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Molecular Characterization of Resistance to Mupirocin in Methicillin-Susceptible and -Resistant Isolates of *Staphylococcus aureus* from Nasal Samples

Fernando Chaves,* Jesus García-Martínez, Sonia de Miguel, and Joaquín R. Otero

Servicio de Microbiología, Hospital Universitario Doce de Octubre, Avenida de Cordoba sn, 28041 Madrid, Spain

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A total of 15 of 101 (14.8%) nasal methicillin-resistant *Staphylococcus aureus* (MRSA) isolates exhibited mupirocin resistance (Mup^r) compared with 1 of 154 (0.6%) methicillin-susceptible *Staphylococcus aureus* isolates. A total of 14 (93%) isolates exhibiting high-level Mup^r belonged to a single clone. Horizontal plasmid transfer and transmission of Mup^r strains contribute to a high incidence of Mup^r MRSA at our institution.

Mupirocin (Mup) is a topical antibacterial agent that interferes with protein synthesis by competitively inhibiting bacterial isoleucyl-tRNA synthetase (11, 19). A 2% Mup calcium ointment (Bactroban Nasal; GlaxoSmithKline) applied topically to the anterior nares eradicates carriage of *Staphylococcus aureus* and prevents infection in certain settings (2, 7, 8, 13). An important concern, however, is the development of Mup resistance (Mup^r) (14, 17), of which there are two types. Low-level Mup^r (MIC, 8 to 256 mg/liter) is usually associated with a mutation in the gene for target enzyme, while high-level Mup^r (Hi-Mup^r) (MIC, >256 mg/liter) is mediated by a plasmid containing the *ileS2* gene that encodes an additional isoleucyl-tRNA synthetase enzyme (3). Such transmissible resistance raises concern about the spread of Mup^r as Mup usage becomes more widespread (9, 10, 17). The objectives of this study were to determine (i) the prevalence of Mup^r in methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) isolates from nasal samples; (ii) the location, if present, of the *ileS2* gene in Mup^r isolates; and (iii) the organism genotype, as defined by pulsed-field gel electrophoresis (PFGE).

Nasal samples submitted to the Clinical Microbiology Laboratory of the Hospital Universitario Doce de Octubre for isolation of *S. aureus* between October 2001 and October 2002 were included in the study. Specimens were obtained from two groups. Group I comprised adults who underwent elective heart surgery, before which a sample from the anterior nares was taken to determine whether the patient was a carrier of *S. aureus*. Group II included all new cases of MRSA infection diagnosed at the hospital that were associated with concurrent nasal carriage. Since 1996, we have instituted the use of topical Mup ointment to reduce the prevalence of nasal MRSA. Samples were inoculated onto phenol-red mannitol salt agar plates that were incubated at 37°C for 48 h. Isolation and identification of *S. aureus* were based upon standard microbiological procedures. All isolates were screened for resistance to Mup on Mueller-Hinton agar with a 5-μg disk (Oxoid). A zone of inhibition ≤ 13 mm in diameter was considered to reflect Mup^r (5). Mup^r organisms underwent MIC analysis by the E-test strip method (AB Biodisk). Susceptibility testing with other antibiotics was performed by disk diffusion (12, 16). All isolates were confirmed as MRSA by PCR detection of the *mecA* gene (6). PCR was also performed on all Mup^r isolates to detect the plasmid-associated *ileS2* gene (1).

Mup^r isolates were typed by PFGE following DNA extraction and digestion with *Sma*I (4). Restriction fragments were separated in a CHEF DRIII PFGE system (Bio-Rad Laboratories). Migration of DNA fragments was normalized using an appropriate size marker. Computer-assisted analysis of PFGE patterns was carried out using GelCompar software (Applied Maths). PFGE types (designated by letters) differed by <7 fragments, while subtypes (designated by Arabic numerals) had indistinguishable patterns (15). Plasmid DNA was extracted by a rapid minipreparation procedure (QIAprep spin plasmid kit from Qiagen) and digested with *Hin*dIII. Southern blot analysis of PFGE-separated *Sma*I digests of genomic DNA and *Hin*dIII plasmid fragments was performed with an ECL system (Amersham) using a 456-bp PCR-amplified *ileS2* gene fragment as a probe.

