SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS AND ANALYSIS OF PLASMIDS IN GENTAMICIN- AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM DUBLIN HOSPITALS

D. C. COLEMAN, H. POMEROY, J. K. ESTRIDGE, C. T. KEANE*, M. T.
CAFFERKEY*, R. HONE† AND T. J. FOSTER

Department of Microbiology, Moyne Institute, Trinity College, Dublin 2,

** Department of Clinical Microbiology, Trinity College, St James's Hospital, James's
Street, Dublin 8, and † Department of Microbiology, The Mater Misericordiae
Hospital, Dublin 9, Ireland*

SUMMARY. Methicillin- and gentamicin-resistant *Staphylococcus aureus* (MGRSA) strains isolated from Dublin Hospitals were classified into two groups (phenotypes). Phenotype-I strains expressed high level resistance to gentamicin and were susceptible to fusidic acid; strains resistant to tetracycline harboured a 3×10^6 -mol. wt plasmid. Strains in phenotype II usually expressed low level resistance to gentamicin, were resistant to fusidic acid and often harboured a $(22-24) \times 10^6$ -mol. wt plasmid that specified resistance to ethidium bromide, tetracycline, kanamycin, neomycin and trimethoprim, or to combinations of these markers. A few phenotype-II strains expressed higher levels of resistance to gentamicin and other aminoglycosides. All MGRSA strains carried a 21×10^6 -mol. wt plasmid conferring resistance to penicillin, ethidium bromide, cadmium and mercury. Gentamicin resistance was invariably chromosomal and all strains carried chromosomal resistance to methicillin, erythromycin, streptomycin and spectinomycin.

Several methicillin-resistant *S. aureus* (MRSA) strains isolated before the emergence of gentamicin resistance harboured a 21×10^6 -mol. wt penicillinase plasmid with the same restriction endonuclease profile as that from some MGRSA strains. Some MRSA strains carried other plasmids related to those found in MGRSA strains.

INTRODUCTION

Methicillin- and gentamicin-resistant strains of *Staphylococcus aureus* (MGRSA) have become an important cause of infection in Dublin hospitals (Hone *et al.*, 1981; Cafferkey *et al.*, 1983). These organisms express resistance to a wide range of antimicrobial agents. They belong to a limited number of phage types and many are untypable by standard typing phages (Cafferkey *et al.*, 1983). Curing and transfer experiments have shown that they harbour plasmids encoding resistance to penicillin

(21×10^6 -mol. wt plasmid) and tetracycline (3×10^6 -mol. wt or 24×10^6 -mol. wt plasmid) (Dowd *et al.*, 1983). In this paper we report a retrospective analysis of MGRSA strains isolated between 1976 and 1984 and of a small number of methicillin-resistant (MRSA) strains isolated between 1970 and 1975 before the emergence of gentamicin resistance.

MATERIALS AND METHODS

Bacterial strains. The strains of *S. aureus* were clinical isolates obtained from the Federated Dublin Voluntary Hospitals, the Mater Misericordiae Hospital, Dublin and from Jervis Street Hospital, Dublin between 1970 and March 1984. *S. aureus* strain RN4220 Nov is a novobiocin-resistant mutant of strain RN4220, a restriction-deficient derivative of strain 8325-4 (Fairweather *et al.*, 1983; Kreiswirth *et al.*, 1983). Strains were stored in 40% v/v glycerol at -20°C .

Chemicals and antibiotics. Chemicals were obtained from Sigma (Poole, Dorset) or BDH Chemicals Ltd (Poole, Dorset). Antibiotics and other inhibitors were obtained as follows: benzyl penicillin, gentian violet, cetyltrimethyl ammonium bromide, ethidium bromide, gentamicin, kanamycin, tetracycline, neomycin and acriflavine were purchased from Sigma. Chlorhexidine was from Imperial Chemical Industries (Alderley Park, Cheshire), tobramycin was a gift from Eli Lilly (Basingstoke, Hampshire), sisomicin and netilmicin were gifts from Schering-Plough (Bloomfield, NJ, USA).

Bacteriophage typing was performed as described by Cafferkey *et al.* (1983).

Culture media. Bacteria were grown at 37° for 18 h in 2-ml volumes of LB broth (Miller, 1972) or Trypticase Soy Broth (BBL, Cockeysville, MD, USA) on an orbital shaker set at 150 rpm. The corresponding agar media were also used.

Screening for plasmid DNA. Isolates were screened as previously described (Dowd *et al.*, 1983).

