Epidemiology of Pseudomonas aeruginosa infections investigated by pyocin typing

IAN B.R. DUNCAN, MD, FRCP[C], FRC PATH; E. VALERIE BOOTH,* LCSLT, FIMLT

Summary: All strains of *Pseudomonas aeruginosa* isolated in a large Canadian hospital over a 3-year period were typed by their pyocin production. Smaller collections of *P. aeruginosa* from other hospitals were also typed. Almost 3000 strains were examined. The typing method did not require use of complex reagents and was successful in subdividing *P. aeruginosa* into numerous types. No single type was restricted to infections of one particular kind. Infections of all kinds were associated with a wide variety of pyocin types. Extensive crossinfection with one particular pyocin type was observed only in urinary infection of patients with urologic disorders. The four pyocin types that were most frequent in our entire series have been reported as the commonest types causing infections in many other parts of the world.

Résumé: *Epidémiologie des infections à Pseudomonas aeruginosa étudiées par typage des pyocines*

Nous avons soumis au typage, d'après la production de pyocine, toutes les souches de *Pseudomonas aeruginosa* isolées dans un grand hôpital canadien au cours d'une période de 3 ans. Nous avons également typés des quantités plus faibles de *P. aeruginosa* provenant d'autres hôpitaux. En tout, 3000 souches ont été examinées. La méthode de typage utilisé n'exigeait pas de réactifs complexes et nous a permis de subdiviser facilement les espèces de *P. aeruginosa* en de nombreux types. Aucun type particulier n'était limité à une infection particulière. Somme toute, des infections de tous genres relevaient d'une grande variété de types de pyocines. Une infection croisée étendue provenant d'un type particulier de pyocine n'a été observée que dans des infections urinaires chez des malades présentant des troubles urologiques. Les quatre types de pyocine qui ont été le plus fréquemment observés dans l'ensemble de l'étude ont été considérés comme les types pathogènes les plus courants dans nombre d'autres parties du monde.

From the department of microbiology, Sunnybrook Medical Centre, Toronto and the department of medical microbiology, University of Toronto

*Present address: Laboratory technology division, Toronto Institute of Medical Technology

Reprint requests to: Dr. I.B.R. Duncan, Microbiology department, Sunnybrook Medical Centre, Toronto, Ont. M4N 3M5

The ease with which *Pseudomonas aeruginosa* infects patients already debilitated by other diseases has become distressingly familiar, particularly in hospital practice. These infections affect patients in all hospital services,[1,2] from the premature infant in a humidified resuscitator to the geriatric patient with an indwelling bladder catheter and pressure ulceration over the sacrum. They may arise sporadically or as serious outbreaks of nosocomial infection. *P. aeruginosa* is found in many locations in hospitals, and numerous different strains can be distinguished. Such infections therefore have a complex epidemiologic background and, if reasoned attempts are to be made to prevent them, it is essential to identify the sources of the infecting organisms by subtyping the strains involved.

Several serologic typing systems have been developed on the basis of O antigens,[3-5] and bacteriophage typing has also been used successfully.[6,7] The third main system is based on the production by most strains of *P. aeruginosa* of antibacterial substances of the bacteriocin class, which inhibit the growth of various other strains of the organism. These bacteriocins, generally referred to as pyocins, form the basis of two distinct typing methods. With one, pyocin preparations from standard laboratory strains are used to type isolates of *P. aeruginosa* by inhibition of their growth;[8] with the other, the isolate to be tested is allowed to produce its own pyocins, which are detected by the inhibition of growth of a series of laboratory "indicator" strains of *P. aeruginosa*.[9-13] A useful feature of this second method for the diagnostic laboratory is that the only reagents required are a number of *P. aeruginosa* strains and these can readily be maintained in any nonspecialized bacteriology laboratory. We therefore chose this second method for the investigation of *P. aeruginosa* infections in our hospital. During the first 3 years of the study every strain of *P. aeruginosa* isolated in the laboratory was typed; in the next 4 years typing was restricted to strains from a limited number of episodes of possible crossinfection. This paper reports the results of the typing.

