecA gene codes for resistance to methicillin and all other β-lactam antibiotics in S. aureus. mecA is also found in other species of staphylococci and is highly conserved except for a silent, single-base mutation in some strains that creates an XbaI site. The mecA gene is regulated by two genes, mecR1 and mecI, located upstream of the mecA gene, and this region, together with mecA, has been referred to as the mec complex. The mecR1 gene encodes a transmembrane inducer of mecA consisting of membrane-spanning (MS) and penicillin-binding (PB) domains. The mecI gene encodes a strong repressor of mecA and consequently strains such as N315 with intact mecR1 and mecI can appear methicillin sensitive in susceptibility tests. The mecR1 and mecI genes have a high degree of homology to the blaR1 and blaI genes, which regulate β-lactamase production, and studies have shown that the blaR1-blaI complex is able to regulate the expression of the mecA gene. MRSA strains that have a dysfunctional regulatory region can either express mecA constitutively, or they can use the β-lactamase regulatory genes to optimally express mecA because BlaR1 is a strong inducer of mecA and BlaI is a weak repressor.

Studies on the mecR1–mecI region have shown that there is considerable genomic diversity in the mec complex. Currently, five different classes of the mec complex have been described. The Class A complex has intact mecR1 and mecI genes. In the Class B complex the PB domain of mecR1 and the complete mecI gene are truncated by a partial copy of IS1272. The Class C complex has two variants, C1 and C2. In the Class C1 complex, the PB domain of mecR1 and the whole of mecI are truncated by IS431, whereas in the Class C2 complex, both the MS and PB domains of mecR1 as well as mecI are truncated by IS431. In Class D mec complex mecI is deleted and the PB domain of mecR1 is truncated. The mec complex is part of a larger region known as the staphylococcal cassette chromosome mec (SCCmec). Four types of SCCmec have been described. The types are different because SCCmec can acquire different genetic elements. Consequently, different SCCmec regions can have the same mec complex. Therefore analysis of the mec complex is more likely to reflect the ancestral origin of mecA because SCCmec can acquire and/or lose elements, whereas the mec complexes are less likely to undergo additional deletions and mutations once mecA is being expressed. The mec complex would therefore appear to be a useful tool to compare the ancestry of MRSA when combined with other molecular methods.

Materials and methods

Thirty-five isolates, comprising 15 English EMRSA, 15 Australian EMRSA and five classic MRSA were analysed. Their mec complexes were analysed by PCR using the following primers: MR116 and MR216 for the mecA gene; mA17 and mecI212 for the Class A mec complex; mA17 and R08f2 (5′-GGCAACCTTAAGCCAGGTA-3′) for the Class B mec complex; mecRA112 and mecRA212 for the MS domain of mecR1; mecRB112 and mecRB212 for the PB domain of mecR1; and mecI112 and mecI212 for mecI. Primers locations are shown in Figure 1. CHEF electrophoresis and Multi-Analyt/PC (Bio-Rad Laboratories, Hercules, CA, USA) were used to determine the overall genetic relatedness of the isolates.

Results and discussion

A summary of the results and details of the isolates are provided in Table 1. All of the isolates amplified with the mecA primers, MR1 and MR2. The mecA PCR products were digested with XbaI to detect the silent mutation. Of the 35 isolates only EMRSA-16 and WBG10267 did not have an XbaI site in their mecA. Nineteen of the 35 isolates amplified with the Class A primers, mA and mecI2. The products were of two sizes, ∼2237 and 2075 bp. PCR for the domains of mecR1, followed by ClaI digestion of the 2075 bp PCR product revealed a deletion in the MS domain of mecR1. Subsequent sequencing of the 2075 bp product (T. T. Lim & W. B. Grubh, unpublished results) showed that the deletion was 166 bp in length. These results indicate a new variant of the Class A mec complex, and will be referred to as the Class A1 mec complex. The other 16 isolates had a Class B mec complex.

Although only a few isolates had identical CHEF patterns (Figure 2) many were found to be related based on the Tenover criteria. Two CHEF pattern clusters were found among the isolates (Figure 2). The CHEF A cluster consisted of 13 isolates with 90% similarity and all but one isolate carried the Class A1 mec complex. The CHEF B cluster consisted of 12 isolates with 75% similarity and all isolates carried the Class B mec complex. The similarity between the two clusters was 66%. Ten isolates that fell outside clusters A and B were not closely related to each other or to the other isolates.

The CHEF A cluster isolates, except for one, were all isolated in the early 1980s and have the 166 bp deletion in the MS domain of mecR1. This deletion was also reported in an American epidemic clone LHH1 and the New Zealand MRSA 85/2082. Both of these strains were also isolated in the same time period as the CHEF A cluster isolates. These results further support the global spread of an MRSA clone in the early 1980s. The CHEF B cluster isolates included the five classic MRSA: COL, WBG512, WBG1434, WBG1438 and WBG1350. They had related CHEF patterns and carried the Class B mec complex. The first MRSA isolated, NCTC10442, has the Class B mec complex. This supports the claim that the early MRSA were clonal.
Genetic organization of \textit{mecA} and \textit{mecA}-regulatory genes

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Schematic diagram of the three classes of \textit{mec} complex showing the location of primers. After Katayama \textit{et al.}\textsuperscript{10} and Kobayashi \textit{et al.}\textsuperscript{12} The bold arrows indicate the direction of the primers and the thin arrows the direction of transcription. MS, membrane-spanning domain of \textit{mecA}; PB, penicillin-binding domain of \textit{mecA}.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Dendrogram of the CHEF patterns of the isolates studied.}
\end{figure}
Nine of the English EMRSA studied carried the Class B mec complex but only six were found in the CHEF B cluster. The other three English EMRSA were only distantly related to the CHEF B cluster isolates. This is especially so for EMRSA-15, which is only 40% related to the CHEF B cluster isolates. The two most likely explanations of these results are that a strain has acquired the mec complex and then some have evolved to give widely different CHEF patterns, or alternatively, strains with different genetic backgrounds have acquired similar mec complexes. Recent studies using MLST and DNA microarray techniques have indicated that the latter hypothesis may better explain this phenomenon.5,6

The Class A and Class B mec complexes were found in both classic MRSA and later MRSA isolates. Although some
of the later isolates were genetically related to the classic isolates and had the same class of mec complex, some of them were not related to each other. In a recent study, an Iberian MRSA was found to be a descendant of an early methicillin-susceptible S. aureus. In another study, evidence has been presented for at least five horizontal transfers of mecA into genetically distinct S. aureus. Both studies have demonstrated the co-existence of descendants of old clones and new clones created by horizontal transfer of the methicillin resistance gene.

The analysis of the mec complex together with the CHEF patterns of these epidemic strains may support the global spread of epidemic clones and their possible ancestry. However, more comprehensive methods such as using MLST or DNA microarray techniques may give a more definite conclusion.

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References


