Epidemiological studies of nosocomial infections with *Pseudomonas aeruginosa* using a DNA probe

A MARK JOFFE, MD, KATHY VOLPEL, PAMELA C KIBSEY, MD, WILLIAM PARANCHYCH, PHD

AM JOFFE, K VOLPEL, PC KIBSEY, W PARANCHYCH. Epidemiological studies of nosocomial infections with *Pseudomonas aeruginosa* using a DNA probe. Can J Infect Dis 1992;3(6):299-306. A DNA probe encoding the *Pseudomonas aeruginosa* pilin gene has been developed in the authors' laboratory and has been shown to be a useful epidemiological tool. In the present study this technology, together with other typing methods, has been used to define relatedness and possible transmission routes of *P aeruginosa* strains isolated in several hospital wards. Clusters of *P aeruginosa* infections, suspected to be the result of nosocomial transmission, developed in a general intensive care unit (ICU) and a neurosurgical ward/ICU, as well as in a burn unit, were studied using antibiograms, lipopolysaccharide-serotyping, and gene probe analysis. Results of these studies demonstrated that each of the general and neurosurgical ICU isolates were different, making nosocomial transmission very unlikely. However, within the burn unit, patient isolates had identical profiles, suggesting that spread between patients was occurring or that a common source of infection was present. Changes in infection control measures within the unit were introduced and may have contributed to eradication of the outbreak. DNA probe studies were valuable in clarifying epidemiological relatedness of isolates that was not evident with the other typing strategies and identified a possible burn-associated strain.

Key Words: Epidemiology, Nosocomial infection, *Pseudomonas aeruginosa*, Typing

Études épidémiologiques sur les infections nosocomiales à *Pseudomonas aeruginosa* à l'aide d'une sonde d'ADN

RÉSUMÉ: Une sonde d'ADN pour l'encodage du gène de *Pseudomonas aeruginosa* a été mise au point dans ce laboratoire et s'est révélée un outil épidémiologique utile. Dans la présente étude, cette technologie, de même que d'autres méthodes de typage, ont été utilisées pour définir les interrelations et les voies de transmission possibles de souches de *P. aeruginosa* isolées dans différentes unités hospitalières. Des infections à *P. aeruginosa*, présumément attribuables à une transmission nosocomiale, ont été observées dans une unité de soins intensifs généraux, dans une unité de soins intensifs neurochirurgicaux et dans une unité de brûlés, où elles ont été étudiées à l'aide d'antibiogrammes, de méthodes de typage sérologique par lipopolysaccharides et d'analyses par sonde génétique. Les résultats de ces études ont démontré que chacun des isolats prélevé dans les unités de soins intensifs généraux et neuro-chirurgicaux était différent, ce qui a permis d'écarter l'hypothèse de la transmission nosocomiale. Cependant, au sein de l'unité des brûlés, les isolats étaient semblables d'un patient à l'autre, ce qui donne à penser qu'il y a eu contamination entre les patients ou qu'il y a eu une source commune à l'infection. Des modifications aux mesures de lutte contre l'infection ont été apportées dans l'unité et peuvent avoir contribué à l'élimination de l'épidémie. Les études par sonde d'ADN ont été utiles pour clarifier les liens épidémiologiques entre les isolats, liens que ne permettaient pas d'identifier les autres stratégies de typage et ont permis la reconnaissance d'une souche possiblement liée à la brûlure.

Department of Medical Microbiology and Infectious Diseases, Faculty of Medicine; Department of Microbiology, Faculty of Science, University of Alberta, Edmonton, Alberta

Correspondence: Dr A Mark Joffe, Department of Microbiology and Immunology, Stanford University School of Medicine, D312 Sherman Fairchild Science Building, Stanford, California 94305-5402, USA. Telephone (415) 723-2671, Fax (415) 725-7282

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PSEUDOMONAS AERUGINOSA IS A MAJOR OPPORTUNISTIC pathogen in immunocompromised individuals, and patients with cystic fibrosis and thermal trauma. Though recognized as a prevalent organism in the hospital environment and a relatively frequent cause of nosocomial disease, the reservoir of the organism and the precise mode of transmission within hospitals are usually unclear.

