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Characterization of a Conjugative Staphylococcal Mupirocin Resistance Plasmid

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We studied conjugative plasmids encoding high-level mupirocin resistance. These plasmids were found in *Staphylococcus aureus* isolates from two geographic locations in the United States. Transfer genes on three mupirocin resistance plasmids with different restriction endonuclease profiles were indistinguishable by DNA hybridization from those on pG01, a conjugative aminoglycoside resistance plasmid representative of similar plasmids that are prevalent in the United States. One mupirocin resistance plasmid, pG0400 (34 kb), was smaller than pG01 (52 kb) because of the absence from pG0400 of DNA, found on pG01, that contained genes encoding resistance to aminoglycosides, trimethoprim, and quaternary ammonium compounds flanked by directly repeated copies of the insertion sequence (IS)-like element IS*431*-IS*257*. The plasmids pG0400 and pG01 were otherwise indistinguishable except for the presence in pG0400 of a 4.5-kb *Hin*dIII fragment encoding mupirocin resistance. The added mupirocin resistance gene was flanked by two directly repeated copies of IS*431*/*257*. The nucleotide sequence of DNA contiguous to the outside of the IS elements, as well as those of the elements themselves, was identical in both pG01 and pG0400, and there were no target site duplications flanking either copy of the element. We conclude that the mupirocin resistance gene was added to an existing conjugative plasmid in conjunction with the deletion of other resistance genes by recombination at IS elements. The construction of conjugative plasmids carrying a mupirocin resistance gene may be a model for the mobility of other resistance genes newly acquired by staphylococci.

Mupirocin (pseudomonic acid A) is a topical antimicrobial agent that is produced by *Pseudomonas fluorescens* (15) and that has activity against staphylococci and streptococci (10, 40). Mupirocin competitively inhibits bacterial isoleucyl-tRNA synthetase and thus interferes with protein synthesis (21). In clinical trials it has been highly successful in eradicating staphylococci from the nares and other sites colonized by staphylococci (11, 19, 23, 34, 37). Staphylococcal isolates resistant to mupirocin are found following topical application (12, 22, 23, 25, 26, 30), and resistance is of two types. First, there is low-level to intermediate resistance, the most common type, usually because of a mutation in the target enzyme. Low-level resistance is usually defined as an MIC of between 8 and 256 μg/ml (17). Second, there is high-level resistance that is mediated by a mupirocin-insusceptible, plasmid-encoded target enzyme acquired from a source other than *Staphylococcus aureus* (12, 13, 17, 20). High-level resistance is defined as an MIC of >500 μg/ml (17). In several reports plasmids encoding high-level mupirocin resistance were shown to be transmissible to *S. aureus* recipients on filter membranes (13, 31, 32), but these plasmids have not been characterized further.

Mupirocin-resistant *S. aureus* isolates were recovered from patients at two different hospitals in the United States; a number of the isolates had high-level resistance (22, 25). Investigators at one institution made the observation that plasmids encoding mupirocin resistance could be transferred to staphylococcal recipients by filter mating (22) and that one of their conjugative mupirocin resistance plasmids was incompatible with a conjugative plasmid belonging to a class of well-described plasmids that are prevalent in North American staphylococci (3, 18, 28, 36). These plasmids are exemplified by pG01 (52 kb), which encodes resistance to aminoglycosides, trimethoprim, and quaternary ammonium compounds, that is capable of transfer to staphylococci of different species (24, 25). pG01 also contains nine copies of an insertion sequence (IS)-like element, IS*431*-IS*257* (4, 8), eight of which are directly repeated. These IS-like sequences, bounding DNA segments that contain antimicrobial resistance or conjugative transfer genes, may have been involved in the cassette-like construction of this plasmid (27). Here we report the acquisition of the gene encoding mupirocin resistance by a pG01-like conjugative plasmid and the apparent horizontal transmission of conjugative mupirocin resistance plasmids in a hospital. DNA sequence data suggest that the gene encoding mupirocin resistance was integrated into the conjugative replicon by recombination at IS-like elements.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *S. aureus* plasmids used in the study are listed in Table 1. *S. aureus* G03000 (LTZ-1 in reference 22) was recovered in 1991 from a patient at the Ann Arbor Veterans Affairs Hospital and has been described previously (22). It was kindly provided by Carol Kauffman. Isolates G03221, G04236, and G03865 (isolates 1, 13, and 20, respectively, in reference 25; Table 1) were recovered from patients seen in the Yale New Haven Hospital in 1991 and have been described previously (25). DNA fragments were cloned in *Escherichia coli* on pUC19 (34), pBR322 (5), pBluescript (7), or pOP302 $(A_2)^+$ (43). All *E. coli* plasmids have been described in a previous publication (29). Clones were propagated in *E. coli* HB101(pBR322 and pOP203 $(A_2)^+$ [6]) and

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TABLE 1. *S. aureus* strains and plasmids evaluated in the study

