Epidemiology of *Pseudomonas aeruginosa* Infections: Determination by Pyocin Typing

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The epidemiology of hospital infections due to *Pseudomonas aeruginosa* was investigated by pyocin typing. The typing method, which determined the pyocin activity of clinical isolates of *P. aeruginosa* on 27 indicator strains, was 43.7% reproducible, but elimination of 9 indicator strains doubled the reproducibility and yielded more readable pyocin inhibition zones. Seventy-eight of 1,084 isolates (7.2%) were untypable. In the second part of the study, *P. aeruginosa* was isolated from 110 patients (5.4% of all admissions) in a 3-month period and typed with the revised method. Twenty pyocin types were identified, 10 of which were obtained from five or more patients. *P. aeruginosa* was isolated from 45 of 353 environmental samples, including water fountains, ice machines, bar soaps, and germicide solutions for toilet brushes. Twenty percent of the environmental samples were untypable but, among typable strains, the five most common environmental strains were the same as the strains most frequently isolated from patients. The organism was frequently isolated from noses (39%), throats (39%), and stools (29%) of patients with *P. aeruginosa* infections or colonizations in urine, sputum, surgical wounds, or skin lesions. Six of eight patients had *P. aeruginosa* in their tracheostomy wounds. Autoinfections by strains already acquired on carrier sites may be significant.

Pseudomonas aeruginosa is often the cause of wound, urinary tract, and respiratory tract infections, many of which are hospital acquired. In our hospital, *P. aeruginosa* is responsible for about 15% of all hospital-acquired infections (19).

The epidemiology of *Pseudomonas* infections in hospitals has not been clearly defined by controlled prospective studies. Most hospitals do not have a single epidemic strain of *P. aeruginosa*, and cross-infections seem to be important. In a preliminary study of pyocin typing, small outbreaks of *Pseudomonas* cross-infection have been documented (22). The purpose of the present studies was to develop an accurate method to survey *P. aeruginosa* infections for epidemiological purposes.

Many methods have been proposed for typing *P. aeruginosa*. Bacteriophage typing is limited because of changes in lytic patterns due to mutation and dissociation (11, 24). Serological typing has been proposed, but the method usually is not sensitive enough when used

alone (23). There have been studies using two or more methods (2, 3). Various methods have been proposed based on either pyocin production or pyocin sensitivity of isolates (7, 11, 12, 24). In the present investigations, a modification of the procedure of Farmer and Herman (11), which tests the pyocin production of *P. aeruginosa* cultures against a set of 27 indicator strains of *P. aeruginosa*, was utilized.

MATERIALS AND METHODS

***P. aeruginosa* cultures.** Specimens of *P. aeruginosa* were obtained from O. Ross, Director of Clinical Laboratories, The Graduate Hospital of the University of Pennsylvania, over a 30-month period. Identification of all cultures for pyocin typing was confirmed by growth on Pseudosel agar (BioQuest, Cockeysville, Md.). *P. aeruginosa* was distinguished from *P. fluorescens* by the ability of the former to grow at 42 C (13).

Typing by pyocin production. The method of Farmer and Herman (11) was used with the following exceptions. Trypticase soy broth cultures of organisms to be typed were grown with constant shaking at 32 C. After 1 h, 1 ml of filter-sterilized mitomycin C, 10 µg/ml, (Sigma Chemical Co., St. Louis, Mo.) was added to induce pyocin production. Shaking was continued for an additional 5 h,

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when 0.3 ml of chloroform was added. The mixture was shaken for 2 min and then allowed to settle at room temperature. The upper layer (pyocin containing) was removed and refrigerated.

A set of 27 indicator strains was obtained from J. J. Farmer, Center for Disease Control, Atlanta, Ga. Pyocin action on each of the 27 indicator strains was recorded as positive or negative according to the formation of a zone of complete inhibition. In recording the result of a pyocin produced from a single clinical isolate on the 27 indicator strains, a classification mnemonic has been followed (10). This mnemonic employs nine sets of triplets for the 27 indicator strains. The lytic pattern for each triplet is represented by a number (Table 1). A test strain that has a pyocin production pattern of (+ - - - + - - + +) would be coded as 564

Epidemiological studies. After modification of the standard typing method, epidemiological studies were made on all clinical units of a 332-bed university hospital with no obstetric or pediatric services.

