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Determination of the Chromosomal Relationship between *mecA* and *gyrA* in Methicillin-Resistant Coagulase-Negative Staphylococci

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***mecA*, the gene that mediates methicillin resistance, and its accompanying *mec* locus DNA, insert near the *gyrA* gene in *Staphylococcus aureus*. To investigate whether there is a similar relationship between *mecA* and *gyrA* in coagulase-negative staphylococci (CNS), *mecA*- and *gyrA*-specific DNA fragments were used to probe methicillin-resistant isolates of *Staphylococcus epidermidis* (MRSE) ($n = 11$) and *Staphylococcus haemolyticus* (MRSH) ($n = 11$). The *gyrA* probe hybridized to the same *Sma*I DNA fragment as the *mecA* probe in all strains tested. However, since the size of the *Sma*I fragments containing *mecA* and *gyrA* varied from 73 to 600 kb, the distance between the two genes was determined more precisely. Cloned *mecA* or *gyrA* fragments plus vector sequences each containing a *Sma*I site were introduced into the chromosome of three isolates each of MRSE and methicillin-resistant *S. aureus* (MRSA), and the sizes of the generated *Sma*I fragments were determined by pulsed-field gel electrophoresis. The distance between *gyrA* and *mecA* was found to be between 38 and 42 kb in both MRSE and MRSA, and the two genes were in the same relative orientation in all strains. Restriction fragment length polymorphism (RFLP) patterns around the *gyrA* gene in CNS were identical, but species specific, for all 10 MRSE and 10 MRSH isolates examined. In contrast, 8 of 11 methicillin-susceptible *S. epidermidis* isolates and 7 of 7 methicillin-susceptible *S. haemolyticus* isolates had different *gyrA* RFLP patterns. These data show that *mecA* is site and orientation specific, relative to *gyrA*, in both MRSE and MRSA. In addition, the local environment around *gyrA* in methicillin-resistant CNS, in contrast to methicillin-susceptible isolates, is similar, suggesting clonality or the requirement for specific DNA sequences with which the *mec* complex must interact for chromosomal integration to occur.**

Penicillin-binding proteins (PBPs), enzymes that cross-link peptidoglycan in the bacterial cell wall, are the targets of β-lactam antibiotics. One of the ways in which staphylococci have become resistant to β-lactam antibiotics is through the acquisition of *mecA*, a gene which is found in the chromosome and which encodes a PBP (PBP2A) with reduced affinity for these antibiotics (12, 33). PBP2A catalyzes all required cell wall cross-linking functions when the β-lactam-susceptible PBPs are bound and inactivated by antibiotics (18). The *mecA* gene is contained within a larger fragment of DNA, typically 32 to 60 kb in size and of unknown origin, known as the *mec* locus or *mec* complex (7). *mec*-associated DNA is not found in methicillin-susceptible staphylococci and, therefore, is assumed to be exogenously acquired. Furthermore, the *mecA* genes and much of the *mec* complexes appear to be identical among all of the methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (CNS) examined (4, 34). Even though the ultimate origin of the *mec* locus is unknown, several investigators have postulated that *S. aureus* acquired methicillin resistance from CNS. One piece of evidence supporting this view is that a single *Staphylococcus haemolyticus*-specific insertion sequence (IS*1272*) was found within the *mec* region of some isolates of *S. aureus* (5). Additionally, Wu and colleagues have identified a *mecA* homolog within *Staphylococcus sciuri* that has a predicted 80% amino acid identity with PBP2A (35). Interestingly, this *mecA* homolog does not confer resistance to methicillin on *S. sciuri*, suggesting that *mecA* may have evolved from a PBP that is not associated with β-lactam resistance.

Sequence analysis of *S. aureus mec* DNA found the integration of the *mec* complex to be site specific (14, 15). The insertion site (*intM*) mapped between *spaA* (protein A) and *gyrA* (the A subunit of DNA gyrase) on the *Sma*I DNA fragment G of the NCTC 8325 chromosome. However, the precise location of the *mec* complex on the chromosome of CNS has not been identified. Since the *gyrA* gene region is highly conserved among eubacteria (20), we investigated the relationship between *gyrA* and *mecA* among methicillin-resistant CNS and compared it to the same relationship in MRSA. Similar chromosomal locations for *mec* among both *S. aureus* and different CNS species would suggest a common mode of acquisition and insertion.

MATERIALS AND METHODS

Bacterial strains and plasmids. The strains and plasmids used in this study are shown in Table 1. We identified isolates as staphylococci by their Gram stain appearance and catalase production. Species identification was performed by coagulation of rabbit plasma (Difco, Detroit, Mich.) and with Staph-Ident strips (Analytab Products, Plainview, N.Y.). The staphylococci examined represent a diverse collection of clinical isolates with unique pulsed-field gel electrophoresis (PFGE) patterns collected from the United States and Canada over a period from the early 1970s to 1987.

