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Tobramycin, amikacin, sisomicin, and gentamicin resistant Gram-negative rods

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Summary

Sensitivities to gentamicin, sisomicin, tobramycin, and amikacin were compared in 196 gentamicin-resistant Gram-negative rods and in 212 similar organisms sensitive to gentamicin, mainly isolated from clinical specimens. Amikacin was the aminoglycoside most active against gentamicin-resistant organisms, *Pseudomonas aeruginosa*, *Klebsiella* spp, *Escherichia coli*, *Proteus* spp, *Providencia* spp, and *Citrobacter* spp being particularly susceptible. Most of the gentamicin-resistant organisms were isolated from the urine of patients undergoing surgery.

Gentamicin was the most active antibiotic against gentamicin-sensitive *E coli*, *Proteus mirabilis*, and *Serratia* spp. *Pseudomonas aeruginosa* and other *Pseudomonas* spp were most susceptible to tobramycin.

Introduction

Since 1964 gentamicin has proved valuable in treating severe infections caused by *Pseudomonas* spp, *Enterobacteria*, and related Gram-negative rods resistant to gentamicin.¹⁻⁴ In one hospital in Los Angeles 20% of clinical isolates of *Pseudomonas aeruginosa* and 50% of *Serratia marcescens* isolates were resistant to gentamicin⁵ and 20% of Gram-negative rods isolated from sputum and blood in a group of hospitals in Japan were resistant to gentamicin.⁶

Gentamicin-resistant organisms have been isolated with increasing frequency at the London Hospital, and we have collected these strains to determine susceptibility to tobramycin, sisomicin, and amikacin. We also compared the activity of the four aminoglycosides against Gram-negative rods sensitive to gentamicin.

Methods

One hundred and ninety-six strains of enterobacteria, *Ps aeruginosa* and other *Pseudomonas* spp, *Acinetobacter* spp, *Alcaligenes* spp, and *Flavobacterium* spp isolated from clinical specimens and which seemed to be resistant to gentamicin using a 10-μg disc were collected over 18 months. Four strains of gentamicin-resistant organisms from environmental sources were included. Another 212 strains of similar organisms sensitive to gentamicin by disc testing were collected over three months.

The minimum inhibitory concentrations (MIC) of gentamicin, sisomicin, tobramycin, and amikacin were measured by the agar dilution technique using doubling dilutions of the antibiotic in DST

TABLE I—Numbers of different species of gentamicin-resistant Gram-negative rods

	No of species		No of species
<i>Providencia</i> spp	35	Indole-positive <i>Proteus</i>	11
<i>Ps aeruginosa</i>	32	<i>Pseudomonas</i> spp	8
<i>Acinetobacter</i> spp	27	<i>Klebsiella</i> spp	8
<i>Enterobacter</i> spp	25	<i>E coli</i>	7
<i>Alcaligenes</i> spp	14	<i>Flavobacterium</i> spp	7
<i>K aerogenes</i>	13	Others	9

TABLE II—Specimens containing Gram-negative rods resistant to gentamicin*

	No of specimens		No of specimens
Urine	119	Pleural fluid	5
Wound swabs	26	Dialysis fluid	4
Sputum	18	Environment	4
Ear swabs	13	CSF	1

*For 6 specimens the sources were unknown

TABLE III—Numbers of gentamicin-resistant Gram-negative rods occurring in different sites in the London Hospital*

	No of isolates		No of isolates
Medical wards	34	Surgical wards:	110
Outpatient clinics:	25	Renal	30
ENT	8	Neurosurgery	26
Surgical	6	Intensive therapy	12
Dermatology	5		

*For 27 isolates the sources were unknown

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agar (Oxoid). An inoculum of about 10^8 log phase bacteria suspended in peptone water was applied to the plates using a multipoint inoculator.

Organisms with an MIC of gentamicin of 8 µg/ml or greater were defined as being resistant. This level was chosen since lower serum concentrations of gentamicin can be maintained throughout most of the treatment of patients suffering from life-threatening infections caused by Gram-negative rods. Sensitive organisms were defined as having an MIC to gentamicin ≤ 4 µg/ml. The distribution of gentamicin-resistant strains in the hospital were studied.

