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# Epidemiology of *Pseudomonas aeruginosa* in a Burns Hospital: Surveillance by a Combined Typing System[1]

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For 3 months, 259 cultures of *Pseudomonas aeruginosa* isolated from nonpatient environmental sources and 262 cultures from 16 infected patients in the Intensive Care Unit (ICU) of Shriners Burns Hospital were typed by a combined system with a high degree of reliability. Sinks were major sources of environmental contamination. Serotypes 1 and 2 were the predominant types found in patients, and they were most prevalent among typable strains from sinks. Strain designations were made on the basis of similarities in data from serological and phage typing. All nontypable strains were typed by pyocin production. Two infected patients carried different strains of *P. aeruginosa* that remained the same type for 45 days, even though their beds in ICU were approximately 6 feet apart. Cross-contamination from patient to patient and spread of infection by nursing personnel were eliminated as major modes of transmission because nasopharyngeal swabs, hair samples, and hands of nursing staff were consistently negative. Splashing of water from contaminated sinks to fomites was suggested as a possible mode of transfer for this infectious agent.

In recent years, a great deal of progress has been made in the management of *Pseudomonas* burn wound sepsis (3, 8, 11, 13), but very little is known about sources and modes of transmission for *Pseudomonas aeruginosa* in the hospital environment. This is due, in part, to lack of a standardized typing method for strain differentiation. In a previous report (6), we demonstrated that serological, phage, and pyocin typing could be combined into a sensitive epidemiological tool for the intraspecific differentiation of *P. aeruginosa* with great reliability. In this article, we show the usefulness of this combined typing system in a prospective study.

## MATERIALS AND METHODS

**Environmental cultures.** A 3-month survey was conducted in the environment of the Intensive Care Unit (ICU), Shriners Burns Institute, Cincinnati, Ohio. Acetamide medium (7) was used to select *P. aeruginosa* from a variety of sources. Two samples per week were taken from counter tops, nurses' hands, sinks, whirlpools, nebulizers (when in use), bed rails, toys, and shoe covers. In addition to these samples, which were taken by contact or a swab, air samples were taken from different locations in the ICU, for 20 min twice each week, with a TDL Particle Slit Sampler (Scientific Products, Evanston, Ill.). Random samples were taken from miscellaneous sources, including one nasopharyngeal swab and a single strand of hair from forty-five members of the nursing staff.

**Patient cultures.** During this 3-month period, all cultures of *P. aeruginosa* isolated from infected patients were obtained from the Clinical Microbiology Laboratory, Shriners Burns Institute.

**Strain identification.** Cultures of *P. aeruginosa* from patients and environmental sources were typed by serology and phage in the combined system as described in the previous report (6).

## RESULTS

**Environmental cultures.** During the 3-month survey of the ICU in Shriners Burns Institute, 259 cultures of *P. aeruginosa* were isolated from nonpatient environmental

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sources (Table 1). Sink drains were consistently positive for this organism, and numerous isolations were made from inside wash basins. The second major source of *P. aeruginosa* was whirlpool drains. Only infrequent isolations were made from other environmental sources. One culture was isolated from each of the following: a toy, a solution bottle (outside), and a records well on a counter surface. Air samples, nurses' hands, and sink faucets were consistently negative.

All hair samples from forty-five members of the nursing staff were negative, but one nasopharyngeal swab was positive for *P. aeruginosa*. However, a second swab culture from the nasopharynx of that subject was negative.

**Patient cultures.** In addition to *P. aeruginosa* cultures isolated from environmental sources, 262 cultures were isolated from 16 infected patients. Hospitalization periods for all burned patients during this 3-month survey are shown in Fig. 1. Thirty-one patients were admitted to the hospital, and 16 of them had positive cultures for *P. aeruginosa* while hospitalized: four were positive when the study began, three others were positive on the date of admission, and nine developed *P. aeruginosa* during their stay in the hospital. The average period of hospitalization prior to infection was 9.9 days. Two infected patients died during the period of this study, but neither death was attributed to *P. aeruginosa* sepsis. The cause of death in one patient (D.J.) was mucormycosis, and the other (A.A.) died as a result of vascular complication.

