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Paleoepidemiologic Investigation of Legionnaires Disease at Wadsworth Veterans Administration Hospital by Using Three Typing Methods for Comparison of Legionellae from Clinical and Environmental Sources

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Multilocus enzyme electrophoresis, monoclonal antibody typing for *Legionella pneumophila* serogroup 1, and plasmid analysis were used to type 89 *L. pneumophila* strains isolated from nosocomial cases of Legionnaires disease at the Veterans Administration Wadsworth Medical Center (VAWMC) and from the hospital environment. Twelve *L. pneumophila* clinical isolates, obtained from patients at non-VAWMC hospitals, were also typed by the same methods to determine typing specificity. Seventy-nine percent of 33 VAWMC *L. pneumophila* serogroup 1 clinical isolates and 70% of 23 environmental isolates were found in only one of the five monoclonal subgroups. Similar clustering was found for the other two typing methods, with excellent correlation between all methods. Enzyme electrophoretic typing divided the isolates into the greatest number of distinct groups, resulting in the identification of 10 different *L. pneumophila* types and 5 types not belonging to *L. pneumophila*, which probably constitute an undescribed *Legionella* species; 7 clinical and 34 environmental VAWMC isolates and 2 non-VAWMC clinical isolates were found to be members of the new species. Twelve different plasmid patterns were found; 95% of VAWMC clinical isolates contained plasmids. Major VAWMC epidemic-bacterial types were common in the hospital potable-water distribution system and cooling towers. Strains of *L. pneumophila* which persisted after disinfection of contaminated environmental sites were of a different type from the prechlorination strains. All three typing methods were useful in the epidemiologic analysis of the VAWMC outbreak.

Epidemiologic analysis of outbreaks of Legionnaires disease is often difficult because of the ubiquity of *Legionella* spp. in the environment. Frequently more than one environmental site contains the same species and serogroup of *Legionella* as that isolated from patients (9, 13, 16). Because eradication of *Legionella* spp. from epidemiologically implicated environmental sites interrupts epidemics of Legionnaires disease, it is important to determine which of potentially many sites should be disinfected. Selective site treatment may be more cost-effective and less hazardous than treatment of many sources (9). The pinpointing of disseminators of *Legionella* spp. also increases our knowledge of engineering errors in the design and construction of water delivery and air-cooling systems.

Several methods have been described for subtyping of *Legionella pneumophila*. These include monoclonal antibody typing schemes, plasmid analysis, DNA digest analysis, polyacrylamide gel electrophoresis of outer membrane proteins, and electromorph (alloenzyme) analysis of enzymes (2, 3, 8, 10, 13, 14, 17, 21, 22). Some of these methods have already been shown to be useful in epidemiologic analysis, but extensive comparative studies of several different methods are lacking (1, 4, 9, 11, 14–16, 21, 22).

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With the goal of elucidating the environmental ecology of *L. pneumophila* at Veterans Administration Wadsworth Medical Center (VAWMC), a site of a major epidemic of Legionnaires disease from 1977 to 1981, we have typed a well-characterized collection of primarily outbreak-associated clinical and environmental isolates of *L. pneumophila* and other *Legionella* spp. The methods employed were multilocus enzyme electrophoresis, monoclonal antibody typing, and plasmid analysis.

MATERIALS AND METHODS

Bacterial strains. A total of 101 isolates of *L. pneumophila* were studied; 89 strains were isolated from patients hospitalized at VAWMC or from VAWMC water samples, and 12 strains were isolated from patients hospitalized elsewhere (Table 1). All these bacteria were isolated at VAWMC, except for some environmental strains which were isolated at the Centers for Disease Control from VAWMC water samples by George Gorman and colleagues. All isolates had been identified as *L. pneumophila* on the basis of growth and biochemical and serological tests (7). In addition, many but not all isolates were characterized for cellular fatty acid and ubiquinone composition by gas-liquid and high-pressure liquid chromatography, respectively (7).

