Laboratory and Epidemiology Communications

Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in a Tokyo Hospital in 2000

Tomoko Fujino, Namiko Mori, Akihiko Kawana, Hisashi Kawabata, Tadatoshi Kuratsuji, Koichiro Kudo, Oichirou Kobori, Yoshio Yazaki and Teruo Kirikae*

International Medical Center of Japan, Toyama 1-21-1, Shinjuku-ku, Tokyo 162-8655

Communicated by Hiroshi Yoshikura

(Accepted June 4, 2001)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen in healthcare facilities (1). Epidemiological analysis of whole MRSA isolates present in a hospital, including restriction fragment length polymorphisms of genomic DNA using pulsed-field gel electrophoresis (PFGE), is essential for assessment of hospital infection controls (2).

Two-hundred-forty-six MRSA isolates were obtained from 74 inpatients in December 2000 in a Tokyo hospital with 27 wards and 925 beds. Among these isolates, 50 isolates, each derived from a single patient, were analyzed for chromosomal DNA typing by using a contour-clamped homogeneous electric field system (CHEF Mapper™, Bio-Rad Laboratories, Hercules, Calif., USA), for 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). In the present study, the isolates showing the same PFGE patterns were considered to have the same strain of MRSA.

Twenty-nine different PFGE patterns of *Sma*I DNA digests were detected (Fig. 1). A band-based cluster analysis of these patterns (Molecular Analyst™, Bio-Rad) revealed that there existed eight clusters of patterns A to I when defined as one cluster of patterns with more than a 70% similarity (Fig. 2A). Patterns B to H showed more than a 50 % similarity to pattern A, and pattern I showed less similarity with others (Fig. 2A). The frequency distribution of MRSA isolates based on PFGE patterns is shown in Fig. 2B. The most frequent pattern (A1) and the second-most frequent one (A2) represented 16% and 10% of the total isolates, respectively. The most frequent cluster of patterns (A1 to A11) made up 56% of the total isolates. A geographic distribution of MRSA isolates in the hospital is shown in Table 1. The isolates with PFGE pattern A was spread over most of wards in the hospital and two isolates with the same PFGE pattern A existed in wards such as 7N and 7S. All isolates with pattern B were from 4N (n = 1) and 4S (n = 3) which are a pediatric ward and an obstetrics

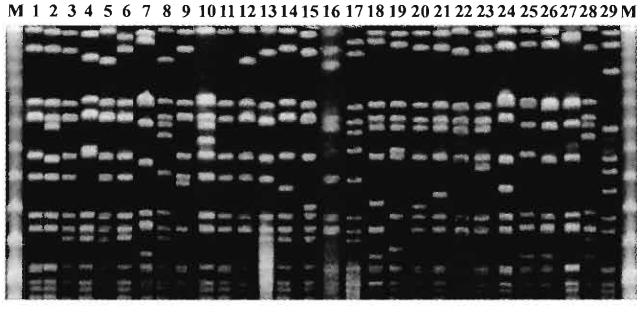

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. M: low range PFG Marker. Lanes 1 to 29: MRSA isolates with different PFGE patterns A1 to I shown in Fig. 2.

*Corresponding author: Fax: 81-3-3202-7364, E-mail: tkirikae@ri.imcj.go.jp

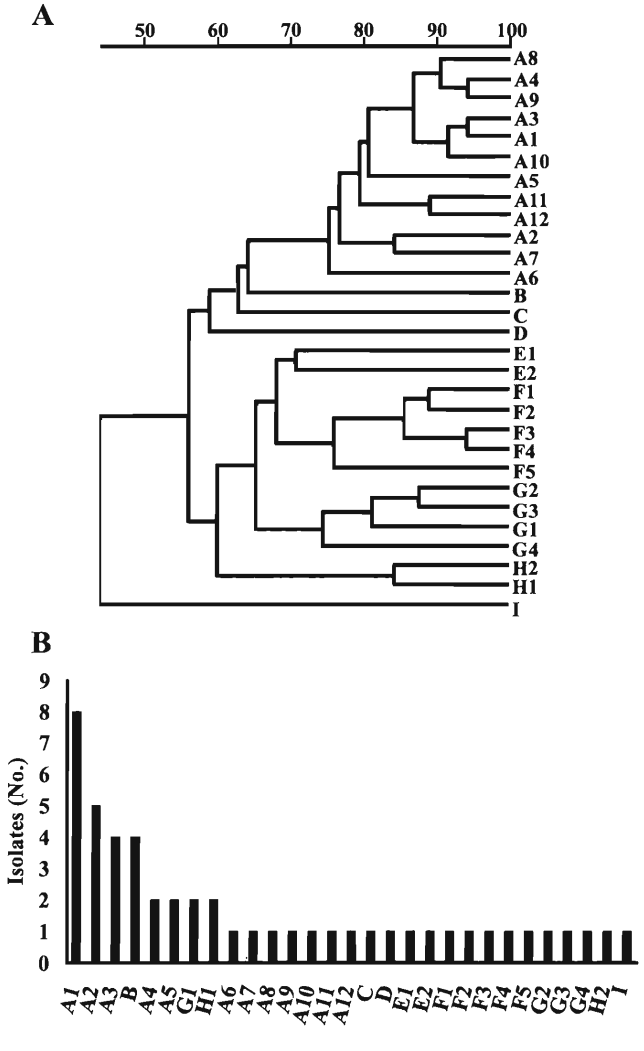

Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns.

