ANTIBIOTIC RESISTANCE IN *STAPHYLOCOCCUS AUREUS* ISOLATED AT AN AUSTRALIAN HOSPITAL BETWEEN 1946 AND 1981

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SUMMARY. A total of 517 strains of *Staphylococcus aureus* isolated at a hospital in Melbourne, Australia between 1946 and 1981 was examined for resistance to a range of antimicrobial agents and for the presence of plasmid DNA. The use of mixed-culture transfer and restriction endonuclease analysis showed that the determinants for resistance to penicillin and to the heavy metals were carried by several related plasmids of $(15\text{--}23) \times 10^6$ mol. wt, and that tetracycline resistance was encoded on a plasmid of 2.8×10^6 mol. wt in strains isolated before 1970. These phenotypes were chromosomally encoded in the majority of strains isolated thereafter. Resistance to chloramphenicol throughout the study period was plasmid-mediated. Of five aminoglycoside-resistance phenotypes, one was plasmid-mediated and three were chromosomally encoded. The remaining phenotype, specifying low-level gentamicin resistance, was found to be located on the chromosome of early isolates, but in later strains was borne by an 18×10^6 mol. wt plasmid which also encoded resistance to quaternary ammonium compounds.

INTRODUCTION

Multiresistant *Staphylococcus aureus* have been important pathogens in Melbourne hospitals during recent years (Pavillard *et al.*, 1982). Molecular analysis of plasmid DNA isolated from such strains of *S. aureus* has identified separate plasmids encoding resistance to chloramphenicol and gentamicin (Lyon, May and Skurray, 1983; Townsend, Grubb and Ashdown, 1983a; Lyon *et al.*, 1984a; Tennent, May and Skurray, 1984), although the latter resistance is chromosomally encoded in some strains. Resistance to erythromycin, kanamycin-neomycin, penicillin, streptomycin, tetracycline, cadmium and mercury is usually mediated by determinants carried by the chromosome in these multiresistant *S. aureus* strains (Lyon *et al.*, 1983, 1984a). In contrast, a study of methicillin-resistant *S. aureus* strains isolated between 1969 and 1981 from a Melbourne hospital (Gillespie, May and Skurray, 1984), revealed that the determinants for penicillinase production and resistance to the heavy metals and tetracycline were plasmid-borne in strains isolated in 1969.

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Although the bacteriophage types of Australian strains of *S. aureus* isolated from the late 1940s to mid 1970s have been well documented (Rountree, Barbour and Thomson, 1951; Rountree, 1953 and 1978; Rountree and Beard, 1958 and 1965; Rountree and Vickery, 1973), little information is available about the plasmids present in *S. aureus* strains before the onset of methicillin resistance. This paper reviews the antibiotic-resistance patterns and plasmid-DNA profiles of clinical isolates of *S. aureus* isolated in a Melbourne hospital during four decades of antibiotic therapy.

MATERIALS AND METHODS

Staphylococcus aureus strains. Clinical isolates of *S. aureus* were collected, identified and stored vacuum-dried in ampoules at the Bacteriology Department, Alfred Hospital, Melbourne and subsequently made available to this laboratory.

S. aureus strains RN2424 and RN2442, carrying the plasmids pT181 and pE194 (Novick *et al.*, 1980) respectively, were kindly provided by R. P. Novick (Public Health Research Institute of the City of New York). Strain SK982, the restriction-deficient recipient strain employed in mixed culture transfers, carries chromosomal resistance to novobiocin and rifampicin and was constructed in this laboratory (Lyon, May and Skurray, 1984b).

General procedures. L-broth and L-agar media, methods for determination of penicillinase production, minimum inhibitory concentrations, antibiotic and heavy metal resistance and plasmid elimination procedures were as described previously (Lyon *et al.*, 1983; Gillespie *et al.*, 1984). Resistance to the quaternary ammonium compounds benzalkonium chloride (Sigma Chemical Company, St Louis, MO 63178 USA) and cetyltrimethylammonium bromide (BDH Chemicals Ltd, Poole, England) was tested in L-agar at concentrations of 4 and 2 mg/L, respectively. Mixed culture transfer by the method of McDonnell, Sweeney and Cohen (1983) was used to transfer resistance to strain SK982.

