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Epidemiological Analysis of a Methicillin-Resistant *Staphylococcus aureus* Outbreak in Surgery Wards by Genomic DNA Polymorphisms

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Hospital infection, especially postoperative infection, caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious problem in surgical wards (1). Analysis of restriction fragment length polymorphisms of genomic DNA using pulsed-field gel electrophoresis (PFGE) is an essential tool for epidemiology of MRSA infection.

In a cardiac surgery ward in a hospital in Tokyo, two patients, P1 and P2, successively contracted severe sepsis. In an orthopedic ward in that hospital, one patient, P3, simultaneously contracted acute arthritis at a left knee joint. MRSA was isolated from the blood or the affected region of each

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patient. The isolates and isolates from carriers in the same wards during that period were tested for chromosomal DNA typing by using a contour-clamped homogeneous electric field system (CHEF Mapper™: Bio-Rad Laboratories, Hercules, Calif., USA), 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).

Seven different PFGE patterns of *Sma*I DNA digests (Fig. 1A), six patterns of *Bgl*I digests (Fig. 1B), and eight patterns of *Bst*X I digests (Fig. 1C) were detected. Sensitivity to antibiotics is shown in Table 1; three different patterns were observed. All isolates except No. 374 produced enterotoxin type C, TSST-1, and type II coagulase (Table 2). No. 374 produced neither enterotoxin nor TSST-1, but did produce type III coagulase (Table 2).

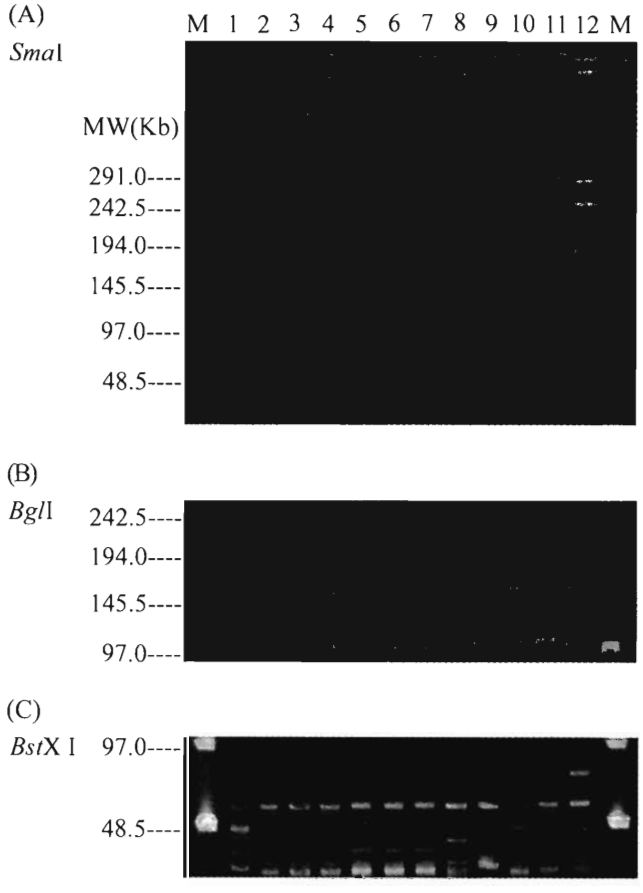

Fig. 1. Pulsed-field gel electrophoresis of genomic DNA from MRSA isolates. Upper panel: *Sma*I-digested genomic DNA; Middle panel: *Bgl*I-digested DNA. Lower panel: *Bst*X I-digested. Lane 1:MRSA isolate No. 374, lane 2: No. 375, lane 3: No. 378, lane 4: No. 356, lane 5: No. 358, lane 6: No. 360, lane 7: No. 379, lane 8: No. 382, lane 9: No. 417, lane 10: No. 418, lane 11: No. 426, lane 12: No. 465. M: low range PFG Marker. PFGE patterns are classified as A-G, *A*-*F*, and α-η, respectively, as shown in Table 2.

