Detection of mecA, mecR1 and mecI genes among clinical isolates of methicillin-resistant staphylococci by combined polymerase chain reactions

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The distribution of the mec genes mecA, mecR1 and mecI that regulate the expression of methicillin resistance was investigated by PCR in 145 staphylococci of hospital origin. Determination of alterations and deletions in parts of the genes was achieved using 11 sets of primers in combined reactions. Methicillin-resistant Staphylococcus epidermidis strains appeared relatively stable, with 57.9% of isolates containing the whole regulatory region. Alterations within the mecA gene were detected more often in other coagulase-negative staphylococci, which also had a higher percentage with deletions of regulatory genes. Among methicillin-resistant S. aureus, a genetically heterogeneous population was identified, with several alterations and deletions of mec genes.

Materials and methods

Bacterial strains

One-hundred and forty-five Staphylococcus isolates from clinical specimens of in-patients at the University Hospital...
of Patras were included in this study. The Clinical Microbiology Laboratory identified 49 isolates as *S. aureus*, 76 as *Staphylococcus epidermidis*, eight as *Staphylococcus hominis*, eight as *Staphylococcus haemolyticus*, three as *Staphylococcus lugdunensis* and one as *Staphylococcus xylosus*, by Gram’s stain, catalase and coagulase production, and by the use of API Staph. System (bioMérieux, La Balme les Grottes, France).

The control strains BB225 (mecillin-sensitive *S. aureus*), BB270 (MRSA, mecA positive) and BB830 [mecillin-resistant *S. epidermidis* (MRSE), mecA/mecR1/mecI positive], kindly provided by Professor B. Berger-Bachi, were used for the establishment of PCR conditions.

**Antibiotic susceptibility testing and β-lactamase production**

Resistance to methicillin was detected by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, using 1 µg oxacillin discs (Difco Laboratories, Detroit, MI, USA), according to NCCLS standards. MICs of methicillin (Sigma, St Louis, MO, USA) were determined by agar dilution method in Mueller–Hinton agar, supplemented with 4% NaCl, after 24 h incubation at 35°C using 10^6 cfu/well. The resistance breakpoint to methicillin was ≥4 mg/L. The production of β-lactamase was tested with nitrocefin discs (Difco) according to the manufacturer’s instructions.

**Preparation of chromosomal DNA**

Cells from an overnight culture in Luria–Bertani broth [5 g/L NaCl, 5 g/L yeast extract, 10 g/L tryptone (Difco)] collected by centrifugation, were suspended in 3 mL buffer (0.1 M Tris–HCl pH 7.5, 0.1 M EDTA pH 8, 0.15 M NaCl) with 1 mg lysozyme (Sigma) and 0.2 mg lysostaphin (Sigma). After incubation of the resulting mixture at 37°C for 30 min, 0.3 mL of 5% SDS in 50% ethanol (Merck, Darmstadt, Germany) was added, followed by phenol:chloroform:isoamylalcohol (25:24:1) extraction. The DNA was precipitated by the addition of two volumes 100% ethanol and was suspended in 2 mL TE buffer (10 mM Tris–HCl pH 8, 1 mM EDTA pH 8) with 1 µL RNase H (10³ U/mL, Boehringer, Mannheim, Germany). The samples were incubated overnight at room temperature, measured for the DNA quantity at 260 nm (Gene Quant II, DNA/RNA calculator, Pharmacia Biotech, CE, Cambridge, England) and kept at 4°C.

