Physical mapping of the *mec* region of an Australian methicillin-resistant *Staphylococcus aureus* lineage and a closely related American strain

DONALD T. DUBIN,¹* SUCHETA G. CHIKRAMANE,¹ BARBARA INGLIS,² PETER R. MATTHEWS²† and PETER R. STEWART²

¹ Department of Molecular Genetics and Microbiology, University of Medicine and Dentistry of New Jersey – Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA

² Division of Biochemistry and Molecular Biology, Faculty of Science, Australian National University, Canberra ACT 2601, Australia

(Received 25 March 1991; revised 29 July 1991; accepted 16 September 1991)

Methicillin-resistant (Mc^r) staphylococci contain chromosomal DNA that is absent from Mc^s cells. This extra DNA harbours the methicillin resistance determinant *mec* and often other resistance determinants. The *mec* region can differ substantially in structure among different isolates. We present studies on the *mec* region of a group of *Staphylococcus aureus* isolates prevalent in Australia and London. Southern hybridization analyses of a prototype Australian isolate, ANS46, and an isogenic Mc^s deletion mutant, ANS62, allowed the physical map of the region to be extended to 55 kb. The DNA corresponding to the deletion, which includes *mec* and resistance determinants for mercury, cadmium (Cd) and tetracycline, amounted to 41 kb. It was bounded precisely at one end by the macrolides–lincosamides–streptogramin B (MLS)-resistance transposon, Tn554. Near the other end was an element with homology to Tn554, ψ Tn554, which carried the Cd^r determinant. The *mec* region of an American Mc^r isolate, R35, was found to be virtually the same as that of ANS46, except that it lacked Tn554. Another class of American Mc^r isolates, prevalent since 1987, differs markedly from ANS46 in *mec* region organization. However, this other American class also contains an insertion of Tn554 in the *mec* region, and the attachment site for this insertion was found to have significant homology to attachment sites for the Tn554 and ψ Tn554 insertions in the *mec* region of the Australian strain. These results suggest possible roles of Tn554 and Tn554-like elements in the evolutionary variation of the *mec* region.

Introduction

In *Staphylococcus aureus*, the structural gene for methicillin resistance, *mec*, resides in a region of the chromosome that is of considerable interest. The *mec* region can harbour various resistance determinants in addition to *mec*, the number, nature and arrangement of which can vary significantly among different isolates (Ubukata *et al.*, 1989; Matthews *et al.*, 1990; Dubin *et al.*, 1991). *mec* is part of a DNA insertion of 20 kb or more that has no allelic counterpart in chromosomes of typical Mc^s *S. aureus* isolates. This DNA is thought to have arisen by transfer from a different organism (Matthews *et al.*,

1987; Beck *et al.*, 1986; Song *et al.*, 1987; Skinner *et al.*, 1988; Dubin, 1990). In the prototype Australian isolate ANS46, which has been extensively characterized (Matthews *et al.*, 1987, 1990), *mec* is associated with *mer*, *tet*, and *cad* genes and four copies of the insertion sequence IS257 (Fig. 1).

The staphylococcal transposon Tn554 encodes macrolides–lincosamides–streptogramin B (MLS) and spectinomycin (Sp) resistance (reviewed in Murphy, 1990). Tn554 is relatively site-specific, entering only the primary chromosomal attachment site in laboratory *S. aureus* strain 8325 (Murphy *et al.*, 1981). However, clinical isolates often have secondary chromosomal insertions of Tn554 (Thakker-Varia *et al.*, 1987; Dubin, 1990). A new group of Mc^r *S. aureus* isolates causing an outbreak in New Jersey in 1987 (the New Jersey group) was found to contain a characteristic secondary insertion designated class 6 (Tillotson *et al.*, 1989). A group of isolates endemic in Australia and England for about 10

† Present address: Rockefeller University, York Avenue, New York, NY 10021, USA.

Abbreviations: MLS, macrolides–lincosamides–streptogramin B; Sp, spectinomycin; Δ ANS46/62, the DNA corresponding to that deleted from strain ANS46 yielding ANS62.

Fig. 1. Maps of the *mec* regions of Australian strain ANS46 and New Jersey strain R35. All sites for *Eco*RI (R), *Pst*I (P), *Sma*I (S), *Xba*I (Xb), and *Xho*I (Xh), and selected sites for *Bgl*II (Bg), *Cla*I (C), *Eco*RV (R5) and *Hind*III (H) are shown. The *Xba*I site in parentheses was partially resistant to cleavage. IS257 insertions are designated 1–4 (Matthews *et al.*, 1990) and Tn554 insertion 6B is designated Tn554.6B. Orientations of transposase reading frames of Tn554 and ψ Tn554 are shown by arrows. Below the ANS46 map we depict cloned fragments MA13 and MA14 with selected restriction sites, and general locations for Hg and Cd resistance determinants (Skinner *et al.*, 1988; Matthews *et al.*, 1987, 1990). The location of the *merA* gene as inferred from the restriction map of Laddaga *et al.* (1987) is indicated by *a*. The positions of several oligonucleotide probes (T prefixes) hybridizing with ANS46 DNA are shown. Those from T53 rightwards hybridized similarly with R35 DNA. Above the R35 map is shown the 0.8 kb *Cla*I fragment corresponding to unoccupied *att*35. Due to the cloning strategy used, *Bgl*II sites of chromosomal DNA correspond to *Hind*III and *Sa*I (Sa) polylinker sites at ends of cloned segments.

years (Pavillard *et al.*, 1982; Cookson & Phillips, 1988) (the Australondon lineage) was found also to contain a distinctive Tn554 insertion, 6B (Chikramane *et al.*, 1991). Insertions 6 and 6B have similar left flanking chromosomal DNA sequences and have been mapped to the *mec* regions of their respective lineages (Chikramane *et al.*, 1991; Dubin *et al.*, 1991).

Study of the Australondon strains has been facilitated by the availability of an *Mcr*^s variant, ANS62, of the prototype Australian *Mcr*^r isolate ANS46. ANS62 was found to have undergone a deletion of at least 35 kb of DNA (Δ ANS46/62) from ANS46 (Matthews *et al.*, 1987). Clones corresponding to this missing DNA were used in mapping a substantial portion of the region (Matthews *et al.*, 1987, 1990; Skinner *et al.*, 1988). The present work extends this map. We show that Tn554 insertion 6B is just beyond the left boundary of Δ ANS46/62; and that an element resembling Tn554 but encoding Cd^r, designated ψ Tn554, is at the right end of Δ ANS46/62. We have also examined a representative strain, R35, of a separate New Jersey *Mcr*^r lineage. R35 was found to have a *mec* region essentially the same as that of the Australondon lineage, but lacking a Tn554 insertion.

