Jpn. J. Infect. Dis., 58, 250-252, 2005

Short communication

Molecular Typing of Methicillin-Resistant *Staphylococcus aureus* by PCR-RFLP and Its Usefulness in an Epidemiological Study of an Outbreak

Noriaki Mitani, Akira Koizumi, Reiko Sano, Takayuki Masutani, Koichi Murakawa, Keiichi Mikasa[1] and Yasuyuki Okamoto*

Central Clinical Laboratory and [1]Center for Infectious Diseases, Nara Medical University, Nara 634-8522, Japan

(Received November 30, 2004. Accepted May 30, 2005)

SUMMARY: A new convenient molecular typing method, simultaneous polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis, for three different genes of methicillin-resistant *Staphylococcus aureus* (MRSA) was evaluated using 35 isolates of MRSA and comparing results with those previously reported for sequencing-based *spa* typing. Twenty-nine isolates of the most frequent protein A (*spa*) type were discriminated into 6 different types by PCR-RFLP. In contrast, *spa* typing could discriminate only 1 of the 19 most frequent PCR-RFLP-type isolates. The discriminatory powers of the two methods were equal for the other isolates. These results suggest that PCR-RFLP has the advantages of both relative easiness and greater discriminatory power than *spa* typing. We also report the case of a suspected outbreak in which PCR-RFLP was sufficient for ruling out the possibility of an outbreak. Thus, PCR-RFLP is preferable as a preliminary screening method for epidemiological studies of nosocomial infection caused by MRSA.

Molecular typing plays a crucial role in epidemiological studies of nosocomial infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Although there are various methods of molecular typing, pulsed-field gel electrophoresis (PFGE) is considered to be the most discriminatory and reliable. However, this method is technically complex, time-consuming and expensive. Hence, it is desirable to find a preliminary screening method that is rapid, simple and highly discriminatory, and that could make PFGE unnecessary. We have previously discussed protein A gene sequencing (*spa* typing), which is a candidate method for the present purposes (1). Following our previous report, we evaluated a new convenient typing method, simultaneous polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis, for three different genes (2), and compared the obtained results with those previously reported for *spa* typing. We describe our results herein, and also report a suspected case of outbreak in which PCR-RFLP was useful for epidemiological study.

Thirty-five MRSA isolates obtained from 26 inpatients who were in our hospital during an 8-month period from March to October 2002 and from 9 inpatients in two nearby hospitals during the same period were used for this comparative study. The backgrounds of the patients (ward, sex, age, etc.) were randomized to reduce the possibility of contact transmission, and various materials were used for MRSA isolation (urine, sputum, feces, etc.). These isolates were the same as those used in our previous study (1). In addition, four isolates of methicillin-susceptible *Staphylococcus aureus* (MSSA) were used. The genes of the *mec*A-associated hypervariable region (HVR), protein A (*spa*), and coagulase (*coa*) in the genomic DNA of MRSA were simultaneously amplified by PCR using the three sets of primers previously reported by Wichelhaus et al. (2). PCR was performed for 35 cycles (denaturation for 1 min at 94°C, annealing for 1 min at 56°C, and extension for 3 min at 72°C), with an initial denaturation for 4 min at 94°C and a final extension for 5 min at 72°C. After digestion with the restriction enzyme *Hha*I at 37°C for 3 h, the PCR products were separated on a 10-20% gradient polyacrylamide gel and stained with ethidium bromide. We used *Hha*I instead of *Hae*II, which was used by Wichelhaus et al. (2), because the sequence which *Hha*I recognizes (5′-GCGC-3′) includes the sequence recognized by *Hae*II (5′-RGCGCY-3′).

As shown in Fig. 1, PCR-RFLP analyses of HVR, *spa* and *coa*, which were detected in the 35 MRSA and 4 MSSA isolates, demonstrated 4 (H1-H4), 8 (S1-S8) and 6 (C1-C6) patterns, respectively. Furthermore, combinations of these PCR-RFLP patterns were able to identify 10 types (R1-R10) of MRSA and 3 types (RS1-RS3) of MSSA (Table 1). The R1 and R4 types of MRSA were found to be relatively frequent. Table 1 also shows a comparison between the two different typing methods, PCR-RFLP and *spa* typing. The most frequent *spa* type, M1, was discriminated into 6 different types by PCR-RFLP (R1-R5 and R10). In contrast, *spa* typing was able to discriminate only one isolate from 19 isolates of the frequent PCR-RFLP type R1. For the other isolates of MRSA, the discriminatory powers of the two methods were equal. These results suggest that PCR-RFLP, which can be carried out more easily, has greater discriminatory power than *spa* typing, though sequencing-based *spa* typing is superior in its precision.

In a suspected case of a nosocomial outbreak, three different methods of molecular typing, PCR-RFLP, *spa* typing and *Sma*I-PFGE, were used for epidemiological study. New isolates of MRSA were detected from 11 patients in two different surgical wards over a period of a few weeks, and molecular typing was carried out (Table 2). In the present epidemiological study, we identified three new types of MRSA

*Corresponding author: Mailing address: Central Clinical Laboratory, Nara Medical University, Shijo-cho 840, Kashihara, Nara 634-8522, Japan. Tel: +81-744-22-3051, Fax: +81-744-22-4810, E-mail: yasuyuki@naramed-u.ac.jp

detected by PCR-RFLP (R11, R12 and R13) (Table 1) and one new type detected by *spa* typing (M9). S9 and C7 (shown in Fig. 1) are new RFLP patterns. M9 is represented as "JMBMGK" following the notation system used in our previous report (1). As shown in Fig. 2, PFGE discriminated seven different patterns (P1 - P7). Although *spa* type M4 is known to be rare (1), the M4 isolates from four patients (X2 - X5) in Ward X were considered to be of the same strain since the results from the three typing methods were consistent. The same strain was also isolated from the nasal discharge of a medical staff member associated with Ward X. These results are strongly suggestive of an outbreak. Nevertheless, each of the isolates from our 6 patients on Ward Y was identified as a different strain based on the combination of results from the three typing methods. In the case of Ward Y, it is noteworthy that PCR-RFLP showed a discriminatory power higher than that of *spa* typing and comparable to that of PFGE.