Antibiotic susceptibility tests were performed by a disk diffusion method. The sensitive control *S. aureus* strain RN4220 Nov was tested on a separate plate. Cultures were diluted 1 in 10^3 in sterile saline and spread on DST Agar (Oxoid) plates. Disks containing the following antibiotics and other inhibitors were placed on the plates: benzylpenicillin 2 units; tetracycline 10 μg ; gentamicin 10 μg ; amikacin 30 μg ; neomycin 20 μg ; kanamycin 20 μg ; fusidic acid 20 μg ; streptomycin 50 μg ; spectinomycin 50 μg ; vancomycin 30 μg ; erythromycin 15 μg ; trimethoprim 2 μg ; tobramycin 10 μg ; HgCl_2 10 μg ; CdNO_3 10^{-6} mols; ethidium bromide 20 μg . Results were read after incubation for 18 h at 37°C . Tests for methicillin resistance were performed at 30°C with 10- μg methicillin strips (Mast).

Minimum inhibitory concentration (MIC) determinations. Stationary-phase broth cultures were diluted 1 in 10^4 and c. 0.01 ml (c. 10^3 cfu) was applied to the surface of DST (aminoglycosides) or LB (cetrimide, chlorhexidine and dyes) plates containing concentrations of inhibitors in the range 0.1–1.0 $\mu\text{g}/\text{ml}$ (0.1 $\mu\text{g}/\text{ml}$ intervals), 1.0–10.0 $\mu\text{g}/\text{ml}$ (1.0 $\mu\text{g}/\text{ml}$ intervals), 10–100 $\mu\text{g}/\text{ml}$ (10 $\mu\text{g}/\text{ml}$ intervals), 100–1000 $\mu\text{g}/\text{ml}$ (100 $\mu\text{g}/\text{ml}$ intervals) or 1000–5000 $\mu\text{g}/\text{ml}$ (1000 $\mu\text{g}/\text{ml}$ intervals).

Transformation. Protoplasts of *S. aureus* RN4220 Nov were prepared as described by Dowd *et al.* (1983) for use as recipients (Chang and Cohen, 1979). Plasmid DNA was prepared by the small scale method used for plasmid screening with the following additional steps: the DNA solution was extracted with aqueous phenol and precipitated with ethanol. Transformants were selected on DM3 agar plates (Chang and Cohen, 1979) containing tetracycline (10 $\mu\text{g}/\text{ml}$) or ethidium bromide (20 $\mu\text{g}/\text{ml}$).

Plasmid elimination experiments. Approximately 5×10^2 cells were inoculated into 2 ml of LB broth and grown for 18 h at 42°C . The cultures were diluted and spread on LB agar plates. Colonies were replica plated on agar containing tetracycline 10 $\mu\text{g}/\text{ml}$ or CdNO_3 0.1 mM. Putative cured colonies were purified from the master plate, examined for resistance markers and screened for the presence of plasmid DNA.

Isolation of plasmid DNA. Plasmid DNA for restriction endonuclease analysis was isolated from transformants of *S. aureus* RN4220 Nov harbouring plasmids from strains of MGRSA

and MRSA as follows: cultures were grown in 500 ml of LB broth in 2-L flasks shaken at 200 rpm for 18 h at 37°C. Cells were harvested by centrifugation at 8000 *g* for 10 min, washed once in 20 ml of 10 mM ethylenediaminetetra-acetic acid (EDTA) at pH 8.0 and resuspended in 10 ml of 10 mM EDTA (pH 8.0); 48 ml of an acetone-ethanol mixture (1:1) was then added. After incubation in an ice bath for 20 min, 120 ml of 10 mM EDTA pH 8.0 was added and incubation in ice was continued for a further 20 min. Cells were centrifuged and resuspended in 20 ml of buffer (150 mM NaCl, 50 mM EDTA; pH 8.0); 250 µl of lysostaphin (Sigma) 1 mg/ml in 50 mM trishydroxymethylaminomethane (Tris-HCl) (pH 7.5) was then added. After incubation at 37°C for 20 min with gentle shaking, 2.8 ml of NaCl (5.0 M), 1.8 ml of EDTA (0.5 M; pH 8.0) and 19.0 ml of sodium dodecyl sulphate (SDS) 2% w/v in 0.7 M NaCl was added and incubation was continued in ice for a further 18 h to lyse the cells. Chromosomal DNA and cell debris were then removed by centrifugation at 36 000 *g* for 45 min. The supernate was decanted into a 250-ml Sorvall centrifuge bucket (Du Pont, Newtown, CT, USA). Pancreatic RNAase (Sigma) and T1 RNAase (Sigma) were added to a concentration of 100 µg/ml. After incubation at 37°C for 30 min with gentle shaking, proteinase K (The Boehringer Corporation Ltd, Dublin, Ireland) was added to achieve a concentration of 50 µg/ml; the tubes were incubated at 37°C for 30 min. An equal volume of 1 M NaCl and polyethylene glycol 6000 (PEG) (Sigma) 10% w/v was added; the PEG was dissolved by gentle shaking and the tubes were incubated at 4°C for 2 h to precipitate the plasmid DNA. The precipitate was recovered by centrifugation at 2000 *g* for 10 min at 10°C and resuspended in 8 ml of 50 mM Tris-HCl (pH 7.5) 10 mM EDTA (pH 8.0); 7.4 g of CsCl and 250 µl of ethidium bromide (10 mg/ml) were added and the plasmid DNA was purified by centrifugation in the 70.1 Ti rotor of a Beckman L8-55 ultracentrifuge (Beckman Instruments, Inc., CA, USA) for 40 h at 42 000 rpm, at 18°C.

