Jpn. J. Infect. Dis., 57, 2004

Laboratory and Epidemiology Communications

Prevalence of Erythromycin-, Tetracycline-, and Aminoglycoside-Resistance Genes in Methicillin-Resistant *Staphylococcus aureus* in Hospitals in Tokyo and Kumamoto

Jun-ichiro Sekiguchi, Tomoko Fujino, Katsutoshi Saruta, Hisami Konosaki, Haruo Nishimura, Akihiko Kawana, Koichiro Kudo, Tatsuya Kondo, Yoshio Yazaki, Tadatoshi Kuratsuji, Hiroshi Yoshikura[1] and Teruo Kirikae*

International Medical Center of Japan, Tokyo 162-8655 and
[1]National Institute of Infectious Diseases, Tokyo 162-8640

Communicated by Hiroshi Yoshikura

(Accepted March 12, 2004)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of their emerging resistance to all current antibiotic classes. Investigating the spread of the drug-resistance genes in MRSA is important for the control of its dissemination (1).

In our previous papers (2-4), a total of 179 MRSA clinical isolates obtained in December 2000, October 2001, and October 2002 from a hospital with 24 wards and 925 beds in Tokyo were assessed using restriction fragment length polymorphism (RFLP) of genomic DNA using pulsed-field gel electrophoresis (PFGE). A band-based cluster analysis of the PFGE patterns of these isolates revealed that 111 of the 179 MRSA isolates formed a cluster of PFGE patterns, called cluster A.

Chromosomal DNA was typed by using a contour-clamped homogeneous electric field system (CHEF Mapper™, Bio-Rad Laboratories, Hercules, Calif., USA). Plasmid DNA was typed by using agarose gel electrophoresis. The antibiotic resistance of MRSA to tetracycline (TC) was analyzed using WalkAway™ (Dade Behring, Deerfield, Ill., USA) and E-test™ (AB BIODISK, Dalvagen, Sweden). PCR was used to detect gentamicin (GM)-resistance genes [*aac6′-aph2″* and *aph*(*3′*)-*III*], erythromycin (EM)-resistance genes (*ermA*, *ermB*, and *ermC*), and TC-resistance genes (*tetK* and *tetM*), while Southern blot used to detect *aac6′-aph2″*, *ermA*, and *tetM*. Some of the PCR products were sequenced for confirmation. Based on these analyses, the isolates were classified into 33 types (Table 1).

Among the 111 MRSA isolates tested, all were resistant to EM, 13 were resistant to GM, and 102 were resistant to TC. The majority of the isolates (97 of 111) were resistant to EM and TC, but sensitive to GM. No isolates were sensitive to all three antibiotics (Table 1). PFGE of *Sma*I digests (Fig. 1A) revealed 23 different patterns. The most frequent pattern was A1, representing 31.5% of the total isolates (Table 1). The profiles of plasmid typing are shown in Fig. 2A. Plasmids of 27 different sizes, ranging from 2.4 kb to 300 kb, were detected. The isolates were classified into 26 plasmid patterns (Table 2). One-hundred-eight of 111 isolates had one or more different-sized plasmids. Three other isolates had no plasmids. Seventy-three isolates accounting for 68% of the total had plasmid pattern I, II, or III, and these isolates had both 50 kb and 35 kb plasmids (Table 2). Among the isolates with PFGE pattern A1, 34 had 50 kb plasmid. Among them, eight had plasmid pattern I, seven had plasmid pattern II, 10 had plasmid pattern III, one had plasmid pattern X, and eight had plasmid pattern XXIV.

The results of PCR analysis are summarized in Table 1. Among the 111 MRSA isolates, 13 were PCR-positive for *aac6′-aph2″*, all isolates were positive for *ermA*, and 103 were positive for *tetM*. No isolate was positive for *aph*(*3′*)-*III*, *ermB*, *ermC*, or *tetK*. The majority of the isolates (103 of 111) were positive for the genes *ermA* and *tetM*, but negative for the others. Twelve isolates with type Nos. 10-12, 24, 28, and 33 were positive for *aac6′-aph2″*, *ermA*, and *tetM*. One isolate with No. 4 was positive for *ermA* and *aac6′-aph2″*, and three with Nos. 3, 7, and 17 were positive for *ermA*.

