Plasmid-mediated resistance to gentamicin in *Staphylococcus aureus*: the involvement of a transposon

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Summary. Resistance to gentamicin, tobramycin and kanamycin (Gm^rTm^rKm^r) in strains of *Staphylococcus aureus* isolated from clinical sources in Australia is mediated by a 4·7 kb transposable element, designated Tn4001. A 2·5 kb *Hind*III fragment which maps symmetrically within Tn4001, and encompasses the aminoglycoside-resistance coding region, has been shown to hybridise with fragments of identical size in *Hind*III digests of three different Gm^rTm^rKm^r plasmids, two of which were self-transmissible, from strains of *S. aureus* isolated in the USA. Examination by electronmicroscopy of self-annealed molecules of the North American Gm^rTm^rKm^r plasmids revealed the presence of stem and loop structures similar to those produced by Tn4001, but with shorter inverted repeats. These results suggest that Gm^rTm^rKm^r in strains of *S. aureus* isolated in the USA is, or once was, transposable, and that transposable elements analogous to Tn4001 may be found in isolates of Gm^rTm^rKm^r *S. aureus* worldwide.

Introduction

Strains of *Staphylococcus aureus* resistant to gentamicin were first isolated from clinical sources in the mid-1970's (Soussy *et al.*, 1975; Speller *et al.*, 1976). Since then, numerous outbreaks of gentamicin-resistant *S. aureus* infection have been reported from hospitals around the world (Crossley *et al.*, 1979; Price *et al.*, 1980; Hone *et al.*, 1981; Pavillard *et al.*, 1982).

In staphylococci, resistance to gentamicin, tobramycin and kanamycin is usually mediated by a bifunctional protein that specifies 6' acetyltransferase [AAC (6')] and 2" phosphotransferase [APH (2")] aminoglycoside modifying activities (Martel *et al.*, 1983; Ubukata *et al.*, 1984) and is plasmid-encoded in the majority of *S. aureus* strains (LeGoffic *et al.*, 1977; Scott *et al.*, 1978; Ubukata *et al.*, 1984). Restriction endonuclease analyses of a number of these plasmids have revealed a high degree of structural relatedness, implying a common origin (Jaffe *et al.*, 1982; Gray *et al.*, 1983; Goering

and Ruff, 1983). Such plasmids range in size from 18 to 57 kilobases (kb) and may specify resistance to other aminoglycosides through 4' adenylyltransferase [AAD (4')] (Wood *et al.*, 1977; Archer and Johnston, 1983; McDonnell *et al.*, 1983) or APH (3') modification (Gray *et al.*, 1983; Ubukata *et al.*, 1984), mediate penicillin resistance via a β lactamase (Cohen *et al.*, 1982; Goering and Ruff, 1983; McDonnell *et al.*, 1983) or carry determinants for resistance to ethidium bromide and quaternary ammonium compounds (Eb^rQa^r) (Asch *et al.*, 1984).

Several of the larger plasmids promote their own transfer, and mobilise non-transferable plasmids, by a process analogous to conjugation (Archer and Johnston, 1983; McDonnell *et al.*, 1983; Asch *et al.*, 1984).

Australian strains of Gm^rTm^rKm^r *S. aureus* manifest a family of structurally related plasmids that uniformly encode resistance to acriflavine, ethidium bromide and quaternary ammonium compounds (Ac^rEb^rQa^r), but may also specify Gm^rTm^rKm^r, resistance to trimethoprim (Tp^r) and β -lactamase production (Lyon *et al.*, 1983; Lyon *et al.*, 1984a; Gillespie and Skurray, 1986). Previous studies indicate that Gm^rTm^rKm^r is encoded by a transposable element designated Tn4001 (Lyon *et al.*, 1984b).

In this paper, we compare the regions encoding Gm^rTm^rKm^r from three distinct plasmids isolated in the USA with Tn4001, using restriction endonu-

Received 21 Apr. 1986; Accepted 24 June 1986.

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clease analysis, DNA-DNA hybridisation and electronmicroscopy to examine the possibility that the one transposon has been responsible for the widespread outbreaks of Gm^rTm^rKm^r in *S. aureus*.