Isolate or strain	Description	Resistance[a]	Reference or source
Strains			
G03221	*S. aureus* isolate 1 from a previous study (25); epidemic isolate from an outbreak on the dermatology ward at the Yale New Haven Hospital	Ox^s Mup^r	25
G04236	*S. aureus* isolate 13 from a previous study (25); isolate from the sputum of a patient seen in the emergency room at the Yale New Haven Hospital	Ox^r Mup^r	25
G03865	*S. aureus* isolate 20 from a previous study (25); isolate from the blood of a patient seen in the emergency room at the Yale New Haven Hospital	Ox^r Mup^r	25
G03000	*S. aureus* isolate LZ-1 from a previous study (22); isolate colonizing a patient at the Ann Arbor Veterans Affairs Hospital	Ox^r Mup^r	22
G0400	Transconjugant of mating between G03221 (donor) and RN4220NR (recipient)	Nov^r Rif^r Mup^r	This study
G0401	Transconjugant of mating between G04236 and RN4220NR	Nov^r Rif^r Mup^r	This study
G0403	Transconjugant of mating between G03000 and RN4220NR	Nov^r Rif^r Mup^r	This study
RN4220NR	Derivative of RN4220 (24), a restriction-deficient host used as a recipient in genetic manipulations	Nov^r Rif^r	This study
Plasmid			
pG0400	A 33.8-kb conjugative mupirocin resistance plasmid resident in G03221 and G03865	Mup^r	This study
pG0401	A 44.1-kb conjugative mupirocin resistance plasmid resident in G04236	Mup^r	This study
pG0403	A 48.2-kb conjugative mupirocin resistance plasmid resident in G03000	Mup^r	This study
pG0402	The 11.1-kb *EcoRI*-A fragment of pG0400 cloned on pOP302 $(A_2)^+$	Tet^r	This study
pG0407	The 2.4-kb *EcoRI*-E fragment of pG0400 cloned on pOP302 $(A_2)^+$	Tet^r	This study
pG0460	The 4.4-kb *EcoRI*-G fragment of pG01 cloned on pOP302 $(A_2)^+$	Tet^r	This study
pG0423	A 7.8-kb *EcoRI-Cla*I fragment of pG01 cloned on pBluescript KS(+)	Amp^r	This study
pG0435	A 4.6-kb *Hin*dIII fragment of pG0400 containing the mupirocin resistance gene cloned on pUC19	Amp^r	This study
pG0435C	pG0435 to which the staphylococcal replicon pSK265 has been added for expression of mupirocin resistance in *S. aureus*	Chl^r Mup^r	This study
pG0171	A 6.2-kb *Eco*RI fragment from the conjugative transfer region (*trs*) of pG01 cloned on pUC19	Amp^r	29
pG0311	A 3.5-kb *Eco*RI-*Sph*I fragment from the conjugative transfer region (*trs*) of pG01 cloned on pUC19	Amp^r	This study
pG0189	A 2.3-kb *Eco*RI-*Hin*dIII fragment flanking the *oriT* site of pG01 cloned on pUC19	Amp^r	This study

[a] Amp, ampicillin; Chl, chloramphenicol; Mup, mupirocin; Nov, novobiocin; Ox, oxacillin; Rif, rifampin; Tet, tetracycline; R, resistant; S, susceptible.

TB1(pUC19 and pBluescript [44]). Recombinant plasmids constructed in *E. coli* were given a pG0 number. When pSK265 (33) carrying chloramphenicol resistance was added as a staphylococcal replicon in order to create a shuttle plasmid, the letter C followed the pG0 number.

Materials and media. Mueller-Hinton agar (MHA; BBL Microbiology Systems, Cockeysville, Md.) was used to culture both *E. coli* and *S. aureus* strains. Lennox L base (GIBCO-Bethesda Research Laboratories, Inc., Gaithersburg, Md.) broth supplemented with 0.2% glucose was used exclusively to culture *E. coli* strains, while brain heart infusion broth (Difco, Detroit, Mich.) or Trypticase soy broth (BBL, Becton, Dickinson & Company, Cockeysville, Md.) was used exclusively to culture *S. aureus* strains. The antibiotic concentrations used were as follows: chloramphenicol, 10 μg/ml, for initial selection of *S. aureus* clones after electroporation; chloramphenicol, 40 μg/ml, for shuttle construction in *E. coli* and maintenance of shuttle plasmids in *S. aureus*; gentamicin, 10 μg/ml, and erythromycin, 20 μg/ml, for plasmid maintenance in *S. aureus*; novobiocin and rifampin, 10 μg/ml each, for selection of plasmids transferred between *S. aureus* pairs by conjugation; ampicillin, 50 μg/ml, for selection of *E. coli* HB101, XL-1 Blue, and TB1 clones; tetracycline, 12.5 μg/ml, in addition to ampicillin, for selecting all XL-1 Blue clones; and mupirocin, 20 μg/ml, both for selection of clones containing the mupirocin resistance gene and for selection of transconjugants in mating experiments. The pBluescript II KS(+) cloning vector and XL-1 Blue host strain were from Stratagene (La Jolla, Calif.). All chemicals and antibiotics with the exception of mupirocin were from Sigma Chemical Co. (St. Louis, Mo.); mupirocin was kindly supplied by SmithKline Beecham (King of Prussia, Pa.). Restriction endonucleases and the other enzymes involved in DNA manipulations were obtained from GIBCO-Bethesda Research Laboratories. Agarose and acrylamide were from International Biotechnologies Inc. (New Haven, Conn.). DNA sequencing was done by using Sequenase version 2.0 (United States Biochemicals, Cleveland, Ohio) with [α-^{35}S]dATP or [α-^{33}P] dATP (New England Nuclear/DuPont, Boston, Mass.). DNA probes for Southern analysis were generated by nick-translation with [α-^{32}P]dCTP and a kit from New England Nuclear/DuPont. Vent polymerase for PCRs was from New England Biolabs, Inc., Beverly, Mass.

Cloning, transformation, and DNA manipulation. Restriction endonuclease digestions, ligations, Klenow reactions, and electrophoresis were performed as described by the manufacturer. Preparation of plasmid DNA and transformation of recombinant plasmids into *E. coli* and *S. aureus* by electroporation were performed as described previously (29).

Sequence analysis. Sequence analysis was performed by the Sanger dideoxy chain termination method with double-stranded DNA as a template. Sequencing reactions were run according to the manufacturer's specifications for sequencing double-stranded DNA with Sequenase version 2.0 as described previously (29). The IS element DNA was sequenced by subcloning *Eco*RI fragments that contained the entire element (see Fig. 3) and by using sequentially synthesized oligonucleotide primers to progress along the cloned DNA. The DNA sequences of the IS elements were analyzed by MacVector Sequence Analysis for potential open reading frames, specific motifs, and comparison with other IS elements.

