

ENDEMIC *PSEUDOMONAS AERUGINOSA* INFECTION IN A NEONATAL INTENSIVE CARE UNIT

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ABSTRACT

Background Nosocomial infections due to *Pseudomonas aeruginosa* have been well described, but the environmental reservoir of the organism varies. We conducted an epidemiologic and molecular investigation of endemic *P. aeruginosa* infection among infants in a neonatal intensive care unit that was associated with carriage of the organisms on the hands of health care workers.

Methods In August 1998, colonization or infection with *P. aeruginosa* was identified in six infants. Surveillance cultures for *P. aeruginosa* were obtained from the other 27 infants in the unit, and possible environmental reservoirs were also assessed. The hands of health care workers were inspected and cultured, and risk factors for *P. aeruginosa* colonization were evaluated. Isolates were analyzed for clonality by pulsed-field gel electrophoresis.

Results Surveillance cultures showed that three additional infants were colonized with *P. aeruginosa*. Cultures of environmental specimens were negative, but cultures of the hands of 10 of 165 health care workers (6 percent) were positive for *P. aeruginosa*. Increasing age ($P=0.05$) and a history of the use of artificial fingernails or nail wraps ($P=0.03$) were both risk factors for colonization of the hands. From January 1997 to August 1998, 49 infants were infected or colonized with *P. aeruginosa*. Pulsed-field gel electrophoresis demonstrated that 17 of these infants and 1 health care worker who had onychomycosis had the same clone. Infants who were exposed to this health care worker in August 1998 were at greater risk of having this clone than infants who were not exposed to this health care worker (odds ratio, 41.2; 95 percent confidence interval, 1.8 to 940.0; $P=0.006$).

Conclusions An increased rate of infection and colonization with *P. aeruginosa* among infants in neonatal intensive care units should be investigated by assessing potential reservoirs, including environmental sources as well as patients and health care workers. (N Engl J Med 2000;343:695-700.)

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PSEUDOMONAS *aeruginosa* is a well-known cause of nosocomial infections among infants in neonatal intensive care units. The clinical syndromes that have been described in affected infants include conjunctivitis and endophthalmitis, skin infections, bloodstream infections, meningitis, pneumonia, and diarrhea and necrotizing enterocolitis.¹⁻³ Environmental sources such as sinks and respiratory-therapy equipment are the most common-

ly described reservoirs of *P. aeruginosa*,⁴⁻⁶ but occasionally, health care workers have been the reservoir.⁷

In January 1997, an increased rate of colonization and infection with *P. aeruginosa* was noted in the neonatal intensive care unit at our hospital. The institution of control measures, such as contact isolation precautions that included the use of gowns and gloves and placement of infants with colonization or infection in a separate room, where they were cared for by designated nurses, temporarily reduced the incidence of this pathogen. Cultures of possible environmental sources did not identify a reservoir. However, in August 1998, *P. aeruginosa* was isolated from six neonates in the neonatal intensive care unit. We describe the epidemiologic and molecular investigation of this endemic *P. aeruginosa* infection.

METHODS

Incident Cases and Infection Rate

The study was initiated when daily review of microbiology-laboratory reports from a neonatal intensive care unit in a university-affiliated pediatric hospital in New York City revealed an increase in the number of positive cultures for *P. aeruginosa* in August 1998. At the time of the study, the unit consisted of 36 beds, a 4-bed transitional nursery, and a 10-bed step-down nursery. An incident case was defined on the basis of the isolation of *P. aeruginosa* in cultures of samples obtained from any body site from January 1997 to September 1999, including cases detected by active surveillance in August 1998 (e.g., from gastric aspirates). The incidence rate per 1000 patient-days was calculated.