Plasmid DNA isolation and restriction endonuclease analysis. DNA was isolated by a rapid procedure and analysed by electrophoresis on 1.0% Tris-acetate-EDTA buffered agarose horizontal gels with standard plasmids of known mol. wt as previously described (Lyon *et al.*, 1983). Preparation of caesium chloride-ethidium bromide purified plasmid DNA and restriction endonuclease analysis with *Eco*RI (Boehringer-Mannheim Australia Pty Ltd, North Ryde, 2113), *Hind*III, *Mbo*I and *Taq*I (New England Biolabs, Beverly, MA 01915, USA) were as reported previously (Lyon *et al.*, 1983).

RESULTS

Characteristics of S. aureus isolates

The percentages of isolates resistant to various antimicrobial agents in each year of the study are presented in table I; < 6% of the total were susceptible to all agents tested. The majority were resistant to penicillin, streptomycin, arsenate, arsenite, cadmium and mercury; resistance to rifampicin and vancomycin was not encountered. During the late 1940s, penicillin- and streptomycin-resistant *S. aureus* were noted and by early 1951 tetracycline or chloramphenicol resistance or both in conjunction with penicillin, streptomycin, arsenate, arsenite, cadmium and mercury resistance was encountered in some isolates. It is interesting to note that resistance to fusidic acid was detected in this period well before clinical use of this agent. Kanamycin- and neomycin-resistant (K^N_R) strains were first observed in 1965, with some of these strains being additionally resistant to tobramycin (TK^N_R); the latter phenotype was not encountered again until 1980. Methicillin resistance was also first observed at this hospital in 1965.

Minimum inhibitory concentrations (MIC) of aminoglycoside antibiotics for these

TABLE I
Antibiotic susceptibilities of S. aureus

Year of isolation	Number of isolates	Percentage of sensitive isolates	Percentage of isolates resistant to																
			Pc	Mc	Sm	Tc	Em	Cl	Cm	Gm	Tm	Km	Nm	Ak	Fa	Asa	Asi	Cd	Hg
1946	7	0	0	0	14	0	0	0	0	0	0	0	0	0	0	57	57	85	14
1947	22	27	13	0	5	0	0	0	0	0	0	0	0	0	5	55	55	73	37
1948	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	100	0
1949	6	0	83	0	33	0	0	0	0	0	0	0	0	0	0	17	17	100	0
1950	10	10	20	0	40	0	0	0	0	0	0	0	0	0	0	90	90	80	60
1951	117	6	36	0	58	3	0	0	2	0	0	0	0	0	3	60	60	85	50
1952	129	7	47	0	53	9	0	0	9	0	0	0	0	0	2	70	70	85	83
1953	17	0	41	0	53	6	0	0	6	0	0	0	0	0	0	53	53	83	35
1954	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
1957	3	0	0	0	67	0	0	0	0	0	0	0	0	0	0	67	67	67	0
1960	9	11	67	0	44	55	0	0	0	0	0	0	0	0	0	67	67	78	78
1961	5	0	60	0	40	40	0	0	20	0	0	0	0	0	0	100	100	100	100
1962	8	63	0	0	13	13	13	0	13	0	0	0	0	0	0	13	13	25	13
1963	1	0	100	0	100	100	0	0	0	0	0	0	0	0	0	100	100	100	0
1964	2	0	50	0	0	0	0	0	0	0	0	0	0	0	0	50	50	100	50
1965	116	1	86	6	73	78	64	10	18	0	4	22	22	0	0	84	84	91	69
1966	20	0	85	0	95	85	70	30	20	0	0	90	90	0	0	70	70	80	65
1967	26	0	96	19	81	23	8	8	12	0	0	8	8	0	0	89	89	100	100
1968	4	0	100	0	75	75	0	0	25	0	0	0	0	0	0	50	50	75	25
1971	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	100	0
1973	2	0	100	50	100	50	50	50	0	0	0	50	50	0	0	50	50	100	100
1976	3	0	100	100	100	100	100	100	0	100	100	100	100	0	0	0	0	0	0
1980	1	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
1981	6	0	100	83	83	100	83	83	50	50	50	50	17	17	0	0	0	67	50