Existing methods for typing strains of *P aeruginosa* include plasmid analysis, bacteriocin (pyocin) typing, phage sensitivity, biotyping, colonial morphology, antibiotic sensitivity patterns and the use of DNA probes. Serotyping generally is accepted as the most practical and reliable for routine use in clinical microbiology laboratories and is based on an agglutination reaction between bacterial lipopolysaccharide (LPS) and type-specific rabbit antisera (1). While most clinical isolates may be typed using this method, strains from cystic fibrosis patients are unique in that the majority polyagglutinate or are nontypable in O-typing sera (2). In addition, it has been reported previously that LPS serotype may not be a stable epidemiological marker and may change over time (3,4).

A DNA probe encoding the *P aeruginosa* strain PAK pilin gene has been developed (5) and is used to probe restriction enzyme digested *P aeruginosa* chromosomal DNA yielding strain-specific restriction fragment length polymorphism (RFLP) patterns. It has been reported that serial *P aeruginosa* isolates from a patient with cystic fibrosis maintained a constant RFLP pattern and pilin gene sequence despite considerable phenotypic variability (4). In addition, recent studies of over 300 *P aeruginosa* isolates from cystic fibrosis patients and a variety of other sources have demonstrated 21 RFLP patterns and have shown the pilin gene probe to be a useful epidemiological tool (6). The occurrence of clusters of *P aeruginosa* infections in hospitalized patients that were closely related epidemiologically provided the unique opportunity to evaluate the pilin gene probe in the study of nosocomial epidemiology. The goals of this study were to determine if nosocomial spread of *P aeruginosa* was occurring and to compare the pilin gene probe with LPS-serotyping as epidemiological typing techniques in this setting.

PATIENTS AND METHODS

Infections with *Pseudomonas aeruginosa*: The University of Alberta Hospitals is a 1250-bed tertiary care facility representing all areas of clinical medicine, including long term care facilities and separate intensive care units (ICUs) for neonates, pediatrics, neurosurgery, coronary care, cardiovascular surgery, burns and a general surgical-medical ICU. Clusters of infections with *P aeruginosa* were observed over a short period of time within three of the hospital's ICUs (Table 1). These three units – the general intensive care unit (GICU), neurosurgical ward/ICU (NWICU) and the burn

intensive care unit (BICU) – are physically remote from each other within the hospital. Clusters included at least three epidemiologically related patients simultaneously or sequentially hospitalized within one of the ICUs, from whom *P aeruginosa* was isolated from an infected site. Pneumonia was defined as the presence of purulent sputum (grossly and microscopically) with or without fever associated with a chest x-ray infiltrate.

The GICU is a 16-bed unit consisting of 10 open beds and six private rooms. Nosocomial *P aeruginosa* pneumonias developed in three mechanically ventilated patients over a 10-day period, with patient GICU-1 also having a sternal wound infection with this organism following coronary artery bypass surgery (Table 1). These three patients occupied three of four adjacent private rooms with patients GICU-1 and GICU-2 side-by-side and with a noncolonized patient in the room separating patients GICU-2 and GICU-3. Patient GICU-X was transferred from another institution during this outbreak and was colonized with *P aeruginosa* upon admission to the GICU.

The NWICU consists of a 24-bed ward with an attached 10-bed ICU. This outbreak involved one ventilated and two unventilated patients, all of whom developed *P aeruginosa* nosocomial pneumonias over a period of one week.

The BICU is a 10-bed self-contained unit in which all necessary care of the burn patient, including ventilation and hydrotherapy, is carried out. The nature of this outbreak is described under 'Results'.

Bacterial strains: *P aeruginosa* strains isolated from patients involved in clusters of nosocomial infections, as well as concurrent isolates from hospitalized patients not involved in outbreaks, were obtained from the clinical microbiology laboratory of the University of Alberta Hospitals. Clinical specimens including sputum, tracheal secretions, and wound and burn swabs were plated on sheep blood and MacConkey agar plates. Blood cultures were inoculated into broth and growth was detected by the BACTEC NR660 system (Becton Dickinson, Maryland). Isolates were identified by conventional methods including standard tests for oxidase, growth at 42°C and fluorescence under ultraviolet light. One isolate from each of patients BICU 1, 2 and 3 was included in the epidemiological survey reported previously (6).