**Oligonucleotides**

Two of the primers used for the detection of the *mecA* gene (named P2 and P3) have already been published and one more sense primer was designed by us (P1). The primer sequences were: P1, 5′-(911)GGTCCCATTAACTCTG-AAG(929)-3′; P2, 5′-(1427)ATCTGATGGTAAAGGTT-GGC(1445)-3′; and P3, 5′-(1923)AATGGCGAAAAAGCACAA-GC(257)-3′. In the second reaction SA15 was used as sense with the antisense primer SA10 (Figure). The primers SA9, SA10, SA13, SA14, SA17, SA18 and SA19 were those published by Suzuki et al. In the first pair, the primer pairs: sense SA9, 5′-(1208)CA-AGCACCGTTACTATCTGC(1227)-3′, and antisense SA15, 5′-(1208)CA-AGCACCGTTACTATCTGC(1227)-3′. For the detection of the 3′ end of the *mecR1* gene, the penicillin binding (PB) domain, we used the sense primer SA15 combined with the antisense primer SA17, with the sequences: SA15, 5′-(1208)CA-AGCACCGTTACTATCTGC(1227)-3′; and SA17, 5′-(1208)CA-AGCACCGTTACTATCTGC(1227)-3′. In the second reaction SA15 was used as sense with the antisense primer SA10 (Figure). The primers SA9, SA10, SA13, SA14, SA17, SA18 and SA19 were those published by Suzuki et al. 

**PCR conditions**

The DNA extract (600 ng) was amplified by PCR in a final volume of 50 µL, containing 0.25 mM of each dNTP, 40 pmol of each primer, 0.5 IU *Taq* DNA polymerase (Promega Corporation, Madison, WI, USA) and buffer provided by the manufacturer. The denaturation was performed for 1 min at 95°C, the annealing was for 1 min at 58°C for the detection of *mecA* gene and for the other reactions at 55°C, and the primer extension for 2 min at 72°C, with a total of 30 cycles. A sample of 10 µL from each reaction was analysed by gel electrophoresis in a 0.8% agarose gel with ethidium bromide. As molecular weight marker, δX174 digested by *Hae*III was used.

**Results**

**Determination of methicillin MIC and β-lactamase production**

The isolates were classified into two groups on the basis of their MIC levels. The first group included 31 *S. aureus*, 51
PCR, mec determinants and staphylococci

S. epidermidis and 11 other CNS for which methicillin MICs ranged from 4 to 128 mg/L. Higher methicillin MICs (>128 mg/L) were found in 18 S. aureus, 25 S. epidermidis and nine other CNS. The majority of isolates in both groups were β-lactamase producers with 39 S. aureus (79.6%), 64 S. epidermidis (84.2%) and 19 other CNS (95%) giving positive results with the nitrocefin test.

Detection of the mecA gene among S. aureus strains

Forty-nine S. aureus strains were identified as mecA positive, yielding PCR products with at least one pair of primers. Of these mecA-positive strains, 43 (87.8%) did not have alterations at the primer-binding sites, since the combinations P1–P3 and P2–P3 gave rise to DNA products of 1046 and 530 bp, respectively, as expected. Four strains (8.2%) showed alterations at the site of the primer P2, giving rise to positive reactions only with the P1–P3 pair. Finally, two strains (4%) presented alterations at the site of the primer P1 (positive PCR deriving only from the pair P2–P3) (Table I).

Detection of the mecA gene among S. epidermidis strains

With the mecA-positive S. epidermidis strains, 72 of 76 (94.7%) did not show any alterations of the specific regions with complementarity to the primers used. The specific DNA bands were detected in both reactions and were the same size as those seen with S. aureus. Three strains (4%) showed alterations at the annealing site of primer P2, while one strain (1.3%) showed alterations at the annealing site of primer P1 (Table I).

Detection of the mecA gene among the other CNS

From the 20 isolates in our collection, 14 (70%) carried intact the specific region of the mecA gene: positive reactions occurred with both pairs of primers and the DNA bands detected were of the same size as those of S. aureus. Five isolates (25%), comprising three S. hominis and two S. lugdunensis, carried the gene with alterations at the binding site of primer P2, while the isolate of S. xylosus showed alterations at the binding site of primer P1 (Table I).

