JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1984, p. 362–364
0095-1137/84/090362-03$02.00/0
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Vol. 20, No. 3

Molecular Epidemiology of *Legionella pneumophila* Serogroup 1

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Received 24 February 1984/Accepted 18 May 1984

The DNA of patient and environmental isolates of *Legionella pneumophila* serogroup 1 was analyzed by restriction endonuclease cleavage. The electrophoretic patterns of the DNA digests of isolates from a group of patients with Legionnaires disease acquired in a hospital were indistinguishable from one another and were identical to the DNA pattern of a strain isolated from the hot water supply of the hospital. On the other hand, they were easily differentiated from strains isolated from patients and hot water supplies in other hospitals in the same city. The homogeneity of populations of *L. pneumophila* serogroup 1 colonizing plumbing systems was also investigated by DNA restriction endonuclease analysis in three hospitals. We distinguished two subtypes in one hospital; the two other hospitals had homogenous populations. Restriction endonuclease digest analysis of *L. pneumophila* serogroup 1 DNA enables subtyping and appears to be a useful method for examining the epidemiology of outbreaks of Legionnaires disease.

In 1983 we were confronted with an epidemic of Legionnaires disease in our new hospital (15). The hot water system appeared to be heavily contaminated with *Legionella pneumophila* serogroup 1. Raising the temperature of the hot water supply resulted in the termination of the epidemic, although sporadic cases still occur, usually associated with technical problems in the hot water supply. All isolates from patients were also *L. pneumophila* serogroup 1.

We were interested in methods for subtyping the isolates to confirm the epidemic spread of Legionnaires disease in the hospital and to ensure that the hot water supply was the source of the epidemic. Subtyping of *L. pneumophila* has been carried out by plasmid analysis (2, 12), peptide profiling (7), and immunochemical methods with absorbed antisera (2, 17) or monoclonal antibodies (6, 10, 12, 16). Restriction endonuclease analysis of bacterial DNA for epidemiological purposes has been employed for *Leptospira* spp. (8, 13), *Corynebacterium diphtheriae* (4, 9), and *Campylobacter jejuni* (1, 11).

We investigated the electrophoretic gel patterns of restriction enzyme digests of total DNA extracted from *L. pneumophila* serogroup 1 isolates.

Three patient isolates and an isolate from the hot water supply originated from the epidemic described above; in addition, two pairs of patient and hot water supply isolates from two other hospitals also in Amsterdam and one strain from a patient who acquired Legionnaires disease in Italy were investigated. All isolates were *L. pneumophila* serogroup 1.

MATERIALS AND METHODS

Cultures. All *L. pneumophila* strains were isolated on *N*-(2-acetamido)-2-aminoethanesulfonic acid-buffered charcoal-yeast-extract agar as modified by Edelstein (3). *L. pneumophila* was identified according to internationally accepted criteria (3), and serogrouping was done with immunofluorescent antisera obtained from the Centers for Disease Control, Atlanta, Ga. Initially, some isolates were sent to R. Weaver at the Centers for Disease Control, who confirmed their identity as *L. pneumophila* serogroup 1.

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Preparation of DNA from bacterial cultures. Cultures (40 ml), grown for 72 h in the liquid medium of Johnson et al. (5), were harvested by centrifugation, washed twice with 10 mM Tris-hydrochloride–10 mM sodium EDTA (pH 8.5), and resuspended in 10 ml of the same buffer as described by Marshall et al. (8). After incubation at 37°C with lysozyme (3 mg/ml) for 15 min, cells were lysed with sodium dodecyl sulfate in a final concentration of 1%. The lysates were incubated for 30 min at 37°C with RNase (50 μg/ml, preheated for 10 min at 80°C) and digested overnight with pronase (0.75 mg/ml, preheated for 10 min at 80°C) at 50°C. Sodium perchlorate was then added to a final concentration of 1 M, and the mixture was incubated for 1 h at 50°C. After dilution with 8 ml of STE (150 mM NaCl, 10 mM Tris-hydrochloride, 1 mM sodium EDTA [pH 8.5]), the DNA was extracted with phenol-chloroform-isoamyl alcohol (25:24:1) and precipitated with ethanol. Precipitated DNA was dissolved in ca. 1 ml of 10 mM Tris-hydrochloride–1 mM sodium EDTA (pH 7.5). After overnight dialysis against the same buffer, the DNA preparations were stored at 4°C. This procedure yielded ca. 50 μg of bacterial DNA per extraction.