Antibiograms: Sensitivity testing of all isolates was performed in the clinical microbiology laboratory of the University of Alberta Hospitals using the automated Vitek microbroth dilution technique (Vitek Systems Inc, Missouri). Table 2 lists the antibiotic susceptibility profiles observed for isolates in this study.

Lipopolysaccharide O-serotyping: All strains were serotyped using the International Antigenic Typing System (DIFCO, Michigan) in either the Provincial Laboratory for Public Health of Northern Alberta or the Department of Pediatrics, University of British Columbia. Isolates

TABLE 1
Results of strain typing studies for *Pseudomonas aeruginosa* isolates from clusters of nosocomial infections

Patient	Date/site of isolate	Antibiogram	Serotype	Pilin	Probe U
General ICU outbreak					
GICU-1	January 11, 1988/sternum	1	Polytypeable	2	—
	January 11, 1988/incision	2	Polytypeable	2	—
	January 14, 1988/sputum	1	Polytypeable	2	—
	February 1, 1988/sputum	1	Polytypeable	2	6.7/2.6
GICU-2	January 27, 1988/sputum	3	6	13	9.5/6.8
	January 27, 1988/sputum	4	3	13	9.5/6.8
GICU-3	January 21, 1988/sputum	5	2	5	—
GICU-X*	January 25, 1988/sputum	6	4	4	—
Neurosurgical ward/ICU outbreak					
NWICU-1	January 11, 1988/sputum	5	5	11	—
NWICU-2†	January 14, 1988/sputum	7	3	9	—
	January 14, 1988/sputum	7	3	9	—
NWICU-3	January 15, 1988/sputum	8	2	5	—
Burn ICU outbreak					
BICU-1	November 9, 1987/back	9	11	7	—
	November 12, 1987/blood	9	11	7	—
BICU-2	February 10, 1988/back	10	11	7	—
	February 11, 1988/abdominal	9	11	7	—
	February 11, 1988/left thigh	9	11	7	—
	February 11, 1988/blood	9	11	7	4.3/9.0
	February 20, 1988/right thigh	9	11	7	—
BICU-3	February 20, 1988/back	9	11	7	4.3/9.0
	April 18, 1988/arm	11	6	6	—
BICU-4	April 27, 1988/burn site	12	—	6	5.3;3.2/23
	April 22, 1988/graft site	1	11	11	9.5/3.0;16
	April 22, 1988/foot	—	11	11	—
BICU-5	March 3, 1989/blood	13	1	3	9.5/5.5
	March 3, 1989/burn biopsy	13	1	3	9.5/5.5

*Patient was colonized with *P aeruginosa* on transfer from another institution (see text); †Different colony morphologies isolated from the same specimen; BICU Burn intensive care unit; GICU General intensive care unit; NWICU Neurosurgical ward intensive care unit; Pilin Pilin gene probe pattern (reference 6)

which agglutinated in more than one typing serum were designated as polytypeable.

DNA probe studies: Chromosomal DNA was prepared from late-log phase bacteria using a modification of the procedure of Coleman et al (7). Purified DNA (1 to 2 µg) was digested with the restriction endonucleases PstI and HindIII (GIBCO Laboratories, New York), electrophoresed in 0.7% agarose and transferred to nitrocellulose paper by the method of Southern (8). A 1.2 kilobase HindIII fragment of PAK chromosomal DNA (5) was nick translated and used to probe the nitrocellulose filters under high stringency conditions (9), washing at 21°C rather than 65°C. Autoradiographs were then exposed at -70°C. Fragments hybridizing with the radiolabelled probe were sized by comparison with molecular weight standards run together with the samples in the agarose gel. Twenty-one RFLP patterns were defined in screening large numbers of isolates and were reported previously (6). Experience with this technique has demonstrated complete reproducibility of RFLP patterns as long as genomic DNA is digested to completion and Southern transfer is adequate (4,6). In this study, a technically satisfactory result was generally obtained

on the first attempt, with occasional isolates requiring a second or confirmatory testing.