PCR amplification and sequencing. Two oligonucleotide primers complementary to the nucleotide sequence of the *gyrA* gene (29) of *S. aureus* were used to generate 450-bp PCR fragments from a methicillin-resistant *Staphylococcus epidermidis* (MRSE) (SE43NR) isolate and a methicillin-resistant *S. haemolyticus*

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TABLE 1. Strains, plasmids, and probes used

Strain, plasmid, or probe	Relevant characteristics[a]	Source or reference
Strains		
MRSE		
SE42NR	Boston, Mass., 1980; Nov^r Rif^r Em^s	This work
SE43NR	Canada, early 1970s; Nov^r Rif^r Em^s	This work
SE22NR	Richmond, Va., 1979; Nov^r Rif^r Em^s	This work
MRSA		
450M	8325-4 transformed with *mec* region; Em^s	23
27216	Boston, Mass., 1980; Em^s	This work
Col	United Kingdom, 1961; Em^s	23
MRSH Y176	Richmond, Va., 1989	3
S. aureus RN4220	Restriction-deficient 8325-4; accepts *E. coli* DNA	17
Plasmids		
pE194(Ts)	Temperature-sensitive gram-positive vector; confers Em^r	25
pROJ6448(Ts)	pE194(Ts) with a 700-bp *Alu*I fragment containing the nick site from pC221 cloned into the *Cla*I site	25
pC221	Mobilizeable staphylococcal plasmid; confers Cm^r	24
pGO1	Conjugative staphylococcal plasmid; confers Gm^r	21
pGO164	1.2-kb *Xba*I-*Bgl*II intragenic fragment from *mecA* cloned into pUC19	This work
pGO567	pGO164 with pROJ6448(Ts) cloned into the *Pst*I site	This work
pGO592	7.5-kb *Sal*I fragment containing *gyrA* (isolated from an SE43NR λ library) cloned into pUC19; clone contains *gyrA* (2.6 kb) plus 2.2 kb of downstream DNA and 2.7 kb of upstream DNA	This work
pGO597	pGO592 with pROJ6448(Ts) cloned into the *Pst*I site	This work
pGO557	735-bp PCR fragment located downstream from *gyrA* in *S. aureus* cloned into the *Hin*cII site of pUC19	This work
pGO634	pGO557 with pE194(Ts) cloned into the *Pst*I site	This work
Probes		
pGO533	450-bp PCR fragment from *gyrA* of SE43NR cloned into the *Sma*I site of pUC19	This work
pGO546	450-bp PCR fragment from *gyrA* of Y176 cloned into the *Sma*I site of pUC19	This work
mecI	439-bp PCR fragment including all of *mecI* from SE42NR	This work
dnaA-dnaN	723-bp PCR fragment including the 3′ end of *dnaA* and the 5′ end of *dnaN* from 450M	1
IS*1272*	1.2-kb *Eco*RI fragment isolated from pGO198	3

[a] Nov, novobiocin; Rif, rifampin; Em, erythromycin; Cm, chloramphenicol; Gm, gentamicin.

(MRSH) (Y176) isolate for use as DNA probes. The two primers synthesized (Oligos Etc., Newtown, Conn.) were (5′)GGGTAAATATCAAAATCATCATGG(3′) and (5′)GCAGTTGGAAATCAGGACC(3′). The primers amplified the sequence encoding amino acids corresponding to nucleotides 76 through 221 of *S. aureus gyrA*. The PCR products were subsequently cloned into pUC19 (36). Sequence analysis was performed with the *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems Inc., Foster City, Calif.) on an Applied Biosystems Inc. 373A sequencer, and both DNA strands were sequenced.

Additional primers used and gene sequences amplified are as follows: (5′)GAAATGGAATTAATATAATG(3′) and (5′)GACTTGATTGTTTCCTC(3′) for the complete *mecI* gene; (5′)GGGCGTGATCATACGACCG(3′) and (5′)CCTGATGTAATTAATGTCTGG(3′) for sequences including the 3′ end of *dnaA* and the 5′ end of *dnaN*; and (5′)CCTAAGCGTAAAGAAGATTCAC(3′) and (5′)GATGGTGGCACCACATAC(3′) for the sequences downstream of *gyrA* in *S. aureus.*

Generation of λ library. Unsheared genomic DNA was isolated from SE43NR by a variation of the Marmur technique as described previously (10). Genomic DNA was partially digested with *Sau*3A, and the fragments were separated by sucrose density gradient centrifugation. Fractions with DNA fragments from 9 to 23 kb long were isolated and subsequently ligated to Lambda DASH II (Stratagene, La Jolla, Calif.) digested with *Bam*HI. Packaging of Lambda DASH II and infection of *Escherichia coli* XL-1 Blue (P2) (Stratagene) was performed according to the manufacturer's recommendations. Plaques containing *gyrA* were isolated after transfer of the denatured phage DNA to nylon membranes (Boehringer Mannheim, Indianapolis, Ind.) and hybridization with *gyrA*-specific probes. DNA from the positive plaques was isolated and mapped by standard methods (27).

