Typing of methicillin-resistant *Staphylococcus aureus* by antibiotic resistance phenotypes

M. T. GILLESPIE*, B. R. LYON† and R. A. SKURRAY‡

Department of Microbiology, Monash University, Clayton, Victoria 3168, Australia

Summary. The identification of new epidemic strains of methicillin-resistant *Staphylococcus aureus* is essential for rapid, effective infection control. We have developed a typing method which uses antibiotic sensitivity patterns to differentiate methicillin-resistant *S. aureus* and which is faster and more cost-effective than biochemical analysis or bacteriophage typing. Characterisation of phenotypes which are chromosomally-encoded, plasmid- or chromosomally-encoded or exclusively plasmid-mediated has enabled us to separate Australian strains of methicillin-resistant *S. aureus* into 11 classes, representatives of which were indistinguishable by bacteriophage type, or plasmid profile alone. The value of this procedure is thus clearly shown.

Introduction

With the increased spread of methicillin-resistant *Staphylococcus aureus* in Australia, Britain and the USA (Lyon and Skurray, 1987), microbiologists have sought typing systems which distinguish new epidemic strains from endemic ones. New *S. aureus* strains are usually detected by their different pattern of antibiotic resistance. The spread of these strains may be monitored by antibiograms, biochemical tests or by bacteriophage typing. Many methicillin-resistant *S. aureus* strains are not typable by bacteriophages of the International Typing Set; supplementary bacteriophages have been isolated to distinguish these non-typable strains of *S. aureus* (Vickery *et al.*, 1983; Richardson *et al.*, 1988).

Recently, electrophoretic separation of cellular proteins has been employed to differentiate methicillin-resistant *S. aureus* (Gaston *et al.*, 1988). Coomassie Blue staining of whole-cell extracts has not been reproducible or has failed to distinguish *S. aureus* strains which could be differentiated by their susceptibility to bacteriophages of the International Set. In contrast, other electrophoretic methods such as Western blot analysis of culture supernate and analysis of cellular proteins with ³⁵S-

methionine have distinguished outbreak strains from epidemic strains (Krikler *et al.*, 1986; Stephenson *et al.*, 1986; Gaston *et al.*, 1988; Lee and Burnie, 1988).

Plasmid typing has been used successfully to trace strains prevalent in the USA (Kozarsky *et al.*, 1986; Rhinehart *et al.*, 1987) and in Ireland (Coleman *et al.*, 1985). We have used plasmid profiles to monitor the spread of epidemic strains of methicillin-resistant *S. aureus* throughout Australia (Lyon *et al.*, 1983, 1984a). This procedure, combined with restriction endonuclease analysis, DNA hybridisation and antibiogram results, has identified the locality of encodement (chromosome- or plasmid-mediated) of antimicrobial resistance determinants in more than 2000 strains isolated in hospitals throughout Australia. These results supported a clonal origin for Australian methicillin-resistant *S. aureus* since isolates had common chromosomally-encoded resistance phenotypes but differed in their plasmid content. The typing scheme described here was adapted to distinguish strains of methicillin-resistant *S. aureus* on the basis of their plasmid-encoded phenotypes and will differentiate current strains of epidemic methicillin-resistant *S. aureus* (EMRSA) from other methicillin-resistant strains detected throughout the late 1960s.

Materials and methods

Strains of Staphylococcus aureus

Bacterial strains were obtained from Australian hos-

Received 30 Jan. 1989; revised version accepted 3 Aug. 1989.

* Present address: St Vincent's Institute of Medical Research, 9 Princes Street, Fitzroy, Victoria 3065, Australia.

† Present address: C.S.I.R.O. Division of Plant Industry, Canberra, A.C.T. 2601, Australia.

‡ Correspondence should be sent to Dr R. A. Skurray, Department of Microbiology, Monash University, Clayton, Victoria 3168, Australia.

pitals between 1946 and 1982 and many have undergone preliminary analysis (Lyon *et al.*, 1983, 1984a; Gillespie *et al.*, 1985, 1986a); relevant strains and plasmids are listed in tables I and II.