All *P. aeruginosa* isolates obtained from the clinical laboratory were pyocin typed during a 3-month period. If possible, nose and throat samples were obtained from patients having *P. aeruginosa* in other body sites. Stool samples and samples from tracheostomies, bedsores, and other skin lesions were collected. Environmental samples were also obtained. Reproducibility of the pyocin typing system was determined by maintaining all strains isolated from the clinical laboratory in stock culture and retyping at least three times during a 4-month period. Strains differing in fewer than three pyocin reactions were retyped simultaneously.

RESULTS

Laboratory studies. Four hundred and thirty-seven clinical specimens from 258 patients were typed for pyocin production; this yielded 280 different pyocin production patterns. The most frequently encountered strains from patients are listed in Table 2. Approximately 6% of the isolates were untypable. Nine other types were isolated from two patients. The reproducibility of the typing method was determined by weekly retyping of the isolates. The percent reproducibility was determined by dividing the number of repeat tests

that yielded consistent pyocin patterns by the number of repeat tests performed. Of the 679 repeat tests performed, 297 tests gave pyocin patterns that were identical to the first test, a reproducibility of 43.7%. When strains were simultaneously typed on the same day, the pyocin patterns were identical.

The frequency of inhibition of each of the indicator strains was determined for 437 tests (Table 3). Indicator strain 4 was inhibited most frequently, 71.7%, and strain 26 was inhibited least frequently, 2.4%. The median percent inhibition was 39.4%. To minimize intertest vari-

TABLE 2. *Pyocin types of P. aeruginosa isolated from patients in order of frequency (27 indicator strains)^a*

Pyocin type	No. of patients
617-664-513	14
111-121-111	7
888-888-888 ^b	7
113-846-513	5
551-833-867	4
113-846-533	4
111-411-111	3
217-866-587	3
317-646-577	3

^a Nine other pyocin types were isolated from two patients.

^b Untypable.

TABLE 3. *Frequency of inhibition of indicator strains by patient isolates of P. aeruginosa*

Indicator strain	% Inhibition
1	44.7
2	49.0
3	34.8
4	71.7
5	55.2
6	63.5
7	28.0
8	22.5
9	65.8
10	23.0
11	18.8
12	20.8
13	34.0
14	58.9
15	46.1
16	44.7
17	43.4
18	42.1
19	35.0
20	25.5
21	14.4
22	46.8
23	45.3
24	48.6
25	38.0
26	2.4
27	49.1

TABLE 1. *Code for reporting the inhibition patterns obtained by pyocin typing of P. aeruginosa*

Results for 3 tests	Representation
+++	1
++-	2
+ - +	3
- + +	4
+ - -	5
- + -	6
- - +	7
- - -	8

ations, nine indicator strains were omitted from the procedure. Criteria for discarding indicator strains were poor reproducibility and formation of inhibition zones that were ill defined and difficult to interpret. Strains 1, 2, 3, 12, 13, 14, 15, 19, and 20 were omitted.

Pyocin typing was repeated, using the 18 indicator strains, and recorded with a six-digit mnemonic. The reproducibility of this modified method was 88%, determined by at least three testings of all isolates at weekly intervals. Isolates that differed in one or two pyocin reactions were retyped simultaneously. If replicate tests were performed on the same day, the reproducibility of the modified method was 98%. No difference in reproducibility was observed when repeat typings were performed 1 week or 1 month after the initial typings. Of 1,084 specimens typed with the modified method, 78 (7.2%) were typable.

This modified method was used in epidemiological studies. Two commonly encountered strains were included in every test as controls.

Epidemiological studies. During the 3-month epidemiological study, *P. aeruginosa* was isolated from 110 patients, or 5.4% of the admissions during that period. *P. aeruginosa* was detected in 45 of 353 environmental samples (13%). A total of 20 different pyocin types was identified (Table 4). Ten pyocin types were isolated from five or more patients, three pyocin types were isolated from two to three patients, and seven pyocin types were found in only one patient. Untypable strains were isolated from 15 patients and comprised 14% of the total of isolates. The pyocin types of strains isolated from patients and environment are compared in Table 4. The 11 major strains in Table 4 were designated strains A through J and U (untypable). Twenty percent of the environmental samples (9/45) were untypable. Among the typable strains, the five most common environmental strains were the same as the strains most frequently isolated from patients.

