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

R. J. Van Ketel

Department of Medical Microbiology, University of Amsterdam, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands

Received 16 February 1988/Accepted 8 June 1988

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 *HpaI* and *HpaII* 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.

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**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 *EcoRI*, *HindIII*, *BamHI*, *HpaI*, and *HpaII* 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
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 EcoRI, HindIII, and BamHI, 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 EcoRI, HindIII, and BamHI (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 EcoRI and HindIII 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 EcoRI, HindIII, and BamHI was poor, DNA was digested with several other enzymes individually (BglII, PstI, SalI, HpaI, and HpaII) and also in double digestions. The results of REA with EcoRI, HindIII, BamHI, HpaI, and the double digestions EcoRI-HindIII and HpaI-HpaII are summarized in Table 1. Double digestions with HpaI and HpaII gave the best results, and the restriction endonuclease profiles of

![FIG. 1. EcoRI 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 HindIII. Presentation is in the same order as for lanes 1 through 5.]

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Serogroup</th>
<th>Restriction endonuclease profile*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA02</td>
<td>Hospital A; patient</td>
<td>1</td>
<td>EcoRI: la, HindIII: la, BamHI: la, HpaI: la, EcoRI-HindIII: la, HpaI-HpaII: la</td>
</tr>
<tr>
<td>HB04</td>
<td>Hospital B; water</td>
<td>1</td>
<td>EcoRI: lb, HindIII: lb, BamHI: la, HpaI: lb, EcoRI-HindIII: lc, HpaI-HpaII: lc</td>
</tr>
<tr>
<td>HC01</td>
<td>Hospital C; patient</td>
<td>3</td>
<td>EcoRI: la, HindIII: lb, BamHI: la, HpaI: la, EcoRI-HindIII: la, HpaI-HpaII: le</td>
</tr>
<tr>
<td>RB01</td>
<td>Residence B; patient</td>
<td>4</td>
<td>EcoRI: la, HindIII: lb, BamHI: la, HpaI: la, EcoRI-HindIII: la, HpaI-HpaII: Id</td>
</tr>
<tr>
<td>HD02</td>
<td>Hospital D; patient</td>
<td>6</td>
<td>EcoRI: lc, HindIII: lc, BamHI: lc, HpaI: lc, EcoRI-HindIII: Ic, HpaI-HpaII: Ie</td>
</tr>
<tr>
<td>HE03</td>
<td>Hospital E; water</td>
<td>1</td>
<td>EcoRI: la, HindIII: la, BamHI: la, HpaI: la, EcoRI-HindIII: le, HpaI-HpaII: If</td>
</tr>
<tr>
<td>HE02</td>
<td>Hospital E; water</td>
<td>6</td>
<td>EcoRI: la, HindIII: lb, BamHI: lc, HpaI: Ic, EcoRI-HindIII: lb, HpaI-HpaII: lg</td>
</tr>
<tr>
<td>HF01</td>
<td>Hospital F; patient</td>
<td>4</td>
<td>EcoRI: I'd, HindIII: I'c, BamHI: I'b, HpaI: I'c, EcoRI-HindIII: I'd, HpaI-HpaII: I'g</td>
</tr>
<tr>
<td>HF02</td>
<td>Hospital F; water</td>
<td>4</td>
<td>EcoRI: I'd, HindIII: I'c, BamHI: I'c, HpaI: I'c, EcoRI-HindIII: I'd, HpaI-HpaII: I'g</td>
</tr>
<tr>
<td>HF04</td>
<td>Hospital F; water</td>
<td>3</td>
<td>EcoRI: I'd, HindIII: I'c, BamHI: I'c, HpaI: I'c, EcoRI-HindIII: I'd, HpaI-HpaII: I'g</td>
</tr>
</tbody>
</table>

* 1. 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.

*The medical history of this patient suggests that the patient had become infected in hospital F, before transfer to hospital E.*
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 1 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 HpaI and HpaII 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 HpaI and HpaII 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,

showed minor differences, especially in double digestions with HpaI and HpaII. 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 HpaI and HpaII, 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 immunochimical 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.

ACKNOWLEDGMENTS
I thank B. de Wever for technical assistance and A. M. Koopmanschap for typing the manuscript.

LITERATURE CITED