GENTAMICIN AND METHICILLIN RESISTANT
STAPHYLOCOCCUS AUREUS IN DUBLIN
HOSPITALS: CLINICAL AND LABORATORY
STUDIES

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SUMMARY. Strains of Staphylococcus aureus resistant to gentamicin and
methicillin first appeared in Dublin hospitals in 1976, and rapidly
became widely disseminated. The number of patients infected or
colonised increased throughout the period of study, especially in 1979
and 1980. Most isolates were from burns, surgical wounds and
traumatic skin lesions. During the 12 months after first isolation of these
multiply antibiotic resistant strains, colonisation or minor infection was
the usual event. Invasive infection such as bacteraemia, deep wound
sepsis and osteomyelitis was rarely seen. Subsequently, as the number
of patients from whom these organisms were isolated increased,
bacteraemia and other severe infection became more common. The
predominant phage type of S. aureus changed with the progression of
the outbreak. Isolates of different phage type were sometimes found in
a single lesion, or in different sites in one patient. By the second half of
1980, most isolates were untypable or typed only with an experimental
phage.

INTRODUCTION

Gentamicin resistance in a clinical isolate of Staphylococcus aureus was first
reported by Lacey and Mitchell (1969). Such resistance was very rare until 1975.
However, as a result of the emergence of neomycin resistance in S. aureus in association
with the topical use of neomycin, Lacey (1975) predicted that gentamicin resistance
would appear in S. aureus. This prediction became a reality in 1975 and 1976 with the
appearance of S. aureus strains resistant to gentamicin and other aminoglycosides in
single unrelated cases and in outbreaks of hospital infection in many centres (Soussy et
al., 1975; Speller et al., 1976; Wyatt et al., 1977; Greenhood et al., 1979). A more
serious development was the appearance of strains of S. aureus resistant to gentamicin
and several other antibiotics including methicillin (Soussy et al., 1976; Shanson et al.,
1976; Crossley et al., 1979; Price et al., 1980).

Received 1st June 1982; revised version accepted 19 Oct. 1982.
In the year from April 1971, 5.4% of *S. aureus* strains isolated from eight Dublin hospitals served by this laboratory were resistant to methicillin (Hone and Keane, 1974). These isolates were also resistant to most of the commonly used antibacterial agents, but all were sensitive to chloramphenicol and gentamicin. Phage typing showed that, in spite of varied patterns, these strains belonged to phage group III; most were sensitive to phages 77 and 84.

A gentamicin and methicillin resistant strain of *S. aureus* (GMRSA) was first isolated from a clinical specimen taken in one of our hospitals in July 1976. This strain was lysed by phages of groups I and III. Subsequently the frequency of isolation of such strains increased and bacteraemias and other invasive infections with these organisms have been observed. Most of these later strains were sensitive to phages of group III, especially 77 and 84. Strains of this phage type have been isolated consistently from these hospitals since then. We report here the distribution of GMRSA in eight hospitals, the types of specimens from which these organisms were isolated and the phage-typing patterns of GMRSA isolates. The period of study was 1 Jul. 1976 to 31 Dec. 1980.

**Materials and methods**

**The Hospitals.** Eight Dublin teaching hospitals participated in this study. These were: (A) The Federated Dublin Voluntary Hospitals (F.D.V.H.), a group of seven hospitals totalling 1231 beds including the National Burns and Plastic Surgery Units and served by a central microbiology laboratory, and (B) the Mater Misericordiae Hospital (M.M.H.), with 449 beds and including the National Cardiac Surgery Unit. In this study the hospitals are designated numbers one to eight.

*S. aureus strains.* *S. aureus* strains were identified by the tube coagulase test with human plasma (standardised normal plasma; Dade Diagnostics Inc., Miami, FA). The ‘Oxford’ strain of *S. aureus* NCTC 6571 was the control organism in antibiotic susceptibility tests.