Surveillance Cultures

Once the increased incidence of colonization and infection with *P. aeruginosa* was noted in August 1998, surveillance cultures were performed to identify all infants with colonization. At that time, there were 33 infants in the neonatal intensive care unit, 6 of whom were identified as being colonized or infected with *P. aeruginosa*; surveillance cultures were obtained from the other 27 infants. Gastric aspirates were obtained for culture by withdrawing 0.5 ml of stomach contents from an existing orogastric tube or a temporary tube. Respiratory tract secretions suctioned from an endotracheal tube were sent for culture, or alternatively, if the infant was not intubated, nasopharyngeal swabs were obtained. Surveillance cultures were obtained twice monthly until all infants in the initial cohort had been discharged and then monthly for a total of three months.

To detect environmental reservoirs for *P. aeruginosa*, we performed cultures of 25 environmental specimens, including tap water, sink drains, liquid medications, respiratory-therapy equipment, hand soaps, hand creams, and water baths used to warm formula. Moist and dry environmental surfaces were swabbed with a cotton-tipped swab (Mini-Tip Culturette Collection and Transport Systems, Becton Dickinson Microbiology Systems, Sparks, Md.).

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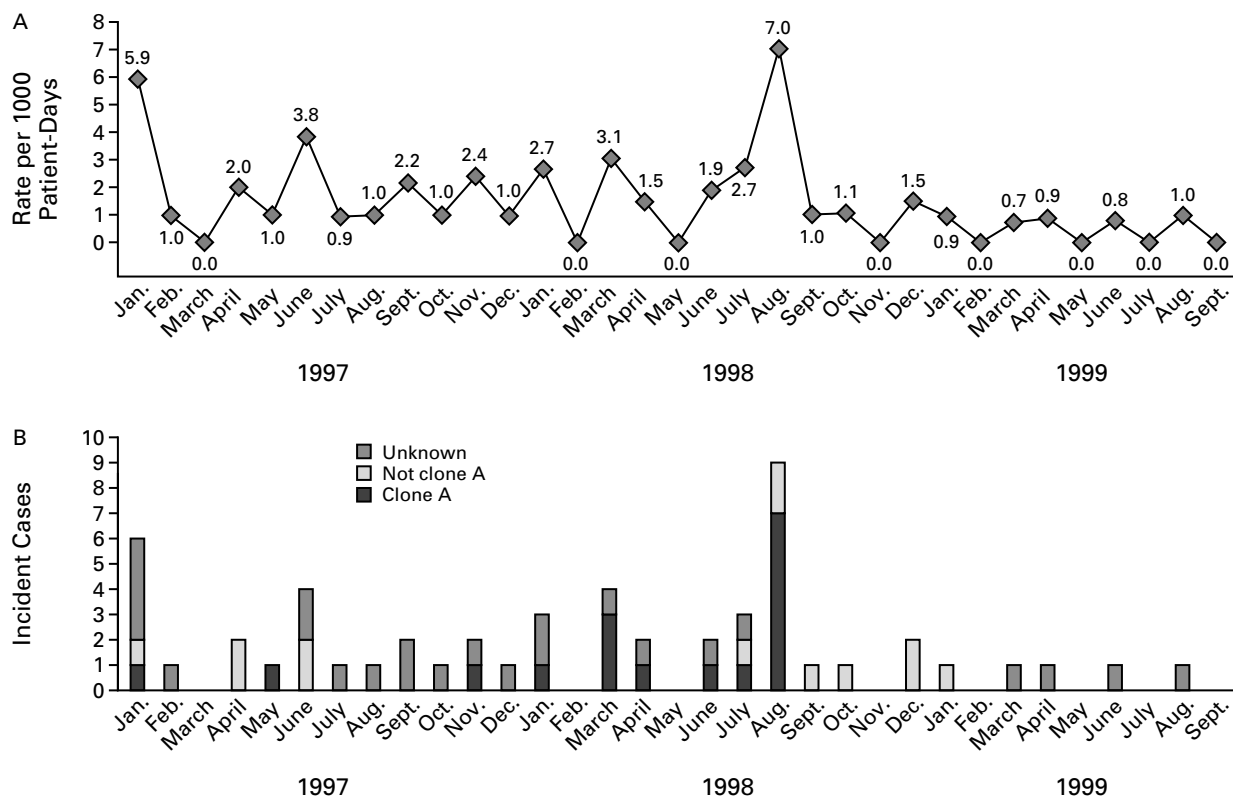


Figure 1. The Incidence Rate of Infection and Colonization with *Pseudomonas aeruginosa* (Panel A) and the Strains Isolated in Incident Cases (Panel B) among Infants in the Neonatal Intensive Care Unit from January 1997 to September 1999.