P. aeruginosa isolations from infective sites (colonization samples not included) were made as follows: sputum, 57 patients; urine, 48 patients; wounds, 10 patients; and other sources (skin lesions, bedsores, blood), 8 patients. Comparison of pyocin types and source showed that 10 of 23 isolations of type B and 6 of 12 isolations of type G were from sputum. Type C and type J were isolated mainly from urine (8 out of 10 samples and 4 out of 5 samples, respectively).

Tables 5, 6, and 7 give the results from 46 of the 110 patients with *P. aeruginosa* who were further evaluated and examined for *P.*

TABLE 4. Pyocin types of *P. aeruginosa* isolated from patients and environmental samples using 18 indicator strains

Type	Pyocin production pattern	Patient samples			Isolations from environmental samples
		No. of patients	No. of sources	No. of isolations	
A	457 213	24	41	51	4
B	458 577	16	23	29	6
C	587 667	10	10	13	7
D	458 533	10	19	27	5
E	158 555	9	13	17	7
F	547 867	7	11	11	1
G	888 887	7	12	12	0
H	458 857	6	9	10	1
I	882 588	5	6	10	1
J	118 333	5	5	5	0
U	888 888 ^a	15	24	33	9
Other (10)		14	14	20	4
Total		128	187	238	45

^a Untypable.

TABLE 5. Infection and colonization with *P. aeruginosa* in the respiratory tract, urine, surgical wound, and skin lesions

Site	Infection	Colonization	Total
Respiratory tract	22	8	30
Urine	14	6	20
Surgical wound	9	0	9
Skin lesion	5	0	5
Total	50	14	64 ^a

^a Sixty-four isolates from 46 patients.

TABLE 6. Carrier rate with *P. aeruginosa* in patients with infection of the urine, sputum, surgical wound, or skin lesion

Colonized site	No. of patients sampled ^a	<i>P. aeruginosa</i> isolation from colonized site	
		No.	% Positive
Nose	46	18	39
Throat	46	18	39
Stool	35	10	29
Bedsores	2	2	
Tracheostomy	8	6	

^a Patients with several infections or colonizations were counted once.

aeruginosa colonization. These 46 patients were all studied during the 3-month epidemiological investigations and were selected on the basis of availability, i.e., patients still in the hospital

TABLE 7. Comparison of pyocin types of *P. aeruginosa* isolated from sputum, urine, surgical wound, and skin lesion cultures isolated from carrier sites

Type of infection	<i>Pseudomonas</i> type on carrier site	Site of infection/colonization				Total
		Respiratory	Urinary	Surgical wound	Skin lesions	
Community acquired	Same type	6	0	0	1	7
	Other type	2	0	0	1	3
	Noncarrier	0	2	0	1	3
Hospital acquired	Same type	12	7	4	1	24
	Other type	4	5	3	1	13
	Noncarrier	6	6	2	0	14
Total	Same type	18	7	4	2	31
	Other type	6	5	3	2	16
	Noncarrier	6	8	2	1	17
Grand total		30	20	9	5	64

and not receiving carbenicillin or gentamycin. More patients were excluded because of antibiotic therapy than because of discharge.

Table 5 lists the site of infection and colonization. Excluding carrier samples, *P. aeruginosa* was isolated from 64 different sources in 46 patients. These patients had 50 infections; isolation of *P. aeruginosa* from 14 sources was not associated with clinical or other signs of infection.

The results of the colonization studies of patients infected with *P. aeruginosa* are presented in Table 6. These patients had *P. aeruginosa* isolated from infections in the urine, sputum, surgical wound, or skin lesions. Colonization of the nose and throat occurred in 39% of the patients; 29% had *P. aeruginosa* in their stools. Six out of eight patients with tracheostomies had *P. aeruginosa* in their tracheostomy wound; these were all different types.