Antibiotic sensitivity testing

L-broth and L-agar media, and methods for the determination of antimicrobial and heavy metal resistance and penicillinase production by use of multipoint inoculation were described by Lyon *et al.* (1983, 1984a). An overnight bacterial suspension was diluted so that approximately 10^4 cfu/inoculum site were delivered on to the surface of an L-agar plate. Plates were incubated at 37°C for 24 h. Antimicrobial agents were incorporated into L-broth and L-agar at the following concentrations: acriflavine (Ac; May and Baker, Australia), 100 µg/ml (low-level) and 250 µg/ml (high-level); amikacin (Ak; Bristol Laboratories, USA), 8 µg/ml; ampicillin (Ap; CSL, Australia), 10 µg/ml; benzalkonium chloride (Bc; Sigma Chemical Co., USA), 2 µg/ml; cadmium nitrate (Cd), 5×10^{-5} M; cetyltrimethylammonium bromide (Ct; BDH Chemicals, England), 1 µg/ml; chloramphenicol (Cm; Boehringer Mannheim, West Germany), 16 µg/ml; clindamycin (Cl; The Upjohn Co, USA), 10 µg/ml; 4'-diamidinodiphenylamine dihydrochloride (Dd; May and Baker, Australia), 50 µg/ml; erythromycin (Em; Abbott Laboratories, Australia), 5 µg/ml; ethidium bromide (Eb; Sigma Chemical Co., USA), 20 µg/ml (low-level) and 100 µg/ml (high-level); fusidic acid (Fa; Leo Pharma-

ceuticals, Denmark), 1 µg/ml; gentamicin (Gm; Schering Corp., USA), 4 µg/ml (low-level) and 500 µg/ml (high-level); kanamycin (Km; Bristol Laboratories, USA), 10 µg/ml (low-level) and 500 µg/ml (high-level); mercuric nitrate (Hg), 1×10^{-5} M; methicillin (Mc; Beecham Australia), 8 µg/ml; neomycin (Nm; The Upjohn Co., USA), 10 µg/ml (low-level) and 500 µg/ml (high-level); paromomycin (Pm; Warner Lambert Co., USA), 8 µg/ml; penicillin G (Pc, Glaxo, Australia) 10 µg/ml; propamidine isethionate (Pi; May and Baker, Australia), 50 µg/ml; rifampicin (Rf; Sigma Chemical Co., USA), 1 µg/ml; sodium arsenate (Asa), 7×10^{-5} M; sodium arsenite (Asi), 4×10^{-4} M; spectinomycin (Sp; The Upjohn Co., USA), 50 µg/ml; streptomycin (Sm; Glaxo, Australia), 10 µg/ml; tetracycline (Tc; Sigma Chemical Co., USA), 5 µg/ml; tobramycin (Tm; Eli Lilly & Co., USA), 4 µg/ml (low-level) and 500 µg/ml (high-level); trimethoprim (Tp; Sigma Chemical Co., USA), 25 µg/ml (low-level) and 500 µg/ml (high-level); vancomycin (Vm; Eli Lilly & Co., USA), 4 µg/ml.

Appropriate care was taken when handling compounds such as cadmium nitrate, mercuric nitrate, sodium arsenate and sodium nitrite as well as the potent mutagen ethidium bromide; protective gloves were worn when handling these agents and they were used only in an exhaust-ventilated safety cabinet. In addition, these agents had to be added to molten agar at a temperature low enough to minimise vaporisation (i.e., 50°C). Procedures for the detoxification of ethidium bromide have been described by Lunn and Sansome (1987).

Table I. Characteristics of *S. aureus* plasmids

Plasmid	Resistance to*	Size (kb)	β-lactamase production	Reference
pSK1	GmTmKmAcEbQaDdPiTp	28.4	—	Gillespie <i>et al.</i> , 1987b
pSK2	Cm	4.5	—	Gillespie <i>et al.</i> , 1987b
pSK3	...	1.5	—	Lyon <i>et al.</i> , 1984a
pSK4	GmTmKmAcEbQaDdPiTpPc	35.1	+	Gillespie <i>et al.</i> , 1987b
pSK7	AcEbQaDdPiTp	23.7	—	Gillespie <i>et al.</i> , 1987b
pSK8	GmTmKmAcEbQaDdPiTpPc	35.1	+	Gillespie <i>et al.</i> , 1987b
pSK9	GmTmKmAcEbQaDdPi	25.7	—	Gillespie <i>et al.</i> , 1987b
pSK11	GmTmKmTpPc	41.8	+	Gillespie <i>et al.</i> , 1987b
pSK14	GmTmKmAcEbQaDdPi	24.4	—	Gillespie <i>et al.</i> , 1987b
pSK15	GmTmKmAcEbQaDdPiPc	31.1	+	Gillespie <i>et al.</i> , 1987b
pSK16	GmTmKmAcEbQaDdPiTpPc	35.1	+	Gillespie <i>et al.</i> , 1987b
pSK17	GmTmKmAcEbQaDdPiTpPc	36.4	+	Gillespie <i>et al.</i> , 1987b
pSK18	AcEbQaDdPi	19.7	—	Lyon <i>et al.</i> , 1984a
pSK21	AcEbQaCdHg	35.3	—	
pSK23	GmTmKmAcEbQaCdHg	38.0	—	Lyon and Skurray, 1987
pSK41	GmTmKmNmPmAkEbQa	47.8	—	Lyon <i>et al.</i> , 1987
pSK51	PcAsaAsiCdHg	29.1	+	Gillespie <i>et al.</i> , 1984
pSK52	Tc	4.4	—	Gillespie <i>et al.</i> , 1986b
pSK57	AcEbQaDdPiPcCdHg	28.8	+	Gillespie <i>et al.</i> , 1986a
pSK58	...	2.4	—	Gillespie <i>et al.</i> , 1984
pSK89	EbQa	2.4	—	Lyon and Skurray, 1987
pSK90	Cm	4.6	—	Gillespie and Skurray, 1988
pUW3626	GmTmKmNmPmAkEbQaPc	54.4	+	Lyon <i>et al.</i> , 1987

* See *Materials and methods* for abbreviations.