Restriction enzymes and electrophoresis. Restriction enzymes (Boehringer) were used according to the manufacturer's instructions. DNA was fractionated in 1.0% agarose gels as described by Aaij and Borst (1972). Fragments of DNA generated by cleavage of bacteriophage lambda with the restriction enzymes *Eco*R1 and *Bam*H1 (Daniels and Blattner, 1982) were used as mol. wt reference markers on agarose gels.

RESULTS

Classification of MGRSA strains

A total of 350 MGRSA strains, isolated from Dublin hospitals between July 1976 and March 1984, were examined. Another 20 MRSA strains isolated between 1970 and 1975 were included to determine whether similar resistance determinants and plasmids were present before the emergence of gentamicin resistance in 1976.

The MGRSA strains were divided into two groups according to their plasmid content and susceptibility to antibiotics and metal ions (table I). Phenotype-I strains were highly resistant to gentamicin, moderately resistant to amikacin and susceptible to fusidic acid. Those that were tetracycline resistant carried a 3.0×10^6 -mol. wt plasmid. Most strains assigned to phenotype II expressed a low level of resistance to gentamicin and were resistant to fusidic acid. Many carried a plasmid of $(22-24) \times 10^6$ mol. wt which could confer resistance to ethidium bromide, tetracycline, kanamycin and neomycin.

Phage types

Most MGRSA strains isolated during 1976 and early 1977 were of phage type 77/84 or 77. In 1976, all isolates of these phage types belonged to phenotype I, but since then strains of both phenotypes have been isolated. Phage type-85 strains were less

TABLE I

Classification of methicillin- and gentamicin-resistant strains of S. aureus into two groups and properties of methicillin-resistant strains

Phenotype	Number of strains tested	Location of resistance determinants		Number of strains carrying plasmid*	Plasmid mol. wt (10 ⁶)
		Chromosome	Plasmid		
I	18	Gm ^H Ak Ka Me	Pc Cd Hg Eb	18	21.0
		Sm Sp Em	Tc	7	3.0
II	325	Gm ^L Ka Me Sm Sp	Pc Cd Hg Eb	146	21.0
		Em Fu			
			Tc Ka Ne Eb Tp	32	22-24
			Tc Ka Ne Eb	15	22-24
			Tc Tp Eb	1	22-24
			Tc Eb	6	22-24
MRSA	20	Me Sm Sp Em	Ka Ne Eb	5	22-24
			Pc Cd Hg Eb	20	21.0
			Tc	7	3.0
			Tc Ka Ne Eb	5	22-24
			Ka Ne Eb	1	22-24

Gm^H, high level resistance to gentamicin (> 100 µg/ml); Gm^L, low level resistance to gentamicin (≤ 30 µg/ml); Me, methicillin; Sm, streptomycin; Sp, spectinomycin; Ak, amikacin; Ka, kanamycin; Em, erythromycin; Pc, penicillin; Cd, cadmium ions; Hg, mercuric ions; Eb, ethidium bromide; Tc, tetracycline; Fu, fusidic acid; Tp, trimethoprim; Ne, neomycin.

* All phenotype-I and MRSA strains and 146 of the 325 phenotype-II strains were screened.

common, although they predominated during the second half of 1977 and in 1978; all of these were of phenotype I. Since 1978, most strains have been of phenotype II and were untypable with the standard phage set. Strains with a broad phage-sensitivity pattern have been encountered regularly since 1980. These include phage types 6/47/54/85 and 6/47/54/77/84/85; all were of phenotype II. No phenotype-I strains have been isolated since 1982.

All the MRSA strains were of phage type 77 or 77/84 except for two isolates, one of which was of type 47/54/75/77/85 and the other of type 47/54/75/77/84/85.