The pyocin types detected in noncontiguous colonized sites and in primary infected sites were compared. Results are presented in Table 7. Identical types were found in approximately half of the cases (31/64). Different pyocin types were found in one-fourth (16/64), and *P. aeruginosa* was not detected in other sites in one-fourth (17/64) of the cases. Patients with *P. aeruginosa* in sputum and urine most frequently had the same type isolated from colonized sites. The majority of the infections were considered hospital acquired, but the frequency of *P. aeruginosa* carriage was similar in hospital- and community-acquired infection. Of the 36 patients with hospital-acquired *P. aeruginosa* infections, 28 had previously been treated with antibiotics. Nine out of 13 patients with urinary tract infections had indwelling urinary catheters, which may have been a predisposing factor.

The results of environmental sampling for *P. aeruginosa* are presented in Table 8. *P. aeruginosa* was frequently isolated from the phenolic germicide used for soaking toilet brushes (10 of 23 samples) and from whirlpool baths (4/9). The organism was frequently detected in water fountains, ice machines, "clean" urinals, and nongermicidal bar soaps used for hand washing.

Four of seven environmental isolations of pyocin type C were from water fountains and ice machines. Type E was frequently detected in nongermicidal bar soaps (five of seven isolates). *P. aeruginosa* was detected in only 2 of 61 food samples, from parsley and a white gravy. *P. aeruginosa* was not isolated from any food that had been cooked in the hospital kitchen. A significant portion, 20%, of the environmental samples was untypable.

There was no specific geographic distribution of most pyocin types. The number of *P. aeruginosa* isolations per bed was highest in the intensive care unit (ICU), 22 isolations per 10 beds, or 2.2 isolations per bed. The isolations per bed for the rest of the hospital were 103 isolations per 310 beds, or 0.33 isolations per bed.

Pyocin type A was frequently isolated from patients in the 4 west (W) and 6W wards. The first isolate was from a patient who was transferred from ICU to 4W with a *P. aeruginosa* infection. The other isolations of type A in 4W occurred within 1 week. Two of the patients from 6W with pyocin type A infections had been staying in ICU simultaneously with other patients infected with *P. aeruginosa* of this type. Eleven of the 13 patient isolations in 4W, 6W, and ICU were made from respiratory tract samples (nose, throat, tracheostomy of sputum). Three of these patients had the same

TABLE 8. Isolation of *P. aeruginosa* from environmental sources

Source	No. of samples obtained	No. positive for <i>P. aeruginosa</i>	Pyocin types isolated ^a
Phenolic germicide for toilet brushes	23	10	A(3), B(2), C(2), other (3)
Ice machine	51	8	B(1), C(2), H(1), U(4)
Nongermicidal bar soap	54	8	D(1), E(5), F(1), U(1)
Water fountain	36	5	A(1), C(2), D(1), U(1)
Urinals (clean)	32	4	B(1), D(1), E(2)
Whirlpool baths	9	4	B(1), D(2), U(1)
Food (uncooked)	61	3	C(1), U(2)
Bedpans (clean)	17	1	Other(1)
Air	24	1	I(1)
Wash basins	12	0	
IPPB ^b machines and ultrasonic nebulizers	22	1	B(1)
O ₂ humidifiers	12	0	
Total	353	45	See Table 4

^a Numbers in parenthesis indicate the number of pyocin types isolated.

^b IPPB, Intermittent positive pressure breathing.

strain isolated from sources other than the respiratory tract.

Pyocin type A *P. aeruginosa* was also isolated from five patients on the ninth floor. These isolations were made from urine or stool samples. Type A was isolated on the same floor from the water fountain faucet and from germicide solutions used for the disinfection of toilet brushes (Table 9). The first isolation of type A was from a patient and 2 days later from the germicide solution. Subsequent isolations were from patients and environment without regularity. No new isolations were made after quality control and cleaning measures eliminated the environmental reservoirs.

Pyocin type B strains were found in 17 patients and seven environmental samples. One patient with a type B respiratory tract infection was transferred from ICU to 4W. Secondary type B respiratory infections occurred in three other patients in the same room within 2 weeks. Five of the six patients in ICU with pyocin type B had respiratory infections. These infections, however, were scattered over the 3-month study period. Type B was isolated from a positive pressure breathing apparatus in the ICU during this time.

Pyocin type G was isolated from seven patients. Three were in ICU over a 3-week period. It was also isolated from another patient 2 days after transfer from ICU during this same 3-

week period. Four of these seven patients had *P. aeruginosa* in their respiratory tract.

