Emergence of Gentamicin- and Carbenicillin-Resistant Pseudomonas aeruginosa in a Hospital Environment

W. GAMAN,* C. CATES, C. F. T. SNELLING, B. LANK, AND A. R. RONALD

Departments of Medical Microbiology and Medicine, University of Manitoba, Manitoba, Canada R3E OW3
and the Infectious Disease Service, Health Sciences Centre, Winnipeg, Manitoba, Canada R3E OZ3

Strains of Pseudomonas aeruginosa resistant to either gentamicin or carbenicillin have been noted since their introduction into clinical use. During a 6-month period, twice-weekly cultures were obtained from all patients treated with either gentamicin or carbenicillin and from all patients with a positive culture for P. aeruginosa. Susceptibility testing to gentamicin and carbenicillin and pyocine typing were performed on all isolates. Organisms with a minimal inhibitory concentration greater than 12.5 µg of gentamicin per ml or greater than 100 µg of carbenicillin per ml were defined as resistant. P. aeruginosa was cultured from 238 patients. One patient was initially infected with a gentamicin-resistant isolate. In 11 other patients, serial cultures revealed the emergence of resistance to gentamicin. All but one of these resistant isolates occurred in patients treated with gentamicin. In eight instances the pyocine and/or serological types before and after the change in sensitivity pattern were the same. Gentamicin-resistant P. aeruginosa emerged significantly more often in patients treated with gentamicin than in those who did not receive gentamicin. Carbenicillin-resistant P. aeruginosa emerged in four of 14 patients treated with carbenicillin. Seventeen of the 238 patients were infected de novo with carbenicillin-resistant P. aeruginosa. Carbenicillin-resistant P. aeruginosa emerged significantly more often in patients treated with carbenicillin than in those who did not receive carbenicillin. No evidence was found for in-hospital spread of resistant P. aeruginosa.

Antimicrobial use has featured changing patterns of bacterial susceptibility (8). In 1972 we designed a prospective study to determine the source of either gentamicin- or carbenicillin-resistant Pseudomonas aeruginosa within the hospital environment.

Gentamicin has been used at the General Centre of the Winnipeg Health Sciences Centre since 1967, and carbenicillin since 1970. Topical gentamicin has been used extensively for the prophylaxis of infection in burns (17). Despite the frequent isolation of gentamicin-resistant P. aeruginosa within this hospital, a previous retrospective study of the epidemiology of these organisms found no persistent hospital reservoir (16).

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* Address reprint requests to: Dr. W. Gaman, Department of Medical Microbiology, Basic Science Building, 730 William Ave., Winnipeg, Manitoba, Canada, R3E OW3.

MATERIALS AND METHODS

The General Centre is a 920-bed, adult, acute care hospital. During the period 1 June to 30 November 1972, all patients from whom P. aeruginosa was isolated and all patients who were treated with either topical or parenteral gentamicin or carbenicillin were identified. Pertinent clinical information including antibiotic therapy, renal status, weight, length of hospital stay, and underlying disease was prospectively collected; serial twice-weekly cultures were obtained as well. The patients were then followed throughout their hospital stay. No attempt was made to influence the use of antimicrobial agents.

Standardized disk susceptibility tests were performed by the Kirby-Bauer technique (3) using a disk containing 10 µg of gentamicin and a disk containing 100 µg of carbenicillin on Mueller-Hinton agar (Baltimore Biological Laboratories). All organisms with a zone size less than 16 mm to gentamicin or 20 mm to carbenicillin were studied further with agar-dilution susceptibility tests (18). A few organisms with a zone size greater than 16 mm also were studied with agar-dilution susceptibility tests. Organisms were grown in broth for 4 h and diluted to 10^8 organisms/ml. Approximately 0.001 ml was

imprinted upon the agar surface, yielding an inoculum at the point of contact of about 10^5 organisms. All studies were performed on a single batch of Mueller-Hinton agar (Baltimore Biological Laboratories). Controls with known susceptibility to carbenicillin and gentamicin were included in all runs. The results were read after 18 h, and the presence of three colonies or less growing at the point of imprint was interpreted as no growth. Unfortunately, the absence of a bimodal distribution makes it difficult to accept strict criteria for separating susceptible from resistant strains (20). Acknowledging these limitations, gentamicin resistance has been defined as an agar-dilution minimal inhibitory concentration (MIC) greater than 12.5 μg/ml and carbenicillin as greater than 100 μg/ml (10).