Selected strains were also typed with a second DNA probe to clarify apparent discrepancies in results between antibiogram, LPS serotype and pilin gene probe. Probe U, a 741 base pair fragment encoding the region upstream of the *P aeruginosa* exotoxin A structural gene (10), was isolated from a 5% acrylamide gel (9) following digestion of plasmid pBRTox with PstI and NruI (GIBCO Laboratories). Southern blots were prepared of BglII- and XhoI- (GIBCO) digested chromosomal DNA and were probed, washed and exposed to x-ray film as described above except that the 741 base pair probe was radiolabelled using the random primer technique (11). Results are expressed as the size of probe reactive fragments in kilobases for the BglII/XhoI digestions in Table 1.

RESULTS

Table 1 summarizes the results of the three clusters investigated in this study, including the antibiotic sensitivity profiles, serotypes and RFLP patterns obtained in DNA probe studies.

TABLE 2
Antibiogram patterns

Pattern*	Amikacin	Gentamicin	Tobramycin	Ticarcillin	Ceftazidime	Piperacillin	Imipenem
1	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
2	Resistant	Resistant	Sensitive	Sensitive	Not applicable	Not applicable	Not applicable
3	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Sensitive
4	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Moderately sensitive	Sensitive
5	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Sensitive	Moderately sensitive
6	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Moderately sensitive
7	Moderately sensitive	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
8	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Moderately sensitive	Resistant
9	Sensitive	Resistant	Resistant	Resistant	Sensitive	Resistant	Sensitive
10	Resistant	Resistant	Resistant	Resistant	Sensitive	Resistant	Sensitive
11	Sensitive	Sensitive	Sensitive	Moderately sensitive	Sensitive	Sensitive	Sensitive
12	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Moderately sensitive
13	Not applicable	Sensitive	Sensitive	Sensitive	Sensitive	Not applicable	Sensitive

*The following groups may not be mutually exclusive: 1, 11 and 13; 2 and 12; 3 and 4

General ICU: DNA probe studies revealed that all four patients involved in this outbreak – including the three patients who sequentially became colonized while in this unit as well as the patient who was transferred from another institution – were colonized with different strains of *P aeruginosa*. This conclusion is supported by the antibiogram patterns and LPS-serotyping.

Four isolates were taken over a three-week period and from two different sites from patient GICU-1. All isolates were identical in DNA probe studies, suggesting that this patient remained colonized with a single strain of *P aeruginosa* throughout the study period. Slight variability in the antibiogram patterns raised the possibility of colonization with two different strains while LPS-serotyping revealed the isolates from patient GICU-1 to be polytypeable.

Two colonies of *P aeruginosa* with different colonial morphologies were isolated from a single sputum specimen for patient GICU-2. While LPS-serotyping suggested that the patient was colonized with two different strains, DNA probe studies with both the pilin gene and probe U demonstrated that this was most probably a single strain.

Neurosurgical ward ICU: All three patients involved in this cluster of infections were colonized by different strains of *P aeruginosa* (indicated by each of the three study techniques). Two colonies with differing morphologies were isolated from the sputum specimen of patient NWICU-2 and, in contrast to patient GICU-2, LPS-serotyping and DNA probe studies concurred that these two colony types represented a single strain.

Burn unit: An outbreak of burn wound sepsis in the burn unit was first recognized when patient BICU-2

developed septic shock from bacteremia with a resistant strain of *P aeruginosa*; within a few days another patient in the burn unit (patient BICU-3) had also become colonized with a similarly resistant strain (Table 1). LPS-serotyping and DNA probe studies using both probes demonstrated that these two patients were probably colonized with the identical strain. In retrospect, there had been a patient in the burn unit three months earlier infected with a resistant strain of *P aeruginosa* (patient BICU-1). Studies of this isolate revealed that it, too, was the identical strain. Environmental sampling was performed and changes were made in the routine care of patients in the burn unit to prevent further transmission (see Discussion).