Among patients in group I, *S. aureus* was isolated from 159 of 689 (23%) nasal swabs. Of these isolates, 154 (96.9%) were MSSA and 5 (3.1%) were MRSA. In contrast, 96 of 137 (70.1%) patients in group II yielded MRSA culture-positive nasal swabs. A total of 15 of 101 (14.8%) MRSA isolates were Mup^r compared with 1 of 154 (0.6%) isolates of MSSA. Of the Mup^r isolates, 14 of 16 (87.5%) exhibited Hi-Mup^r and gave a positive result by *ileS2* PCR (Table 1).

Among the 16 Mup^r isolates, PFGE identified one major clone (PFGE type A) containing 14 of 15 (93%) isolates of MRSA and 13 of 14 (93%) Hi-Mup^r isolates. The remaining two isolates belonged to two other PFGE types (Fig. 1). All nasal carriers of type A Mup^r MRSA also provided other specimens from which MRSA of the same PFGE subtype was isolated. Patients with PFGE type A were located in different hospital wards, with the exception of those who yielded isolates 12 and 15 (both subtype A1), who were resident in the Trauma Ward. Southern analysis of *Hin*dIII-digested plasmid DNA

* Corresponding author. Mailing address: Servicio de Microbiología, Hospital Universitario Doce de Octubre, Avenida de Cordoba sn, Madrid 28041, Spain. Phone: (34) 91-3908239. Fax: (34) 91-5652765. E-mail: fchaves.hdoc@salud.madrid.org.

TABLE 1. Mupirocin-resistant isolates of *S. aureus* from the nostrils

Isolate	Mupirocin resistance pattern (MIC [μg/ml])[a]	*ileS2* PCR result	Resistance pattern[b]	*mecA* PCR result	PFGE pattern	*ileS2* locus polymorph
1	R (>1,024)	+	Met/Cip/Ery/Gen	+	A1	I
2	R (>1,024)	+	Met/Cip/Ery/Clin	+	A4	II
3	R (>1,024)	+	Met/Cip/Ery/Gen/Fus	+	A2	I
4	I (8)	−	Met/Cip/Ery/Clin/Gen	+	A3	
5	R (>1,024)	+	Met/Cip/Ery/Gen	+	A1	II
6	R (>1,024)	+	Met	+	A5	II
7	I (16)	−	Met/Cip/Ery/Clin/Gen	+	B	
8	R (>1,024)	+	Met/Cip/Ery/Clin/Gen	+	A2	II
9	R (>1,024)	+	Met/Cip/Ery/Gen	+	A2	I
10	R (>1,024)	+	Met/Cip/Ery/Clin/Gen	+	A1	II
11	R (>1,024)	+	Met/Cip/Ery	+	A2	I
12	R (>1,024)	+	Met/Cip/Ery	+	A1	II
13	R (>1,024)	+	Susceptible	−	C	II
14	R (>1,024)	+	Met/Cip	+	A1	II
15	R (>1,024)	+	Met/Cip/Ery	+	A1	II
16	R (>1,024)	+	Met/Cip	+	A1	II

[a] R, resistant; I, intermediate.
[b] Cip, ciprofloxacin; Clin, clindamycin; Fus, fusidic acid; Ery, erythromycin; Gen, gentamicin; Met, methicillin.

confirmed the plasmid location of the *ileS2* gene in all 14 Hi-Mup^r isolates. Different sizes of hybridizing *Hin*dIII plasmid fragments distinguished two *ileS2* gene polymorphs. Regions homologous to the *ileS2* probe were found on *Hin*dIII fragments of 6.1 kb (polymorph I; 10 isolates) or 4.5 kb (polymorph II; 4 isolates) (Fig. 2). Most Hi-Mup^r MRSA isolates of PFGE type A (9 of 13, 69%) carried the *ileS2* probe region on the 4.5-kb fragment, as did the only Hi-Mup^r MSSA isolate (PFGE type C) (Fig. 1 and 2). No positive results were observed in Southern blot analysis of PFGE-separated *Sma*I-digested genomic DNA. This is because the *ileS2*-containing plasmids (even prior to digestion with *Sma*I) are smaller than can be detected at the lower limit of resolution and migrate out of the gel during the course of electrophoresis.

