Environmental monitoring and clinical surveillance for Legionella species were done for 12 months as recommended by the Allegheny County Health Department (Pittsburgh). The water system of a hospital was found to be colonized with Legionella pneumophila serogroup 5. Three patients with nosocomial L. pneumophila serogroup 5 disease were subsequently diagnosed after laboratory tests for legionellae were made available for all patients with nosocomial pneumonia. All serogroup 5 isolates from the hospital water matched the 3 patient isolates by pulsed-field gel electrophoresis (PFGE). Furthermore, isolates found in the water supply dating back 10 years showed the same PFGE pattern. In contrast, 12 L. pneumophila serogroup 5 isolates from eight other institutions had different PFGE patterns. Routine environmental cultures were important in stimulating the application of Legionella laboratory testing, which subsequently identified unsuspected patients with nosocomial legionnaires’ disease.

Of patients infected with pneumonia caused by Legionella pneumophila, >80% are due to serogroup 1 or 6 [1]. In contrast, only 6 patients with legionnaires’ disease caused by L. pneumophila serogroup 5 were reported to the Centers for Disease Control and Prevention between 1980 and 1989 [1]. In a review of the literature, we found only 5 reported patients with L. pneumophila serogroup 5 disease [2–5]. Accordingly, little is known about the epidemiology and clinical manifestations of infection caused by L. pneumophila serogroup 5.

Colonization of hospital water distribution systems with L. pneumophila serogroup 1 has been repeatedly linked to acquisition of nosocomial legionnaires’ disease [6]. With application of the Allegheny County Health Department (Pittsburgh) guidelines in 1994, one hospital was found to have L. pneumophila serogroup 5 as the predominant organism in its water supply. Would colonization with a serogroup rarely linked to disease result in nosocomial legionnaires’ disease? We instituted a prospective clinical surveillance for legionnaires’ disease for 1 year in this hospital to address this issue.

Materials and Methods

The Allegheny County Health Department issued guidelines in 1993 for the control and prevention of nosocomial legionnaires’ disease [7]. These guidelines were instituted in hospital A and included annual environmental surveillance for Legionella species by culturing all hot-water tanks and a representative number of distal sites (faucets or shower heads). If colonization of L. pneumophila was documented, the guidelines recommended specialized laboratory testing for legionellae for all patients with nosocomial pneumonia, either in-house or through a reference laboratory.

Prospective clinical surveillance. Surveillance for legionnaires’ disease in hospitalized patients with fever and pulmonary infiltrates was done at hospital A between May 1994 and April 1995 based on the above guidelines. The infection control practitioner ensured that for every patient with nosocomial pneumonia, the following additional tests for legionellae would be done: urinary antigen, sputum culture, and serology.

Sputum specimens were plated on nonselective and selective buffered yeast extract agar (BCYE) for the isolation of legionellae as previously described [8]. Direct fluorescent antibody (DFA) staining of samples was done using a monoclonal antibody reagent (Genetic Systems, Redmond, WA) that reacts with all serogroups of L. pneumophila. Serogroup-specific polyclonal reagents (SciMedX, Denville, NJ) were used for definitive identification and serogrouping.

Acute and convalescent sera were analyzed for the presence of anti-Legionella antibodies by ELISA. Sera were tested with separate IgM and IgG conjugates. Patients experiencing 4-fold seroconversion to titers ≥256 were classified as “definitive” cases. Patients with single elevated titers to ≥256 were classified as “presumptive” cases.

Legionella antigen (L. pneumophila serogroup 1) in urine was detected by RIA (Binax, South Portland, ME) per manufacturer’s instructions. A ratio of ≥3.0 of the counts per minute (cpm) of the test sample to the cpm of the negative control urine is considered positive.

Environmental surveillance. Environmental culturing for legionellae was done in patient care areas and the hot-water storage
tanks of the hospital [8]. *Legionella*-like colonies were subcultured to BCYE agar and serotyped by DIPA staining.

Pulsed-field gel electrophoresis (PFGE). PFGE was used to determine concordance between patient and environmental isolates of *L. pneumophila* serogroup 5 and to demonstrate genetic diversity among isolates of serogroup 5 from different geographic areas. Hospital A isolates included the 3 patient and 6 environmental strains (2 concurrent and 4 from previous years). Epidemiologically linked patient (n = 1) and environmental isolates (n = 3) were also available from the Pittsburgh VA Medical Center [5].