Strains identified as clone A and other strains are indicated. Isolates that were not available for pulsed-field gel electrophoresis are labeled unknown.

Cultures of the Hands of Health Care Workers

The hands of health care workers who came in contact with infants hospitalized in the neonatal intensive care unit during August 1998 were cultured for *P. aeruginosa* with use of a modification of the "glove juice" method.⁸ Both hands of each worker were sequentially put into a sterile polyethylene bag containing 50 ml of sampling solution (0.075 M phosphate buffer, pH 7.9, containing 0.1 percent polysorbate 80, 0.1 percent sodium thiosulfate, 0.3 percent lecithin, and an inhibitory concentration of vancomycin). One bag was used for each worker. Each hand was massaged by an infection-control practitioner through the wall of the bag for 15 to 30 seconds, and samples were delivered to the microbiology laboratory within 1 hour for processing.

Processing of Specimens

Specimens were processed on arrival at the clinical-microbiology laboratory. Enriched and selective mediums (trypticase soy agar with 5 percent sheep's blood and MacConkey agar plates, BBL, Sparks, Md.) were inoculated, incubated at 35°C, and examined for growth at 24 and 48 hours. Isolates of *P. aeruginosa* were identified (MicroScan identification system, Dade Behring, West Sacramento, Calif.) and then frozen at -70°C for further analyses.

Molecular Epidemiology

Pulsed-field gel electrophoresis (BioRad GenePath Strain Typing System, Hercules, Calif.) was performed with use of *SpeI* to analyze the clonality of *P. aeruginosa* strains isolated from the cohort of patients in August 1998, from the hands of health care work-

ers, and from patients from January 1997 to December 1998 when isolates were available.^{9,10} We interpreted the banding patterns according to the manufacturer's criteria: isolates with identical restriction patterns were considered identical, those that differed by one to three bands were considered to be closely related, those that differed by four or five bands were considered to be possibly related, and those that differed by six or more bands were considered to be genetically different.

Risk Factors for Colonization of the Hands with *P. aeruginosa*

The hands of all health care workers were inspected by the infection-control practitioner at the time of culturing. The presence of false nails, nail polish, and cracked or inflamed nail beds was noted. Health care workers also completed a questionnaire that included questions about sex, age, occupation, and the length of time they had worked in the neonatal intensive care unit. Possible exposures to *P. aeruginosa* and risk factors for infection, such as use of antibiotics and a history of otitis externa, swimming in the preceding year, skin lesions or dermatitis, latex allergy, nail or nail-bed infections, and the use of artificial nails or nail wraps, were assessed.

Statistical Analysis

Risk factors for colonization of the hands of health care workers with *P. aeruginosa* were determined by logistic-regression analysis with the use of SAS software (SAS Institute, Cary, N.C.). The association between exposure to a specific nurse in August 1998 and infection or colonization with the endemic clone of *P. aeruginosa* was assessed. Confidence intervals were obtained by the

TABLE 1. SITES FROM WHICH *PSEUDOMONAS AERUGINOSA* WAS ISOLATED AMONG INFANTS IN THE NEONATAL INTENSIVE CARE UNIT, JANUARY 1997 TO DECEMBER 1998.

SITE	NO. OF POSITIVE CULTURES (%)*	NO. OF INFANTS WITH INITIALLY POSITIVE CULTURES (%)
Blood	9 (12)	9 (18)
Eye	21 (29)	15 (31)
Respiratory tract†	22 (30)	14 (29)
Wound	3 (4)	1 (2)
Urine	5 (7)	4 (8)
Gastrointestinal tract‡	10 (14)	4 (8)
Catheter tip or insertion site of Broviac catheter	3 (4)	2 (4)
Total	73	49

*One infant may have had more than one positive site.