Although other methods may provide more precise discrimination of frequent types, PCR-RFLP is preferable as a preliminary screening method because it is rapid, simple, highly discriminatory, and occasionally sufficient for ruling

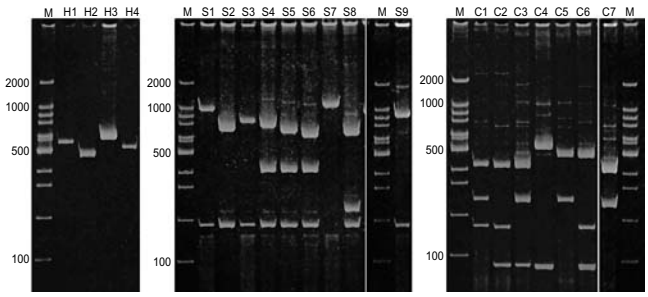

Fig. 1. PCR-RFLP patterns of HVR, *spa*, and *coa*. H1 - H4 , S1 - S9, and C1-C7 indicate the pattern names of HVR, *spa*, and *coa*, respectively. M, molecular marker. Molecular sizes (in bases) are indicated on the left.

Table 1. Comparison between RCR-RFLP-based typing of MRSA and MSSA, and sequencing-based *spa* typing

	RFLP typing	RFLP pattern			*spa* typing									No. of isolates
		HVR	*spa*	*coa*	M1	M2	M3	M4	M5	M6	M7	M8	M9	
MRSA	R1	H1	S1	C1	18		1							19
	R2	H1	S1	C3	1									1
	R3	H1	S1	C5	1									1
	R4	H2	S1	C1	7									7
	R5	H4	S1	C1	1									1
	R6	H1	S2	C4					2					2
	R7	H1	S3	C1								1		1
	R8	H3	S4	C5				1						1
	R9	H1	S5	C2						1				1
	R10	H2	S7	C1	1									1
	R11	H1	S1	C7	(2)									(2)
	R12	H1	S6	C1							(1)			(1)
	R13	H1	S9	C1									(1)	(1)
				Total	29(2)	0	1	1	2	1	(1)	1	(1)	35(4)
MSSA	RS1	n.d.	S4	C2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1
	RS2	n.d.	S6	C6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2
	RS3	n.d.	S8	C6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1
													Total	4

R1 - R13 and RS1 - RS3 indicate the type names of MRSA and MSSA, respectively. *spa* typing (M1 - M8) has been described in our previous report (Ref. 1). M9 is a new type. R1 - R10 and M1 - M8 were identified among 35 collected isolates. R11 - R13 and M9 were newly identified among four isolates detected in a suspected outbreak (Table 2). Numbers of isolates belonging to one of these new types are indicated in parentheses. n.d., not determined.

Table 2. Molecular typing of MRSA isolates detected in a suspected outbreak

Ward	Patient	Sample	PCR-RFLP typing	*spa* typing	PFGE pattern
X	X1	Sputum	R11	M1	P2
	X2	Pus	R4	M3	P1
	X3	Pus	R4	M3	P1
	X4	Pus	R4	M3	P1
	X5	Pus	R4	M3	P1
Y	Y1	Drainage	R11	M1	P4
	Y2	Drainage	R12	M7	P6
	Y3	Drainage	R4	M1	P3
	Y4	Drainage	R1	M1	P3
	Y5	Venous catheter	R13	M9	P5
	Y6	Drainage	R4	M1	P7

Each designation of P1-P7 indicates a different *Sma*I-PFGE pattern.

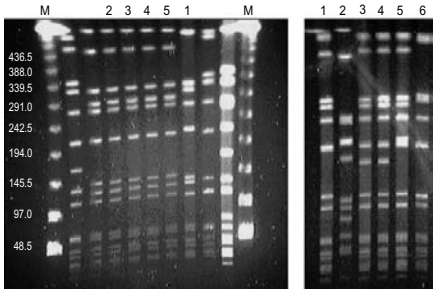

Fig. 2. *Sma*I-PFGE patterns of MRSA isolates in a suspected case of outbreak. Numbers on the top of the left and the right figures indicate 5 patients in Ward X (X1-X5) and 6 patients in Ward Y (Y1-Y6), respectively. M, molecular marker. Molecular sizes (in kilo bases) are indicated on the left.

out the possibility of an outbreak.

REFERENCES

1. Mitani, N., Ohnishi, M., Masutani, T., Murakawa, K. and Okamoto, Y. (2002): Molecular typing of methicillin-resistant *Staphylococcus aureus* by protein A gene sequencing. Jpn. J. Infect. Dis., 55, 179-180.
2. Wichelhaus, T. A., Hunfeld, K-P., Böddinghaus, B., Kraiczy, P., Schäfer, V. and Brade, V. (2001): Rapid molecular typing of methicillin-resistant *Staphylococcus aureus* by PCR-RFLP. Infect. Control Hosp. Epidemiol., 22, 294-298.