All bacteria had been frozen in 2% skim milk at −70°C for periods ranging from several weeks to 7 years. The frozen

TABLE 1. Source and number of strains classified by enzyme ET

Species	ET	No. of strains[a]					
		VAWMC				Other hospitals	
		CL1	ENV1	CL4	ENV4	CL1	CL4
L. pneumophila	1	0	0	0	0	1	0
	2	0	0	0	0	1	0
	3	1	5	0	0	1	0
	4	1	0	0	0	1	0
	5	1	0	0	0	3	0
	6	0	0	0	0	0	2
	7	0	0	0	0	1	0
	8	0	1	0	0	0	0
	9	23	12	1	2	0	0
	10	1	0	0	0	0	0
Undescribed species[b]	13	0	0	0	0	1	0
	14	1	0	0	0	0	0
	15	0	2	1	29	0	1
	16	4	1	0	0	0	0
	17	1	2	0	0	0	0

[a] *L. pneumophila* serogroup and source: CL, clinical; ENV, environmental.
[b] Species 1 of reference 17.

bacteria were thawed quickly at room temperature (15 to 37°C), then passed twice on buffered charcoal-yeast extract medium supplemented with 0.1% alpha-ketoglutaric acid (BCYE), incubated at 35°C in a humidified air incubator (7). In some cases, typing of a strain which had been multiply passaged on BCYE medium was compared with that of the same isolate at low passage to determine the stability of types associated with passage history.

Multilocus enzyme electrophoresis. The application of multilocus enzyme electrophoresis to analysis of the genetic structure of *Legionella* populations has recently been described (17). Briefly, starch-gel electrophoresis was employed to detect mobility variants for each of 22 metabolic enzymes. For each enzyme, distinctive mobility variants were designated as electromorphs, which were equated with alleles at the corresponding structural gene locus. Because virtually all isolates of *Legionella* show activity for all 22 enzymes assayed, it is presumed that the structural genes are located on the chromosome rather than on plasmids.

Each isolate was characterized by its combination (profile) of electromorphs for the 22 enzymes, and distinctive combinations were designated as electrophoretic types (ETs), which correspond to multilocus chromosomal genotypes.

Monoclonal antibody typing. All *L. pneumophila* serogroup 1 stains were serotyped at VAWMC using monoclonal antibodies made in Oxford, England (22). The reactions obtained in Los Angeles were confirmed by retesting most of the strains in Oxford; this retesting was performed without knowledge of results obtained in Los Angeles. Some of the serogroup 1 strains were also typed by one of us (J.R.J.) using a different set of murine monoclonal antibodies produced in Quebec, Canada (10). Both the Quebec and Oxford antibodies have been found to give similar results. Monoclonal typing was performed by indirect immunofluorescent microscopy.

Plasmid analysis. Plasmid analysis was performed on all 101 strains of *L. pneumophila* using two different alkaline sodium dodecyl sulfate lysis procedures; each strain was tested with both procedures (12, 20). Restriction enzyme analysis was done with *Eco*RI and, in some cases, *Hin*dIII enzyme (Bethesda Research Laboratories, Bethesda, Md.). Plasmid molecular weights were approximated by reference to bands of *Legionella bozemanii*, WIGA strain, and *Hin*dIII-digested lambda phage DNA as standards. Each strain was tested at least three times to ensure reproducibility of plasmid and restriction enzyme patterns.

The Legionnaires disease epidemic at VAWMC. A total of 219 cases of nosocomial Legionnaires disease occurred at VAWMC between the spring of 1977 and the spring of 1982. Continuous chlorination of the potable water system was initiated in July 1980, after which the number of nosocomial cases of Legionnaires disease gradually declined (18). Fewer than 10 of 1,500 environmental cultures taken in the postchlorination period were positive for *L. pneumophila*. No nosocomial case of Legionnaires disease was detected from March 1982 until February 1984. Two nosocomial cases did occur in March, 1984, temporally related to interruption of continuous chlorination for 1 week, at which time multiple water site cultures were positive for *L. pneumophila*.

Analysis of results. All bacterial strains were identified by code numbers only, and all classifications by each of the typing methods were made without knowledge of strain identity or relatedness. Once all strains were typed, this information was entered into a computer spreadsheet (Microsoft Multiplan, Bellevue, Wash.) containing detailed information about each isolate. Statistical tests of association, by chi-square analysis, were performed by standard methods as well as with a computer-assisted statistical software program (Stats Plus; Human Systems Dynamics, Northridge, Calif.). Contingency coefficients were calculated by the method of Siegel (19).

RESULTS

Multilocus enzyme typing. Fifteen different multilocus profiles or ETs were identified among the 101 *L. pneumophila* strains examined (Table 1). Two major findings were made. The first was that 43 of the strains were a new, undescribed species of *Legionella*, closely related to but different from *L. pneumophila* (species 1 in reference 17). This new species was represented by ETs 13 through 17 and contains organisms apparently phenotypically identical to *L. pneumophila*; 11 of these had been identified as *L. pneumophila* serogroup 1, and 31 were identified as *L. pneumophila* serogroup 4. The other major finding was the concentration of VAWMC isolates in two ETs.