Materials and methods

Hospitals from which P. aeruginosa strains were obtained

1. Sunnybrook Medical Centre, Toronto, which is a 1000-bed university general hospital with approximately equal numbers of acute- and extended-care patients. The acute-care patients are of all ages beyond the pediatric range; the extended-care patients are virtually all elderly males requiring permanent hospital care. The centre has also a major ambulatory-care unit in which the staff see patients from the community outside the hospital.

2. Lyndhurst Lodge Hospital, Toronto, which is a specialized 52-bed hospital for the rehabilitation of paraplegic patients. Bacteriologic specimens from this hospital are examined in the microbiology laboratory at Sunnybrook Medical Centre.

3. Other hospitals (Table IV), all of which are active general hospitals in Ontario. In size they range from 400 to 600 beds.

P. aeruginosa strains examined

All of 2781 strains of *P. aeruginosa* isolated in the microbiology laboratory or received from other hospitals from November 1967 to December 1970 were pyocin-typed. Of the total, 961 were reisolates of strains previously isolated from the same infection, so that only 1820 were distinct strains. Most of the strains (1246) were from inpatients at Sunnybrook Medical Centre; 582 strains were isolated from urine specimens, 282 from sputum specimens, 332 from other clinical specimens and 50 from fecal specimens examined during a survey of intestinal carriage of *P. aeruginosa*. A further 158 strains were isolated from various environmental samples taken at Sunnybrook Medical Centre, and 147 came from infections in patients from the surrounding community who were seen in the ambulatory-care unit. The remaining 269 strains were from infections of patients in other hospitals — 152 from Lyndhurst Lodge Hospital and 117 from the four other hospitals.

Pyocin typing

All strains were pyocin-typed within a few days of being isolated. The method of typing was that of Gillies and Govan[11,12] with minor modifications.[14] Eight primary and five secondary indicator strains of *P. aeruginosa* were obtained from Dr. R.R. Gillies, University of Edinburgh, Scotland. We used the notation of Gillies and Govan for designating types and subtypes of type 1. In our analysis we also included as separate types or subtypes strains giving patterns of inhibition not described by Gillies and Govan.

Results

Distribution of pyocin types in total series

In the total series of 1820 *P. aeruginosa* strains 95 different pyocin inhibition patterns were observed by typing the strains with the eight primary indicator organisms. Apart from type 1, which accounted for 46% of the total, the only common types were type 10 (10%) and type 3 (8%). Type 1 strains were readily subdivided by means of the five supplementary indicator organisms into 21 different subtypes. The most common subtypes of type 1 were 1c (12% of the total series), 1b (11%), 1d (10%) and 1h (6%). No one type or subtype was therefore so common as to make this typing method unsuitable for hospital epidemiologic investigations. In the entire series 115 distinct pyocin inhibition patterns were observed. Untypable strains that did not produce pyocins inhibiting any of the eight primary indicator organisms accounted for 9.7% of the series.

The distribution of types in the three hospital series and in that from the general community is shown in Table I. The distribution of types was similar

Table I—Percentage distribution of pyocin types from infections in various hospitals and in the community

Pyocin type	Hospitals			Patients at home
	Sunnybrook	Lyndhurst Lodge	Other	
1b	11	3	17	10
1c	10	13	15	12
1d	10	26	3	3
1h	4	3	9	2
Other type 1	9	8	7	8
3	8	6	8	15
5	3	2	3	8
10	12	7	12	9
11	3	1	0	2
22	2	6	3	3
All other	19	12	12	20
Untypable	9	13	11	8
Total	100	100	100	100

Table II—Percentage distribution of pyocin types isolated from clinical specimens and fecal carriers at Sunnybrook Medical Centre

Pyocin type	Clinical specimens			Fecal carriers
	Urine	Sputum	Other	
1b	8	14	16	8
1c	11	11	10	12
1d	18	3	5	6
1h	5	3	6	4
Other type 1	6	8	4	16
3	6	12	9	6
5	2	5	4	4
10	15	5	11	14
11	2	1	6	0
22	1	2	1	0
All other	18	27	16	20
Untypable	8	9	12	10
Total	100	100	100	100

Table III—Percentage distribution of common pyocin types in urinary infections in different services at Sunnybrook Medical Centre

Pyocin type	Clinical service		
	Extended care	Urology	All others
1b	3	9	8
1c	9	15	10
1d	29	29	8
1h	6	6	4
3	2	6	7
5	1	2	2
10	26	9	11
11	2	3	1
22	1	3	1
Total	79	82	52

in each group except for the higher frequency of 1d among *P. aeruginosa* isolated from patients at Lyndhurst Lodge Hospital. There was a wide range of types in all four groups.

