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Usefulness of PCR-Restriction Fragment Length Polymorphism Typing of the Coagulase Gene To Discriminate Arbekacin-Resistant Methicillin-Resistant *Staphylococcus aureus* Strains▽

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We compared the results of two typing methods for 678 strains of methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *S. aureus*. PCR-restriction fragment length polymorphism typing of the coagulase gene was a more reliable method than coagulase serotyping from the viewpoint of arbekacin resistance.

Molecular typing plays an important role in epidemiological studies of nosocomial infection, such as methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Pulsed-field gel electrophoresis and multilocus sequence typing are considered the most discriminatory and reliable methods of typing, although they are technically complex, time consuming, and expensive. Coagulase serotyping is widely used in Japan in addition to conventional and genetic methods for distinguishing *S. aureus* strains. On the other hand, it has been reported that PCR-restriction fragment length polymorphism (RFLP) typing of

TABLE 1. Correlation between the coagulase serotype and the *coa*-RFLP type in MRSA and MSSA[a]

RFLP type	No. of strains with serotype									Total no. of strains
	I	II	III	IV	V	VI	VII	VIII	Other	
XS15			2 (0/2)							2 (0/2)
SS01		1 (0/1)								1 (0/1)
SS21			1 (0/1)							1 (1/0)
S12		4 (1/3)					2 (0/2)			6 (1/5)
S15		1 (1/0)								1 (1/0)
S23			14 (6/8)							14 (6/8)
S24		2 (1/1)								2 (1/1)
S25				8 (0/8)						8 (0/8)
M11								13 (0/13)		13 (0/13)
M12		23 (1/22)					10 (0/10)			33 (1/32)
M22							29 (16/13)		1 (1/0)	30 (17/13)
M23		3 (3/0)								3 (3/0)
M31			12 (3/9)							12 (3/9)
M41							1 (0/1)			1 (0/1)
L01		1 (0/1)					17 (2/15)		2 (0/2)	20 (2/18)
L11		2 (2/0)								2 (2/0)
L16	10 (8/2)					2 (0/2)			1 (0/1)	13 (8/5)
L21		371 (356/15)					1 (0/1)		7 (7/0)	379 (363/16)
L22				42 (37/5)					1 (1/0)	43 (38/5)
L23							26 (0/26)			26 (0/26)
L24					12 (0/12)				4 (0/4)	16 (0/16)
L31		27 (24/3)								27 (24/3)
L35							1 (0/1)			1 (0/1)
L51							1 (0/1)			1 (0/1)
LL01			1 (0/1)							1 (0/1)
LL21							1 (0/1)			1 (0/1)
LL27			4 (1/3)							4 (1/3)
LL31							1 (0/1)			1 (0/1)
XL21					13 (0/13)					13 (0/13)
XL22						1 (0/1)			1 (0/1)	2 (0/2)
XXL41		1 (0/1)								1 (0/1)
Total	10 (8/2)	436 (389/47)	34 (11/23)	50 (37/13)	25 (0/25)	3 (0/3)	90 (18/72)	13 (0/13)	17 (9/8)	678 (472/206)

[a] Results are shown as no. of strains (no. of MRSA strains/no. of MSSA strains).

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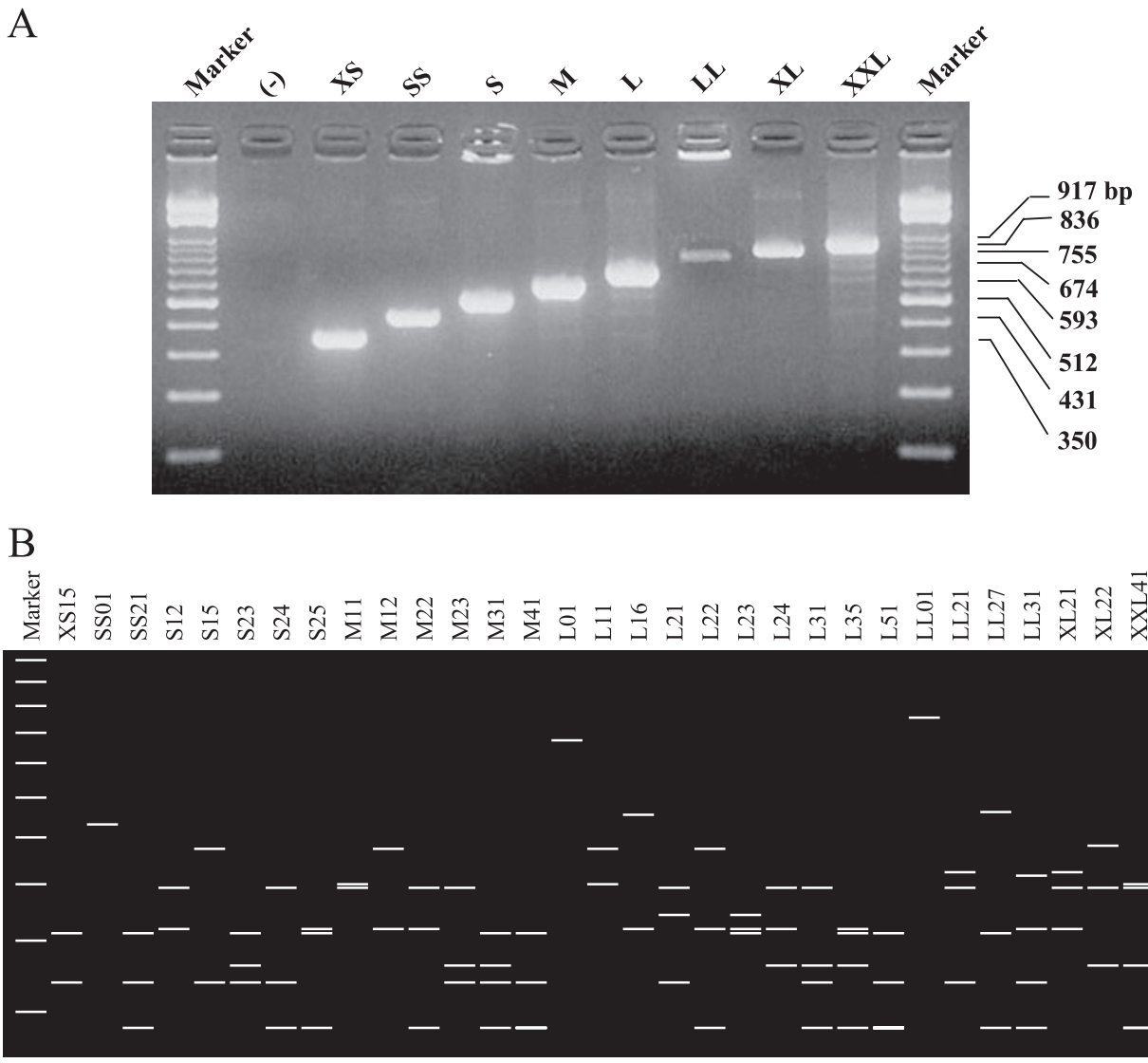