Most VAWMC clinical and environmental isolates belonged to a few major types (Table 1). Of 33 *L. pneumophila* serogroup 1 clinical strains isolated from VAWMC, 73% were ET 9, as were 67% of 18 environmental strains. Thirty of 33 serogroup 4 clinical and environmental strains from VAWMC were ET 15. Of these 33 serogroup 4 strains, 1 of the 2 clinical isolates was of ET 15, as were 94% of 31 environmental isolates; the other serogroup 4 clinical strain was ET 9. These distributions differed significantly from that found for non-VAWMC isolates ($P < 0.001$, chi-square test with correction for continuity).

The two types representing the majority of VAWMC strains, numbers 9 and 15, were almost exclusively isolated in the prechlorination period. Some ETs were detected either exclusively (ET 5) or more frequently (ET 3) postchlorination in environmental samples. Also, a few ET 15 strains were isolated postchlorination.

Five ETs were isolated from more than one hospital population. ET 3 contained the most geographically diverse isolates, obtained from VAWMC, another Los Angeles area hospital, and a hospital in New York City. ETs 5 and 15

contained isolates from both VAWMC and other Los Angeles area hospitals. ET 6 contained isolates from two Los Angeles area hospitals, but not from VAWMC. ET 4 contained isolates from VAWMC and from a Seattle area hospital.

Monoclonal antibody typing. Sixty-four of 65 *L. pneumophila* serogroup 1 strains were typable with the Oxford monoclonal antisera (Table 2). Fifty-four were identified as the Pontiac type; of these, 48 were identified as the Pontiac 1a and 1b subgroups. Of all the VAWMC serogroup 1 clinical isolates, 79% were of the Pontiac 1b subgroup, as were 70% of VAWMC environmental isolates. These results were confirmed by analysis of some of the strains in Quebec and by independent repeat testing in Oxford. Serial passage of bacteria did not affect these results.

The clustering of VAWMC clinical and environmental isolates in the Pontiac 1b subgroup was significantly different from the pattern observed for the non-VAWMC isolates ($P < 0.005$, chi-square test with correction for continuity). No significant differences in this clustering were detected when the distribution of VAWMC clinical subgroups was compared with that of environmental subgroups. All but one of the Pontiac 1b subgroup strains were isolated from nosocomial cases of Legionnaires disease at VAWMC in the period from November 1978 to July 1980, which corresponds with the duration of the major hospital epidemic of Legionnaires disease. This subgroup was again isolated from one of two nosocomial cases of Legionnaires disease at VAWMC, which occurred coincident with interruption of chlorination in March 1984.

All of the Pontiac 1a subgroup clinical and environmental strains were isolated in the period from March to July 1980. The two clinical strains of *L. pneumophila* that were typable only to the Pontiac group level were isolated in December 1978 and June 1980.

VAWMC clinical isolates of *L. pneumophila* serogroup 1 belonging to the Pontiac 3a, OLDA 1a, and Bellingham subgroups were isolated only after initiation of water chlorination in July 1980. The only *L. pneumophila* serogroup 1 environmental strains isolated from VAWMC after initiation of water chlorination were of the OLDA 1a subgroup; these were isolated in the period from October 1978 to April 1984.

The limited number of isolates from other hospitals made analysis of geographic differences or hospital-specific subgroups difficult to perform. No VAWMC isolate subgroups were unique; clinical isolates of *L. pneumophila* bacteria from six other hospitals were of subgroups identical to those found for VAWMC strains. These six other hospitals were as close as 1 mile and as far away as 1,500 miles from VAWMC. Three of the four Pontiac 3a bacteria strains, all of which were clinical isolates, were from one university hospital. Two of these strains were isolated from different patients with community-acquired pneumonia over a 2-day period; the third had been isolated 2 years previously.

TABLE 2. Distribution of *L. pneumophila* serogroup 1 monoclonal types

Monoclonal designation		Source and total no. of strains (no. of new species)[a]		Others (clinical)
		VAWMC		
Group	Subgroup	Clinical	Environmental	
Pontiac	1a	2 (1)	2	1
	1b	26 (5)	16 (5)	1
	3a	1	0	3
	Unclear	2	0	0
OLDA	1a	1	5	1
Bellingham		1	0	2
Nontypable		0	0	1 (1)

[a] The numbers in parentheses indicate the number of strains that belong to undescribed *Legionella* species 1 (17) of the designated monoclonal type.