DNA isolation and manipulation. Recombinant plasmids were generated in *E. coli* DH5α (23) with either pUC18 or pUC19 as the vector (36). The temperature-sensitive gram-positive plasmid pROJ6448(Ts) was subsequently ligated onto each clone (25). *E. coli* plasmid DNA was isolated by the boiling method of Holmes and Quigley (16). *S. aureus* and *S. epidermidis* plasmid DNA was prepared by the cetyltrimethylammonium bromide lysis method of Townsend et al. (32). Electroporation of recombinant plasmids containing pROJ6448(Ts) into *S. aureus* RN4220 was performed by the method of Schenk and Laddiga (28). Transduction was utilized to move recombinant plasmids between strains of *S. aureus* by using phage 80α according to previously described methods (8).

DNA hybridization. Genomic DNA was extracted from staphylococcal isolates by a variation of the Marmur technique as previously described (10). Genomic digests were then transferred by alkaline capillary transfer to Zeta-Probe (Bio-Rad, Hercules, Calif.) nylon membranes ([α-^{32}P]dCTP-labeled probes) or nylon membranes purchased from Boehringer Mannheim (digoxigenin-labeled probes) according to the method of Sambrook et al. (27). Southern hybridization was performed with DNA probes that were either labeled with ^{32}P (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) by nick translation or with digoxigenin-11-dUTP (Boehringer Mannheim) by random primed labeling.

PFGE. Genomic DNA was prepared for PFGE by previously described methods (6). If large fragments were to be visualized, the following parameters were used: 6 V/cm; initial pulse time, 1 s; final pulse time, 30 s for 22 h at 14°C. If small fragments (<200 kb) were to be visualized, the parameters were changed to 6 V/cm; initial pulse time, 0.5 s; final pulse time, 12 s for 24 h at 14°C. PFGE size standards were purchased from New England Biolabs (Beverly, Mass.). Band sizes were estimated with an IS1000 digital imaging system (Alpha Innotech Corp., San Leandro, Calif.).

Conjugative mobilization and allelic replacement. pROJ6448(Ts)-containing clones were electroporated into RN4220, which contained both pGO1 (conjugative; gentamicin resistant [Gm^r]) and pC221 (mobilizeable; chloramphenicol resistant [Cm^r]) to create the donor. pROJ6448(Ts) (erythromycin resistant [Em^r]) contains the nick site from pC221 cloned into the unique *Cla*I site. Therefore pROJ6448(Ts)-containing plasmids can be mobilized by pGO1 if

FIG. 1. Comparison of the *gyrA* DNA sequences from nucleotides 246 to 642 in *S. aureus*, *S. epidermidis*, (*S. epi*), and *S. haemolyticus* (*S. haem*). Nucleotides in *S. epidermidis* and *S. haemolyticus* which are dissimilar to those in *S. aureus* are shown in bold.

mobA and *mobB* from pC221 are supplied in *trans*, as previously described (25, 27). *S. epidermidis* isolates, chosen for their susceptibility to erythromycin, were selected for resistance to both rifampin and novobiocin by serial passage in the antibiotics. The mating procedure was performed by the syringe method as described previously (18). Transconjugants were selected on phenol red mannitol agar (or MRY agar) (Difco) supplemented with 1% yeast extract (Difco), 10 μg of rifampin/ml, 1 μg of novobiocin/ml, and 10 μg of erythromycin/ml. Phenol red mannitol agar was used to differentiate between *S. epidermidis* and *S. aureus. S. aureus* ferments mannitol, which creates a yellow colony, while *S. epidermidis* colonies remain red. Transconjugants were confirmed by restriction endonuclease analysis of plasmid DNA. Transfer frequencies were determined by dividing the number of transconjugants by the number of donor cells. Staphylococcal strains harboring plasmids containing pROJ6448(Ts) were cured by three rounds of growth at the nonpermissive temperature (43°C) in order to detect homologous recombination of the plasmid into either *gyrA* or *mecA*.