Southern analysis. Plasmid DNA from *S. aureus* was isolated by cesium chloride density gradient centrifugation as described previously (16). Samples were analyzed by restriction endonuclease digestion and agarose gel electrophoresis.

Southern blot procedures were performed under conditions described previously (41). Briefly, DNA was separated by electrophoresis in 0.7% agarose and was then depurinated, denatured, and transferred to nitrocellulose overnight in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Nitrocellulose was baked for 2 h in a vacuum oven and was prehybridized in Denhardt's solution for 4 h at 42°C. Blots were then hybridized overnight at 42°C with Denhardt's solution containing the appropriate [α-^{32}P]dCTP-labelled probes in 50% formamide, washed, and exposed to X-ray film at 70°C for a time appropriate for optimum visualization of signals. Radiolabelled DNA probes were generated by nick-translation.

Filter mating. Overnight brain heart infusion (BHI) agar cultures of donor and recipient strains were suspended in saline to a no. 3 McFarland standard, mixed in a 1:1 ratio (2 ml of donor strain to 2 ml of recipient strain) and forced through a syringe onto a 25-mm-diameter, 0.45-μm-pore-size nitrocellulose filter. The filter was placed on BHI agar, bacteria side up, and was incubated at 37°C overnight. Cells were vortexed off of the filters in 1 ml of saline and were plated onto MHA containing appropriate antibiotics, selecting for donors, recipients, and transconjugants. The recipient was always *S. aureus* RN4220NR, which was resistant to novobiocin and rifampin. Mating frequency was expressed as the

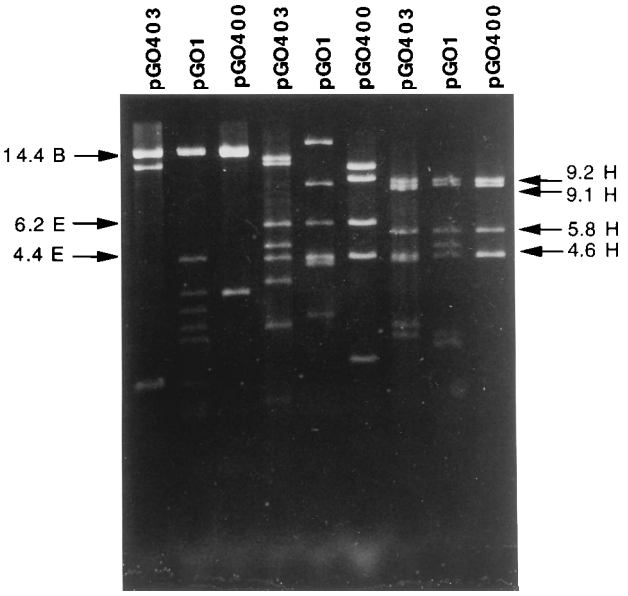


FIG. 1. Restriction endonuclease digests of three conjugative plasmids. The pG0403 and pG0400 plasmids, from patients in Michigan and Connecticut, respectively, encoded mupirocin resistance, while pG01 encoded resistance to aminoglycosides, trimethoprim, and quaternary ammonium compounds. The plasmids in the first three lanes were digested with *Bgl*II, the plasmids in next three lanes were digested with *Eco*RI, and the plasmids in last three lanes were digested with *Hin*dIII. The numbers to the right and left indicate the sizes of the specific fragments (in kilobases) that are common to all three plasmids. The letters designate lanes containing *Eco*RI (E), *Hin*dIII (H), or *Bgl*II (B).

number of transconjugants (Nov^r Rif^r Mup^r) divided by the number of donors (Mup^r).

RESULTS

Mating experiments. Wild-type donor isolates had the following plasmid contents: G03221, three plasmids; G03865, two plasmids; and G04236 and G03000, a single large plasmid each. Filter mating was performed with each donor and the RN4220NR recipient by selecting for transconjugants resistant to mupirocin, novobiocin, and rifampin. Transfer frequencies were similar for each mating, ranging from 1×10^{-5} to 5×10^{-5} transconjugants per donor. These transfer frequencies were the same as those in matings performed simultaneously with RN4220 containing pG01 as the donor, selecting for transconjugants resistant to gentamicin, novobiocin, and rifampin. Transconjugants from each mating contained a single plasmid with sizes as follows: pG01, 52 kb; pG0403 from G03000, 48.2 kb; pG0401 from G04236, 44.1 kb; and pG0400 from both G03221 and G03865, 33.8 kb. Restriction digests of pG01, pG0400, and pG0403 are shown in Fig. 1.

DNA hybridization and cloning of the mupirocin resistance gene. The restriction endonuclease digests shown in Fig. 1 were probed with DNA fragments cloned from pG01 (Table 1). Two probes, pG0171 and pG0311, were from the pG01 conjugative transfer region, *trs* (32); the third probe, pG0189, was from another region of pG01 required for conjugative transfer (38). These probes (see Fig. 3) were chosen because they contained no repeated IS-like sequences. All probes hybridized with fragments from pG01, pG0400, and pG0403 (Fig. 2A to C) and pG0401 (data not shown). Multiple fragments of these plasmids also hybridized with pG0156, a probe containing sequences internal to the IS-like element IS*431*-IS*257* (41; data not shown).