Table II. Characteristics of *S. aureus* strains

Strain	Resistant to*	Plasmids carried	Reference
SK20	PcMcSmTcCdHgEmClSp	pSK3	
SK413	PcMcSmTcCdHgEmClSpGmTmKmNmPmAceEbQaTp ^L	pSK21	
SK430	PcMcSmTcCdHgEmClSpAcEbQaDdPiTp ^L	pSK18	Tennent <i>et al.</i> , 1985
SK445	PcMcSmTcCdHgEmClSpGmTmKmNmPmAceEbQa	pUW3626	Lyon <i>et al.</i> , 1987
SK449	PcMcSmTcCdHgAcEbQaDdPi	pSK52, pSK57	Gillespie <i>et al.</i> , 1986a
SK456	PcMcSmTcCdHgEmClSpGmTmKmAcEbQaDdPiTp ^H	pSK4	Lyon <i>et al.</i> , 1984a
SK457	PcMcSmTcCdHgEmClSpGmTmKmTp ^L	...	Gillespie <i>et al.</i> , 1984
SK460	PcMcSmTcCdHgEmClSpGmTmKmNmPmAceEbQaDdPiTp ^H	pSK3, pSK7	Lyon <i>et al.</i> , 1984a
SK480	PcMcSmTcCdHgEmClSpGmTmKmAcEbQaDdPiTp ^H CmFa	pSK2, pSK3, pSK8	Lyon <i>et al.</i> , 1983
SK501	PcMcSmTcCdHgEmClSpGmTmKmAcEbQaDdPiTp ^H Cm	pSK2, pSK3, pSK11	
SK529	PcMcSmTcCdHgEmClSpGmTmKmAcEbQaDdPiTp ^H Cm	pSK1, pSK2, pSK3	Lyon <i>et al.</i> , 1984a
SK565	PcMcSmTcCdHgEmClSpGmTmKmAcEbQaDdPiTp ^L Cm	pSK2, pSK3, pSK15	Lyon <i>et al.</i> , 1984a
SK604	PcMcSmTcCdHgEmClSpGmTmKmAcEbQaDdPiTp ^L Cm	pSK2, pSK9	Lyon <i>et al.</i> , 1984a
SK605	PcMcSmTcCdHgEmClSpGmTmKmNmPmAceTp ^L	...	
SK612	PcMcSmTcCdHgEmClSpGmTmKmNmPmAceEbQa	pSK41	Lyon <i>et al.</i> , 1987
SK634	PcMcSmTcCdHgEmClSpGmTmKmAcEbQaDdPiTp ^H	pSK3, pSK16	Lyon <i>et al.</i> , 1984a
SK654	PcMcSmTcCdHgEmClSpGmTmKmNmPmAceEbQaTp ^L	pSK23	
SK656	PcMcSmTcCdHgEmClSpGmTmKmAcEbQaDdPiTp ^L Cm	pSK2, pSK3, pSK14	Lyon <i>et al.</i> , 1984a
SK707	PcMcSmTcCdHgEmClSpGmTmKmAcEbQaDdPiTp ^H	pSK3, pSK17	
SK1448	PcSmTcAsaAsiCdHgEbQa	pSK89	
SK1651	PcMcSmTcCdHgAsaAsiCm	pSK51, pSK52 pSK58, pSK90	Gillespie <i>et al.</i> , 1984

* See *Materials and methods* for abbreviations.

Results and discussion

Differentiation of methicillin-resistant S. aureus

Genetic analysis of *S. aureus* strains from Australian hospitals has shown that the outbreaks of methicillin (Mc)-resistant *S. aureus* seen after 1980 resulted from the spread of a single epidemic strain or closely-related derivatives of that strain (Lyon *et al.*, 1984a; Skurray *et al.*, 1988). All of these isolates were resistant to penicillin (Pc), streptomycin (Sm) and tetracycline (Tc), and 95% were resistant to erythromycin (Em) and spectinomycin (Sp). They differed, however, in their susceptibility to the aminoglycosides amikacin (Ak), gentamicin (Gm), kanamycin (Km), neomycin (Nm) and tobramycin (Tm). Resistance to Gm, Km and Tm was shown to be encoded by a 4.7-kb transposon, Tn4001, which could occupy either a plasmid or a chromosomal locus (Lyon *et al.*, 1984b, 1987; Gillespie *et al.*, 1987b). Tn4001 was most frequently (77% of Gm^r isolates) associated with one of a group of structurally-related plasmids (known as the pSK1 family), 24–42 kb in size, which may confer resistance to a high level of trimethoprim (Tp), to antiseptic agents such as acriflavine (Ac) and quaternary ammonium compounds (Qa; e.g., benzalkonium chloride, (Bc),

