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Gentamicin Use and *Pseudomonas* and *Serratia* Resistance: Effect of a Surgical Prophylaxis Regimen

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An outbreak of prosthetic valve endocarditis due to methicillin-resistant *Staphylococcus epidermidis* prompted a change in antimicrobial prophylaxis for open heart surgery in a general hospital from a regimen of aqueous penicillin G, methicillin, and kanamycin to a 5-day regimen of cefazolin and gentamicin. As a result, total gentamicin use in the hospital more than doubled. Increased resistance of pseudomonas and serratia isolates paralleled the increased total use of gentamicin. For pseudomonas species, the incidence of gentamicin resistance increased from 3 to 15%; for serratia species, from 8 to 88%; and for the total of both organisms, from 4 to 28%. Resistance decreased rapidly after removal of gentamicin from the prophylaxis regimen. Review of serratia isolates from the urinary tract showed that gentamicin resistance was associated with prior antibiotic therapy, especially with gentamicin, care on the surgical services, especially the surgical intensive care unit, and presence of indwelling bladder catheters. Gentamicin use in a 5-day antimicrobial prophylaxis regimen for open heart surgery can represent a large proportion of the total hospital use of that antibiotic, with potential adverse effects on hospital flora.

Studies of bacterial endocarditis involving prosthetic heart valves have stressed the considerable morbidity and mortality of this infection (5, 11, 17, 18, 20, 34, 39). Wilson et al. noted a high incidence of gram-negative bacterial infection in both early and, in contrast to other studies, late infections (39). They recommended consideration of a prophylactic regimen consisting of methicillin and gentamicin at the time of prosthetic valve surgery, since this combination would be effective against most bacteria causing early-onset prosthetic valve infection. Further, they suggested that the short duration of the prophylactic regimen would be unlikely to induce resistant bacterial flora and superinfection.

We have observed the effects of a similar prophylactic regimen at our hospital. In contrast to the reasonable expectations of Wilson et al., we found substantial changes in bacterial flora. Our data show that prophylactic surgical antibiotic use can represent a large proportion of a hospital's total use of that antibiotic, correlating with induction of resistant bacterial flora. We report this experience to make others aware of such potential adverse effects, with particular reference to open heart surgery antibiotic regimens.

An outbreak of prosthetic valve endocarditis due to methicillin-resistant *Staphylococcus epidermidis* recently occurred at Strong Memorial Hospital, a 706-bed referral and primary care facility, whose Cardiovascular Surgery Service perform an average of six open heart procedures weekly. All open heart surgery patients receive initial postoperative care in the surgical intensive care unit. The six patients involved in the outbreak underwent surgery between February and April 1974, and received a routine 5-day prophylactic antibiotic regimen consisting of aqueous pencillin (4×10^6 U/day), methicillin (4 g/day), and kanamycin (15 mg/kg per day). The *S. epidermidis* strains isolated from the patients were uniformly susceptible to cephalosporins, gentamicin, and vancomycin. Details of this outbreak of prosthetic valve endocarditis are presented elsewhere (Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. no. 192, 1975). With recognition of the outbreak in June 1974, the prophylactic antibiotic regimen for open heart surgery was changed to a 5-day course of cefazolin (21 mg/kg per day) and gentamicin (4.5 mg/kg per day), with some surgeons adding penicillin. The criteria for therapeutic use of gentamicin remained constant during the entire period: infection with gram-negative bacilli, proven or strongly suspected of being resistant to kanamycin.

The Hospital Infection Control Program is regularly informed of isolation of unusual or

multiply antibiotic-resistant microorganisms. An increased incidence of gentamicin resistance, most notable for pseudomonas and serratia species, was evident by November and December 1974. As a result of this increase, coupled with an end to the earlier outbreak of prosthetic valve endocarditis, gentamicin was removed from the prophylactic antibiotic regimen for open heart surgery. Coincident with the change to a 5-day regimen of cefazolin (21 mg/kg per day) and kanamycin (15 mg/kg per day) on 25 February 1975, the entire patient population was moved to locations in a new hospital building recently constructed. Continued monitoring of resistance patterns of the hospital bacterial flora revealed an initial rapid decrease in incidence of resistance, followed by further gradual decrease in the months subsequent to the change in regimen and movement to a new building.

