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Similar DNA Restriction Endonuclease Profiles in Strains of *Legionella pneumophila* from Different Serogroups

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DNA of strains of *Legionella pneumophila* serogroups 1, 3, 4, and 6, isolated from patients and environmental sources, was examined by restriction endonuclease analysis (REA). Major differences in profiles enabled subtyping in many strains with the same serogroup antigen. However, a cluster of *L. pneumophila* strains, originating from all the examined serogroups, had similar restriction endonuclease profiles, sometimes with minor differences. This suggests that the genetic similarity between strains of *L. pneumophila* of different serogroups is sometimes closer than in strains with the same serogroup antigen. Seven environmental sources harbored two *L. pneumophila* strains with various serogroup antigens; six sources had similar restriction endonuclease profiles. The resolution of small differences in profiles is hampered in REA by the great magnitude of DNA fragments; even upon extensive analysis, these differences are not always readily visualized. Double digestions with the restriction enzymes *Hpa*I and *Hpa*II showed the best results and sometimes revealed differences not evident by digestions with a single endonuclease. REA has a great capacity for accurate epidemiological typing of *L. pneumophila*, in addition to classical serogrouping; it appeared that the results of the two techniques do not necessarily correlate. On the other hand, it should be stressed that small differences in profiles are not easily detected by REA.

Restriction endonuclease analysis (REA) of genomic DNA is now an established technique for molecular epidemiology of bacterial infections (2, 7, 8, 11, 13, 17, 19). Typing and subtyping of *Legionella pneumophila* strains has been carried out by plasmid analysis (1, 3, 10, 12, 15), peptide profiling (9), immunochemical methods with absorbed antisera (3, 21) and monoclonal antibodies (5, 6, 10, 14), alloenzyme analysis (16, 17), and REA of whole-cell DNA (17, 20).

In 1984, we reported the utility of REA for examining the epidemiology of *L. pneumophila* infections in Amsterdam (20). Recently, Tompkins et al. (17) confirmed this observation and extended this method for infections by *Legionella dumoffii*. They also reported a good correlation of the technique with the results of alloenzyme analysis and noted that the REA typing results did not necessarily correlate with typing by monoclonal antibodies, an observation that was also evident from the results of our group regarding a typing method using absorbed antisera (21). Concerning the relation between genetic and immunochemical typing methods, Selander et al. (16) reported a close genetic relation between some strains of *L. pneumophila* of different serogroups, as demonstrated by alloenzyme analysis, and a more distant relation between some *L. pneumophila* strains of the same serogroup.

We have investigated the restriction endonuclease profiles of defined strains of *L. pneumophila* serogroups 1, 3, 4, and 6. It appeared that although the restriction enzyme profiles differed substantially within one serogroup of *L. pneumophila*, as reported earlier for *L. pneumophila* serogroup 1 (20), similar REA fingerprints among a group of *L. pneumophila* strains with different serogroup antigens were observed; strains isolated from the same environmental source also shared common fingerprints.

MATERIALS AND METHODS

Strains. All strains of *L. pneumophila* were isolated from patients with Legionnaires disease and the putative environmental source for their infections, usually the hot-water supplies of hospitals and residences in the region of Amsterdam (18, 19). *L. pneumophila* was isolated as described by Edelstein (4), and serogrouping was carried out with immunofluorescent antisera obtained from the Centers for Disease Control, Atlanta, Ga. Some isolates were also sent to the Centers for Disease Control for confirmation of the serogrouping results.

A total of 13 pairs of patient and environmental strains of *L. pneumophila* serogroup 1 were examined; 4 pairs of *L. pneumophila* serogroup 3, 5 pairs of *L. pneumophila* serogroup 4, and 3 pairs of *L. pneumophila* serogroup 6 were examined as well. Each pair of strains contains an isolate from a patient and the strain isolated from the most probable environmental source of infection and was epidemiologically unrelated to the other pairs. The only epidemiological link between the strains was the Amsterdam municipal water supply, which all the environmental sources shared.

Reference strains of *L. pneumophila* of various serogroups were obtained, courtesy of the Centers for Disease Control.