Materials and methods

Bacterial strains and plasmids

All *S. aureus* Gm^r plasmids were transferred from clinical isolates to the laboratory strains SK982 (rifampicin and novobiocin resistant; Rf^r, Nv^r) (Lyon *et al.*, 1984b) or SK983 (streptomycin and fusidic acid resistant; Sm^r, Fa^r) (Tennent *et al.*, 1985). The plasmids used, along with relevant characteristics, are listed in table I.

General methods

Standard culture media and methods for the determination of antimicrobial susceptibilities and minimum inhibitory concentrations (MICs) were as previously described (Lyon *et al.*, 1983; Tennent *et al.*, 1985).

Plasmid transfer

Mixed-culture transfer and filter matings were conducted essentially as described by McDonnell *et al.* (1983). Resistant progeny in transfers with the recipient strains SK982 or SK983 were selected on Brain Heart Infusion Agar (Oxoid) containing (μ g/ml) Gm 8 and Rf 10, Nv 1 or Sm 100, Fa 10, respectively.

Plasmid DNA isolation, restriction analysis and cloning procedures

The isolation of purified plasmid DNA from *S. aureus* cultures, digestion with the restriction endonucleases Bg¹/III, EcoRI, HaeIII, HindIII and PvuII (New England Biolabs), agarose gel electrophoresis, and the estimation of DNA fragment sizes were performed as published (Lyon *et al.*, 1983). HindIII digests of lambda (λ) virus DNA (Miles Laboratories) were employed as standards with fragment sizes (in kb) taken from Daniels *et al.* (1983). The procedure for *E. coli* plasmid DNA isolation, together with all recombinant DNA techniques, have been described previously (Ray and Skurray, 1983).

DNA-DNA hybridisation

DNA was transferred to nitrocellulose filters by the bidirectional transfer procedure of Smith and Summers (1980). DNA for use as a radiolabelled probe was electroeluted from an agarose gel (Smith, 1980) and nick translated with [α^{32} P] dATP essentially as described by Maniatis *et al.* (1982). Hybridisation was conducted in the presence of formamide 50%, 5 $\times$ SSC (1 $\times$ SSC was 0.15M NaCl, 0.015M sodium citrate), SDS 0.1% w/v and

Denhardt's solution 2% (Maniatis *et al.*, 1982) at 45°C for 16 h. Hybridised filters were washed at least five times in 4 $\times$ SSC, SDS 0.1% w/v, dried, and exposed to X-Omat-AR X-ray film (Kodak) with an intensifying screen for 24–48 h at –70°C.

Electronmicroscopy

Self-annealed molecules were formed from purified plasmid DNA and mounted for electronmicroscopy essentially as described by Davis *et al.* (1971). Molecules were visualised and photographed using a Philips EM300 at $\times 20\,000$. Plasmid pC194 (2.91 kb; Horinouchi and Weisblum, 1982) was cleaved at the single HindIII site and included as a double-stranded size standard, and ϕ X174 viral DNA (New England Biolabs, 5.375 kb; Sanger *et al.*, 1977) was used as a single-stranded size standard. Contour lengths (expressed as an average with standard deviation in kb) were determined by measurement of photographic enlargements with a MOP-1 digital analyser (Carl Zeiss).

Results

Physical and genetic analysis of an Australian Gm^rTm^rKm^r plasmid

Plasmid pSK1 is the prototype of a family of structurally related plasmids detected in strains of Gm^rTm^rKm^r *S. aureus* isolated from Australian hospitals (Lyon *et al.*, 1983; Lyon *et al.*, 1984a). Transfer of pSK1 to SK982 in mixed cultures has revealed that pSK1 encodes Ac^rEb^rQa^r, Gm^rTm^rKm^r and Tp^r (table I), and comparative restriction mapping of this plasmid with other members of the plasmid family has enabled the construction of a physical-genetic map as illustrated in fig. 1. The genetic determinant for Gm^rTm^rKm^r has been mapped by comparison with the Gm^sTm^sKm^s plasmid pSK7 (table I), which was found to be identical to pSK1 except that it does not possess the 4.7-kb Gm^rTm^rKm^r transposon, Tn4001 (fig. 1).