**Strain identification of patient cultures.** The distribution of serological types in patients for the 3-month period is shown in Fig. 2. Monospecific reactions in types 1 and 2 were most prevalent. One patient (S.C.) was infected with type 2 continuously throughout the study. Only two patients (D.J. and J.W.) had cultures that were monospecific for type 5. One patient (J.W.) also had many cultures that were nontypable. Monospecific reactions for types 3, 4, 6, and 7 were conspicuously absent.

Serological types and phage patterns for all typable cultures are shown in Tables 2, 3, and 4. Monospecific reactions were predominant among serotypes. Cultures from only one patient (T.J.) showed cross-reactions (Table 2), and these reactions were in types 3 and 7. Data from phage typing were more variable. Phage types are seldom monospecific, and pattern reactions were used for strain differentiation. From our data, specific correlations cannot be made between phage and serological types. However, lysis of cultures with phages 68, 7, and M4 were more predominant in strains belonging to serotype 1, whereas serotype 2 cultures were lysed more frequently by a different group of phages, among which phage 24 was more prevalent. Although other lytic reactions are common in cultures belonging to both serotypes 1 and 2, phage reactions that are more frequent for each serotype were used to support the differences in strain designations.

Cultures that were nontypable by phage and serology were typed by pyocin production. In May 1971, twenty-three cultures were isolated from one patient (J.W.): five cultures were serotype 5, and eighteen were nontypable by serology (Table 3). Among these 23 cultures, 12 were lysed by phage (188/1) and 11 were nontypable. Subsequently, the eighteen cultures that were nontypable by serology were differentiated by pyocin production into a single group with a mnemonic designation of

TABLE 1. *Environmental sources of Pseudomonas aeruginosa in 1971*[a]

| Source | April (week) | | | | May (week) | | | | June (week) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Air | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |
| Nebulizer $H_2O$ | 0/2 | 1/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | NT | NT | NT |
| Counter surface | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 1/6 | 2/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| Bed rails | 0/4 | 0/4 | 0/4 | 1/4 | 1/4 | 1/4 | 0/4 | 0/4 | 4/4 | 0/4 | 1/4 | 0/4 |
| Nurses' hands | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| Sinks: | | | | | | | | | | | | |
| Drain | 14/14 | 13/14 | 12/14 | 12/14 | 12/14 | 14/14 | 14/14 | 14/14 | 14/14 | 14/14 | 13/14 | 14/14 |
| Basin | 1/14 | 9/14 | 3/14 | 3/14 | 2/14 | 9/14 | 2/14 | 5/14 | 4/14 | 0/14 | 3/14 | 3/14 |
| Faucet | 0/14 | 0/14 | 0/14 | 0/14 | 0/14 | 0/14 | 0/14 | 0/14 | 0/14 | 0/14 | 0/14 | 0/14 |
| Whirlpool: | | | | | | | | | | | | |
| Drain | 1/4 | 4/4 | NT | 2/4 | 1/4 | 4/4 | 3/4 | 1/4 | 1/4 | NT | 1/4 | 2/4 |
| Inside | 1/4 | 1/4 | NT | 0/4 | 0/4 | 1/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 |

[a] Expressed as the ratio of positive cultures per total cultures. NT = not tested.