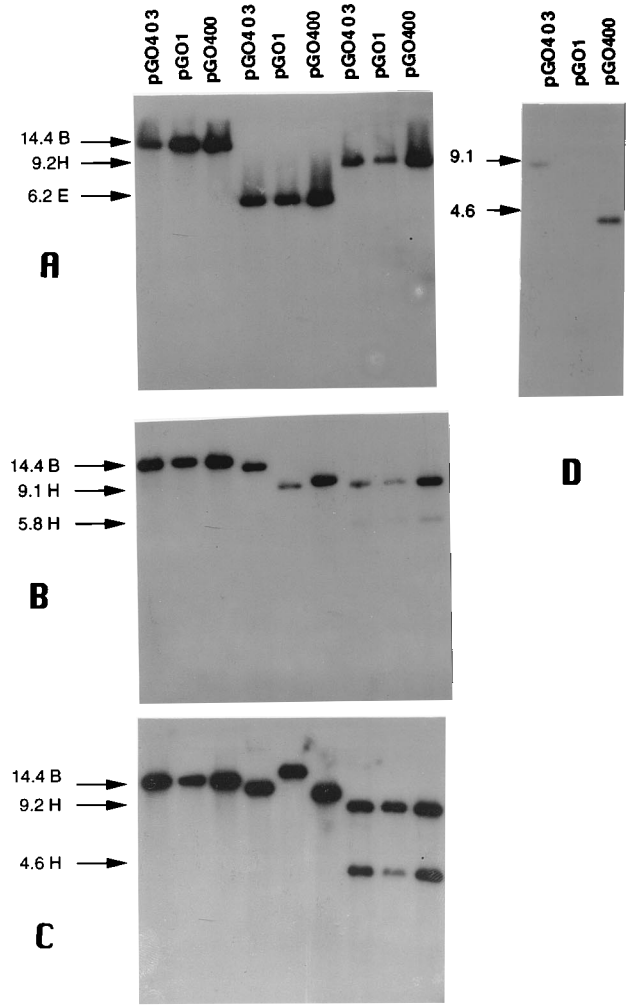


FIG. 2. Southern blots of the gel shown in Fig. 1 after transfer of the DNA to a nitrocellulose membrane and hybridization with a variety of ^{32}P-labelled DNA probes. Each panel contains the same target DNA that was stripped of its probe and rehybridized. All lanes correspond to plasmids cut with the same enzymes described in the legend to Fig. 1. (A) All digests were probed with pG0171, a 6.2-kb fragment cloned from the pG01 *trs* transfer region; (B) all digests were probed with pG0189, a 2.3-kb fragment cloned from an undefined region of pG01 required for transfer; (C) all digests were probed with pG0311, a 3.7-kb fragment that was also from the pG01 *trs* region; (D) only the *Hin*dIII digests of the three plasmids were probed with the 3.6-kb *Bgl*II fragment from pG0400.

On the basis of the plasmid map of pG0400 (see below) we felt that a portion of the mupirocin resistance gene was contained in a 3.1-kb *Bgl*II fragment. When this fragment was used as a probe against *Hin*dIII digests of pG0403, pG0400, and pG01, only the mupirocin resistance plasmids hybridized with the probe (Fig. 2D). The 4.6-kb *Hin*dIII fragment from pG0400 that hybridized with the *Bgl*II fragment probe was then cloned in *E. coli* to yield pG0435, and the pSK265 staphylococcal replicon was added to produce the shuttle plasmid pG0435C. Following electroporation of pG0435C into staphylococcal strain RN4220, transformants were able to grow on plates containing 500 μg of mupirocin per ml, confirming the location of the mupirocin resistance gene on pG0400.

Construction of plasmid maps. The smallest mupirocin resistance plasmid, pG0400, was chosen for more detailed study and was mapped by using both the hybridization data described