Pc, penicillin; Mc, methicillin; Sm, streptomycin; Tc, tetracycline; Em, erythromycin; Cl, clindamycin; Cm, chloramphenicol; Gm, gentamicin; Tm, tobramycin; Km, kanamycin; Nm, neomycin; Ak, amikacin; Fa, fusidic acid; Asa, arsenate; Asi, arsenite; Cd, cadmium; Hg, mercury.

strains revealed two distinct levels of gentamicin resistance. Strains resistant to gentamicin, tobramycin, kanamycin and neomycin (GTKN^R), such as the 1976 isolates, or to gentamicin, tobramycin and kanamycin (GTK^R) displayed low-level gentamicin resistance (128 mg/L). Only one isolate in this collection, SK460 from 1981, displayed high-level gentamicin resistance (>4096 mg/L) and was additionally resistant to tobramycin, kanamycin, neomycin and amikacin (GTKNA^R); SK460 has been described previously (Lyon *et al.*, 1984a). The aminoglycoside MICs for GTK^R, GTKN^R and GTKNA^R strains in this study were equivalent to those displayed by strains of similar phenotypes isolated from another Melbourne hospital (Gillespie *et al.*, 1984). The MICs of tobramycin, kanamycin and neomycin for TKN^R strains were 64, 64 and 128 mg/L, respectively.

Among the strains that were bacteriophage typed, group I, II, III and mixed group I/III lytic patterns were represented; the majority of strains were either group III or non-typable. The bacteriophage types and antibiotic-resistance phenotypes of 16 selected isolates from the period 1947 to 1981 are presented in table II.

Plasmid composition of S. aureus and location of resistance determinants

A total of 206 isolates was screened for the presence of plasmid DNA; fig. 1 shows the agarose-gel electrophoresis of DNA isolated from the representative strains in table II. The use of mixed culture transfer and plasmid elimination procedures permitted the identification of a number of plasmid-encoded phenotypes. The

FIG. 1.—Agarose gel (1.0% w/v) electrophoresis of rapidly isolated *S. aureus* DNA. The chromosomal band is labelled Chr. Lanes contain: (a) molecular weight standards with sizes (10^6) shown on the left; (b) SK1011; (c) SK1020; (d) SK1027; (e) SK1073; (f) SK1215; (g) SK1064; (h) SK1373; (i) SK1396; (j) SK1499; (k) SK1529; (l) SK1585; (m) SK 1582; (n) SK1597; (o) SK462; (p) SK460; and (q) SK1592. Characteristics of these strains are presented in table II; their order across the gel corresponds to that down the table.

plasmids detected in this study, along with the resistance phenotypes which they confer and their sizes, are summarised in table III.

The plasmids pSK60 (SK1011), pSK75 (SK1073), pSK79 (SK1215), pSK74 (SK1064), pSK76 (SK1373) and pSK80 (SK1529) (fig. 1, lanes b, e, f, g, h and k) encode resistance to the heavy metals arsenate, arsenite, cadmium and mercury and carry the determinants for penicillinase production. Plasmids pSK64 (SK1020) and pSK59 (SK1396) (fig. 1, lanes c and i), also mediate resistance to these four heavy metals but not to penicillin, while the plasmids pSK67 (SK1027; fig. 1, lane d) and