cetyltrimethylammonium chloride (Ct)) and to ethidium bromide (Eb) (Gillespie *et al.*, 1987b; Skurray *et al.*, 1988). The last-named phenotypes are generally plasmid-mediated; strains which harbour such plasmids are easily identified, a fact which forms the basis of the following typing scheme.

Since all the strains of methicillin-resistant *S. aureus* examined encoded resistance to Pc, Sm and Tc and the heavy metals cadmium (Cd) and mercury (Hg) (table II), this was considered the reference point for the identification system (figure). The Hg, Mc and Tc resistance determinants have been mapped in close association on the chromosomes of a number of isolates of methicillin-resistant *S. aureus* by hybridisation and molecular cloning analyses (Gillespie *et al.*, 1987a; Matthews *et al.*, 1987), thereby strengthening the claim for a clonal origin of methicillin-resistant strains of *S. aureus*. One feature that distinguished methicillin-resistant isolates detected after 1970 from those detected prior to this date was their susceptibility to arsenate (Asa) and arsenite (Asi). Methicillin-resistant *S. aureus* strains isolated in the 1960s frequently possessed heavy-metal resistance (β -lactamase or penicillinase) plasmids which mediated resistance to Pc, Asa, Asi, Cd and Hg, whereas the majority (>99%) of methicillin-resistant isolates after 1970

Figure. Steps involved in the differentiation of methicillin-resistant *S. aureus* on the basis of antibiotic resistance phenotypes. Representative strains along with their plasmids are indicated. Abbreviations are as in the text.

did not carry such a plasmid (Gillespie *et al.*, 1985); in strains isolated after 1970 that carried a heavy-metal resistance plasmid, the plasmid did not confer Asa^r or Asi^r.

Once the methicillin-resistant isolate has been shown to be of the post-1970s type, the strains can be divided by phenotypes which were found to be plasmid-encoded in isolates from the 1980s (see figure). The first such differentiating phenotype is that of resistance towards Ac, Eb and Qa. Resistant strains fell into one of two classes; those with high-level resistance possessed minimum inhibitory concentrations (MICs) of 340, 6, 4 and 180 µg/ml for Ac, Bc, Ct and Eb, whereas those with low-level resistance demonstrated MICs of 6, 4 and 30 µg/ml, for Bc, Ct and Eb, respectively, but were sensitive to Ac. This point effectively differentiated the strains by their plasmid complement into those which carried (i) a pSK1-type or heavy-metal resistance plasmid (high-level), or (ii) a self-transmissible plasmid (low-level), such as pSK41 or pUW3626 (Cohen *et al.*, 1982; Lyon *et al.*, 1987),

or (iii) neither plasmid type (sensitive strain); the only exception to this was a strain carrying pSK11, a pSK1-family plasmid, which did not express resistance to Ac, Eb or Qa (see below).

Strains sensitive to acriflavine, ethidium bromide and to quaternary ammonium compounds

Strains sensitive to AcEbQa may be further differentiated by their ability to encode gentamicin resistance (Gm^r) (figure). Strains which are sensitive to Gm, such as SK20 (table II and figure), do not possess a large molecular weight plasmid species and may be devoid of extrachromosomal DNA. Such strains represent the "core" strain of contemporary Australian isolates since all resistance determinants are chromosomally-determined. As with Ac^rEb^rQa^r strains, resistance to Gm can be of two levels—high-level (MIC > 4096 µg/ml) and low-level (MIC 32 µg/ml). High-level resistant strains, such as SK605 (table II and figure), were additionally resistant to high levels of Tm, Km,