Methods

Bacterial strains and susceptibility determinations. *S. aureus* strains and pertinent characteristics are summarized in Table 1. Antibiotic susceptibilities were determined by disc diffusion (Barry & Thorns-

berry, 1980). Cd susceptibility was assessed by plating onto CY agar containing 100 μ M-CdCl₂ (Skinner *et al.*, 1988). Hg susceptibility was determined with discs containing 20 and 100 nmol HgCl₂ (Weiss *et al.*, 1977).

Plasmid pEM715 was provided by E. Murphy, in *S. aureus* RN4932 (Thakker-Varia *et al.*, 1987).

DNA methods. Total cellular DNA was prepared after lysing cells with lysozyme, lysostaphin and SDS (Chikramane *et al.*, 1991). Southern blots were prepared as before (Tillotson *et al.*, 1989). In most cases 0.7% agarose gels were used, with size standards consisting of *Hind*III fragments of bacteriophage λ , end-labelled by filling in with the Klenow fragment of *Escherichia coli* DNA polymerase. To analyse small fragments 1.2% or 2% (w/v) gels were used, with a '1 kb ladder' (BRL/Life Technologies), similarly labelled, as standard. Filters were hybridized, and washed, as described previously (Berent *et al.*, 1985; Chikramane *et al.*, 1991).

PCR amplification was performed using Amplitaq (Cetus Corp), following the supplier's recommended protocol.

DNA sequencing was performed by a dideoxynucleotide procedure (Sanger *et al.*, 1977).

Nomenclature of Tn554 insertions and their attachment sites. Tn554 insertions in staphylococci have been classified on the basis of restriction pattern of adjacent chromosomal DNA (Tillotson *et al.*, 1989). The most common insertion in *S. aureus* is designated the primary insertion or insertion 1. Commonly found secondary insertions have been numbered 2–8 (Tillotson *et al.*, 1989). Appended letters (e.g., insertion 2B) generally denote restriction site polymorphisms. For insertion 6B, the B reflects a more marked difference from insertion 6, namely a different right adjacent chromosomal arm.

The attachment (*att*) site for the primary insertion is designated *att*554 after Tn554 (see Murphy, 1990). *att* sites for secondary insertions are named after prototype strains, e.g. *att*155 corresponds to insertion 6 in strain R155 and *att*46 corresponds to insertion 6B in strain ANS46. *att*35, in which no Tn554 insertion has been detected, refers to the locus

Table 1. *Bacterial strains*

Strain	Antimicrobial agent susceptibilities*						Description
	Sp	MLS	Hg	Tc	Mc	Cd	
WJ137	R	R	S	S	S	R	New Jersey Mc ^s isolate with Tn554 insertions 1 and 2 (Thakker-Varia <i>et al.</i> , 1987).
R155	R	R	S	S	R	S	New Jersey Mc ^r outbreak isolate with Tn554 insertions 1B (similar to 1) and 6 (Tillotson <i>et al.</i> , 1989; Chikramane <i>et al.</i> , 1991).
ANS46	R	R	R	R	R	R	Australian Mc ^r isolate (Matthews <i>et al.</i> , 1987) containing Tn554 insertions 1, 2B (similar to 2), and 6B (Chikramane <i>et al.</i> , 1991).
ANS62	R	R	S	(R)†	S	S	Mc ^s deletant of ANS46 (Matthews <i>et al.</i> , 1987) with Tn554 insertions 1, 2B, and 6B' (Chikramane <i>et al.</i> , 1991).
R35	S	R‡	R	R	R	R	1987 New Jersey Mc ^r isolate lacking Tn554 (Tillotson <i>et al.</i> , 1989).

* Abbreviations: Sp, spectinomycin; MLS, macrolides-lincosamides-streptogramin B; Hg, mercury; Tc, tetracycline; Mc, methicillin; Cd, cadmium.

† Decreased Tc^r due to the absence of *tetA*, but presence of another Tc^r determinant.

‡ MLS^r due to *ermC* in the plasmid pNE131.

in strain R35 that is homologous to *att46*. When an *att* site is occupied, DNA to the left (or right) of the transposon is referred to as the left (or right) *att* site arm. This terminology is extended to unoccupied *att* sites by reference to the position of the predicted transposon-mediated cleavage of the site.

The designations insertion 6B' and *att46'* for ANS62 are special cases. As explained in experiments now to be described, these are the same as insertion 6B and *att46* except for the fact that Δ ANS46/62 abuts the insertion.

The term pseudotransposon for ψ Tn554 reflects the fact that a portion of this element (about half) was found to be homologous to Tn554, while the rest lacked homology. Further, DNA adjacent to the left end of ψ Tn554 (orientation as in Fig. 1) proved to be homologous to DNA adjacent to Tn554 insertion 6 of strain R155.

Results

General organization of the Australondon mec region, mapping rationale, and comparison to New Jersey strain R35

Fig. 1 shows a physical map covering 55 kb of the *mec* region of the ANS46 chromosome. DNA in the central portion of this map amounting to approximately 35 kb has been characterized in detail (Matthews *et al.*, 1990; Inglis *et al.*, 1990). This 35 kb stretch extends between the regions of the leftmost and rightmost cloned fragments from Δ ANS46/62, clones MA13 and MA14. In addition to *mec*, this 35 kb region contains an Hg resistance operon and an integrated copy of the Tc^r plasmid pT181, both flanked by copies of the insertion

sequence IS257 (Skinner *et al.*, 1988; Matthews *et al.*, 1990).

The clone MA13 includes part of Tn554 insertion 6B (Chikramane *et al.*, 1991). As shown below, MA14 includes part of what we refer to as the pseudotransposon ψ Tn554, which hybridized with some of the probes derived from Tn554. Thus, Tn554-related probes helped refine the map of *mec*-associated DNA covered by MA13 and MA14, and permitted extension of the map beyond the cloned segments. These probes are summarized in Figs 1 and 2, and Table 2.