A 2.5-kb HindIII fragment, which maps symmetrically within Tn4001 and is believed to encompass the gene(s) for Gm^rTm^rKm^r (fig. 1), has been cloned from pSK31, a Tn4001-inserted derivative of pII147 (Lyon *et al.*, 1984b), into the unique HindIII site in the *E. coli* vector plasmid pACYC184 (fig. 2). The resulting recombinant plasmid, designated pSK310, mediates Gm^rTm^rKm^r in the *E. coli* host, thus confirming the location of the aminoglycoside resistance determinant on the 2.5-kb HindIII fragment. Digestion of pSK310 with HindIII (fig. 3A, lane c) revealed a fragment with identical mobility to that of the 2.5-

Fig. 1. Linear restriction maps of the plasmids pSK1, pSK7, pSH6, pSK41, pUW3626 and pCRG1600. These maps, with the exception of that for pSK7, are aligned relative to the common 2.5-kb *Hind*III fragment within the Gm^rTm^rKm^r region; the map for pSK7 is aligned to that of pSK1; coordinates are in kb. Restriction endonuclease sites are indicated by ■ (*Bgl*II), ○ (*Eco*RI), ◆ (*Hind*III), ● (*Hae*III) and ▽ (*Pvu*II); only relevant *Hae*III sites are shown. Gm, Tm, Km, Ac, Eb, Qa, Tp, Nm, Pm and Pc designate the plasmid DNA segments that mediate resistance to gentamicin, tobramycin, kanamycin, acriflavine, ethidium bromide, quaternary ammonium compounds, trimethoprim, neomycin, paromomycin and penicillin, respectively. Tra indicates the DNA segment that mediates plasmid self-transmissibility. Inverted repeats are represented by the thick lines. The map of pCRG1600 is adapted from Asch *et al.* (1984); it should be noted that although the sizes of the *Eco*RI fragments as used by the latter authors to map pCRG1600 differ from those we have determined for pUW3626, both plasmids appear to possess five, out of six, common-sized *Eco*RI fragments (Goering and Ruff, 1983).

kb *Hind*III fragment of pSK1 (fig. 3A, lane b) together with the 4.2-kb *Hind*III linear fragment of pACYC184. When the 2.5-kb *Hind*III fragment of pSK310 was used as a radiolabelled probe, it was found to hybridise to itself (fig. 3B, lane c) and to the 2.5-kb *Hind*III fragment of pSK1 (fig. 3B, lane b), thus confirming the identity of these fragments.

Physical and genetic analysis of North American Gm^rTm^rKm^r plasmids

The three plasmids pSK41, pSH6 and pUW3626 (see table I for details) were transferred to the recipient strains SK982 or SK983 to enable their further characterisation. Plasmid pSH6 was found,

Table I. *Staphylococcus aureus* plasmids and their characteristics

Plasmid	Phenotype	Modifying enzymes	Size (kb)	Clinical isolate*	Reference
pSK1	Gm ^r Tm ^r Km ^r Ac ^r Eb ^r Qa ^r Tp ^r	ND	28.4	SK529	Lyon <i>et al.</i> , 1983, 1986
pSK7	Ac ^r Eb ^r Qa ^r Tp ^r	ND	23.7	SK460	Lyon <i>et al.</i> , 1984a
pSK41	Gm ^r Tm ^r Km ^r Nm ^r Pm ^r Eb ^r Qa ^r Tra ⁺	AAC(6')APH(2'')AAD(4')	47.8	Spratlin	Wood <i>et al.</i> , 1977
pSH6	Gm ^r Tm ^r Km ^r	AAC(6')APH(2'')	20.8	SH6	McDonnell <i>et al.</i> , 1983
pUW3626	Gm ^r Tm ^r Km ^r Nm ^r Pm ^r Eb ^r Qa ^r Pc ^r Tra ⁺	ND	54.4	L3626	Cohen <i>et al.</i> , 1982

ND; not determined.

