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

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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) × 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 methi-
cillin, 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

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(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), sissomicin 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°C 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; HgCl2 10 μg; Cd(NO3)2 10^{-5} 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 0-1-10 μg/ml (0-1 μg/ml intervals), 1-0-10 μg/ml (1-0 μg/ml intervals), 10-100 μg/ml (10 μg/ml intervals), 100-1000 μg/ml (100 μg/ml intervals) or 1000-5000 μg/ml (1000 μg/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 μg/ml) or ethidium bromide (20 μg/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 μg/ml or Cd(NO3)2 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 digested 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 EcoR1 and BamH1 (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 × 10⁶-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) × 10⁶ 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
Above the table:

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 $21 \times 10^6$-mol. wt plasmid. Seven of the 18 phenotype-I strains were resistant to tetracycline and carried a $3.0 \times 10^6$-mol. wt plasmid. A $3.0 \times 10^6$-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

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### Table I

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

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Number of strains tested</th>
<th>Location of resistance determinants</th>
<th>Number of strains carrying plasmid*</th>
<th>Plasmid mol. wt ($10^6$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>18</td>
<td>GmH Ak Ka Me Sm Sp Em</td>
<td>18</td>
<td>21.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tc</td>
<td>7</td>
<td>3.0</td>
</tr>
<tr>
<td>II</td>
<td>325</td>
<td>GmL Ka Me Sm Sp Em Fu</td>
<td>146</td>
<td>21.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tc Ka Ne Eb Tp</td>
<td>32</td>
<td>22-24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tc Ka Ne Eb</td>
<td>15</td>
<td>22-24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tc Tp Eb</td>
<td>1</td>
<td>22-24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tc Eb</td>
<td>6</td>
<td>22-24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ka Ne Eb</td>
<td>5</td>
<td>22-24</td>
</tr>
<tr>
<td>MRSA</td>
<td>20</td>
<td>Me Sm Sp Em</td>
<td>20</td>
<td>21.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tc</td>
<td>7</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tc Ka Ne Eb</td>
<td>5</td>
<td>22-24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ka Ne Eb</td>
<td>1</td>
<td>22-24</td>
</tr>
</tbody>
</table>

GmH, high level resistance to gentamicin (>100 µg/ml); GmL, low level resistance to gentamicin (<30 µg/ml); Me, methicillin; Sm, streptomycin; Sp, spectinomycin; Ak, amikacin; Ka, kanamycin; Em, erythromycin; Pe, 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.
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 $\times$ 10^6-mol. wt element was eliminated from tetracycline-resistant strains of phenotype I, whereas a 24 $\times$ 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 $\times$ 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 $\times$ 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 $21 \times 10^6$-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 $21 \times 10^6$-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 $21 \times 10^6$-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 $3 \times 10^6$-mol. wt. plasmid was transferred.

*Resistance to ethidium bromide, cetrimide, 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 $21 \times 10^6$-mol. wt plasmid specifying penicillin resistance (pPC128) or a $24 \times 10^6$-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 (cetrimide), 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, cetrimide and dyes*