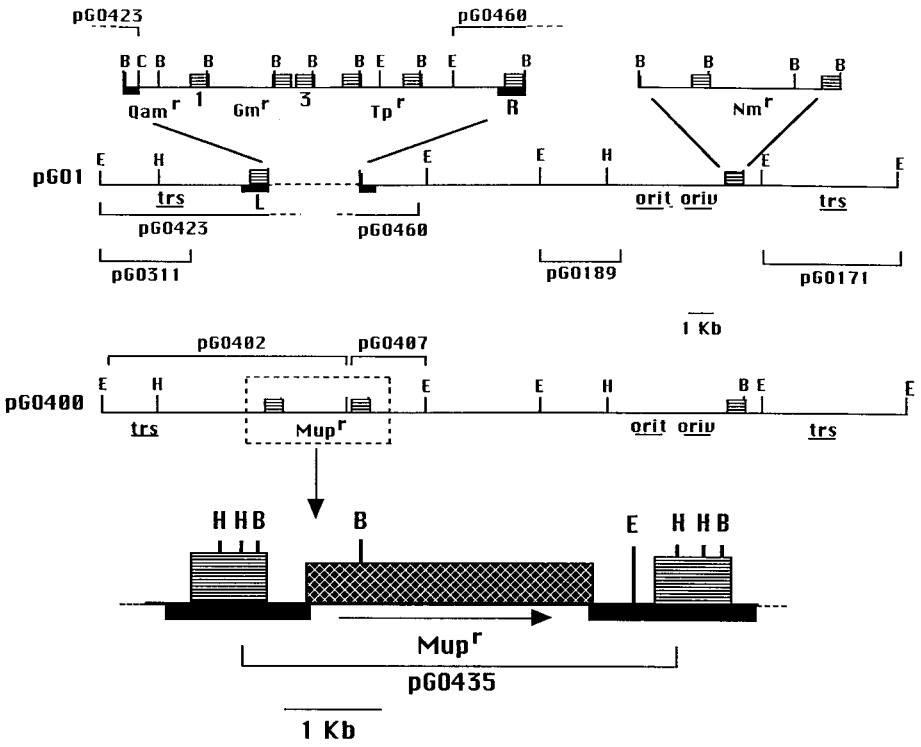


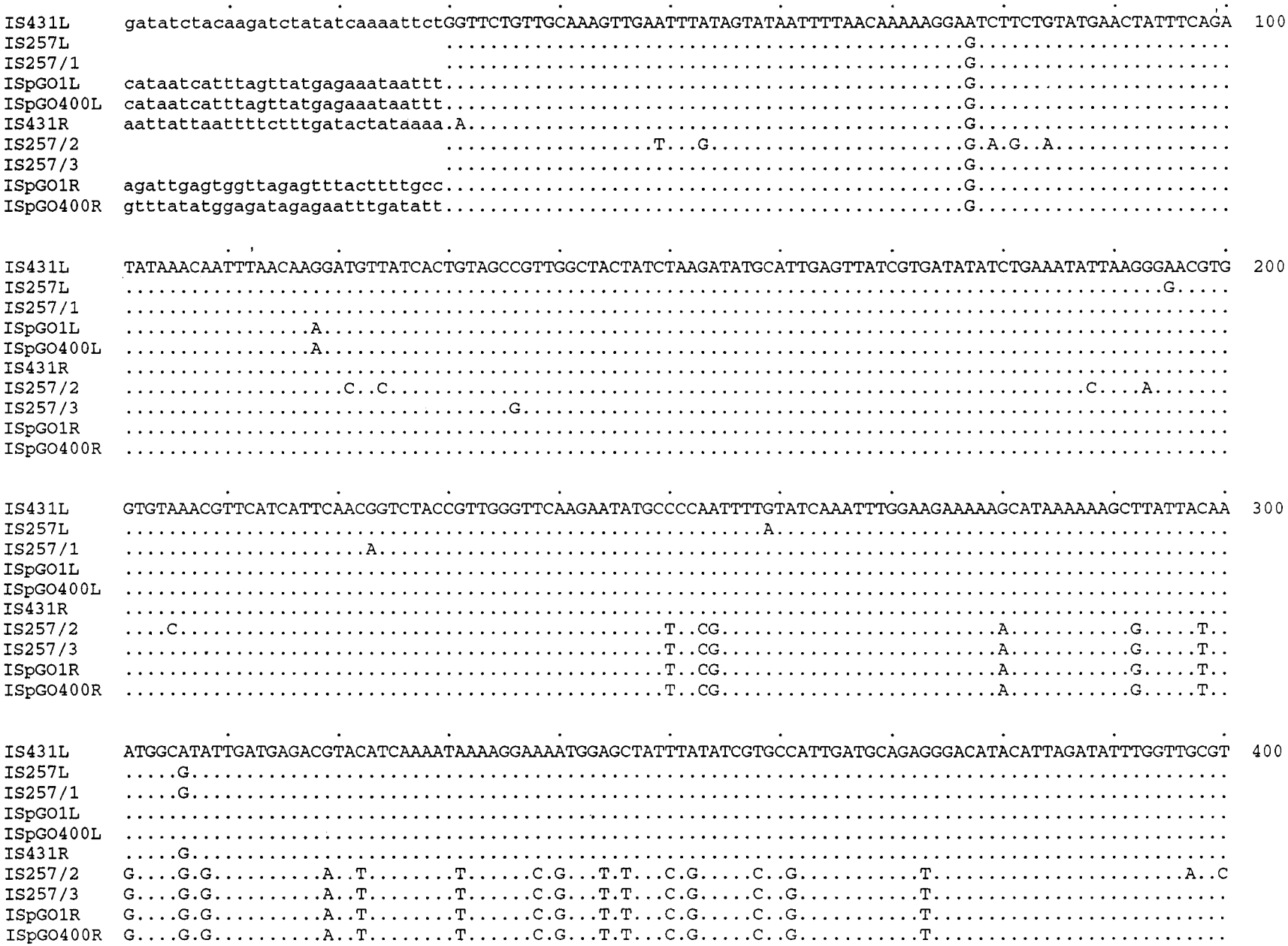
FIG. 3. Restriction site maps of conjugative plasmids pG01 and pG0400 and their common genes. Antibiotic resistance genes are identified as follows: Qam^r, quaternary ammonium resistance; Gm^r, gentamicin resistance; Tp^r, trimethoprim resistance; Nm^r, neomycin resistance; Mup^r, mupirocin resistance. The major transfer gene complex is *trs*; *oriT* and *oriV* designate the origins of conjugative transfer and vegetative replication, respectively. The restriction endonuclease cleavage sites are as follows: E, *Eco*RI; H, *Hin*dIII; B, *Bgl*II; and C, *Cla*I. Brackets indicate the fragments cloned as probes or used to determine nucleotide sequence or gene phenotype as described in the text. The dark boxes underline segments of the plasmids for which the nucleotide sequence was determined. The horizontally striped boxes above the line designate the sites for directly repeated copies of the insertion sequence IS*431*/*257*. The arrow under the hatched mupirocin resistance gene, enlarged at the bottom of the figure, indicates the direction of transcription of the gene. L and R under IS sequence boxes on pG01 indicate ISpG01L and ISpG01R, respectively, as described in the text. The numbers 1 and 3 designate the IS elements that are probably identical to IS*257*1 and IS*2573*, respectively (28).

above and single and double digests with restriction endonucleases. The map of pG0400 was compared with the previously published map of pG01 (27). As illustrated in Fig. 3, pG0400 was identical to pG01 by DNA hybridization and analysis of restriction endonuclease cleavage sites in the regions containing transfer genes (*trs*), the origins of transfer and vegetative replication (*ori*t and *ori*v), and a 4.5-kb *Eco*RI fragment containing DNA of unknown function. However, large DNA fragments present on pG01 were deleted from pG0400. These fragments in pG01 contained genes encoding resistance to quaternary ammonium compounds (Qam^r or *qacD* [8]), gentamicin resistance (Gm^r or *aacA-aphD* [8]), trimethoprim resistance (Tp^r or *dfrA* [40]), and neomycin and paromomycin resistance [8]), (Nm^r or *aadD* [9]) as well as numerous directly repeated copies of IS*431*-IS*257*. The fragments in pG01 that were not found in pG0400 were precisely those between directly repeated IS copies. Similarly, the additional DNA in pG0400 that was not found in pG01 and that carried the mupirocin resistance gene was flanked by directly repeated IS copies; each copy was similar in relative location to the position of each of two elements present on pG01.

