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A Clonal Expansion of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in a Tuberculosis Ward

Masayoshi Hosoda, Junji Toyama, Kayo Hasada, Tomoko Fujino[1], Ryuji Ohtsuki[1] and Teruo Kirikae[1]*

Aichi Prefectural Owari Hospital, Ichinomiya, Aichi 491-0934 and
[1]International Medical Center of Japan, Toyama 1-21-1, Shinjuku-ku, Tokyo 162-8655

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important nosocomial pathogens, which are spread primarily by direct or indirect person-to-person contact. Genome typing using pulsed-field gel electrophoresis (PFGE) is a powerful tool for investigating the source, transmission, and spread of MRSA (1).

In August 2001, an MRSA outbreak occurred in a tuberculosis ward in a 350-bed hospital in the Chubu District. The tuberculosis ward had 50 beds with 32 regular medical staff members, including three doctors, 20 nurses, and two assistant nurses. Microbiologists in the hospital's Laboratory Department noticed that bacteria except for *Mycobacterium* spp., suspected of MRSA, were frequently isolated in sputum samples from tuberculosis patients, even when the samples were decontaminated for 15 min with the same volume of a 1:1 mixture of 4% sodium hydroxide and 2.9% sodium citrate supplemented with 12 mg/ml of N-acetyl L-cysteine prior to mycobacterial culture. After MRSA surveillance, 17 MRSA isolates were obtained from 15 inpatients.

These 17 isolates and four MRSA isolates obtained in other wards at the same time were tested for chromosomal DNA typing by using a contour-clamped homogeneous electric field system (CHEF Mapper™, Bio-Rad Laboratories, Hercules, Calif., USA), antibiotic susceptibility (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).

PFGE patterns of the *Sma*I DNA digests of the 21 isolates are shown in Fig. 1. Figure 2 shows the results of cluster band-based analysis of PFGE patterns (Molecular Analyst™, Bio-Rad) of these isolates together with all the MRSA isolates obtained from a hospital in Tokyo in October 2002 (1). Isolates from this hospital consisted of eight PFGE patterns; A2, A3, A9, M2, M7, Y3, AO, and J6. Isolates from the hospital in Tokyo consisted of 32 PFGE patterns, which included all the patterns found in the hospital in the Chubu

*Corresponding author: Fax: +81-3-3202-7181, E-mail: tkirikae@ri.imcj.go.jp

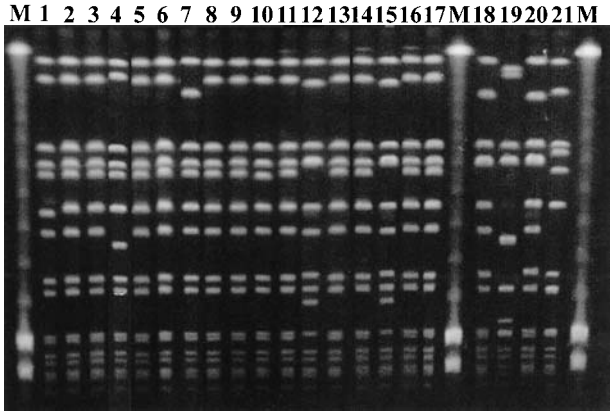

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. M: low range PFG Marker. Lanes 1 to 21are in the same order as the MRSA isolates listed in Table 2.

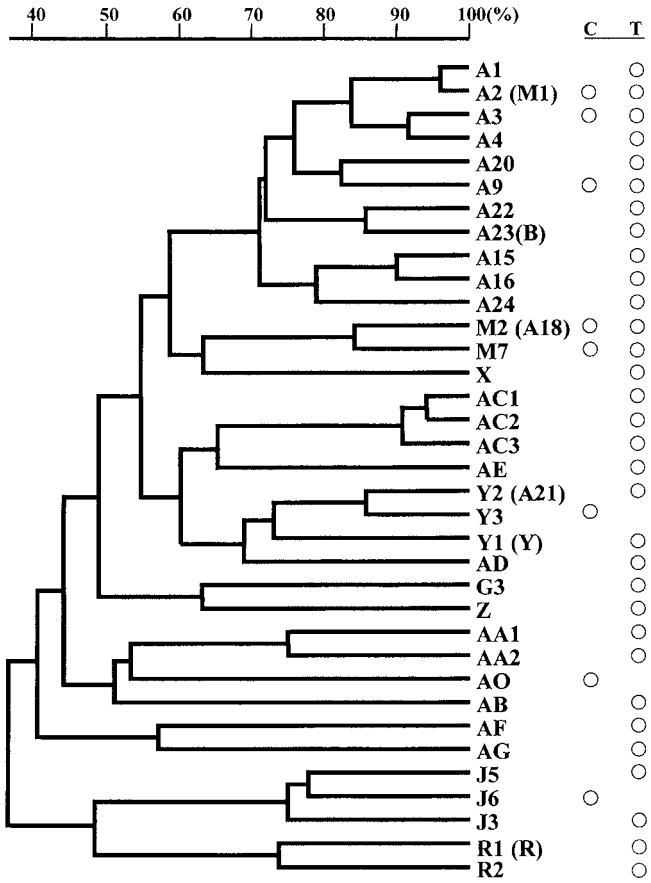

Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns. C: Chubu hospital, T: Tokyo hospital.