TABLE 3. Plasmid types

Plasmid type	No. of bands	Mol wt (10^6)	Serogroup
H1	1	100	1
H2A	1	115	4
H3	1	45	1
H4	1	25	1
H5	1	125	1
H6	1	40	1
H8	1	30	1
H9	1	85	1
H10	1	55	4
H12	1	90	1
S1	1	20	1, 4
S2	1	25	1, 4

Plasmid analysis. We found that there were marked differences between strains in terms of susceptibility to cell lysis. For example, the WIGA strain of *L. bozemanii*, which was used to produce molecular weight standards, reproducibly yielded plasmids regardless of the lysis method used. However, other strains, including many *L. pneumophila* serogroup 1 isolates, yielded negative results with one plasmid extraction method and positive results with another. The Kado-Liu plasmid extraction method (12), which uses 3% alkaline sodium dodecyl sulfate for cell lysis, often yielded good plasmid bands when the Takahashi method (20), which used 2% alkaline sodium dodecyl sulfate, did not. However, it was much more difficult to obtain good restriction enzyme digests with the Kado-Liu method than with the Takahashi method. We found that modifying the Takahashi method by substituting the Kado-Liu lysing reagent significantly improved our results. Regardless of the method used, false-negative results were occasionally observed, which were resolved with repeated analyses. Serial passage of bacteria was not found to alter plasmid content.

Twelve different plasmid patterns were demonstrated (Table 3). All differed from each other after digestion by *Eco*RI restriction endonuclease. Restriction enzyme digestion of the S group of plasmids gave two to three bands on gel electrophoresis which appeared in two patterns, called S1 and S2; these were clearly different with both *Eco*RI and *Hin*dIII digestions.

Distribution of the plasmid types is shown in Table 4. The three most common patterns were H1, S1, and S2. Of 35 VAWMC clinical isolates, 34 contained plasmids, versus 8 of 12 for non-VAWMC clinical isolates ($P = 0.015$ by chi-square test). The frequency of plasmid-containing strains for VAWMC environmental and clinical isolates was not significantly different ($P > 0.1$ by chi-square test, $1 - \beta = 0.2$ with $\alpha_2 = 0.05$).

Correlation of typing methods. It was difficult to make strain-by-strain comparisons of the various typing methods, as only serogroup 1 monoclonal antibodies were used. Regardless, some major correlations existed.

Chi-square analysis comparing the distribution of monoclonal subgroups with that of ETs showed high correlation ($P < 0.0001$ by chi-square testing, contingency coefficient =

TABLE 4. Plasmid types according to source and serogroup

Plasmid	Total no. of strains (no. of new species)[a]					
	VAWMC				Other	
	CL1	ENV1	CL4	ENV4	CL1	CL4
H1	1	5	0	0	1	0
H2a	0	0	0	0	0	1
H3	0	0	0	0	1	0
H4	0	0	0	0	2	0
H5	1	0	0	0	0	0
H6	0	0	0	0	0	1 (1)
H8	0	0	0	0	1	0
H9	1	0	0	0	0	0
H10	0	0	0	0	0	1
H12	1	0	0	0	0	0
S1	7 (6)	4 (4)	1 (1)	28 (27)	0	0
S2	21	14 (1)	1	2 (1)	0	0
None	1	0	0	1 (1)	4 (1)	0

[a] *L. pneumophila* serogroup and source: CL, clinical; ENV, environmental. For an explanation of the numbers that appear in parentheses, see the footnote to Table 2.

0.86). Within the high overall group correlation, there were close correlations observed between specific monoclonal types and ETs.

The closest correlation was between the OLDA 1a monoclonal subgroup and ET 3. All seven OLDA 1a isolates were ET 3 strains and vice versa. The most frequent *L. pneumophila* serogroup 1 VAWMC monoclonal type, Pontiac 1b, was closely correlated with ET 9. Of 35 *L. pneumophila* serogroup 1 ET 9 isolates, 30 were in the Pontiac 1b monoclonal subgroup, and the remainder were in the Pontiac monoclonal group ($P < 0.0001$ by chi-square testing). However, 12 Pontiac 1b strains, six of which were VAWMC clinical isolates, were classified in other ETs; four of these six were ET 16 strains. Similarly, all but one of four Pontiac-3a strains were also in ET 5 and vice versa ($P < 0.001$ by chi-square testing). The other close association was between ET 4 and the Bellingham monoclonal type; both ET 4 strains were Bellingham monoclonal types, and two of three Bellingham types were ET 4 ($P < 0.01$ by chi-square testing).