DNA sequence analysis of IS elements and flanking DNA. In order to further investigate possible mechanisms by which the mupirocin resistance gene was acquired by a core pG01-like conjugative plasmid, the IS*431*-IS*257* elements flanking the mupirocin resistance gene and elements in a similar relative position on pG01 were sequenced as diagrammed in Fig. 3. The comparative nucleotide sequences are shown in Fig. 4. The nucleotide sequence of the left IS element on pG01 (ISpG01L in Fig. 4) differed from ISpG0400L only at nucleotides 769 and 768 (AC for ISpG01L and CT for ISpG0400L) and 804 (the addition of an A residue to ISpG0400L). The sequences of ISpG01R and ISpG0400R were identical. In contrast, ISpG01L and ISpG01R differed from each other by 48 nucleotides, and ISpG0400L and ISpG0400R differed from each other by 46 nucleotides.

The IS sequences on pG01 and pG0400 were also compared with the published sequences of similar elements found at a number of different genetic loci. The left copy of IS*431* (IS*431*L) and the right copy of IS*431* (IS*431*R) were initially cloned from the *S. aureus* chromosome and were located within DNA flanking *mecA*, the gene mediating methicillin resistance (24). The left and right copies of ISpG01 differed from the IS*431* sequences at 28 and 25 nucleotides, respectively, with different nucleotide changes in each copy. Likewise, two copies of IS*257* (IS*257*L and IS*257*/2), which were found on staphylococcal plasmids in association with trimethoprim and gentamicin resistance genes (8), differed from both ISpG01L and ISpG01R and ISpG0400L and ISpG0400R at more than 25 nucleotides. In contrast, IS*257*/3 differed from ISpG01R and ISpG0400R at only one nucleotide and IS*257*/1 differed from ISpG01L and ISpG0400L at only three nucleotides. IS*257*/1 and IS*257*/3 flank the 5′ and 3′ ends, respectively, of the *aacA-aphD* gentamicin resistance gene on staphylococcal plasmid pSH6. These IS*257* sequences correspond to those numbered 1 and 3 on pG01 in Fig. 3; ISpG01L and ISpG01R are IS sequences L and R in Fig. 3.

We also determined the nucleotide sequence of DNA inter-


IS431L gatatctacaagatctatatcaaaattctGGTTCTGTTGCAAAGTTGAATTTATAGTATAATTTTAACAAAAAGGAATCTTCTGTATGAACTATTTCAGA 100
IS257L G
IS257/1 G
ISpGO1L cataatcatttagttatgagaaataattt G
ISpGO400L cataatcatttagttatgagaaataattt G
IS431R aattattaattttctttgatactataaaa.A G
IS257/2 T...G G.A.G..A
IS257/3 G
ISpGO1R agattgagtggttagagtttacttttgcc G
ISpGO400R gtttatatggagatagagaatttgatatt G
IS431L TATAAACAATTTAACAAGGATGTTATCACTGTAGCCGTTGGCTACTATCTAAGATATGCATTGAGTTATCGTGATATATCTGAAATATTAAGGGAACGTG 200
IS257L G
IS257/1
ISpGO1L .A
ISpGO400L .A
IS431R
IS257/2 C..C C....A
IS257/3 G
ISpGO1R
ISpGO400R
IS431L GTGTAAACGTTCATCATTCAACGGTCTACCGTTGGGTTCAAGAATATGCCCCAATTTTGTATCAAATTTGGAAGAAAAAGCATAAAAAAGCTTATTACAA 300
IS257L A
IS257/1 A
ISpGO1L
ISpGO400L
IS431R
IS257/2 ....C T..CG A G....T..
IS257/3 T..CG A G....T..
ISpGO1R T..CG A G....T..
ISpGO400R T..CG A G....T..
IS431L ATGGCATATTGATGAGACGTACATCAAAATAAAAGGAAAATGGAGCTATTTATATCGTGCCATTGATGCAGAGGGACATACATTAGATATTTGGTTGCGT 400
IS257L .....G
IS257/1 .....G
ISpGO1L
ISpGO400L
IS431R .....G
IS257/2 G....G.G.....A..T.....T.....C.G...T.T...C.G.....C..G.....T.....A..C
IS257/3 G....G.G.....A..T.....T.....C.G...T.T...C.G.....C..G.....T.....
ISpGO1R G....G.G.....A..T.....T.....C.G...T.T...C.G.....C..G.....T.....
ISpGO400R G....G.G.....A..T.....T.....C.G...T.T...C.G.....C..G.....T.....

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IS431L      AAGCAACGAGATAATCATTCGGCATATGCGTTTATCAAACGTCTCATTAAACAATTTGGTAAACCTCAAAAGGTAATTACAGATCAGGCACCTTCAACGA  500
IS257L      ....................A..............T.....................................................................
IS257/1     ....................A.....................................................................................
ISpGO1L     ....................A.....................................................................................
ISpGO400L   ....................A.....................................................................................
IS431R      .....................................................................................................
IS257/2     ..AA........C....A..A..............T..G..C..T..........C.............T.A.............A.................
IS257/3     ....................A.....................................................................................
ISpGO1R     ....................A.....................................................................................
ISpGO400R   ....................A.....................................................................................