District, except for Y3, AO, and J6. In Fig. 2, we recognize 16 clusters, if patterns with more than a 70% similarity are postulated to form a cluster. In the hospital in Chubu, there were five clusters. In the hospital in Tokyo there were four clusters among the five detected in the hospital in Tokyo. These data indicate the clonal expansion of MRSA not only within hospitals but also nationwide.

Eight different antibiotic sensitivity patterns, a to h, were seen among Chubu isolates (Tables 1 and 2). Nineteen Chubu isolates produced enterotoxin type C, TSST-1, and coagulase type II (Table 2), while one strain (No. 1160) produced enterotoxin B and coagulase type II, but not produce TSST-1.

It was remarkable that 10 isolates from 11 inpatients in the tuberculosis ward (Nos. 1123, 1124, 1125, 1127, 1128, 1129, 1130, 1137-1, 1134, and 1159) had the same character, i.e., enterotoxin type C, TSST-1 positive, coagulase type II, the same PFGE patterns of *Sma*I DNA digests, A2(M1), and the same spectrum of antibiotic susceptibility, pattern f. Two other isolates, Nos. 1126 and 1133, had almost identical characters except for a minor difference in antibiotic susceptibility (Tables 1 and 2). Two other isolates from the tuberculosis ward (Nos. 1132 and 1137-2) had the same PFGE pattern, A3. Four isolates obtained from other wards at the same period had PFGE patterns and antibiotic resistance patterns different from those from the tuberculosis ward (Table 2). These results indicate that clonal expansion of MRSA occurred within the tuberculosis ward.

The main mode of transmission of MRSA is via hands, which may become contaminated by direct or indirect contact with colonized or infected persons. Most patients in a tuberculosis ward could exercise a higher level of daily activity as compared to other patients, and had a greater chance of direct or indirect contact with each other. This could have brought about the MRSA outbreak in the tuberculosis ward as in another case reported previously (2). Infection control measures against contact transmission as well as air-borne infection, including education to inpatients, are necessary in tuberculosis wards.

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

Antibiotic pattern	Antibiotics									
	FOM	TC	LVFX	MINO	AMK	GM	ST	ABK	TEIC	VCM
a	R	R	R	R	S	S	S	S	S	S
b	R	R	S	R	S	S	S	S	S	S
c	R	R	I	R	S	S	S	S	S	S
d	R	R	I	I	S	S	S	S	S	S
e	R	R	I	I	I	S	S	S	S	S
f	R	S	R	S	S	S	S	S	S	S
g	S	S	R	S	S	S	S	S	S	S
h	R	S	I	S	S	S	S	S	S	S

All the isolates were resistant to PCG, MPIPC, IPM, EM, CLDM, CCL, CMZ.
PCG: benzyl-penicillin, MPIPC: oxacillin, CEZ: cefazolin, IPM: imimenem, EM: erythromycin, CLDM: clindamycin, CCL: cefaclor, CMZ: cefmetazole, FOM: fosfomycin, TC: tetracycline, LVFX: levofloxacin, MINO: minocyclin, AMK: amikacin, GM: gentamicin, ST: trimethoprim/sulfamethoxazole, ABK: arbekacin, TEIC: teicoplanin, VCM: vancomycin, R: resistant, S: susceptible, I:intermediate.

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

Ward	Patient No.	Isolate No.	Colonization/ Infection	Specimen	PFGE pattern	Antibiotic patern	Enterotoxin	TSST-1	Coagulase
TB	P1	1122	Colonization	gastric juices	M2(A18)	f	C	+	II
	P2	1123	Colonization	sputum	A2(M1)	f	C	+	II
	P3	1124	Colonization	sputum	A2(M1)	f	C	+	II
	P4	1135	Colonization	sputum	AO	h	C	+	II
	P5	1125	Colonization	sputum	A2(M1)	f	C	+	II
	P6	1126	Colonization	sputum	A2(M1)	g	C	+	II
	P7	1136	Colonization	sputum	M7	f	C	+	II
	P8	1127	Colonization	sputum	A2(M1)	f	C	+	II
	P9	1128	Colonization	sputum	A2(M1)	f	C	+	II
	P10	1129	Colonization	sputum	A2(M1)	f	C	+	II
	P11	1130	Colonization	sputum	A2(M1)	f	C	+	II
	P12	1132	Colonization	sputum	A3	d	C	+	II
	P13	1133	Colonization	sputum	A2(M1)	h	C	+	II
	P14	1137-1	Colonization	sputum	A2(M1)	f	C	+	II
	P14	1137-2	Colonization	sputum	A3	c	C	+	II
	P15	1134	Colonization	sputum	A2(M1)	f	C	+	II
	P15	1159	Colonization	sputum	A2(M1)	f	C	+	II
Other	P16	1150	Infection	pharynx	A9	d	C	+	II
	P17	1151	Colonization	pharynx	J6	a	C	+	II
	P18	1152	Infection	wound	A9	e	C	+	II
	P19	1160	Colonization	sputum	Y3	b	B	–	II

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