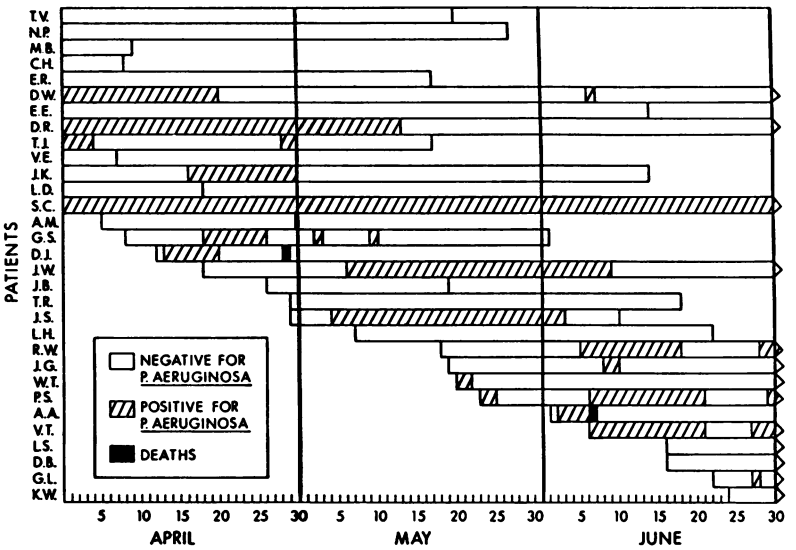


FIG. 1. *Hospitalization period of infected and noninfected patients.*

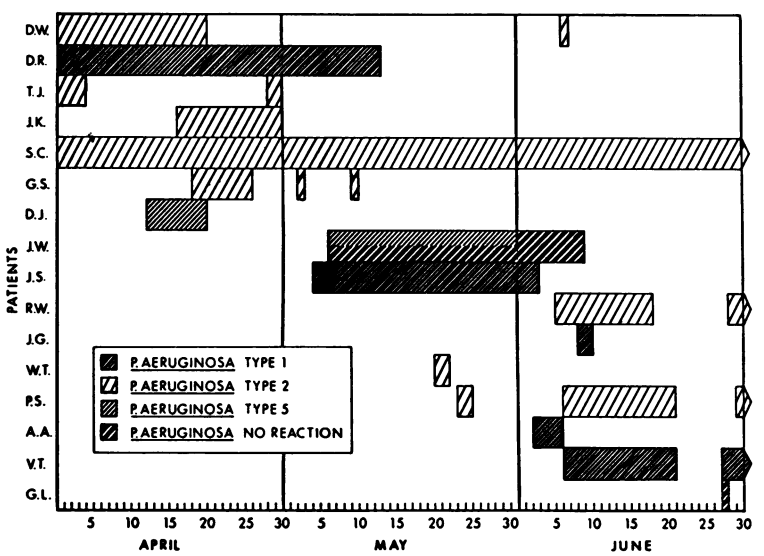


FIG. 2. *Distribution of serotypes over a three-month period.*

612 216 313.

**Strain identification of environmental cultures.** Cultures from sink and whirlpool drains were less reactive than cultures from patients. During any month, 33 to 48% of cultures from sinks were nontypable by serology, and cross-reactions were numerous (Tables 5–7). However, serotypes 1 and 2 were predominant among typable cultures. Many cultures that were nontypable by serology were also nontypable by phage. In May 1971, 13 cultures from two sinks (C and G) were nontypable by phage: three were serotype 1, one was serotype 2, and two showed cross-reactions with serotypes 3 and 7. Typable cultures from three other sinks (A, E, and F) were lysed by only phage (188/1).

All nontypable cultures from sink drains produced pyocins, but their reactions with indicator strains were highly variable.

## DISCUSSION

Infected patients, sink drains, and whirlpools were shown to be reservoirs of *P. aeruginosa* in the hospital environment (Table 1).