Restriction endonuclease digestions of DNA and gel electrophoresis. Whole-cell DNA was prepared and purified as described earlier (20). Approximately 2 μg of bacterial DNA was digested to completion with 10 to 20 U of *Eco*RI, *Hin*dIII, *Bam*HI, *Hpa*I, and *Hpa*II under conditions specified by the manufacturer (Boehringer GmbH, Mannheim, Federal Republic of Germany), sometimes in double digestions as well. Double digestions were carried out sequentially or, when incubation buffer conditions permitted, simultaneously. Each double digestion was carried out sequentially at least once. The incubation was carried out for 6

TABLE 1. Epidemiological, serological, and REA data of 10 *L. pneumophila* strains

Strain	Source	Serogroup	Restriction endonuclease profile[a]					
			*Eco*RI	*Hin*dIII	*Bam*HI	*Hpa*I	*Eco*RI-*Hin*dIII	*Hpa*I-*Hpa*II
HA02	Hospital A; patient	1	Ia	Ia	Ia	Ia	Ia	Ia
HB04	Hospital B; water	1	Ib	Ia	Ia	Ia	Ib	Ib
HC01	Hospital C; patient	3	Ia	Ib	Ia	Ib	Ia	Ic
RB01	Residence B; patient	4	Ia	Ia	Ia	Ia	Ia	Id
HD02	Hospital D; patient	6	Ic	Ia	Ia	Ia	Ic	Ie
HE03	Hospital E; water	1	Ia	Ia	Ia	Ia	Ia	Ie
HE02	Hospital E; water	6	Ia	Ia	Ia	Ia	Ia	If
HF01	Hospital F; patient[b]	4	I'd	I'c	I'b	I'c	I'd	I'g
HF02	Hospital F; water	4	I'd	I'c	I'b	I'c	I'd	I'g
HF04	Hospital F; water	3	I'd	I'c	I'c	I'c	I'd	I'g

[a] I, Type I restriction endonuclease profile (minor differences are indicated by small letters); I', strains contain plasmid DNA producing dense bands in all digestions and bring about an altered type I restriction endonuclease profile.
[b] The medical history of this patient suggests that the patient had become infected in hospital F, before transfer to hospital E.

h at 37°C for single digestions and 12 h at 37°C for double digestions.

The DNA fragments were separated by 35-V (1.7-V/cm) overnight electrophoresis in 0.7% agarose gels containing ethidium bromide (1 μg/ml) and then photographed with a Polaroid 5-mm camera.

REA patterns were visually inspected.

RESULTS

REA revealed major differences in profiles with *Eco*RI, *Hin*dIII, and *Bam*HI, both in strains of *L. pneumophila* serogroup 1, as reported earlier (20), and in strains of *L. pneumophila* serogroups 3 and 4.

However, 4 of the 13 pairs of patient and environmental strains of *L. pneumophila* serogroup 1, 2 of the 4 pairs of *L. pneumophila* serogroup 3, 3 of the 5 pairs of *L. pneumophila* serogroup 4, and all 3 pairs of *L. pneumophila* serogroup 6 shared a restriction endonuclease profile that was indistinguishable from one another or had only minor differences in digestions with the restriction endonucleases *Eco*RI, *Hin*dIII, and *Bam*HI (restriction endonuclease profile type I). In addition to a single strain isolated from the patients, multiple strains of different serogroups were recovered from seven environmental sources. For six sources, the two isolated *L. pneumophila* serogroups (always a combination of *L. pneumophila* serogroups 1, 3, 4, and 6) had similar type I restriction endonuclease profiles.

All other pairs of strains had unique restriction endonuclease profiles (type II, III, etc.) in all digestions examined.

The restriction endonuclease profiles of reference *L. pneumophila* serogroup 1 (Philadelphia 1) and reference *L. pneumophila* serogroup 6 (Chicago 2) are related to the type I restriction endonuclease profile, although differences in the restriction endonuclease profiles of these strains are more easily detected and show more variation in profiles than the *L. pneumophila* strains with the type I restriction endonuclease profile, isolated in Amsterdam; reference *L. pneumophila* serogroup 3 (Bloomington 2) and reference *L. pneumophila* serogroup 4 (Los Angeles 1) are unrelated.

Ten *L. pneumophila* strains with the type I restriction endonuclease profile are now discussed in more detail, and the data of these strains are presented in Table 1.

The *Eco*RI and *Hin*dIII digests of the strains 1 through 5 from Table 1 are shown in Fig. 1. Identical or nearly identical restriction endonuclease profiles are observed in these epidemiologically unrelated strains of different *L. pneumophila* serogroups.