Resistance to antimicrobial agents

All the MGRSA and MRSA strains were resistant to penicillin, erythromycin, methicillin, streptomycin, spectinomycin, mercuric ions, cadmium ions and ethidium bromide. They were all susceptible to arsenate and vancomycin. All MGRSA strains were resistant to gentamicin and kanamycin (table II).

Plasmid screening experiments

All MRSA strains and phenotype-I MGRSA strains and 146 of 350 phenotype-II MGRSA isolates were screened for plasmids. All harboured a 21×10^6 -mol. wt plasmid. Seven of the 18 phenotype-I strains were resistant to tetracycline and carried a 3.0×10^6 -mol. wt plasmid. A 3.0×10^6 -mol. wt plasmid was also observed in seven of 12 tetracycline resistant MRSA strains.

Of the 146 phenotype-II strains tested, 59 harboured another plasmid in the size range $(22-24) \times 10^6$ mol. wt. The presence of this type of plasmid correlated with

TABLE II
Susceptibility to antimicrobial agents of MGRSA and MRSA isolates

Antimicrobial agent	Percentage of strains resistant	
	MGRSA (350)	MRSA (20)
Methicillin	100	100
Streptomycin	100	100
Spectinomycin	100	100
Gentamicin	100	0
Kanamycin	100	25
Neomycin	34	25
Amikacin	100*	0
Penicillin G	100	100
Cadmium nitrate	100	100
Mercuric chloride	100	100
Ethidium bromide	100	100
Tetracycline	44	60
Trimethoprim†	26	0
Erythromycin	100	10
Fusidic acid	95	0
Vancomycin	0	0

* Includes phenotype II strains that were of reduced sensitivity to amikacin (MIC 2 µg/ml).

† Only 120 of the MGRSA strains were tested against trimethoprim.

various patterns of resistance as shown in table I. A similar plasmid was found in six of 20 MRSA strains and its presence correlated with expression of resistance to neomycin, kanamycin and ethidium bromide.

Plasmid elimination

Representative strains from the two phenotypes of MGRSA were grown at 42°C to promote plasmid loss. Up to half of the cells of tetracycline-resistant strains of both phenotypes were tetracycline sensitive after growth at 42°C. Plasmid-screening experiments revealed that a 3×10^6 -mol. wt element was eliminated from tetracycline-resistant strains of phenotype I, whereas a 24×10^6 -mol. wt element was lost from tetracycline-resistant strains of phenotype II. Phenotype-II strains that were resistant to neomycin or trimethoprim also lost these markers.

The 21×10^6 -mol. wt plasmid was eliminated from only one strain of each group. In both cases resistance to cadmium, mercury, penicillin and ethidium bromide were lost concomitantly. Plasmid-free variants still expressed resistance to methicillin, gentamicin, kanamycin, erythromycin, streptomycin and spectinomycin; phenotype-II strains were also resistant to fusidic acid.

Plasmid transfer

DNA from tetracycline-resistant phenotype-I strains yielded tetracycline-resistant transformants of *S. aureus* RN4220 Nov at a high frequency. Screening tests showed that a 3×10^6 -mol. wt plasmid had been transferred.

When resistance to ethidium bromide was selected, a lower frequency of

transformation was obtained. In each case the transformants had also acquired resistance to cadmium, mercury and penicillin associated with a 21×10^6 -mol. wt plasmid.

DNA from phenotype-II strains was used to transform *S. aureus* RN4220 Nov with selection for resistance to tetracycline or ethidium bromide. Two classes of ethidium bromide-resistant transformants were obtained: those in which resistance to cadmium, penicillin and mercury was linked on a 21×10^6 -mol. wt plasmid; and those that had also acquired resistance to kanamycin, neomycin, trimethoprim and tetracycline on a $(22-24) \times 10^6$ -mol. wt plasmid. Similar experiments with MRSA strains revealed that resistance to ethidium bromide was associated with a 21×10^6 -mol. wt plasmid carrying cadmium, penicillin and mercury resistance, and, in some cases, with a large plasmid carrying kanamycin, neomycin and tetracycline resistance similar to those found in phenotype-II MRSA strains. In some tetracycline-resistant strains, a 3×10^6 -mol. wt. plasmid was transferred.

Resistance to ethidium bromide, cetrимide, chlorhexidine, and dyes

All MGRSA were resistant to ethidium bromide (table II). To examine the involvement of plasmids in this phenotype, strain SA20 (phenotype II) was cured of plasmids conferring resistance to tetracycline and penicillin. It was not possible to obtain a penicillin-sensitive, tetracycline-resistant variant.