TABLE 2. *Intraspecific identification of Pseudomonas aeruginosa cultures isolated from patients during April 1971*[a]

| Patient | Serological type | No. of cultures | Phage pattern | No. of cultures |
|---|---|---|---|---|
| T.J. | 2 | 2 | 119X/(188/1)[b] | 3 |
| | CR | 3 | (188/1) | 2 |
| D.R. | 1 | 19 | 68/(188/1) | 19 |
| | NR | 1 | NR | 1 |
| S.C. | 2 | 32 | 24/(188/1) | 20 |
| | NR | 1 | 24/119X/(188/1) | 11 |
| | | | NR | 2 |
| J.K. | 2 | 7 | 24/119X/(188/1) | 1 |
| | | | 24/(188/1) | 4 |
| | | | NR | 2 |
| G.S. | 2 | 9 | 24/119X/(188/1) | 9 |
| D.J. | 5 | 1 | 24/119X/(188/1) | 3 |
| | NR | 2 | | |
| D.W. | 2 | 5 | 24/119X/(188/1) | 6 |
| | NR | 1 | | |
| | | Total: 83 | | Total: 83 |

[a] CR = cross reaction; NR = no reaction
[b] A single phage reaction.

TABLE 3. *Intraspecific identification of Pseudomonas aeruginosa cultures isolated from patients during May 1971*

| Patient | Serological type | No. of cultures | Phage pattern | No. of cultures |
|---|---|---|---|---|
| D.R. | 1 | 4 | NR | 5 |
| | NR[a] | 1 | | |
| S.C. | 2 | 62 | 24/119X/(188/1)[b] | 51 |
| | | | 24/119X/352/1214/C11 | 5 |
| | | | 24/119X/M4 | 6 |
| G.S. | 2 | 2 | 24/(188/1) | 2 |
| J.W. | 5 | 5 | (188/1) | 12 |
| | NR | 18 | NR | 11 |
| J.S. | 1 | 10 | 7/119X/M4/(188/1)/2 | 10 |
| | NR | 2 | (188/1) | 1 |
| | | | NR | 1 |
| W.T. | 2 | 2 | 24/(188/1) | 2 |
| P.S. | 2 | 4 | | |
| | NR | 1 | 44/1214/C11 | 5 |
| | | Total: 111 | | Total: 111 |

[a] NR = no reaction.
[b] A single phage reaction.

TABLE 4. *Intraspecific identification of Pseudomonas aeruginosa cultures isolated from patients during June 1971*

| Patient | Serological type | No. of cultures | Phage pattern | No. of cultures |
|---|---|---|---|---|
| S.C. | 2 | 16 | 24/(188/1)[a] | 16 |
| J.W. | NR[b] | 6 | (188/1) | 6 |
| J.S. | 1 | 2 | 7/119X/M4/2 | 2 |
| R.W. | 2 | 13 | 24/(188/1) | 13 |
| J.G. | 1 | 2 | (188/1) | 2 |
| P.S. | 2 | 10 | 44/1214/C11 | 5 |
| | | | 44/1214 | 4 |
| | | | NR | 1 |
| A.A. | 1 | 4 | M4 | 4 |
| V.T. | 1 | 3 | 119X/M4/C11 | 6 |
| | 2 | 1 | M4/C11 | 2 |
| | NR | 9 | 44/(188/1) | 1 |
| | | | 119X/C11 | 2 |
| | | | 119X | 1 |
| | | | NR | 1 |
| G.L. | NR | 1 | NR | 1 |
| D.W. | 2 | 1 | (188/1) | 1 |
| | | Total: 68 | | Total: 68 |

[a] A single phage reaction.
[b] NR = no reaction.

When this study was initiated, four patients were infected with *P. aeruginosa* (Fig. 1). One of them (T.J.) was only sporadically infected, and two others (D.W. and D.R.) had cultures positive for *P. aeruginosa* for 20 and 45 days, respectively. The fourth patient (S.C.) was positive throughout the study.

Our combined system typing data indicated that patients D.R. and S.C. had populations of *P. aeruginosa* that were of different strains. This is significant because both patients were housed in close proximity to each other, and both patients were highly infected. Cultures from both patients were representative and included samples from burned wounds, urine, and body surfaces, but their strains of *P. aeruginosa* remained monospecific by serological and distinctly different by phage typing. Cross-contamination was ruled out for these two patients during this period.

