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Molecular Typing of Methicillin-Resistant *Staphylococcus aureus* by Protein A Gene Sequencing

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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′-AGACGATCCTTC GGTGAGC-3′ and 5′-CAGCAGTAGTGCCGTTTGC-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 “JFKBPE”, type 6 “EJCMBPB”, and type 7 “JMEMDαGGK” 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

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

Number of type	Size of PCR products (bp)	*SpaA* type	In our hospital		Inpatients in a nearby hospital	Inpatients in another nearby hospital
			Inpatients	Outpatients		
1	392	JMBMDMGMK	32	3	3	5
2	368	MBMDMGMK	–	1	–	–
3	368	JMMDMGMK	1	–	–	–
4	416	GFMFMBQBLO	1	–	–	–
5	320	JFKBPE	2	2	–	–
6	344	EJCMBPB	1	–	–	–
7	392	JMEMDαGGK	2	–	–	–
8	224	MK	–	–	–	1

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′-AAAAAAGACGGCAACAAGCCTGGT-3′.

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 MRSAs. *SpaA* typing will prove useful in epidemiological studies of MRSA.

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