Infections at Sunnybrook Medical Centre

A detailed analysis was made of the types of *P. aeruginosa* isolated from infections of inpatients at Sunnybrook Medical Centre. Many different pyocin types were isolated in cases of infections of all kinds. There was no evidence that particular types caused specific kinds of infection. The distribution of types in urinary infections, respiratory infections and in infections of other sites is shown in Table II. Apart from a somewhat greater frequency of types 1d and 10 in urinary infections and type 3 in respiratory infections, the various types were similarly represented in these three groups.

The distribution of *P. aeruginosa* types in different hospital wards was investigated for evidence of crossinfection with particular strains. The types associated with respiratory infections and with all other infections except those of the urinary tract were distributed similarly in the various groups of wards. However, the distribution of types causing urinary tract infections varied with respect to the different services (Table III). On the extended-care wards 55% of *P. aeruginosa* strains were types 1d and 10. A special survey was made of urinary infection in 126 extended-care patients who had permanent indwelling bladder catheters. One third of these patients were infected with *P. aeruginosa;* of the strains isolated from the patients 45% were type 1d and 30% were type 10. Table III shows that there was a high incidence of type 1d also on the urology ward and that type 1c was fairly common. Type 1d was the prevalent strain during the 1st year of the investigation, whereas type 1c appeared and became as frequent as 1d during the 2nd and 3rd years.

In most areas of the hospital, including medical wards, surgical wards and intensive care units, individual episodes of crossinfection were observed, but there was no evidence of continuing infection by highly prevalent strains. Only in the extended-care and urology wards, where in many patients bladder catheters or other instruments were passed on single occasions or indwelling catheters were inserted for permanent management, was there clear evidence of important crossinfection by a few common pyocin types.

One small-scale outbreak of infection comprised several urinary infections in catheterized patients on an extended-care ward and a variety of infections on a surgical ward. The same uncommon subtype of type 1 was grown from all the infections, from cultures of rubber urine collection bags worn by the extended-care patients, and from the surroundings of the physiotherapy treatment pool. Improved methods of disinfecting urine collection bags and of cleaning and disinfecting the pool area were then introduced. Either as a result of this or by chance, infections by this type of *P. aeruginosa* disappeared completely.

Another type of epidemiologic study in which typing proved useful was an investigation of a minor increase in the number of gentamicin-resistant *P. aeruginosa* isolated in the hospital during 1973 and 1974 as compared with earlier years. All strains resistant at a concentration of 4 μg/ml were typed to determine whether this was a chance finding or whether a single resistant strain was being spread by crossinfection. Typing showed that half the strains were of the same type and that the remainder were a mixture of different types. The mechanism of spread of the identical strains appeared to be bladder catheterization.

Table IV—Percentage distribution of the commonest pyocin types reported in different areas

Location of hospital and reference no.	Pyocin types				
	1	3	5	10	UT*
Canada (Ontario)					
Toronto (Sunnybrook)†	44	8	3	12	9
Various†	51	8	3	12	11
Kingston[17]	43	11	4	9	11
United States					
Albany, New York[18]	39	12	17	10	10
Milwaukee, Wisconsin[19]	52	7	3	11	11
Scotland[12]	33	28	5	3	7
Norway[20]	32	20	14	6	3
Israel[21]	41	7	5	12	4
Australia					
Victoria[22]	30	21	4	11	9
New South Wales[22]	37	16	2	23	6
Singapore[22]	47	10	0	17	10