Southern blotting detected *aac6′-aph2″* on 30 kb, 38 kb, 190 kb, or 200 kb plasmids carried by six isolates and on the chromosomes of 12 isolates. On the chromosomes, it was present in 110 kb and 220 kb *Sma*I fragments (two isolates), in a 220 kb *Sma*I fragment (one isolate), and in a 500 kb *Sma*I fragment (five isolates) (Fig. 1 and Table 1). The *ermA* was found on the chromosomes of all the isolates, mostly on 220 kb and 580 kb *Sma*I fragments. The *tetM* was found on the chromosomes of 104 isolates, mostly in the 290 kb *Sma*I fragment.

The PCR analysis gave data consistent with resistance pattern of the bacteria in all the cases except three, which were types Nos. 2, 5, and 14. An isolate of type No. 2 was sensitive to GM, resistant to EM, and intermediately resistant to TC, while negative for *aac6′-aph2″*, but positive for *ermA* and *tetM* in PCR. An isolate of type No. 5 was resistant to GM and EM, but sensitive to TC, while being PCR-negative for *aac6′-aph2″* and *tetM*, but positive for *ermA*. An isolate of type No. 14 was sensitive to GM and TC, but resistant to EM, while PCR-negative for *aac6′-aph2″*, but positive for *ermA* and *tetM*. This discordance may probably be brought about by mutations in the coding or promoter region of the PCR-detected genes.

Among 111 MRSA isolates obtained from a hospital in Tokyo and whose PFGE patterns showed A clusters, 34 isolates showing PFGE pattern A1 were sensitive to GM, and

*Corresponding author: Mailing address: International Medical Center of Japan, Toyama 1-21-1, Shinjuku-ku, Tokyo 162-8655, Japan. Fax: +81-3-3202-7364, E-mail: tkirikae@ri.imcj.go.jp

Table 1. PFGE pattarns of MRSA isolates; MICs of GM, EM and TC from these isolates; and distribution of GM-, EM-, and TC-resistance genes among these isolates

Typing no.[1)]	PFGE pattern[2)]	No. of isolates in			MIC(μg/ml) of			PCR product							Southern blot		
		2000	2001	2002	GM	EM	TC	A[3)]	B	C	D	E	F	G	*aac6′-aph2″*	*ermA*	*tetM*
															plasmid/chromosome (kb)		
1	A1	7	10	17	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
2		0	0	1	<1(S)	>4(R)	5(I)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
3	A2(M1)	2	0	0	<1(S)	>4(R)	≦4(S)	–	–	+	–	–	–	–	—/—	—/220, 580	—/—
4		1	0	0	>8(R)	>4(R)	≦4(S)	+	–	+	–	–	–	–	38, 200/110, 220	—/220, 580	—/—
5		0	1	0	>8(R)	>4(R)	≦4(S)	–	–	+	–	–	–	–	—/500	—/220, 580	—/—
6		1	0	0	>8(R)	>4(R)	≦4(S)	+	–	+	–	–	–	–	—/500	—/220, 580	—/—
7		1	0	0	<1(S)	>4(R)	≦4(S)	–	–	+	–	–	–	–	—/—	—/220, 580	—/—
8		0	0	1	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/580
9	A3	3	8	0	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
10		0	1	0	>8(R)	>4(R)	≧16(R)	+	–	+	–	–	–	+	30, 38/—	—/220, 580	—/290
11		1	0	0	>8(R)	>4(R)	≧16(R)	+	–	+	–	–	–	+	—/220	—/220, 580	—/290
12		0	0	1	>8(R)	>4(R)	≧16(R)	+	–	+	–	–	–	+	—/500	—/220, 580	—/290
13	A4	2	7	4	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
14		0	1	0	<1(S)	>4(R)	≦4(S)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
15	A5	2	1	0	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 550	—/290
16	A6	1	0	0	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
17	A7	1	0	0	<1(S)	>4(R)	≦4(S)	–	–	+	–	–	–	–	—/—	—/220, 580	—/—
18	A8	1	0	0	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	–	—/—	—/220, 580	—/680
19	A9	1	3	1	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/100, 220, 580	—/290
20	A10	1	0	0	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 630	—/290
21	A11	1	1	0	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
22	A12	1	0	0	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
23	A13	0	1	0	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
24	A14	0	1	0	>8(R)	>4(R)	≧16(R)	+	–	+	–	–	–	+	—/500	—/220, 580	—/290
25	A15	0	2	1	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
26	A16	0	1	4	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
27	A17	0	1	0	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
28	A18(M2)	0	1	4	>8(R)	>4(R)	≧16(R)	+	–	+	–	–	–	+	—/500	—/210, 590	—/70, 590
29	A20	0	0	1	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
30	A21	0	0	1	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 230, 580	—/290
31	A22	0	0	2	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 580	—/290
32	A23	4	0	1	<1(S)	>4(R)	≧16(R)	–	–	+	–	–	–	+	—/—	—/220, 550	—/290
33	A24	0	0	1	>8(R)	>4(R)	≧16(R)	+	–	+	–	–	–	+	38, 190/110, 220	—/220, 580	—/290