Detection of mecR1–mecI genes among S. aureus strains

For the detection of the regulator genes mecR1–mecI eight primers were used, which were combined in five pairs (see Figure). All the strains were examined for the presence of the regulator region. From the 49 mecA-positive strains, 13 (26.5%) carried intact mecR1–mecI genes, at least with regard to the regions complementary to the primers used. The PCRs were positive with all primers and the DNA bands showed the expected sizes relative to the control strain. Seven isolates (14.3%) carried the whole mec regulator region with an alteration at the binding site of the primer SA15 and a positive result when using the pair SA9–SA10. Three isolates (6.1%) also carried the region which presented an alteration at the site complementary to the primer SA9, with a positive result when the pair SA15–SA10 was used and one strain (2.1%) revealed an alteration at the annealing site of primer SA17. From the remaining isolates, 11 (22.4%) carried only the mecR1 gene (deletion of the mecI gene and a positive result with the pair SA15–SA17), seven (14.3%) carried only the MS part of the mecR1 gene (positive PCR results when we used at least one of the pairs SA13–SA14 and SA18–SA19), and seven (14.3%) did not carry the mec regulator region at all (Table II). The PCR products were the same size as that of the control strain.

Detection of the mecR1–mecI genes among S. epidermidis strains

The distribution of the regulator genes in these strains was studied by the application of the same reactions. Forty-four of the 76 mecA-positive S. epidermidis isolates (57.9%) carried the whole regulator region without alterations in the binding sites for the primers used. Three isolates (3.9%) revealed alterations at the annealing site of primer

Table I. Detection of the mecA gene among clinical strains of staphylococci by the application of sense primers P1, P2 and antisense P3 in two PCRs (P1–P3 and P2–P3)

<table>
<thead>
<tr>
<th>Isolates</th>
<th>mecA PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive with primers P1–P3/P2–P3 no. (%)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>43 (87.8)</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>72 (94.7)</td>
</tr>
<tr>
<td>Other CNS</td>
<td>14 (70)</td>
</tr>
<tr>
<td>Total</td>
<td>129 (89)</td>
</tr>
</tbody>
</table>
SA15, six isolates (7.9%) at the site of primer SA9 and four isolates (5.3%) at the annealing site of primer SA17. Deletion of the mecl gene was detected in only one isolate (1.3%), while deletion of the PB domain of the mecR1 gene was detected in 16 isolates (21.1%). Two of the 76 isolates (2.6%) lacked the regulator gene (Table II). All the DNA bands detected were of the same size as those of S. aureus.

Detection of the mecR1–mecI genes among the other CNS

Two of the 20 mecA-positive CNS carried the whole regulator region without alterations. Eight isolates (40%) showed a deletion of the PB domain of the mecR1 gene and 10 isolates (50%) lacked the regulator genes (mecR1/mecI negative) (Table II). The PCR products were the same size as those produced with S. aureus.

Correlation of MIC levels with the presence of regulator genes

Twenty-five S. aureus isolates were found to be negative for the presence of mecl, 23 of them (46.9%) with lower MIC values (Table III). From the mecI-positive isolates, four of eight with lower MICs and seven of 16 with MICs > 128 mg/L, carried alterations at the PB domain of mecR1. Most of the S. epidermidis isolates were positive for the presence of mecl but 17 (22.4%) mecl-negative isolates had low MICs. Eight of the 34 isolates with low methicillin MICs and five of the 23 showing high methicillin MICs had alterations at the PB site of mecR1. Among the other CNS, only two isolates were found to carry the mecl gene and had low methicillin MICs, while none of these isolates had any alterations detected with the primers used.

Discussion

The mecA gene is highly conserved among staphylococcal species. Selection of primers for the amplification of the mecA gene is significant for the accuracy of test results.

The A+T content of mecA is high (c. 70%) and in order to minimize non-specific amplification of unrelated DNA regions, three primers were chosen to contain c. 50% G+C and were combined in two reactions in order to identify altered mecA DNA. The majority (89%) of this strain collection carried intact the mecA gene with the MRSE possessing mecA sequences that were more conserved (94.7%).

