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Comparison of Pulsed-Field Gel Electrophoresis and PCR Analysis of Polymorphisms on the *mec* Hypervariable Region for Typing Methicillin-Resistant *Staphylococcus aureus*

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Two hundred fifty-four methicillin-resistant *Staphylococcus aureus* (MRSA) strains typed by pulsed-field gel electrophoresis (PFGE) were tested by PCR for the *mec*-associated hypervariable region (HVR-PCR) to determine their number of direct repeat units (DRUs). Eight different groups of repeats were found among the MRSA strains and compared to 28 pulsotypes classified by PFGE. Some MRSA strains belonging to the same pulsotype showed different numbers of DRUs. HVR-PCR was rapid, easy to perform, and reproducible and has the ability to obtain an unambiguous positive result for each isolate analyzed. However, this technique shows a discriminatory power inferior to that of PFGE. We conclude that PFGE is a more reliable method of typing MRSA than HVR-PCR.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as an important cause of hospital-acquired infections worldwide (3, 7). Owing to high mortality and increased cost of treatment of MRSA infections (4), the control of these infections must be improved by using molecular typing methods. The ideal technique must be rapid, easy to perform, reproducible, and capable of demonstrating differences between strains of MRSA with great discriminatory power (2). Toward that end, several molecular methods have been used with varying success (16, 18). Among the techniques put forward for typing MRSA, pulsed-field gel electrophoresis (PFGE) of *Sma*I restriction fragments has been the one most applied in epidemiological studies (1, 12, 18). PFGE has proven itself to be robust enough to type strains with great resolution, is highly reproducible (5), and is considered the "gold standard" technique for typing MRSA (16). However, PFGE is time-consuming and requires both specialized electrophoretic equipment and software. A new PCR-based method has been proposed for typing MRSA which appears to be easier and more rapid than other molecular methods (14). The proposed method is PCR based and relies upon the length of polymorphisms of the hypervariable region of the staphylococcal methicillin resistance gene (*mec*) for strain resolution. The *mecA* gene is responsible for the intrinsic resistance of MRSA to all beta-lactam antibiotics (Fig. 1) (6, 11, 14). The DNA sequence between IS*431mec* and *mecA* is called a hypervariable region (HVR) because of the lengths of the polymorphisms of different staphylococcal isolates (15). HVR is composed of direct repeat unit elements (DRUs), each of 40 bp (Fig. 1). The proposed method (14) is based on the length polymorphisms of HVR-PCR products among different staphylococcal isolates. In order to assess the applicability of this method, a comparative study between the HVR-PCR method and PFGE seemed to be required. Thus, in the present study, we tried to address the correlation between methods by using a PFGE-typed MRSA collection.

Two hundred fifty-four MRSA strains isolated from Porto Alegre (south of Brazil) and Montevideo (Uruguay) hospitals between 1996 and 1998 were used in this study. PFGE analysis of macrorestriction patterns with *Sma*I was performed as described previously (5, 9). Dendrograms were constructed by using the Dice coefficient (8) and the unweighted pair group method with arithmetic average (13). Interpretation of PFGE patterns was based on the previously proposed criteria for restriction patterns produced by PFGE (17). The HVR-PCR assay was performed as described by Nishi et al. (14) with modifications. DNA preparations from single MRSA colonies cultured overnight at 37°C in Luria agar were suspended in 500 μl of TE buffer (10 mmol of Tris-HCl per liter, 1 mmol of EDTA per liter; pH 8.0), centrifuged, washed, and resuspended in 100 μl of TE buffer. Lysostaphin (4 U; Sigma, St. Louis, Mo.) was added, and the resulting solution was incubated at 37°C for 30 min. Samples were then boiled for 10 min and diluted in TE buffer to 500 μl for storage at 4°C. The primers used for amplification of the *mec*-associated HVR were 5′-ACTATTCCCTCAGGCGTCC-3′ (HVR1; 338 to 356) and 5′-GGAGTTAATCTACGTCTCATC-3′(HVR2; 892 to 912) (14). DNA extract (2 μl) was added to a 48-μl PCR mixture containing 0.25 mmol of each deoxynucleoside triphosphate, 2 U of *Taq* polymerase (Cenbiot, Porto Alegre, Rio Grande do Sul, Brazil), 50 mmol of KCl per liter, 10 mmol

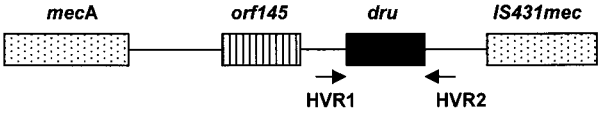

FIG. 1. Representation of the *mecA* determinant, showing the positions of *mecA*, *orf145*, the DRU region, and *IS431*, according to Ryffel et al. (15). Arrows show the region of primers designed for PCR.

