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# Physical Mapping of the *mec* Region of an American Methicillin-Resistant *Staphylococcus aureus* Strain

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**We mapped part of the *mec* region of a locally prevalent strain of *Staphylococcus aureus*. The *mec* region was found to harbor an insert of the transposon Tn*554*, which encodes spectinomycin and macrolide-lincosamide-streptogramin B resistance, and a 4.6-kb segment of DNA that contains the kanamycin resistance gene *aadD*. This 4.6-kb segment appears to be an integrated form of a previously described plasmid, pUB110, and is flanked by copies of the insertion sequence IS*257*. The integration event may be an example of processes that have led to accretion of resistance determinants in the *mec* region of *S. aureus*.**

We have reported some properties of a group of methicillin-resistant ($Mc^r$) *Staphylococcus aureus* isolates responsible for an outbreak of infections in New Jersey hospitals in 1987 (30). A characteristic of this group is the correlation between the methicillin resistance phenotype and the presence of a particular secondary insert (insert 6) of the transposon Tn*544*, which contains determinants for macrolide-lincosamide-streptogramin B (MLS) and spectinomycin resistance ($Sp^r$). Southern hybridization indicated that $Mc^s$ *S. aureus* isolates uniformly lacked DNA corresponding to the chromosomal attachment site for Tn*554* insert 6, designated *att155* after prototype strain R155. In the present study, we demonstrate that the epidemiologic association between Tn*554* insert 6 and *mec* in the New Jersey outbreak group reflects close physical linkage. In addition, we show that the *mec* region contains an element that resembles the kanamycin resistance plasmid pUB110 and that is flanked by copies of the insertion sequence IS*257*.

The *S. aureus* strains used, R155 and ANS46, have been described previously. R155 is a prototype of $Mc^r$ isolates prevalent in New Jersey since 1987 that are resistant to methicillin, the MLS group, and kanamycin but that are susceptible to tetracycline and gentamicin (7, 30). Strain ANS46 is a prototype of isolates that have been prevalent in eastern Australia for about a decade and that are resistant to methicillin, MLS, kanamycin, tetracycline, and gentamicin (18, 21).

Chromosomal DNA was isolated after lysing cells with lysozyme, lysostaphin, and sodium dodecyl sulfate (30). DNA was digested with restriction endonucleases and subjected to electrophoresis through 0.7% agarose gels, followed by transfer to membrane filters as described previously (30). Filters were probed with labeled DNA segments as follows: MA12 and MA14 from ANS46 (Fig. 1), segments of Tn*554* and Tn*554* insert 6 junctions (7, 30), segments of plasmid pT181 (13), and a "3.5-kb *Bgl*II fragment" overlapping *mec* (Fig. 2). The oligonucleotide probes used in this study are listed in Table 1. They were designed to have $T_D$'s of 48 to 52°C (4) and were synthesized by the cyanoethyl phosphoramidite method (25) on an ABS 380B DNA synthesizer. Hybridization was performed as described previously (30), except that oligonucleotide hybridization was conducted at 45°C. Filters were washed as described previously (7).

Analyses were performed in parallel on chromosomal DNA from strains R155 and ANS46. Figure 1 shows the mapped region of R155 and compares it with a corresponding region of ANS46 chromosomal DNA. Figure 2 shows a higher-resolution map, including a portion of the R155 *mec* region that differs from ANS46. These maps were obtained by digesting samples of DNA with the nine restriction endonucleases described in the legends to Fig. 1 to 3, singly and sometimes in pairs. Southern blots were then hybridized sequentially with one or more of the probes described above. Examples of Southern hybridization patterns are shown in Fig. 3.