Nm and Ak (MICs >4096, 256–4096, 2048 and 512 µg/ml, respectively); this high level of resistance is unlikely to be due to a novel aminoglycoside modifying enzyme, but is more probably attributable to a chromosomal mutation leading to altered cell-wall permeability (Gillespie *et al.*, 1984). The low-level Gm^r phenotype is encoded by Tn4001 which additionally confers resistance to tobramycin (Tm) and kanamycin (Km) (MICs 64 and 256 µg/ml; Lyon *et al.*, 1987). Strains with low-level Gm^r (MIC 128 µg/ml) can be further subdivided on the basis of their susceptibility to trimethoprim (Tp). High-level Tp^r strains (MIC >1000 µg/ml), e.g., SK501 (table II), may carry a plasmid such as pSK11 (Skurray *et al.*, 1988). This plasmid is related to the pSK1 family of plasmids, but possesses an additional copy of Tn4002, a transposon specifying β-lactamase production, which has inserted within the Ac^rEb^rQa^r gene, thereby abolishing this resistance phenotype (Gillespie *et al.*, 1988). Low-level Tp^r strains (MIC <100 µg/ml), e.g., SK457 (table II), carry a chromosomal copy of the Gm^r transposon Tn4001 as established by DNA hybridisation analysis (Gillespie *et al.*, 1987b). Thus the lack of plasmid-associated phenotypes, such as Ac^rEb^rQa^r or high-level Tp^r, indicates a chromosomal location for the Gm^r determinant, and in the 34 strains examined which demonstrate this phenotype this has held true. The low-level Tp^r determinant in these and all contemporary methicillin-resistant *S. aureus* strains is chromosomally-encoded and is unrelated to the plasmid-encoded Tp^r determinant *dfrA* (Young *et al.*, 1987; Tennent *et al.*, 1988; Rouch *et al.*, 1989).

Strains with high-level resistance to acriflavine, ethidium bromide and to quaternary ammonium compounds

Two related determinants encoding high-level Ac^rEb^rQa^r are present in *S. aureus* strains. The first determinant, *qacA*, additionally encodes resistance to the diamidines 4'4'-diamidinodiphenylamine dihydrochloride (Dd) and propamidine isethionate (Pi) (Lyon and Skurray, 1987; Tennent *et al.*, 1989). This determinant is predominantly carried by pSK1-family plasmids but has been detected on a heavy-metal resistance plasmid, pSK57 (Gillespie *et al.*, 1986a). Furthermore, it is entirely likely that chromosomal resistance to Qa now reported in some isolates (Rahman *et al.*, 1988) results from the integration of a pSK1-family plasmid into the *S. aureus* chromosome (Gillespie *et al.*, 1989). The second determinant, *qacB*, does not confer resistance to Dd or Pi (Lyon and Skurray, 1987). *qacB*

has only been found on heavy-metal resistance plasmids in contemporary isolates (e.g., pSK21 and pSK23 in the isolates SK413 and SK654, respectively; tables I and II), but was encoded by plasmids of unknown family origin in isolates from 1973 to 1975 and as early as 1950. Apart from the minor phenotypic differences of these determinants, *qacA* and *qacB* share substantial restriction map identity and DNA homology (unpublished results).

High-level Ac^rEb^rQa^r (*qacA*) strains may be subdivided by their level of resistance towards the aminoglycoside Gm. Strains susceptible to Gm carry a pSK1-type plasmid which does not carry the Gm^rTm^rKm^r transposon Tn4001. The only pSK1-type plasmid detected in a Gm^s host has been pSK18 (SK430; table II); this plasmid additionally lacks the Tp^r determinant *dfrA* and consequently strains harbouring this plasmid demonstrate low-level Tp^r. High-level Gm^r strains (MIC >4096 µg/ml) are additionally resistant to high levels of Tm, Km, Nm and Ak and carry the pSK1-related plasmid pSK7 (e.g., SK460; table II and figure). pSK7 is structurally identical to pSK1 except that it lacks Tn4001 and hence the aminoglycoside resistance exhibited by such strains must be chromosomally-encoded. The only distinguishable difference between SK460 and other high-level Gm^r strains (e.g., SK605, see above) was the carriage of pSK7. Presumably, this difference reflects the loss of such a plasmid from a host, such as SK460, thereby constructing plasmid-less progeny such as SK605.

In our studies, the majority of Australian strains that were resistant to Gm conferred low-level Gm^rTm^rKm^r (97%) and in 77% of these, resistance was plasmid-mediated. These low-level Gm^r strains can be divided into two groups based on their susceptibility to Tp. Strains which demonstrate low-level Tp^r carry one of three plasmid types, e.g., SK604 (pSK9), SK565 (pSK15) and SK656 (pSK14) (tables I and II and figure), as differentiated by restriction endonuclease analysis. All three plasmids are deleted for the Tp^r determinant, *dfrA*, and pSK15 carries a single insertion of Tn4002 but is otherwise identical to pSK14. High-level Tp^r strains comprise those that carry the prototype plasmid, pSK1, such as SK529 (table II and figure), or otherwise identical plasmids that carry a copy of Tn4002 where the insert does not abolish any function of pSK1, e.g., SK456 (pSK4), SK480 (pSK8) and SK634 (pSK16). These Tn4002-harbouring plasmids differ from one another by the locality or orientation of insertion of Tn4002 in the pSK1 vector as determined by restriction endonuclease analysis. Since Tn4002 or a Tn4002-like

element preferentially occupies a chromosomal site in methicillin-resistant *S. aureus*, (Gillespie *et al.*, 1988) the occurrence of this transposon at a secondary site, particularly on a plasmid whose copy number is more than one per bacterial chromosome, may result in increased expression of β -lactamase production (i.e., effectively increasing the host's MIC towards penicillin). However, no differential MIC was observed between strains that exhibited a single chromosomal copy of Tn4002 compared with strains that possessed a chromosomal copy together with a copy of Tn4002 on a pSK1-family plasmid. Thus, strains bearing plasmids such as pSK4, pSK8 or pSK16 could not be differentiated from pSK1-harbouring isolates.