TABLE II
Characteristics of selected S. aureus strains

Year of isolation	Strain number	Resistance to*	Penicillinase production	Bacteriophage type†
1947	SK 1011	Pc, Asa, Asi, Cd, Hg	+	83A
1947	SK 1020	Asa, Asi, Cd, Hg	—	52/52A/79/80/81/6/42E/47/53/54/75/77/83A/85
1949	SK 1027	Pc, Cd	+	42E/47/53/54/75/77/83A
1951	SK 1073	Pc, Asa, Asi, Cd, Hg	+	75/77/85
1953	SK 1215	Pc, Sm, Tc, Asa, Asi, Cd, Hg	+	6/47/85
1953	SK 1064	Pc, Sm, Tc, Asa, Asi, Cd, Hg	+	53/54/75/77
1960	SK 1373	Pc, Sm, Cm, Asa, Asi, Cd, Hg	+	80/81
1965	SK 1396	Sm, Tc, Em, Cm, Asa, Asi, Cd, Hg	—	42E/47/53/54/75/77/84
1965	SK 1499	Pc, Sm, Tm, Km, Nm, Cd	+	95; 100 RTD
1965	SK 1529	Pc, Mc, Sm, Tc, Em, Asa, Asi, Cd, Hg	+	77/84/85; 100 RTD
1973	SK 1585	Pc, Mc, Sm, Tc, Em, Cl, Km, Nm, Cd, Hg	+	NT
1976	SK 1582	Pc, Mc, Sm, Tc, Em, Cl, Gm, Tm, Km, Nm	+	NT
1980	SK 1597	Tm, Km, Nm	—	47
1981	SK 462	Pc, Mc, Sm, Tc, Em, Cl, Cm, Gm, Tm, Km, Cd, Hg, Eb, Qa	+	83A/85
1981	SK 460	Pc, Mc, Sm, Tc, Em, Cl, Cm, Gm, Tm, Km, Nm, Ak, Cd, Hg, Eb, Qa	+	83A; 100 RTD
1981	SK 1592	Pc, Sm, Tc, Cm, Cd	+	53/54/85/90

*Abbreviations as in table 1, with the addition of Eb=ethidium bromide; Qa=quaternary ammonium compounds.

†Unless otherwise indicated, bacteriophage types were determined at routine test dilution (RTD); NT = not typable at either RTD or 100 RTD.

TABLE III
Properties of plasmids detected in S. aureus

Plasmid	Isolate no.	Resistance phenotype	Size (mol. wt $\times 10^6$)	Year detected
pSK 1	SK 462	Gm ^R Tm ^R Km ^R Eb ^R Qa ^R	18.0	1981
pSK 2	SK 462	Cm ^R	3.0	1981
pSK 7	SK 460	Eb ^R Qa ^R	15.0	1981
pSK 50	SK 1597	Tm ^R Km ^R Nm ^R	3.0	1980
pSK 52	SK 1215	Tc ^R	2.8	1953
pSK 59	SK 1396	Asa ^R Asi ^R Cd ^R Hg ^R	18.9	1965
pSK 60	SK 1011	Pc ^R Asa ^R Asi ^R Cd ^R Hg ^R	22.7	1947
pSK 61	SK 1396	Em ^R	2.4	1965
pSK 64	SK 1020	Asa ^R Asi ^R Cd ^R Hg ^R	22.5	1947
pSK 67	SK 1027	Pc ^R Cd ^R	17.0	1949
pSK 68	SK 1592	Cm ^R Sm ^R	3.0	1981
pSK 70	SK 1064	Cm ^R	3.0	1953
pSK 71	SK 1396	Cm ^R	3.0	1965
pSK 74	SK 1064	Pc ^R Asa ^R Asi ^R Cd ^R Hg ^R	21.7	1953
pSK 75	SK 1073	Pc ^R Asa ^R Asi ^R Cd ^R Hg ^R	21.5	1951
pSK 76	SK 1373	Pc ^R Asa ^R Asi ^R Cd ^R Hg ^R	15.1	1960
pSK 77	SK 1499	Pc ^R Cd ^R	17.5	1965
pSK 79	SK 1215	Pc ^R Asa ^R Asi ^R Cd ^R Hg ^R	24.7	1953
pSK 80	SK 1529	Pc ^R Asa ^R Asi ^R Cd ^R Hg ^R	20.8	1965

Abbreviations as in tables I and II.

pSK77 (SK1499; fig. 1, lane j) encode resistance only to penicillin and to the heavy metal cadmium.