The results for ANS46 are in agreement with earlier findings (7, 9, 17, 18). The left end, as shown in Fig. 1, of the mapped portion of the ANS46 *mec* region is occupied by an element partially homologous to Tn*554*, which we call ΨTn*554* (9). As shown in Fig. 1, the left end of the mapped region of R155 was also found to be occupied by a Tn*554*-related element, but in this case it was classical Tn*554*, in *att155* (i.e., insert 6). DNA whose restriction map was found to be very similar to that of the ANS46 DNA rightward of ΨTn*554* was found beginning at the right transposon boundary and extending for approximately 11 kb rightward. This 11 kb of DNA was rich in *Bgl*II sites; hence, *Bgl*II digests probed with MA12 are especially useful in demonstrating the similarity to ANS46 (data not shown). This stretch also includes the *mec* gene, which is only 8 kb from the Tn*554* insert. Thus, *mec* is closely linked to Tn*554* insert 6 on the R155 chromosome, explaining the epidemiologic correlation between these elements (30). Since *mec* and adjacent DNA are absent from $Mc^s$ strains, it also explains the absence of the *att155*-related sequence from all naturally occurring $Mc^s$ *S. aureus* isolates we examined (8, 30). It should be noted, however, that ANS46 is capable of giving rise in vitro to $Mc^s$ variants which lack *mec* and some other resistance genes nearby, but which retain an *att155* equivalent (12).

Within the 11-kb conserved region of ANS46 and R155 DNA is a 2.2-kb *Hin*dIII fragment (*mecR* in Fig. 1) that appears to correspond to a fragment shown by Tesch et al. (28) to overlap a regulatory element. This fragment was

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TABLE 1. Oligonucleotide probes used in this study

| Oligonucleotide | Sequence | Element/gene | Position (reference) |
|---|---|---|---|
| T6B | TATTTATTAT ACTATGGTTA A | Tn*554* | 21–1 (20) |
| TEM | ACCTTCCACT TCAAATAC | Tn*554* | 6610–6627 (20) |
| T26 | CACTAATCTC TTTTATTCTC | *att155* R Arm | 166–147 (30) |
| TMA14 | GGATGGCCTG GAGTG | *att155* R Arm[a] | |
| T43B | GTTCCACTTA TTTTAATAG | *mec* | 19–37 (27) |
| T43 | GTACTGCAGA ACTCAAA | *mec* | 1922–1939 (27) |
| T45A | GCACCAATTT TATATCGA | IS*257*.1-IS*257*.2 | 219–236 (17) |
| T45B | GCCCCAATTT TGTATCAA | IS*257*.3-IS*257*.4 | |
| T46 | CTGTCATTGT ACATCGAA | IS*257* | 518–535 (17) |
| T46B | GCTAGCAAGT TAAGCG | IS*257* | 719–734 (17) |
| TK | TTATGGCTCT CTTGGTC | *aadD* | 108–124 (16) |
| TKB | GACCATCTGT GCCAG | *aadD* | 616–630 (16) |
| TMER | GAATGGACAA AACATCAC | *merA* | 3765–3782 (14) |

[a] From a sequence determined for a region near the right end of ANS46 cloned segment MA14 (Fig. 1) (6, 17), which is about 2.5 kb from ΨTn*554*.

noted in two of eight *S. aureus* strains examined by those workers: ANS46 and, interestingly, an American isolate.

Investigators at the same laboratory earlier isolated a 3.5-kb *Bgl*II fragment from a 1967 European Mc$^r$ *S. aureus* isolate, which contained part of an IS*257* element and part of *mec* (3). This fragment has provided a convenient probe for *mec* in various strains (18, 29, 30) and is rather highly conserved. Indeed, all of the six disparate Mc$^r$ isolates examined initially were found to have a homologous 3.5-kb *Bgl*II fragment (3), and ANS46 does as well (18). However, as illustrated in Fig. 2, the homology between R155 and ANS46 dissipates within this fragment, between its right *Pst*I

