

## High-Level Mupirocin Resistance within Methicillin-Resistant *Staphylococcus aureus* Pandemic Lineages

Eduardo Pérez-Roth,<sup>1</sup> Celeste López-Aguilar,<sup>1</sup> Julia Alcoba-Florez,<sup>2</sup> and Sebastián Méndez-Álvarez<sup>1,3,4\*</sup>

Laboratorio de Biología Molecular, Unidad de Investigación,<sup>1</sup> and Unidad de Microbiología,<sup>2</sup> Hospital Universitario Nuestra Señora de Candelaria, and Departamento de Biología Celular y Microbiología, Universidad de La Laguna,<sup>3</sup> Santa Cruz de Tenerife, and Investigador Asociado, Centro de Investigaciones Biológicas del Consejo Superior de Investigaciones Científicas, Madrid,<sup>4</sup> Spain

Received 13 January 2006/Returned for modification 24 March 2006/Accepted 2 July 2006

**The methicillin-resistant *Staphylococcus aureus* (MRSA) population in the Hospital Universitario Nuestra Señora de Candelaria over a 5-year period (1998 to 2002) was marked by shifts in the circulation of pandemic clones. Here, we investigated the emergence of high-level mupirocin resistance (Hi-Mup<sup>r</sup>). In addition to clonal spread, transfer of *ileS2*-carrying plasmids played a significant role in the dissemination of Hi-Mup<sup>r</sup> among pandemic MRSA lineages.**

Most hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) isolates are members of five MRSA pandemic lineages or clonal complexes (CCs), namely, CC5, CC8, CC22, CC30, and CC45 (6, 14). Mupirocin constitutes the cornerstone of avoidance of MRSA carriage and ulterior infection, but resistance has emerged, and its spreading is worrisome, with transferable high-level mupirocin resistance (Hi-Mup<sup>r</sup>) being of clinical significance (3, 4, 5, 11, 23, 25). Hi-Mup<sup>r</sup> is associated with an additional isoleucyl-tRNA synthetase that is encoded by the *ileS2* gene (8, 10). The *ileS2* gene was commonly reported on plasmids that differed in size, restriction patterns, and ability to be transferred in conjugation experiments (12, 13, 16, 21, 24, 26).

We reported that 95.5% of the 375 MRSA isolates obtained from patients at Hospital Universitario Nuestra Señora de Candelaria (located in Tenerife, Canary Islands, Spain) between 1998 and 2002 belonged to six clones fitting in pandemic lineages (i.e., CC5, CC8, CC22, and CC30) (20). In the present work, we investigated Hi-Mup<sup>r</sup> in such a staphylococcal population.

(Parts of this work were presented during the 1st International Workshop for Origin and Evolution of Bacterial Pathogens, Baeza, Spain, October 2004, and during the XX Congreso Nacional de Microbiología, Cáceres, Spain, September 2005.)

Isolates were screened for mupirocin resistance by the disk

TABLE 1. Amplification and sequencing primers used in this study

Target gene	Primer name <sup>a</sup>	Sequence (5'-3')	Reference or source	GenBank accession no.	Predicted PCR product size (bp)	
Amplification <i>ileS2</i>	MupA	TATATTATGCGATGGAAGGTTGG	1		458	
	MupB	AATAAAATCAGCTGGAAGTGTG				
	<i>traD-traE</i>	TrAD-E.F	GTAGACAATTAGATCATGCAGTG	Thisstudy	NC_005024	212
	TrAD-E.R	TGCCATAATCATAAACTCCCTTC				
	<i>traK</i>	TraK-F	TTGCCGAAGATAGCGAATTG	Thisstudy	NC_005024	1,888
TraK-R	CTGCAATACCTTCGGTCAGTTC					
Sequencing <i>traK</i>	TraK-R1	CGCCACTAGGGTCAGTTACAAC	Thisstudy	NC_005024		
	TraK-F2	GTCAGCTGGGTATGTTATTCCTAATG	Thisstudy	NC_005024		
	TraK-R2	GGGTGGTCTTTAGATAATTCATTAAGC	Thisstudy	NC_005024		
	TraK-F3	AACCTAATCAAGATGAAGAAGGATCAG	Thisstudy	NC_005024		
	TraK-R3	ATATTTGGCCATTCGTCTAG	Thisstudy	NC_005024		
	TraK-F4	CTTCTCTCAAATGTTCCAACAGC	Thisstudy	NC_005024		
	TraK-R4	GCTTTATCCTGTTGCATATTAC	Thisstudy	NC_005024		
	TraK-F5	GCTACACTGGAAGGGCATTACTAAATG	Thisstudy	NC_005024		
	TraK-R5	TTCGTAGAATGCTTTCTTAG	Thisstudy	NC_005024		

<sup>a</sup> TraK-F and TraK-R primers were used in amplification and sequencing.