* We are grateful to G. K. Best, Medical College of Georgia, USA, S. Cohen, University of Chicago, USA and M. L. Cohen, Center for Infectious Diseases, Atlanta, USA for providing strains Spratlin, SH6 and L3626, respectively.

Fig. 2. Strategy for cloning the central coding region of *Tn4001*. A simplified restriction map of the 28.4-kb *Ac^rEb^rQa^r*, *Tp^r* and *Gm^rTm^rKm^r* plasmid pSK1 showing the inverted repeats of the 4.7-kb aminoglycoside resistance transposon *Tn4001* (thick lines). During mixed-culture transfer experiments, *Tn4001* was transposed to the chromosome of a strain which carried the 32.6-kb penicillin (*Pc*), arsenate (*Asa*), cadmium (*Cd*) and mercury (*Hg*) resistance plasmid pII147 (Shalita *et al.*, 1980), constructing the strain SK2230. A bacteriophage 80 α lysate was prepared from SK2230 and used to transduce *S. aureus* strain NCTC 8325 for resistance to gentamicin and cadmium. One such plasmid resulting from this transduction was pSK31, which arose from the transposition of *Tn4001* from the *S. aureus* chromosome to pII147. Purified pSK31 DNA was digested with *Hind*III and "shotgun" cloned into the *Hind*III site of the *E. coli* vector pACYC184 (double open lines) to produce the chloramphenicol-resistant (*Cm^r*) and tetracycline-sensitive (*Tc^s*) 6.7-kb plasmid pSK310. Restriction endonuclease sites are indicated by B (*Bam*HI), E (*Eco*RI), Ha (*Hae*III), and H (*Hind*III); only the relevant *Hae*III sites are shown.

Fig. 3. (A) Agarose gel (1.2% w/v) electrophoresis of purified *S. aureus* and *E. coli* plasmid DNA cleaved with *Hind*III. Lane a, λ virus DNA cleaved with *Hind*III, fragment sizes (in kb) are shown at left; b, pSK1; c, pSK310; d, pSK41; e, pSH6; f, pUW3626. (B) DNA from the above gel transferred to nitrocellulose and hybridised with a ^{32}P nick-translated probe consisting of the 2.5-kb *Hind*III fragment from pSK310.

like the pSK1-related plasmids, to readily transfer to SK982 in broth mixed cultures, however pSK41 and pUW3626 failed to transfer under these conditions. The latter two plasmids did, however, transfer to SK983 at high frequency in filter matings, a result consistent with these plasmids being self-transmissible (Tra^+ ; McDonnell *et al.*, 1983). Indeed, pUW3626 has previously been reported to be Tra^+ (Goering and Ruff, 1983).

All three plasmids were shown to encode $\text{Gm}^r\text{Tm}^r\text{Km}^r$, a phenotype which in the case of pSK41 and pSH6 is mediated by AAC (6') and APH (2'') activities (Scott *et al.*, 1978; Vogel *et al.*, 1978). Plasmids pSK41 and pUW3626 also encoded resistance to neomycin (Nm^r) and paromomycin (Pm^r), a phenotype presumed to be mediated by the AAD(4') activity of pSK41 (Scott *et al.*, 1978), and to ethidium bromide and quaternary ammonium compounds. Plasmid pUW3626 additionally mediated Pc^r via a β lactamase (table I).

The minimum inhibitory concentrations (MICs)

of gentamicin, tobramycin and kanamycin were 32, 64 and 256 $\mu\text{g/ml}$, respectively, for pSK41 and pUW3626, while pSH6 gave MICs of 128, 64 and 512 $\mu\text{g/ml}$. These results compare with MICs of 32, 16 and 256 $\mu\text{g/ml}$ for the three aminoglycosides with strain SK982 containing pSK1. The MICs of ethidium bromide, benzalkonium chloride and cetyltrimethylammonium bromide were 40, 6 and 4 $\mu\text{g/ml}$, respectively, for pSK41 and pUW3626, compared with 180, 6 and 4 $\mu\text{g/ml}$ for pSK1. Unlike pSK1, plasmids pSK41, pSH6 and pUW3626 did not mediate resistance to acriflavine or trimethoprim.