A minority (2.8%) of the isolates showed alterations at the annealing site of primer P1, including two MRSA. At the complementary region of primer P2, which includes the ClaI restriction site, more strains showed alterations, including four MRSA, three MRSE and five MRCNS other than S. epidermidis (25%). S. hominis and S. lugdunensis, which expressed this alteration, have c. 35% DNA relatedness. After digestion of their chromosomal DNA by ClaI and hybridization with mecA probe, all these strains showed two hybridization bands (data not shown). The MIC of methicillin for these isolates ranged from 8 to 500 mg/L. These findings lead us to conclude that the alterations in those sites are restricted without affecting the ClaI restriction sequence or the enzymic activity of PBP2a.

It has been reported that considerable variation in the presence of the other genes involved in the expression of methicillin resistance exists. The regulatory elements mecR1 and mecI have been detected in 60–95% of MRSA. Clinical isolates before 1970 had deletions of the PB domain of mecR1, while more recent isolates tend to have mecl polymorphism and mecA promoter mutations. Intact and fully functional mec regulator genes appear to strongly repress PBP2a production. In contrast, mecA-positive strains with deletions of the PB site of mecR1 and of the downstream mecl, express PBP2a constitutively, provided that the strain does not contain inducible β-lactamase and the regulatory elements blaR1–blal, which coregulate the enzyme production.

Previous studies have detected mecR1 and mecl among different species of MRCNS. Evidence that mutations in mecl play a role in derepression of mecA in CNS has not as yet been discovered.
Table II. Detection of alterations at the specific annealing sites of primers and deletions of mecR1–mecI regulator genes, detected by PCRs

<table>
<thead>
<tr>
<th>Isolates</th>
<th>mecR1/mecI intact no. (%)</th>
<th>mecR1/mecI deletion no. (%)</th>
<th>mecI alt. SA17 no. (%)</th>
<th>mecI alt. SA9 no. (%)</th>
<th>mecI alt. SA15 no. (%)</th>
<th>mecI del. PB deletion no. (%)</th>
<th>Total no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>13 (26.5)</td>
<td>11 (22.4)</td>
<td>1 (2.1)</td>
<td>3 (6.1)</td>
<td>7 (14.3)</td>
<td>7 (14.3)</td>
<td>49 (100)</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>44 (57.9)</td>
<td>1 (1.3)</td>
<td>4 (5.3)</td>
<td>6 (7.9)</td>
<td>3 (3.9)</td>
<td>16 (21.1)</td>
<td>76 (100)</td>
</tr>
<tr>
<td>Other CNS</td>
<td>2 (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>59 (40.7)</td>
<td>12 (8.3)</td>
<td>5 (3.5)</td>
<td>9 (6.2)</td>
<td>10 (6.9)</td>
<td>31 (21.3)</td>
<td>145 (100)</td>
</tr>
</tbody>
</table>

alt, alterations; del, deletions; PB, penicillin-binding domain.

Table III. Detection of regulator genes mecR1/mecI among different staphylococcal species in relation to the methicillin MIC of the strains

<table>
<thead>
<tr>
<th>Isolates</th>
<th>mecR1/mecI positive no. (%)</th>
<th>mecR1/mecI negative no. (%)</th>
<th>mecI positive no. (%)</th>
<th>mecI negative no. (%)</th>
<th>Total no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>4 (8.2)</td>
<td>23 (46.9)</td>
<td>9 (18.3)</td>
<td>7 (14.3)</td>
<td>49 (100)</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>26 (34.2)</td>
<td>17 (22.4)</td>
<td>18 (23.7)</td>
<td>5 (6.6)</td>
<td>76 (100)</td>
</tr>
<tr>
<td>Other CNS</td>
<td>2 (10)</td>
<td>9 (45)</td>
<td>0</td>
<td>0</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>32 (22.1)</td>
<td>49 (33.8)</td>
<td>27 (18.6)</td>
<td>13 (8.9)</td>
<td>145 (100)</td>
</tr>
</tbody>
</table>