The penicillin-resistant, tetracycline-sensitive strain retained resistance to ethidium bromide, whereas the strain that was sensitive to penicillin and tetracycline was also susceptible to the inhibitor. *S. aureus* strain RN4220 carrying a 21×10^6 -mol. wt plasmid specifying penicillin resistance (pPC128) or a 24×10^6 -mol. wt plasmid specifying tetracycline resistance (pTC128) was shown to be resistant to ethidium bromide. Thus, both plasmids in the phenotype-II strain SA128 conferred resistance to ethidium bromide. These findings were confirmed by transforming *S. aureus* RN4220 Nov with plasmids that specified penicillin and tetracycline resistance from several other strains.

Ethidium bromide resistance was also associated with resistance to cetyltrimethyl ammonium bromide (cetrимide), chlorhexidine, acriflavine and gentian violet (table III). The presence of two copies of the ethidium bromide-resistance marker in strain SA20 did not cause an increase in resistance over that of strains carrying a single copy.

TABLE III
Minimum inhibitory concentrations of chlorhexidine, cetrимide and dyes

<i>S. aureus</i> strain	Minimum inhibitory concentration ($\mu\text{g/ml}$) of				
	Ethidium bromide	Cetyltrimethyl ammonium bromide	Chlorhexidine	Acriflavine	Gentian violet
RN4220	0.75	0.5	0.25	20	0.075
RN4220 (pPC128)	170	2.0	0.75	250	0.25
RN4220 (pTC128)	170	2.0	0.75	250	0.25
SA20 (pPC20, pTC20)	170	2.0	0.75	250	0.75
SA20Tc ^s (pPC20)	170	2.0	0.75	250	0.75
SA20Tc ^s Pc ^s	0.75	0.5	0.25	30	0.1

Resistance to aminoglycosides

The MICs of several aminoglycosides for phenotype-I and -II MGRSA strains are shown in table IV. Derivatives which were cured of plasmids and transformants of strain RN4200 Nov carrying individual plasmids from these strains were also tested.

Phenotype-I strains were more resistant to all the aminoglycosides (except neomycin and kanamycin) than were most phenotype-II strains. Loss of the 3×10^6 -mol. wt or the 21×10^6 -mol. wt plasmid did not affect resistance.

The phenotype-II strains that harboured a $(22-24) \times 10^6$ -mol. wt plasmid were, like phenotype-I strains, highly resistant to kanamycin (MIC 1000 $\mu\text{g/ml}$); they were also resistant to neomycin. Strains lacking this plasmid, including those from which the plasmid had been eliminated, were susceptible to neomycin and were less resistant to kanamycin (MIC 100 $\mu\text{g/ml}$). Furthermore, transformants of *S. aureus* RN4220 carrying this plasmid expressed resistance to kanamycin (MIC 200 $\mu\text{g/ml}$) and to neomycin.

Eight of 338 phenotype-II strains were more resistant to gentamicin, amikacin, tobramycin, sisomicin and neomycin than the rest. Three levels of resistance to gentamicin were detected: three strains were inhibited by 80–100 $\mu\text{g/ml}$, three strains by 250–300 $\mu\text{g/ml}$ and two strains by 1500–1600 $\mu\text{g/ml}$.

The MRSA strains that carried a $(22-24) \times 10^6$ -mol. wt plasmid similar to the phenotype-II MGRSA strains were resistant to kanamycin and neomycin.

Restriction endonuclease analysis of plasmid DNA

Plasmid DNA was purified from ethidium bromide- and tetracycline-resistant transformants of *S. aureus* RN4220 Nov harbouring plasmids from representative

TABLE IV
Minimum inhibitory concentrations of aminoglycosides for MGRSA and MRSA strains

<i>S. aureus</i> strain	Phenotype	Minimum inhibitory concentrations ($\mu\text{g/ml}$) of						
		Ak	Gm	Ka	Ne	Si	To	Nt
SA614 Tc ^r Pc ^r	I	10	200	1000	<1	30	80	20
SA614 Tc ^s Pc ^r	I	10	200	1000	<1	30	80	20
SA614 Tc ^s Pc ^s	I	10	200	1000	<1	30	80	20
SA128 Tc ^r Pc ^r	II	2	20	1000	30	0.8	10	2
SA128 Tc ^s Pc ^r	II	2	20	100	<1	0.8	10	2
RN4220		0.6	0.3	<1	<1	0.2	0.2	0.2
RN4220 (pPC128)*		0.6	0.3	<1	<1	0.2	0.2	0.2
RN4220 (pTC128)*		0.6	0.2	200	20	0.2	0.2	0.2
SA229	Atypical II	40	1600	5000	20	100	200	110
SA49	Atypical II	6	100	2000	20	10	50	10
SA724	Atypical II	15	300	3500	20	50	120	40
SA150 Ne ^r	MRSA	0.6	0.2	200	10	0.2	0.2	0.2
RN4220 (pNE150)†		0.4	0.2	200	10	0.2	0.2	0.2

Ak, amikacin; Gm, gentamicin; Ka, kanamycin; Ne, neomycin; Si, sisomicin; To, tobramycin; Nt, netilmicin.

