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Investigations of the Occurrence of Gentamicin-Resistant *Staphylococcus aureus*

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During the 19-month period from June 1976 to December 1977, 90 patients became colonized or infected with gentamicin-resistant *Staphylococcus aureus* (GRS). Of 63 adults, 56 had hospital-acquired GRS, whereas only 9 of 27 children had hospital-acquired GRS ($P < 0.001$). The other 7 adults and 18 children had GRS present on admission. More than half of those who acquired GRS in the hospital had received prior aminoglycoside therapy. Attack rates were higher in adults than in children and significantly higher on the plastic surgery service than on any other adult service. Phage typing revealed a single-strain outbreak on the plastic surgery ward involving 11 patients, whereas other isolates were of several phage types. Community-acquired GRS occurred more frequently in rural native communities ($P < 0.02$) and may be related to the use of topical gentamicin. Of 17 native children, 10 were from the same area but there was no common phage type. Agar dilution minimal inhibitory concentration (MIC) testing confirmed that all isolates were gentamicin resistant (MIC $\geq$ 8 μg/ml) and almost all were tobramycin resistant (MIC $\geq$ 8 μg/ml). Although the MIC distribution between gentamicin disk-susceptible and -resistant strains was significantly different, MIC's for 90% of gentamicin disk-resistant strains were $\leq$8 μg of amikacin per ml, and MIC's for 92% of the strains were $\leq$4 μg of netilmicin per ml.

Recently, reports of clinical isolates of gentamicin-resistant *Staphylococcus aureus* (GRS), mostly of single isolates or outbreaks associated with a single phage type, have appeared in the literature (2, 9, 10, 13, 15-18, 20). In June 1976, we became concerned with the possible occurrence of GRS in our institution and began testing all clinical isolates of *S. aureus* for susceptibility to gentamicin. When it became apparent that GRS were being isolated with increasing frequency, more detailed clinical, epidemiological, and microbiological investigations were undertaken. Our observations on the occurrence of GRS in hospitalized patients from June 1976 to December 1977 are reported below. To our knowledge, this is the first report of the simultaneous occurrence of GRS in both a large community and a hospital population.

MATERIALS AND METHODS

The Health Sciences Centre is a 1,298-bed teaching hospital which is geographically divided into a number of centers: the General Centre (728 beds) provides acute adult medical, surgical, and psychiatric care; the Children's Centre (204 beds) provides care to children and adolescents; the Rehabilitation Centre (148 beds) provides facilities for rehabilitation medicine; the Respiratory Centre (58 beds) provides care for pulmonary diseases and tuberculosis; and the Women's Centre (160 beds) provides obstetrical and gynecological care. All of these centers are serviced by a single clinical microbiology laboratory.

Organisms were identified as *S. aureus* if they had typical colonial and microscopic morphology and produced coagulase. All isolates were routinely tested for antimicrobial susceptibility by using a modified Kirby-Bauer technique (3). Isolates were considered gentamicin resistant if the zone of inhibition around a 10-μg gentamicin disk was 15 mm or less.

The resistance patterns of all isolates were reviewed on a daily basis by one of the infection control nurses (J.L. and R.H.). Patient charts were reviewed on a regular basis by two of the authors (F.B. and W.A.) for compilation of clinical and epidemiological data.

Acquisition of GRS was defined as community acquired if GRS was present on admission. The definition for hospital acquisition included the absence of GRS on admission cultures with appearance of GRS in subsequent cultures from the same site.

Each decision on the role played by the GRS was based on the assessment of the treating physician(s). When this was not clearly stated in the chart of a patient, the following criteria were used. A site was classified as colonized if there was no evidence of local inflammation, if there was no polymorphonuclear pleocytosis on Gram stain, or if the presence of the

organisms had no bearing on the clinical course of the patient. A site was classified as clinically infected if there were signs of inflammation or if the presence of the organisms affected the patient's course. Urinary tract infection was defined as the presence of $\geq 10^5$ organisms per ml of urine on a midstream or catheterized specimen.

Minimal inhibitory concentrations (MIC's) of gentamicin, tobramycin, amikacin, and netilmicin were determined by an agar dilution method, using Mueller-Hinton agar. Organisms were grown to a concentration of 10^8 organisms per ml in Mueller-Hinton broth, diluted to 10^6 organisms per ml in saline, and inoculated with a Steers replicator. Reliability of the method was monitored by inclusion of a strain of *Escherchia coli* of known susceptibility (ATCC 25922).