Results

EPIDEMIOLOGY OF GENTAMICIN-RESISTANT ORGANISMS

Table I shows the range of gentamicin-resistant organisms and the number of isolates of each particular species. *Providencia* spp, *Ps*

aeruginosa, *Acinetobacter* spp, and *Enterobacter* spp were the most common organisms. "Other species" included one strain of *S marcescens* and four strains each of *Citrobacter freundii* and *Proteus mirabilis*. The isolation rate of some species was not constant over the 18 months of collection. Most *Ps aeruginosa* strains were collected over the first six months, most *Providencia* spp over the second six months, and most *Enterobacter* spp only over the last six months.

Specimens yielding gentamicin-resistant organisms are shown in table II. Most isolates came from catheter or midstream specimens of urine, and only 26 strains came from the next most common source—wound swabs. The four environmental specimens were samples of water from Winchester bottles used for 24-hour urine collection on the renal ward.

Table III shows the distribution of gentamicin-resistant bacteria in the London Hospital. The isolation rate from surgical wards was over three times that from medical wards. The three main surgical areas affected are shown.

Gentamicin-resistant Gram-negative rods amounted to 1-2% of all Gram-negative rods isolated in this hospital; this represented at least a twofold increase over the previous 18 months. The incidence varied considerably between species. For instance, the overall resistance of *Ps aeruginosa* was around 5%, of *Klebsiella* and *Enterobacter* spp 7%, of *Acinetobacter* spp 20%, and of *Alkaligenes* spp 25%. *E coli* and *P mirabilis* had a very low incidence of gentamicin resistance (around 0.1%). *Providencia* spp and *Flavobacterium* spp isolated from this hospital were invariably resistant to gentamicin.

The use of parenteral gentamicin has more than doubled over the last four years (table IV). The most gentamicin is used in the three

TABLE IV—Amount of gentamicin used in London Hospital from 1972-5

Year	Gentamicin (g) used				Total (g)
	Injectable	Drops	Ointments	Powder	
1972	320	10	45	15	390
1973	540	7	12	6	565
1974	560	5	31	3	599
1975	760	4	27	9	800

TABLE V—MICs of gentamicin, sisomicin, tobramycin and amikacin for Gram-negative rods resistant to gentamicin

Species	No of strains	Antibiotic	No of strains with MICs (µg/ml) of:										
			0.5	1	2	4	8	16	32	64	128	256	> 256
<i>Ps aeruginosa</i>	32	G					8	5	3	4	7	2	3
		S		7	3	4	8	5	3	2			
		T		6	10	7	6	2					
		A	1	5	7	1	6	8	2	1	2		
<i>Pseudomonas</i> spp	8	G					2						2
		S				3	1	1	1	1		1	1
		T			1	4	1	2	1	2	1		2
		A					1						1
<i>K aerogenes</i>	13	G					6	3	1				3
		S					3	6	1			1	2
		T		1		1	4	4	2	1			
		A		7	3		1	1	1				
<i>Klebsiella</i> spp	8	G					2				3	2	1
		S				1	1	1	1		1	2	1
		T		1		4	2	2	1	1			
		A		2	1	2	1	1	1				
<i>E coli</i>	7	G						3	1		1	2	
		S						3				3	
		T					1	2	4				
		A		4		1		1	1		1		
<i>Serratia</i> spp	1	G						1					
		S											
		T					1	1					
		A						1					
<i>Enterobacter</i> spp	25	G									2		23
		S									2	17	6
		T											
		A	1	24				2	20	3			
<i>Citrobacter</i> spp	4	G											4
		S											
		T							3	1			
		A		4									
Indole-positive <i>Proteus</i>	11	G						2	1	3	3	2	
		S						4	2	2	1	2	1
		T					3	3	1	2	1	1	
		A		2	2	2	4	1					
<i>P mirabilis</i>	4	G						1	1	2			
		S						1	2				
		T					1	1	2				
		A		2		1				1			
<i>Providencia</i> spp	35	G					1	10	6	4	4	4	6
		S						4	2	2		9	8
		T						9	6	5	4	3	1
		A		6	10	4	2	2	1		2		
<i>Acinetobacter</i> spp	27	G						6	5	3	2	4	7
		S			1			2	8	5	3	2	6
		T			2	6	1	3	3	3	2	4	3
		A			3	5	1		3	4	3	4	4
<i>Alcaligenes</i> spp	14	G					1	1		1	2	2	7
		S								3	1	3	5
		T					2	2			1	1	4
		A						3	3		1	1	6
<i>Flavobacterium</i> spp	7	G									4	2	1
		S										1	6
		T											7
		A											1