Construction of strains. RN4220 donor strains containing pGO1, pC221, and pGO567 or pGO597 were mated into SE42NR, SE43NR, and SE22NR. Mobilization of recombinant plasmids into *S. epidermidis* strains occurred at a frequency of 10^{-8} per donor cell as previously described (31). The recombinant plasmids pGO567 and pGO634 were transferred from RN4220 into *S. aureus* strains by transduction with phage 80α. The presence of undeleted recombinant plasmids was confirmed by agarose gel electrophoresis of plasmid DNA. All appropriate strains were subsequently cured of pGO634, pGO597, or pGO567 and analyzed by PFGE to determine whether homologous recombination had occurred within the appropriate *Sma*I band (the band containing both *gyrA* and *mecA*). Homologous recombination of the recombinant plasmid into the appropriate gene was confirmed by PCR or Southern analysis. Strains containing pGO567, pGO634, or pGO597 within the appropriate gene were named by first listing the strain and then adding "/*mecA*::pGO567", "/*gyrA*::pGO597", or "/*gyrA*::pGO634".

Nucleotide sequence accession numbers. The GenBank accession numbers for the nucleotide sequences presented are AF005934 for the 450-bp fragment of the *gyrA* gene of *S. epidermidis* (SE43NR) and AF005935 for the 450-bp fragment of the *gyrA* gene of *S. haemolyticus* (Y176).

RESULTS

Nucleotide sequence comparisons of *gyrA* genes. The nucleotide sequences of the *gyrA* genes obtained from *S. epidermidis* (SE43NR) and *S. haemolyticus* (Y176) compared to the sequence of *S. aureus gyrA* are presented in Fig. 1. The three nucleotide sequences are highly similar: the *S. epidermidis* and the *S. haemolyticus gyrA* nucleotide sequences each show 83% identity to the nucleotide sequence of *gyrA* of *S. aureus*. The sequence determined for *S. epidermidis* is identical, in its region of overlap, to that published by Sreedharan et al. (29) and extends the known sequence of *S. epidermidis gyrA* from nucleotides 1 to 642. The encoded proteins are also highly similar, with the *S. haemolyticus* GyrA showing 90% identity and the *S. epidermidis* GyrA showing 93% identity to the amino acid sequence of *S. aureus* GyrA.

RFLPs among CNS. All 22 *S. epidermidis* and 18 *S. haemolyticus* isolates were unique, as assessed from both differing geographic origins and unrelated *Sma*I fragment patterns. The results of Southern blot hybridization with either pGO533 (*S. epidermidis*) or pGO456 (*S. haemolyticus*) *gyrA* DNA probes are presented in Table 2. All 11 MRSE isolates examined had identical 12-kb *Cla*I, 10-kb *Eco*RI, and 4-kb *Hin*dIII fragments that hybridized with the *gyrA* probe. In contrast, there were several restriction fragment length polymorphisms (RFLPs) seen among methicillin-susceptible *S. epidermidis* (MSSE) isolates. Only 3 of 11 isolates had RFLPs identical to that seen among MRSE. Ten of the 11 MRSH isolates hybridizing with the *gyrA* probe had identical 2-kb *Cla*I fragments, while all 11 isolates hybridized with an approximately 20-kb *Eco*RI fragment and a 2-kb *Hin*dIII fragment. Again, in contrast to the methicillin-resistant isolates, there were several RFLPs seen among methicillin-susceptible *S. haemolyticus* (MSSH); none was the same as the MRSH pattern when all three restriction enzymes were compared. While MRSE and MRSH had identical RFLPs within each species, none of the RFLP patterns was the same between species.

Chromosomal location of *gyrA* in relation to *mecA*. Both the *gyrA* probe and the *mecA* probe hybridized on the same *Sma*I fragment in each of 25 isolates tested (3 MRSA, 11 MRSH, and 11 MRSE isolates). The size of the *Sma*I fragment to which the *gyrA* and *mecA* probes hybridized ranged from 73 to 600 kb (Table 2).

Introduction of plasmids into CNS. Even though DNA hybridization localized *gyrA* and *mecA* to the same *Sma*I fragment in all isolates examined, we could say nothing about the distance between the genes because of the variable sizes of the fragments (Table 2). We sought to estimate the intergenic *mecA-gyrA* distances in CNS by introducing new *Sma*I sites into or near each of these genes and measuring the sizes of the resulting fragments by PFGE. Three erythromycin-susceptible MRSE isolates (SE42NR, SE43NR, and SE22NR) were used as recipients for mobilizeable plasmids from *S. aureus* donors. Plasmids for chromosomal integration were created as follows. An intragenic 1.2-kb *mecA* fragment was ligated into pUC19 (pGO164), and the mobilization plasmid [pROJ6448(Ts)] was added to create pGO567 (Table 1). Similarly, a 7.5-kb fragment was obtained from a SE43NR λ library that contained the 3′ end of *gyrA* and downstream sequences. It was similarly ligated into pUC19, and the mobilization plasmid was added to

