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An Outbreak of Enteritis Induced by Methicillin-Resistant *Staphylococcus aureus* Producing Enterotoxin Types A and C, Toxic Shock Syndrome Toxin-1 and Coagulase Type II

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common pathogens causing hospital infections (1). Enteritis caused by MRSA is a serious problem, especially for postoperative patients (2).

In a hospital with 420 beds, 4 patients successively contracted

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serious acute enteritis and 2 patients contracted mild enteritis. MRSA was isolated from the stools of the patients. The isolates from patients with MRSA infection or from its carriers in the same hospital just before the outbreak were tested for chromosomal DNA typing by using pulsed-field gel electrophoresis (PFGE) (CHEF Mapper™: Bio-Rad Laboratories, Hercules, Calif., USA), plasmid DNA typing on agarose gel electrophoresis, antibiotic resistance pattern (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).

Six different PFGE patterns of *Sma*I DNA digests (Fig. 1) were detected. A band-based cluster analysis of these patterns (Molecular Analyst™: Bio-Rad) revealed that patterns B to G shared more than an 89% similarity, while sharing a relatively lower similarity with pattern A (Fig. 2). There were three different kinds of plasmid with sizes of >10 kb, 5 kb, and 2 kb (Fig. 3). Twelve isolates had one, two, or three of them (from the carriage pattern of the plasmids, the isolates were classified into four groups), while six had none. Sensitivity to antibiotics is shown in Table 1; there were four different patterns. All isolates produced enterotoxin type C, TSST-1, and type II coagulase (Table 2). Numbers 407 and 414 produced enterotoxin type B, and Nos. 412, 416, 520, 521, 522, and 524 produced enterotoxin type C in addition.

As summarized in Table 2, four isolates (Nos. 520-522 and 524) from serious enteritis patients in either ward 5E or the ICU shared the same character with the one (No. 412) obtained before the outbreak. They all produced enterotoxin types A and C, TSST-1, and coagulate type II and had the same PFGE pattern of *Sma*I DNA digests, *Bgl*I digests and *Bst*XI digests (Fig. 4), the same spectrum of antibiotic susceptibility and the same plasmid patern (no plasmid). The

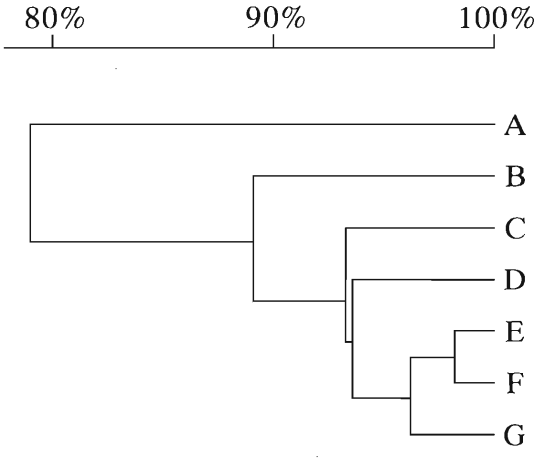

Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns of *Sma*I-digested genomic DNA.

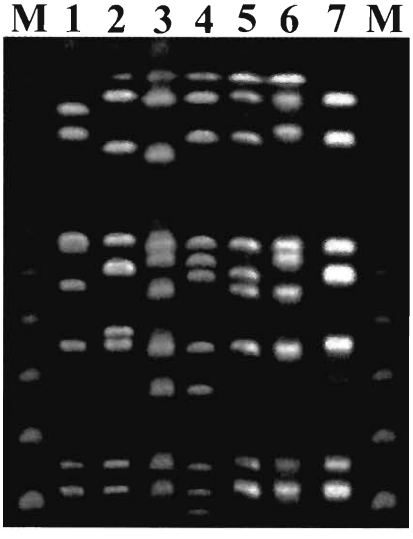

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates.
Lane 1: PFGE pattern A, lane 2: B, lane 3: C, lane 4: D, lane 5: E, lane 6: F, lane 7: G, M: low-range PFG Marker.