There was no discernible correlation between antibiotic sensitivity and pyocin type during this study. Ninety-five and 97% of *P. aeruginosa* isolates were sensitive to gentamycin and colistin, respectively. Ten percent or less of the types were sensitive to eight other antibiotics tested.

DISCUSSION

Reports have been published on the effectiveness of pyocin typing by the Farmer method (9, 11). Our experience has been less encouraging. Reproducibility with the 27 indicator strains was poor, 44%, but was increased by elimination of selected strains. Our modified method that utilized 18 indicator strains was satisfactory for use in an epidemiological study. Certain pyocin zones were difficult to interpret, which has also been observed by others (11, 23). Bergan noted that calcium supplements resulted in inhibition of more indicator strains and inhibition reactions that were easier to read (2). Merrikin and Terry demonstrated instability in 19% of the pyocin type in 274 strains (18), and Jacob et al. showed the lytic patterns of individual pyocins were dependent on their concentration (14).

TABLE 9. Isolations of *P. aeruginosa* from environmental samples by type and nursing unit

Nursing unit	Pyocin types isolated ^a	Total no. of isolations
9W	A(3), D(2), E(2), U(1), other (1)	9
8W	B(1), E(2), U(1)	4
8 East	B(2), E(1)	3
Ice machine, 8th floor ^b	U(1)	1
7W	C(1)	1
Ice machine, 7th floor	C(2), H(1)	3
6W	C(2), other (3)	5
Ice machine, 6th floor	C(1), U(1)	2
5W	A(1), E(1)	2
Ice machine, 5th floor	U(1)	1
4W	B(2), D(1), E(2), U(1)	6
Ice machine, 4th floor	B(1)	1
CRC ^c	I(1)	1

^a Numbers in parentheses indicate the number of pyocin types isolated.

^b The ice machines were shared by the two nursing units on each floor.

^c Clinical Research Center.

Bobo et al. have reported that the pyocin sensitivity is liable to some variations (3). However, these workers reported a high reproducibility of the pyocin production pattern within a single outbreak strain. Variation in the method implies that the typing results may change due to variation in the sensitivity of the indicator strain when the typing is performed on different days. This was controlled in the present study by the inclusion of control cultures and by repetitive testing of 255 specimens. Some variations in pyocin typing results are frequent (22). In a study of the "scrape and streak" method of pyocin typing, Edmonds et al. report reproducibilities poorer than in the present study (8). Some of the variation in the present study may be due to strains being maintained in the refrigerator. The use of freeze-dried strains may eliminate this potential difficulty.

One advantage of this method over previous typing methods is the ability to differentiate large numbers of strains. However, the tendency of *P. aeruginosa* to dissociate makes it possible that the strains may also change during transfer from one patient to another, and this should be considered. If this method is widely used, the test conditions must be carefully standardized. Control strains should be included, and strains to be compared must be typed simultaneously. Because of the variation found in repeat tests, the method, as it now exists, could only be used for short-term epidemiological studies. Furthermore, typing results from different laboratories cannot be compared, unless isolates are typed in the same laboratory. It may be possible to combine pyocin typing with other methods, e.g., serology, antibiotic sensitivity or biotype. This has been reported by several investigators (2, 3, 23). Type differences or similarities of antibiotic sensitivity should be investigated. In one study, Jones et al. screened indicator strains to obtain a set that eliminated reactions that are difficult to read, minimized zones due to bacteriophage lysis, and was most sensitive in differentiation (15).

Nevertheless, the modified pyocin typing method described here has been of value in the present study in defining episodes of cross-infection. The importance of colonization and the environment in hospital-acquired *P. aeruginosa* infection has also been demonstrated.

In the epidemiological study, 110 patients, 5.4% of the total admissions over a 3-month study period, yielded *P. aeruginosa*. Of these 110 patients, 46, or 42%, of those infected with *P. aeruginosa* were selected for further study. Patient selectivity for this study was influenced by: (i) difficulties in obtaining follow-up sam-

ples, especially at the beginning and end of the study, (ii) administration of anti-*Pseudomonas* antibiotics, and (iii) patient discharge or death.