Fig. 3 shows Southern hybridization patterns illustrating relationships between DNA contained in clones MA13 and MA14, Tn554 insertion 6B, and the ψ Tn554 insertion. We ran, in parallel, samples of a *Hind*III plus *Pst*I digest of an MA13-containing plasmid (lane 1), a *Hind*III plus *Pst*I digest of chromosomal DNA from an Australondon strain (lane 2), and a *Hind*III digest of an MA14-containing plasmid (lane 3). (a) shows ethidium bromide-stained bands; patterns are as expected from earlier work (see Fig. 3 legend and Matthews *et al.*, 1990).

Fig. 3(b) shows a blot hybridized with oligonucleotide TEM. This probe is specific for Tn554, and hybridizes with right junction fragments of the three Tn554 insertions of the Australondon chromosome (Chikramane *et al.*, 1991). The 4.6 kb *Pst*I/*Hind*III band from Tn554 insertion 6B comigrates with a band from MA13, showing that MA13 includes the right junction of Tn554 insertion 6B (Fig. 1). MA14 did not hybridize with the TEM probe (Fig. 3), nor did it hybridize with

Fig. 2. Restriction maps of Tn554-related portions of the *mec* region. Maps of the ψ Tn554 insertion of ANS46 and R35 (lowest extended line), Tn554 insertion 6 of R155, insertion 6B of ANS46, insertion 6B' of ANS62, and the region of R35 corresponding to insertion 6B are shown. These are denoted by strain and *att* site designations. Restriction sites within ≈ 5 kb of junctions are shown for *Cla*I (C), *Eco*RI (R), *Eco*RV (R5), *Hind*III (H), *Pst*I (P), *Xba*I (Xb) and *Xho*I (Xh), and selected sites are shown for *Bgl*II (Bg), *Hpa*I (Hp), *Rsa*I (Rs) and *Alu*I (A). In addition, we show *Fnu*4HI (F) sites bounding Tn554 segments of plasmid pEM9715 (Thakker-Varia *et al.*, 1987) used as probes. The orientation of ψ Tn554 is reversed relative to that shown in Fig. 1. The *Cla*I site in parentheses is partially resistant to digestion in R35. The boundaries of Tn554 and ψ Tn554 are shown as heavy vertical lines. Cloned Tn554 fragments (A, B, B1, B2, C, and D) are shown by bold letters. Cloned junction fragments from insertion 6 (H57, H40; Tillotson *et al.*, 1989) are shown as shaded rectangles. Bars above these rectangles define the *Eco*RI/*Cla*I and *Eco*RV/*Hind*III fragments used as *att* arm-specific probes. Below the map of the ψ Tn554 insertion, we indicate regions of this insertion that are homologous to Tn554 fragments C, B1 and B2, to two Tn554-related oligonucleotides, to the *Sal*I/*Eco*RI fragment of MA14, and to H40. Dashed lines indicate that the precise extent of homologous sequence is not known. For R35 we interrupt the map over the region presumed to correspond to the (absent) Tn554 insertion.

Tn554 cloned fragment probes A–C (Fig. 2 and Table 2) (data not presented). Thus, this clone must lack homology to Tn554, and the portion of ψ Tn554 included in MA14 (the left half as drawn in Fig. 1, the right half as drawn in Fig. 2) also lacks homology to Tn554.

Fig. 3c presents results with an oligonucleotide probe, T26, based on the sequence of the right flanking chromosomal DNA of Tn554 insertion 6 (i.e. the right arm of attachment site *att*155 as it occurs in the 1987 New Jersey outbreak group (Tillotson *et al.*, 1989). T26 failed to hybridize with MA13 (lane 1), confirming the finding (Chikramane *et al.*, 1991) that the right flanking chromosomal DNA of insertion 6B (i.e., the right arm of the attachment site in ANS46 referred to as *att*46) differs from that of *att*155. However, oligonucleotide T26 did hybridize with an MA14 *Hind*III fragment of approximately 2.4 kb (lane 3). A similar fragment had previously been found in New Jersey strain R35 (Tillotson *et al.*, 1989). The 2.4 kb *Hind*III fragments from ANS46 and R35 also hybridized with an *Eco*RV/*Hind*III fragment of clone H40 specific for the right adjacent chromosomal DNA of insertion 6 of strain R155 (Table 2 and Fig. 2). This *Hind*III fragment was absent from ANS62 (data not

shown). Thus, the *mec* regions of ANS46 and R35 carry a substantial length of DNA similar to the right arm of *att*155. Sequence analysis (S. G. Chikramane & D. T. Dubin, unpublished results) has shown that both the T26 and H40 probes hybridize to DNA that abuts ψ Tn554 (on the left as drawn in Fig. 1 and on the right as drawn in Fig. 2). Thus this DNA can be considered to be an arm of a putative *att* site, '*att* ψ Tn554', which is partially homologous to *att*155. The 2.4 kb *Hind*III fragment of MA14 corresponds to a junction fragment of ψ Tn554 (approx. coordinates 39.5 to 42 kb in Fig. 1).

To further explore the relationship between R35 and ANS46 we used a series of probes spaced along the ANS46 map in Southern hybridizations with DNA digested with the nine endonucleases cited in the legend to Fig. 1. Several of these probes are oligonucleotides corresponding to sequence within Δ ANS46/62 (Fig. 1 and Table 2), as follows: T45A and T45B are specific for the left and right pair of IS257 elements, respectively (Matthews *et al.*, 1990); TMER is based on the sequence of the pI258 *merA* gene (Laddaga *et al.*, 1987); TPL and TPR are specific for the left and right IS257–pT181 junctions of ANS46 (Matthews *et al.*, 1990); T43 and

Table 2. Probes derived from Tn554 elements used for mapping mec-associated DNA

Probe*	Tn554-related elements† (prototype strain)				
	Insertion			ψ Tn554 (ANS46, R35)	att35 (R35)
	6 (R155)	6B (ANS46)	6B' (ANS62)		
Left att155					
T41	+	+	+	—	+
H57 <i>EcoRI/ClaI</i> segment	+	+	+	—	+
Tn554 (internal)					
T6B (left end)	+	+	+	+	—
Fragment C	+	+	+	+	—
Fragment B	+	+	+	+	—
Fragment A	+	+	+	—	—
Fragment D	+	+	+	—	—
TEM (right penultimate)	+	+	+	—	—
T54 (Australondon right end)	—	+	+	—	—
ψ Tn554					
T59	—	—	—	+	—
MA14 <i>EcoRV/SaI</i> segment	—	—	—	+	—
Right att155					
T26	+	—	—	+	—
H40 <i>EcoRV/HindIII</i> segment	+	—	—	+	—
Right att46					
T53	—	+	—	—	+
MA13 <i>HindIII/EcoRI</i> segment‡	—	+	—	—	+

* Oligonucleotide probes (T prefixes) have been described previously (Chikramane *et al.*, 1991; see also Fig. 1) except for T59. This is the reverse complement of GAATTCGCTAGAGC, which corresponds to Tn554 residues 2212 to 2229 (Murphy *et al.*, 1985), except that the underlined residue, which is a C in Tn554, was converted to a T to create an *XbaI* site (see Results). DNA fragment probes are shown in Figs 1 and 2.