G = Gentamicin. S = Sisomicin. T = Tobramycin. A = Amikacin.

surgical wards listed in table III as having the highest yield of gentamicin-resistant Gram-negative rods.

MICS OF GENTAMICIN-RESISTANT BACTERIA

The MICs of gentamicin, sisomicin, tobramycin, and amikacin are shown in table V. The percentage of strains of gentamicin-resistant organisms with MICs of 8 µg/ml to tobramycin and sisomicin or MICs of 16 µg/ml to amikacin is shown in table VI. The level of 8 or 16 µg/ml were chosen as lower serum levels may be attained throughout most of a course of treatment with these antibiotics. This gives some indication of the in-vitro "susceptibility" or "resistance" of the organisms.

All strains of indole-positive *Proteus* spp, *Enterobacter* spp, and *Citrobacter* spp were sensitive only to amikacin. Amikacin was the most active antibiotic against *E coli*, *K aerogenes*, *Providencia* spp, *Klebsiella* spp, and *Ps aeruginosa*, although tobramycin showed considerable activity over the latter two groups. *Alcaligenes* spp and *Acinetobacter* spp were seldom sensitive to any of the aminoglycosides studied and *Flavobacterium* spp were constantly resistant to all the antibiotics. Sisomicin showed little activity against any of the organisms studied and was most active against *Ps aeruginosa*, 44% of these being susceptible.

TABLE VI—Susceptibility of gentamicin-resistant Gram-negative rods to sisomicin, tobramycin, and amikacin

Species	No of strains	% Susceptible to various concentrations of antibiotics		
		Sisomicin (8 µg/ml)	Tobramycin (8 µg/ml)	Amikacin (16 µg/ml)
<i>Ps aeruginosa</i>	32	44	75	85
<i>Pseudomonas</i> spp	8	40	57	40
<i>K aerogenes</i>	13	0	15	94
<i>Klebsiella</i> spp	8	12	50	75
<i>E coli</i>	7	0	0	72
<i>Serratia</i> spp	1	0	0	100
<i>Enterobacter</i> spp	25	0	0	100
<i>Citrobacter</i> spp	4	0	0	100
Indole-positive <i>Proteus</i>	11	0	0	100
<i>P mirabilis</i>	4	0	0	75
<i>Providencia</i> spp	35	0	14	92
<i>Acinetobacter</i> spp	27	4	30	33
<i>Alcaligenes</i> spp	14	7	14	22
<i>Flavobacterium</i> spp	7	0	0	0

TABLE VII—Mode MICs of gentamicin, sisomicin, tobramycin, and amikacin for gentamicin-sensitive Gram-negative rods

Species	No of strains	Mode MIC (µg/ml) to:			
		Gentamicin	Sisomicin	Tobramycin	Amikacin
<i>Ps aeruginosa</i>	17	1	1	0.25	2
<i>Pseudomonas</i> spp	7	1	2	0.50	4
<i>E coli</i>	52	0.5	1	1	2
<i>K aerogenes</i>	19	0.5	0.5	0.5	1
<i>Klebsiella</i> spp	16	0.5	1	0.5	1
<i>Enterobacter</i> spp	18	0.5	1	0.5	1
<i>P mirabilis</i>	32	0.5	1	1	2
Indole-positive <i>Proteus</i>	23	0.5	1	0.5	1
<i>Serratia</i> spp	7	0.5	2	2	2
<i>Citrobacter</i> spp	14	0.5	1	0.5	1
<i>Acinetobacter</i> spp	7	0.5	1	0.5	2