Strains of *P. aeruginosa* were typed by the pyocine identification method of Govan and Gillies (9).

Selected isolates were submitted as well to the University of Toronto (courtesy of S. Duncan and N. Hinton of the Department of Microbiology, Faculty of Medicine) for serotyping and phage typing.

RESULTS

P. aeruginosa was isolated from 238 patients of whom 110 received gentamicin and 128 did not (Table 1). Both groups were comparable with regard to primary disease. The mean age was 52 and 51 years, respectively. Table 2 summarizes the predominant site from which *P. aeruginosa* was isolated. Figure 1 plots the zone of inhibition around a disk containing 10 μg of gentamicin against the agar-dilution MIC of *P. aeruginosa* isolates. All isolates are from individual patients. Fourteen isolates had an agar-dilution MIC greater than 12.5 μg/ml. However, only 11 of these isolates were pure growths; three isolates all with zone sizes greater than 16 mm had isolated resistant mutant colonies that were selected out by the agar-dilution MIC test. Excluding the mutant colonies, these three isolates had MICs less than 12.5 μg of gentamicin per ml and thus were not considered resistant.

One isolate had a zone of inhibition of only 6 mm, but unfortunately did not have an MIC performed on it (patient no. 12, Table 3). This isolate was, however, considered resistant.

Thus, resistant *P. aeruginosa* were isolated

from 12 patients. Ten of the 110 patients who were treated with gentamicin had resistant isolates, whereas only two of the 128 patients who

TABLE 2. *Predominant sites of Pseudomonas aeruginosa isolation*

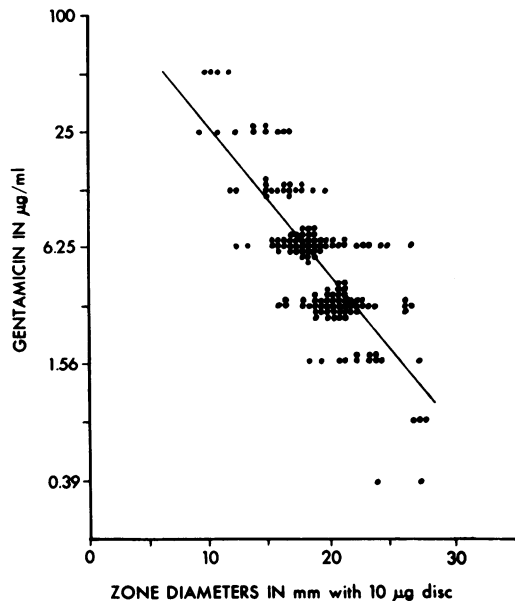| Site	Not treated with gentamicin	Treated with gentamicin	%
Urinary tract	65	39	43.7
Wounds	33	46	33.1
Operative	11	14	
Traumatic	11	24	
Burns	11	8	
Upper respiratory tract	26	19	18.9
Ears	0	3	1.3
Blood	1	2	1.3
Rectum	2	1	1.3
Cervix	1	0	0.4

FIG. 1. *Zone of inhibition around a disk containing 10 μg of gentamicin against the agar-dilution MIC of P. aeruginosa isolates.*

TABLE 1. *Gentamicin and Pseudomonas*

Patient from whom:	No. of patients who:[a]						Total
	Received gentamicin			Did not receive gentamicin			
	Total	S	R	Total	S	R	
Pseudomonas was isolated	110	100	10	128	126	2	238
Pseudomonas was not isolated	111						111

[a] S, Sensitive initially and on subsequent culture; R, resistant initially or became resistant.