Phage typing was performed by the Canadian Staphylococcus Phage-Typing Reference Centre of the Laboratory Centre for Disease Control, Ottawa, Canada.

Statistical comparison of mutually exclusive events was by contingency table chi-square analysis. The distribution of MIC's for each antibiotic between gentamicin disk-susceptible and -resistant strains was analyzed by the Kolmogorov-Smirnov two-sided test.

RESULTS

Clinical and epidemiological data. During the 19 months of observation, 90 hospitalized patients were found to be colonized or infected with GRS; 63 were adults, and 27 were children. An epidemic graph of the number of colonized or infected patients each month is shown in Fig. 1. The number of cases per month increased steadily over this period. More than half of the cases (59%) occurred during the last 6 months of the study. This increase occurred in both children and adults.

Of the 63 adults, 56 acquired GRS during their hospitalization, and seven were colonized or infected on admission. Of the 27 children, 9 acquired the organism during their hospitalization, and 18 were colonized or infected on admission ($\chi^2 = 26.37$, $P < 0.001$).

Hospital-acquired GRS. The 56 adults ranged in age from 17 to 85 years, and the 9 children ranged in age from 7 days to 15 years. Reasons for admission for the adults were trauma (17 patients), surgery (16 patients), burns (12 patients), chronic illness (10 patients), and myocardial infarction (1 patient). Duration of hospitalization before acquisition of GRS varied from 1 day to 17 months (median, 4 weeks) in adults, and from 3 days to 5 months (median, 7 days) in children. A total of 35 of the adults and 2 of the children underwent surgical procedures before colonization or infection with GRS; 31 adults and 5 children had received an aminoglycoside antibiotic before acquisition of GRS.

Of the adults, 43 became colonized in 44 sites

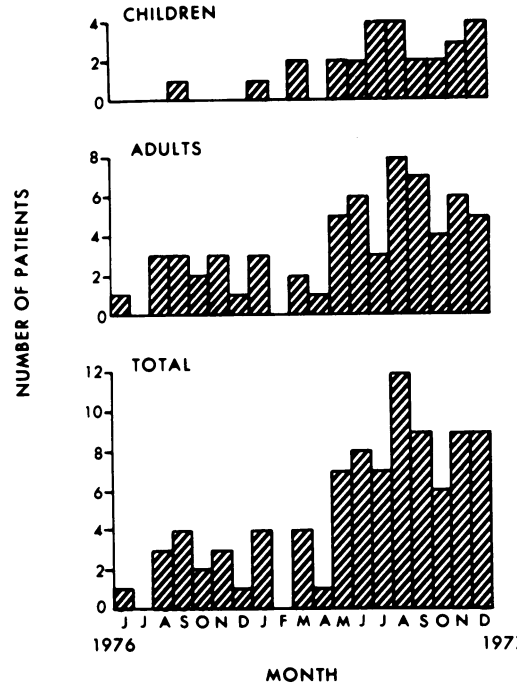

FIG. 1. *Epidemic graph of gentamicin-resistant S. aureus. June 1976 through December 1977.*

but did not become infected. Colonized sites included surgical wounds (20 patients), burn wounds (9 patients), chronic skin ulcerations (6 patients), oropharynx (4 patients), urine (3 patients), skin abrasion (1 patient), and anterior nares (1 patient).

Thirteen adults became infected with GRS. Five developed urinary tract infection, four developed skin infection, two developed bacteremia, and one developed pneumonia. Another patient with a chronically infected femoral head prosthesis and an open wound became infected with GRS after multiple courses of antibiotics, including gentamicin. Two of these patients died of their infection. One patient with bacteremia developed shock and died in spite of appropriate anti-staphylococcal therapy. Postmortem examination revealed evidence of acute staphylococcal endocarditis. The other patient died in spite of appropriate therapy for postoperative staphylococcal pneumonia. Postmortem examination revealed extensive bilateral staphylococcal pneumonia with cavitation and abscess formation.

Six children became colonized but did not become infected. Colonized sites included burn wounds (three), skin lesions (two), and a surgical wound (one).

Three children became infected with GRS.

Acute lymphadenitis developed in one patient, a surgical wound abscess developed in one patient, and acute conjunctivitis developed in one patient. The child with the surgical wound abscess died in spite of appropriate anti-staphylococcal therapy. Postmortem findings included a cerebellar staphylococcal abscess.