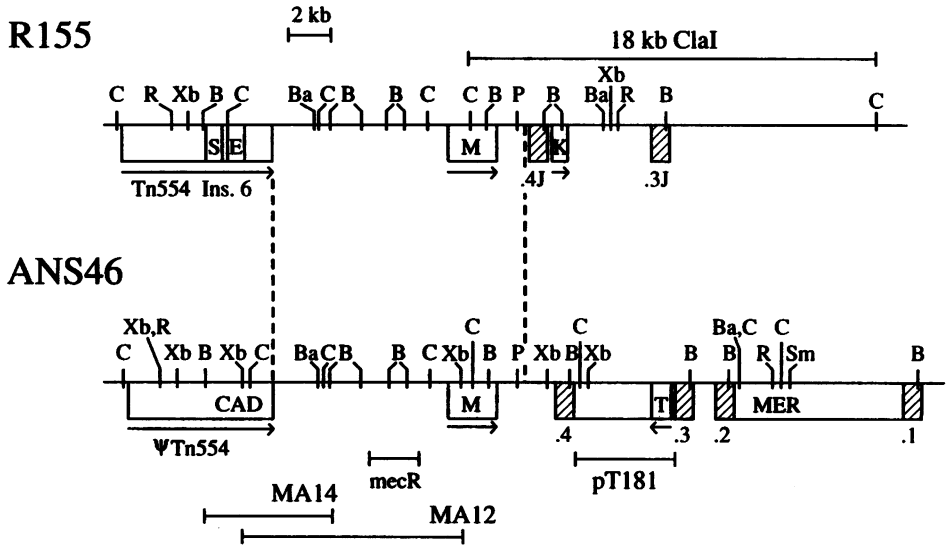


FIG. 1. The *mec* region of New Jersey strain R155. Abbreviations are as follows: Ba, *Bam*HI; B, *Bgl*II; C, *Cla*I; R, *Eco*RI; P, *Pst*I; Sm, *Sma*I; Xb, *Xba*I. The open rectangles represent resistance determinants for spectinomycin (S), tetracycline (*ermA*) (E), methicillin (*mec*) (M), kanamycin (*aadD*) (K), and tetracycline (T). Regions containing determinants for resistance to cadmium or mercury and mercurial agents are designated CAD or MER, respectively. Hatched rectangles represent copies of IS*257*, which are numbered as described in the text. For R155, we show all *Cla*I sites in the mapped region and all sites leftward of the *Bgl*II site of IS*257*.3J, corresponding to *Bam*HI, *Bgl*II, *Eco*RI, and *Xba*I. For ANS46, some *Bgl*II and *Cla*I sites in the mercury resistance region are omitted. We show one of several *Pst*I sites, that which is near the right boundary of the stretch (flanked by dashed lines) of high similarity between the two *mec* regions.

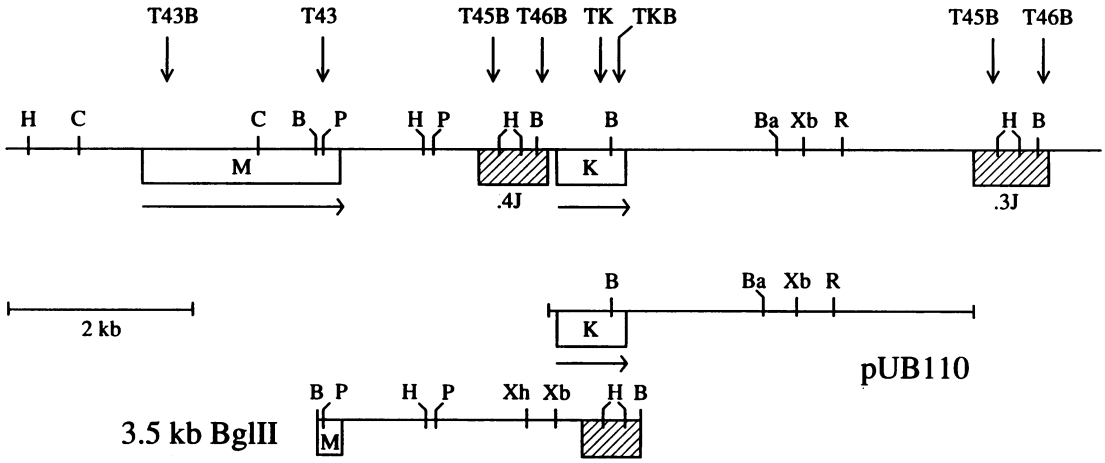


FIG. 2. Map of the *mec*-IS*257*-*aadD* portion of R155 DNA. Abbreviations are as described in the legend to Fig. 1; in addition, H designates *Hin*dIII and Xh designates *Xho*I. Above the map we indicate locations of oligonucleotides. Below the map we diagram the 3.5-kb *Bgl*II fragment (3), which is aligned with R155 on the basis of the *Bgl*II site in *mec*, and plasmid pUB110, which is aligned with R155 on the basis of the *Bgl*II site of the *aadD* gene and linearized at residue 3100 (19).

site (present in R155) and its *Xho*I site (absent from R155), and the corresponding *Bgl*II fragment of R155 is 2.5 kb in size.