TABLE 2. Hybridization of CNS isolates with *gyrA* probes

Isolate (no.)	Restriction enzyme fragment							
	*Cla*I[a]		*Eco*RI		*Hin*dIII		*Sma*I[b]	
	Size (kb)	No.	Size (kb)	No.	Size (kb)	No.	Size (kb)	No.
MRSE (11)	12	11	10	11	4	11	600	4
							280	1
							130	1
							115	2
							100	2
							92	1
MSSE (11)[c]	15	3	15	1	12 + 6	1		
	12	5	12	4	8	1		
	7	1	10	6	6	1		
					5	1		
	2.5	1			4	6		
					2	1		
MRSH (11)	2	10	20	11	2	11	218	1
							194	1
	0.5	1					177	1
							130	1
							125	1
							97	3
							93	3
MSSH (7)[d]	6	2	20	4	2	5		
	5	2	20 + 9	1				
	4.8	2	12	1	4	1		
	4	1	9	1				

[a] MRSH and MSSH isolates were probed with pGO546 (*gyrA*). MRSE and MSSE isolates were probed with pGO533 (*gyrA*).
[b] MRSH isolates were probed with both pGO164 (*mecA*) and pGO546. MRSE isolates were probed with both pGO164 and pGO533.
[c] Only 10 of 11 MSSE isolates were digested with *Cla*I and probed with *gyrA*.
[d] Only six of seven MSSH isolates were digested with *Hin*dIII and probed with *gyrA*.

produce pGO597 (Table 1). Each of these plasmids, containing a unique *Sma*I site, could be introduced into each of the three MRSE recipients at low frequency (5 to 10 colonies per plate) by conjugative mobilization. However, attempts to introduce these plasmids into two erythromycin-susceptible MRSH isolates were unsuccessful. Growing cells at the nonpermissive temperature for pROJ6448(Ts) replication allowed detection of cells containing *mec* and *gyr* sequences integrated into the chromosome by homologous recombination. Integration was confirmed by PFGE as shown in Fig. 2. The same *mecA* plasmid described above was used for three MRSA isolates (Col, 450M, and 27216), but a different *gyrA* vector (pGO634), containing a 735-bp PCR fragment 3′ to *gyrA*, was constructed by referring to published sequences in the database (29).

Determination of the distance between *mecA* and terminal *Sma*I sites. The distance between *mecA* and the terminal *Sma*I sites was estimated by comparison to a PFGE size standard. The wild-type *Sma*I fragments that contained both *mecA* and *gyrA* were 96 kb for SE42NR, 115 kb for SE22NR, and 280 kb for SE43NR. However, when a new *Sma*I site was introduced into *mecA* by homologous recombination, a band of similar size was found in each isolate after digestion with *Sma*I. This new *Sma*I band was estimated to be 62 kb in SE42NR, 66 kb in SE43NR, and 62 kb in SE22NR. When a new *Sma*I site was introduced into *mecA* in *S. aureus* 450M, Col, and 27216, the wild-type *Sma*I fragments that contained both *mecA* and *gyrA* (218 kb for 450M and Col and 206 kb for 27216) were cleaved into two bands by *Sma*I (142 and 76 kb for 450M and Col and 130 and 76 kb for 27216). Southern blot analysis with *gyrA* as a probe revealed that *gyrA* was present on the 62-kb *Sma*I fragment of SE42NR/*mecA*::pGO567 and SE22NR/*mecA*::pGO567, the 66-kb fragment of SE43NR/*mecA*::pGO567, and the 76-kb fragment of 450M/*mecA*::pGO567, Col/*mecA*::pGO567, and 27216/*mecA*::pGO567.

In order to orient *mecA* in relation to *gyrA*, probes for DNA 5′ to *mecA* were used. This was the *mecI* repressor gene for the three MRSE isolates and a copy of IS*1272* for the three MRSA isolates. Since the new *Sma*I site was introduced on the 3′ end of *mecA*, if *mecI* or IS*1272* hybridized with the same new *Sma*I fragment as *gyrA*, then *gyrA* was upstream of *mecA*. If *mecI* or IS*1272* hybridized with the fragment opposite from the one hybridized by *gyrA*, then *gyrA* was downstream of *mecA*. In all isolates *gyrA* was found downstream (3′) of *mecA* (data not shown).