Restriction enzyme digestions of DNA and gel electrophoresis. Approximately 2 μg of bacterial DNA was digested to completion in 2 h with 6 U of *Eco*RI and *Hin*dIII in, respectively, 100 mM Tris-hydrochloride–50 mM NaCl–5 mM $MgCl_2$ (pH 8.0) and 6 mM Tris-hydrochloride–60 mM NaCl–6 mM $MgCl_2$–6 mM β-mercaptoethanol (pH 7.5). Digests were electrophoresed at 30 to 40 V overnight in buffered (40 mM Tris-acetate [pH 7.7] with 20 mM sodium acetate and 2 mM sodium EDTA) 0.7% agarose containing ethidium bromide (1 μg/ml) and then photographed with a Polaroid 5-mm camera. The size of the gel was 19 by 23 cm.

RESULTS

We prepared DNA digests of isolates derived from three patients in hospital A (Fig. 1 and 2, lanes 1, 2, and 3), an isolate from the hot water supply of hospital A (Fig. 1 and 2, lanes 4), and an isolate from a patient who was transferred from hospital B to hospital A most probably during the incubation period of Legionnaires disease (Fig. 1 and 2, lanes 5). The DNA digest from an isolate of the hot water supply in hospital B is presented in lanes 6 of Fig. 1 and 2. Figures 1 and 2, lanes 7 show the strain isolated from a

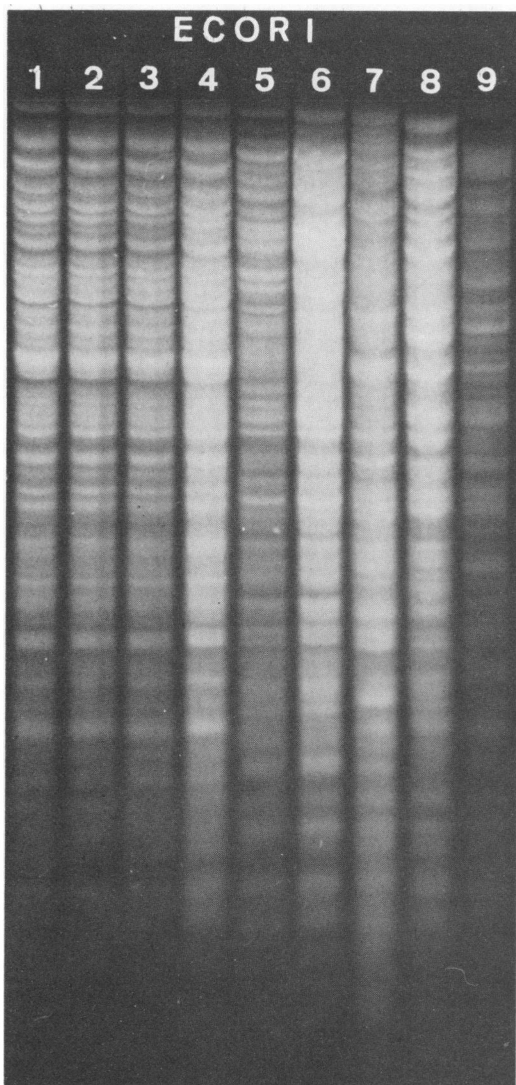

FIG. 1. *Eco*RI digestion of DNA from *L. pneumophila* strains isolated from (lanes) 1, patient 1, hospital A; 2, patient 2, hospital A; 3, patient 3, hospital A; 4, hot water-derived strain, hospital A; 5, patient transferred from hospital B to hospital A; 6, hot water-derived strain, hospital B; 7, Italian patient; 8, hot water-derived strain, hospital C; 9, patient, hospital C.

patient from Italy, and lanes 8 and 9 are, respectively, a hot water supply-derived isolate and a patient isolate originating in hospital C, also located in the city of Amsterdam.

The endonuclease electrophoretic digest patterns of the three patient isolates and the hot water supply strain from hospital A are indistinguishable from one another both in the *Eco*RI (Fig. 1) and *Hin*dIII (Fig. 2) digests. In both digests the profiles of the strain from the patient transferred from hospital B to hospital A are easily differentiated from the hot water-derived strain of hospital A and are identical to the profile of the hot water-derived strain from hospital B (Fig. 1 and 2, lanes 6). The Italian isolate has a unique pattern in both digests (Fig. 1 and 2, lanes 7). Finally, the hot water-derived and the patient strain from hospital C both have unique profiles in the *Eco*RI and *Hin*dIII digests and are easily differentiated from one another. A study of the homogeneity of *L. pneumophila* colonizing the plumbing system of the three hospitals mentioned above was undertaken by restriction endonuclease analysis of the DNA of 10 separate isolates. In the water supply of hospital A, we detected only one subtype, i.e., the one presented in lanes 4 of Fig. 1 and 2. In hospital B we distinguished two subtypes: eight isolates had restriction endonuclease electrophoretic patterns identical to the one presented in lanes 6, but in both digestions, two were indistinguishable from the strain isolated in hospital A (lanes 4). The 10 strains isolated from the plumbing system of hospital C were all of the *L. pneumophila* serogroup 1 subtype that is presented in Fig. 1 and 2, lanes 8.