Rightward of *mec* in the R155 DNA are two copies of the insertion sequence IS*257*, about 4.6 kb apart. This conclusion is based on comparison of the restriction map shown in Fig. 2 with published maps (e.g., reference 17), hybridization with IS*257*-specific probes (Table 1), and hybridization with the IS*257* moiety of the 3.5-kb *Bgl*II fragment shown in Fig. 2 (data not shown). ANS46 DNA also contains two IS*257* elements rightward of *mec*, designated IS*257*.3 and IS*257*.4, and a second pair further rightward (Fig. 1), IS*257*.1 and IS*257*.2 (17). Sequencing of these insertion sequence copies showed that IS*257*.1 and IS*257*.2 are the same, as are IS*257*.3 and IS*257*.4; but IS*257*.1-IS*257*.2 differ from IS*257*.3-IS*257*.4 at five positions (17). Three of these differences are clustered, forming the basis for probes T45A and T45B (Table 1), which distinguish between the two pairs. The IS*257* copies in the R155 DNA hybridized with T45B (specific for IS*257*.3-IS*257*.4) (Fig. 3), but not with T45A (data not shown). This, plus their proximity to *mec*, suggests that the R155 IS*257* elements are analogous to IS*257*.3 and IS*257*.4; hence, we designate them IS*257*.3J and IS*257*.4J, respectively (for Jersey). In ANS46, IS*257*.3 and IS*257*.4 flank an integrated copy of pT181 (10, 17, 26). In contrast, R155 DNA failed to hybridize with pT181 DNA; rather, it hybridized with probes (TK, TKB; Table 1) designed on the basis of the kanamycin resistance gene *aadD*, which has been shown to be near *mec* in several other staphylococcal isolates (28, 31).

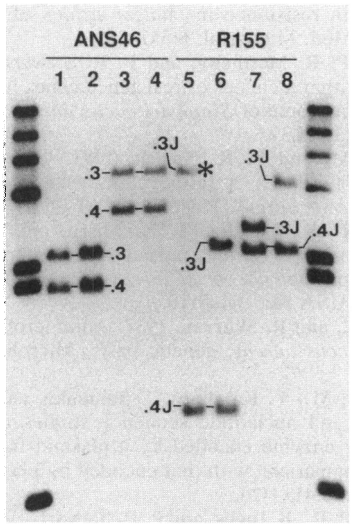


FIG. 3. Southern hybridization patterns: *Hin*dIII, *Hin*dIII plus *Bam*HI, *Bgl*II, and *Bgl*II plus *Bam*HI fragments hybridizing with an IS*257* probe. DNA samples from ANS46 and R155 were processed in parallel, and a Southern blot was hybridized with probe T45B (Table 1). The outermost lanes show marker bands from a *Hin*dIII digest of phage lambda: 23, 9.4, 6.6, 4.4, 2.3, 2.0, and 0.59 kb. Other lanes are as follows: 1 and 5, *Hin*dIII; 2 and 6, *Hin*dIII plus *Bam*HI; 3 and 7, *Bgl*II plus *Bam*HI; 4 and 8, *Bgl*II. The IS*257* copy that served as a source of each band is designated as in Fig. 1.

Our restriction analysis placed the *aadD* equivalent (referred to below simply as *aadD*) between IS*257*.3J and IS*257*.4J (Fig. 1 and 2). In addition, the *aadD*-containing segment between the IS*257* copies resembles in size and restriction map an *aadD*-containing *S. aureus* plasmid, pUB110 (11, 19). Figure 3 shows fragments that hybridized with IS*257* oligonucleotide T45B, after chromosomal DNA was digested with *Hin*dIII or *Bgl*II, with and without *Bam*HI. *Bam*HI digestion decreased the sizes of the hybridizing *Hin*dIII and *Bgl*II fragments from IS*257*.3J (Fig. 3, lane 5 versus lane 6 and lane 8 versus lane 7), reflecting the presence of the *Bam*HI site of the putative integrated pUB110-like plasmid (Fig. 2). In contrast, integrated pT181 lacked a *Bam*HI site and all of the hybridizing ANS46 bands were *Hin*dIII (Fig. 3, lanes 1 and 2) or *Bgl*II (Fig. 3, lanes 3 and 4) products.