Digestion of these heavy metal-resistance plasmids with *Eco*RI, revealed a close relatedness between pSK60, pSK64, pSK75, pSK79, pSK74, pSK59 and pSK80 (fig. 2, lanes b-h); each possessed common *Eco*RI fragments of 6.6, 2.5, 2.3 and 1.0 kb. On this basis, these plasmids were assigned to the α family of penicillinase plasmids, as described by Shalita, Murphy and Novick (1980). Of similar phenotype, pSK76 (fig. 2, lane i) belongs to the γ family since it lacks the 2.5 and 1.0 kb *Eco*RI fragments (Shalita *et al.*, 1980). The remaining penicillinase plasmids pSK77 and pSK67 (fig. 2, lanes j and k) appear unrelated to either of these families and resemble members of the 'orphan'

FIG. 2.—Agarose gel (1.0% w/v) electrophoresis of purified *S. aureus* heavy metal resistance- and penicillinase-plasmid DNA cleaved with the restriction endonuclease *Eco*RI. Lanes contain: (a) lambda phage DNA cleaved with *Hind* III; (fragment sizes in kilobases (kb) taken from Daniels *et al.*, 1983 are shown on the left); (b) pSK60; (c) pSK64; (d) pSK75; (e) pSK79; (f) pSK74; (g) pSK59; (h) pSK80; (i) pSK76; (j) pSK77; and (k) pSK67. Properties of these plasmids are presented in table III.

family of penicillinase plasmids which, like pSK67 and pSK77, mediate resistance only to penicillin and cadmium (Shalita *et al.*, 1980).

Tetracycline resistance (Tc^R) in the isolates SK 1215, SK 1373, SK 1396 and SK 1592 (fig. 1, lanes f, h, i and q) was shown by mixed culture transfer and restriction endonuclease analysis to be mediated by a common 2.8×10^6 mol. wt plasmid, pSK52. The presence of this plasmid was inferred in the Tc^R isolate SK 1529 (fig. 1, lane k) which possesses extrachromosomal DNA of the same mol. wt, although the Tc^R phenotype could not be transferred or eliminated from this strain. On the basis of restriction endonuclease fingerprinting with *Hind* III and *Mbo*I, which generate three and four fragments, respectively, the Tc^R -plasmid pSK 52 (fig. 3A, lanes b and d) was found to be indistinguishable from pT181 (fig. 3A, lanes a and c), a *S. aureus* tetracycline resistance plasmid of known nucleotide sequence (Khan and Novick, 1983). The Tc^R isolates SK 1585, SK 1582, SK 462 and SK 460 (fig. 1, lanes l, m, o and p) did not contain pSK52, and Tc^R could not be associated with plasmid DNA of a different mol. wt; therefore, in these strains, Tc^R was assumed to be chromosomally encoded.

Chloramphenicol resistance (Cm^R) was found to be mediated by plasmids of approximately 3×10^6 mol. wt, such as pSK2, pSK68, pSK70 and pSK71 (fig. 1, lanes o, q, g and i), which were distinguished on the basis of restriction endonuclease analysis (M. Gillespie, J. May and R. Skurray, unpublished data). The Cm^R -plasmid pSK68 also specified streptomycin resistance (Sm^R) and was the only plasmid encountered to which Sm^R could be assigned. The Sm^R isolate SK 1373 (fig. 1, lane h) possessed only the penicillinase plasmid pSK 76 and the Tc^R -plasmid pSK 52; plasmid free derivatives of this strain retained Sm^R , suggesting chromosomal encodement of this determinant. Similar results obtained with other strains are in accord with a chromosomal locus for this determinant.

Erythromycin resistance (Em^R) in SK 1396 (fig. 1, lane i) was mediated by the 2.4×10^6 mol. wt plasmid pSK61. This plasmid was compared with pE194, an Em^R plasmid of similar mol. wt, the nucleotide sequence of which is known (Horinouchi and Weisblum, 1982). Digestion of both plasmids with *Mbo*I and *Taq*I showed that pSK61 (fig. 3B, lanes c and e) differs from pE194 (fig. 3B, lanes b and d). Other Em^R isolates, such as SK 1529, SK 1585, SK 1582, SK 462 and SK 460 (fig. 1, lanes k, l, m, o and p) did not possess extrachromosomal DNA of this mol. wt and no association of this phenotype could be made with any plasmids in these strains. The finding that Em^R transcipts from mixed culture transfer experiments between SK 1529 and SK 982 lacked extrachromosomal DNA provides support for a chromosomal locus for this determinant as has previously been suggested with other Melbourne isolates (Lyon *et al.*, 1983; Gillespie *et al.*, 1984).