† Tn554 and ψ Tn554 insertions include adjacent att arms. Hybridization (+) or lack of it (—) of the insertions and of att35 to the listed probes is shown.

‡ Around the 10 kb coordinate on the map of Fig. 1.

T43B are from the *mec* sequence (Song *et al.*, 1987); and TMA14 is based on sequencing (S. G. Chikramane & D. T. Dubin, unpublished results) of MA14. All of these probes hybridized strongly with ANS46 and R35 and yielded the same Southern hybridization patterns with both strains (data not shown).

Southern hybridization using Tn554 insertion-related probes: extension of the map through and beyond MA13 and MA14

The presence in ANS46 of two other copies of Tn554 (the primary insertion and a secondary insertion, 2B), in addition to Tn554 6B and ψ Tn554 (Chikramane *et al.*, 1991), led to complex Southern hybridization patterns with some Tn554-related probes. To facilitate interpretation, in most experiments four strains were processed in parallel with ANS46. ANS62, the Mc^s deletion mutant of ANS46, indicated whether a particular ANS46 band was all or partly within Δ ANS46/62 and hence could be

considered part of the *mec* region. WJ137, a recent New Jersey Mc^s isolate, provided markers for Tn554 insertions 1 and 2B (Thakker-Varia *et al.*, 1987). New Jersey isolate R35 proved especially valuable in that its *mec* region closely resembled that of ANS46, but it lacked Tn554. Finally, it was of interest to compare New Jersey outbreak prototype strain R155 with an Australondon strain.

As an example, Fig. 4 shows hybridizations of *EcoRI*-digested DNA. The complexity of patterns obtained for ANS46 with an internal Tn554 probe (B) which spans the single *EcoRI* site of the transposon is shown in Fig. 4(a), lane 5. The complexity is due to the presence of three Tn554 insertions, as well as ψ Tn554. This last element also has a single *EcoRI* site, and yields two *EcoRI* fragments (4.6 and ca 25 kb) which hybridize with probe B. The R35 sample illustrates the utility of this strain. The fragments of R35 that hybridize with internal Tn554-related probes such as probe B can be attributed to the pseudotransposon. Two hybridizing bands from R35 (Fig. 4a, lane 6) migrated with the 4.6 and $\approx$ 25 kb

Fig. 3. Southern blot analysis of fragments MA13 and MA14. *Bgl*II fragments of ANS46 DNA were cloned into the *Bam*HI site of pGEM-1 (Skinner *et al.*, 1988; Matthews *et al.*, 1990). The end lanes of each panel show markers from a *Hind*III digest of bacteriophage λ . (a) shows ethidium bromide-induced fluorescence patterns. Lane 1 shows a *Hind*III plus *Pst*I digest of the MA13-containing plasmid. The major bands are a 3.6 kb *Hind*III/*Pst*I product that includes the 2.9 kb vector moiety, a 4.6 kb *Pst*I/*Hind*III product corresponding to a right junction fragment of insertion 6B, and a 2.8 kb *Hind*III/*Pst*I product just downstream (the latter two as diagrammed in Fig. 1). Lane 2 shows a *Hind*III plus *Pst*I digest of DNA from a prototype London isolate of the Australondon lineage (Chikramane *et al.*, 1991). Lane 3 shows a *Hind*III digest of the MA14-containing plasmid. The major bands are a 4.3 kb product that includes the vector moiety, and 2.4 and 2.0 kb products corresponding to those diagrammed in Fig. 1. (b) and (c) represent Southern blots of the separation of (a) and of a parallel separation, hybridized with Tn554-related oligonucleotides TEM (b) or T26 (c) (see Table 2). Designations 1, 2B and 6B, and ψ refer to right junction fragments of Tn554 insertions or of the ψ Tn554 insertion, respectively.

bands of ANS46, indicating that the latter bands arose from ψ Tn554.

ANS62 lacked bands corresponding to the pseudo-transposon (Fig. 4a, lane 4), showing that this element is part of Δ ANS46/62. In contrast, ANS62 retained bands corresponding to Tn554 insertion 6B. We designate the 6B equivalent in ANS62 insertion 6B'. The band corresponding to the right *Eco*RI fragment (6B'R, lane 4) migrated differently from its counterpart in ANS46, reflecting the fact that insertion 6B' is near the Δ ANS46/62 boundary (Chikramane *et al.*, 1991).

Fig. 4(b) illustrates the homology between the DNA flanking the left end of insertion 6B of the Australondon lineage (i.e. the left arm of *att*46), and the left arm of *att*155. Oligonucleotide T41, based on the *att*155 sequence of strain R155 (Tillotson *et al.*, 1989), recognized

*Eco*RI fragments in ANS46 DNA (6BL, lane 5) and ANS62 DNA (6B'L, lane 4), as well, of course, as a fragment in R155 DNA (6L, lane 3).

The comigration of 6B'L with 6BL (Fig. 4b, lanes 4 and 5) is in accord with our previous mapping (Chikramane *et al.*, 1991), and reflects the fact that the deletion does not extend leftwards into Tn554 insertion 6B. The marked difference in migration between the 6B'L/6BL bands, and that from R155 (6L), reflects the fact that sequences near *att*46 and *att*155 become divergent beginning a few hundred residues leftwards of the insertion sites. The ≈ 7 kb band generated in R35 DNA by probe T41 (Fig. 4b, lane 6) indicates the presence in this strain of DNA homologous to left arms of *att*155 and *att*46, but the band size differs from those of the other strains (lanes 3, 4 and 5) because R35 lacks

Fig. 4. Southern Blot analysis of *Eco*RI digests. A blot was hybridized sequentially with Tn554 segment B (a), oligonucleotide T41 (b) and a 2.4 kb pT181 probe [equivalent to residues 3130–4437/0–1081 in the circular map of Khan & Novick (1983)] (c). Markers (lane 1) are as for Fig. 3; their positions are drawn in for (c) as the marker 32 P had decayed by the time of this hybridization. Lanes 2–6 correspond to strains WJ137, R155, ANS62, ANS46, and R35, respectively (Table 1). Bands are designated according to their element of origin, R and L specifying whether right or left junction fragment, and ψ specifying ψ Tn554.