Digestion with the restriction endonucleases *Eco*RI (data not presented) and *Hind*III revealed similarities amongst the North American plasmids. Plasmids pSK41 (fig. 3A, lane d) and pUW3626 (fig. 3A, lane f) possessed up to nine *Hind*III fragments of common size, while pSH6 (fig. 3A, lane e) had at least six fragments in common with the other two plasmids. All three North American plasmids possessed a *Hind*III fragment of identical electrophoretic mobility to the 2.5-kb *Hind*III fragment of pSK1 and pSK310 (fig. 3A, lanes b and c). As this fragment contains the $\text{Gm}^r\text{Tm}^r\text{Km}^r$ encoding region of Tn4001, the possibility that the same fragment exists in the North American plasmids was tested by probing the digests with the 2.5-kb *Hind*III fragment of pSK310. The probe was shown to hybridise the 2.5-kb *Hind*III fragments of each of the North American plasmids (fig. 3B, lanes d, e and f), thereby indicating that $\text{Gm}^r\text{Tm}^r\text{Km}^r$ in both Australian and North American isolates of *S. aureus* may be encoded by the same genetic determinant.

Physical maps of pSH6, pSK41 and pUW3626 were prepared by analysis of restriction digests of the three plasmids (fig. 1). The maps were aligned relative to one another and to the map of pSK1 by use of the common 2.5-kb *Hind*III fragment associated with $\text{Gm}^r\text{Tm}^r\text{Km}^r$ in each plasmid. From this comparison it became obvious that pSH6, pSK41 and pUW3626 were structurally related to each other and to the previously characterised 52.9-kb plasmid pCRG1600 (Asch *et al.*, 1984) which was also isolated in the USA (fig. 1). Use of DNA probes specific for the determinants of $\text{Tm}^r\text{Km}^r\text{Nm}^r\text{Pm}^r$, Eb^rQa^r and Pc^r , indicated that the regions associated with these resistances in pSK41 and pUW3626 are consistent with the locations of the analogous resistance determinants on pCRG1600 (fig. 1), further testifying to the relatedness of the North American plasmids (M. Gillespie, J. May and R. Skurray, unpublished data).

Comparison of the *Gm^rTm^rKm^r* encoding regions

The *Gm^rTm^rKm^r* encoding region from the plasmids isolated in the USA was subjected to more extensive analysis to determine if it comprises a transposable element such as *Tn4001*.

Restriction mapping of pSK1 has revealed the presence of recognition sites for *Hae*III within the inverted repeats of *Tn4001* that result in a unique 3.9-kb *Hae*III fragment which maps symmetrically within the transposon (fig. 1). The North American plasmids did not possess this fragment, a finding that was verified by hybridisation with the *Tn4001*-specific probe (data not presented); the 2.5-kb *Hind*III fragment of pSK310 hybridised to an 11.7-kb *Hae*III fragment of both pSK41 and pUW3626, while in the *Hae*III digest of pSH6, it hybridised with a 6.6-kb *Hae*III fragment. The respective *Hae*III fragments have been mapped on the three North American plasmids and the *Hae*III sites were found to be located well outside the positions expected if they were present in *Tn4001*-like inverted repeats (fig. 1). Further evidence which suggests that the *Gm^rTm^rKm^r* region of the North American plasmids differs from *Tn4001* resulted from the restriction mapping of the plasmids with *Bgl*III. The North American plasmids possessed symmetrical *Bgl*III sites, on either side of the *Gm^rTm^rKm^r* encoding region, which produced a unique 3.15-kb *Bgl*III fragment, whereas no *Bgl*III sites were shown to be present in *Tn4001* (fig. 1).

To investigate if there was any homology between the inverted repeats of *Tn4001* and the flanking sequences of the North American *Gm^rTm^rKm^r* determinant, *Hind*III digests of the North American plasmids were probed with a 0.7-kb *Hae*III-*Hind*III fragment derived from the inverted repeat of *Tn4001* (data not presented). The 0.5- and

1.9-kb *Hind*III fragments which, in these three plasmids, map on either side of the 2.5-kb *Gm^rTm^rKm^r*-associated *Hind*III fragment (fig. 1), were found to hybridise with this probe, suggesting that sequences homologous to the inverted repeat of *Tn4001* are present in the *Gm^rTm^rKm^r* region of these North American plasmids.