Because many variables may influence the

TABLE 5. *Intraspecific identification of Pseudomonas aeruginosa cultures isolated from sink drains during April 1971*

| Sink | Serological type | No. of cultures | Phage pattern | No. of cultures |
|---|---|---|---|---|
| A | 1 | 1 | (188/1)[a] | 3 |
| | 2 | 1 | 7/M4/C11/(188/1)/2 | 1 |
| | 7 | 1 | | |
| | NR[b] | 1 | NR | 2 |
| | CR[c] | 2 | | |
| B | 1 | 1 | (188/1) | 1 |
| | 2 | 4 | C11/(188/1) | 2 |
| | NR | 1 | NR | 3 |
| C | NR | 2 | (188/1) | 4 |
| | CR | 3 | NR | 1 |
| D | 1 | 2 | (188/1) | 3 |
| | 5 | 1 | 7/16/21/44/68/(188/1)/2 | 1 |
| | NR | 2 | NR | 1 |
| E | 5 | 2 | (188/1) | 2 |
| | NR | 1 | NR | 2 |
| | CR | 1 | | |
| F | 2 | 1 | (188/1) | 3 |
| | 5 | 1 | C11/(188/1) | 1 |
| | NR | 1 | | |
| | CR | 1 | | |
| G | 1 | 1 | NR | 3 |
| | NR | 2 | | |
| | | Total: 33 | | Total: 33 |

[a] A single phage reaction.
[b] NR = no reaction.
[c] CR = cross-reaction.

TABLE 6. *Intraspecific identification of Pseudomonas aeruginosa cultures isolated from sink drains during May 1971*

| Sink | Serological type | No. of cultures | Phage pattern | No. of culture |
|---|---|---|---|---|
| A | 2 | 3 | (188/1) | 7 |
| | NR[b] | 2 | | |
| | CR[c] | 2 | | |
| B | 1 | 1 | (188/1) | 5 |
| | 2 | 1 | M4/C11 | 1 |
| | NR | 2 | | |
| | CR | 2 | | |
| C | 1 | 1 | NR | 6 |
| | 2 | 2 | | |
| | NR | 2 | | |
| | CR | 1 | | |
| D | 1 | 2 | 1214 | 1 |
| | 2 | 1 | NR | 5 |
| | 5 | 1 | | |
| | CR | 2 | | |
| E | 1 | 3 | (188/1) | 3 |
| | NR | 2 | NR | 4 |
| | CR | 2 | | |
| F | NR | 6 | (188/1) | 3 |
| | CR | 1 | NR | 4 |
| G | 1 | 2 | NR | 7 |
| | NR | 4 | | |
| | CR | 1 | | |
| | | Total: 46 | | Total: 46 |

[a] A single phage reaction.
[b] NR = no reaction.
[c] CR = cross-reaction.

presence or absence of one reaction in a phage pattern, phage data were used to support serological differentiation of cultures. In April 1971, two patients (S.C. and J.K.) had 39 cultures that were serotype 2 and one nontypable culture. Among these cultures, 24 had identical phage patterns, 24/(188/1); 12 others had a phage pattern that differed by only one phage, 24/119X/(188/1); and three were nontypable by phage. On the basis of similarities in typing data for the combined system as previously described (6), these 39 cultures are considered to be identical. In contrast, one patient (V.T.), during June 1971, had three cultures that were serotype 1, one serotype 2 culture, and 9 nontypable cultures. Among these 13 cultures, five different phage patterns were obtained. Because of differences in serotypes and phage patterns, one patient (V.T.) was considered to be infected with a mixed population of *P. aeruginosa*.

Self-contamination from *P. aeruginosa* in the gastrointestinal tract is a possibility, but we did not study fecal carriage among patients. Therefore, subsequent isolations of this organism from stools may represent either fecal carriage or fecal contamination. A range of 3 to 29% has been reported for the incidence of fecal carriage for *P. aeruginosa* (2, 12, 14, 16, 17), but the status of this organism as a member of the normal microflora in healthy subjects remains questionable (5, 15).

