A recently developed molecular method for typing methicillin-resistant Staphylococcus aureus (MRSA) spaA is based on the DNA sequencing of the protein A gene polymorphic X region (1,2). The polymorphic X region consists of a variable number of 24-bp repeats that appear to arise from the deletion and duplication of the repetitive units, and also by point mutation. A previous report showed that spaA typing is adequate for outbreak investigations, but should be complemented with other techniques for long-term surveillance or studies comparing distant clonal lineages (3). We here report the spaA types of MRSA isolates obtained from 39 inpatients and six outpatients at Nara Medical University Hospital, and from nine inpatients at two other hospitals nearby, over the 6-month period from March to August, 2002. Patient backgrounds were various, and various materials were used for MRSA isolation (urine, sputum, feces, etc.).

The polymorphic X region in the genomic DNA of MRSA was amplified by the polymerase chain reaction (PCR) with the previously designed set of primers 5’-AGACGATCCTTCGGTGAGC-3’ and 5’-CAGCAGTAGTGCCGTTTG-3’ (3). PCR was performed for 35 cycles (denaturation for 30 s at 95°C, annealing for 30 s at 60°C, and extension for 45 s at 72°C), with an initial denaturation for 10 min at 95°C, and a final extension for 10 min at 72°C. Direct sequencing of the PCR product was performed using the Thermo Sequenase Core Sequencing kit (Amersham Biosciences, Buckinghamshire, UK) in a Hitachi SQ5500E sequencer. For the sequence primer, the previously designed inner forward primer 5’-CAAGCACCAAAAGAGGAA-3’ (1) was labeled with Texas Red. The consensus sequences were expressed by the previously defined nomenclature for 24-bp repeat polymorphism (2).

The spaA typing of the MRSA isolates from patients in our hospital identified one frequent and six less frequent types (Table 1). In two other hospitals nearby, frequent type 1 and another less frequent type 8 were identified. Types 1, 2, and 3 shared the motif “MDMGMK”, which is found in the New York/Tokyo clone spaA type (TJMBMDMGMK) (3). This type was frequently recovered over a 2-year period from inpatients at the Mayo Clinic (4). Type 4 possessed the previously reported motif “BQBLO” (2,3). However, type 5 “JKPBPE”, type 6 “EJCMBPB”, and type 7 “JMEMDafGGK” had little similarity with any of previously reported spaA types (2,3). These MRSAs may be endemic to our area.

Type 7 was isolated from two patients with low-level
MRSA infections. The isolates showed a relatively low antibiotic resistance; i.e., they were able to grow on an oxacillin screening plate, though poorly. They were confirmed as MRSA by a latex agglutination test to detect penicillin-binding protein 2’ (PBP2’). This type contained a new 24-bp repeat sequence (described in the legend for Table 1) that has not been previously documented. Type 8 “MK”, found in a nearby hospital, was a novel type with a very short 24-bp repeat sequence. It is possible that this type could have been produced by a large deletion of the repetitive units from the frequent type.

The above data suggested that long-standing globally distributed MRSA clones including the frequent type 1 circulated in our hospital along with several area-specific MRSA. SpaA typing will prove useful in epidemiological studies of MRSA.

REFERENCES


Table 1. SpaA typing of MRSA isolates obtained from patients in our hospital and two nearby hospitals

<table>
<thead>
<tr>
<th>Number of type</th>
<th>Size of PCR products (bp)</th>
<th>SpaA type</th>
<th>In our hospital</th>
<th>Inpatients in a nearby hospital</th>
<th>Inpatients in another nearby hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inpatients</td>
<td>Outpatients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>392</td>
<td>JMBDMGMK</td>
<td>32</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>368</td>
<td>MBMDGMK</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>368</td>
<td>JMMDMGMK</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>416</td>
<td>GFMFMBOBLO</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>320</td>
<td>JFKBPE</td>
<td>2</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>344</td>
<td>EJCMDPB</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>392</td>
<td>JMEMDGK</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>224</td>
<td>MK</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
</tbody>
</table>

The PCR products except 5’-end 76 bp was sequenced. The SpaA type was expressed by the previously defined nomenclature for 24-bp repeat polymorphism (2). α denotes the new 24-bp repeat sequence as 5’-AAAAAAGACGGCAACAGCCTGGT-3’.