The close linkage of *mec*, *aadD*, and the two IS*257* copies of R155 was confirmed by detailed examination of some of the larger hybridizing fragments that were detected. Particularly useful was *Cla*I, which yielded a labeled fragment of about 18 kb (Fig. 1) when blots were probed with the *mec* oligonucleotide T43, the IS*257* oligonucleotide T45B, and the *aadD* oligonucleotide TK (Table 1). Sequential hybridization of the same blot yielded single bands, which were indistinguishable after migration, for each oligonucleotide

(data not shown). Similarly, probes T46B, TK, TKB, and T45B (Fig. 2) yielded *Hin*dIII bands that were indistinguishable after migration, at approximately 5.4 kb (band marked with an asterisk in Fig. 3).

IS*257*.1 and IS*257*.2 of the ANS46 *mec* region flank a mercury resistance operon (17). Southern hybridization of R155 DNA with T45A (specific for these IS*257* versions) and with T46 and T46B (generic IS*257* probes) (Table 1) yielded no additional bands. R155 is $Hg^s$ and failed to hybridize with a probe on the basis of the *merA* sequence (Table 1); thus, it lacks the mercury operon and the IS*257* copies that flank it in ANS46.

The sequence of the IS*257* element (IS*431mec*) of the original 3.5-kb *Bgl*II fragment is the same as that of ANS46 IS*257*.3-IS*257*.4 except for one residue (1, 2, 17). This similarity and our results for R155 with oligonucleotide probe T45B suggest that IS*431mec*, IS*257*.3-IS*257*.4, and IS*257*.3J-IS*257*.4J constitute a subfamily within the large IS*257* family (17, 23, 24). We propose a scenario for R155 like that proposed for ANS46 (17). A primitive chromosomal IS*257*-like element, IS*431mec* or a close relative, entered a resident plasmid by replicational transposition. In the case of the Australian lineage, the plasmid was pT181, and in the case of the New Jersey lineage, it was pUB110. Homologous recombination between the plasmid and chromosomal copies of IS*257* then yielded the integrated configurations that were so strikingly similar in the two *S. aureus* strains.

Our restriction map suggests that the pUB110-like plasmid linearized and then became integrated at about residue 3100 of the pUB110 sequence (19). This site does not interrupt any genes, being just upstream of *aadD* and downstream of a plasmid maintenance gene, α (19). Similarly, the site of linearization of pT181 as it is integrated in the ANS46 chromosome is just upstream of *tet* and downstream of a maintenance gene, *repC* (13, 15, 17). Both sites appear to be compatible with continued expression of the respective maintenance genes (in addition to expression of the resistance genes). Expression of maintenance genes in the case of chromosomally integrated plasmids would seem redundant; indeed, recent studies have indicated that the *repC* gene of integrated pT181 in ANS46 is defective (22). However, if the integration scenario described above is valid, it is possible that an intermediate stage, in which the circular plasmid harbors a copy of IS*257*, is sufficiently prolonged to favor (under antibiotic selection pressure) cointegrates that remain independent replicons over cointegrates defective in their replication.

The *mec*-IS*257*-*aadD* gene order has been reported to occur in the chromosome of a recent Swiss *Staphylococcus epidermidis* isolate (28), and restriction maps suggest its occurrence in Japanese *S. aureus* isolates (31). Perhaps these other isolates will also prove to have the Tn*554*-*mec*-IS*257*-pUB110 (like)-IS*257* configuration of the R155 *mec* region. Sequences facilitating the chromosomal integration of resistance determinants may have played an important role in the evolution of the *mec* region (7, 12, 26, 30). IS*257*, as well as *att155* (which would facilitate entry of Tn*554*), appear to be examples of such sequences.

After the initial submission of the manuscript, the report of Byrne et al. (5) appeared, which describes integrated forms of pUB110 in two *S. aureus* plasmids, pSK41 and pUW3626. These copies of pUB110 are also flanked by copies of IS*257*. However, their sites of linearization appear to differ from that of the pUB110 in the R155 chromosome, and the orientation of the IS*257* elements is opposite to those of R155.

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