Electronmicroscopy analysis of the *Gm^rTm^rKm^r* plasmids

Because restriction mapping and hybridisation with the 2.5-kb *Hind*III fragment of pSK310 had located the *Gm^rTm^rKm^r* determinant to the second largest *Eco*RI fragment of pSK1 and the largest *Eco*RI fragments of each of the North American plasmids, we examined self-annealed molecules of the relevant *Eco*RI fragments for the production of secondary structures by electronmicroscopy.

Molecules of self-annealed, *Eco*RI cleaved pSK1 plasmid DNA demonstrated the characteristic stem and loop structure of *Tn4001* (fig. 4A). Measurements of four molecules determined that the 4.74-kb transposon comprised a 1.37-kb double-stranded stem and a 2.0-kb single-stranded loop (table II). Examples of self-annealed molecules formed from pSK41 and pSH6 DNA are illustrated in figs. 4B and 4C, respectively. Such molecules exhibited a long single-stranded region of DNA from end (a) and a shorter single-stranded segment of DNA from end (b), interrupted by two stem and loop structures, marked (c) and (d), separated by a short single stranded link. The average contour length measurements of self-annealed molecules of pSK41, pSH6 and pUW3626 are presented in table II. These measurements indicate that the single-stranded loop marked (c) is comparable in size to the loop formed by *Tn4001*, but that the length of

Table II. Contour length measurements of self-annealed DNA from *Gm^rTm^rKm^r* plasmids

Feature	Plasmid size*			
	pSK1	pSK41	pSH6	pUW3626
SS end (a) to DS stem (c)	2.34 ± 0.06	11.33 ± 0.27	9.85 ± 0.88	10.63 ± 0.78
DS stem (c)	1.37 ± 0.05	0.76 ± 0.03	0.69 ± 0.11	0.73 ± 0.13
SS loop (c)	2.00 ± 0.14	2.09 ± 0.17	2.33 ± 0.12	2.05 ± 0.25
SS link between (c) and (d)	—	0.15	0.02	0.15
DS stem (d)	—	0.57 ± 0.08	0.55 ± 0.04	0.65 ± 0.09
SS loop (d)	—	0.20	0.20	0.20
DS stem (d) to SS end (b)	2.29 ± 0.06	1.58 ± 0.24	1.62 ± 0.22	1.61 ± 0.25

SS, single-stranded DNA; DS, double-stranded DNA; (a), (b), (c), (d) refer to the features indicated in fig. 4.

* Measurements are in kb; at least four molecules were measured for each feature.

Fig. 4. Electronmicrographs of A, pSK1; B, pSK41; C, pSH6 plasmid DNA which had been cleaved with *EcoRI* and treated to enhance the formation of secondary structures. Tracings of each molecule are located at right for easier interpretation. The ends of each molecule are denoted by (a) and (b). The arrow marked (c) indicates a structure with a large single-stranded loop; the arrow marked (d) indicates a structure with a small single-stranded loop.

the associated double-stranded stem is considerably shorter than that seen for Tn4001.

The stem and loop structures have been mapped by relating their contour measurements to the restriction maps of the Gm^rTm^rKm^r plasmids and, as expected, correspond to the position of the common 2.5-kb *Hind*III fragment associated with aminoglycoside resistance (fig. 1). The additional stem and loop structures (d) found on the North American plasmids have also been located on these physical maps, but there is no indication of their possible role or relationship with the Gm^rTm^rKm^r determinant, which maps immediately to their left (fig. 1).