Two other patients became colonized with *P aeruginosa* in the two months subsequent to the recognition of this outbreak. Patient BICU-4 was colonized with a different strain as demonstrated by all three typing techniques. Patient BICU-5 was colonized by a strain with the same serotype (O11) as the earlier outbreak strains. However, DNA probe studies using both probes in addition to the antibiogram patterns indicated that this represented a different and unique strain. Typing of *P aeruginosa* isolates from the first bacteremic patient following recognition of this outbreak (patient BICU-6) also revealed a strain which was different from the epidemic strain.

Background strains: Two sporadic clinical isolates were available from *P aeruginosa*-infected patients hospitalized during this period but unrelated to the identified outbreaks. One, an eye isolate from a patient with conjunctivitis, was typed as RFLP pattern 6 with the pilin gene probe, while the second, a sputum isolate

from a mechanically ventilated patient in the cardiovascular ICU, could not be typed with this probe despite repeated attempts. This was unusual as less than 5% of isolates have been found to be nontypeable in this system (6). It does, however, suggest that this isolate was different from others studied in this report. Isolates obtained from a GICU patient one year prior to the recognition of the cluster were also analyzed and found to be type 5. No environmental isolates obtained from hospital sources during this period were available for typing.

DISCUSSION

P aeruginosa is a ubiquitous organism found in soil, plants, water and food. Indeed, 5 to 29% of normal individuals are colonized in their gastrointestinal tracts (12). A recent study (13) found that 22.6% of patients were colonized with *P aeruginosa* on admission to a general hospital, with an additional 10.8% acquiring the organism during their hospitalization. Within the hospital, potential sources of pathogenic organisms are numerous and include the patient's own microflora, those in the hospital environment or carried by medical personnel, contaminated equipment and other patients. It generally is agreed that colonized and infected patients are the major reservoir of resistant Gram-negative organisms within hospitals (14,15). There is also considerable evidence that spread of such resistant strains between hospitalized patients occurs on the hands of health care personnel, 30 to 40% of whom carry Gram-negative organisms on their hands (some of them continuously) (16-20). Gastrointestinal acquisition and carriage of Gram-negatives appears to be an important intermediate step in certain situations (14), while some patients are self-infected from their own gastrointestinal microflora (21). A gastrointestinal reservoir was felt to be important in one prospective study in which transfer of *P aeruginosa* between surgical patients in a hospital ward was demonstrated (22).

Previous studies of ICUs have demonstrated conflicting results related to cross-infection of *P aeruginosa* between patients. Large epidemics related to contaminated respiratory therapy equipment have been reported in the past (23), but are rarely a problem today. Cross-infection of *P aeruginosa* between patients in one prospective ICU study was attributed to health care personnel, as 24% of hand cultures and 30% of uniform cultures grew this organism (24). On the other hand, prospective studies in a spinal cord unit (25) and a surgical-medical ICU (26) concluded that cross-infection with *P aeruginosa* is not a major problem in these settings. Clearly, the validity of all such studies depends on the reliability of the strain typing systems employed.

The present authors have investigated clusters of nosocomial infections with *P aeruginosa* in three ICUs in which transmission between patients was strongly

suspected on clinical grounds. The results demonstrated that the patients involved in the GICU and NWICU outbreaks were each infected with a different and unique strain, revealing that transmission between patients was not a factor in these two outbreaks. From an infection control standpoint it is important to ensure that potentially virulent and multiply resistant Gram-negative organisms are not being spread between hospitalized patients. It was not possible in this study to examine potential sources of the *P aeruginosa* colonizing the patients.

Of the currently available typing schemes for *P aeruginosa*, LPS-serotyping generally is considered to be the most practical, while colony morphology and antibiogram patterns are the least accurate (27,28). While reproducibility in testing serial isolates has been questioned, antimicrobial susceptibility data assessment is likely the most readily available typing technique for most clinicians and was therefore included in this investigation. In this study, multiple isolates from individual patients revealed slight variability in antibiograms, making interpretation confusing. As such, it is difficult to establish firmly epidemiological relatedness between strains on this basis. Furthermore, rapid changes in antibiogram patterns frequently are seen in subpopulations of *P aeruginosa* in response to antimicrobial therapy, further adding to the difficulty in interpretation.