Tn554 insertions. This R35 band corresponds to a locus similar to *att46*; we designate the locus *att35* and discuss it in greater detail in connection with Fig. 5.

Fig. 4(c) shows the same blot hybridized to a probe containing plasmid pT181 DNA. This probe hybridized to large fragments in ANS46 and R35 DNA (pT181, lanes 5 and 6). The pT181-hybridizing bands proved to be the same *Eco*RI fragment as hybridized to Tn554-related probes (a). This was shown by using the array of probes described above. The large *Eco*RI fragment of ANS46 and R35 thus contains the integrated copy of pT181, *mec*, and most of ψ Tn554 (Fig. 1). The faster migrating bands in ANS62 and ANS46 (Fig. 4c, lanes 4 and 5) are probably due to the presence of a *Cm*^r plasmid resembling pSK2 (Lyon & Skurray, 1987) that contains replication DNA homologous to that of pT181 (Projan *et al.*, 1985).

The experiment of Fig. 5 shows *Cla*I digests probed to illustrate some properties of left ends (orientation as in Fig. 2) of Tn554-related elements in the *mec* region, and of the left arms of corresponding attachment sites. A

filter was hybridized with probe T6B (a), an oligonucleotide based on the left end of Tn554 and which also recognizes ψ Tn554 (Table 2); and then (b) with the 0.5 kb *Eco*RI/*Cla*I fragment of H57 from the New Jersey strain R155, which contains only sequence to the left of the *att155* insertion site (Fig. 2). A similar filter was hybridized with the entire 1.4 kb *Eco*RI/*Hind*III H57 fragment illustrated in Fig. 2 (c). This last probe recognized all the bands shown in Fig. 5(a) and (b), but also hybridized to a 0.8 kb fragment in R35 (Fig. 5c, lane 6). This agrees with the previous conclusion that the left end of the mapped region of R35 is similar to that of ANS46, except that it lacks a Tn554 insertion. The absence of the insertion would bring the *Cla*I site of the right arm of the putative *att35* site to within 0.8 kb of the *Cla*I site of the left arm (see Fig. 1). The resulting *Cla*I fragment would hybridize with the 1.4 kb H57 fragment but not the 0.5 kb *Eco*RI/*Cla*I fragment derived from it, or with oligonucleotide T6B. Thus, R35 appears to contain an unoccupied Tn554 *att* site.

Verification of this inference was provided by a

Fig. 5. Southern blot analysis of *Cla*I digests. For (a) and (b), a blot was hybridized sequentially with oligonucleotide T6B and the 0.5 kb *Eco*RI/*Cla*I fragment of cloned insertion 6 left junction fragment H57 (Fig. 2). A parallel blot was hybridized with the entire 1.4 kb H57 fragment (c). Lanes are as for Fig. 4, and bands are identified as for that figure (all being left as drawn in Fig. 2), with the following elaborations: 1* designates a restriction fragment length polymorph of the primary insertion of WJ137; ψ^* designates a band arising due to partial resistance of the left-adjacent *Cla*I site (Fig. 2) of the R35 pseudotransposon insertion; and *att* designations are applied to bands containing solely attachment site arm moieties.

Southern blot of a *Cla*I/*Eco*RV digest of R35 DNA, probed with *att*155-specific oligonucleotide T41. This probe should hybridize with a $\approx$ 130 bp fragment from *att*35. A *Cla*I cleavage point occurs 113 residues to the left of the putative *att*35 cleavage site (as determined for *att*155; see Tillotson *et al.*, 1989) and an *Eco*RV site occurs 19 residues to the right (as determined for *att*46; Chikramane *et al.*, 1991). Such a fragment was observed, but hybridization of R35 blots with an oligonucleotide, GTGGGGATATAACCCC, that should recognize *att*46 (see Chikramane *et al.*, 1991) yielded faint bands, suggesting that *att*35 and *att*46 are similar but not identical.

All hybridizing chromosomal segments in Fig. 5(b) correspond to attachment site arm (as opposed to transposon) sequences. The designation *att*46' refers to the hypothetical attachment site of ANS62 insertion 6B', the right arm of which is quite different from the right arm of *att*46 (i.e. the right adjacent chromosomal DNA of insertion 6B of ANS46). The results of Fig. 5(b) show that the similarity between the left arm of *att*155 and the

left arms of *att*46, *att*46' and *att*35 extend substantially beyond their shared *Cla*I sites. All hybridized with the 0.5 kb *Eco*RI/*Cla*I fragment of strain R155 clone H57 (Fig. 2).

Fig. 5 demonstrates the only difference noted between the R35 and ANS46 *mec* regions besides those related to insertion 6B. The *Cla*I site adjacent to the ψ Tn554 element in R35 (but not that in ANS46) was partially resistant to digestion (Fig. 5a and c, lane 6). This resistance was unaffected by prolonging the digestion time or by increasing the concentration of enzyme. The *Cla*I site in question may be methylatable. Perhaps in R35 it is partially methylated while in ANS46 it is unmethylated.

Hybridization properties of ψ Tn554

Fig. 2 presents maps of Tn554 insertions 6 of R155, 6B of strain ANS46, and 6B' of ANS62, and the analogous chromosomal region of strain R35. These are compared to the ψ Tn554 insertion of ANS46 and R35. The ψ Tn554

Table 3. Hybridization analysis of the left end of the ANS46/ANS62 deletion

Oligonucleotide probes correspond to the right chromosomal junction of ANS46 Tn554 insertion 6B. A slash precedes the chromosomal sequence. The three chromosomal nucleotides adjacent to the right ends of insertions 1, 2B and 6B are shown beneath the corresponding headings. All the listed oligonucleotides have a T_D (Berent *et al.*, 1985) of 48 °C. The hybridization temperature was held at 45 ± 0.5 °C. (+) indicates the presence of a band at least 50% the intensity of insertion 6B bands and (–) indicates no band or a band of less than 10% the intensity of insertion 6B bands, determined by Southern blot hybridizations of *Hind*III plus *Pst*I digests.