We identified four MRSA-infected patients with nasal colonization by Mup-susceptible (Mup^s) organisms; Mup^r bacteria were subsequently isolated (isolates 5, 12, 14, and 15) from these patients 7 to 30 days after intranasal application of Mup ointment (Table 1). The Mup^s and Mup^r isolates from three of the four patients were of the same PFGE subtype, while in the case of the fourth patient there was a difference of a single band (Fig. 1). In these patients, Mup treatment probably exerted selective pressure for organisms which had preexisting high-level resistance and which subsequently recolonized their nasal passages (18).

We detected a much higher percentage of Mup^r among isolates of MRSA (14.8%) than among isolates of MSSA (0.6%). Two epidemiological phenomena probably contribute to Hi-Mup^r in *S. aureus*. First, Southern blots of plasmid DNA located the *ileS2* resistance gene on two different plasmid fragments, indicating that at least two plasmids or plasmid variants harbor this gene. One of these variants was implicated in horizontal gene transfer and spread of Hi-Mup^r between MRSA and MSSA. This was demonstrated by the identification of a 4.5-kb *ileS2*-hybridizing plasmid fragment in two isolates (one of MRSA and the other of MSSA) with distinctly different PFGE genotypes. Second, identification of the same PFGE subtypes and *ileS2* hybridization and antibiotic resistance patterns among Hi-Mup^r isolates (Table 1) suggests that patient-patient transmission also occurs. Mup treatment should therefore be used cautiously to avoid the emergence of Hi-Mup^r

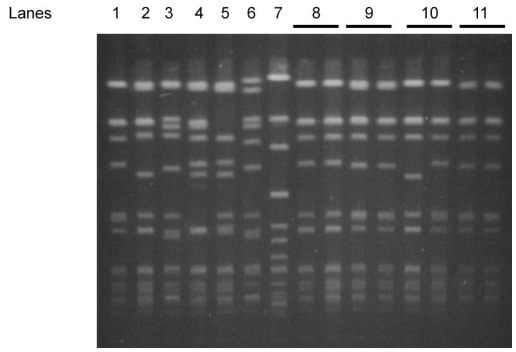

FIG. 1. PFGE DNA patterns of Mup^r *S. aureus* isolates from nasal samples. Lanes 1 to 7 represent genotypes A1 (isolate 1), A2 (isolate 3), A3 (isolate 4), A4 (isolate 2), A5 (isolate 6), B (isolate 7), and C (isolate 13), respectively. Lanes 8 to 11 represent the banding patterns of paired Mup^s and subsequently recovered Mup^r *S. aureus* isolates (isolates 5, 12, 14, and 15) from nasal samples of four patients.

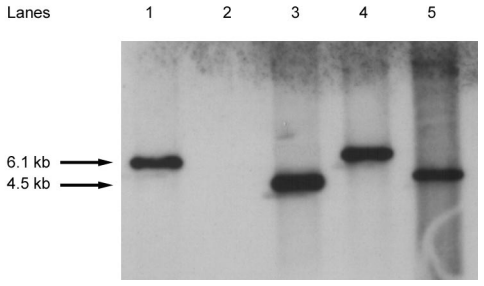

FIG. 2. Southern blot of *Hin*dIII-digested plasmid DNA from selected Mup^r *S. aureus* isolates hybridized with an *ileS2* probe. Lane 1, MRSA isolate 1 (Hi-Mup^r); lane 2, MRSA isolate 4 (low-level Mup^r); lane 3, MRSA isolate 5 (Hi-Mup^r); lane 4, MRSA isolate 8 (Hi-Mup^r); lane 5, MSSA isolate 13 (Hi-Mup^r).

MRSA and the spread of resistance in hospitals in which MRSA is frequently isolated.

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