Since the division of these strains is based solely on their plasmid-encoded phenotypes, the loss of a plasmid from a host dramatically affects this typing system. For instance, if a strain such as SK529 spontaneously lost the plasmid pSK1, the strain would no longer demonstrate the pSK1-encoded phenotypes—resistance to Gm, Tm, Km, Ac, Eb, Qa and Tp—and would be typed as a SK20-type strain (see figure). We have observed the loss of this plasmid from strain SK529 and by this typing scheme the resultant derivative strain was typed as SK20. Furthermore, loss of the pSK1-family plasmids pSK4, pSK8, pSK9, pSK14 and pSK16 from the strains SK456, SK480, SK604, SK656 and SK634, respectively, resulted in derivative strains with a phenotype equivalent to SK20 (see figure); such a result helped to verify this typing scheme.

Strains with low-level resistance to ethidium bromide and to quarternary ammonium compounds

The *qacC* determinant encoding low-level resistance to Eb and to the quaternary ammonium compounds, Bc and Ct (MICs 30, 6 and 4 μ g/ml, respectively) was originally detected on a 2.4-kb plasmid, pSK89, from a methicillin-sensitive strain isolated in 1960 (table II; Lyon and Skurray, 1987). *qacC* shared DNA homology with the Eb^rQa^r determinant on a small molecular weight plasmid from *S. epidermidis* and with the *qacD* determinant on self-transmissible aminoglycoside resistance plasmids isolated from *S. aureus* and *S. epidermidis* strains from North America which conferred an identical resistance phenotype (e.g., SK445, harbouring pUW3626; table II and figure; M. T. Gillespie, J. M. Tennent and R. A. Skurray, unpublished results). Since the small molecular weight Eb^rQa^r plasmids have been detected only in methicillin-sensitive strains, low-level Eb^rQa^r is considered to be indicative of a self-transmissible

plasmid in methicillin-resistant strains. To verify the involvement of a self-transmissible plasmid in mediating this resistance phenotype, the strains can be tested for resistance towards Nm and paromomycin (Pm); both antibiotics, along with Ak and Km are inactivated by the 4'adenyltransferase encoded by members of the self-transmissible plasmid family (Lyon *et al.*, 1987).

Other useful phenotypes for strain identification

In addition to the phenotypes used within this scheme, other antibiotic resistance phenotypes may be encountered and may be of value in differentiating local strains. Chloramphenicol (Cm) resistance is mediated exclusively by a diverse family of plasmids of 3.5–5.5 kb in size (Gillespie and Skurray, 1988). These plasmids may belong to one of a number of different incompatibility groups and, therefore, could co-exist with pSK1-family, heavy-metal resistance family or self-transmissible-type plasmids, the former two belonging to incompatibility group 1 (Lyon and Skurray, 1987). It should be noted, however, that Cm^r is an unstable phenotype as Cm^r plasmids can be easily lost from a strain when grown in the absence of Cm; this effect is commonly seen in the laboratory and has also been observed in the clinical environment (Fisher, 1960). Resistance to erythromycin (Em) and clindamycin (Cl) is chromosomally-determined, most probably via Tn554 or a related element since strains that display these resistances are also resistant to spectinomycin (Sp; table II) (Murphy *et al.*, 1985). This phenotype is commonly encountered in Australian *S. aureus* strains. Other chromosomally-encoded phenotypes include resistance towards fusidic acid and rifampicin, both of which were rarely detected in *S. aureus* strains, and resistance to the fluoroquinolones (Lyon *et al.*, 1983; Ubukata *et al.*, 1989).

Conclusions

Traditional methods of typing have failed to differentiate the strains of methicillin-resistant *S. aureus* isolated from Australian hospitals over the last decade. All Australian-type isolates were either non-typable, or only weakly typable at 100 $\times$ RTD with bacteriophages of the International Typing Set (Lyon *et al.*, 1984a), although some differentiation of Australian strains has been achieved with experimental bacteriophages (Vickery *et al.*, 1983). Biochemical analyses have also failed to discriminate Australian-type methicillin-resistant *S. aureus* strains since they possess similar biochemical

properties (Gedney and Lacey, 1982). Plasmid DNA analysis has shown that some Australian hospitals harbour particular plasmid-bearing strains and this has provided limited epidemiological data (Lyon *et al.*, 1984a). By use of the scheme described here we have been able to differentiate these particular plasmid-bearing strains from other hospital isolates, and trace the spread of methicillin-resistant *S. aureus* within a given hospital, between different hospitals of one city and between hospitals of different cities. The identity of these strains was subsequently confirmed by plasmid DNA analysis and bacteriophage typing. The full potential of plasmid typing procedures, however, is not realised until plasmids are fingerprinted by restriction endonucleases and the results linked to phenotypic data.