[1)]: Typing no. is corresponding to the lane No. shown in Fig. 1.
[2)]: The PFGE patterns was reported in ref 2, 3, and 4.
[3)]: A: *aac6′-aph2″*, B: *aph(3′)-III*, C: *ermA*, D: *ermB*, E: *ermC*, F: *tetK*, G: *tetM*.

resistant to EM and TC. They had *ermA* in 220 kb and 580 kb *Sma*I chromosomal digests and *tetM* in a 290 kb *Sma*I chromosomal digest, but they did not have plasmids harboring *ermA*, *tetM*, or any other of the drug-resistance genes tested. Previous studies (5,6) showed that MRSA isolates having the PFGE pattern A1 were wide spread in hospitals in Tokyo and in Kumamoto. Both the Kumamoto and the Tokyo isolates had *ermA* in 220 kb and 580 kb *Sma*I chromosomal fragments and *tetM* in 290 kb *Sma*I fragments. However, their antibiotic resistance patterns were different (1). Most Kumamoto isolates were resistant to GM, EM, and TC; they had a multidrug resistant 40 kb plasmid harboring *aac6′-aph2″*, *ermA*, and *tetM*, and 200 kb plasmid harboring *aac6′-aph2″*. They also had *aac6′-aph2″* in a 110 kb *Sma*I chromosome fragment. The Tokyo isolates, meanwhile, were found to be GM-sensitive, and had no 40 kb or 200 kb plasmids and no *aac6′-aph2″* in their chromosomes. In summary, there appears to have been a clonal expansion of closely related MRSAs in hospitals in Tokyo and in Kumamoto, but the MRSA in Kumamoto appeared to have recently acquired the GM-resistance gene, *aac6′-aph2″*, which was not found in the Tokyo isolates.

REFERENCES

1. Sekiguchi, J., Fujino, T., Saruta, K., Kawano, F., Takami, J., Miyazaki, H., Kuratsuji, T., Yoshikura, H. and Kirikae, T. (2003): Spread of erythromycin-, tetracycline-, and aminoglycoside-resistance genes in methicillin-resistant *Staphylococcus aureus* clinical isolates in a Kumamoto hospital. Jpn. J. Infect.Dis., 56, 133-137.
2. Fujino, T., Mori, N., Kawana, A., Kawahata, H., Kuratsuji, T., Kudo, K., Kobori, O., Yazaki and Kirikae, T. (2000): Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a Tokyo hospital in 2000. Jpn. J. Infect. Dis., 54, 91-93.
3. Fujino, T., Mori, N., Kawana, A., Naiki, Y., Kawahata, H., Kuratsuji, T., Kudo, K., Kobori, O., Yazaki and Kirikae, T. (2001): Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a Tokyo hospital in 2001. Jpn. J. Infect. Dis., 54, 240-242.
4. Fujino, T., Sekiguchi, J., Kawana, A., Konosaki, H.,