FIG. 1. Agarose gel electrophoresis pattern. (A) PCR amplification of *coa* genes. (B) Schematic representation of PCR-amplified *coa* genes digested with AluI.

the coagulase gene (*coa*) can be used to discriminate *S. aureus* strains on the basis of sequence variation within the 3′ end coding region of the gene (1, 2, 8). Arbekacin is effective, even in gentamicin-resistant MRSA strains, and has been used extensively in Japan since its approval as an anti-MRSA agent in 1990 (9, 10). We have studied arbekacin resistance in MRSA and recently reported the Japanese trend of arbekacin-resistant MRSA strains in the last two decades by using *coa*-RFLP typing (10). The results showed that arbekacin-resistant MRSA strains were distributed over only a few *coa*-RFLP types and remained at a low level. In this study, we assessed the clinical usefulness of *coa*-RFLP typing, especially from the viewpoint of arbekacin resistance, by comparing coagulase serotyping in both clinically isolated MRSA and clinically isolated methicillin-susceptible *S. aureus* (MSSA) in Japan.

Coagulase serotyping was performed with a coagulase-typing reagent kit (Denka Seiken Co. Ltd., Tokyo, Japan) according to the manufacturer's instructions. Based on the results, a total of 678 isolates, including 206 MSSA and 472 MRSA strains from unrelated clinical sources in Japan between 1979 and 2000 (4, 6, 7), were classified into nine serotypes, I to VIII and "other" (Table 1). MRSA strains were divided into serotypes I, II, III, IV, and VII, and most (82%) of the MRSA strains belonged to serotype II. A similar trend was previously observed in MRSA isolated in Japan (3). The serotype distribution of MSSA strains differed from that of MRSA strains. MSSA strains contained all serotypes. However, the majority of the MSSA strains were classified into serotypes II (28%) and VII (35%).

We next carried out *coa*-RFLP typing according to the method of Hookey et al., with some modifications (2). *coa* gene fragments were amplified from colonies grown on agar plates with the primers coaF (ATAGAGATGCTGGTACAGG) and coaR (GCTTCCGATTGTTCGATGC). As shown in Fig. 1A, PCR products of eight sizes were obtained from 678 isolates and named XS, SS, S, M, L, LL, XL, and XXL according to

their sizes. The sizes of the products ranged from 350 to 917 bp in increments of 81 bp, reflecting the number of 81-bp repeat units contained in the *coa* gene. After digestion with AluI, 31 *coa*-RFLP types were detected and numbered to allow them to be distinguished from each other (Fig. 1B). The majority (77%) of MRSA strains belonged to type L21, indicating the spread of a specific type of MRSA in Japan. In contrast, there was no such tendency in MSSA. This result was in agreement with the results of serotyping.

A comparison of the results of the two typing methods may illuminate the advantages of *coa*-RFLP typing from the viewpoint of arbekacin resistance. Serotype II contains 11 *coa*-RFLP types, including type L31. Previously, we reported strain PRC104 as a highly arbekacin-resistant strain (128 μg/ml) (5). Although the typing data for this strain were not incorporated in Table 1 because of its history of isolation, the strain belongs to type L31 (unpublished data). This implies that this important *coa*-RFLP type cannot be recognized by coagulase serotyping alone. Types M22 and M31 are also important due to their high incidence of arbekacin-resistant strains (10). The *coa*-RFLP typing clearly distinguished type M22 or M31 isolates from other strains belonging to serotype VII or III, respectively. Thus, our results demonstrated that *coa*-RFLP typing has higher discriminatory power than coagulase serotyping and may be useful for discriminating groups that may be potential reservoirs of arbekacin-resistant MRSA.

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