More detailed review of gentamicin use and resistance patterns of gram-negative bacterial flora was then undertaken, as outlined below. Pseudomonas and serratia species were evaluated, since these had been the most prominent and clinically important organisms during that period.

MATERIALS AND METHODS

Pharmacy records were reviewed for total monthly unit charges to hospital patients for gentamicin from 1971 through 1975. This provided an indirect, although reasonably accurate, appraisal of gentamicin received by the patients.

All cultures of blood, urine, and exudates (including other body fluids, such as cerebrospinal fluid, joint fluid, wound drainage, surgical specimens, transtracheal specimens, and eye and ear specimens) were reviewed for the period of study. Data were specifically collected on all isolates of pseudomonas and serratia species. More than one isolate of either organism from a single patient was recorded only if these isolates had different antibiograms or were cultured from different clinical sites, such as urine and wound. Susceptibilities were determined by standard Kirby-Bauer disk susceptibility methods (3). Susceptibility testing by tube dilution methods was performed on a number of isolates of both pseudomonas and serratia, in instances of clinical infection meriting antimicrobial therapy.

Two gentamicin-resistant blood culture isolates of *Serratia marcescens* were available for further bacteriological investigation at the time of this study. Conjugation experiments were performed with both isolates to determine whether R factors could be implicated as a basis for their gentamicin resistance. The methods of Cooksey et al. (8) for such assays involving *S. marcescens* were used. *Klebsiella pneumonia* strain KB5-B1, very kindly provided by W. E. Farrar, Jr., served as the recipient strain. Incubation for conjugation was maintained for up to 72 h at 22 and 37°C, with mating mixtures plated at 24-h intervals on gentamicin-containing media. Subsequently, with availability of agarose gel electrophoretic methods, examination of the serratia strains for plasmid deoxyribonucleic acid was performed (26) in parallel with known positive and negative control organisms.

Detailed chart reviews were done on patients with urinary tract isolates of serratia during the period of study. Available records (78 of the total 116 cases) of patients with either gentamicin-susceptible or -resistant isolates were analyzed.

RESULTS

The data on gentamicin use are presented in Table 1. Total use of this antibiotic was fairly constant for both the 3 years and the 6 months before the period under consideration. Thus, there was no steady gradual increase in gentamicin use that might obscure the findings of the study period. It can be seen, however, that total use of gentamicin more than doubled during the period from July 1974 to February 1975, the period when gentamicin was part of the 5-day prophylactic antibiotic regimen for open heart surgery. Calculation of monthly gentamicin use for patient days on the surgical prophylaxis regimen accounts almost completely for the increased use of that antibiotic.

The data accumulated from review of clinical bacterial isolates during the period of study are summarized in Fig. 1. The results from May and June 1974 are representative of isolates of the preceding years. Increases in incidence of gentamicin resistance for pseudomonas, serratia, and for the total of both organisms paralleled increases in the total use of gentamicin. Increased resistance was most evident with serratia isolates. There was an initial marked decrease in resistance in serratia species following the discontinuation of gentamicin prophylaxis, with a more gradual decline in resistance of pseudomonas species apparent even months afterwards. Eight months after these changes, despite

TABLE 1. *Total monthly gentamicin use (g)*

Period	Mean + SE[a]	Range
Preceding 3 years (July 1971–June 1974)	19.36 ± 1.75[b]	6.48–32.88
Preceding 6 months (Jan.–June 1974)	21.81 ± 3.12[b]	12.48–32.88
Study period (July 1974–Feb. 1975)	50.70 ± 4.72	28.98–70.56
Subsequent 4 months (March–June 1975)	29.36 ± 5.26[c]	17.50–42.98

[a] Values represent mean ± standard error. Statistical analysis is based upon comparison of value indicated to that of the study period (July 1974 to Feb. 1975) by *t* test.