Probe	Insertion			6B'
	1 (/ACG)	2B (/CAA)	6B (/ACC)	
T56 TACTCTTGATAATACATC/A	+	+	+	+
T57 CTCTTGATAATACATC/AC	+	–	+	–
T58 TCTTGATAATACATC/ACC	+	–	+	–

insertion is shown in opposite orientation to that of Fig. 1, to illustrate the similarity between the pseudotransposon and Tn554. In particular, the spacing between the internal *Hpa*I, *Eco*RI and *Xba*I sites of Tn554 was indistinguishable from that of corresponding sites in ψ Tn554. However, there was a discrete difference between these regions of pseudotransposon and transposon, namely, the second ψ Tn554 *Xba*I site apparently coincident with the *Eco*RI site. Examination of the sequence of Tn554 (Murphy *et al.*, 1985) revealed the string CCTAGA just to the right of the *Eco*RI site. We tested the idea that the corresponding sequence in ψ Tn554 was TCTAGA (the *Xba*I recognition sequence) using oligonucleotide T59 (Table 2). T59 hybridized with ψ Tn554 (data not shown), implying that the *Eco*RI site of ψ Tn554 does correspond to that of Tn554. Also, internal fragments of ψ Tn554 hybridized to 1.2 and 2 kb internal *Fnu*4HI/*Eco*RI fragments B1 and B2 of Tn554 (Fig. 2) as follows: ψ Tn554 fragments leftwards of the *Eco*RI/*Xba*I locus hybridized with probe B1 but not B2, while fragments to the right of this reference point hybridized with probe B2 but not B1.

The relationship between Tn554 and ψ Tn554 was examined with other probes summarized in Table 2 and Fig. 2. There was extensive sequence similarity between the left 2–3 kb of ψ Tn554 and the corresponding part of Tn554 (Fig. 2). No homology was detected between the right portions of the two elements.

Left boundary of the ANS46/ANS62 deletion

It was inferred from earlier results (Chikramane *et al.*, 1991) that the left boundary of Δ ANS46/62 was within 21 residues of the right end of Tn554 insertion 6B. This was based on the facts that insertions 6B of ANS46 and 6B' of ANS62 both hybridize with the right transposon-terminal oligonucleotide T54 (Table 2), and that ANS62

lacks an *Eco*RV site that occurs at residues 16–21 rightwards of ANS46 insertion 6B (Fig. 2 and Chikramane *et al.*, 1991). To localize more precisely the left boundary of the deletion, DNA from ANS46 and ANS62 was hybridized with a set of overlapping oligonucleotides corresponding to the right transposon–chromosome junction of insertion 6B (Table 3). A single 3' terminal mismatch did not prevent hybridization (see the T56 insertion 2B result), but two 3' terminal mismatches did (T57 insertion 2B). The results for insertion 6B' of ANS62 indicated that the chromosomal sequence directly to the right of the transposon differs from the corresponding sequence for insertion 6B at the first residue beyond the transposon. We infer that the left boundary of the deletion begins precisely at the end of the Tn554 sequence of insertion 6B.

Right boundary of the ANS46/ANS62 deletion

The location of the right boundary of Δ ANS46/62 was studied with oligonucleotide T6B, which corresponds to the left end of ψ Tn554 (Fig. 2). To map more precisely the site of hybridization of this oligonucleotide, PCR was used to amplify the DNA between T6B and oligonucleotide T59 (Fig. 2). Major discrete products running at $\approx$ 1.2 kb resulted when R35 or ANS46 DNA was used as template (Fig. 6a, lanes 1–4). The $\approx$ 2.2 kb PCR product from ANS46 DNA (Fig. 6a, lane 2) probably arises from false priming by T59 in the region of the *Eco*RI site of Tn554. The corresponding region of Tn554, but not ψ Tn554, contains a *Hind*III site (Fig. 2), and the $\approx$ 2.2 kb product was virtually undetectable when ANS46 template DNA was treated with *Hind*III prior to amplification (Fig. 6a, lane 1). The PCR product from the pseudotransposon was $\approx$ 0.1 kb smaller than the *Xho*I/*Xba*I junction fragment, placing the left end of ψ Tn554 $\approx$ 0.1 kb from the *Xho*I site (Fig. 2). More precise values

Fig. 6. PCR sizing of terminal and junction fragments of ψ Tn554. For (a) we subjected DNA samples to PCR amplification using as primers oligonucleotide T59 and an oligonucleotide corresponding to T6B, oriented towards T59 (Fig. 2 and Table 2). Samples (10%) of the products were subjected to electrophoresis through a 1.2% agarose gel and stained with ethidium bromide. Lanes: 1, ANS46 DNA previously treated with *Hind*III; 2, intact ANS46 DNA; 3, R35 DNA previously treated with *Hind*III; 4, intact R35 DNA; 5, 1 kb ladder. For (b), 1% portions of two of the PCR products were digested with *Hpa*I, and subjected to electrophoresis as for (a). *Hpa*I plus *Xho*I digests of chromosomal DNA preparations were run in parallel. After blotting, the filter was hybridized with Tn554 probe B1 (Fig. 2). Lanes: 1, '1 kb ladder'; 2 and 3, *Hpa*I-treated PCR product of *Hind*III-digested ANS46 and R35 DNA, respectively; 4-6, *Hpa*I/*Xho*I digests of ANS62, ANS46, and R35 chromosomal DNA, respectively. Sizes of pertinent marker bands are in bp; a 617 bp band arising from an internal *Hpa*I/*Xho*I fragment of Tn554 (Murphy *et al.*, 1985) was also used as a size marker. Relevant experimental bands are designated by arrows: namely, the *Hpa*I fragments of the PCR products, which were estimated to be 820 and 410 bp; and the *Hpa*I/*Xho*I junction fragments of ψ Tn554, which were estimated to be 900 bp.

were obtained by comparing *Hpa*I digests of the PCR products with *Xho*I/*Hpa*I digests of chromosomal DNA (Fig. 6b). The difference between the appropriate PCR fragments and corresponding chromosomal fragments was 80 bp, indicating that the *Xho*I site is located this distance (± 10 bp) from the pseudotransposon.