Because of the large number of gentamicin and methicillin resistant strains of *S. aureus* isolated, only selected representative strains were stored for further study; these included all isolates from invasive infection, sites normally sterile and epidemiological specimens and isolates from a single specimen showing differences in cultural characteristics or antibiotic susceptibility. Isolates selected for plasmid studies were stored in 40% glycerol at −20°C; other isolates were stored on nutrient agar slopes at room temperature.

**Antibiotic Susceptibility Tests.** Antibiotic susceptibility tests were performed by the Stokes’ disk diffusion method (Stokes and Waterworth, 1972) on Diagnostic Sensitivity Test Agar (DST, Oxoid Ltd) with the following disks: penicillin G 2 units, tetracycline 10 μg, erythromycin 15 μg, trimethoprim 1-25 μg, sulphamethoxazole 100 μg, gentamicin 10 μg, amikacin 30 μg, neomycin 30 μg, kanamycin 30 μg, tobramycin 30 μg, chloramphenicol 30 μg, clindamycin 2 μg, fusidic acid 10 μg, rifampicin 30 μg, and vancomycin 30 μg. Single disks and “Mastrings” (Mast Laboratories Ltd, Liverpool) were used. The plates were incubated overnight at 37°C. Methicillin resistance was tested at 30°C using a 10 μg methicillin disk (Annear, 1968).

Minimum inhibitory concentrations (MICs) of gentamicin, neomycin, kanamycin, tobramycin, amikacin and vancomycin were performed by the agar dilution method on DST agar. A multipoint inoculator was used to deliver 10^3–10^4 cfu of an overnight broth culture; this allowed 27 strains to be inoculated on a plate. Minimum bactericidal concentrations (MBCs) of gentamicin, kanamycin and amikacin, were determined by the tube dilution method. MICs for methicillin were determined by the tube dilution method incubated overnight at 30°C.

**Bacteriophage Typing.** This was performed on all stored isolates. The method used was that of Blair and Williams (1961) using the routine set of phages together with the experimental phages 88A, 89 and 90. These, and their propagating strains were kindly supplied by the Director of the Staphylococcal Reference Laboratory, Colindale, London.
RESULTS

Isolation of GMRSA

The frequency of isolation of GMRSA from clinical specimens, or the proportion of all *S. aureus* isolates that were gentamicin and methicillin resistant was not known for all eight hospitals because records were kept of selected *S. aureus* isolates only. Every patient from whom GMRSA was isolated, and the affected sites, was recorded. However, for gentamicin-sensitive *S. aureus*, records were kept only of patients in whom the organism had caused significant infection or who were bacteraemic, and patients from whom *S. aureus* was isolated from the nose before cardiac surgery.

GMRSA strains were isolated from clinical specimens from 849 patients. Fig. 1 shows the numbers of patients from whom GMRSA were isolated in each 3-month period. GMRSA were isolated from some patients for several months and, from seven patients, were isolated from an infected site for more than 12 months. The total of 849 is certainly an underestimate of the number of patients infected or colonised, as the indications for taking specimens varied greatly within and between hospitals. Fig. 1 also shows episodes of bacteraemia and other severe infection for each 3-month
period throughout the study. The incidence of bacteraemia and other severe infection increased especially in 1979.

Spread of GMRSA in the hospitals

The numbers of patients in the different hospitals from whom GMRSA were isolated during each 3-month period are shown in fig. 2. The number of patients

![Histograms showing the numbers of patients in different hospitals from whom GMRSA were isolated each 3-month period from July 1976 to December 1980.]

Fig. 2. Numbers of patients in the different hospitals from whom GMRSA were isolated in each 3-month period from 1 Jul. 1976 to 31 Dec. 1980.
infected or colonised varied greatly between the different hospitals, but, in all cases showed a progressive increase during the study.