\* Corresponding author. Mailing address: Unidad de Investigación, Hospital Ntra. Sra. de Candelaria, Ctra. del Rosario s/n, 38010 Santa Cruz de Tenerife, Spain. Phone: 34-922-600080. Fax: 34-922-600562. E-mail: smenalv@gobiernodecanarias.org.

TABLE 2. Phenotypic and genotypic characteristics of high-level mupirocin-resistant MRSA isolates

MRSA clone (lineage) <sup>b</sup> and isolate no.	Isolation date (day/mo/yr)	Hospital ward <sup>c</sup>	Sample origin <sup>d</sup>	PFGE subtype <sup>e</sup>	HindIII-REAP pattern <sup>e</sup>	<i>ileS2</i> locus polymorph	<i>ileS2</i> -carrying plasmid type	Resistance transferred to standard recipient <sup>f</sup>
ST125-IVA (CC5)								
HUNSC-356	06/11/2001	Internal medicine	Wound	C1	XI	E	pMUP2	+
HUNSC-364	29/11/2001	Internal medicine	Sputum	C1	XI	E	pMUP2	ND
HUNSC-414	22/03/2002	Internal medicine	Nasal exudate	C1	XI	E	pMUP2	ND
HUNSC-453	22/05/2002	Internal medicine	Wound	C1	XII	E	pMUP2	+
HUNSC-464	20/06/2002	Internal medicine	Sputum	C1	XI	E	pMUP2	ND
HUNSC-485	28/08/2002	Vascular surgery	Wound	C1	XII	E	pMUP2	ND
HUNSC-488	03/09/2002	ICU	BAS	C1	XIII	E	pMUP2	+
HUNSC-492	13/09/2002	Internal medicine	Nasal exudate	C1	XI	E	pMUP2	ND
HUNSC-505	07/10/2002	Vascular surgery	Nasal exudate	C1	XIII	E	pMUP2	ND
HUNSC-510	23/10/2002	Vascular surgery	Wound	C1	XIII	E	pMUP2	ND
HUNSC-523	13/11/2002	Vascular surgery	Wound	C1	XIV	E	pMUP2	+
HUNSC-525	19/11/2002	Vascular surgery	Wound	C1	XIV	E	pMUP2	ND
HUNSC-534	12/12/2002	Internal medicine	Nasal exudate	C1	XV	E	pMUP2	+
HUNSC-535	12/12/2002	Internal medicine	Nasal exudate	C1	XV	E	pMUP2	ND
HUNSC-537	17/12/2002	Internal medicine	Nasal exudate	C1	XV	E	pMUP2	ND
HUNSC-538	17/12/2002	Internal medicine	Nasal exudate	C1	XV	E	pMUP2	ND
HUNSC-540	18/12/2002	Internal medicine	Nasal exudate	C1	XV	E	pMUP2	ND
HUNSC-541	31/12/2002	Internal medicine	Nasal exudate	C1	XV	E	pMUP2	ND
ST146-IVA (CC5) HUNSC-521	09/11/2002	Hematology	Wound	D1	V	A	pMUP4	ND
ST247-IA (CC8)								
HUNSC-229	04/05/2000	Vascular surgery	Sputum	A1	I	D	pMUP1	+
HUNSC-246	08/04/2000	ICU	Blood	A1	I	D	pMUP1	ND
HUNSC-267	07/11/2000	Neurology	BAS	A1	I	D	pMUP1	ND
HUNSC-268	14/08/2000	Neurology	Wound	A1	I	D	pMUP1	ND
HUNSC-182	19/01/1999	ICU	BAS	A2	II	D	pMUP1	+
HUNSC-183	09/02/1999	ICU	BAS	A2	II	D	pMUP1	ND
HUNSC-242	29/06/2000	General surgery	Wound	A6	I	D	pMUP1	ND
HUNSC-243	04/07/2000	General surgery	Blood	A6	I	D	pMUP1	ND
HUNSC-244	07/07/2000	General surgery	Catheter	A6	I	D	pMUP1	ND
HUNSC-264	16/10/2000	Digestive surgery	Wound	A8	III	E	pMUP2	+
ST22-IV (CC22)								
HUNSC-684	21/10/2002	Dermatology	Wound	E1	XVI	E	pMUP2	+
HUNSC-799	06/04/2002	Vascular surgery	Wound	E1	XVI	E	pMUP2	ND
ST36-II (CC30)								
HUNSC-234	06/06/2000	Vascular surgery	Wound	B1	IV	B	pMUP3	+
HUNSC-238	14/06/2000	Vascular surgery	Wound	B1	IV	B	pMUP3	ND
HUNSC-256	11/08/2000	Internal medicine	Wound	B1	V	A	pMUP4	ND
HUNSC-277	14/02/2001	Internal medicine	Nasal exudate	B1	V	A	pMUP4	+
HUNSC-298	04/04/2001	Traumatology	Wound	B1	VI	E	pMUP5	+
HUNSC-319	14/05/2001	Internal medicine	Wound	B1	VI	E	pMUP5	ND
HUNSC-326	25/06/2001	Internal medicine	Nasal exudate	B1	V	A	pMUP4	ND
HUNSC-339	30/07/2001	Traumatology	Wound	B1	VII	E	pMUP6	ND
HUNSC-363	01/12/2001	Vascular surgery	Blood	B1	VIII	C	pMUP7	+
HUNSC-393	11/02/2002	Thoracic surgery	Sputum	B1	IX	A	pMUP8	+
HUNSC-396	19/02/2002	Internal medicine	Wound	B1	IX	A	pMUP8	ND
HUNSC-401	02/03/2002	Traumatology	Wound	B1	VI	E	pMUP5	ND
HUNSC-491	10/09/2002	General surgery	Wound	B1	X	D	pMUP9	+
HUNSC-499	30/09/2002	Traumatology	Catheter	B1	I	D	pMUP1	ND
HUNSC-503	07/10/2002	Traumatology	Wound	B1	I	D	pMUP1	ND
HUNSC-459	06/06/2002	U. medicine	Wound	B4	VII	E	pMUP6	+
HUNSC-489	03/09/2002	Cardiology	Wound	B4	VII	E	pMUP6	ND