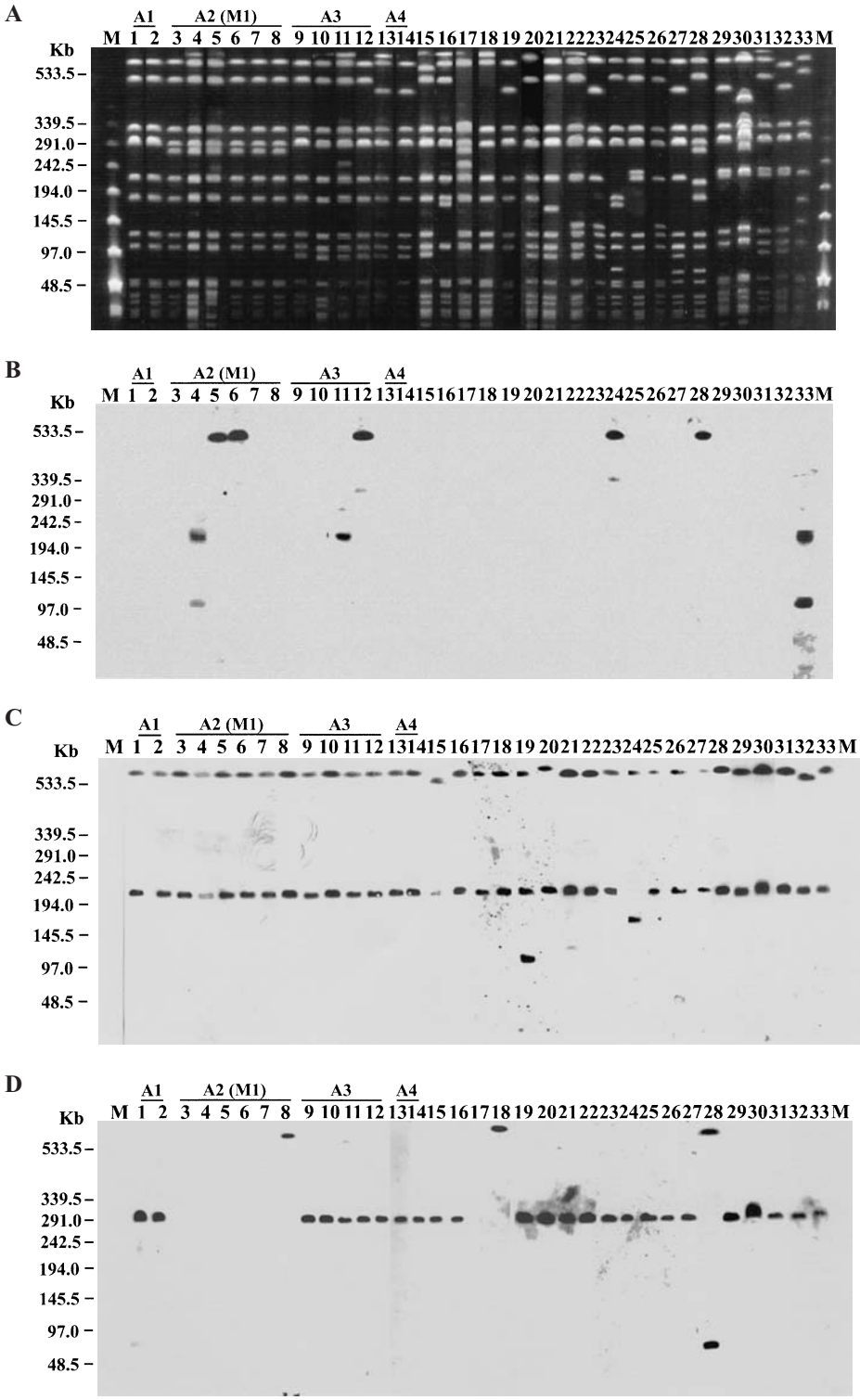

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates (A) and Southern blotting hybridized with *aac6′-aph2″* (B), *ermA* (C), and *tetM* (D). M: low range PFG Marker. Lanes 1 to 33: Lane Nos. is corresponding to the typing Nos. of MRSA isolates listed in Table 1.

Nishimura, H., Saruta, K., Kudo, K., Kobori, O., Yazaki, Y., Kuratsuji, T. and Kirikae, T. (2002): Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a Tokyo hospital in 2002. Jpn. J. Infect.Dis., 55, 210-213.

5. Kawano, F., Miyazaki, Y., Takami, J., Fujino, T., Saruta, K. and Kirikae, T. (2002): Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a Kumamoto hospital in 2001. Jpn. J. Infect. Dis., 55, 29-30.
6. Kawano, F., Miyazaki, H., Takami, J., Fujino, T., Sekiguchi, J., Saruta, K., Kuratsuji, T. and Kirikae, T. (2003): Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a Kumamoto hospital in 2002. Jpn. J. Infect. Dis., 56, 129-132.

