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Isoleucyl-tRNA Synthetase Mutations in *Staphylococcus aureus* Clinical Isolates and In Vitro Selection of Low-Level Mupirocin-Resistant Strains

Mupirocin-resistant strains of methicillin-resistant *Staphylococcus aureus* (MRSA) have already been reported in Europe (3, 7; B. Cookson, H. Farrelly, M.-F. Palepou, and R. George, Letter, Lancet **339:**625, 1992; I. Hudson, Letter, Lancet **339:**56, 1992; M. Rahman, W. C. Noble, and B. Cookson, Letter, Lancet **ii:**387-388, 1987). Additionally, a low-level resistance to mupirocin (LLR-Mup) in *S. aureus* emerged as the result of independent, spontaneous mutational events within the usual chromosomal isoleucyl-tRNA synthetase (*ileS*) gene, with the mutations occurring only with unsuitable usage of mupirocin (5). Hence, we compared the *ileS* gene sequences obtained from mupirocin-susceptible MRSA (SA-7S) and those obtained from mupirocin-resistant MRSA (SA-7R) (4) where the resistance was acquired by in vitro selection from the original strain (SA-7S).

In recent studies using isolates from Europe and North America, *ileS* gene mutation points correlating with LLR-Mup were reported (1). In this study, we report on *ileS* gene full-length sequences from LLR-Mup MRSA strains, which were isolated in Japan and compared with those of the reported *ileS* gene mutation for molecular heterogeneity in isolates (1).

A total of 258 MRSA isolates from different patients in 15 hospitals at the Tohoku area, Japan, were investigated. The MIC of mupirocin was determined by E-test (AB Biodisk, Solna, Sweden) (9). By the results of this susceptibility test, six strains (SA-1 to -6) were LLR-Mup (Table 1).

The SA-7R strain was initially a mupirocin-susceptible strain (SA-7S; MIC, 0.025 μg/ml) but developed LLR-Mup (MIC, 16 μg/ml) following seven cycles of serial passage while being exposed to one-half the MIC of mupirocin by the agar dilution method (4). Genomic DNAs of these eight strains (SA-1 to SA-6, 7S, and 7R) were extracted by a method described elsewhere (6). The entire *ileS* gene was amplified by PCR as previously described (1). IleS-1 (5′-TACCGCGAGCAATCGTCCCT-3′) and IleS-2 (5′-TGTTGGCATCGTGGGCATAG-3′) were designed to amplify the entire coding sequence of the *ileS* gene (2). PCR templates of all strains were sequenced by the Big-Dye terminator cycle sequencing method using an ABI PRISM 377XL sequencer (Applied Biosystems, Foster City, Calif.), and the sequences were compared with those of the published *S. aureus ileS* gene sequence (GenBank accession no. X74219). The missense mutation points of all strains are presented in Table 1. All strains contained the A637G mutation and either the G1762T or the G1891T mutation from seven LLR-Mup strains including SA-7R. In the SA-7R strain, only the G1762T mutation was detected, but this mutation was not present in SA-7S. On the other hand, the newly confirmed A2412T mutation was detected from the *ileS* gene only in the SA-5 strain.

In the present study, there was no A2330G mutation in any of the LLR-Mup MRSA strains from Japan. However, LLR-Mup strains from Europe and North America had this mutation. Although Antonio et al. did not mention a correlation between the A2330G mutation and LLR-Mup, we believe that the A2330G mutation is not essential for LLR-Mup. In addition, the A637G mutation may not be involved in LLR-Mup, because the mupirocin-susceptible strain (SA-7S) possessed the A637G mutation. In the SA-5 strain, the new A2412T mutation was confirmed. However, this A2412T mutation may not be the essence of mupirocin resistance. In the case of the SA-6 strain, there is no G1762T mutation, but SA-6 possessed the G1891T mutation. It is known that both G1762T and G1891T mutations affect the Rossman fold, which forms a complex with mupirocin (1, 6, 8). Therefore, the SA-6 strain, with the G1891T mutation, may have shown LLR-Mup.

From the results of this study, mupirocin-susceptible *S. aureus* acquired LLR-Mup through a single mutation, G1762T. This result supports the possibility that mutations affecting the Rossman fold of IleS are correlated with a low level of resistance to mupirocin.

TABLE 1. Mutation points in full-length *ileS* genes

Strain	Source	MIC (μg/ml)	Mutation point (amino acid change) relative to X74219[a]			
			A637G (Asn→Asp)	G1762T (Val→Phe)	G1891T (Val→Phe)	A2412T (Lew→Phe)
SA-1	Sputum	64	+	+		
SA-2	Pus	12	+	+		
SA-3	Urine	16	+	+		
SA-4	Sputum	8	+	+		
SA-5	Sputum	8	+	+		+
SA-6	Sputum	6	+		+	
SA-7S	Sputum	0.025	+			
SA-7R		16	+	+		

[a] X74219 is the GenBank accession number for the the published *ileS* gene sequence from *S. aureus*. +, presence of indicated mutation.

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