We were interested in the minor differences in the type I restriction endonuclease profiles and since resolution of these minor differences in digestions with *Eco*RI, *Hin*dIII, and *Bam*HI was poor, DNA was digested with several other enzymes individually (*Bgl*II, *Pst*I, *Sal*I, *Hpa*I, and *Hpa*II) and also in double digestions. The results of REA with *Eco*RI, *Hin*dIII, *Bam*HI, *Hpa*I, and the double digestions *Eco*RI-*Hin*dIII and *Hpa*I-*Hpa*II are summarized in Table 1. Double digestions with *Hpa*I and *Hpa*II gave the best results, and the restriction endonuclease profiles of

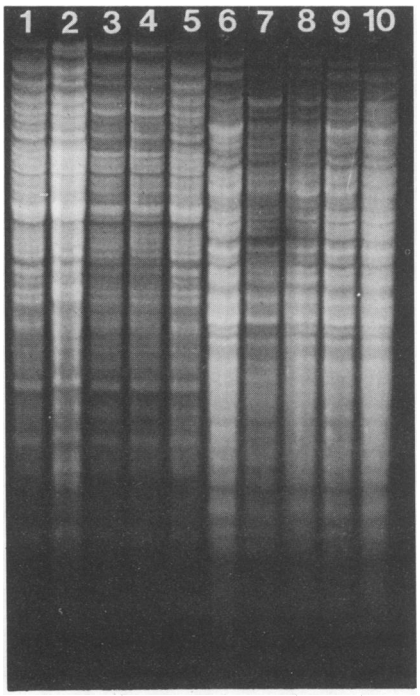

FIG. 1. *Eco*RI digestion of DNA isolated from strains of *L. pneumophila*. Lanes: 1, strain HA02 (hospital A; patient; *L. pneumophila* serogroup 1); 2, strain HB04 (hospital B; water; *L. pneumophila* serogroup 1); 3, strain HC01 (hospital C; patient; *L. pneumophila* serogroup 3); 4, strain RB01 (residence B; patient; *L. pneumophila* serogroup 4); 5, strain HD02 (hospital D; patient; *L. pneumophila* serogroup 6). Lanes 6 through 10 contain DNA isolated from the same strains digested with *Hin*dIII. Presentation is in the same order as for lanes 1 through 5.

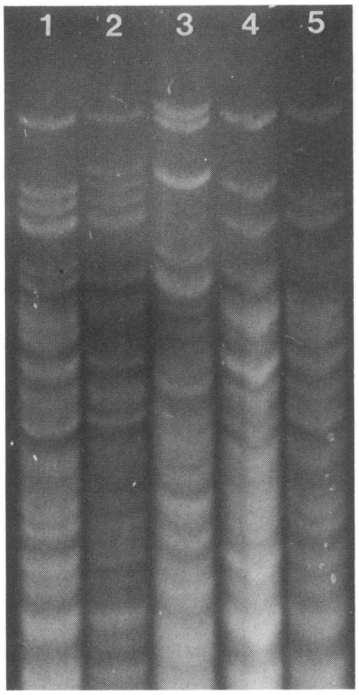

FIG. 2. *Hpa*I and *Hpa*II double digestion of DNA isolated from *L. pneumophila*. Lanes 1 through 5: *L. pneumophila* strains are the same as those described in the legend to Fig. 1 and are presented in the same order.

strains 1 through 5 from Table 1 are shown in Fig. 2. The fragments with the highest molecular weights are now well separated. Distinct small and sometimes multiple fragment (lane 3) differences were now rather easily detected between the profiles of all five strains.

An example of similar type I profiles in *L. pneumophila* strains isolated from the same environmental source is shown in Fig. 3. The data of the strains are presented in Table 1 (strains 6 through 10). Lanes 1 and 2 show the *Hpa*I and *Hpa*II double-digest restriction endonuclease profiles of environmental strains of *L. pneumophila* serogroup 1 (lane 1) and serogroup 6 (lane 2), isolated from the hot-water supply of hospital E; lane 3 represents the profile of a *L. pneumophila* strain, isolated from a patient who had been transferred from hospital F to hospital E; in lanes 4 and 5, the profiles of strains isolated from the hot-water supply of hospital F are presented (lane 4, *L. pneumophila* serogroup 4; lane 5, *L. pneumophila* serogroup 3). The dense band (indicated by an arrow) seen in lanes 3, 4, and 5 appeared to be plasmid derived, as determined by comparison of *Hpa*I and *Hpa*II double-digest profiles of whole-cell DNA and isolated plasmid DNA and also coelectrophoresis of these digests; these experiments also suggested that some other dense bands present in lanes 3, 4, and 5 but lacking in lane 1 and 2 are, in fact, plasmid derived. Small differences are visible in the *L. pneumophila* serogroup 1 and 6 strains from hospital E (arrows); the profiles in lanes 3, 4, and 5 are indistinguishable.