KN^R strains isolated in 1965 were of phage type 84/85 at RTD and showed a plasmid-DNA profile similar to the KN^S isolate SK 1529 (fig. 1, lane k), which possessed penicillinase, Tc^R and cryptic (1×10^6 mol. wt) plasmids. Resistance to these aminoglycosides could not be associated with any plasmid in these strains nor in subsequent KN^R isolates. In contrast, TKN^R isolates, such as SK 1499 and SK 1597 (fig. 1, lanes j and n) carry a 3×10^6 mol. wt plasmid which encodes this resistance phenotype; this plasmid in the latter strain is designated pSK 50 and its identity to the plasmid seen in SK 1499 is yet to be established.

In the 1976 gentamicin-resistant isolates as first described by Perceval, McLean and

FIG. 3.—A. Agarose gel (1.2% w/v) electrophoresis of tetracycline-resistance plasmids from *S. aureus*. Lanes contain: (a) and (c) pT181; (b) and (d) pSK52; (e) lambda phage DNA cleaved with *Eco*RI and *Hind* III with fragment sizes in kb taken from Daniels *et al.* (1983) shown on the right. Lanes a and b contain *Hind* III digested DNA, whereas lanes c and d contain DNA digested with *Mbo*I. B. Agarose gel (1.2% w/v) electrophoresis of erythromycin-resistance plasmids from *S. aureus*. Lanes contain: (a) lambda phage DNA cleaved with *Eco*RI and *Hind* III with fragment sizes in kb shown on the left; (b) and (d) pE194; (c) and (e) pSK61. Lanes b and c contain *Mbo*I digested DNA; lanes d and e contain DNA digested with *Taq*I.

Wellington (1976), such as SK1582 (fig. 1, lane m), low-level gentamicin resistance was found to be chromosomally determined, as was the high-level gentamicin resistance of strain SK460 (fig. 1, lane p and Lyon *et al.*, 1984a). However, strain SK462 (fig. 1, lane o) possesses the plasmid pSK1 which mediates the low-level GTK^R phenotype (Lyon *et al.*, 1983) in this strain as well as resistance to ethidium bromide (Eb) and to quaternary ammonium compounds (Qa), such as benzalkonium chloride and cetyltrimethylammonium bromide (Lyon *et al.*, 1984b). The plasmid pSK7 present in strain SK460 (fig.

1, lane p) is related to pSK1 (Lyon *et al.*, 1984a) and encodes resistance to quaternary ammonium compounds and to ethidium bromide but not to gentamicin (B. Lyon, J. May and R. Skurray, unpublished data); gentamicin-resistant strains from 1976 (e.g. SK1582, fig. 1, lane m) which lack pSK1, pSK7 or other related plasmids (Lyon *et al.*, 1984a) were susceptible to Eb and Qa.

No antibiotic resistance phenotype could be ascribed to the plasmids smaller than 1.8×10^6 mol. wt in the isolates SK1020, SK1215, SK1064, SK1396, SK1529, SK1585, SK1582, SK1597, SK462 and SK460 (fig. 1, lanes c, f, g, i, k, l, m, n, o and p).

DISCUSSION

The *S. aureus* isolates collected from this Melbourne Hospital, resemble strains recovered during the equivalent period from the Royal Prince Alfred Hospital, Sydney with respect to antibiotic resistance and bacteriophage type (Rountree *et al.*, 1951; Rountree, 1953 and 1978; Rountree and Beard, 1958 and 1965; Rountree and Vickery, 1973). At both hospitals, penicillin-resistant strains of various bacteriophage types were detected in the late 1940s. In Melbourne strains, penicillin resistance was mediated by one of two plasmids distinguishable by resistance to arsenate, arsenite and mercury and *EcoRI* restriction endonuclease patterns. These two plasmids, pSK60 and pSK67, belong to the α and 'orphan' families of penicillinase plasmids, respectively. One further type of penicillinase plasmid, pSK76, detected in the bacteriophage type 80/81 isolate SK1373, belongs to the γ family; an association of γ family penicillinase plasmids and strains of bacteriophage type 80/81 has been previously reported (Shalita *et al.*, 1980).