Determination of the distance between *gyrA* and terminal *Sma*I sites. Integration of pGO597, containing the *S. epidermidis gyrA* gene and flanking sequences, into the chromosome of the three MRSE isolates introduced a new *Sma*I site that generated a fragment of 24 kb in each isolate. Integration of pGO634, containing the *S. aureus gyrA* intragenic fragment, into the three MRSA isolates generated a new 36-kb *Sma*I fragment. The chromosomal map distance between *mecA* and *gyrA* was calculated by subtracting the size of the small *gyrA* *Sma*I fragment (24 to 36 kb) from the size of the chromosomal *Sma*I fragment 3′ to *mecA* (62 to 76 kb), shown above to contain *gyrA*. As with *mecA*, the orientation of *gyrA* relative to *mecA* was determined by using a probe for a gene 5′ to *gyrA* (*dnaA-dnaN*), since the new *Sma*I site was introduced 3′ of *gyrA*. In each case, *dnaA-dnaN* hybridized to the small (24- to 36-kb) *Sma*I fragment while *mecA* hybridized to the larger

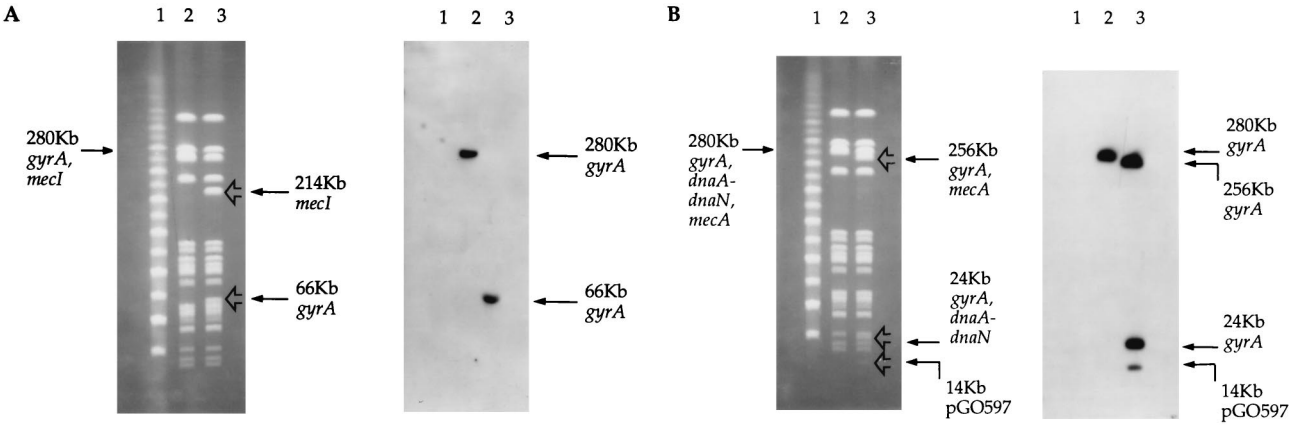

FIG. 2. PFGE (left) and corresponding Southern blots (right) of SE43NR (A and B), SE43NR with a *Sma*I-containing plasmid integrated into *mecA* (SE43NR/*mecA*::pGO567) (A), and SE43NR with a *Sma*I-containing plasmid integrated near *gyrA* (SE43NR/*gyrA*::pGO597) (B). (A) Gel probed with *gyrA* PCR fragment. Lanes: 1, PFGE ladder; 2, SE43NR; 3, SE43NR/*mecA*::pGO567. The arrows indicate bands that hybridized with *gyrA* in both SE43NR (280-kb) and SE43NR/*mecA*::pGO567 (66 kb). A 214-kb fragment hybridized with the *mecI* probe in SE43NR/*mecA*::pGO567, while *mecI* hybridized to the 280-kb fragment in SE43NR (see Results) (data not shown). (B) Gel probed with the entire plasmid pGO592, which includes pUC19 as well as 7.5 kb of SE43NR sequences (*gyrA* plus 3′ and 5′ sequences). Lanes: 1, PFGE ladder; 2, SE43NR; 3, SE43NR/*gyrA*::pGO597. The arrows indicate bands that hybridized with pGO592 (*gyrA*) in both SE43NR (280 kb) and SE43NR/*gyrA*::pGO597 (256, 24, and 14 kb). Due to insertion and duplication of the entire 7.5-kb *gyrA* chromosomal fragment in the chromosome of SE43NR, both new *Sma*I fragments (256 and 24 kb) hybridized with pGO592. Additionally, a percentage of the population of SE43NR/*gyrA*::pGO597 contained uncured copies of pGO597 (pGO592 plus the pROJ6448 (Ts) staphylococcal replicon) that hybridized to pGO592 (14 kb). A probe for sequences 5′ to *gyrA* (*dnaA-dnaN*) hybridized with the 24-kb fragment in SE43NR/*gyrA*::pGO597, while *mecA* hybridized with the 256-kb fragment. Both *mecA* and *dnaA-dnaN* hybridized to the 280-kb fragment in SE43NR (see Results) (data not shown).

fragment. This oriented the direction of transcription of the *gyr* operon away from the nearest natural *Sma*I site toward *mecA*. Southern blot analysis of both SE43NR/*mecA*::pGO567 and SE43NR/*gyrA*::pGO597 is shown in Fig. 2. Maps of the distances between the *mecA* and *gyrA* genes for the isolates studied are shown in Fig. 3.