[b] $P < 0.001$.

[c] Not significant.

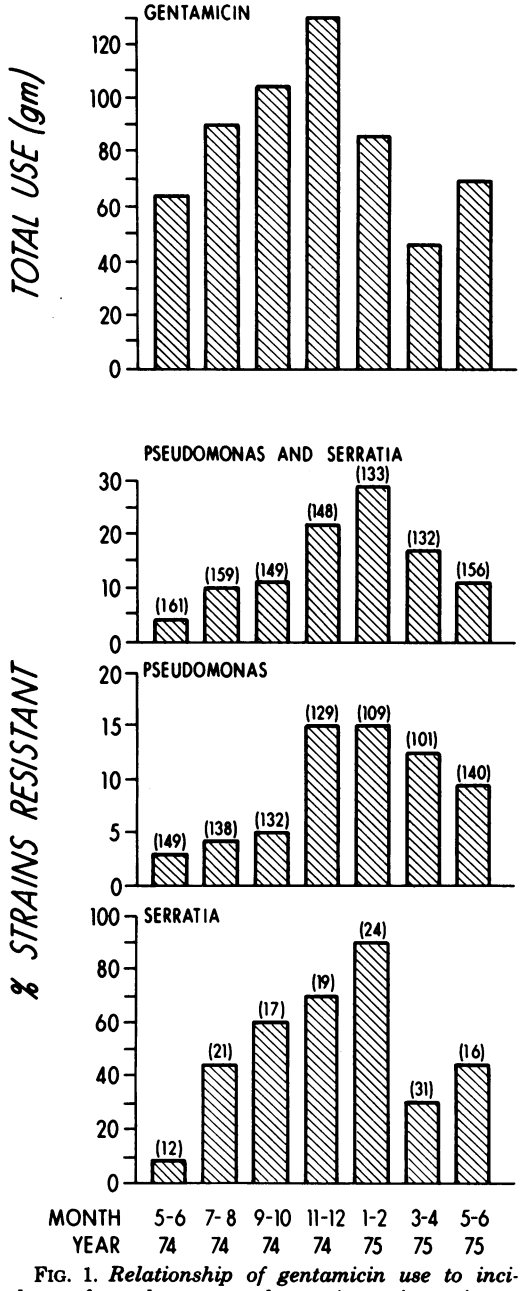

FIG. 1. *Relationship of gentamicin use to incidence of pseudomonas and serratia strains resistant to gentamicin. Total number of isolates is expressed in parentheses. Increased incidence of resistant pseudomonas and, to a greater extent, serratia strains was associated with increased use of gentamicin.*

an intermediate degree of gentamicin use (mean, 38.9 g/month), the incidence of pseudomonas and serratia resistance remained decreased at 11 and 30%, respectively (combined incidence, 16%), with none of the blood isolates showing gentamicin resistance. Use in the latter period was therapeutic, not prophylactic.

The source and resistance patterns of the isolates are further described in Table 2. Most resistant isolates were from urine cultures. Whereas pseudomonas isolated from the blood remained susceptible to gentamicin, this was not the case for serratia; three blood isolates in the periods prior and subsequent to the study period were susceptible to gentamicin, whereas the two isolates during the time of prophylactic gentamicin use were resistant. These serratia isolates were susceptible to amikacin by broth dilution studies. Transfer of R factor-mediated resistance to gentamicin could not be demonstrated with either of the resistant blood culture isolates of *S. marcescens* that were tested. Furthermore, plasmid deoxyribonucleic acid could not be demonstrated using agarose gel electrophoresis. Although resistant pseudomonas species did not produce bacteremia that was detected, they were responsible for several clinically significant cases of tracheobronchitis, wound infection, and urinary tract infection.

Since the increased incidence of gentamicin resistance was more pronounced with serratia strains, with the majority of isolates recovered from the urinary tract, records of 78 patients with serratia urinary isolates during the period from May 1974 to June 1975 were reviewed (Table 3).