In summary, the phenotypic typing scheme we describe is easily performed in a routine clinical laboratory since it only requires media plates and

in that respect is far less expensive and less labour intensive than procedures employing ³⁵S-protein analysis or immunoblotting. It should be noted that Australian methicillin-resistant *S. aureus* have also been reported in London hospitals (Townsend *et al.*, 1984) and from the characteristics (plasmid sizes and phenotypes and the location of antibiotic resistance determinants) of the EMRSA strain detected in London and South-East London hospitals (Cookson and Phillips, 1988), this strain is also equivalent to the Australian strains we describe here. We hope that this paper will form a useful reference for clinical microbiologists and others attempting to monitor the spread of EMRSA in the UK and for those who wish to devise protocols for the identification of new or endemic strains of *S. aureus*.

This work was supported by a Project Grant from the National Health and Medical Research Council of Australia.

REFERENCES

- Coleman D C *et al.* 1985 Susceptibility to antimicrobial agents and analysis of plasmids in gentamicin- and methicillin-resistant *Staphylococcus aureus* from Dublin hospitals. *Journal of Medical Microbiology* **20**: 157–167.
- Cohen M L, Wong E S, Falkow S 1982 Common R-plasmids in *Staphylococcus aureus* and *Staphylococcus epidermidis* during a nosocomial *Staphylococcus aureus* outbreak. *Antimicrobial Agents and Chemotherapy* **21**: 210–215.
- Cookson B D, Phillips I 1988 Epidemic methicillin-resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* **21** Suppl C: 57–65.
- Fisher M W 1960 The susceptibility of staphylococci to chloramphenicol: a survey of experimental and clinical experiences. *Archives of Internal Medicine* **105**: 413–423.
- Gaston M A *et al.* 1988 Evaluation of electrophoretic methods for typing methicillin-resistant *Staphylococcus aureus*. *Journal of Medical Microbiology* **26**: 189–197.
- Gedney J, Lacey R W 1982 Properties of methicillin-resistant staphylococci now endemic in Australia. *Medical Journal of Australia* **1**: 448–450.
- Gillespie M T, Lyon B R, Loo L S L, Matthews P R, Stewart P R, Skurray R A 1987a Homologous direct repeat sequences associated with mercury, methicillin, tetracycline and trimethoprim resistance determinants in *Staphylococcus aureus*. *FEMS Microbiology Letters* **43**, 165–171.
- Gillespie M T, Lyon B R, Messerotti L J, Skurray R A 1987b Chromosome- and plasmid-mediated gentamicin resistance in *Staphylococcus aureus* encoded by Tn4001. *Journal of Medical Microbiology* **24**: 139–144.
- Gillespie M T, Lyon B R, Skurray R A 1988 Structural and evolutionary relationships of β -lactamase transposons from *Staphylococcus aureus*. *Journal of General Microbiology* **134**: 2857–2866.
- Gillespie M T, Lyon B R, Skurray R A 1989 Gentamicin and antiseptic resistance in epidemic methicillin-resistant *Staphylococcus aureus*. *Lancet* **1**: 503.
- Gillespie M T, May J W, Skurray R A 1984 Antibiotic susceptibilities and plasmid profiles of nosocomial methicillin-resistant *Staphylococcus aureus*: a retrospective study. *Journal of Medical Microbiology* **17**: 295–310.
- Gillespie M T, May J W, Skurray R A 1985 Antibiotic resistance in *Staphylococcus aureus* isolated at an Australian hospital between 1946 and 1981. *Journal of Medical Microbiology* **19**: 137–147.
- Gillespie M T, May J W, Skurray R A 1986a Plasmid-encoded resistance to acriflavine and quaternary ammonium compounds in methicillin-resistant *Staphylococcus aureus*. *FEMS Microbiology Letters* **34**: 47–51.
- Gillespie M T, May J W, Skurray R A 1986b Detection of an integrated tetracycline resistance plasmid in the chromosomes of methicillin-resistant *Staphylococcus aureus*. *Journal of General Microbiology* **132**: 1723–1728.
- Gillespie M T, Skurray R A 1988 Structural relationships among chloramphenicol-resistance plasmids of *Staphylococcus aureus*. *FEMS Microbiology Letters* **51**: 205–210.
- Kozarsky P E, Rimland D, Terry P M, Wachsmuth K 1986 Plasmid analysis of simultaneous nosocomial outbreaks of methicillin-resistant *Staphylococcus aureus*. *Infection Control* **7**: 577–581.
- Krikler S J, Pennington T H, Petrie D 1986 Typing of strains of *Staphylococcus aureus* by Western blot analysis of culture supernates. *Journal of Medical Microbiology* **21**: 169–171.
- Lee W, Burnie J P 1988 Fingerprinting methicillin-resistant *Staphylococcus aureus* by the immunoblot technique. *Journal of Medical Microbiology* **25**: 261–268.
- Lunn G, Sansone E B 1987 Ethidium bromide: destruction and decontamination of solutions. *Analytical Biochemistry* **162**: 453–458.
- Lyon B R, Gillespie M T, Byrne M E, May J W, Skurray R A 1987 Plasmid-mediated resistance to gentamicin in *Staphylococcus aureus*: the involvement of a transposon. *Journal of Medical Microbiology* **23**: 101–110.
- Lyon B R, Iuorio J L, May J W, Skurray R A 1984a Molecular epidemiology of multiresistant *Staphylococcus aureus* in Australian hospitals. *Journal of Medical Microbiology* **17**: 79–89.
- Lyon B R, May J W, Skurray R A 1983 Analysis of plasmids in nosocomial strains of multiple-antibiotic-resistant *Staphy-*