<sup>a</sup> Each Hi-Mup<sup>r</sup> isolate showed a MIC of  $\geq 1,024$   $\mu\text{g/ml}$ .

<sup>b</sup> Results of PFGE, multilocus sequence, and staphylococcal chromosome cassette typing were already included in the analysis of MRSA clones (20).

<sup>c</sup> ICU, intensive care unit; U. medicine, urgency medicine.

<sup>d</sup> BAS, bronchial aspirate.

<sup>e</sup> Patterns obtained from total plasmid DNA.

<sup>f</sup> ND, not determined; +, isolate produced transconjugants.

diffusion and Etest methods (7, 15). The *ileS2* gene was detected by a multiplex PCR (17, 18). Plasmid DNA was extracted with the QIAprep spin plasmid kit (QIAGEN, Hilden, Germany) with the addition of lysostaphin (Sigma Chemical

Co., St. Louis, Mo.) and digested with EcoRI and HindIII. Curing of plasmids and conjugation experiments were performed as previously described (24). The primers used are listed in Table 1.

TABLE 3. Structural group, *ileS2* locus polymorph, and clonal complex distribution of Hi-Mup<sup>r</sup> plasmid types

Structural group	<i>ileS2</i> locus polymorph	Size(s) (kb) of restriction fragments hybridizing with the <i>ileS2</i> probe <sup>a</sup>	<i>ileS2</i> -carrying plasmid type (kb) <sup>b</sup>	No. of isolates (%)	MRSA clone(s)	Clonal complex(es)
S1	A	4.3	pMUP4 (26)	4 (8.3)	ST146-IVA, ST36-II	CC5, CC30
			pMUP8 (41)	2 (4.2)	ST36-II	CC30
S2	B	5.0	pMUP3 (34)	2 (4.2)	ST36-II	CC30
	C	4.7, 5.0	pMUP7 (39)	1 (2.1)	ST36-II	CC30
S3	D	5.2	pMUP1 (33)	11 (22.9)	ST247-IA, ST36-II	CC8, CC30
			pMUP9 (35)	1 (2.1)	ST36-II	CC30
S4	E	5.5	pMUP2 (40)	21 (43.8)	ST125-IVA, ST247-IA, ST22-IV	CC5, CC8, CC22
			pMUP5 (47)	3 (6.2)	ST36-II	CC30
			pMUP6 (46)	3 (6.2)	ST36-II	CC30

<sup>a</sup> Aproximated sizes by comparison with a lambda HindIII marker.

