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Molecular Epidemiology of a Methicillin-Resistant *Staphylococcus aureus* Infection

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Nosocomial infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious problem in immunocompromised hosts such as cancer patients (1).

In a ward for chemotherapy in a children's hospital with 40 beds, a patient with leukemia contracted acute dermatitis. MRSA (No.516) was isolated from the affected part of the body. The patient was repeatedly in and out of this and another hospital. MRSA had not been isolated from patients in the wards in the previous 2 years. However, nine MRSA isolates (Nos. 388-393 and 517-519) had been obtained from patients with carriage in other wards in the same hospital, before and during the time the patient was in the hospital. All the isolates were subjected to chromosomal DNA typing by using pulsed-field gel electrophoresis (PFGE) (CHEF Mapper™: Bio-Rad Laboratories, Hercules, Calif., USA), typing by length polymorphism of ribosomal DNA spacer regions by a polymerase chain reaction (PCR) assay (2), plasmid DNA typing by using agarose gel electrophoresis, antibiotic resistance (WalkAway™, Dade Behring, Deerfield, Ill., USA), enterotoxin serotyping (SET-RPLA: Denka Seiken Co., Tokyo), toxic shock syndrome toxin-1 (TSST-1) production (TST-RPLA: Denka Seiken), and coagulase serotyping (Denka Seiken).

As shown in Fig. 1, six different PFGE patterns of *Sma*I DNA digests were observed. Four *Bgl*I, four *Bst*XI and six *Cop*I PFGE patterns were detected (Fig. 2). As for ribosomal DNA spacer regions, bands with 220 bp, 270 bp and 300 bp were detected. Isolate No. 391 had all three bands, and isolate Nos. 518 and 519 had 270 and 300 bp bands (Fig. 3). Four different sized plasmids of 2 kb, 2.5 kb and 4.3 kb were detected (Fig. 4). Isolate Nos. 389 and 391 had no plasmid. Isolate Nos. 388, 390, 392, 393, 516 and 517 had a plasmid sized 2 kb. Isolate Nos. 518 and 519 had plasmids sized 2.5 kb and 4.3 kb. Sensitivity to antibiotics is shown in Table 1;

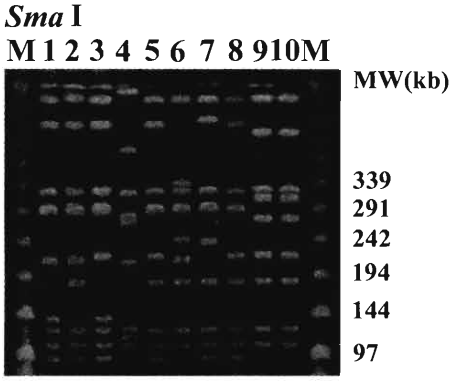

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates.
Lane 1-6: No.388-393 and Lane 7-10: No. 516-519, respectively.
M: low range PFG Marker.

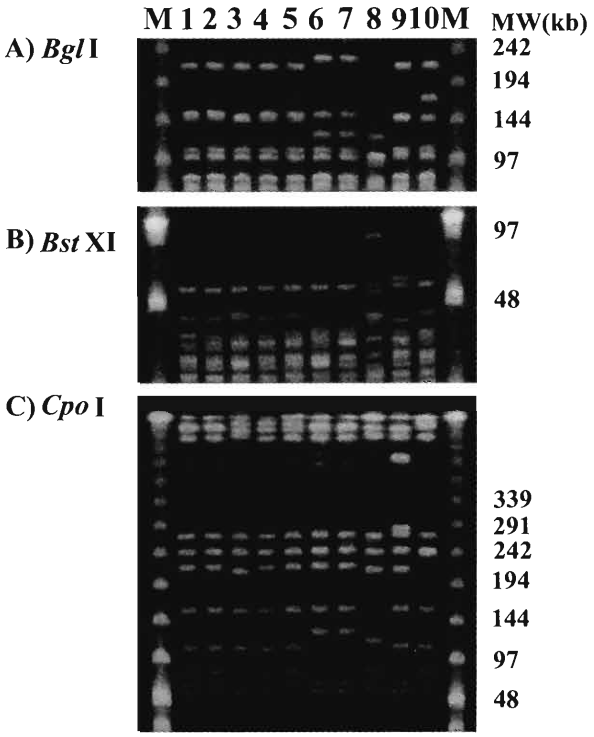

Fig. 2. Pulsed-field gel electrophoresis of genomic DNA. Genomic DNA was digested by *Bgl*I (A), *Bst*XI (B) or *Cpo*I (C). Lane 1: No. 388, Lane 2: No. 390, Lane 3: No. 389, Lane 4: No. 392, Lane 5: No. 517, Lane 6: No. 518, Lane 7: No. 519, Lane 8: No. 391, Lane 9: No. 393, Lane 10: No. 516. M: low range PFG Marker.