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TABLE 1. Comparison of the number of DRUs obtained by HVR-PCR and the pulsotypes (PFGE)

Pulsotype	No. of strains showing no. of DRUs								
	3	5	7	8	9	10	11	12	Total
A		1	11	33		68	16		129
B						6	4	3	13
F				4		2	1		7
G				2		2	15	1	20
H			2	6		13	1		22
I						3			3
J					3	4			7
K				3	2	1			6
L	2								2
M							2		2
N						2			2
O			1			2			3
P							3		3
Q				1		1			2
R						1	1		2
S						2			2
T							2		2
U				1			1		2
V			1			2			3
NC[a]			2	1		8	10		21
Total	2	1	17	51	5	118	56	4	254

[a] NC, strains not clustered by PFGE.

of Tris-HCl (pH 9.0) per liter, 1.5 mmol of $MgCl_2$ per liter, and MilliQ water to a 50-μl final volume. Each sample was subjected to an initial step of 5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C, in a PTC 200 thermocycler (MJ Research, Inc., Watertown, Mass.). The amplicons were run in a 3% agarose gel with ethidium bromide and recorded in an UV-Gel Doc system (Bio-Rad). A 50-bp DNA ladder (Gibco BRL Products) was used to estimate the amplicon sizes.

PFGE distinguished 19 MRSA pulsotypes, and 21 strains were not clustered. HVR-PCR showed 8 types, with 3, 5, 7, 8, 9, 10, 11, and 12 DRUs. All the MRSA strains generated amplification fragments ranging from 290 to 650 bp, indicating a 100% capacity to obtain interpretable results by the HVR-PCR method. The number of HVR was estimated according to the following formula: number of DRUs = (amplicon base pair length − 171)/40, where 171 is the number of base pairs not repeated inside the amplified sequence and 40 is the number of base pairs present in each repetitive unit fragment. The reproducibility was assayed as follows: two MRSA strains were amplified several times, as internal controls, showing constant amplification fragment sizes in all reactions. MRSA strains with 10 DRUs were predominant in this study, present in 118 cases (46%), representing 52.7% of pulsotype A, 46% of pul-