*UT = untypable
†Present series

Fecal carriage of P. aeruginosa

A survey of the prevalence of intestinal carriage of *P. aeruginosa* by patients on admission to hospital was done between June 1968 and July 1969. Stool samples were obtained from random patients in the medical wards within 24 hours of admission. No attempt was made to assess the carriage rate of patients who had been in hospital for longer than 1 or 2 days. Stool samples were cultured for *P. aeruginosa* by inoculation to selective media containing cetrimide.[15] Each sample was inoculated to a plate of Mueller-Hinton agar (BBL*) containing 0.03% cetrimide and to a trypticase soy broth (BBL) with 0.03% cetrimide. Stools from 216 patients were tested; 51 yielded strains of *P. aeruginosa* (fecal carrier rate, 24%). The distribution of pyocin types among these fecal strains is shown in Table II. Although the number of fecal isolates was not large, the type distribution was similar to that of isolates from clinical infections in the hospital.

Discussion

The most striking feature of the epidemiology of *P. aeruginosa* infections at Sunnybrook Medical Centre was the great diversity of pyocin types. In almost all wards, infections occurred either singly or as very small clusters of cases caused by the same type of *P. aeruginosa.* Widespread infection in a ward by one pyocin type was limited to urinary infections in patients with indwelling catheters or who were undergoing repeated catheterization in the extended-care wards and the urology ward. The epidemiology of hospital infections due to *P. aeruginosa* is quite different from that of hospital staphylococcal infections. A large proportion of the latter are caused by a few resident types of "hospital" staphylococci,[16] and prevention can be based on the isolation of all patients infected with these types. By blocking the spread of these strains to other patients the incidence of staphylococcal infection in a hospital can be greatly reduced. Such an approach is not successful in preventing *P. aeruginosa* infections in hospital because of the many different types of *P. aeruginosa,* each causing only one or two infections.

There was a remarkably similar distribution of pyocin types among the *P. aeruginosa* strains from Sunnybrook Medical Centre, from other hospitals and from the community outside hos-

*Baltimore Biological Laboratories, division of Bioquest Limited. Products are marketed in Canada by Becton, Dickinson and Company, Ltd., Mississauga, Ont.

pital. The only exception was the higher frequency of type 1d in the series from Lyndhurst Lodge Hospital, where unavoidable urinary crossinfection with one strain might be expected. Our findings are similar to those of other investigators from many parts of the world.[12,17-22] All these investigators reported that *P. aeruginosa* infections were caused by a wide range of different types but that the most prevalent pyocin types always included types 1, 3, 5 and 10. The distribution of pyocin types in different geographic areas is shown in Table IV.

Our studies showed that the proportions of pyocin types in the feces of *P. aeruginosa* carriers were similar to those of infections in both community and hospital patients. Although the likely reason for this is that many infections are caused by strains derived directly from fecal carriage, both may simply mirror the distribution of types in nature. We found that 24% of patients on admission to hospital were fecal carriers of *P. aeruginosa;* other workers have reported fecal carriage rates of 18 to 24%.[23-25] It is tempting to conclude from all these findings that endogenous infection is a common epidemiologic pattern in *P. aeruginosa* infections and that patient-to-patient crossinfection is a limited phenomenon except in the special case of urinary infections in wards with many catheterized patients.

The Gillies and Govan pyocin-typing method proved reasonably satisfactory in our hands so long as the subtyping of type 1 strains was done routinely. We examined multiple isolates of *P. aeruginosa* from more than 500 patients and we were impressed by the consistency of the typing patterns of repeat isolates from the same infection. Provided that we used an expanded system of nomenclature for types with pyocin patterns not described by the original investigators, we found the method sufficiently discriminating for assessing the epidemiology of *P. aeruginosa* infections in our hospital. We continue to use it to investigate minor episodes of *P. aeruginosa* crossinfection, and it proved most useful for studies carried out in the laboratory on the antibiotic sensitivity of *P. aeruginosa.*[26] We believe that it is the most suitable method for typing *P. aeruginosa* in a hospital diagnostic laboratory when reference laboratory facilities elsewhere are not available. The typing of *P. aeruginosa* in a reference laboratory is probably best done with serotyping as the primary method and an additional method for further subtyping. Either pyocin typing or phage typing can be used as the secondary method, and a cooperative study between this and another laboratory of the medical microbiology department of the University of Toronto is currently under way to determine which of the two is preferable.

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