GENTAMICIN-SENSITIVE BACTERIA

The mode MICs for the main groups of gentamicin-sensitive organisms is shown in table VII. Tobramycin was more active against *Ps aeruginosa* and *Pseudomonas* spp than the three other aminoglycosides. Gentamicin was marginally more active against *E coli*, *Proteus mirabilis*, and *Serratia* spp, but the MICs of gentamicin and tobramycin against other species were comparable. The MICs of sisomicin and amikacin were generally higher than those of gentamicin.

Discussion

The MIC of gentamicin for most enterobacteria is in the range of 0.5-2 µg/ml and for *Ps aeruginosa* is slightly higher at 0.5-4

µg/ml.⁷ Tobramycin has been reported to be two to four times more active than gentamicin against *Ps aeruginosa*.⁸ Our results with gentamicin-sensitive organisms agree with this finding, but there are differing reports from other laboratories on the activity of sisomicin and amikacin. Sisomicin is said to be more active than gentamicin or tobramycin against *E coli*,⁹ but inferior activity has been reported by another group.¹⁰ *Serratia* spp have been said to be most sensitive to sisomicin¹¹ and amikacin.¹² This wide divergence in results from different groups may reflect differences in techniques or even a difference in strains isolated from various sources.

We found that gentamicin was most active against all gentamicin-sensitive rods except *Pseudomonas* spp, which were more sensitive to tobramycin. Gentamicin seems to be suitable for treating infections caused by these organisms, with the possible exception of pseudomonal infections, which might respond more rapidly to tobramycin.

Sisomicin MICs were generally higher than those of gentamicin and since attainable serum levels of these two antibiotics are similar there seems little point in choosing sisomicin.

The amikacin MICs were generally two to four times greater than those for gentamicin, but amikacin is a kanamycin derivative and much higher serum levels can be maintained with relatively few toxic effects.¹³ Both ototoxic and nephrotoxic effects have been reported, however, although such effects are mainly linked with particularly high doses or prolonged courses of amikacin.^{13 14} Further clinical trials are required to compare the therapeutic efficacy and toxic effects of amikacin with those of other aminoglycosides. Amikacin has been reported to be active against gentamicin-resistant *Ps aeruginosa*, *Providencia* spp, and *S marcescens*¹⁵ and showed considerable activity against our gentamicin-resistant Gram-negative rods. Clinical trials with amikacin have given promising results,^{13 16} and this antibiotic may have a place in future chemotherapy, especially if infections with gentamicin-resistant organisms become more common.

The high activity of amikacin against these resistant organisms can be explained by its low susceptibility to enzyme degradation. The ability to produce these aminoglycoside-inactivating enzymes is controlled by plasmids which may be passed to gentamicin-sensitive Gram-negative rods during bacterial conjugation. While gentamicin and sisomicin are susceptible to five enzymes and tobramycin to four, amikacin is inactivated by only one of these enzymes.¹⁷

The isolation rate of gentamicin-resistant Gram-negative rods is increasing in this hospital in parallel with the increased use of parenteral gentamicin. Areas where the most gentamicin is used yield most resistant organisms. There have been reports¹⁸ of resistance developing in bacteria in patients on gentamicin treatment but we have had no experience of this. Several patients acquired resistant organisms during treatment but the infections were such that these organisms might have been introduced from the environment or might have been selected by treatment with gentamicin.

We cannot explain the many *Ps aeruginosa* and *Providencia* spp organisms isolated in the first and second six-monthly periods. Both groups of organisms occurred in widely separated areas of the hospital and the *Pseudomonas* strains belonged to several pyocine types. Many of the *Enterobacter* spp isolated in the last six-months occurred in one area of the hospital, indicating possible cross-infection.