The chromosomal restriction sites near the right junction of insertion 6B' of ANS62 are a mirror image of those near the left junction of ψ Tn554 (as drawn in Fig. 2). Thus, when both regions are viewed in the same orientation relative to the chromosome (Fig. 1), the restriction patterns are essentially the same. In particular, the right junction *Rsa*I/*Xho*I fragment of insertion 6B' is ≈ 160 bp (Fig. 2). Since the rightmost *Rsa*I site in Tn554 is 80 bp from the end (Murphy *et al.*, 1987), the *Xho*I site adjacent to insertion 6B' of ANS62 (Fig. 2) must be ≈ 80 bp beyond the transposon. These results indicate that the chromosomal sequences rightwards from the *Xho*I site flanking insertion 6B' and leftwards from the *Xho*I site flanking ψ Tn554 (Fig. 2) are similar. Thus, the right boundary of Δ ANS46/62 is located between the right end of ψ Tn554 (as drawn in Fig. 1) and

the chromosomal *Xho*I site, and extends no further than ≈ 80 bp past the pseudotransposon.

Discussion

General features of the *mec* region of ANS46 and comparison to New Jersey *Mc*^r lineages

The present studies have mapped all the sites for five restriction endonucleases and many of the sites for another seven over a stretch of 55 kb of chromosomal DNA corresponding to the *mec* region of the prototype Australian *Mc*^r isolate ANS46 (Figs 1 and 2). In addition to *mec*, this region contains determinants for spectinomycin and MLS-resistance (in Tn554), Hg^r, Tc^r (in pT181), and Cd^r (in a novel element, ψ Tn554).

The *mec* region of the New Jersey isolate R155 differs substantially from that of ANS46. It lacks the integrated plasmid pT181, the pseudotransposon ψ Tn554 (with its Cd^r determinant) and the Hg^r locus present in ANS46. However, the homologies between the left arms of *att*155 and *att*46, and between the right arms of *att*155

and *attψTn554*, imply that strains R155 and ANS46 have evolved from a common *Mc^r* ancestor. Complex rearrangements in the regions flanking the attachment sites must have occurred during the divergence of the two lineages.

The relationship between the Australondon group and New Jersey *Mc^r* isolate R35 appears to be much closer. Our initial hybridization results led us to suggest that R35 contained the equivalent of *att155*, but that sequences different from *Tn554* were inserted in this attachment site (Tillotson *et al.*, 1989). The present work shows that this 'insertion' constitutes more than 30 kb of *mec* region DNA, and that the *att155* right arm occurs in the wrong orientation relative to the left. It is simpler to view the *mec* region of R35 as a virtual copy of that of ANS46, without a *Tn554* insertion in its version (*att35*) of *att46*. Also, one can view the region of R35 corresponding to the right arm of *att155* as an arm of *attψTn554*. We propose that R35 and the Australondon strains share a relatively recent common ancestry, and that integration of the *Tn554* insertions into the DNA of an ANS46 ancestor occurred in Australia or England after the geographical separation of the Australondon and R35 lineages.

Strain R35 was collected in a 1987 survey of two New Jersey hospitals, and was the only one of its kind among 35 *Mc^r* *S. aureus* isolates characterized (most being similar to strain R155) (Tillotson *et al.*, 1989). This seemed to belie the apparent ability of Australondon strains to spread and displace other *Mc^r* strains (Pavillard *et al.*, 1982; Cookson & Phillips, 1988). Interestingly, a more recent (1990) survey showed that in one of these New Jersey hospitals, R35-like *Mc^r* strains now predominate, although the original New Jersey outbreak (R155-like) strains continue to predominate at the other hospital (D. T. Dubin & W. D. Janssen, unpublished results).

The ANS46/ANS62 deletion

The deletion of *mec* DNA in ANS62 was shown here to be 41 kb (Fig. 1), which is in accord with a recent estimate (≈ 50 kb) based on field inversion gel electrophoresis (Inglis *et al.*, 1990).

The proximity of the left boundary of Δ ANS46/62 to the right boundary of insertion 6B led earlier to the suggestion that the transposon might have played a role in generating the deletion (Dubin, 1990). The present work, which demonstrates the precise coincidence of these boundaries, strengthens this idea. The fact that the right boundary of the deletion is now shown to be at or near an end of *ψTn554* suggests that the pseudotransposon was also involved in the deletional event. The exact nature of this event remains to be determined.

Strain ANS62 was recovered by screening ≈ 3000 colonies for methicillin sensitivity after treatment of ANS46 with acriflavine. The ANS46/ANS62 type of deletion is probably not a rare event, and indeed may occur even more frequently with other types of stress, for example ultraviolet irradiation (P. R. Matthews & P. R. Stewart, unpublished observations). Similar types of deletion involving the *mec* region can occur upon storage, or under conditions of starvation (Matthews *et al.*, 1990; Inglis *et al.*, 1990). The *Tn554* and *ψTn554* insertions of ANS46 DNA as well as the copies of IS257 may contribute to the instability and the evolutionary variation of this region.

Nature of ψTn554

The portion of *Tn554* to which *ψTn554* shows extended sequence similarity (roughly the left half as drawn in Fig. 2) is that which encodes the *Tn554* transposases (Murphy *et al.*, 1985). There is good evidence that the other region of *ψTn554* contains the *Cd^r* determinant that is present in ANS46. A probe containing sequences from the *cadA* gene of plasmid p1524 hybridized with the 10 kb *Xba*I fragment of ANS46 corresponding to coordinates 34–44 kb of Fig. 1 (Matthews *et al.*, 1990). This, coupled with the present results showing that the left portion of MA14 (orientation as for Fig. 1) corresponds to an arm of *attψTn554*, supports the placement of the *Cd^r* determinant of ANS46, as shown in Fig. 1, in the *EcoRV*/*Sal*I fragment of MA14. DNA from all of the *Cd^r* strains listed in Table 1 hybridized to this fragment, whereas DNA from the *Cd^s* strain R155, like ANS62, did not (data not shown). Ongoing sequence analysis will identify the *Cd^r* gene of *ψTn554* and will determine if reading frames corresponding to *Tn554* transposases remain intact. This will help ascertain whether *ψTn554* is a mobile element and what its evolutionary relationship to *Tn554* might be.

This work was supported in part by the National Health and Medical Research Council of Australia (P.R.S.). We thank Lotus Moon-McDermott and Sheila Mazar for their technical assistance and JoAnn Humphrey and Michael Russo for their help in preparing illustrations.