* Plasmid pPC128 was the 21×10^6 -mol. wt plasmid from *S. aureus* strain SA128; pTC128 was the $(22-24) \times 10^6$ -mol. wt plasmid from the same isolate.

† Plasmid pNE150 was the $(22-24) \times 10^6$ -mol. wt plasmid from the MRSA strain SA150.

FIG.—Agarose gel electrophoresis of purified *S. aureus* plasmid DNA cleaved with restriction enzymes. Lanes A and J contain DNA fragments of bacteriophage lambda cleaved by both *EcoRI* and *BamHI*, as mol. wt reference markers. Fragment sizes (in kb) are shown on the right. (Lanes B–E: $(22-24) \times 10^6$ -mol. wt plasmids from MRSA (lane B) and phenotype-II MGRSA strains (lanes C, D and E) cleaved with *Hind III*. Lanes F–I: 21×10^6 -mol. wt plasmids encoding resistance to penicillin, cadmium, mercury and ethidium bromide from phenotype-I MGRSA (lane F), phenotype-II MGRSA (lanes G and H) and MRSA strains (lane I) cleaved with *EcoRI*. The resistance determinants encoded by the $(22-24) \times 10^6$ plasmids were: lane B: ethidium bromide, kanamycin, neomycin; lane C: ethidium bromide, tetracycline, trimethoprim; lanes D and E: ethidium bromide, kanamycin, neomycin, tetracycline and trimethoprim.

MRSA and MGRSA strains and was cleaved by the restriction enzymes *EcoRI* and *Hind III*. Agarose gel electrophoresis showed that fragment patterns of all the plasmids conferring penicillin resistance were identical (fig., lanes F–I; *Hind III* data not shown). Cleavage by *Hind III* of the $(22-24) \times 10^6$ -mol. wt plasmids harboured by some MRSA and phenotype-II MGRSA strains revealed that the plasmids had several fragments in common but some differences were also observed (fig. lanes B–E).

DISCUSSION

The MGRSA strains described here were divided into two groups based on plasmid profiles and resistance determinants. The strains had several features in common with the MRSA strains isolated in Dublin hospitals between 1970 and 1975: they all expressed chromosomal resistance to methicillin, erythromycin, spectinomycin and streptomycin and carried a 21×10^6 -mol. wt plasmid conferring resistance to penicillin, cadmium, mercury and ethidium bromide. Furthermore, restriction enzyme analysis showed the penicillinase plasmids, as well as the $(22-24) \times 10^6$ -mol. wt plasmid found in MRSA and phenotype-II MGRSA strains, to be closely related. Differences observed could be partly due to variations in resistance determinants.

It seems likely that the MRSA strains encountered earlier acquired gentamicin resistance by gene transfer events which resulted in the determinant being incorporated

in the chromosome, possibly by transposition (Lyon *et al.*, 1984b). The relationship between phenotype-I and -II MGRSA strains is being investigated.

The MGRSA strains isolated in Dublin differ from similar *S. aureus* strains that have been reported from France, Australia, the UK and the USA (Soussy *et al.*, 1975; Shanson *et al.*, 1976; Crossley *et al.*, 1979; McGowan *et al.*, 1979; Price *et al.*, 1980; Jaffe *et al.*, 1982; Archer and Mayhall, 1983; Lyon *et al.*, 1983; Naidoo *et al.*, 1983; Townsend *et al.*, 1983a) by invariably exhibiting chromosomal resistance to gentamicin. In strains reported from the USA, resistance to gentamicin is often encoded by large conjugative plasmids (Archer and Johnston, 1983; Forbes and Schaberg, 1983; McDonnell *et al.*, 1983). Plasmid and chromosomal resistance has been found in Australian strains (Gillespie *et al.*, 1984; Lyon *et al.*, 1983 and 1984a; Townsend *et al.*, 1983a and 1984a), the variable location possibly being due to a transposon (Lyon *et al.*, 1984b; Townsend *et al.*, 1984a). The Australian strains also differ from those reported here by exhibiting chromosomal resistance to penicillin (Lyon *et al.*, 1983; Townsend *et al.*, 1983a; Gillespie *et al.*, 1984). Chromosomal resistance to penicillin is unusual in European and American isolates.