<sup>b</sup> Plasmid sizes were determined by summation of restriction fragments using agarose gel electrophoresis following separate digestions with EcoRI and HindIII.

The *ileS2* locus was associated with conjugative plasmids in each Hi-Mup<sup>r</sup> isolate. Hi-Mup<sup>r</sup> was manifested by 48 MRSA isolates (12.8%) (Table 2) and increased significantly from 0% in 1998 to 15.6% in 2002 ( $P < 0.001$ ). Each Hi-Mup<sup>r</sup> isolate belonged to one of the six major MRSA clones and amplified the *ileS2* gene fragment. Non-Hi-Mup<sup>r</sup> isolates did not show the *ileS2* amplicon. Pulsed-field gel electrophoresis (PFGE) subtypes shown by Hi-Mup<sup>r</sup> isolates, excepting PFGE subtype B4, also included mupirocin-susceptible isolates, suggesting intrahospital acquisition of Hi-Mup<sup>r</sup> by circulating clones.

HindIII restriction endonuclease analysis of plasmid DNA (REAP) revealed 16 patterns (i.e., I to XVI) (Table 2), each hybridizing with the *ileS2*-specific probe. Thus, the *ileS2* gene was harbored by plasmids; this result was consistent with data from previous studies (12, 21). The different sizes of *ileS2*-hybridizing plasmid fragments permitted five polymorphs of the *ileS2* locus (i.e., A to E) to be distinguished. Polymorphs A, B, D, and E were characterized by a single HindIII-hybridizing

fragment, while polymorph C was defined by two hybridizing fragments (Tables 2 and 3).

One Hi-Mup<sup>r</sup> isolate of each restriction endonuclease analysis pattern ( $n = 16$ ) was randomly selected for conjugation assays. The Hi-Mup<sup>r</sup> was transferred from each isolate (Table 2). Overall, nine different *ileS2*-carrying plasmids (i.e., pMUP1 to pMUP9) were identified in transconjugants (Fig. 1). Susceptibility tests and curing experiments support that idea that these plasmids mediate Hi-Mup<sup>r</sup>. Plasmids differed from each other by at least one HindIII restriction fragment, but some bands in common permitted four structural groups (i.e., S1 to S4) to be distinguished (Table 3). EcoRI digestions corroborated these results (data not shown). Plasmids included in each group displayed the same *ileS2* locus polymorph (Fig. 1A1). The exception was group S2, given that plasmids pMUP3 and pMUP7 showed different polymorphs due to an additional *ileS2*-hybridizing fragment in pMUP7, which appeared as a double-intensity ca. 4.7-kb HindIII band, suggesting the exis-