DISCUSSION

Restriction endonuclease analysis of *L. pneumophila* serogroup 1 DNA seems to be a useful method for studying the epidemiology of outbreaks of Legionnaires disease. The differences in restriction endonuclease patterns are striking and convincing. Digestions with the restriction endonuclease *Bam*HI confirmed the data presented above (data not shown). Our findings with the patient and the hot water isolates from hospital A are compatible with the clinical

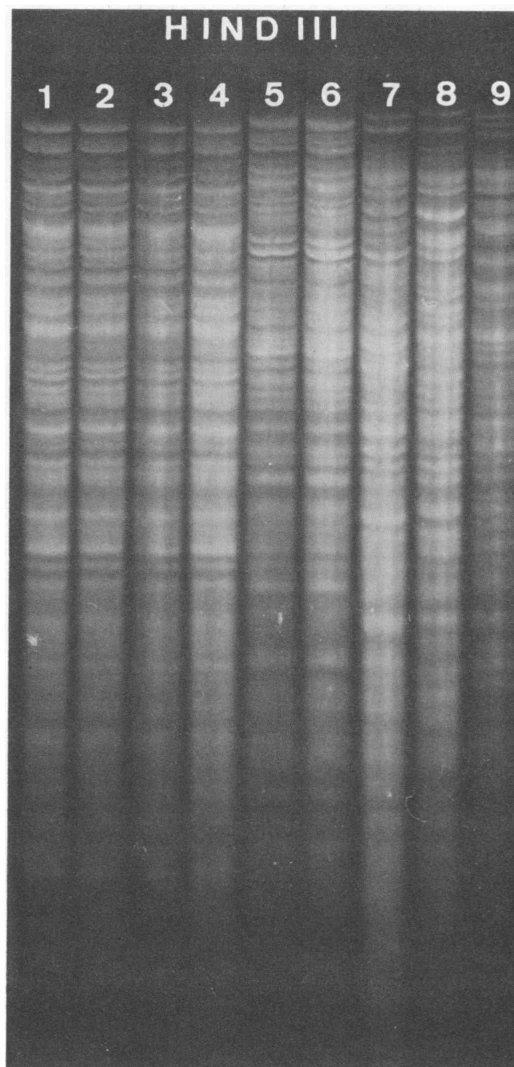

FIG. 2. *Hin*dIII digestion of DNA from *L. pneumophila* strains. Lanes and sources are the same as those described in the legend to Fig. 1.

epidemiological data concerning the source of the epidemic (15). With regard to the patient who had been transferred from hospital B to hospital A, the data suggest that the hot water supply of hospital B was the source of his infection, which is in accordance with the fact that he developed pneumonia within 48 h of admission to hospital A; the patient was most probably in the incubation period of Legionnaires disease during transfer. Lastly, it was not very likely that the patient from hospital C was infected in that hospital; he was readmitted to hospital C with symptoms of beginning Legionnaires disease after having been discharged from that hospital 8 days before. Restriction endonuclease analysis of the DNA of the patient and hot water isolates from hospital C seems to confirm this observation. Apparently, as noted by others (12, 14), different serogroups and subtypes of serogroups of *L. pneumophila* can colonize the same plumbing system; hospital A and C harbor other serogroups in addition to serogroup 1 (respectively, serogroup 4 and 6 and serogroup 4), and the hot water supply of hospital B has at least two subtypes of *L. pneumophila* serogroup 1. Analysis of restriction endonuclease digests of chromosomal DNA is a useful method in examining the epidemiology of *L. pneumophila* serogroup 1. In addition, it seems likely that this method might also be useful for studying the epidemiology of other bacterial diseases.

ACKNOWLEDGMENTS

We thank P. J. G. M. Rietra for making strains available and for the epidemiological data used in this study, O. G. Zanen-Lim for the immunological analysis of strains, and J. van der Noordaa for useful discussions.

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