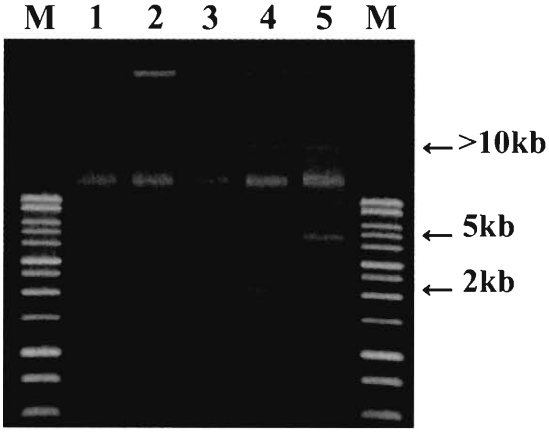

Fig. 3. Agarose gel electrophoresis of plasmid DNA.
Bands of four different sizes were found. A band of the second largest size was derived from fragments of genomic DNA and other bands from different plasmids. Lane 1: MRSA with no plasmid, lanes 2-5: plasmid patterns I-IV, respectively. M: molecular marker.

Table 1. Antibiotic pattern classified by antibiotic pattern of 18 antibiotics against MRSA

Antibiotics pattern	Antibiotics listed in footnote#	CLDM	GM	MINO	ABK	VCM	ST
a	R	R	S	I	S	S	S
a′	R	R	S	S	S	S	S
b	R	R	R	I	S	S	S
c	R	S	R	I	S	S	S
d	R	R	R	S	S	S	S

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

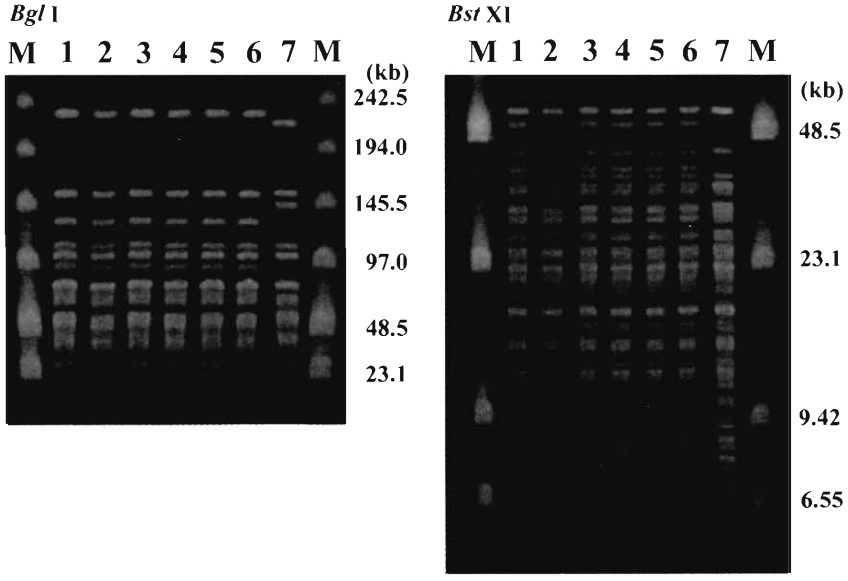

Fig. 4. Pulsed-field gel electrophoresis of *Bgl*I-digested or *Bst*XI-digested genomic DNA from MRSA isolates producing enterotoxins A and C.
Lanes 1 to 6: enterotoxin A/C producing MRSA Nos. 412, 416, 520-522, and 524. Lane 7: enterotoxin B producing MRSA No. 525. M: low range PFG Marker.

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

Month of isolation (2000)	Symptoms	No. of isolates	Ward	Isolation part	PFGE (*Sma*I) Pattern	Antibiotic pattern	Plasmid pattern	Enterotoxin	TSST-1	Coagulase type
July	carrier	407	3W	nasal cavity	C	b	I	BC	+	II
		408	4W	sputum	B	c	IV	C	+	II
		409	ICU	pharynx	C	d	I	C	+	II
		410	ICU	sputum	D	a	II	C	+	II
		411	3E	pus	B	c	III	C	+	II
		412	5E	abdomen	A	a	-	AC	+	II
		413	ICU	sputum	E	a'	-	C	+	II
		414	2W	sputum	F	a	I	BC	+	II
		415	5W	trachea	G	b	III	C	+	II
		416	6E	trachea	A	a'	I	AC	+	II
August	watery diarrhea	520	5E,ICU	feces	A	a	-	AC	+	II
	watery diarrhea	521	5E	feces	A	a	-	AC	+	II
	watery diarrhea	522	5E,ICU	feces	A	a	-	AC	+	II
	mild diarrhea	523	4W	feces	D	a	II	C	+	II
	watery diarrhea	524	ICU	feces	A	a	-	AC	+	II
	mild diarrhea	525	6W	feces	B	d	IV	BC	+	II

results indicated that an enterotoxins A and C- and TSST-1-producing MRSA strain caused enteritis in the outbreak. Toxin production is a useful marker in predicting MRSA enteritis outbreak.

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