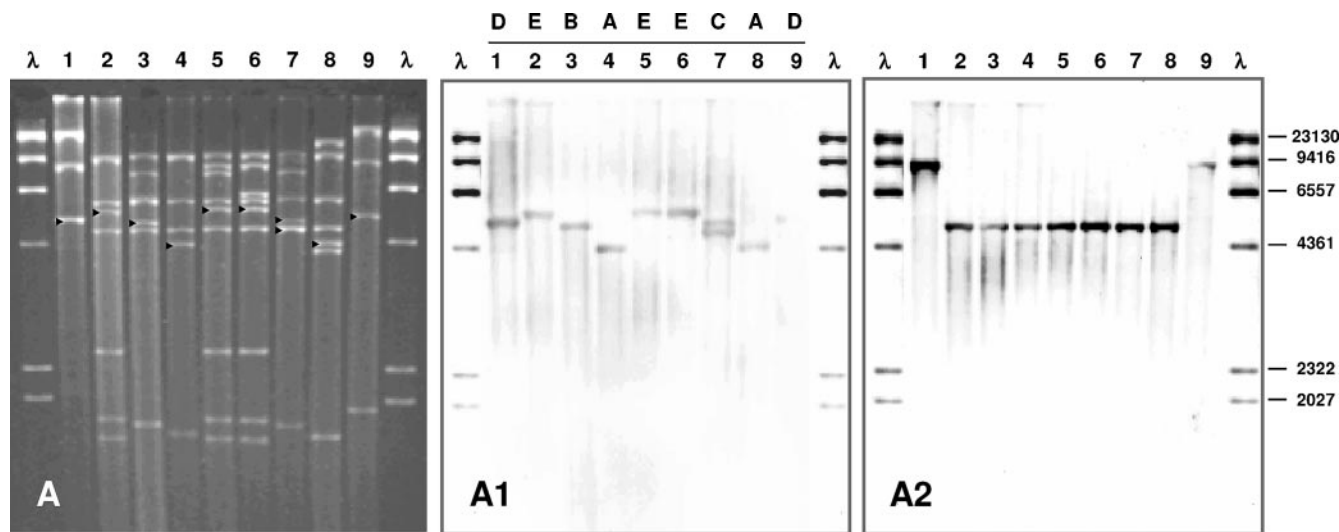


FIG. 1. Analysis of *ileS2*-carrying plasmid types by restriction endonuclease analysis and Southern hybridization. (A) HindIII restriction patterns of plasmid types extracted from *S. aureus* transconjugants of Hi-Mup<sup>r</sup> MRSA isolates. ►, *ileS2*-hybridizing fragments. (A1) Southern hybridization with the *ileS2*-containing DNA as a probe, showing the different *ileS2* polymorphs (A to E). (A2) Southern hybridization using the *traK* DNA fragment as probe. Lanes 1 through 9, plasmid types pMUP1 to pMUP9, lambda, lambda phage DNA digested with HindIII and digoxigenin labeled as a molecular size standard. Numbers on the right correspond to the molecular sizes (in base pairs) of lambda DNA HindIII restriction fragments.

tence of two copies of the *ileS2* gene in pMUP7 (Fig. 1A, lane 7). Plasmids belonging to distinct groups differed from each other by at least five bands and showed different polymorphisms.

***ileS2*-carrying plasmids are related to the pSK41 family of staphylococcal multiresistance plasmids.** It has been suggested that mupirocin resistance plasmids belong to the pSK41 family of staphylococcal conjugative plasmids (12) (the pSK41 sequence was obtained from GenBank, accession no. NC\_005024). The putative relationship of pSK41 to *ileS2*-carrying plasmids was examined at the *traK* gene level. The *traK* gene of pSK41 is involved in the *tra* gene system for conjugation (2, 9). The *traK* gene was detected in each *ileS2*-carrying plasmid (Fig. 1A2). The nucleotide sequence of the open reading frame (1,641 bp) in the ca. 1.8-kb *traK* PCR products from plasmids included in groups S1 and S2 was identical to the pSK41 *traK* gene sequence. Group S3 plasmids showed 41 nucleotide differences, plus the insertion of 6 nucleotides, compared to the pSK41 *traK* sequence (>97% identity). The three plasmid types included in group S4 showed one nucleotide difference (>99% identity). These results indicated that the *traK* gene of pSK41 was conserved in *ileS2*-carrying plasmids. The plasmids included in groups S1, S2, and S4 had a single 4.7-kb *traK*-hybridizing fragment. The plasmids in group S3 presented a ca. 9-kb hybridization band. Additionally, the *traD-traE* region was detected in each Hi-Mup<sup>r</sup> isolate and transconjugants. Our results are consistent with the concept of *ileS2*-carrying plasmids being members of the pSK41 family of conjugative plasmids.

**Distribution of Hi-Mup<sup>r</sup> plasmids among pandemic lineages (CCs).** Some of the *ileS2*-carrying plasmids were detected in the background of different MRSA clones belonging to different CCs (Tables 2 and 3), indicating plasmid transfer. Furthermore, we found plasmids of the four structural groups in the collection of Hi-Mup<sup>r</sup> ST36-II epidemiologically related isolates. The emergence of Hi-Mup<sup>r</sup> in ST36-II is of health concern because it is the predominant clone, completely replacing the Iberian clone (ST247-IA) (19, 20, 22). Unlike ST36-II isolates, Hi-Mup<sup>r</sup> isolates belonging to clones ST125-IVA and ST22-IV shared a single, unique *ileS2*-carrying plasmid, which indicates clonal dissemination in the spread of Hi-Mup<sup>r</sup>.

**Conclusions.** All major MRSA pandemic clones already circulating in the hospital acquired *ileS2*-carrying plasmids, resulting in a multiclonal population structure. In addition to clonal spread and plasmid transfer, the finding of the *ileS2* gene in plasmids differing in size and restriction patterns show that gene transposition constitutes an important mode of dissemination of Hi-Mup<sup>r</sup>. Intervention strategies in the current scenario should identify dissemination of MRSA clones and/or *ileS2*-carrying mobile elements.