Table 1. Distribution of MRSA in a hospital

Ward	PFGE pattern																										
	A												B	C	D	E		F					G				H		I
	1	2	3	4	5	6	7	8	9	10	11	12				1	2	1	2	3	4	5	1	2	3	4	1	2	
4N				1#									1																
4S				1									3																
5N								1						1															
5S		1																											
6N			1																										
6S																1													
7N	2																												
7S	2																												
8N																											1		
8S		1																											
9N	1				1										1														
9S																										1			
10N	1																	1										1	
10S			1																										
11N	1	1								1							1			1			2				1		
11S			1																										
12S					1																								1
13N																			1		1			1					
13S		1																											
14			1						1		1	1																	
15	1					1																1							
16		1					1																		1				

#Number of patients with MRSA

Table 2. Antibiotic pattern classfield by antibiotic pattern of 17 antibiotics against MRSA

Antibiotic pattern	Antibiotics								
	EM	LVFX	CLDM	FOM	GM	ABK	MINO	VCM	ST
a	R	R	R	R	R	R	I	S	S
b	R	I	R	R	R	R	S	S	S
c	R	R	R	R	R	S	I	S	S
d	R	R	R	R	R	S	S	S	S
e	R	I	R	R	R	S	I	S	S
f	R	R	R	I	R	S	S	S	S
g	R	I	R	R	R	S	S	S	S
h	R	S	R	R	R	S	S	S	S
i	R	R	S	R	R	S	S	S	S
j	R	R	R	R	S	S	I	S	S
k	R	R	R	R	S	S	S	S	S
l	R	I	R	R	S	S	I	S	S
m	R	I	R	R	S	S	S	S	S
n	R	R	R	S	S	S	I	S	S
o	R	R	R	S	S	S	S	S	S
p	R	R	R	I	S	S	S	S	S
q	R	R	S	S	R	S	S	S	S
r	S	R	R	R	S	S	I	S	S
s	S	R	S	S	R	S	S	S	S

#All the isolates were resistant to PCG, MPIPC, ABPC, CEZ, CTM, CFDN, FMOX, IPM.
PCG: benzyl-penicillin, MPIPC: oxacillin, ABPC: ampicillin, CEZ: cefazolin, CTM: cefotiam, CFDN: cefdinir, FMOX: flomoxef, IPM: imipenem/cilastatin, EM: erythromycin, LVFX: levofloxacin, CLDM: clindamycin, FOM: fosfomycin, GM: gentamicin, ABK: arbekacin, MINO: minocycline, VCM: vancomycin, ST:streptomycin, R: resistant, S: susceptible, I: intermediate.

and gynecology ward, respectively. Isolates with pattern F were from 10N, 11N, 13N, and 15, and isolates with pattern G were from 9S, 11N, 13N, and 16.

Sensitivity to antibiotics is shown in Table 2. The MRSA isolates had a wide spectrum of drug-resistance showing 19 different patterns. Those isolates were resistant to 10-15 of 17 tested drugs. No isolate was resistant either to vancomycin or streptomycin. No isolate with a specific antibiotic pattern spread in a specific ward. No correlation was found between the antibiotic patterns and PFGE patterns (data not shown).

Among 50 MRSA isolates, 49 isolates produced coagulase type II, and one produced an unknown type of coagulase. Forty-three isolates produced enterotoxin type C, four isolates enterotoxin types B and C, one enterotoxin type A, one enterotoxin type B, and one enterotoxin other than types A, B, C, and D. Forty-eight isolates produced TSST-1, but 2 did not produce it. Collectively, among 50 MRSA isolates, 42 isolates produced coagulase type II, enterotoxin type C, and TSST-1.

PFGE analysis revealed that clonal expansion of MRSA isolates occurred in a hospital. Phenotyping based on antibiotic sensitivities and other biological properties was found to be an unsuitable method of assessing MRSA isolates in healthcare facilities.

REFERENCES

1. Baltimore, R. S. (1998): Neonatal nosocomial infections. Semin. Perinatol., 22, 25-32.
2. Tharavichitkul, P., Fujino, T., Mori, N., Kaneko, A., Kirikae, F., Sirisanthana, T. and Kirikae, T. (2001): Molecular epidemiology of methicillin-resistant *Staphylococcus* aureus in a hospital in Chiang Mai. Jpn. J. Infect. Dis., 54, 82-83.