Analysis of correlation between plasmid type and ET distributions showed a significant plasmid type-ET association ($P < 0.0001$ by chi-square test, contingency coefficient = 0.94). This association was related in part to the high number of H1 plasmid type strains which were also ET 3 strains. All seven ET 3 strains carried the H1 plasmid and vice versa. Since, as stated above, the distribution of the OLDA 1a monoclonal subgroup was closely correlated with that of ET 3, the same close association was true between plasmid group H1 and the OLDA 1a subgroup. The other close associations between plasmid types and enzyme types were for plasmid groups S1 and S2 with ETs 15 and 9, respectively. Of 33 ET 15 strains, 29 carried the S1 plasmid, and 29 of 40 S1 strains were in ET 15 ($P < 0.0001$ by chi-square test). Similarly, 89% of the 38 ET 9 strains contained the S2 plasmid, and an equal proportion of 38 S2-containing isolates were in ET 9 ($P < 0.0001$ by chi-square test). Because there was also a close correlation between ET 9 and the Pontiac 1b monoclonal type, the distribution of plasmid type S2 was also closely correlated with the distribution of this monoclonal type.

Epidemiologic correlations of typing findings. Bacterial strains of ET 9, the major VAWMC epidemic type, were isolated from multiple environmental sites in the prechlorination period, including several cold water storage tanks, multiple water fixtures such as shower heads and faucets, and the hospital cooling tower. Using Pontiac 1b as a marker of the epidemic strain added one additional environmental site to this list, a hot-water return pump; otherwise, there was no difference between monoclonal and ET analysis in environmental sites positive for the marker strain.

The environmental distribution of the main *L. pneumophila* serogroup 4 epidemic strain, ET 15, included multiple sites, almost identical to the environmental site distribution of ET 9.

Typing analysis of VAWMC clinical and environmental *L. pneumophila* isolates from the postchlorination period revealed no clear-cut matches between clinical and environmental strains. Three nosocomial cases of Legionnaires disease occurred in this period, all caused by *L. pneumophila* serogroup 1, one in November 1981 and two in April 1984. The two April cases occurred 1 week after continuous chlorination was stopped. The 1981 isolate was characterized as a Bellingham/ET 4 strain with no detectable plasmid. The two 1984 isolates were a Pontiac 1b/plasmid group H2 (ET not determined) strain and a Pontiac 3a/ET 5/plasmid group H12 strain. No environmental strains matching the clinical isolates were isolated. Strains of *L. pneumophila* serogroup 1/OLDA 1a/ET 3/plasmid group H1 were isolated from VAWMC environmental sites in August 1981, October 1982, and March 1984. Several VAWMC environmental isolates of *L. pneumophila* serogroup 4/ET 15/plasmid group S1 were also obtained in March 1984.

DISCUSSION

This study revealed that most cases of endemic-epidemic Legionnaires disease at VAWMC were caused by a single clone of *L. pneumophila* which is distinct from other strains of *L. pneumophila*. Also demonstrated were nosocomial and community-acquired cases of Legionnaires disease caused by a new species of *Legionella* yet to be named.

Multiple locus enzyme analysis yielded the greatest number of types of *L. pneumophila* and also identified five clones of a new *Legionella* species. Considerably more experience will be needed to determine whether such extreme division of *L. pneumophila* is any more helpful in epidemiologic analysis than less discriminating methods. For example, several VAWMC clinical isolates were classified in ETs 16 and 17, each of which had environmental correlates. All of these strains were characterized as monoclonal type Pontiac 1b, except one which was a Pontiac 1a strain; all contained the S1 plasmid. They were also clustered in time over a 3-month period in 1980. Thus, using ET methods, these cases would be defined as a minicluster of Legionnaires disease cases caused by two closely related ETs, superimposed on endemic cases caused by other ETs. Using monoclonal antibody typing, plasmid analysis, or both, these cases would not have been recognized as a separate event. Whether this distinction holds practical epidemiologic importance awaits further study.

In the present study, 22 enzymes were analyzed to estimate the overall genetic relatedness of strains. A comparable analysis would not be feasible in a small laboratory, but for many epidemiologic purposes, the examination of large numbers of enzymes may not be required. A degree of strain discrimination similar to that achieved by monoclonal antibody typing or plasmid analysis could have been obtained in the present study by electrophoresing only 4 or 5 of the 22 enzymes studied.