†Samples included tracheal aspirates, sputum, nasopharyngeal swabs, throat swabs, swabs of tracheostomy sites, and pleural fluid.

‡Samples included gastric aspirates, stool, rectal swabs, and swabs of gastrostomy sites.

exact method with use of a Pascal program, and P values were calculated by the two-sided Fisher's exact test (Epi Info 6, version 6.04b, Centers for Disease Control and Prevention, Atlanta).

RESULTS

Incident Cases and Infection Rate

The incidence rate of colonization and infection with *P. aeruginosa* was 7.0 cases per 1000 patient-days in August 1998 (Fig. 1A). There were 49 incident cases in the neonatal intensive care unit from January 1997 to December 1998 (Fig. 1B), including those among 9 infants in the 33-infant cohort of August 1998; 6 of the 9 were identified by clinical cultures, and 3 by surveillance cultures. The organism was isolated from numerous sites, but the eyes and the respiratory tract were the most common sites (Table 1). All strains of *P. aeruginosa* were susceptible to all anti-pseudomonas antimicrobial drugs except trimethoprim-sulfamethoxazole.

Surveillance Cultures

None of the cultures of environmental specimens grew *P. aeruginosa*. Three of the 27 infants from whom surveillance cultures were obtained had *P. aeruginosa* isolated from gastric aspirates.

Cultures of the Hands of Health Care Workers

In all, 166 health care workers who came in contact with infants in the neonatal intensive care unit in August 1998 were identified for hand culturing, and cultures of the hands were obtained from 165 (99 percent). Cultures were obtained from the majority of these health care workers (96 percent) within the first week of the study. Cultures were obtained from seven workers who had been on vacation or leave

TABLE 2. RESULTS OF CULTURES OF HEALTH CARE WORKERS' HANDS FOR *PSEUDOMONAS AERUGINOSA*.

JOB CATEGORY*	NO. CULTURED	NO. WITH POSITIVE CULTURES (%)
NICU clinicians	41	2 (5)
NICU nurses	82	8 (10)
Consulting physicians	8	0
Respiratory therapists	13	0
Technologists	11	0
Ancillary staff	10	0
Total	165	10 (6)

*NICU denotes neonatal intensive care unit. The NICU clinicians consisted of 12 attending physicians, 9 fellows, 3 nurse practitioners, and 17 members of the house staff. The consulting physicians were from cardiology, ophthalmology, and surgery. The technologists consisted of two personnel from radiology, two from electrocardiography, two from echocardiology, one from electroencephalography, and four phlebotomists. The ancillary staff consisted of six nurses' aides and four housekeepers.

during the first week when they returned to work. The hands of 10 health care workers (6 percent) were positive for *P. aeruginosa* (Table 2). Hand cultures were repeated in these 10 workers within the next week; 7 had negative cultures, but in 3, the cultures were again positive for *P. aeruginosa*. These three workers were furloughed with full pay until cultures of their hands were negative for *P. aeruginosa*. All had underlying risk factors for persistent colonization. The first health care worker wore nail extenders; when the extenders were removed, hand cultures were subsequently negative. The second health care worker had candida onychomycosis that was first noted in August 1998 (including during the initial hand inspection), and hand cultures were positive for *P. aeruginosa* before and after chlorhexidine gluconate scrubbing. The third health care worker had otitis externa and persistently positive cultures of specimens obtained from the hands, ear canal, and anterior nares. The second and third health care workers were both treated for their underlying conditions and returned to work after their conditions resolved and hand cultures were negative for *P. aeruginosa*.