All isolates in 1976 were from in-patients in Hospital 7. This hospital includes the National Burns and Plastic Surgery Units. Throughout the study, 404 of the 849 patients from whom GMRSA were isolated were in Hospital 7. Strains of GMRSA were isolated from only three patients in Hospital 4 and from only eight patients in Hospital 5. Episodes of bacteraemia and other severe infections were uncommon in Hospital 7 when the total number of patients colonised or infected is taken into account. The greatest incidence of severe infections was in Hospital 3.

Relationship of GMRSA to duration of hospital stay. This was not studied formally. It was, however, noted that in burns patients, swabs taken at admission were usually negative for GMRSA; swabs taken subsequently (48 or 72 h later) frequently yielded GMRSA and the likelihood that the lesions would be colonised or infected with these organisms increased with duration of hospital stay.

Sites of GMRSA infections

Sites from which GMRSA was isolated have been divided into two groups—superficial and deep. Specimens from nose, throat, perineum, axilla and hair, which were considered to be carrier sites, were excluded. Tables I and II show the numbers of patients from whom GMRSA were isolated in specimens from individual sites. Patients from whom GMRSA were isolated from more than one similar site are included once only, even though GMRSA isolates from these sites sometimes showed minor differences in morphology, antibiotic susceptibility pattern or phage type. When the results were analysed by these methods, 849 patients were colonised or infected at 924 sites of which 680 sites (73.6%) were superficial.

Wounds—surgical and non-surgical including pressure sores and ulcers—and burns, were the sites from which GMRSA were isolated most frequently. The urinary

<p>| TABLE I |</p>
<table>
<thead>
<tr>
<th>Isolation of GMRSA from superficial sites of 849 patients (1976–1980)</th>
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<tbody>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Wound</td>
</tr>
<tr>
<td>Burn</td>
</tr>
<tr>
<td>Foreign body†</td>
</tr>
<tr>
<td>Ulcer</td>
</tr>
<tr>
<td>Pressure sores</td>
</tr>
<tr>
<td>Sinus</td>
</tr>
<tr>
<td>Tracheostomy</td>
</tr>
<tr>
<td>Conjunctivitis</td>
</tr>
<tr>
<td>Arterial fistula</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

* One isolate only was recorded per site per patient; patients were sometimes colonised at more than one site.
† Site of drain, drain tip, fluid from drain, pin, rod, pacemaker wire.
TABLE II

Isolation of GMRSA from deep sites in 849 patients (1976–1980)

<table>
<thead>
<tr>
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<th></th>
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</thead>
<tbody>
<tr>
<td>Urinary tract</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>23</td>
<td>58</td>
<td>108</td>
</tr>
<tr>
<td>Urine Catheter tip</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>Sputum</td>
<td>1</td>
<td>7</td>
<td>17</td>
<td>20</td>
<td>29</td>
<td>74</td>
</tr>
<tr>
<td>Blood</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>11</td>
<td>15</td>
<td>31</td>
</tr>
<tr>
<td>Abscess</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Osteomyelitis†</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>13</td>
<td>39</td>
<td>65</td>
<td>118</td>
<td>238</td>
</tr>
</tbody>
</table>

* One isolate only was recorded per site per patient; patients were sometimes colonised at more than one site.
† All were post-operative infections and five were also post-traumatic.

tract was the site of infection in 12.7% of patients; the organisms were isolated from sputum or tracheal aspirate from 8.7%. Bacteraemia was documented in 3.5% of infected patients.

Presence of other organisms

Superficial sites. GMRSA isolated from burns and graft wounds were usually in mixed culture, most commonly with various gram-negative bacilli. Infected surgical wounds usually gave a pure growth of GMRSA, and the appearance of the gram-stained smears was compatible with the presence of staphylococci only. The majority (>90%) of specimens from the sites of “foreign body” implants, drains and sinuses yielded pure cultures as did specimens from conjunctivae, arterial fistulas and intravenous infusion sites. In specimens from pressure sores and ulcers, a mixed flora was usually demonstrated by both gram-stained smear and culture; however, GMRSA was the predominant organism.