alt. PB, alterations within the penicillin-binding domain of mecR1; mecR1/mecI(+), positive PCRs with the primers used for the regulatory region.
In this study, we tried to locate alterations or deletions of regulator genes among methicillin-resistant clinical isolates from a large teaching hospital and to correlate these findings with methicillin MIC and species, using a combination of several PCR primers. Deletion of mecl alone was identified in 11 (22.4%) MRSA and only one MRSE, while seven more MRSA and 16 MRSE were found with deletion of mecl and of the PB domain of mecR1, carrying only the MS part of the gene. Loss of both regulator elements was detected in seven MRSA, two MRSE and 10 of 20 MRCNS. It is interesting that the MRSE strains seem to be stable, while the majority of the other MRCNS (18 of 20) have deletions of mecl together with the PB domain of mecR1 or of both regulator genes. None of the MRCNS isolates was found to carry the PB domain with alterations, as far as we can ascertain from the primers used. The absence of regulator genes has been discussed for methicillin-resistant S. haemolyticus isolates.12,14

It has already been reported that the old epidemic MRSA carried only the mecA gene, but investigators considered that it was more likely that these strains lost their regulator genes by a deletional event after they had acquired the original mec component.14

According to the results presented, we postulate that MRSE seem to be the source of mec determinants in the hospital environment and that the other staphylococcal species have lost or altered parts of the genes after acquisition of mec elements. Allelic variation of chromosomal enzyme loci, as well as hybridization and sequence analysis, of strains isolated in different or in the same geographical areas, support the hypothesis of continuous horizontal gene transfer among staphylococci.12,14,33

Correlation of the deletions and alterations identified with the methicillin MICs revealed that 23 (46.9%) MRSA, even though mecl negative, had MICs = 128 mg/L. This fact might be partially explained by the possibility that these strains carry an inducible β-lactamase element, since 16 of them were β-lactamase producers and that mecA repression is due to the function of blaI.13 For the remaining seven isolates, which were β-lactamase negative, another mechanism must be responsible for the regulation of resistance.34,35 The presence of the regulatory genes with or without alterations at the PB domain of mecrI among 16 MRSA with high MICs (>128 mg/L) leads us to the conclusion that either the alterations at the PB domain are not essential for β-lactam binding at the protein, or that other mechanisms are also involved in the expression of high-level resistance.12,14,34,36

Among the MRSE group, 26 strains (34.2%) that carried intact the regulator genes and eight strains (10.5%) with alterations at the PB domain of mecrI, had lower MICs (≤128 mg/L) indicating a slow induction of mecA.35 Of the 17 mecl-negative isolates with low MIC, 16 were β-lactamase producers, leading us to the hypothesis that blar1-blaI could be responsible for the regulation of mecA.

These results can classify the MRSE strains, as pre-MRCNs, that are phenotypically susceptible (MIC 4–8 mg/L), mecA positive and are more common than pre-MRSA.11 Among the MRCNS other than S. epidermidis, the two strains that carry intact the regulatory elements had low MICs, as expected, while eight of the nine mecl-negative isolates expressing low MICs were β-lactamase positive. The remaining MRCNS (nine isolates) with high MICs lacked regulator genes (Table III).

In summary, this study has revealed the presence of pre-MRSA isolates that might be the reservoir of mec-related resistance determinants. The population of mecA-positive S. aureus strains seems to have emerged after acquisition of mec elements followed by genetic alterations. Evidence was also found that mecrI and mecl may be deleted often among the other staphylococcal species, and not only in S. haemolyticus. The expression of methicillin resistance is not only the result of interaction between mecA, mecrI and mecl, but is a more complex mechanism that involves several regulatory elements.

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References

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