Nursing personnel did not appear to be carriers or vectors for the transmission of this organism because cultures from their nasopharynx, hair, and hands were consistently negative for *P. aeruginosa*. Because of the questionable status of *P. aeruginosa* as a member of the intestinal flora of healthy subjects (5, 15), we did not examine stool specimens from nursing personnel.

Definitive correlations between serotypes

TABLE 7. *Intraspecific identification of Pseudomonas aeruginosa cultures isolated from sink drains during June 1971*

| Sink | Serological type | No. of cultures | Phage pattern | No. of cultures |
|---|---|---|---|---|
| A | 2 | 4 | (188/1)[a] | 7 |
| | NR[b] | 1 | | |
| | CR[c] | 2 | | |
| B | 1 | 1 | (188/1) | 4 |
| | 2 | 1 | M4/C11 | 1 |
| | NR | 4 | NR | 1 |
| C | 1 | 1 | (188/1) | 1 |
| | 5 | 1 | 44/(188/1) | 1 |
| | NR | 4 | NR | 4 |
| D | 2 | 1 | 24/44/68/(188/1) | 2 |
| | NR | 3 | 44/(188/1) | 2 |
| | CR | 3 | NR | 3 |
| E | 1 | 3 | (188/1) | 2 |
| | NR | 3 | 44/68/(188/1) | 1 |
| | CR | 1 | 68 | 1 |
| F | 1 | 1 | (188/1) | 3 |
| | NR | 3 | M4 | 1 |
| | CR | 1 | NR | 1 |
| G | 1 | 1 | (188/1) | 1 |
| | NR | 4 | 7/73/119X/M4/C11/(188/1)2 | 1 |
| | CR | 2 | NR | 5 |
| | | Total: 45 | | Total: 45 |

[a] A single phage reaction.
[b] NR = no reaction.
[c] CR = cross-reaction.

and phage types were not observed, but strain designations were made on the basis of similarities between serotypes and phage types in the combined system. Serotypes 1 and 2 and phage pattern 24/(188/1), 24/119X/(188/1), and 68/(188/1) with deviations in one or more phage reactions were prevalent among patient cultures. Typable cultures from sink drains were predominantly serotypes 1 and 2 and phage pattern (188/1). Total cultures from sinks were fewer in number than those from patients, and their typing reactions were more variable. Several phages, 24, 68, 119X, and M4, had one-plus (+) lytic patterns in cultures from sinks which were nonreportable, as described in our previous paper (6). If all phage reactions were reportable, instead of only strongly positive three-plus (+++) and two-plus (++) reactions, our data from sinks and patients would reveal cultures with more similar typing patterns. Perhaps only reporting strongly positive reactions for phage typing is an inherent fallacy that needs reconsideration. Because of variation among phage patterns, our findings were not conclusive, but we suggest that environmental reservoirs may serve as a source from which patients are contaminated. As a result of the constant interaction between personnel, patients, fomites, and sinks, splashing of water may serve as a "ping pong" type of indirect contamination of patients in the hospital environment. This hypothesis deserves further testing.

Kohn (9, 10) proposed sterilization of sink drains by heating water with steam or an immersion heater. In this hospital, Maley (*unpublished data*) obtained preliminary data which suggest that sink drains can be sterilized for 24 hr by treatment with formaldehyde.

A combined typing system has been demonstrated to be highly sensitive and reliable for studying the epidemiology of *P. aeruginosa*. Serological typing is a useful tool when used separately, but when used in conjunction with phage typing, the combined system is more discriminating, and it increases the reliability of strain identification. Subsequently, when nontypable cultures were typed by the pyocin production, a label could be attached to 100% of the cultures. Similar approaches have been used recently by others (4, 16).

The predominant strains that we detected may be common to this geographical location, or they may represent organisms that are highly resistant to antibacterial agents and other prophylactic measures. In this hospital, patient mortality has decreased significantly since the introduction of a vaccination program with a polyvalent *Pseudomonas* vaccine (1).

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