Serratia urinary tract infections, both susceptible and resistant to gentamicin, were associated with surgery, presence of an indwelling bladder catheter and prior antibiotic therapy. The first gentamicin-resistant serratia isolate and two of the first three such isolates during this period were from patients who received gentamicin in the open heart surgery prophylaxis regimen and underwent postoperative care on the surgical intensive care unit. Throughout the period of study, if a patient underwent open heart surgery or received gentamicin for other purposes, serratia isolated from the urinary tract was always gentamicin resistant.

The initial seven resistant isolates were identified in patients of the surgical intensive care unit, who were eventually transferred to several private and nonprivate surgical wards. Gentamicin-resistant isolates continued to be predominantly associated with patients in the surgical intensive care unit. However, within 2 months of the emergence of these isolates, they were identified in patients who never received care on the intensive care unit, but who shared regular ward facilities with patients who had previously done so and who had been identified as harboring gentamicin-resistant serratia in their urinary tracts. Common features to both groups of in-

fected patients included prior antibiotic therapy and the presence of indwelling bladder catheters. Five or 6 months after the onset of increased resistance to gentamicin, up to 40% of such isolates were in patients who had never been in the surgical intensive care unit, but who shared ward facilities and staff with patients who had been. Only toward the end of the outbreak were gentamicin-resistant serratia isolated from patients never receiving care on either the surgical intensive care unit or the surgical ward services. After the change in surgical antibiotic prophylaxis,

TABLE 2. *Isolates of pseudomonas and serratia by site and incidence of resistance*

Duration	Site	Pseudomonas			Serratia			Combined pseudomonas and serratia		
		Total no. of isolates	No. R[a]	% R	Total no. of isolates	No. R	% R	Total no. of isolates	No. R	% R
May–June 1974	Blood	3	0	0	1	0	0	4	0	0
	Urine	66	4	6	11	1	9	77	5	6
	Exudates	80	1	1	0	0	0	80	1	1
	Total	149	5	3	12	1	8	161	6	4
July–Aug. 1974	Blood	4	0	0	0	0	0	4	0	0
	Urine	69	2	3	17	7	41	86	9	10
	Exudates	65	3	7	4	2	50	69	5	7
	Total	138	5	4	21	9	43	159	14	9
Sept.–Oct. 1974	Blood	8	0	0	1	1	100	9	1	11
	Urine	66	0	0	12	8	67	78	8	10
	Exudates	58	7	12	4	1	25	62	8	13
	Total	132	7	5	17	10	59	149	17	11
Nov.–Dec. 1974	Blood	2	0	0	0	0	0	2	0	0
	Urine	79	16	20	18	13	72	97	29	30
	Exudates	48	3	6	1	0	0	49	3	6
	Total	129	19	15	19	13	68	148	32	22
Jan.–Feb. 1975	Blood	4	0	0	1	1	100	5	1	20
	Urine	50	10	20	19	16	84	69	26	38
	Exudates	55	6	11	4	4	100	59	10	17
	Total	109	16	15	24	21	88	133	37	28
March–April 1975	Blood	8	0	0	1	0	0	9	0	0
	Urine	56	11	20	26	9	35	82	20	24
	Exudates	37	1	3	4	0	0	41	1	2
	Total	101	12	12	31	9	29	132	21	16
May–June 1975	Blood	3	0	0	1	0	0	4	0	0
	Urine	76	7	9	13	6	46	89	13	15
	Exudates	61	5	8	2	1	50	63	6	10
	Total	140	12	9	16	7	44	156	19	12

[a] R, Resistant.