Plasmid-mediated resistance to tetracycline in *S. aureus* is usually conferred by a 3×10^6 -mol. wt plasmid (Lacey, 1975 and 1984) such as that detected in our phenotype-I MGRSA strains and in some American and Australian isolates (McDonnell *et al.*, 1983; Gillespie *et al.*, 1984). Resistance to tetracycline specified by large plasmids such as those found in our phenotype- II strains have been reported previously in *S. aureus* in strains from France that were also resistant to streptogramin B antibiotics (El Solh *et al.*, 1980).

Another unusual feature of the MGRSA strains described here was the occurrence of plasmid-borne resistance to trimethoprim. This resistance marker is usually chromosomal in *S. aureus* (Lewis and Lacey, 1973; Lacey, 1975) although such plasmids have been found in MGRSA strains in Australia (Townsend *et al.*, 1984a). Furthermore, in Australian strains, tetracycline resistance was usually chromosomal (Gillespie *et al.*, 1984).

The pattern of aminoglycoside resistance displayed by strains from Dublin that were highly resistant to gentamicin suggests the involvement of APH(2'') and AAC(6'), enzymes previously shown to be responsible for gentamicin resistance in *S. aureus* (Phillips and Shannon, 1984). Resistance to kanamycin and neomycin specified by the $(22-24) \times 10^6$ -mol. wt plasmid is most likely due to APH(3') (Phillips and Shannon, 1984).

Plasmid-mediated resistance to ethidium bromide in *S. aureus*, first reported by Johnston and Dyke (1969), is common in MGRSA strains (Jaffe *et al.*, 1982; Townsend *et al.*, 1983b; Lyon *et al.*, 1984b) and was present in all Dublin isolates. Resistance to quaternary ammonium compounds and to cationic surfactive agents specified by such plasmids was first reported by Townsend *et al.* (1983b and 1984b). It seems possible that resistance to these compounds, which are commonly used as antiseptics and disinfectants, is specified by the same, or closely linked, determinants.

We are indebted to the Medical Research Council of Ireland for a Hospital Infection Unit Programme grant. We thank Professor J. P. Arbuthnott, for his valuable guidance, Dr M. Storrs for performing antibiotic titrations, Dr Lesley Baxter for antibiograms, Miss Lindsay Jones for the strains, Mrs M. de Saxe of the Staphylococcal Reference Laboratory, Colindale, London, for typing phages and Gillian Johnston and Mairead Daly for help in preparing the manuscript.