Deep sites. A pure growth of GMRSA was isolated from urinary catheter tips, intravenous catheter tips, abscesses, pleural fluid and specimens from osteomyelitis. Gram-stained smears of the specimens revealed pus cells and gram-positive cocci in clusters.

GMRSA isolates from urine were obtained in pure culture in c. 90% of cases. Sputum was cultured by a dilution method; most specimens yielded >10^7 colonies of GMRSA/ml. In all cases, the gram-stained smear of the specimen showed profuse pus cells and intra- and extra-cellular gram-positive cocci in clusters.

Isolation of GMRSA from out-patients

GMRSA were frequently isolated from infected patients for many months after discharge from hospital. This was not studied formally and isolates were from specimens taken on attendance at the out-patient or casualty department. Specimens from decubitus ulcers, pressure sores, operative wounds, stitch sinuses or wounds
overlying irradiated areas accounted for the great majority of isolates. The \textit{S. aureus} carriage sites were not studied in these patients.

One specimen received from a general practitioner yielded GMRSA. The patient was a 3-year-old child with burns who attended two children's hospital casualty departments for dressings. The burns were being treated with topical fusidic acid.

\textit{Antimicrobial sensitivity tests}

Results of disk tests with 59 strains of GMRSA are shown in table III. All strains were sensitive to rifampicin and vancomycin. Most were resistant to sulphonamides (Su) and trimethoprim (Tmp). However, Su and Tmp sensitivities were variable; of otherwise indistinguishable colonies picked from a seemingly pure strain, some were resistant while others were sensitive to these two antibiotics. When Su and Tmp susceptibility testing was performed on isolates from successive specimens from the same site, similar variation was seen.

Fig. 3 shows the MICs of gentamicin, tobramycin, kanamycin, neomycin,
amikacin and vancomycin for 33 isolates from bacteraemia and severe infections. MICs of methicillin were determined for 75 strains from many sources (wounds, burns, bacteraemia, serious infections and samples of air). The MICs of methicillin were ≥200 mg/L for all strains.

**Bacteriophage typing of GMRSA isolates**

The initial GMRSA isolate was sensitive to a wide range, including Group I and Group III phages; it gave a type profile of 52/52A/6/42E/47/54/75/77/84/81/94 at 100 RTD. No *S. aureus* with this broad range was detected before or since. Subsequent isolates were of various phage types. Most isolates in 1976 and during the first 6 months of 1977 were type 77/84 or 77/+. Such isolates were sometimes found throughout the remainder of the study. GMRSA isolates of phage type 85 appeared in January 1977 and were the predominant type isolated during the second 6 months of 1977 and most of 1978. Strains that were untypable with the routine set of phages and some that gave inhibition reactions with a small number of group III phages at 100 RTD also emerged at the beginning of 1977. The latter isolates usually exhibited confluent lysis with the experimental phage 90 at 100 RTD. Most GMRSA isolates in 1979 and 1980 gave this pattern. From 6–30% of isolates tested at various times (1978–1980) were completely untypable.

The phage types isolated from blood cultures and from other severe infection are shown in fig. 4. The different types found paralleled the overall phage typing pattern. No type was associated with bacteraemia more often than with severe infection without bacteraemia.

The typability of all strains of *S. aureus* isolated in one hospital (MMH) was assessed over a 6-year period (1975–1980). The results are shown in table IV. Before
TABLE IV

Typability of S. aureus isolates (M.M.H.), 1975–1982

<table>
<thead>
<tr>
<th>Typing phages</th>
<th>Percentage of strains typable* with the given phages in</th>
</tr>
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<tbody>
<tr>
<td>Standard phage set</td>
<td>84</td>
</tr>
<tr>
<td>Standard phages at RTD</td>
<td>60</td>
</tr>
<tr>
<td>Standard phages at RTD x 100</td>
<td>24</td>
</tr>
<tr>
<td>Experimental phages</td>
<td></td>
</tr>
<tr>
<td>Phage 90</td>
<td>0</td>
</tr>
<tr>
<td>Phage 88</td>
<td>0</td>
</tr>
<tr>
<td>Phage 89</td>
<td>0</td>
</tr>
</tbody>
</table>