Attack rates (number of patients colonized or infected/number of discharges × 100%) were calculated for the various areas of the Health Sciences Centre. The attack rate for hospitalized children was 0.08%, whereas that for hospitalized adults was 0.15%. There were no resistant isolates from adults hospitalized in the Women's Centre or in the psychiatric units of the General Centre and, eliminating these patients from the denominator, the attack rate for adults becomes 0.23% ($\chi^2 = 10.05$, $P \leq 0.01$, compared to children).

Attack rates for adults, broken down by services, are shown in Table 1. There was a strikingly higher attack rate for the plastic surgery service (1.47%) than for any other service ($\chi^2 \geq 22.19$, $P < 0.001$).

Analysis of phage typing of strains from 43 of the 65 patients with hospital-acquired GRS revealed that, except for isolates from plastic surgery patients, there were no common phage types. However, of 16 plastic surgery patients whose isolates were phage typed, 11 were the same type (53/42E). These patients became colonized between May and October 1977. Eight had colonized burn wounds, and three had colonized surgical wounds. One patient developed skin infections subsequent to burn wound colonization. During this same period, only one other plastic surgery patient became colonized with GRS, and phage typing indicated his isolate to be a different strain (type 29). During November and December 1977, four plastic surgery patients became colonized with four different GRS strains.

A review of Infection Control Unit surveillance records indicated that the overall rate of colonization with *S. aureus* had been considerably higher on the plastic surgery ward than on other wards in the hospital. This is felt to be related to the type of patient admitted to this service, which includes patients with major burns and with large open wounds. Topical gentamicin is rarely administered to burn patients in our institution because of previous experience with development of gentamicin-resistant *Pseudomonas aeruginosa* (6, 14), and none of the patients in this series had received topical gentamicin.

TABLE 1. *Gentamicin-resistant S. aureus attack rates for hospitalized adults*

Location and service	No. of patients colonized or infected	Attack rate (%)
General Centre	52	0.23
Internal medicine	4	0.05
General surgery	19	0.35
Plastic surgery	21	1.47[a]
Other surgical services	7	0.12
Psychiatry	0	0.00
Rehabilitation Centre	3	0.23
Respiratory Centre	1	0.08
Women's Centre	0	0.00

[a] $\chi^2 \geq 22.19$, $P < 0.001$ compared to any other service.

A recent surveillance of the plastic surgery ward staff, including physicians, revealed an *S. aureus* carriage rate of 19% in the anterior nares, whereas the carriage rate by staff of a similar-size general surgical ward in the same wing of the hospital was 34%. None of these isolates were gentamicin resistant. *S. aureus* has never been grown from environmental surveillance cultures of the plastic surgery ward.

Community-acquired GRS. Patients with community-acquired GRS ranged in age from 22 to 76 years for adults and 1 month to 8 years for children. Reasons for admission for the adults were chronic skin ulcers (four), infected skin lesions (two), and lung abscess (one). Reasons for admission for the children were acute infections (eight), gastroenteritis (six), burn (one), trauma (one), leukemia (one), and family situation (one). A number of the children had received topical antibiotics, type unknown, for skin or ear lesions.

Of 7 adults, 5 were from an urban area, whereas 16 of 18 children were from rural areas ($\chi^2 = 6.35$, $P < 0.02$). Only 2 of 7 adults were native Indian or Inuit (Eskimo), whereas 17 of 18 children were native Indians ($\chi^2 = 8.65$, $P < 0.01$). Ten of the 17 native children were from two reserves in close geographic proximity, and each of these 10 had colonized or infected skin or ears. The other seven native children were from other rural areas without geographic clustering.

Three of the adults were colonized only, each with chronic skin ulcerations. Four adults were infected at the time of admission with GRS. Infected sites included chronic skin ulcer (one), traumatic wound (one), psoriatic skin lesion (one), and lung abscess (mixed with gentamicin-susceptible *S. aureus*) secondary to obstructing bronchogenic carcinoma (one).

Six of the children were colonized, and 12 were infected. Colonized sites included external ear canals (four), oropharynx (one), and skin (one). Infected sites included skin (seven), external ear canals (three), the eye (one), and a burn wound (one).

Strains from 16 of 25 patients with community-acquired GRS were phage typed. There was no common phage type among the strains, including the strains from 6 of the 10 natives from the same area.

Microbiology. MIC's of gentamicin, tobramycin, amikacin, and netilmicin were determined for 72 strains of GRS. MIC's of 72 strains of gentamicin disk-susceptible *S. aureus* from separate patients were also determined for comparison (Table 2). The MIC's of gentamicin for all 72 strains resistant by disk testing were 8 μg or greater per ml, and results were similar for tobramycin. Although the MIC distribution for amikacin and netilmicin between gentamicin disk-susceptible and -resistant strains was significantly different ($P < 0.001$), 90% of the strains were susceptible to amikacin (≤8 μg/ml), and 92% of the strains were susceptible to netilmicin (≤4 μg/ml).