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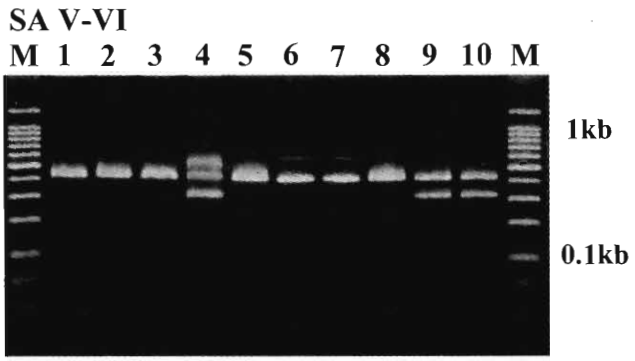

Fig. 3. Detection of *S. aureus*-specific ribosomal DNA spacer regions by PCR. DNA coding for *S. aureus*-specific ribosomal DNA spacer regions was amplified by PCR using a pair of oligonucleotide primers detecting *S. aureus* 16S ribosomal DNA and 23S ribosomal DNA regions (ref. 2, SA V and SA VI). Lane 1-6: No. 388-393 and Lane 7-10: No. 516-519, respectively. M: 100 bp DNA Ladder Marker.

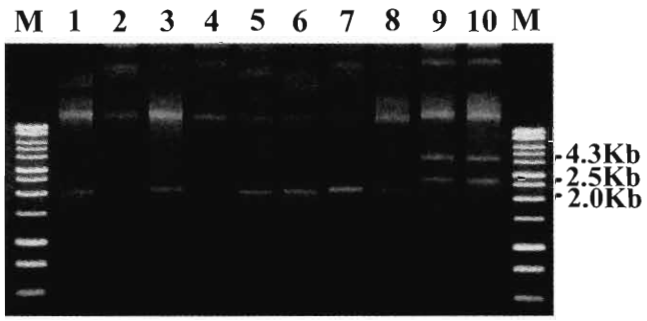

Fig. 4. Agarose gel electrophoresis of plasmid DNA.
Lane 1-6: No. 388-393 and Lane 7-10: No. 516-519, respectively.
M: 1 Kb DNA Ladder Marker.

Table 1. Antibiotic pattern classified by antibiotic pattern of 24 antibiotics against MRSA

Antibiotics pattern	Antibiotics listed in footnote#	PIPC	FOM	CLDM	EM	GM	MINO	AMK	ABK	RFP	ST	VCM
a	R	R	R	R	R	S	I	S	S	S	S	S
b	R	S	R	R	R	R	S	I	S	S	S	S
c	R	R	S	S	R	R	S	S	S	S	S	S
d	R	R	R	R	I	R	I	I	S	S	S	S
e	R	R	R	R	R	R	I	S	S	S	S	S

#Listed antibiotics are ampicillin, clarithromycin, cefditoren, cefazolin, cefpodoxime, cefpirome, cefotiam, cefotaxime, cefozopran, flomoxef, imipenem, oxacillin, benzyl-penicillin. PIPC: piperacillin, FOM: fosfomycin, CLDM: clindamycin, EM: erythromycin, GM: gentamicin, MINO: minocycline, AMK:amikacin, ABK: arbekacin, RFP: rifampicin, ST: sulfamethoxazole-trimethoprim, VCM: vancomycin, R: resistant, S: susceptible, I: intermediate

Table 2. Phenotypic and genotypic characterization of the *S. aureus* isolates

Isolate month (2000)	Isolate No.	Ward	Isolation part	PFGE				PCR with V&VI	Antibiotic pattern	Plasmid pattern	Enterotoxin	TSST-1	Coagulase type
				*Sma*I	*Bgl*I	*Bst*XI	*Cpo*I						
July	388	A	sputum	D	d	*A*	ϕ	1	a	1	C	+	II
	389	NICU	nasal cavity	C	d′	*A*	β	1	b	2	C	+	II
	390	A	sputum	D	d	*A*	ϕ	1	a	1	C	+	II
	391	B	trachea	F	c	*X*	δ	2	c	2	–	–	III
	392	NICU	skin	C	d	*A*	β	1	d	1	C	+	II
	393	B	nasal cavity	B	d	*Δ*	ε	1	a	1	C	+	II
September	516	C	skin	A	a	*A*	α	1	a	1	BC	+	II
	517	D	trachea	C	d	*A*	β	1	a	1	C	+	II
	518	NICU	carity	E	b	*B*	χ	3	e	3	BC	+	II
	519	B	nasal cavity	E	b	*B*	χ	3	a	3	BC	+	II

there were five different patterns. Isolate No. 391 produced none of enterotoxin types A to D. Isolate Nos. 388-390, 392, 393 and 517 produced enterotoxin type C. Isolate Nos. 516, 518 and 519 produced enterotoxin types B and C. Isolate No. 391 produced coagulase type III but no TSST-1; other isolates produced TSST-1 and coagulase type II (Table 2).

The data are summarized in Table 2. Isolate No. 516 from the child chemotherapy unit (ward C) was different from other isolates with respect to various markers. Isolate No. 388 from ward A was closely related to No. 390 from the same ward. Isolate No.518 from Neonatal Intensive Care Unit (NICU) resembled No. 519 from ward B. Two MRSA isolates from NICU, Nos. 389 and 392, were almost identical to each other, but isolate No. 518 which was isolated from the same ward 4 months later was distantly related to the two isolates.

In this study, PFGE analysis clearly showed that MRSA No. 516 isolated in the child chemotherapy ward was not introduced from other wards in the hospital (Table 2). The combination of PFGE with other genotypings and phenotypings appears to be useful for epidemiological analysis.

REFERENCES

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2. Saruta, K., Matsunaga, T., Kono, M., Hoshina, S., Ikawa, S., Sakai, O. and Machida, K. (1997): Rapid identification and typing *Staphylococcus aureus* by nested PCR amplified ribosomal DNA spacer region. FEMS Microbiol. Lett., 146, 271-278.