As summarized in Table 2, in patient P1, two different PFGE patterns of *Sma*I DNA digests were detected among twelve MRSA isolates obtained from different sources on different days. In patient P2, all eight MRSA isolates showed the same pattern (pattern A). Isolate No. 358 from patient P1, No. 357 from P2, and No. 379 from P3 showed the same PFGE pattern of *Sma*I DNA digests (pattern A), the same pattern of *Bgl*I digests (pattern *A*), the same pattern of *Bst*X I digests (pattern α), and the same spectrum of antibiotic susceptibility (pattern a). In an orthopedic surgery ward, another MRSA isolate was obtained from another patient (P4), in addition to P3. The PFGE patterns of the isolate (No. 382: patterns C*B*ε) were different from those of No. 379 from P3 (patterns A*A*α). In a cardiac surgery ward, 17 doctors and 21 nurses were working during the outbreak. MRSA surveys of personnel were performed, and detected three carriers, including a doctor (M3) and two nurses (M1 and M2). Isolate No. 375 from M2 showed the same PFGE patterns as those of No. 356 from P1 (patterns B*A*β), though the antibiotic resistance pattern was slightly different. Isolate No. 374 was different from other MRSA isolates in PFGE pattern, antibiotic susceptibility, and other biological properties. From the end of July through August, four additional MRSA isolates were obtained from three patients (P5, P6, and P7) in the cardiac surgery ward. The PFGE patterns were different from those of isolates from patients P1, P2, and P3.

The above events could be summarized as follows. The infections of patients P1 and P2 in the cardiac surgery ward and possibly that of P3 in the orthopedic surgery ward were caused by the same or closely related MRSA, given that the isolates shared the same molecular markers. During the same period, three medical staff in the cardiac surgery ward were found to be MRSA carriers. The isolates were, however, different from one another and also different from the isolates from patients P1, P2, and P3. Later, three more patients in the same ward were infected by MRSA, though the strains were distinct from any of the previous isolates. This finding suggests that new MRSAs were constantly introduced into the ward.

REFERENCE

1. Baltimore, R. S. (1998): Neonatal nosocomial infections. Semin. Perinatol., 22, 25-32.

Table 1. Antibiotic pattern classified by MIC pattern of 15 antibiotics against MRSA

Antibiotic pattern	Antibiotics listed in footnote#	EM	CLDM	LVFX	GM	MINO	ABK	ST	VCM	FOM
a	R	R	R	R	S	S	S	S	S	S
a′	R	R	R	R	S	I	S	S	S	S
a″	R	R	R	I	S	I	S	S	S	S
b	R	R	R	R	R	S	S	S	S	S
c	R	S	S	S	R	S	S	S	S	S

Listed antibiotics are benzyl-penicillin, ampicillin, piperacillin, cefazolin, cefotiam and imipenem/cilastatin.
EM: erythromycin, CLDM: clindamycin, LVFX: levofloxacin, GM: gentamicin, MINO: minocycline, ABK: arbekacin, ST: streptomycin, VCM: vancomycin, FOM: fosfomycin, R: resistant, S: susceptible, I: intermediate

Table 2. Phenotypic and genotypic characterization of the MRSA isolates

Patient (P)/ Medical personnel (M)	Symptoms	Isolation No.	Date of isolation	Isolation ward	Source	PFGE pattern (*Sma*I)	PFGE pattern (*Bgl*I)	PFGE pattern (*Bst*X I)	Antibiotic pattern	Enterotoxin pattern	TSST-1	coagulase type
P1	sepsis	358	June 19	cardiac surgery	catheter	A	*A*	α	a′	C	+	II
		356	June 19		sputum	B	*A*	β	a	C	+	II
		359	June 19		urine	A				C	+	II
		363	June 30		sputum	A				C	+	II
		364	June 30		nose	A				C	+	II
		370	July 5		nose	A				C	+	II
		371	July 5		sputum	A				C	+	II
		378	July 7		sputum	A				C	+	II
		380	July 21		sputum	A				C	+	II
		419	July 28		blood	A				C	+	II
		420	July 31		sputum	A				C	+	II
		421	July 31		nose	B				C	+	II
		427	August 4		sputum	A				C	+	II
P2	sepsis	357	June 19	cardiac surgery	blood	A	*A*	α	a	C	+	II
		360	June 19		surgical wound	A				C	+	II
		361	June 19		surgical wound(1)	A				C	+	II
		362	June 19		surgical wound(2)	A				C	+	II
		365	June 30		surgical wound(3)	A				C	+	II
		366	June 30		sputum	A				C	+	II
		372	July 3		sputum	A				C	+	II
		373	July 3		sputum	A				C	+	II
P3	arthritis	379	July 10	orthopedic surgery	knee joint	A	*A*	α	a′	C	+	II
P4		382	July 28		nose	C	*B*	ε	a′	C	+	II
M1		377	July 5	cardiac surgery	nose	B	*B*	δ	a	C	+	II
M2		375	July 5		nose	B	*A*	β	a″	C	+	II
M3		374	July 5		nose	G	*C*	χ	c	−	−	III
P5		417	July 28	cardiac surgery	nose	B	*D*	φ	a′	C	+	II
		465	August 21		nose	E	*F*	η	a′	C	+	II
P6		418	July 28		sputum	F	*E*	γ	a′	C	+	II
P7		426	August 1		nose	D	*A*	β	b	C	+	II