Molecular Analysis of *P. aeruginosa* Strains

Isolates were available for molecular analysis in 29 of the 49 incident cases (59 percent). Seven of the nine infants who were infected or colonized with *P. aeruginosa* in August 1998 had the same clone, clone A (Fig. 1B and 2). From January 1997 through August 1998, this clone was found in 17 infants. Two additional infants had clone B (isolated from both in April 1997) (Fig. 2), two had clone L (isolated in June 1997), and the remaining eight clones were unique. None of the *P. aeruginosa* strains detected after Au-

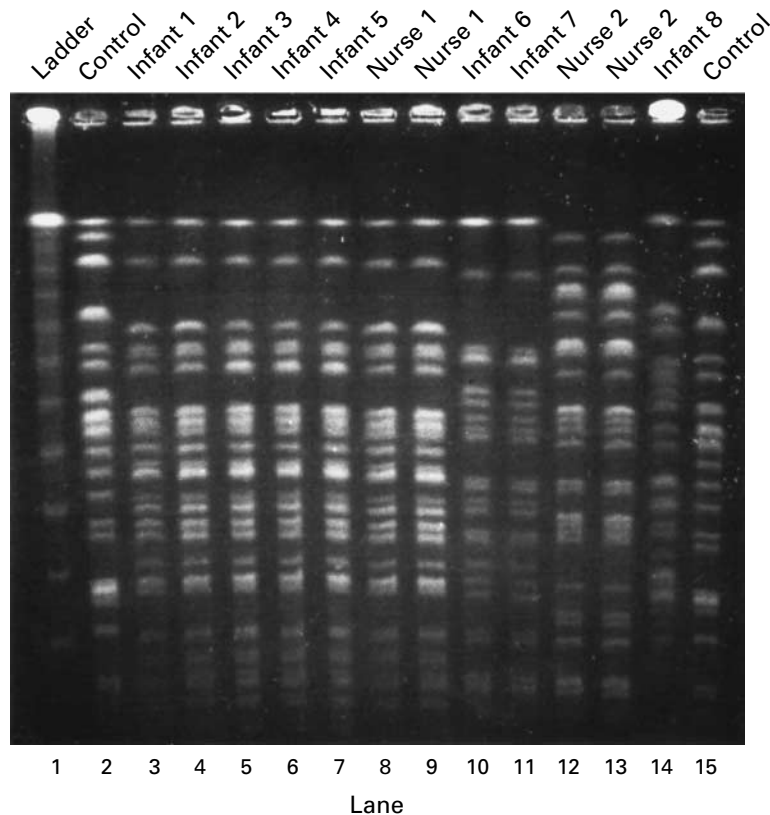


Figure 2. Results of Pulsed-Field Gel Electrophoresis of Representative Strains of *Pseudomonas aeruginosa* Isolated from Infants in the Neonatal Intensive Care Unit and from Health Care Workers.

Lane 1 shows the molecular-size ladder; lane 2, a control strain (BioRad Laboratories); lane 3, blood from Infant 1; lane 4, blood from Infant 2; lane 5, blood from Infant 3; lane 6, gastric aspirate from Infant 4 obtained for surveillance culture; lane 7, eye swab from Infant 5; lane 8, hands of Nurse 1; lane 9, hands of Nurse 1 (sample was obtained two weeks after the sample shown in lane 8); lane 10, tracheal aspirate from Infant 6; lane 11, blood from Infant 7; lane 12, ear swab from Nurse 2; lane 13, hand of Nurse 2; lane 14, blood from Infant 8; and lane 15, a control strain (BioRad Laboratories).

Lanes 3 through 9 show clone A. Lanes 10 and 11 show clone B. Nurse 1 had candida onychomycosis. Nurse 2 had otitis externa.

gust 1998 were clone A. Only the health care worker with candida onychomycosis was colonized or infected with clone A. None of the other nine workers with colonization had a clone that was also isolated from an infant (data not shown).