Gentamicin-resistant Gram-negative rods are becoming more numerous in many hospitals. Although many of the infections caused by our strains were relatively mild, these organisms are available as a source of infection for groups at risk. They may also colonise the environment and may exist as a potentially dangerous reservoir of infection. Many of these infections may be susceptible to treatment with amikacin, but the problem of treating infections caused by gentamicin-resistant *Flavobacterium* spp, *Acinetobacter* spp, and *Alcaligenes* spp remains unsolved.

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Hypertension after renal transplantation

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Summary

The incidence of hypertension (mean diastolic pressure above 90 mm Hg) was evaluated in 85 patients with renal transplants whose follow-up ranged from 3 to 84 months. Bilateral nephrectomy had been performed in 80 recipients. The proportion of hypertensive subjects rose during the first three months, subsequently stabilised around 50-60% for up to five years, and then decreased slightly during the next two years. Over the years hypertension fluctuated so that one-third of the initially hypertensive patients became normotensive, and over one-third of the initially normotensive patients became hypertensive.

The main single aetiological factor was renal failure. A significant relation between steroid dosage and blood pressure was found in only a quarter of the hypertensive patients, and in another quarter no cause could be found.

Introduction

Although hypertension is a well-known complication of renal transplantation, its incidence and causes have been evaluated only in short-term studies soon after the operation.¹⁻⁶ We undertook this study, firstly, to assess the incidence of chronic hypertension up to seven years after renal transplantation and, secondly, to define its causal factors.

Patients and methods

From 1 January 1968 to 1 January 1973 152 transplantations were performed in 142 patients. At three months 80% of the grafts were functioning. Immunosuppressive treatment consisted basically of

azathioprine (2 mg/kg body weight) and prednisolone. Actinomycin C and antilymphocyte globulins were given to some patients during the first six months.⁷

We studied 85 patients (52 males and 33 females) whose ages ranged from 7 to 55 years (mean 35.5). They had survived with their first graft for more than three months and were regularly followed up at our clinic. Patients were seen twice weekly during the first six months, weekly during the next six months, then twice monthly, and eventually monthly thereafter. A twice weekly schedule was resumed when rejection occurred.

The transplant originated from a cadaver in 82 cases and from a living relative in three cases. Bilateral nephrectomy was performed in 80 recipients.

Patients' charts were reviewed up to January 1976, so that the potential follow-up ranged from a minimum of 36 months to a maximum of 96 months. Blood pressure at the end of 1, 2, 3, 6, 12, 18, 24, 36, 48, 60, 72, and 84 months was calculated by averaging the last five blood pressure readings obtained in the supine position. Mean diastolic pressures exceeding 90 mm Hg without treatment or 85 mm Hg with a diuretic and a salt-free diet were considered hypertensive. Mean diastolic pressures exceeding 100 mm Hg without treatment or 95 mm Hg with a diuretic and a salt-free diet were considered severely hypertensive.

Graft function was assessed by averaging the values of the last five serum creatinine determinations obtained at the end of each period. Total prednisolone dosage during each time interval was calculated for every patient.

Renal failure had been caused by chronic glomerulonephritis (50 patients), chronic interstitial nephritis including pyelonephritis and analgesic abuse (29 patients), polycystic kidneys (3 patients), malignant hypertension (2 patients), and renal tuberculosis (1 patient).

Results

INCIDENCE OF HYPERTENSION

The incidence of hypertension increased over the first three months and stabilised subsequently at around 50-60% for up to five years after transplantation. It fell thereafter to 40%. Slightly fewer than half the hypertensive patients suffered from severe hypertension (table I).

EVOLUTION OF HYPERTENSION

The stability of hypertension was assessed in 58 patients whose grafts functioned for at least 36 months. Of 31 patients who were normotensive at three months, 14 had become hypertensive at 36 months. Conversely, of the 27 patients who were hypertensive at three months, nine had become normotensive at 36 months.

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