TABLE 3. *Factors associated with serratia urinary tract isolates*

Factor	Total no. of isolates (%)	No. gentamicin sensitive (%)	No. gentamicin resistant (%)	*P* value[a]
Total number (%)	78 (100)	31 (40)	47 (60)	
Surgery	70 (90)	25 (81)	45 (96)	<0.05
Surgical intensive care unit	44 (56)	11 (35)	33 (70)	<0.01
Open heart surgery	4 (5)	0	4 (9)	NS
Prior antibiotics	60 (77)	21 (68)	39 (83)	NS
Prior gentamicin	12 (15)	0	12 (26)	<0.01
Isolate from second site	6 (8)	3 (10)	3 (6)	NS
$\geq 10^5$ Organisms/ml	53 (68)	20 (65)	33 (70)	NS

[a] χ^2 analysis; NS, not significant.

gentamicin-resistant isolates decreased steadily, though least rapidly in those patients associated with the surgical intensive care unit.

Review of the medical records revealed no difference between gentamicin-susceptible and -resistant serratia strains in regard to virulence, as judged by frequency with which each type reached significant ($\geq 10^5$/ml) colony counts in the urine and, further, with which each type of urinary isolate was associated with blood stream invasion. Both *S. marcescens* and *S. liquefaciens* were isolated among the gentamicin-resistant organisms. Both resistant blood culture isolates were *S. marcescens*. These two strains had identical API characterizations. The patient associated with the first isolate was discharged from the hospital 3 months before the recovery of the second gentamicin-resistant blood culture isolate. Both gentamicin-resistant and -susceptible serratia isolates persisted with bacterial concentrations of $\geq 10^5$ organisms/ml of urine after catheter removal.

DISCUSSION

The present study demonstrates an association between antibiotic use and emergence and spread of microbial resistance to the antibiotic. The data further provide insight into the effect that addition of gentamicin to an open heart surgery prophylaxis regimen can have on a hospital's total gentamicin use and the incidence of gentamicin resistance of its microbial flora. To our knowledge, this is an as yet generally unrecognized source of adverse effect on hospital flora. Whereas this effect was notable at our hospital, other centers with differing patient populations, less open heart surgery, or different postoperative facilities might notice less change in the incidence of microbial resistance to gentamicin. It would appear from our study, however, that monitoring of antimicrobial susceptibility is warranted for all centers considering such a regimen.

Although the association of increased use of an antibiotic and emergence of generalized resistance of the hospital flora to it is commonly accepted, there are few data to support this relationship in the literature. Most studies have involved special units within a general hospital, such as an intensive care nursery (10, 13) or a burn unit (33). Incidence of resistance in other areas of the hospital remained unchanged or was not reported. Emergence of gentamicin resistance has been associated with topical (33) and oral (14) therapy. It has been described with gentamicin use in cancer centers (7, 14). The hospital of the present study is a general referral and primary care facility and, as such, should be expected to reflect the situation in general hospitals more closely than do these prior studies. In the present study, an increase in gentamicin resistance of patients' flora occurred also in those who had not received the antibiotic, but who shared the intensive care setting with postoperative open heart surgery patients who did. This flora was eventually transmitted to patients in other wards, and was reflected in an increased incidence of gentamicin resistance of isolates of pseudomonas and serratia species for the entire hospital flora.

Rennie and Duncan (29), reporting on emergence of gentamicin-resistant *Klebsiella* in a general hospital, showed that an R factor, demonstrated in the first such strain that persisted, appeared to lose the capacity to transfer its gentamicin resistance after infecting several patients. Other strains of R factor-mediated gentamicin-resistant *Klebsiella* infected few patients and did not persist in the hospital. Spread of the resistant organisms occurred when patients were transferred from one ward to another, as appeared to be the case in the present study. The serratia isolates tested for R factor transfer in the current study were isolated 3 to 7 months after the emergence of gentamicin-resistant strains, and may likewise have lost their ability to transfer resistance.