Risk Factors for Colonization of the Hands

The 10 health care workers with colonization were significantly older than the workers without colonization (mean age, 47 vs. 41 years, $P=0.04$). Increasing age (odds ratio for each year of age, 1.1; 95 percent confidence interval, 1.0 to 1.2; $P=0.05$) and a history of the use of artificial nails or nail wraps (odds ratio, 7.0; 95 percent confidence interval, 1.2 to 38.3; $P=0.03$) were risk factors for *P. aeruginosa* carriage. None of the other factors assessed, including sex, occupation, antibiotic use, a history of otitis externa, and a history of skin lesions and dermatitis, could be used to predict hand carriage.

Risk of Acquiring Clone A after Exposure to Health Care Workers

In August 1998, 7 infants were infected or colonized with clone A, 24 infants had negative cultures, and 2 infants were colonized with another clone of *P. aeruginosa*. Nursing assignments for August 1998 revealed that on average, 17 nurses (range, 1 to 34) cared for each of the 33 infants in the cohort. Exposure to three nurses — the health care worker with candida onychomycosis and two nurses with negative cultures — was associated with an increased risk of colonization with clone A among the infants (Table 3).

Infection-Control Measures

To control the infection, contact isolation procedures were used for infants who were colonized or infected with *P. aeruginosa*: gown and gloves were used during any contact with these patients, and the patients were placed in a separate room and cared

TABLE 3. RISK OF INFECTION OR COLONIZATION WITH *PSEUDOMONAS AERUGINOSA* CLONE A AMONG INFANTS IN A NEONATAL INTENSIVE CARE UNIT AFTER EXPOSURE TO FOUR HEALTH CARE WORKERS IN AUGUST 1998.

HEALTH CARE WORKER No.	STRAIN COLONIZING HAND OF HEALTH CARE WORKER	COLONIZATION WITH CLONE A (N=7)	NEGATIVE CULTURES OR COLONIZATION WITH ANOTHER CLONE (N=26)*	ODDS RATIO (95% CI)†	P VALUE
		no. of infants (%)			
1‡	Clone A	3 (43)	0	41.2 (1.8–940.0)	0.006
2§	Not clone A	2 (29)	7 (27)	1.1 (0.1–9.1)	1.00
3	Negative	5 (71)	4 (15)	13.8 (1.5–164.9)	0.009
4	Negative	3 (43)	1 (4)	18.8 (1.2–625.0)	0.02

*Two infants were colonized with a clone other than clone A, and 24 infants had negative cultures.

†CI denotes confidence interval.

‡This health care worker had candida onychomycosis.

§This health care worker had otitis externa.

for by designated nurses. At the beginning of each shift, health care workers washed their hands with a preparation containing 4 percent chlorhexidine gluconate for two minutes; during their shifts, the workers washed their hands with a preparation containing 2 percent chlorhexidine gluconate. Staff members were asked to wear no jewelry other than wedding bands and wristwatches. Cosmetic nail treatments were not permitted. In addition, several care practices were changed: water baths were no longer used to heat formula, and the number of supplies kept by the patients' bedsides was minimized.

DISCUSSION

The role of *P. aeruginosa* as an important pathogen in children, especially in premature infants, has been known since 1960.¹⁻³ Numerous sites of colonization with *P. aeruginosa* have been reported, but the respiratory and gastrointestinal tracts have been the most common.¹¹ Premature infants appear to be at increased risk for invasive disease after colonization.¹² We found that cultures of gastric aspirates were particularly helpful in identifying infants with colonization. Although previous studies have not reported culturing this site, it is not surprising that potential pathogens are present in the stomachs of hospitalized neonates. In premature infants, the gastric pH is often high,¹³ thereby permitting bacterial growth. As a result of frequent suctioning, possible aspiration, and the use of oropharyngeal tubes, the respiratory tract and the gastrointestinal tract may share colonizing flora. The eyes may become colonized if they are not protected during suctioning and during removal of feeding tubes.