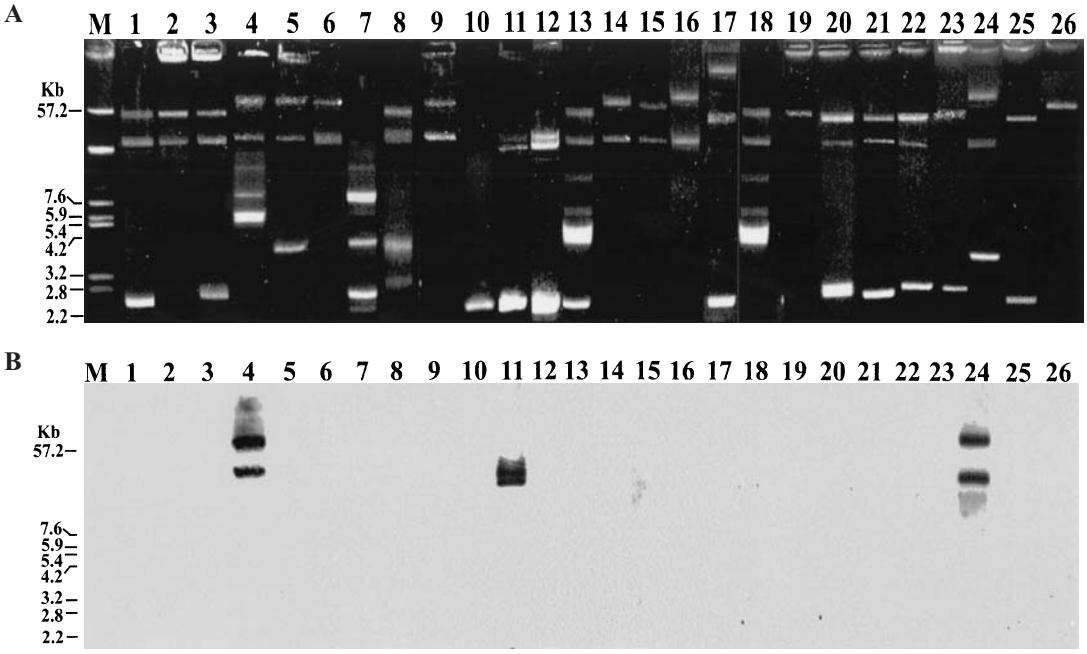

Fig. 2. Agarose gel electrophoresis of plasmid DNA from MRSA isolates (A) and Southern blotting hybridized with *aac6′-aph2″* (B). M: Marker plasmid derived from *E. coli* V517. Lanes 1 to 26: Lane Nos. is corresponding to the plasmid typing Nos. of MRSA isolates listed in Table 2.

Table 2. Plasmid typing pattern classified by plasmid size and its frequency

Plasmid pattern	Frequency in			Plasmid size (kbp)																									
	2000	2001	2002	300	200	190	180	170	160	90	85	50	40	38	35	33	30	11	10	8	6	5.5	4.5	4	3.5	2.9	2.8	2.7	2.6	2.4
I	10	26	0									○			○														○
II	6	3	7									○			○														
III	7	0	14									○			○														○	
IV	1	0	0		●[1]									●					○	○	○	○								
V	1	0	0		○[2]										○										○					
VI	1	0	0		○										○															
VII	1	0	0																○	○				○					○	○
VIII	1	0	0									○			○										○	○				
IX	1	0	0							○			○																	
X	0	3	0																											○
XI	0	1	0											●			●													○
XII	0	1	0										○			○														○
XIII	0	1	0									○			○			○			○		○	○						○
XIV	0	1	0					○					○																	
XV	0	1	0						○				○																	
XVI	0	1	0				○						○																	
XVII	0	1	0	○								○																○		
XVIII	0	1	0									○			○			○			○		○	○						
XIX	0	0	1									○																		
XX	0	0	3									○			○											○	○			
XXI	0	0	1									○			○												○			
XXII	0	0	1									○			○											○				
XXIII	0	0	1									○														○				
XXIV	0	0	1			●								●											○					
XXV	0	0	9									○																	○	
XXVI	0	0	1								○																			

[1]: Plasmid harboring *aac6′-aph2″*.
[2]: Plasmid not harboring any of the drug-resistant genes tested.