TABLE 3. *Patients with gentamicin-resistant Pseudomonas aeruginosa*[a]

Patient	Site of infection	Wt (kg)	Aminoglycoside therapy	Initial isolation						Subsequent isolation				
				Date	Pyocine type	Serological type	Phage type	MIC	Date	Pyocine type	Serological type	Phage type	MIC
1	Amputation stump	42	Gentamicin 8/25 to 9/12, 1972 80 mg i.m. oh8 10/10 to 10/27, 1972 80 mg i.m. oh8	8/23	10	H 8	No lysis	1.5	11/24	10	H 8	119X	25
2	Wound infection 83 (incision)	83	Gentamicin 10/5 to 10/26, 1972 60 mg i.v. q8h	10/13	10	H 11	44/352/1214/M4	6.25	10/27	10	H 11	44/352/1214/COL11	50
3	Urinary tract	75	Gentamicin 7/8 to 7/16, 1972 80 mg i.v. t.i.d.	7/6	1A	H 10	16/68	3.1	7/27	10	5cPs11	21/31/44/68/109/1214	50
4	Burn	85	Gentamicin 10/10 to 24/10, 1972 80 mg i.m. oh8 and topical gentamicin cream 10/26 to 11/13, 1972	10/31	u/t	H 1	7	6.25	11/9	5	AA	No lysis	25
5	Urinary tract	54	Gentamicin 9/2 to 9/6, 1972 80 mg i.m. oh8	9/1	u/t	H 4	24/68/M4	6.25	9/9	5	H 4	24/31/68/M4	25
6	Urinary tract	62	Gentamicin 8/22 to 8/28, 1972 50 mg i.m. t.i.d.	8/31	u/t	ND	ND	3.1	9/1	u/t	ND	ND	25
7	Urinary tract	98	Gentamicin 9/30 to 10/18, 1972 80 mg i.m. b.i.d.	9/26	u/t	H 16910	7	3.1	10/6	u/t	H 16910	31	25
8	Stasis ulcer	47	Topical gentamicin cream 6/19 to 7/3, 1972 Gentamicin 6/21 to 6/24, 1972 40 mg i.m. oh12 6/25 to 6/27, 1972 60 mg i.m. oh8 6/28 to 7/3, 1972 60 mg i.m. oh12	6/1	1J	H 4	ND	6.25	6/26	1J	H 4	31	25

9	Urinary tract	36	Gentamicin 6/30 to 7/1, 1972 80 mg i.m. oh8 7/1 to 7/6, 1972 30 mg i.m. b.i.d. 7/11 to 7/16, 1972 30 mg i.m. q8h Kanamycin 6/22 to 6/29, 1972 500 mg i.m. b.i.d.	7/10	59	ND	ND	6.25	7/17	59	ND	50
10	Urinary tract	45	Gentamicin 7/5 to 7/12, 1972 40 mg i.m. oh12 7/13 to 7/17, 1972 80 mg i.m. q8h Kanamycin 6/27 to 7/5, 1972 500 mg i.m. b.i.d.	6/29	3	H 10	7/31/119X/COL11	6.25	7/20	3	H 10	7/31/119X/COL11	25
11	Wound infection (incision)	68	Neomycin 7/27 to 7/31, 1972 No gentamicin	7/14	3	H 10	352/1214/M4/COL11	6.25	12/7	3	H 10	352/1214/M4/COL11	25
12	Urinary tract	80	No gentamicin	9/25	1J	ND	ND	ND				

[a] i.m., Intramuscular; i.v., intravenous; t.i.d., three times a day; u/t, untypable; ND, not done; b.i.d., two times a day.

were not treated with gentamicin had resistant isolates ($P < 0.025$ by χ^2 test) (Table 1).