Active surveillance of infants who are at high risk is recommended to monitor nosocomial infections. In

neonatal intensive care units, bloodstream infections were the most common nosocomial infection reported to the National Nosocomial Infection Surveillance system from 1986 to 1994, but *P. aeruginosa* was a relatively rare cause of these infections.¹⁴ Likewise, in a 12-year single-center survey of neonatal sepsis, *P. aeruginosa* caused 8 of 433 bloodstream infections (2 percent) and 8 of the 110 documented gram-negative infections (7 percent).¹⁵ In contrast, we detected nine bloodstream infections with *P. aeruginosa* over a period of 18 months, and these infections represented 47 percent of infections caused by gram-negative pathogens. Furthermore, investigators at a newborn intensive care unit reported that *P. aeruginosa* was isolated from 6 percent of cultures of eye swabs (18 of 307) over a 10-year period¹⁶ — a value similar to the 7 percent prevalence reported by the National Nosocomial Infection Surveillance system in 1996.¹⁴ In contrast, we detected *P. aeruginosa* in 12 percent of conjunctival cultures (21 of 177). This finding strongly suggests that *P. aeruginosa* has been a relatively rare pathogen among patients in neonatal intensive care units and therefore that any increase in the rate of infection and colonization should be promptly investigated.

Transient colonization of the hands with *P. aeruginosa* has been described,^{17,18} and dermatitis of the hands has been associated with an increased rate of carriage of *P. aeruginosa*.¹⁹ Although dermatitis was not a risk factor in our study, older age was associated with an increased risk of colonization with *P. aeruginosa*. We speculate that skin changes associated with aging may lead to an increased rate of colonization with *P. aeruginosa*. (Since most of the health care workers from whom we obtained cultures were women, sex could not be assessed as a risk factor.) We also

confirmed previous observations that hands with artificial nails were more likely to be colonized with gram-negative bacteria, including *P. aeruginosa*, than were hands with natural nails.^{20,21}

Previously described outbreaks of *P. aeruginosa* in nurseries have focused on possible reservoirs of infection, including contaminated incubators, respiratory-therapy equipment, sinks, and hand lotion.⁴⁻⁶ However, outbreaks of *P. aeruginosa* have rarely been attributed to hand carriage. In an outbreak of *P. aeruginosa* in a surgical intensive care unit that was linked to hand carriage by a single staff member,⁷ the same clone of *P. aeruginosa* was isolated from nine patients.

In our study, complementary epidemiologic and molecular analyses were performed to determine the mode of transmission of *P. aeruginosa* in the neonatal intensive care unit. Not only did we document that a dominant clone was present in the unit for 18 months, but we also demonstrated that two other clones were each shared by two infants, providing further evidence of the occurrence of cross-infection with *P. aeruginosa*.

Initially, we focused on an assessment of traditional reservoirs and used well-validated strategies to control colonization and infection with *P. aeruginosa*. However, these strategies were effective only temporarily. Our findings suggest that cultures of the hands of health care workers should be considered early in such investigations.

Despite the links provided by molecular epidemiology, there are some gaps in our understanding of the sustained transmission of clone A. We documented the presence of this clone in the neonatal intensive care unit beginning in January 1997, but the reservoirs of the clone were not fully understood for 18 months. Although the hands of health care workers were most likely responsible for the transmission of clone A in August 1998, we speculate that infants with colonization who were hospitalized for prolonged periods served as the previous reservoir. At least one infant with clone A was hospitalized throughout 1998, but there were too many missing isolates from patients in 1997 for us to confirm this hypothesis. Jellard and Churcher made a similar observation²²: using phage typing, these investigators demonstrated that an endemic strain in stool samples from infants in a neonatal intensive care unit was present over a 16-month period. In that study, cultures of environmental specimens and swabs of the throats and rectums of health care workers were negative, but hand cultures were not performed.

In conclusion, an increase in the rate of infection and colonization with *P. aeruginosa* among infants in a neonatal intensive care unit should be promptly investigated. All potential reservoirs should be identified, including environmental sources, equipment used in patient care, patients, and the hands of health

care workers. Underlying conditions such as otitis externa or onychomycosis may be associated with persistent carriage of *P. aeruginosa* on the hands of health care workers and should be detected and eradicated. It is likely that our observations are applicable to other intensive care units as well.

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