                    .         .         .         .         .         .         .         .         .         .
IS431L      AGGTAGCAATGGCTAAAGTAATTAAAGCTTTTAAACTTAAACCTGACTGTCATTGTACATCGAAATATCTGAATAACCTCATTGAGCAAGATCACCGTCA  600
IS257L      ..................................................C..................................................
IS257/1     .A........A.........................................................................................
ISpGO1L     .A........A.........................................................................................
ISpGO400L   .A........A.........................................................................................
IS431R      .....................................................................................................
IS257/2     .......C.........A.........TG.....GT.G......A..........C.....T........C......T.......A........T.....
IS257/3     ..........................................G..........................................................
ISPGO1R     ..........................................G..........................................................
ISpGO400R   ..........................................G..........................................................

                    .         .         .         .         .         .         .         .         .         .
IS431L      TATTAAAGTAAGAAAGACAAGGTATCAAAGTATCAATACAGCAAAGAATACTTTAAAAGGTATTGAATGTATTTACACTCTATATAAAAAGAACCGCAGG  700
IS257L      ..................................................................................G..................
IS257/1     ....................A.................G.....A.....AC.C.....C.............G.....................A
ISpGO1L     ....................A.................G.....A.....AC.C.....C.............G.....................A
ISpGO400L   ....................A.................G.....A.....AC.C.....C.............G.....................A
IS431R      .....................................................................................................
IS257/2     C.....G..G..........A.........G.......G.....A.....AC.C.....C.............GGAT...........-....T...
IS257/3     ..................................................................................G..................
ISpGO1R     ..................................................................................G..................
ISpGO400R   ..................................................................................G..................