<table>
<thead>
<tr>
<th><em>S. aureus</em> strain</th>
<th>Ethidium bromide</th>
<th>Cetyltrimethyl ammonium bromide</th>
<th>Chlorhexidine</th>
<th>Acriflavine</th>
<th>Gentian violet</th>
</tr>
</thead>
<tbody>
<tr>
<td>RN4220</td>
<td>0.75</td>
<td>0.5</td>
<td>0.25</td>
<td>20</td>
<td>0.075</td>
</tr>
<tr>
<td>RN4220 (pPC128)</td>
<td>170</td>
<td>2.0</td>
<td>0.75</td>
<td>250</td>
<td>0.25</td>
</tr>
<tr>
<td>RN4220 (pTC128)</td>
<td>170</td>
<td>2.0</td>
<td>0.75</td>
<td>250</td>
<td>0.25</td>
</tr>
<tr>
<td>SA20 (pPC20, pTC20)</td>
<td>170</td>
<td>2.0</td>
<td>0.75</td>
<td>250</td>
<td>0.75</td>
</tr>
<tr>
<td>SA20Tc2 (pPC20)</td>
<td>170</td>
<td>2.0</td>
<td>0.75</td>
<td>250</td>
<td>0.75</td>
</tr>
<tr>
<td>SA20Te3 (pC63)</td>
<td>170</td>
<td>2.0</td>
<td>0.75</td>
<td>250</td>
<td>0.75</td>
</tr>
<tr>
<td>SA20Te3 (pC63)</td>
<td>170</td>
<td>2.0</td>
<td>0.75</td>
<td>250</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>0.5</td>
<td>0.25</td>
<td>30</td>
<td>0.1</td>
</tr>
</tbody>
</table>
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 $3 \times 10^6$-mol. wt or the $21 \times 10^6$-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 $1000 \mu g/ml$); 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 $100 \mu g/ml$). Furthermore, transformants of \textit{S. aureus} RN4220 carrying this plasmid expressed resistance to kanamycin (MIC $200 \mu g/ml$) 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 $80-100 \mu g/ml$, three strains by $250-300 \mu g/ml$ and two strains by $1500-1600 \mu g/ml$.

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 \textit{S. aureus} RN4220 Nov harbouring plasmids from representative

<table>
<thead>
<tr>
<th>S. aureus strain</th>
<th>Phenotype</th>
<th>Minimum inhibitory concentrations (pg/ml) of Ak Gm Ka Ne Si To Nt</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA614 Tc' Pc' I</td>
<td>1</td>
<td>10 200 1000 &lt;1 30 80 20</td>
</tr>
<tr>
<td>SA614 Tc' Pc' I</td>
<td>1</td>
<td>10 200 1000 &lt;1 30 80 20</td>
</tr>
<tr>
<td>SA614 Tc' Pc' I</td>
<td>1</td>
<td>10 200 1000 &lt;1 30 80 20</td>
</tr>
<tr>
<td>SA128 Tc' Pc' II</td>
<td>2</td>
<td>2 20 1000 30 0-8 10 2</td>
</tr>
<tr>
<td>SA128 Tc' Pc' II</td>
<td>2</td>
<td>2 20 1000 30 0-8 10 2</td>
</tr>
<tr>
<td>RN4220</td>
<td></td>
<td>0-6 0-3 &lt;1 &lt;1 0-2 0-2</td>
</tr>
<tr>
<td>RN4220 (pPC128)*</td>
<td></td>
<td>0-6 0-3 &lt;1 &lt;1 0-2 0-2</td>
</tr>
<tr>
<td>RN4220 (pTC128)*</td>
<td></td>
<td>0-6 0-2 200 0-2 0-2 0-2</td>
</tr>
<tr>
<td>SA229</td>
<td>Atypical II</td>
<td>40 1600 5000 20 100 200 110</td>
</tr>
<tr>
<td>SA49</td>
<td>Atypical II</td>
<td>6 100 2000 20 10 50 10</td>
</tr>
<tr>
<td>SA724</td>
<td>Atypical II</td>
<td>15 300 3500 20 50 120 40</td>
</tr>
<tr>
<td>SA150 Ne'</td>
<td>MRSA</td>
<td>0-6 0-2 200 10 0-2 0-2</td>
</tr>
<tr>
<td>RN4220 (pNE150)†</td>
<td></td>
<td>0-4 0-2 200 10 0-2 0-2</td>
</tr>
</tbody>
</table>

Ak, amikacin; Gm, gentamicin; Ka, kanamycin; Ne, neomycin; Si, sisomicin; To, tobramycin; Nt, netilmicin.
* Plasmid pPC128 was the $21 \times 10^6$-mol. wt plasmid from \textit{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.
Fw.-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 \times 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 \times 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 $3 \times 10^6$-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.

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