In summary, the results of single and double digestions in strains with type I REA indicated that isolates containing the same *L. pneumophila* serogroup antigen that were epidemiologically related (pairs) were indistinguishable in all digestions, whereas some of those sharing the same serogroup antigen but which were not epidemiologically linked,

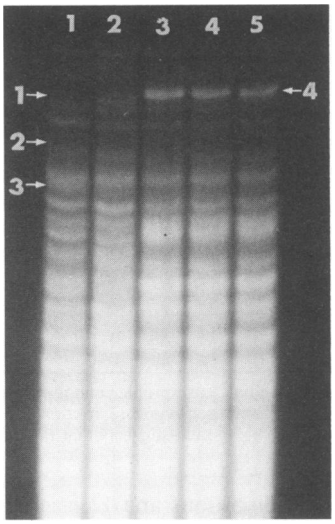

FIG. 3. *Hpa*I and *Hpa*II double digestions of DNA isolated from *L. pneumophila*. Lanes: 1, strain HE03 (hospital E; water; *L. pneumophila* serogroup 1); 2, strain HE02 (hospital E; water; *L. pneumophila* serogroup 6); 3, strain HF01 (hospital F; patient; *L. pneumophila* serogroup 4); 4, strain HF02 (hospital F; water; *L. pneumophila* serogroup 4); 5, strain HF04 (hospital F; water; *L. pneumophila* serogroup 3). Arrow 1 shows an extra band in lane 2 that is not present in lane 1; arrows 2 and 3 mark extra brands in lane 1 that are not present in lane 2; arrow 4 marks a plasmid-derived band in lanes 3, 4, and 5.

showed minor differences, especially in double digestions with *Hpa*I and *Hpa*II. However, in two strains with different serogroup antigens, we could not visualize differences using any combination of endonucleases; these strains (*L. pneumophila* serogroups 1 and 4) were isolated from the same hot-water supply.

DISCUSSION

By REA, major differences in DNA profiles of *L. pneumophila* strains can be distinguished, and this technique enables subtyping of these strains. On the other hand, the differences in REA are sometimes more pronounced in strains that share the same serogroup antigen than in strains with different serogroup antigens.

Apparently, the genetic similarity between some strains with different serogroup antigens is greater than between strains from the same serogroup. This observation is in accordance with the results of Selander et al. (16), who designated several groups of genetic similarities in *L. pneumophila* on the basis of alloenzyme analysis and concluded that strains of different serogroups sometimes belong to the same alloenzyme group. *Legionella* strains of the same serogroup, on the other hand, belong to several alloenzyme groups. One can ask whether grouping of *L. pneumophila* by means of REA (or alloenzyme typing) is more profitable for epidemiological purposes than classical serotyping.

In six of seven environmental sources that harbor more than one serogroup of *L. pneumophila*, the restriction endonuclease profiles of these strains were quite similar. This genetic similarity indicates that they may have been derived from the same clone. In my opinion, one can speculate that the genotype and phenotype of *L. pneumophila* strains in environmental sources may not be as stable as usually presumed.

Differences in restriction endonuclease profiles of bacterial strains may be difficult to detect as a result of the

production of a large number of fragments. It is known that by using conventional REA, toxigenic strains of *Corynebacterium diphtheriae* are indistinguishable from nontoxigenic strains (13). As a consequence, small differences in REA are not readily detected. Even after extensive REA, it is impossible to calculate how many restriction site changes have occurred in strains that seem to have only minor differences in REA.

Although I observed that some differences in these strains not apparent by digestions with a single endonuclease are readily detected in double digestions with *Hpa*I and *Hpa*II, only a minor part of the genome is visualized with this method. Other techniques are needed to explore how "small" small differences in REA really are.

I conclude that REA is at least a very useful method for studies on the epidemiology of *L. pneumophila*, in addition to immunochemical analysis, and I agree with Tompkins et al. (17) that small differences in strains of the same serogroup and with different serogroup antigens are not always readily detected with REA.

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