References

- BARRY, A. L. & THORNSBERRY, C. (1980). Susceptibility testing: diffusion test procedures. In *Manual of Clinical Microbiology*, 3rd edn, pp. 463–474. Edited by E. A. Lennette. Washington: American Society for Microbiology.
- BECK, W. D., BERGER-BACHI, B. & KAYSER, F. H. (1986). Additional DNA in methicillin-resistant *Staphylococcus aureus* and molecular cloning of *mec*-specific DNA. *Journal of Bacteriology* **165**, 373–378.
- BERENT, S. L., MAHMOUDI, M., TORCZYNSKI, R. M., BRAGG, P. W. & BOLLON, A. P. (1985). Comparison of oligonucleotide and long DNA fragments as probes in DNA and RNA dot, Southern, Northern, colony and plaque hybridization. *Biotechniques* **3**, 208–220.

- CHIKRAMANE, S. G., MATTHEWS, P. R., NOBLE, W. C., STEWART, P. R. & DUBIN, D. T. (1991). Tn554 inserts in methicillin-resistant *Staphylococcus aureus* from Australia and England: comparison with an American methicillin-resistant group. *Journal of General Microbiology* **137**, 1303–1311.
- COOKSON, B. D. & PHILLIPS, I. (1988). Epidemic methicillin-resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* **21** (suppl. C), 57–65.
- DUBIN, D. T. (1990). Tn554 and related DNA: mapping and tracking the staphylococcal chromosome. In *Molecular Biology of the Staphylococci*, pp. 85–98. Edited by R. Novick. New York: VCH Publishing.
- DUBIN, D. T., MATTHEWS, P. R., CHIKRAMANE, S. G. & STEWART, P. R. (1991). Physical mapping of DNA from the *mec* region of an American methicillin *Staphylococcus aureus* lineage. *Antimicrobial Agents and Chemotherapy* **35**, 1661–1665.
- INGLIS, B., MATTHEWS, P. R. & STEWART, P. R. (1990). Induced deletions within a cluster of resistance genes in the *mec* region of the chromosome of *Staphylococcus aureus*. *Journal of General Microbiology* **136**, 2231–2239.
- KHAN, S. A. & NOVICK, P. R. (1983). Complete nucleotide sequence of pT181, a tetracycline-resistance plasmid from *Staphylococcus aureus*. *Plasmid* **10**, 251–256.
- LADDAGA, R. A., CHU, L., MISRA, T. K. & SILVER, S. (1987). Nucleotide sequence and expression of the mercurial resistance operon from *Staphylococcus aureus* p1528. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 5106–5110.
- LYON, B. R. & SKURRAY, R. (1987). Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. *Microbiological Reviews* **51**, 88–134.
- MATTHEWS, P. R., REED, K. C. & STEWART, P. R. (1987). The cloning of chromosomal DNA associated with methicillin and other resistances in *Staphylococcus aureus*. *Journal of General Microbiology* **133**, 1919–1929.
- MATTHEWS, P. R., INGLIS, B. & STEWART, P. R. (1990). Clustering of resistance genes in the *mec* region of the chromosome of *Staphylococcus aureus*. In *Molecular Biology of the Staphylococci*, pp. 59–83. Edited by R. Novick. New York: VCH Publishing.
- MURPHY, E. (1990). Properties of the site-specific transposable element Tn554. In *Molecular Biology of the Staphylococci*, pp. 123–136. Edited by R. Novick. New York: VCH Publishing.
- MURPHY, E., HUWYLER, L. & BASTOS, M. C. (1985). Transposon Tn554: complete nucleotide sequence and isolation of transposition-defective and antibiotic-sensitive mutants. *EMBO Journal* **4**, 3357–3365.
- MURPHY, E., PHILLIPS, S., EDELMAN, I. & NOVICK, R. P. (1981). Tn554: isolation and characterization of plasmid insertions. *Plasmid* **5**, 292–305.
- PAVILLARD, R., HARVEY, K., DOUGLAS, D., HEWSTONE, A., ANDREW, J., COLLOPY, B., ASCHE, V., CARSON, P., DAVIDSON, A., GILBERT, G., SPICER, J. & TOSOLINI, F. (1982). Epidemic of hospital-acquired infection due to methicillin-resistant *Staphylococcus aureus* in major Victorian hospitals. *Medical Journal of Australia* **1**, 451–454.
- PROJAN, S. J., KORNBLUM, K., MOGHAZEK, S. L., EDELMAN, I., GENNARO, M. L. & NOVICK, R. P. (1985). Comparative sequence and functional analysis of pT181 and pC221, cognate plasmid replicons from *Staphylococcus aureus*. *Molecular and General Genetics* **199**, 452–464.
- SANGER, F., NICKLEN, S., & COULSON, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 5463–5467.
- SKINNER, S., INGLIS, B., MATTHEWS, P. R. & STEWART, P. R. (1988). Mercury and tetracycline resistance genes and flanking repeats associated with methicillin resistance on the chromosome of *Staphylococcus aureus*. *Molecular Microbiology* **2**, 289–298.
- SONG, M. D., WACHI, M., DOI, M., ISHINO, F. & MATSUHASHI, M. (1987). Evolution of an inducible penicillin-target protein in methicillin-resistant *Staphylococcus aureus* by gene fusion. *FEBS Letters* **221**, 167–171.
- THAKKER-VARIA, S., JENSSEN, W. D., MOON-MCDERMOTT, L., WEINSTEIN, M. P. & DUBIN, D. T. (1987). Molecular epidemiology of macrolides–lincosamides–streptogramin B resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *Antimicrobial Agents and Chemotherapy* **31**, 735–743.
- TILLOTSON, L. E., JENSSEN, W. D., MOON-MCDERMOTT, L. & DUBIN, D. T. (1989). Characterization of a novel insertion of the macrolides–lincosamides–streptogramin B resistance transposon Tn554 in methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrobial Agents and Chemotherapy* **33**, 541–550.
- UBUKATA, K., NONOGUCHI, R., MATSUHASHI, M., DONG SONG, M. & KONNO, M. (1989). Restriction maps of the regions coding for the methicillin and tobramycin resistances on chromosomal DNA in methicillin-resistant staphylococci. *Antimicrobial Agents and Chemotherapy* **33**, 1624–1626.
- WEISS, A. A., MURPHY, S. D. & SILVER, S. (1977). Mercury and organomercurial resistances determined by plasmids in *Staphylococcus aureus*. *Journal of Bacteriology* **132**, 197–208.