Table 3 summarizes the relevant data on the 12 patients from whom gentamicin-resistant *P. aeruginosa* were isolated. None of the 10 patients (no. 1 through 10), treated with gentamicin during the study, had been treated previously with gentamicin. Resistant organisms were cultured from two patients who were not treated with gentamicin during this admission. Patient no. 11 had been treated with neomycin compresses. Patient no. 12 was treated with gentamicin 1 year previously for a *P. aeruginosa* urinary tract infection. On this admission an initial urine culture was positive for a resistant *P. aeruginosa*.

The use of topical gentamicin preceded the appearance of resistant organisms in only two patients (no. 4, 8). Both were treated also with parenteral gentamicin. Nine other patients with cultures positive for susceptible *P. aeruginosa* were treated with topical gentamicin alone. Five of these nine were burn patients. Five additional patients were treated prophylactically with topical gentamicin without colonization with *P. aeruginosa*.

Table 3 lists pyocine types, serotypes, and phage types of gentamicin-resistant pseudomonas. Any organism with one of these three parameters identical for the susceptible and resistant isolates were defined as the same strain. In two patients (no. 6, 9) only pyocine typing was performed. In patient no. 12 only one of the cultures was positive during the study period. In eight patients (no. 1, 2, 5, 7–8, 9, 10, 11) serial cultures showed a sensitive strain become resistant. In two patients, (no. 3, 4), the resistant pseudomonas was a new strain. Both the susceptible and the resistant isolates from patient no. 6 were untypable. Seven of the 10 patients treated with gentamicin had a resistant organism of the same strain as the initial susceptible isolate. In four of these seven patients, the organism reverted back to a susceptible strain after cessation of gentamicin therapy. In one patient the resistant strain was replaced by a different susceptible strain, while in two others the resistant organism persisted throughout the patient's hospital stay. One patient's resistant organism was eradicated at limb amputation. At no time during the 6-month period of the study were two patients with identical strains of *P. aeruginosa* hospitalized together on the same ward.

Ninety patients in the study who received parenteral gentamicin had normal renal function (blood urea nitrogen <20 mg%, serum creatinine <1.5 mg%). In this group, 10 patients were treated with less than 2 mg gentamicin/kg per day. Four of these 10 (no. 2, 7, 8, 10) had a resistant organism emerge with the same markers as the initial susceptible isolate. In contrast, only six of the 80 patients who were treated with 2 mg of gentamicin/kg per day or more had resistant organisms emerge (no. 1, 3, 4, 5, 6, 9) ($P < 0.01$ by χ^2 test).

Seventeen patients were treated with carbenicillin during the 6-month study period (Table 4). None had been treated with carbenicillin previously. Of these, 15 had positive cultures for *P. aeruginosa*. One of the patients was infected with an initially resistant isolate. In 10 of 14 patients the *P. aeruginosa* was eradicated or remained susceptible to carbenicillin. In four patients, resistant *P. aeruginosa* emerged. Two of these patients died with their resistant organism, whereas in the others the organism reverted to a susceptible strain after discontinuation of carbenicillin therapy. Two hundred twenty-three patients with positive cultures with *P. aeruginosa* were not treated with carbenicillin. A carbenicillin-resistant pseudomonas was isolated from 19 patients (8%). In 16 of these 19 the organism was isolated de novo. There was a significantly greater proportion of resistant organisms occurring in the carbenicillin-treated group ($P < 0.025$ by χ^2 test).

DISCUSSION

P. aeruginosa isolates resistant to gentamicin were reported initially in 1967 by Müeller (14) in burn patients treated with both topical and systemic gentamicin. The resistant organisms became fully susceptible when gentamicin therapy was discontinued in the burn unit. Shulman et al. (15) and Stone and Kolb (19) studied an outbreak of gentamicin-resistant pyocine type 5 *P. aeruginosa* in a burn unit and found a point source in the hydrotherapy equipment. The spread of the resistant strain appeared to relate directly to the use of topical

TABLE 4. *Carbenicillin and Pseudomonas aeruginosa*

Patient from whom:	No. of patients who:[a]					
	Received carbenicillin			Did not receive carbenicillin		
	Total	S	R	Total	S	R
Pseudomonas was isolated	15	10	5	223	204	19
Pseudomonas was not isolated	2					