In the present study, nose, throat, and stool carriage were common, and most patients with tracheostomies contained *P. aeruginosa* in their tracheostomy wound. Half of the patients infected with *P. aeruginosa* had identical pyocin types isolated from other sites. Although colonization may be secondary to the infection, these results suggest that *P. aeruginosa* infections can be autoinfections from strains already acquired on sites of colonization. Isolation of *P. aeruginosa* from six out of eight patients with tracheostomies suggests this to be an important site of acquisition, as also observed by others (16).

Identical pyocin types were frequently isolated from patients and environmental samples. Water fountains, ice machines, bar soaps, and germicide solutions used for toilet brushes were reservoirs. One small episode of type A cross-infection was terminated after the elimination of this type from environmental reservoirs, which may have been coincidental. The isolation of four out of seven environmental strains of type C from water fountains and ice machines may be due to a special affinity of this strain for water, whereas pyocin type E strains predominate on bars of soap (Table 8). Similarly, pyocin type B and type G were found in sputum, whereas type C and J were associated with urinary tract infection. More studies would be needed to confirm this type of relationship.

The importance of the hands of the personnel as a vehicle for the transfer of *P. aeruginosa* infections has been stressed (16), although not specifically examined in these studies. The frequent contamination of bars of soap suggests that organisms may be obtained during the very procedure performed to prevent transfer. In one study (4), Brannan and Judge have shown that bacteria inoculated onto bar soap are not readily transferable to subsequent users. We have shown that bacteria can contaminate and reproduce in liquid detergents and antiseptics (5, 17). Small bars of soap that are used quickly with free-draining, easy to clean soap racks may be preferable for routine handwashing. Soap leaflets or powder would also be acceptable, although more expensive. If liquid preparations are used, sterility tests must be done.

The association of five pyocin type A infections on the ninth floor with the isolation of the same type from the germicide used for toilet brushes and from water fountains suggests a possible transfer through bedpans and

by colonization of the gastrointestinal tract from the drinking water (6). Although food has been suggested as an important vehicle for transmission of *P. aeruginosa* infections (6), the organism was rarely isolated from food in this study.

The majority of infections with type A, B, and G probably originated in the 10-bed ICU. The isolation rate of *P. aeruginosa* from patients was higher in ICU than in all other hospital units, 2.2 isolations per bed compared with 0.33 isolations per bed for the rest of the hospital. Cross-infection due to the concentration of debilitated patients, a high frequency of tracheostomied and urinary-catheterized patients, a high level of activity, the use of different kinds of special treatment and equipment, and the nursing of these patients in close proximity to each other is a possible explanation. The ICU has been renovated, and quality control testing of inhalation therapy and urinary catheter equipment has been initiated since the end of these studies. These decisions were based on epidemiological results of this study and of a computerized infection surveillance program (20).

The majority of the infections associated with ICU were respiratory tract infections. *P. aeruginosa* was not found in any of the air samples obtained from ICU during this study, although we have in the past isolated this organism, using slit and Andersen air samplers. Contact spread, especially by the hands of the attending personnel, is generally thought to be the major mode of transmission for *P. aeruginosa* infections (16). In patients with respiratory tract infections and tracheostomies, *P. aeruginosa* aerosols can be generated during coughing, suctioning, etc., and *P. aeruginosa* is most likely spread short distances by droplets and droplet nuclei (21), but this mode of transmission could not be demonstrated in this study. Further transmission to other patients is probably by other persons and fomites through contact.

Only a small percentage of clinical isolates of *P. aeruginosa* was untypable, 6.0%, by the original method and 7.2% by the modified method. A higher incidence of untypables was detected in environmental sampling, 20% of 45 isolates in this study. In previous studies, 33 isolates of *P. aeruginosa* isolated from contaminated children's soap bubble solutions from three manufacturers were untypable (17). Using other pyocin typing and serological methods, Al-Dujaili and Harris also noted a higher proportion of untypable strains among environmental isolates of *P. aeruginosa* (1). These workers also noted a tendency of un-

typable strains to produce smaller amounts of hemolysin. Such a correlation, if true, would offer a partial explanation of relatively sporadic hospital *P. aeruginosa* infections accompanied by widespread environmental *P. aeruginosa* contamination.

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