DISCUSSION

The amount of DNA within *mec* varies among MRSA isolates (9, 15, 30). The *mec* region was initially identified in isogenic *S. aureus* strains, one of which was susceptible while the other was the same strain made methicillin resistant by transformation with DNA from a clinical methicillin-resistant isolate (7). In those experiments, the methicillin-resistant transformant contained approximately 32 kb of DNA not found in the susceptible parent. Subsequent mapping and sequencing experiments performed by Hiramatsu et al. have identified clinical isolates with from 32 to 63 kb of DNA flanking *mecA* that were not found in methicillin-susceptible isolates (15). Mapping and sequence data from a number of investigators have shown features common to *S. aureus mec* regions (3,

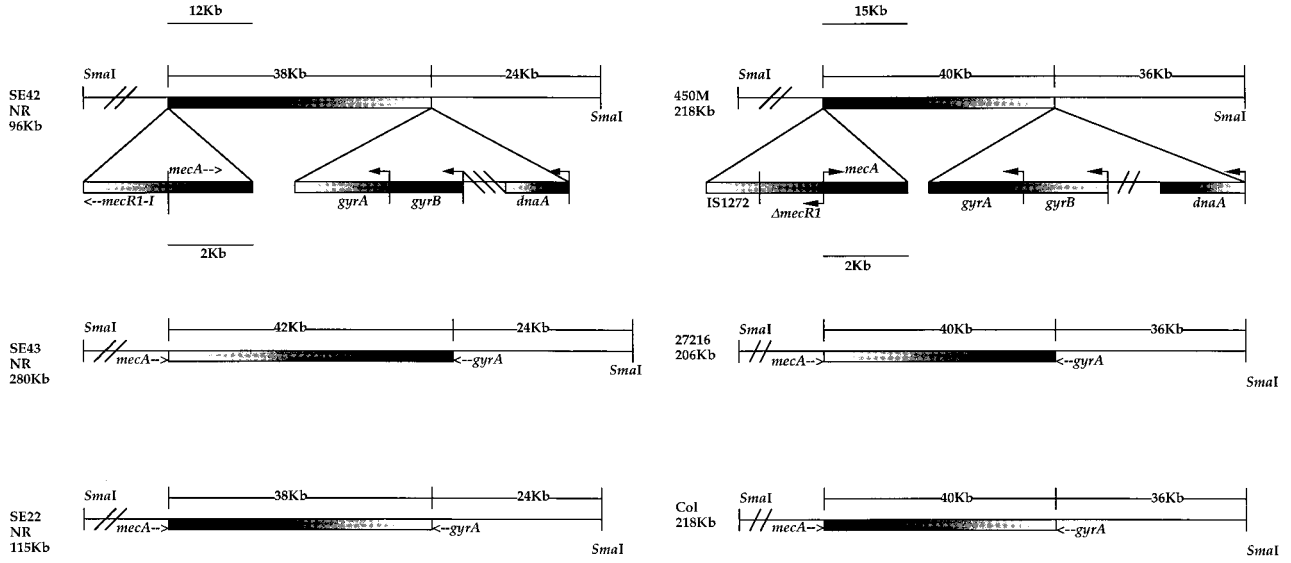

FIG. 3. Chromosomal map distance between *mecA* and *gyrA* in strains studied. The distances below the strain names represent the sizes of the wild-type *Sma*I fragments containing both *mecA* and *gyrA*. The distances between the two genes and the terminal *Sma*I sites, as indicated above the linear maps, was determined by the addition of new *Sma*I restriction sites at the 3′ end of *mecA* (nucleotide 1904 of 2,144) in both MRSE and MRSA, 2.2 kb downstream of *gyrA* in MRSE and 735 bp downstream of *gyrA* in MRSA. The triangular schematics below SE43NR and 450M represent the organization of the *mec* and *gyr* regions within the strains studied.

4, 9, 30). First, 3′ to *mecA* is a region that varies in length, containing a variable number of 40-bp repeated sequences and an open reading frame always flanked by a copy of IS*431* (26). Second, on the other side of IS*431*, farther downstream of *mecA*, investigators have found a number of integrated plasmids and resistance genes that are usually flanked by another copy of IS*431* (30). Presumably, the terminus of *mec* DNA 3′ to *mecA* is close to the second copy of IS*431* and contains sequences that are repeated in inverted order at the other terminus (14). Therefore, the DNA 3′ to *mecA* in most *S. aureus* isolates varies from 10 to 20 kb in length, depending upon the size of the IS*431*-flanked integrated elements, but has been reported to be as little as 5 or as much as 30 kb (15).