Isolates of *P aeruginosa* from noncystic fibrosis patients can be reproducibly serotyped in 92 to 99% of cases (2,29). In contrast, 68 to 80% or more of isolates from cystic fibrosis patients are nontypeable or polytypeable by LPS-serotyping (2,30,31). Development of O-antigen specific monoclonal antibodies (MAbs) for serotyping may circumvent this problem and provide improved discrimination between strains (32-35), although reproducibility for the MAbs was only 75% in a recent study (36). As noted earlier, changes in LPS serotype may occur in single strains over time (4,37), and some strains are now known to synthesize more than one type of chemically and antigenically distinct LPS molecule (38). The reproducibility and discriminatory power of several typing methods has been compared, and use of more than one typing system has been advocated, at least for cystic fibrosis isolates (39, 40).

It is unusual that *P aeruginosa* isolates from patient GICU-1 could not be serotyped while DNA probe studies demonstrate conclusively that this patient was colonized with a single unique strain throughout the three-week study period. Furthermore, serotyping isolates of different colony morphologies in patient GICU-2 suggested the coexistence of two different strains, while DNA probe studies indicated that this reflected phenotypic variability in a single strain as has been demonstrated previously (1). Rapid adaptation with alteration in an organism's phenotype in response to environmental stimuli through modulation of gene ex-

pression is well-established (41). Recently genetic rearrangement in *P aeruginosa* has been described as a mechanism for altering expression of virulence factors including LPS serotype (42).

The burn unit outbreak was initially recognized when patients BICU-2 and BICU-3 were sequentially infected with a resistant strain of *P aeruginosa*. Antibigrams, DNA probe studies and serotyping demonstrated that a patient who had died of pseudomonas sepsis in the burn unit three months earlier (patient BICU-1) was infected with the same strain. This suggested nosocomial transmission through cross-infection or, more likely, a common source of infection was present as there was no temporal overlap between patients BICU-1 and BICU-2 and -3. Multiple environmental cultures from the burn unit, including water sources and the hydrotherapy tank, failed to grow *P aeruginosa* except for a single culture on one occasion from the scale used to monitor weights of all patients in the unit. Unfortunately, this isolate was inadvertently discarded and could not be typed to determine if this was a potential reservoir of *P aeruginosa* within the burn unit. However, use of the scale was discontinued, as was the hydrotherapy routine for burn victims, in the hope of preventing further spread of this organism. Two patients became colonized with *P aeruginosa* shortly after recognizing this cluster (BICU-4 and BICU-5); antibiogram and DNA probe studies of these, as well as those from a subsequent bacteremic patient (BICU-6), were consistent with eradication of the outbreak strain. LPS-serotyping, however, could not differentiate BICU-5 isolates from the earlier outbreak strain. This serotype (O11) is known to be prevalent (23) and the discriminatory power of LPS-serotyping may not be sufficient for such high frequency types (35). Monitoring of antibigrams and RFLP patterns in burn unit isolates has confirmed the eradication of the multi-resistant epidemic strain, while elimination of hydrotherapy in the routine care of the burn patient has had a major impact on the frequency and pattern of *P aeruginosa* colonization and the morbidity and mortality related to it.

While no definite associations between RFLP types and disease states have been identified, the epidemic strain detected in the burn unit cluster is of particular interest because of a possible predilection of this RFLP pattern for burn patients. While other types have been isolated from burn patients, RFLP type 7 appears to be a relatively infrequent type and has been isolated only from burn patients. Furthermore, this type has now been implicated in burn wound sepsis both in this outbreak as well as in a prolonged outbreak involving a virulent multiresistant *P aeruginosa* isolate spanning 18 months in the burn unit of another major city (6). While the possibility that RFLP type 7 may have an affinity for burns or that it may have increased virulence in this setting is intriguing, it remains specula-

tive at present. In support of this possibility is the recognition that serious disease is caused by a small number of clones within a pathogenic species (43).

