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 10^4 – 10^5 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

FIG. 1. Numbers of patients from whom GMRSA were isolated in each 3-month period from 1 Jul. 1976 to 31 Dec. 1980. — = Total patients, --- = patients with bacteraemia or other severe infections.

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

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

TABLE I
Isolation of GMRSA from superficial sites of 849 patients (1976–1980)

Site of infection	Number of GMRSA strains isolated* in					Total
	1976	1977	1978	1979	1980	
Wound	7	28	72	118	174	399
Burn	13	25	28	36	60	162
Foreign body†	0	2	3	10	18	33
Ulcer	0	1	3	11	20	35
Pressure sores	0	0	4	8	15	27
Sinus	0	0	1	3	5	9
Tracheostomy	0	1	1	1	6	9
Conjunctivitis	0	0	2	0	3	5
Arterial fistula	0	0	0	0	1	1
Total	20	57	114	187	302	680

* 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)

Site of infection	Number of GMRSA strains isolated* in					Totals
	1976	1977	1978	1979	1980	
Urinary tract						
Urine	1 }	3 }	15 }	23 }	55 }	97 }
Catheter tip	0 }	3 }	2 }	3 }	3 }	11 }
Sputum	1	7	17	20	29	74
Blood	1	0	4	11	15	31
Abscess	0	0	1	3	6	10
Osteomyelitis†	0	0	0	1	8	9
Pleural fluid	0	0	0	4	2	6
Total	3	13	39	65	118	238

* 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 >10⁷ 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

TABLE III

Antibiotic sensitivity of 58 GMRSA isolates from patients with severe infections

Antibiotic	Percentage sensitivity	Antibiotic	Percentage sensitivity
Penicillin	0	Tobramycin	0
Methicillin	0	Neomycin	56
Erythromycin	0	Amikacin	92
Clindamycin	24	Sulphonamides	Variable
Fusidic Acid	44	Trimethoprim	Variable
Tetracycline	60	Chloramphenicol	92
Kanamycin	0	Rifampicin	100
Gentamicin	0	Vancomycin	100

overlying irradiated areas accounted for the great majority of isolates. The *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.

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,

FIG. 3. Percentage inhibition of 33 strains of *S. aureus* by increasing concentrations of antibiotic. □ = Amikacin, ■ = neomycin, ● = gentamicin, ○ = vancomycin, ▲ = tobramycin, △ = kanamycin.

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

FIG. 4. Phage types of GMRSA associated with bacteraemia and severe infections without bacteraemia.

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

Typing phages	Percentage of strains typable* with the given phages in					
	1975	1976	1977	1978	1979	1980
Standard phage set	84	78	74.5	63	66	40.5
Standard phages at RTD	60	47	50	43	52	25
Standard phages at RTD × 100	24	31	24.5	20	14	15.5
Experimental phages						
Phage 90	0	0.5	0	5	10	27.5
Phage 88	0	1	0	0	0	0
Phage 89	0	1	1	0	0	0

* 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 × 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 × 100 which was introduced into routine typing at RTD × 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 *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

- ANNEAR, D. I. 1968. The effect of temperature on resistance of *Staphylococcus aureus* to methicillin and some other antibiotics. *Medical Journal of Australia*, **1**, 444–446.
- BINT, A. J., GEORGE, R. H., HEALING, D. E., WISE, R. AND DAVIES, M. 1977. An outbreak of infection caused by a gentamicin-resistant *Staphylococcus aureus*. *Journal of Clinical Pathology*, **30**, 165–167.
- BLAIR, J. E. AND WILLIAMS, R. E. O. 1961. Phage typing of staphylococci. *Bulletin of the World Health Organization*, **24**, 771–784.
- CROSSLEY, K., LOESCH, D., LANDESMAN, B., MEAD, K., CHERN, M. AND 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.
- FADEN, H., NETER, E., McLAUGHLIN, S. AND GIACOIA, G. 1979. Gentamicin-resistant *Staphylococcus aureus*: Emergence in an Intensive Care Nursery. *Journal of the American Medical Association*, **241**, 143–145.
- GREENHOOD, G. P., HILL, D. L., DIXON, R. E., CARTER, M. J. AND KANTO, W. P. 1979. Changing phage typing patterns of epidemic gentamicin-resistant *Staphylococcus aureus*. Evidence for transmission of gentamicin resistance. *Lancet*, **1**, 289–291.
- HONE, R. AND KEANE, C. T. 1974. Characteristics of methicillin resistant *Staphylococcus aureus*. *Irish Journal of Medical Sciences*, **143**, 145–154.
- HONE, R., CAFFERKEY, M. T., KEANE, C. T., HARTE-BARRY, M., MOORHOUSE, E., CARROLL, R., MARTIN, F. AND RUDDY, R. 1981. Bacteraemia in Dublin due to gentamicin-resistant *Staphylococcus aureus*. *Journal of Hospital Infection*, **2**, 119–126.
- LACEY, R. W. 1975. Antibiotic resistance plasmids of *Staphylococcus aureus* and their clinical importance. *Bacteriological Reviews*, **39**, 1.
- LACEY, R. W. AND MITCHELL, A. A. B. 1969. Gentamicin-resistant *Staphylococcus aureus*. *Lancet*, **2**, 1425–1426.

- PRICE, E. H., BRAIN, A. AND 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.
- PUBLIC HEALTH LABORATORY SERVICE. 1977. Unusual antibiotic-resistant staphylococcus in a hospital. *British Medical Journal*, **2**, 325.
- SHANSON, D. C., KENSIT, J. G. AND 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. AND DUVAL, J. 1975. A gentamicin resistance plasmid in *Staphylococcus aureus*. *Annals of Microbiology (Paris)*, **126b**, 91-94.
- SOUSSY, C. J., DUBLANCHET, A., CORMIER, M., BISMUTH, R., MIZON, F., CHARDON, H., DUVAL, J. AND FABIANI, G. 1976. Nouvelle resistances plasmidiques de *Staphylococcus aureus* aux aminosides, (Gentamicine, tobramycin, amikacine). *La Nouvelle Presse Médicale*, **5**, 2599-2602.
- SPELLER, D. C. E. and 7 others. 1976. Epidemic infection by a gentamicin resistant *Staphylococcus aureus* in three hospitals. *Lancet*, **1**, 464-466.
- STOKES, E. J. AND WATERWORTH, P. M. 1972. Antibiotic sensitivity tests by diffusion methods. *Association of Clinical Pathologists, Broadsheet No. 55*.
- VOGEL, L., NATHAN, C., SWEENEY, H. M., KABINS, S. A. AND COHEN, S. 1978. Infections due to gentamicin-resistant *Staphylococcus aureus* strain in a nursery for neonatal infants. *Antimicrobial Agents and Chemotherapy*, **13**, 466-472.
- WARREN, R. E. AND ROBERTS, S. O. B. 1976. Gentamicin-resistant staphylococci. *Lancet*, **2**, 543-544.
- WYATT, T. D., FERGUSON, W. P., WILSON, T. S. AND McCORMICK, E. 1977. Gentamicin-resistant *Staphylococcus aureus* associated with the use of topical gentamicin. *Journal of Antimicrobial Chemotherapy*, **3**, 213-217.