Wilson et al. (39) suggested that since their proposed use of gentamicin in open heart surgery antimicrobial prophylaxis regimens involved a short duration of treatment, little effect on bacterial flora might occur. A prior study (6) did address itself to effects of the duration of antibiotic prophylaxis for cardiac surgery, using the agent cephalothin in a single-dose or a 5-day, multiple-dose regimen. The multiple-dose prophylaxis was felt to have the adverse effect of shifting the bacteria causing wound infections to a more resistant group of organisms. However, sufficient data to support that finding, as well as effect on the general hospital flora, were not reported. A 5-day antibiotic regimen was used in the present study; it would be difficult to infer that less change in microbial flora would have occurred if a similar regimen had been followed for one less day, as proposed by Wilson et al. (39). Again it must be noted that any patient in the present study who had serratia isolated from the urinary tract after undergoing open heart surgery or receiving gentamicin for other reasons harbored a gentamicin-resistant strain of serratia.

Antibiotic use in an open heart surgery antimicrobial prophylaxis regimen thus must be considered as a potentially large and unrecognized proportion of that antibiotic's use in the hospital setting, with the ability to produce adverse effects on hospital bacterial flora. The importance

of gram-negative organisms in terms of morbidity from nosocomial infection has been established, notably for pseudomonas (4, 27) and serratia (9, 21, 30, 31), including postoperative and intensive care unit settings (1, 38). This has included infections due to gentamicin-resistant serratia (24, 32, 36) and other (12, 15, 16, 19) gram-negative bacteria. Resistance of *S. marcescens* is often due to the presence of R factors (8, 23), with transfer of gentamicin resistance more difficult to demonstrate than for other antibiotics (8, 32). In their very exhaustive investigation of R factor-mediated antibiotic resistance of this organism, Cooksey and co-workers (8) were able to transfer antibiotic resistance in only 17 of 39 attempts, using *Klebsiella* as the recipient organism. We were unable to demonstrate presence or transfer of an R factor by the blood isolates of *S. marcescens* available to us.

In the present study, the gentamicin-resistant urinary tract isolates of serratia appeared to have a virulence equal to that of susceptible strains. This is suggested by the frequency with which each type was associated with blood stream invasion, and the frequency with which each type reached significant bacterial concentrations in the urine and persisted after removal of the indwelling bladder catheters. Weinstein et al. (37), in studying isolates of pseudomonas as well as other gram-negative bacteria, found that resistant isolates developed in the laboratory frequently lacked virulence; however, those found clinically can be virulent and, in fact, indistinguishable from susceptible isolates except for their resistance to the antibiotic. Other studies have supported the virulence of gentamicin-resistant clinical isolates of pseudomonas and serratia for patients (2, 25) and in animal models of infection (35).

The association of resistant gram-negative bacterial isolates and either or both indwelling bladder catheters and previous antibiotic therapy is well established (15, 19, 29, 32, 36). The risk of a catheterized patient being colonized or infected with such an isolate in the present study appeared to vary directly with the extent of that patient's proximity to or clustering with other catheterized patients already so colonized, a finding also suggested by others (22).

Factors involved in the reversal of the trend of the hospital flora toward greater gentamicin resistance are less easily determined. It is likely that the change in surgical antimicrobial prophylaxis contributed to the initial marked decrease in incidence of resistance noted in March and April, 1975. Continued decline in incidence of resistance in the subsequent months might be attributed to removal of the selective pressure of a high degree of antibiotic use. This concept would be supported by the experience of others (13). We cannot exclude the possibility that the concomitant move to the new facilities may have contributed to the decline, but no other changes in patterns of antibiotic resistance were observed. Phair et al. (28) have analyzed the prevalence of nasal carriage or hospital acquisition of *S. aureus*, and found no apparent influence exerted by major changes in the hospital environment.

Finally, the experience reported here supports the role of a hospital infection control program. Initial recognition of the problem of gentamicin-resistant isolates, as well as corrective measures, could have been significantly delayed without the routine monitoring of resistant clinical isolates. In reporting experience with detailed surveillance of nosocomial infection, Mulholland et al. (27) felt that the data on isolates from the urinary tract set the pattern for hospital isolates as a whole. It can be seen from our data (Table 2) that this was generally true. Thus, monitoring of resistance of urine isolates might provide a relatively simple screen for increased incidence of resistance when a major change in antibiotic usage takes place.

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