Monoclonal typing was a reliable method and was easy to perform and interpret. This sort of testing could be used in any clinical microbiology laboratory accustomed to serotyping bacteria; although we used immunofluorescent methods, monoclonal typing could also be performed using coagglutination or enzyme-linked immunoabsorbent methods as well. The major drawback of monoclonal subgrouping is the lack of extensive typing sera for other *Legionella* serogroups and species. Also, since at least two new cryptic *Legionella* species exist which react with monoclonal antibodies directed against what were previously thought to be exclusively *L. pneumophila* serogroup antigens, a major potential for misclassification exists (17). Though its epidemiologic importance is unknown, such a misclassification would not have influenced the conclusions of an investigation of the VAWMC outbreak, if such typing methods had been available during the outbreak.

Plasmid analysis provided less discrimination among VAWMC *Legionella* isolates than did the monoclonal typing or enzyme methods. Regardless, it was sufficient to distinguish between the major VAWMC types. Like enzyme analysis, its utility is not confined to a single serogroup, and as with all the other methods, geographic diversity of plasmid types creates the potential for wrong conclusions about the source of a particular isolate. We found this method of typing to be the most tedious and technically difficult system. Because of the differences we and others have noted in the ease of lysis of some strains, we consider that the possibility of falsely concluding that a certain strain contains no plasmid is high (2). Because of this, and because many clinical isolates of *L. pneumophila* have been reported to contain no plasmid, plasmid typing may be less useful than other typing methods (3, 13).

Our results showing that most nosocomial cases of Legionnaires disease at VAWMC were caused by a single type of *L. pneumophila* are in agreement with studies of other outbreaks of the disease (4, 9, 11, 14–16, 21, 22). However, perhaps because we were able to study a much larger sample of same-source clinical and environmental isolates than have others, it is apparent that there can be considerable genetic and serologic diversity among *Legionella* strains causing outbreaks of Legionnaires disease. Thus, although *L. pneumophila* serogroup 1/ET 9/Pontiac 1b/plasmid type S2 caused the majority of cases of Legionnaires disease at VAWMC, other well-documented nosocomial cases of Legionnaires disease at VAWMC were caused by other subgroups of *L. pneumophila*, and indeed by another *Legionella* species (yet unnamed).

This diversity of *Legionella* subgroups causing the VAWMC outbreak is probably a reflection of the environmental diversity of *Legionella* spp. at VAWMC and more globally (1, 8, 10, 14, 16, 17). It is possible that a small cluster of cases of Legionnaires disease could be falsely dismissed as being unrelated to a common source exposure because of the finding of more than one type of clinical bacterial isolate, unless the possibilities of environmental diversity of the types and sampling errors are kept in mind.

One intriguing finding of this study is the postchlorination replacement of ET 15 and 9 environmental strains with primarily ET 3/OLDA 1a environmental strains. It is possible that this latter type of *L. pneumophila* is more resistant to chlorine than were the earlier types, or that chlorination induced mutagenesis. Perhaps disinfection not only reduces *Legionella* bacterial density in the environment but also causes a shift in the resident strains, which may differ in their virulence characteristics.

The value of combining typing methods is not clear in this study because of the close correlations observed between the typing methods. Except for plasmid typing, each of the other methods used multiple reactions in arriving at a classification. Combining the results of enzyme electrophoretic and monoclonal typing would probably increase the ability to discriminate between strains, although the practical importance of and need for this are unanswered by this study. Because of the possibility of plasmid transfer between different strains, species, or even genera, it is unclear whether the linkage of plasmid type and other typing methods would remain constant during the period of other outbreaks (5, 6). Maher and colleagues found dissociation between plasmid and monoclonal type in an investigation of a nosocomial epidemic at their hospital (13).

We conclude that the VAWMC outbreak of Legionnaires disease was caused primarily by one type of *L. pneumophila*, which was ubiquitous in the hospital environment. Another major resident of the hospital water distribution system and cooling towers was an undescribed *Legionella* species which occasionally caused human disease. Use of any one of the three typing methods would have provided adequate epidemiologic information, and combining these methods did not necessarily result in a significantly greater amount of practical information. Because of its ability to discriminate between species of *Legionella* and among *L. pneumophila*, multilocus enzyme electrophoresis is probably the preferred single method to use in epidemiological analysis, although the simpler-to-use monoclonal antibody typing scheme would be a close second choice. Plasmid analysis is not necessarily essential for epidemiologic analysis and may at times be the most difficult test to perform of the three.

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