**Nucleotide sequence accession numbers.** The nucleotide sequences of the entire *traK* homolog genes from mupirocin resistance plasmids pMUP1, pMUP2, pMUP3, pMUP4, pMUP5, pMUP6, pMUP7, pMUP8, and pMUP9 identified in this study have been deposited in GenBank under accession numbers DQ232628, DQ232629, DQ232630, DQ232631, DQ232632, DQ232633, DQ232634, DQ232635, and DQ232636, respectively.

We are grateful to Mark Enright and Manuel Espinosa for critical reading of the manuscript, Warren B. Grubb for providing the *S.*

*aureus* WBG541 strain, and Ninive Batista and Francisco Javier González-Paredes for their technical assistance in susceptibility tests and *traK* gene sequencing, respectively. We thank SmithKline Beecham Pharmaceuticals (United Kingdom) for the gift of mupirocin powder.

This study was supported by grants 2001/020 from the Consejería de Educación, Cultura y Deportes, FUNCIS 2002/038 and 2005/50 from the Canary Islands Autonomous Government, 2001/3150 from the Fondo de Investigación Sanitaria, and BIO2002/00953 from the Ministerio de Ciencia y Tecnología, Government of Spain, to S.M.-A. E.P.-R. was supported by a grant from the Consejería de Educación, Cultura y Deportes, and C.L.-A. was supported by a grant from FUNCIS, Canary Islands Autonomous Government. S.M.-A. was partially supported by Fondo de Investigación Sanitaria contract 99/3060.

## REFERENCES

1. Anthony, R. M., A. M. Connor, E. G. M. Power, and G. L. French. 1999. Use of the polymerase chain reaction for rapid detection of high-level mupirocin resistance in staphylococci. *Eur. J. Clin. Microbiol.* **18**:30–34.
2. Berg, T., N. Firth, S. Apisiridej, A. Hettiaratchi, A. Leelaporn, and R. A. Skurray. 1998. Complete nucleotide sequence of pSK41: evolution of staphylococcal conjugative multiresistance plasmids. *J. Bacteriol.* **180**:4350–4359.
3. Bradley, S. F., M. A. Ramsey, T. M. Morton, and C. A. Kauffman. 1995. Mupirocin resistance: clinical and molecular epidemiology. *Infect. Control Hosp. Epidemiol.* **16**:354–358.
4. Cookson, B. D. 1998. The emergence of mupirocin resistance: a challenge to infection control and antibiotic prescribing practice. *J. Antimicrob. Chemother.* **41**:11–18.
5. Eltringham, I. 1997. Mupirocin resistance and methicillin-resistant *Staphylococcus aureus* (MRSA). *J. Hosp. Infect.* **35**:1–8.
6. Enright, M. C., D. A. Robinson, G. Randle, E. J. Feil, H. Grundmann, and B. Spratt. 2002. The evolutionary history of methicillin resistant *Staphylococcus aureus* (MRSA). *Proc. Natl. Acad. Sci. USA* **99**:7687–7692.
7. Finlay, J. E., L. A. Miller, and J. A. Poupard. 1997. Interpretative criteria for testing susceptibility of staphylococci to mupirocin. *Antimicrob. Agents Chemother.* **41**:1137–1139.
8. Gilbert, J., C. R. Perry, and B. Slocombe. 1993. High-level mupirocin resistance in *Staphylococcus aureus*: evidence for two distinct isoleucyl-tRNA synthetases. *Antimicrob. Agents Chemother.* **37**:32–38.
9. Grohmann, E., G. Muth, and M. Espinosa. 2003. Conjugative plasmid transfer in gram-positive bacteria. *Microbiol. Mol. Biol. Rev.* **67**:277–301.
10. Hodgson, J. E., S. P. Curnock, K. G. H. Dyke, R. Morris, D. R. Sylvester, and M. S. Gross. 1994. Molecular characterization of the gene encoding high-level mupirocin resistance in *Staphylococcus aureus* J2870. *Antimicrob. Agents Chemother.* **38**:1205–1208.
11. Hurdle, J. G., A. J. O'Neill, L. Mody, I. Chopra, and S. F. Bradley. 2005. In vivo transfer of high-level mupirocin resistance from *Staphylococcus epidermidis* to methicillin-resistant *Staphylococcus aureus* associated with failure of mupirocin prophylaxis. *J. Antimicrob. Chemother.* **56**:1166–1168.
12. Morton, T. M., J. L. Johnston, J. Patterson, and G. L. Archer. 1995. Characterization of a conjugative staphylococcal mupirocin resistance plasmid. *Antimicrob. Agents Chemother.* **39**:1272–1280.
13. Needham, C., M. Rahman, K. G. H. Dyke, and W. C. Noble. 1994. An investigation of plasmids from *Staphylococcus aureus* that mediate resistance to mupirocin and tetracycline. *Microbiology* **140**:2577–2583.
14. Oliveira, D. C., A. Tomasz, and H. de Lencastre. 2002. Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. *Lancet Infect. Dis.* **2**:180–189.
15. Palepou, M. F. I., A. P. Johnson, B. D. Cookson, H. Beattie, A. Charlett, and N. Woodford. 1998. Evaluation of disc diffusion and Etest for determining the susceptibility of *Staphylococcus aureus* to mupirocin. *J. Antimicrob. Chemother.* **41**:577–583.
16. Pawa, A., W. C. Noble, and S. A. Howell. 2000. Co-transfer of plasmids in association with conjugative transfer of mupirocin or mupirocin and penicillin resistance in methicillin-resistant *Staphylococcus aureus*. *J. Med. Microbiol.* **49**:1103–1107.
17. Pérez-Roth, E., F. Claverie-Martín, J. Villar, and S. Méndez-Álvarez. 2001. Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. *J. Clin. Microbiol.* **39**:4037–4041.
18. Pérez-Roth, E., F. Claverie-Martín, N. Batista, A. Moreno, and S. Méndez-Álvarez. 2002. Mupirocin resistance in methicillin-resistant *Staphylococcus aureus* clinical isolates in a Spanish hospital. Co-application of multiplex PCR assay and conventional microbiology methods. *Diagn. Microbiol. Infect. Dis.* **43**:123–128.
19. Pérez-Roth, E., F. Lorenzo-Díaz, and S. Méndez-Álvarez. 2003. Establishment and clonal dissemination of the methicillin-resistant *Staphylococcus*