Isolation of bacterial DNA and preparation of plugs were done as previously described [9]. Restriction digestion of chromosomal DNA was done with either 10 U of SfiI or NotI (New England Biolabs, Beverly, MA) for 15 h at the appropriate temperature. The plugs were loaded into a 1% PFGE-certified agarose gel prepared and run in 0.5× TRIS borate–EDTA buffer. PFGE was done by using the contour-clamped homogenous electric field system (CHEF-DR II; Bio-Rad, Hercules, CA) at 14°C and 200 V, with an increasing switch time from 1 to 35 s for SfiI-restricted genomic DNAs and from 2 to 70 s for NotI-restricted genomic DNAs, for 22 h. Bacteriophage λ concatemers (48.5 kb; New England Biolabs) were used as DNA size markers. After ethidium-bromide staining, the gels were photographed with a UV light source.

### Results

*Legionnaires' disease caused by L. pneumophila serogroup 5.* During the study period, 102 patients with nosocomial pneumonia were identified in hospital A. The proportion of nosocomial pneumonia caused by *L. pneumophila* serogroup 5 was 3% (3/102). The total number of patient samples evaluated for legionellae during the study period in hospital A included 75 sputum cultures, 164 urine antigen tests, and 90 serologic tests. Three patients with legionnaires' disease caused by *L. pneumophila* serogroup 5 were diagnosed by culture (table 1). Sputum from 2 of these patients was positive by DFA stain; 1 had a positive urinary antigen test and another seroconverted.

Patient 1, a 52-year-old white man, had fever, pleuritic chest pain, cough with purulent sputum, and hemoptysis for 4 days. He had been admitted to hospital A and had received corticosteroids for 13 days and a cephalosporin for 11 days and had been discharged 5 days prior to this admission. On readmission, he had fever (38.5°C), pulse of 160/min, respiration 24/min, and altered mental status. His white blood cell count (WBC) was 27,600/mm³, with 92% polymorphonuclear leukocytes. Chest radiographs showed bilateral, multiple, poorly developed rounded opacities. *L. pneumophila* serogroup 5 was identified by DIPA and sputum culture. The *L. pneumophila* serogroup 1 urinary antigen test was also positive (ratio to negative, 5.32).

Despite intravenous erythromycin for 5 days and rifampin for 2 days, he died on the 7th day. The autopsy showed bilateral pulmonary consolidation with abscess formation, pulmonary edema, and congestion. Lung specimens were not examined for *Legionella* species.

Patient 2, a 70-year-old white man, was transferred from hospital A to the Pittsburgh VA Medical Center due to new onset of fever and poor response of pneumonia to a 4-week antibiotic treatment. He had received oral prednisone and erythromycin in the 3 weeks prior to transfer. The chest radiograph showed bilateral infiltrates with pleural effusion. *L. pneumophila* serogroup 5 was identified by DIPA and sputum culture. He received 1 g of erythromycin intravenously every 6 h and 600 mg of rifampin orally every day for 2 weeks. The pulmonary infiltrates resolved over 2 months. His urinary antigen test was negative. Seroconversion (>1:512) was demonstrated.

Patient 3, a 91-year-old white man, underwent a partial gastrectomy and partial colectomy 3 days after admission. Pneumonia developed 3 days postoperatively, as evidenced by fever (39°C), tachycardia (138/min), tachypnea (30/min), confused mental status, and productive cough. A pulmonary infiltrate with right base consolidation, small left-sided pleural effusion, and moderate right-sided pleural effusion were seen on his chest radiographs. His WBC was 14,000/mm³ with 38% bands. The sputum culture yielded *L. pneumophila* serogroup 5. The patient received 500 mg of erythromycin intravenously every 6 h for 10 days, followed by 500 mg of oral clarithromycin every 12 h for 7 days. He was discharged on the 32nd hospital day.
Environmental investigations. *L. pneumophila* serogroup 5 was recovered from 4 of 5 hot-water storage tanks (10–1000 cfu/mL) of hospital A. Colonization of the hospital water supply (distal sites and hot-water tank) by *L. pneumophila* serogroup 5 had been documented over a 10-year period, and isolates of *L. pneumophila* serogroup 5 were available from 1984, 1986, and 1994. No other serogroups were isolated.