From June 1976 to December 1977, 4% of all isolates of *S. aureus* reported from the clinical microbiology laboratory were gentamicin resistant by disk susceptibility testing. All of these isolates were susceptible to penicillinase-resistant penicillins. During this period, the rates of isolation of gentamicin-resistant strains of *S. aureus* rose from 2.5% in the last 6 months of 1976 to 5.3% in the last 6 months of 1977 ($\chi^2 = 24.9$, $P < 0.001$).

Seven of the initial isolates were sent to Julian Davies at the University of Wisconsin. All seven strains were found to have a gentamicin-phosphorylating enzyme and some also contained an adenylating enzyme of low activity and an acetylating enzyme. These enzymes are typical of similar resistant strains that have been previously reported and are similar to those commonly found in aminoglycoside-resistant gram-negative bacilli (2, 4, 10, 12, 16). Our strains were also found to contain a variety of plasmids. Further work to determine which, if any, of these plasmids determine the resistance enzymes is presently underway.

DISCUSSION

We have described the occurrence of both hospital- and community-acquired GRS in a large teaching hospital. More than half of the hospital-acquired GRS occurred in patients who had previously received an aminoglycoside antibiotic during their hospitalization. This finding is similar to recent reports of hospital outbreaks of GRS in Britain and Australia (2, 9, 17).

Previous studies on other strains (2, 4, 10, 12, 16), and preliminary studies on our strains, have shown that the aminoglycoside resistance may be mediated by drug-inactivating enzymes. Antibiotic resistance in *S. aureus* is often plasmid determined, with spread of resistance from strain to strain by bacteriophage-mediated transduction (17). Transduction of gentamicin resistance from a resistant to a susceptible strain has been demonstrated by Bint et al. (2). Soussy and associates have demonstrated that the gentamicin resistance plasmid locus is distinct from that imparting penicillin resistance (19).

Phage typing and epidemiological study of our isolates has shown that, with the exception of the outbreak on the plastic surgery ward, the increasing occurrence of GRS was associated with a number of different strains. We believe that this may be due to the selective pressure of the large amounts of aminoglycosides administered to hospitalized patients.

The outbreak on the plastic surgery ward with the same strain occurred over a 6-month period in long-stay patients with either major burns or large open wounds requiring extensive daily dressing changes. It is likely that spread of this strain was from patient to patient via hand carriage by ward staff. This is supported by the absence of GRS in the ward staff surveillance. The absence of this strain on this ward in the last 2 months of the study may have been related to our awareness of the problem and stricter adherence to aseptic techniques by the ward staff. Since the end of the study period, isolates

TABLE 2. *Agar-dilution MIC's of four aminoglycosides for 144 strains of S. aureus*[a]

Antibiotic	Gentamicin disk susceptibility	No. of strains for which MIC (μg/ml) was:									
		≤0.125	0.25	0.5	1	2	4	8	16	32	≥64
Gentamicin	Susceptible	10	55	5	2						
	Resistant							2	26	31	13
Tobramycin	Susceptible	13	58								1
	Resistant						3	13	46	3	7
Amikacin	Susceptible			26	32	12	2				
	Resistant			1		43	18	3	4	2	1
Netilmicin	Susceptible		47	25							
	Resistant			2	4	54	6	3	2		1

[a] Of these strains, 72 were gentamicin disk susceptible and 72 were disk resistant.

of GRS from this ward have continued to be of differing phage types.

The community-acquired GRS isolates were also found by phage typing to be different strains. The high proportion of native Indians with community-acquired GRS is of particular interest. There has been widespread use of topical gentamicin preparations, especially for treatment of skin and ear infections, in the native population. Since most patients had colonization or infection involving the skin or ears, it is probable that the occurrence of community-acquired GRS is due to the selective pressure of the use of topical gentamicin. Association of the appearance and spread of GRS with the use of topical aminoglycoside preparations has been previously reported (18, 20).

A number of authors now accept the use of carbenicillin and an aminoglycoside alone for initial empirical management of febrile episodes in the compromised host (3, 7, 15), assuming that the aminoglycoside will provide initial anti-staphylococcal coverage (11). The presence of GRS in an institution may require the addition of an appropriate anti-staphylococcal antibiotic for the initial management of febrile episodes in the compromised host.

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