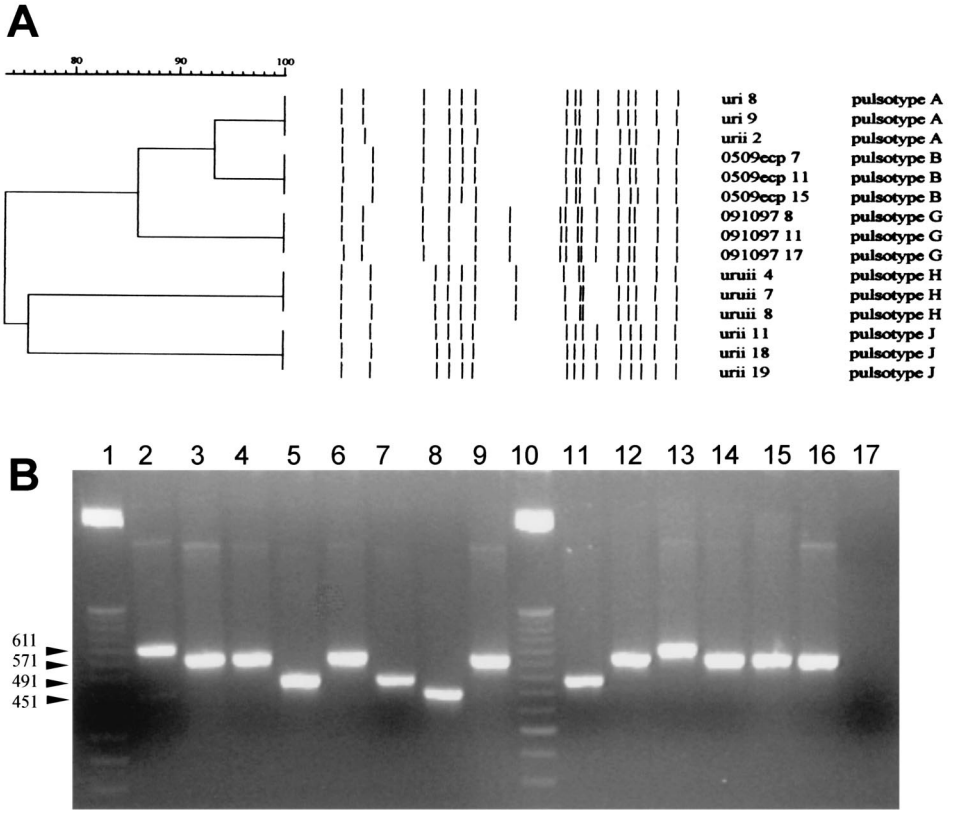

FIG. 2. Representation of some MRSA strains typed by PFGE and HVR-PCR. (A) Dendrogram showing estimates of the percent similarities among some of the principal pulsotypes identified by PFGE. (B) Gel electrophoresis showing amplicons generated by HVR-PCR. Estimated sizes of the generated amplicons are on the left. Lanes 1 and 10, molecular markers (50 bp); lane 2, 0509ecp 7, pulsotype B (11 DRUs); lane 3, 0509ecp 11, pulsotype B (10 DRUs); lane 4, 0509ecp 15, pulsotype B (10 DRUs); lane 5, urii 2, pulsotype A (8 DRUs); lane 6, uri 8, pulsotype A (10 DRUs); lane 7, uri 9, pulsotype A (8 DRUs); lane 8, urii 4, pulsotype H (7 DRUs); lane 9, urii 7, pulsotype H (10 DRUs); lane 11, urii 8, pulsotype H (8 DRUs); lane 12, 091097 8, pulsotype G (10 DRUs); lane 13, 091097 11, pulsotype G (11 DRUs); lane 14, 091097 17, pulsotype G (10 DRUs); lane 15, urii 11, pulsotype J (10 DRUs); lane 16, urii 18, pulsotype J (10 DRUs); lane 17, negative control.

sotype B, 59% of pulsotype H, and 38% of the pulsotypes not clustered. The distribution of the remaining MRSA strains shows that 56 (22%) and 51 (20%) exhibit 11 and 8 DRUs, respectively. Among the 129 strains classed by PFGE as pulsotype A, 68 showed 10 DRUs, 33 showed 8 DRUs, 16 showed 11 DRUs and 11 showed 7 DRUs (Table 1). These results show that there is no good correlation between the results obtained by PFGE and HVR-PCRs. Some MRSA strains typed by PFGE and HVR-PCR are shown in Fig. 2A and B, respectively. It can be seen that the HVR-PCR method failed to equal the discriminatory power of PFGE, in particular for differentiating between epidemic and sporadic (strains classed as not clustered by PFGE) MRSA strains. Several isolates with identical pulsotypes showed different patterns with HVR-PCR. On the other hand, HVR-PCR and PFGE methods show Gaston and Hunter discrimination indices (10) of 0.697 and 0.726, respectively. However the combined use of both methods increase the index to 0.899. Our results are consistent with those obtained by Schmitz et al. (16), who analyzed 183 MRSA strains typed by PFGE, RAPD, 16S-23S rDNA, protein A PCR, HVR-PCR, and coagulase gene PCR and reported similar results with PFGE and HVR-PCR. They concluded that HVR-PCR was more discriminant only than the coagulase gene PCR. In spite of the fact that the HVR-PCR method appears to be reproducible, rapid, easy to perform, and capable of demonstrating differences between all MRSA strains analyzed, our results showed that it exhibits a lower discriminatory power than the PFGE method. Although PCR-HVR should not be totally discarded, we conclude that the use of this method to type MRSA strains is not warranted.

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