* Average number of strains typed per year = 300.
† Strains that were untypable with the standard phage set were tested with the experimental phages.

the appearance of GMRSA in the hospital in 1978, >74% of all S. aureus strains isolated were typed by the routine set of phages at RTD or RTD x 100. Subsequently the percentage typability decreased and, in 1980, 59-5% were untypable. Some of the untypable strains were lysed by an experimental phage (90) at RTD x 100 which was introduced into routine typing at RTD x 100 only in 1979. No antibiotic sensitive strains have been found that were lysed by phage 90; most were typable with the routine set of phages at RTD.

DISCUSSION

This survey shows that gentamicin and methicillin resistant S. aureus (GMRSA) have emerged as a serious problem both in the number and severity of infections in our hospitals. Most previously reported outbreaks of infection with gentamicin resistant S. aureus have been small and invasive infections rare. (Shanson et al., 1976; Speller et al., 1976; Public Health Laboratory Service, 1977; Wyatt et al., 1977; Faden et al., 1979; Price et al., 1980). However, in a large outbreak of infection with strains of S. aureus resistant to methicillin and aminoglycosides, invasive infection was more frequently seen (Crossley et al., 1979). In contrast to the findings of Crossley et al. (1979), the multiply antibiotic resistant strains of S. aureus encountered in Dublin hospitals, in this study, were most frequently isolated from superficial sites. This experience was similar to that of other authors (Shanson et al., 1976; Speller et al., 1976; Warren and Roberts, 1976; Bint et al., 1977; Public Health Laboratory Service, 1977; Wyatt et al., 1977; Vogel et al., 1978; Price et al., 1980). Bacteraemia and other severe infections were uncommon in the first 2 years of this study but were more frequently seen subsequently. This increase occurred as the organisms became more widespread in the hospitals. Infection or colonisation with GMRSA was not confined to these hospitals, but was an emerging problem in other Dublin hospitals throughout the study period (Hone et al., 1981). Additionally, patients sometimes transferred from hospitals in different parts of the country were already colonised or infected with GMRSA on admission. Within the Dublin area, some services such as cardiac surgery, plastic surgery and neurosurgery, are located only in certain hospitals so
transfer of patients between hospitals is inevitable. Thus GMRSA may have been introduced or reintroduced into a hospital from any of these sources.

Because of the wide dissemination of GMRSA throughout the Irish Republic, it was interesting that, with the exception of one outbreak of gentamicin resistant infection (Wyatt et al., 1977), strains of \textit{S. aureus} resistant to gentamicin were rarely isolated in Belfast hospitals throughout the study period (Wyatt, personal communication). It is not known whether these organisms are commonly found in other hospitals in Northern Ireland.

In this study, as in the study of Greenhood et al. (1979), the predominant phage type changed with the progression of the outbreak. GMRSA isolates of different phage types were sometimes found in a single lesion, or in different sites in a patient, as had also been reported previously (Wyatt et al., 1977). Phage typing became less helpful as an epidemiological tool as the outbreak progressed. By the second half of 1978, most isolates were untypable with the routine set of phages, some of which typed only with the experimental phage 90 at 100 RTD. Such isolates were essentially untypable. In three other outbreaks, most strains were lysed by the experimental phage 90 in addition to other standard phages in groups I and III (Speller et al., 1976; Bint et al., 1977; Wyatt et al., 1977). New phages or different typing methods are now needed to detect differences between strains in these hospitals. It is intended to investigate the possibility that serotyping and biotyping may detect differences between strains.

We wish to thank Mrs Mary Foody for valuable assistance with preparation of the manuscript.

REFERENCES