- lococcus aureus*. *Antimicrobial Agents and Chemotherapy* **23**: 817–826.
- Lyon B R, May J W, Skurray R A 1984b Tn4001: a gentamicin and kanamycin resistance transposon in *Staphylococcus aureus*. *Molecular and General Genetics* **193**: 554–556.
- Lyon B R, Skurray R A 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.
- Murphy E, Huwyler L, Bastos M C F 1985 Transposon Tn554: complete nucleotide sequence and isolation of transposon-defective and antibiotic-sensitive mutants. *EMBO Journal* **4**: 3357–3365.
- Rahman M, Naidoo J, George R C 1988 New genetic location of gentamicin-resistance in methicillin-resistant *Staphylococcus aureus*. *Lancet* **2**: 1256.
- Rhinehart E *et al.* 1987 Nosocomial clonal dissemination of methicillin-resistant *Staphylococcus aureus*: elucidation by plasmid analysis. *Archives of Internal Medicine* **147**: 521–524.
- Richardson J F, Chittasobhon N, Marples R R 1988 Supplementary phages for the investigation of strains of methicillin-resistant *Staphylococcus aureus*. *Journal of Medical Microbiology* **25**: 67–74.
- Rouch D A, Messerotti L J, Loo L S L, Jackson C A, Skurray R A 1989 Trimethoprim resistance transposon Tn4003 from *Staphylococcus aureus* encodes genes for a dihydrofolate reductase and thymidylate synthase flanked by three copies of IS257. *Molecular Microbiology* **3**: 161–175.
- Skurray R A *et al.* 1988 Multiresistant *Staphylococcus aureus*: genetics and evolution of epidemic Australian strains. *Journal of Antimicrobial Chemotherapy* **21** Suppl C: 19–39.
- Stephenson J R, Crook S J, Tabaqchali S 1986 New method for typing *Staphylococcus aureus* resistant to methicillin based on sulphur-³⁵methionine labelled proteins: its application in an outbreak. *British Medical Journal* **293**: 581–583.
- Tennent J M, Lyon B R, Gillespie M T, May J W, Skurray R A 1985 Cloning and expression of *Staphylococcus aureus* plasmid-mediated quaternary ammonium resistance in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* **27**: 79–83.
- Tennent J M, Young H-K, Lyon B R, Amyes S G B, Skurray R A 1988 Trimethoprim resistance determinants encoding a dihydrofolate reductase in clinical isolates of *Staphylococcus aureus* and coagulase-negative staphylococci. *Journal of Medical Microbiology* **26**: 67–73.
- Tennent J M, Lyon B R, Midgley M, Jones I G, Purewal A S, Skurray R A 1989 Physical and biochemical characterization of the *qacA* gene encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. *Journal of General Microbiology* **135**: 1–10.
- Townsend D E, Ashdown N, Bradley J M, Pearman J W, Grubb W B 1984 “Australian” methicillin-resistant *Staphylococcus aureus* in a London hospital? *Medical Journal of Australia* **141**: 339–340.
- Ubukata K, Itoh-Yamashita N, Konno M 1989 Cloning and expression of the *norA* gene for fluoroquinolone resistance in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **33**: 1535–1539.
- Vickery A M, Beard-Pegler M A, Rountree P M 1983 Strain differentiation in methicillin-resistant *Staphylococcus aureus*. *Pathology* **15**: 235–240.
- Young H-K, Skurray R A, Amyes S G B 1987 Plasmid-mediated trimethoprim-resistance in *Staphylococcus aureus*: characterization of the first gram-positive plasmid dihydrofolate reductase (type S1). *Biochemical Journal* **243**: 309–312.