REFERENCES

- Archer G L, Johnston J L 1983 Self-transmissible plasmids in staphylococci that encode resistance to aminoglycosides. *Antimicrobial Agents and Chemotherapy* **24**:70-77.
- Archer G L, Mayhall C G 1983 Comparison of epidemiological markers used in the investigation of an outbreak of methicillin-resistant *Staphylococcus aureus* infections. *Journal of Clinical Microbiology* **18**:395-399.
- Aaji C, Borst P 1972 The gel electrophoresis of DNA. *Biochimica et Biophysica Acta* **269**: 192-200.
- Cafferkey M T, Hone R, Falkiner F R, Keane C T, Pomeroy H 1983 Gentamicin and methicillin resistant *Staphylococcus aureus* in Dublin Hospitals: clinical and laboratory studies. *Journal of Medical Microbiology* **16**:117-127.
- Chang S, Cohen S N 1979 High frequency transformation of *Bacillus subtilis* protoplasts by plasmid DNA. *Molecular and General Genetics* **168**:111-115.
- Crossley K, Loesch D, Landesman B, Mead K, Chern M, Strate R 1979 An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides. I. Clinical studies. *Journal of Infectious Diseases* **139**: 273-279.
- Daniels D L, Blattner F R 1982 Nucleotide sequence of the Q gene and the Q to S intergenic region of bacteriophage lambda. *Virology* **117**:81-92.
- Dowd D, Cafferkey M, Dougan G 1983 Gentamicin and methicillin resistant *Staphylococcus aureus* in Dublin hospitals: molecular studies. *Journal of Medical Microbiology* **16**:129-138.
- El Solh N, Fouace J M, Shalita Z, Bouanchaud D H, Novick R P, Chabbert Y A 1980 Epidemiological and structural studies of *Staphylococcus aureus* R plasmids mediating resistance to tobramycin and streptogramin. *Plasmid* **4**:117-120.
- Fairweather N, Kennedy S, Foster T J, Kehoe M, Dougan G 1983 Expression of a cloned *Staphylococcus aureus* α -hemolysin determinant in *Bacillus subtilis* and *Staphylococcus aureus*. *Infection and Immunity* **41**:1112-1117.
- Forbes B A, Schaberg D R 1983 Transfer of resistance plasmids from *Staphylococcus epidermidis* to *Staphylococcus aureus*: Evidence for conjugative exchange of resistance. *Journal of Bacteriology* **153**:627-634.
- 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.
- Hone R *et al.* 1981 Bacteraemia in Dublin due to gentamicin-resistant *Staphylococcus aureus*. *Journal of Hospital Infection* **2**:119-126.
- Jaffe H W, Sweeney H M, Weinstein R A, Kabins S A, Nathan C, Cohen S 1982 Structural and phenotypic varieties of gentamicin resistance plasmids in hospital strains of *Staphylococcus aureus* and coagulase-negative staphylococci. *Antimicrobial Agents and Chemotherapy* **21**:773-779.
- Johnston L H, Dyke K G H 1969 Ethidium bromide resistance, a new marker on the staphylococcal penicillinase plasmid. *Journal of Bacteriology* **100**:1413-1414.
- Kreiswirth B N *et al.* 1983 The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. *Nature* **305**:709-712.
- Lacey R W 1975 Antibiotic resistance plasmids of *Staphylococcus aureus* and their clinical importance. *Bacteriological Reviews* **39**:1-32.
- Lacey R W 1984 Antibiotic resistance in *Staphylococcus aureus* and streptococci. *British Medical Bulletin* **40**:77-83.
- Lewis E L, Lacey R W 1973 Present significance of resistance to trimethoprim and sulphonamides in coliforms, *Staphylococcus aureus*, and *Streptococcus faecalis*. *Journal of Clinical Pathology* **26**:175-180.
- Lyon B R, May J W, Skurray R A 1983 Analysis of plasmids in nosocomial strains of multiple-antibiotic-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **23**:817-826.
- 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 1984b Tn4001: A gentamicin and kanamycin resistance transposon in *Staphylococcus aureus*. *Molecular and General Genetics* **193**:554-556.

- McDonnell R W, Sweeney H M, Cohen S 1983 Conjugational transfer of gentamicin resistance plasmids intra- and interspecifically in *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrobial Agents and Chemotherapy* **23**:151-160.
- McGowan J E, Terry P M, Huang T-SR, Houk C L, Davies J 1979 Nosocomial infections with gentamicin-resistant *Staphylococcus aureus*: plasmid analysis as an epidemiologic tool. *Journal of Infectious Diseases* **140**:864-872.
- Miller J H 1972 Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, p 433.
- Naidoo J, Noble W C, Weissmann A, Dyke K G H 1983 Gentamicin-resistant staphylococci: genetics of an outbreak in a dermatology department. *Journal of Hygiene* **91**:7-16.
- Phillips I, Shannon K 1984 Aminoglycoside resistance. *British Medical Bulletin* **40**:28-35.
- Price E H, Brain A, Dickson J A 1980 An outbreak of infection with a gentamicin and methicillin-resistant *Staphylococcus aureus* in a neonatal unit. *Journal of Hospital Infection* **1**:221-228.
- Shanson D C, Kensit J G, Duke R 1976 Outbreak of hospital infection with a strain of *Staphylococcus aureus* resistant to gentamicin and methicillin. *Lancet* **2**: 1347-1348.
- Soussy C J, Bouanchaud D H, Fouace J, Dublanchet A, Duval J 1975 A gentamicin resistance plasmid in *Staphylococcus aureus*. *Annales de Microbiologie (Paris)* **126b**:91-94.
- Townsend D E, Grubb W B, Ashdown N 1983a Genetics of drug resistance in methicillin-resistant *Staphylococcus aureus* from Australian hospitals. *Journal of Hospital Infection* **4**:331-337.
- Townsend D E, Greed L, Ashdown N, Grubb W B 1983b Plasmid-mediated resistance to quaternary ammonium compounds in methicillin-resistant *Staphylococcus aureus*. *Medical Journal of Australia* **2**:310.
- Townsend D E, Ashdown N, Greed L C, Grubb W B 1984a Analysis of plasmids mediating gentamicin resistance in methicillin-resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* **13**:347-352.
- Townsend D E, Ashdown N, Greed L C, Grubb W B 1984b Transposition of gentamicin resistance to staphylococcal plasmids encoding resistance to cationic agents. *Journal of Antimicrobial Chemotherapy* **14**:115-124.