Molecular biology has recently contributed novel techniques for the identification of a wide variety of microorganisms and the differentiation of strains within species (44). Chromosomal DNA fingerprinting of *P aeruginosa* by restriction enzyme digestion with rare cutting endonucleases and pulsed-field electrophoresis has been used to type polyagglutinable cystic fibrosis isolates (45) and to monitor the epidemiology of *P aeruginosa* in patients attending a cystic fibrosis clinic (46). A DNA probe consisting of a 741 base pair PstI-NruI fragment encoding the nucleotide sequence upstream of the *P aeruginosa* exotoxin A gene has been described (10) and used in studying epidemiology in cystic fibrosis (3,47,48), acute leukemia (49) and in distinguishing relapse from reinfection in a variety of infections due to *P aeruginosa* (37). Another DNA probe encoding the N-terminus of the exotoxin A structural gene and its 5' flanking region has been described and used with a probe encoding the PAK pilin gene to type a small number of isolates (50). Most recently, a combination of esterase electrophoretic typing and ribotyping has been applied to cystic fibrosis isolates highlighting the complexity of *P aeruginosa* colonization in this disease (51).

Few studies have used these new and potentially more reliable techniques in hospital epidemiology to evaluate reservoirs and mechanisms of nosocomial transmission of *P aeruginosa*. Pulsed-field electrophoresis has been used to monitor airway colonization in an ICU setting and has demonstrated a lack of contamination from environmental sources or between patients (52) while, more recently, possible cross-contamination between immunocompromised children in a hematological ICU with a gastrointestinal reservoir has been shown (53). Multilocus enzyme electrophoresis (54) has been used in a prospective study of a general medicine-oncology unit which demonstrated that 10% of 283 patients admitted over a six-month period acquired *P aeruginosa* in hospital (55). Of these, 15% were likely cross-contamination from other patients and 27% were linked to environmental sources, while the source for the remaining 60% of nosocomially colonized patients was not established.

In summary, by using DNA probe technology, persistence of a common strain of *P aeruginosa* has been demonstrated in a burn unit infecting three patients over a three-month period, suggesting possible cross-infection or infection from a common source. While cross-infection between patients in the GICU and NWICU was felt to be likely by virtue of the proximity in time and space of those involved, the data have shown that patients involved in these two clusters were each infected with a different strain. When multiple isolates from individual patients were typed, the hybridization pattern remained constant, suggesting that each

patient was colonized with a single strain at all sites and throughout the study period, in spite of considerable phenotypic variability including antibiotic susceptibility and LPS serotype. Results of RFLP typing of selected isolates with a second DNA probe always concurred with results obtained using the pilin gene probe. In general, LPS-serotyping and antibiograms in this study allowed for discrimination between unrelated isolates obtained from different patients, but variability in sequential isolates from individual patients would make conclusions based on these techniques difficult. The pilin gene probe in this and previous studies of cystic fibrosis, ICU and burn patients has been found to be a consistent and reliable technique. While its use may not yet be readily applicable in the general clinical microbiology laboratory, it particularly is useful in outbreak investigation for the unambiguous delineation of relationships between strains. Although based on a limited number of isolates, this study suggests a poten-

tial role for DNA probe studies in establishing relationships between isolates which are epidemiologically related but appear to be different (based on antibiogram and serotyping), while also distinguishing between unrelated isolates exhibiting highly prevalent serotypes such as type O11. Discrimination between strains with the pilin gene probe may be less than ideal, however, as 80% of strains belong to types 1 to 5 (6). Of further concern is the possibility that genomic rearrangements may lead to instability of RFLP patterns (56), although the use of two independent probes in this study directed at different regions of the genome strengthens the conclusions regarding relatedness between strains. A direct comparison of conventional typing methods with the newly developed ones described here is to define the optimal use of the various techniques singly or in combination so that the epidemiology and pathogenesis of *P aeruginosa* may be addressed definitively.

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