Discussion

Nosocomial infections caused by strains of *S. aureus* resistant to gentamicin have been reported with increasing frequency over the past decade. Resistance has been correlated with the presence of plasmids in isolates from the USA (Wood *et al.*, 1977; Jaffe *et al.*, 1982; Gray *et al.*, 1983), Europe (Soussy *et al.*, 1975; Naidoo *et al.*, 1983) and Japan (Ubukata *et al.*, 1984), although in some instances a chromosomal locus seems more likely (El Solh *et al.*, 1981; Kayser *et al.*, 1981; Dowd *et al.*, 1983). In strains of *S. aureus* isolated from Australian hospitals, Gm^rTm^rKm^r can be encoded on plasmids or the chromosome (Lyon *et al.*, 1983; Townsend *et al.*, 1983; Gillespie *et al.*, 1984), and we have demonstrated that the genetic determinant for this resistance is carried by a transposon designated Tn4001 (Lyon *et al.*, 1984b).

Restriction mapping of several Gm^rTm^rKm^r plasmids, such as pSH8 (McDonnell *et al.*, 1983), a 45-kb relative of pSH6 that is structurally similar to pSK41, and pG02 (Archer *et al.*, 1985), a 50-kb self-transmissible plasmid isolated from *S. epidermidis*, has indicated that resistance to these aminoglycosides is associated with the presence of a *Hind*III fragment of c. 2.4-kb in size. Gm^rTm^rKm^r plasmids isolated from European sources exhibited similar *Hind*III fragments (Naidoo *et al.*, 1983), and the gene(s) for the AAC(6') and APH(2'') that mediate Gm^rTm^rKm^r has reportedly been cloned into *E. coli* and *Bacillus subtilis* on a 2.3-kb *Hind*III fragment from a plasmid isolated in the USA (Gray *et al.*, 1983). The demonstration that a 2.5-kb *Hind*III fragment which maps symmetrically within the transposon Tn4001 hybridises with fragments of identical size in *Hind*III digests of three different North American plasmids, implies that the determinants of Gm^rTm^rKm^r carried by Australian and

North American plasmids are homologous. Like the Australian determinant, the Gm^rTm^rKm^r determinant present on North American plasmids is flanked by inverted repeat sequences. Although these inverted repeats were some 0.65-kb shorter than the repeats of Tn4001, they nonetheless shared homology on the basis of DNA-DNA hybridisation, suggesting the possibility of a common evolutionary pathway for Tn4001 and these "Tn4001-like" elements on the North American plasmids.

The transposition of Tn4001 to multiple plasmid and chromosomal sites has been achieved *in vitro*, and transposon-specific sequences have been detected by DNA-DNA hybridisation on the chromosomes of Gm^rTm^rKm^r *S. aureus* isolated from Australian hospitals (M. Gillespie, B. Lyon and R. Skurray, unpublished observation). Despite the fact that chromosomal Gm^rTm^rKm^r has not been reported in the USA, an indication that Gm^rTm^rKm^r in strains of *S. aureus* from the USA is, or once was, transposable, is seen in the carriage of a determinant for AAC(6') and APH(2'') activities by plasmids of different incompatibility groups (Gray *et al.*, 1983). Attempts in this laboratory to demonstrate the transposition of the Gm^rTm^rKm^r determinant from the North American plasmids by techniques used to identify Tn4001 (Lyon *et al.*, 1984b) have been unsuccessful, however, and it is possible that the inverted repeats with which this determinant is associated do not encode the necessary functions for transposition.

Although the Australian and North American Gm^rTm^rKm^r plasmids appear to be unrelated, it is significant that both plasmid families carry similar resistance determinants. Apart from mediating the common phenotype of Gm^rTm^rKm^r, members of each family have been shown to carry determinants for Eb^rQa^r and to code for a β lactamase. Hybridisation analysis has demonstrated that the Eb^rQa^r determinants of the Australian and USA plasmids are distinct (Gillespie and Skurray, 1986), although similar comparisons have shown that the β lactamase determinants share substantial homology. From this evidence, one might conclude that, during the last decade, strains of *S. aureus* in Australian and USA hospitals faced very similar selection pressures, but that quite diverse plasmid vectors were chosen to disseminate the resistance genes required for survival.

We thank Jan Tennent for helpful discussions and Linda Messerotti and Khim Hoe for skilful technical assistance. This work was supported by a Project Grant from the National Health and Medical Research Council (Australia).

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