DNA 5′ to *mecA* is much more extensive. The two-gene regulatory operon, *mecR1-mecI*, is immediately 5′ to the *mecA* promoter-operator in most isolates, but in some isolates these sequences are partially deleted and replaced by a truncated copy of IS*1272* (5). The DNA 5′ to *mecI* or IS*1272* has been completely sequenced in one Japanese isolate, N315, and is said to contain an open reading frame with homology to a site-specific recombinase gene (15).

There is less known about *mec* DNA in CNS, but DNA hybridization and PCR amplification in both MRSE and MRSH suggest that the *mec* sequences 3′ to *mecA* are essentially the same (2, 4). In addition, the DNA at least 4 kb 5′ to *mecA* in MRSE is the same, as shown by hybridization, as that in MRSA (4). Other elements, such as Tn*554* and pUB110, have been identified in the *mec* DNA of both MRSE (4, 34) and MRSA (9, 30) as well. The DNA 5′ to *mecA* in MRSH seems to be arranged somewhat differently, with only 20 bp of *mecR1* remaining before the insertion of unidentified DNA (4). Thus, there is sufficient evidence to conclude that substantial portions of *mec* are homologous among these staphylococci and, therefore, that all three species acquired the DNA from a common source.

We found the chromosomal distances from the *gyrA* to the *mecA* genes to be very similar among all of the six staphylococci (three MRSA and three MRSE isolates) that we mapped, and therefore, we documented site specificity for *mec* insertion in two different staphylococcal species. It has been reported that there is a putative *mec* insertion site common to all *S. aureus* isolates and that the *mec* termini contain inverted repeats, suggesting that, in MRSA, *mec* is or has been a site-specific mobile element (14, 15). However, there are no target site duplications flanking *mec* in MRSA (15). The putative *S. haemolyticus* insertion site is reported to be different from that of *S. aureus* (14), and none has been reported for *S. epidermidis.*

One would expect that, since there is variability in the distances between *mecA* and the *mec* terminus 3′ to *mecA*, we would have found diversity in the distances between *mecA* and *gyrA*. However, in our isolates we found the distance between the two genes to be highly conserved. This may be due to the fact that we chose isolates that were susceptible to erythromycin and, thus, were less likely to be multiresistant. Therefore, these isolates may not contain integrated plasmids or resistance elements within 3′ *mec* DNA. On the basis of our data on the distance from *gyrA* to *mecA* in MRSE, the orientation of *gyrA* 3′ to *mecA*, and the reported lengths of *mec* DNA 3′ to *mecA* in MRSA, we can estimate the distance from *gyrA* to the 3′ *mec* terminus at 18 to 32 kb, but it could be as little as 8 or as much as 37 kb, depending on the amount of *mec* DNA 3′ to *mecA*. It should be relatively straightforward to locate the *S. epidermidis mec* insertion site in *S. epidermidis* genomic libraries or genome sequences. Since our data show that *S. epidermidis* and, probably, *S. haemolyticus* have the same relative chromosomal insertion site specificity as *S. aureus*, identification of specific insertion site sequences in different staphylococcal species may help define the mechanism of acquisition and insertion of *mec.*

Site-specific insertion of *mec* DNA in staphylococci may be due to the presence of target insertion sites for a phage or mobile element. However, our RFLP data show that there is a lack of polymorphism around the *gyrA* genes among MRSE and MRSH isolates, in contrast to extensive RFLP heterogeneity in these areas among methicillin-susceptible isolates. This apparent *gyrA* RFLP homogeneity in the context of extensive genome diversity, as determined by PFGE, suggests that *mec* may have entered the chromosome by homologous recombination, bringing a portion of the *gyr* locus or intervening DNA from a donor organism. The availability of genome sequences from a variety of related organisms may also help resolve this question.

Finally, it has been extremely difficult to introduce plasmid DNA into random clinical *S. epidermidis* isolates. Techniques such as electroporation, protoplast fusion, protoplast transformation, and nonspecific mobilization have been successful for only a few isolates (2, 11, 13, 19, 22). However, we have shown that conjugative mobilization can be used to introduce recombinant plasmids into virtually any *S. epidermidis* isolate and that allelic replacement mutagenesis can be successfully performed as a result. The only limitation that we faced was the requirement that the recipient be erythromycin susceptible. This requirement may have been the cause of our failure to introduce plasmids into *S. haemolyticus*, since we could find only two isolates in our extensive *S. haemolyticus* collection that were susceptible to erythromycin. However, this problem can be easily overcome by construction of mobilizeable plasmids containing a variety of resistance markers. Systems for conjugative mobilization of plasmids into CNS should expand studies on the genetic basis for pathogenesis in these species.

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