[a] S, Sensitive to carbenicillin; R, resistant to carbenicillin.

gentamicin. In a cancer hospital Greene et al. (10) found 12 isolates of gentamicin-resistant *P. aeruginosa*. Eight of these organisms emerged while the patient was being treated with oral prophylactic gentamicin. The present study provides similar results. In a 6-month prospective study, 10 of 12 gentamicin-resistant organisms were found to emerge in association with gentamicin therapy with no evidence of spread within the hospital. On the other hand, Chadwick (4) found little association between gentamicin therapy and the emergence of resistant organisms. Other investigators have reported a significant increase in gentamicin resistance over the past 5 years but have not attempted to relate this to antimicrobial use (1, 2, 6). Holmes et al. (12) have described two different patterns of gentamicin resistance. Strains with stable resistance were limited to three pyocine types and seemed to originate from the Intensive Care Unit. Strains with unstable resistance represented a wide variety of pyocine types and came from many different hospital wards.

The association in the present study between the emergence of gentamicin resistance and the use of low-dose therapy was not expected. This observation needs to be confirmed in other studies.

Before the introduction of carbenicillin into clinical use, about 10% of *P. aeruginosa* were not inhibited by 100 μg of carbenicillin per ml (7). This "background" of carbenicillin resistance has persisted unchanged in several recent surveys. Of the 238 patients in the present series, 93% were initially colonized with a susceptible organism. However, in four of the 15 patients treated with carbenicillin, a resistant isolate emerged with at least a fourfold increase in resistance.

In 1969 Darrell and Waterworth (5) found resistance emerging during the course of carbenicillin therapy in five patients. They postulated from their experience that a previous history of penicillin use, particularly ampicillin, was also associated with the occurrence of *P. aeruginosa* resistant to carbenicillin. We are unable to relate therapy with any other penicillins other than carbenicillin to the emergence of resistant *P. aeruginosa*. Holmes et al. (11) found that eight of 13 nonurinary pseudomonas isolates developed a fourfold increase in resistance to carbenicillin and persisted throughout therapy. Greene et al. (10) also noted that seven of their 13 carbenicillin-resistant organisms were associated with bacteremia, evidence that resistant organisms can be virulent. However, in spite of these reports of the development of resistance, the failure of these organisms to

become predominant in the hospital environment suggests that they may be comparable to resistant organisms emerging during gentamicin therapy. The resistance is relatively unstable, frequently does not persist in the absence of carbenicillin therapy, and has not been associated with an increased ability to colonize or survive in the hospital environment. In contrast to this, in a burn unit Lowbury et al. (13) experienced an epidemic of carbenicillin-resistant *P. aeruginosa* due to several different resistant pyocine types. These organisms destroyed carbenicillin with a carbenicillinase and possessed an R factor that could transfer the enzyme to susceptible pseudomonas and *Enterobacteriaceae*. An antimicrobial control policy on the burn unit resulted in the disappearance of resistant strains of *P. aeruginosa*. They concluded from this experience that even R factor-mediated resistance is not highly communicable except in the presence of a strong selective force and the opportunity for cross-infection.

The evidence from the current study suggests that the use of either gentamicin or carbenicillin will be associated in a significant number of patients with the emergence of resistant *P. aeruginosa*. The resistance is often transient during the course of therapy, does not appear to be associated with increased virulence, and does not spread in the hospital environment. Except in a closed unit, in association with the widespread use of a selective antimicrobial agent, the organisms have not become ascendant over susceptible strains. To ensure the continuing therapeutic efficacy of these agents in the treatment of pseudomonas infection, the use of both gentamicin and carbenicillin should be monitored and the antimicrobial susceptibility of *P. aeruginosa* should be tabulated to detect the emergence of resistance. Topical and oral use of gentamicin should be discouraged, particularly on closed units. The available information does suggest that widespread resistance is preventable with conservative antimicrobial policies and the prevention of intrahospital spread of infection.

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