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IS431L      TCTCTTCAGATCTACGGATTTTCGCCATGCCACGAAATTAGCATCATGCTAGCAAGTTAAGCGAACACTGACATGATAAATTAGTGGTTAGCTATATTTT  800
IS257L      .....................................................................................................
IS257/1     ..........................................GGAT...T....C.......G.....AC...........A.G..A.............
ISpGO1L     ..........................................GGAT...T....C.......G.....AC...........A.G..A.............
ISpGO400L   ..........................................GGAT...T....C.......G...................A.G..A.............
IS431R      .....................................................................................................
IS257/2     ........................A.................TTAT...T....TG....G.....G.......CA.....A.G...A.............
IS257/3     .....................................................................................................
ISpGO1R     .....................................................................................................
ISpGO400R   .....................................................................................................
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                    .          .          .          .          .          .          .
IS431L      T---ACTTTGCAACAGAACCagaattaatataacgagatatcaa
IS257L      .-T-...............
IS257/1     .CTA...............
ISpGO1L     .CTA...............aaactttgtatacaacttagttgtttttacagtgatttaatatatgg
ISpGO400L   .CT-...............ctactaatgatataatttgcagtagtttgtattataactttacttta
IS431R      .-TT...............cctaaaattcatttattgggcatacaatgctttt
IS257/2     .CTA...............
IS257/3     .-T-...............
ISpGO1R     .---...............aaataaaagataaaataacatcttggcttgataaagataacaacaaa
ISpGO400R   .---...............aaataaaagataaaataacatcttggcttgataaagataacaacaaa
```

FIG. 4. Comparison of the nucleotide sequences of IS-like sequences that were either determined as a result of the present study (ISpG01L and ISpG01R; ISpG0400L and ISpG0400R) or contained in previous publications (IS*431*L and R [4] and IS*257* [8]). Flanking sequences are indicated by lowercase letters, while IS sequences are indicated by capital letters. Dots beneath IS nucleotides designate sequence identity to that of IS431L; substituted nucleotides are indicated.

nal to the IS elements, within the fragment encoding mupirocin resistance, and compared it with the sequence recently reported for DNA flanking a gene mediating high-level mupirocin resistance cloned from a conjugative plasmid resident in an *S. aureus* isolate from England (pJ2947 [20]). A total of 158 bases 5′ to the translational start site and 466 bases 3′ to the translational stop of the resistance gene were identical between pG0400 and pJ2947. The reported sequence from pJ2947 5′ to the mupirocin resistance gene was interrupted 319 bases from an *Eco*RI site by the IS element on pG0400, and both the interrupted sequence and restriction site were missing from our plasmid. However, DNA 3′ to the gene on pG0400 continued for 285 bases beyond the *Eco*RI site at which sequencing stopped on pJ2947. The nucleotide sequence on pG0400 was interrupted by the insertion of the right-hand copy of the IS element. Although we did not sequence the entire mupirocin resistance gene on pG0400, the nucleotides encoding the amino- and carboxy-terminal amino acids were identical to those reported for pJ2947. Thus, if the entire 3,072 nucleotides encoding mupirocin resistance were the same on both pJ2947 and pG0400, as seems likely, there would be 3,984 bp between the IS elements on pG0400.

Hospital transmission of mupirocin resistance plasmids. An epidemiologic study of the phenotypes and both the hospital sites and dates of isolation of *S. aureus* isolates resistant to mupirocin recovered from patients at the Yale New Haven Hospital has been published previously (25). We examined three isolates described in that study. We found that those isolates contained conjugative mupirocin resistance plasmids. The isolates were recovered from patients in different areas of the hospital and had different chromosomal *Sma*I restriction endonuclease fragment sizes and overall plasmid contents. However, the conjugative plasmids from two of the isolates (G03221 and G03865) were identical by restriction mapping and hybridization with the probes used in the Southern blots shown in Fig. 2. This suggests horizontal transfer of these conjugative plasmids.

DISCUSSION

In the present study, we characterized conjugative plasmids carrying a gene encoding mupirocin resistance that were initially found in *S. aureus* isolates from two different geographic locations. The plasmid from an isolate in Michigan and plasmids from two isolates in Connecticut differed in their overall restriction endonuclease fragment profiles but had identical conjugative transfer and mupirocin resistance genes, as determined by DNA-DNA hybridization. The probes for conjugative transfer genes came from pG01, a well-characterized conjugative plasmid from *S. aureus* similar to conjugative replicons from staphylococci that have been isolated in the United States and that have been described by other investigators (18, 28, 36). These conjugative plasmids all appear to share the same conjugative transfer genes, as determined by analysis of restriction endonuclease fragment sizes, which were common (2, 18, 28, 36), DNA-DNA hybridization analyses (3, 36), or nucleotide sequence analysis (14, 29). Most plasmids encode resistance to some combination of aminoglycosides, quaternary ammonium compounds, or trimethoprim, with some also containing a gene for β-lactamase (8, 18, 27, 35). The prevalence of plasmids with these transfer genes in a geographic sample of staphylococci from U.S. hospitals was assessed by DNA-DNA hybridization with a probe from the pG01 conjugative transfer region (3). This survey found that 48% of methicillin-resistant *S. aureus* and 17% of methicillin-resistant coagulase-negative staphylococci contained plasmids with conjugative transfer genes identical to those on pG01. In addition, Janssen et al. (22) have previously shown that pG0403 from their isolate LTZ-1 was incompatible with conjugative plasmids similar to pG01 isolated in the 1980s from patients at the University of Michigan Hospital. All of these data provide convincing evidence that conjugative mupirocin resistance plasmids are the same basic replicons as the class of staphylococcal conjugative gentamicin resistance plasmids that were first described in the early 1980s.

The present study also provides evidence for the manner in which related staphylococcal conjugative plasmids are constructed and evolve. The core replicon common to pG01 and the smallest mupirocin resistance plasmid, pG0400, consists of the 14-kb *trs* region (29), a 4.5-kb *Eco*RI fragment previously found by insertional mutagenesis to be nonessential for conjugation or replication (41), and a 6.3-kb region containing a 2-kb open reading frame required for conjugation, the origin of conjugative transfer, and, by inference, the origin of replication (38). The *trs* region and the rest of the core replicon on pG0400 are joined by directly repeated copies of the insertion sequence IS*431*-IS*257*. The DNA present in pG01 that is missing from pG0400 contains antimicrobial resistance genes and an integrated copy of the small plasmid, pUB110 (9), that also encodes resistance to aminoglycosides. These resistance genes and the integrated plasmid are also bounded on pG01 by directly repeated IS*431*-IS*257* copies (27). The DNA in pG01 that is missing from pG0400 appears to be precisely deleted at several of these elements. These data suggest that pG0400 was rearranged by recombination between copies of the IS elements, which deleted the intervening DNA. We also have data that pG01 deletion derivatives appear following sequential conjugative transfer at a frequency of 1 to 10%; all deletions occur precisely at IS copies (1).

We also provided evidence in the present study supporting the hypothesis that the addition to pG0400 of the gene encoding mupirocin resistance occurred at one or more copies of the IS element by recombination rather than by IS-mediated transposition. This hypothesis is supported by several lines of evidence. First, the nucleotide sequences of DNA flanking the outsides of the left and right IS elements on pG0400 exactly matched the sequences flanking two IS elements on pG01. A transpositional event would be expected to interrupt target DNA at random sites unrelated to the location of preexisting IS elements. Second, the nucleotide sequences of the IS elements themselves in the same relative positions on pG01 and pG0400 were virtually identical. There was enough of a difference between the sequences of the left and right IS copies and published sequences of these elements found in different genetic contexts to suggest that IS sequence identity could only have occurred by recombination. Third, there were no target site duplications characteristic of a replicative transposition event. Target site duplications have been found around some copies of IS*431*-IS*257*, suggesting that in certain contexts the elements are transpositionally active and that they transpose by standard mechanisms (39). Further support of the mechanism of IS-mediated recombination as a common means by which genes are acquired and exchanged in staphylococci is provided by an earlier study in which we showed that the gentamicin and trimethoprim resistance genes of pG01 could be added to the penicillinase plasmid pI258 in exchange for mercury resistance genes (42). The exchange occurred precisely between directly repeated copies of IS*431*-IS*257*.

The mechanism by which recombinational integration of mupirocin resistance gene sequences occurred is not clear. However, because ISpG0400L interrupts sequences that are 5′ to the mupirocin resistance gene on a different replicon (20), it is likely that one or both of the IS elements originally transposed to their locations flanking the gene. The nucleotide changes in neither ISpG0400L nor ISpG0400R interrupt or significantly change the amino acid composition of the single IS*431*-IS*257* open reading frame that is thought to encode a transposase (4).

Our study offers insight into the potential for the spread of mupirocin resistance genes among staphylococci. We demonstrated the horizontal transfer of one conjugative mupirocin plasmid in the Yale New Haven Hospital by finding the same plasmid in different staphylococcal isolates from patients in different areas of the hospital. Furthermore, the same resistance gene was found on two different but related conjugative plasmids in *S. aureus* from two geographically unconnected hospitals. This suggests that at least two independent integrative events occurred and that these events resulted in the acquisition of a new resistance gene by a widely prevalent conjugative plasmid. Since these conjugative plasmids move readily between coagulase-negative and coagulase-positive staphylococci, one can also assume that a large reservoir of conjugative mupirocin resistance plasmids is probably present in coagulase-negative staphylococci. Thus, given the ease and rapidity by which the mupirocin resistance gene can move onto a conjugative plasmid and the large potential reservoir for these plasmids in coagulase-negative staphylococci, any large increase in mupirocin use in the United States would be predicted to select an increased number of mupirocin-resistant *S. aureus*. Careful monitoring of mupirocin usage and more testing of *S. aureus* for mupirocin resistance would seem to be indicated. Finally, the model proposed for the integration of the mupirocin resistance gene into conjugative plasmids and the gene's subsequent horizontal and clonal dissemination could apply to any resistance gene newly acquired by *S. aureus.*

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