Before the early 1970s, penicillinase production, in association with heavy metal resistance, was mediated most commonly by closely related plasmids belonging to the α family. In strains isolated thereafter, the majority of which were methicillin-resistant, penicillinase production and resistance to cadmium and mercury were chromosomally encoded. These observations are similar to those made with methicillin-resistant strains from another Melbourne hospital (Gillespie *et al.*, 1984 and unpublished data). Likewise, tetracycline resistance, which had exclusively been plasmid-mediated since its detection in 1951, was chromosomally determined in the majority of isolates during the 1970s and 1980s. These results, coupled with changes in bacteriophage type from extended group-III lytic patterns evident in the 1960s to limited group III lytic patterns or non-typable reactions in the 1970s, suggest that isolates from the 1970s were new endemic hospital strains.

By 1950, chloramphenicol was widely used clinically and had become accepted as a promising broad spectrum antibiotic (Shaw, 1983); however, Cm^R *S. aureus* were first noted at this Melbourne hospital late in 1951. In these, and subsequent isolates, Cm^R was mediated by a variety of plasmids of 3×10^6 mol. wt. In this respect, the Cm^R determinant, which persisted on a plasmid, is unlike the determinants for resistance to tetracycline, penicillin and heavy metals all of which altered with time in their locality of encodement from plasmid to chromosome.

Erythromycin-resistant *S. aureus* were noted at this hospital during the early 1960s. In these strains, resistance was found to be encoded either on a 2.4×10^6 mol. wt plasmid distinguishable from pE194 (Horinouchi and Weisblum, 1982) or on the chromosome. In the latter strains, Em^R may be encoded on a transposon, such as

Tn554, which preferentially occupies a chromosomal site (Novick *et al.*, 1981); the transfer of chromosomally encoded Em^R during mixed culture with strain SK1529 could well be accounted for by transposition of such an element.

Neomycin was used as a prophylactic agent against staphylococcal infection during the late 1950s and early 1960s in Australian hospitals and resistance to this antibiotic, by strains of bacteriophage type 84/85, was detected at the Royal Prince Alfred Hospital, Sydney in 1965 (Rountree and Beard, 1965). Strains of this bacteriophage type which were resistant to neomycin and kanamycin were also among isolates collected from this hospital in 1965, although it should be noted that KN^S strains of the same bacteriophage type were detected (Willis, Smith and O'Connor, 1966; M. Gillespie, J. May and R. Skurray, unpublished data). The KN^R phenotype in strains throughout this study was chromosomally determined. In contrast, linked resistance to tobramycin, kanamycin and neomycin was mediated by a plasmid such as pSK50. The level of resistance that this plasmid confers is equivalent to that of a previously described *S. aureus* plasmid of similar size which encodes a nucleotidyltransferase (Le Goffic *et al.*, 1976).

Low-level gentamicin resistance was found to be chromosomally determined in 1976 isolates but to be mediated by the plasmid pSK1 in the isolates from 1981. The determinant for gentamicin resistance on this plasmid has been shown to be a transposable element, designated Tn4001; additionally this plasmid carries determinants for resistance to ethidium bromide and to quaternary ammonium compounds (Lyon *et al.*, 1984b). If the chromosomal low-level gentamicin resistance seen in the 1976 isolates was due to Tn4001, this may have transposed to a Qa^REb^R-plasmid, such as pSK7, allowing more rapid dissemination of gentamicin resistance. Strains possessing such a gentamicin- and quaternary ammonium-resistance plasmid may be endowed with a survival advantage over Qa^S isolates (Townsend *et al.*, 1983b). Such an assumption would explain the high frequency with which pSK1 and related plasmids have been encountered in Australian strains (Lyon *et al.*, 1984a).

Future studies with the strains described in this paper, and with those in a collection of methicillin-resistant *S. aureus* we have recently described (Gillespie *et al.*, 1984), should provide further insights into the molecular mechanisms whereby resistance determinants have been progressively sequestered by *S. aureus* over a period of four decades so enabling these organisms to persist as important nosocomial pathogens in environments of high antibiotic usage.

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