- aureus* UK-16 epidemic strain in a Spanish hospital. *J. Clin. Microbiol.* **41**:5353.
20. **Pérez-Roth, E., F. Lorenzo-Díaz, N. Batista, A. Moreno, and S. Méndez-Álvarez.** 2004. Tracking methicillin-resistant *Staphylococcus aureus* clones during a 5-year period (1998 to 2002) in a Spanish hospital. *J. Clin. Microbiol.* **42**:4649–4656.
  21. **Rahman, M., S. Connolly, W. C. Noble, B. Cookson, and I. Phillips.** 1990. Diversity of staphylococci exhibiting high-level resistance to mupirocin. *J. Med. Microbiol.* **33**:97–100.
  22. **Santos-Sanches, I., M. Ramirez, H. Troni, M. Abecassis, M. Papua, A. Tomasz, and H. de Lencastre.** 1995. Evidence for the geographic spread of methicillin-resistant *Staphylococcus aureus* clone between Portugal and Spain. *J. Clin. Microbiol.* **33**:1243–1246.
  23. **Schmitz, F. J., E. Lindenlauf, B. Hofmann, A. C. Fluit, J. Verhoef, H. P. Heinz, and M. E. Jones.** 1998. The prevalence of low- and high-level mupirocin resistance in staphylococci from 19 European hospitals. *J. Antimicrob. Chemother.* **42**:489–495.
  24. **Udo, E. E., and L. E. Jacob.** 1998. Conjugative transfer of high-level mupirocin resistance and the mobilization of non-conjugative plasmids in *Staphylococcus aureus*. *Microb. Drug Resist.* **4**:185–193.
  25. **Upton, A., S. Lang, and H. Heffernan.** 2003. Mupirocin and *Staphylococcus aureus*: a recent paradigm of emerging antibiotic resistance. *J. Antimicrob. Chemother.* **51**:613–617.
  26. **Woodford, N., A. P. Watson, S. Patel, M. Jevon, D. J. Waghorn, and B. D. Cookson.** 1998. Heterogeneous location of the *mupA* high-level mupirocin resistance gene in *Staphylococcus aureus*. *J. Med. Microbiol.* **47**:829–835.