Molecular typing of patient and environmental isolates by PFGE. The *L. pneumophila* serogroup 5 isolates from the 3 patients with nosocomial pneumonia acquired in hospital A had the same PFGE pattern as the *L. pneumophila* serogroup 5 isolates (including 2 concurrent and 4 from previous years) from the water supply of hospital A (figure 1). In contrast, serogroup 5 isolates from patients (*n* = 3) and environmental sources (*n* = 8) from other institutions had different PFGE patterns. Nine different PFGE patterns were demonstrated among unrelated isolates. One environmental isolate of *L. pneumophila* serogroup 5 isolated from VAMC-2 and serogroup 5 isolates from hospital A showed an identical PFGE pattern by using *NotI* restriction (data not shown); however, a 1-band difference was shown by *SfiI* restriction (figure 1).

A longitudinal study of *L. pneumophila* serogroup 5 isolates from both hospital A and the Pittsburgh VA Medical Center for up to 10 years demonstrated that isolates from each hospital had a unique and stable PFGE pattern (figure 1).

Discussion

Prospective clinical surveillance for 1 year found 3 patients with culture-confirmed nosocomial legionnaires’ disease due to *L. pneumophila* serogroup 5 in a hospital with a water system colonized with *L. pneumophila* serogroup 5 (table 1). It should be emphasized that prior to the initiation of this study, endemic legionellosis was not suspected in this hospital. *Legionella di-
agnostic testing was subsequently introduced as a result of positive environmental cultures. The 3 cases of legionellosis were shown to be hospital acquired; PFGE genotyping showed that the 3 patient isolates were identical to environmental isolates from the hospital water supply.

Cleavage of genomic DNA with restriction enzymes and separation by PFGE has proven useful in the investigation of nosocomial infections caused by *L. pneumophila* serogroups 1, 6, and 10 [10–12]. The discriminatory power of PFGE varies, depending on the restriction enzyme used. *Sst*I appears to be more discriminatory than *Not*I for subtyping *Legionella* strains since *Not*I produced fewer bands than *Sst*I in the macrorestriction analysis [10, 12].

For 1 patient, the urinary antigen test (specific for only *L. pneumophila* serogroup 1 [13]) was positive. Mixed infection with serogroup 1 in this patient could not be shown when 18 different colonies from the primary culture were tested and confirmed to be *L. pneumophila* serogroup 5. Persistent antigenuria from a previous *L. pneumophila* serogroup 1 infection [13] appeared unlikely, given the absence of previous pneumonia. Furthermore, we have previously shown that urinary antigen positivity usually converts to negative within 2 months in 59 culture-confirmed patients with legionnaires’ disease due to serogroup 1 [14]. On the other hand, urinary antigen cross-reactivity has been reported for *L. pneumophila* serogroups 4 and 10 [15]. Therefore, cross-reactivity by *L. pneumophila* serogroup 5 with the *L. pneumophila* serogroup 1 urinary antigen test may have occurred.

Nasogastric tubes have been linked to nosocomial legionellosis [16]; microaspiration of contaminated water or colonized oropharyngeal secretions was the presumed mode of entry. Of interest, 2 of 3 patients in our study had a nasogastric tube in place.

Four of 5 patients with *L. pneumophila* serogroup 5 disease previously died [2–5]. 2 sisters with underlying systemic lupus erythematosus treated with prednisone developed pulmonary nodules and microabscesses; 1 patient with carcinoma of esophagus developed pleural empyema, and 1 chronic lymphocytic leukemia.

In summary, we showed that colonization of *L. pneumophila* serogroup 5 in the hospital water supply can result in nosocomial legionnaires’ disease. *L. pneumophila* serogroup 5 disease may be overlooked because some diagnostic tests, such as for urinary antigen, will usually not detect this serogroup. PFGE proved useful in demonstrating that the hospital water supply was the source. We demonstrated that sufficient genetic diversity exists among strains of *L. pneumophila* serogroup 5 such that PFGE can be used in epidemiologic investigations for this serogroup. The stability of *L. pneumophila* colonization in the water environment, as shown in the results, may help unravel the epidemiology of previous outbreaks in which the source was unknown; for example, single unique genotypes of *L. pneumophila* serogroup 5 were consistently isolated from two hospitals over a 10-year period.

Finally, routine environmental cultures are useful in stimulating the introduction of specialized *Legionella* testing into the clinical laboratory. Such testing may be required to diagnose patients with nosocomial legionnaires’ disease that may have been previously overlooked.

References