

Laboratory and Epidemiology Communications

A Case of *Legionella* Pneumonia Linked to a Hot Spring Facility in Gunma Prefecture, Japan

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Communicated by Masahiko Makino

(Accepted December 18, 2009)

Legionnaires' disease, which manifests as pneumonia or the less severe Pontiac fever, has been associated with hot spring facilities and public bath houses in Japan (1). Recent studies suggest the incidence of *Legionella* pneumonia in Japan is increasing (2). Here, we describe a case of *Legionella* pneumonia and identify the probable source of infection as the water from a hot spring facility in Maebashi-shi, Gunma Prefecture, Japan.

The case involves a 64-year-old Japanese male with diabetes mellitus. In February 2008, he often used the same hot spring facility near his home. On February 20, he developed symptoms including a low-grade fever (37.0°C) and a cough. He presented at Maebashi Red Cross Hospital with a high

fever (39.6°C) on February 26 (hospital day 1), with the following clinical data: leukocyte count, $11.3 \times 10^3/\mu\text{L}$ (normal range, $4.0\text{--}9.0 \times 10^3/\mu\text{L}$); platelet count, $1.36 \times 10^5/\mu\text{L}$ ($1.8\text{--}3.5 \times 10^5/\mu\text{L}$); and C-reactive protein level, 24.3 mg/dL (<0.5 mg/dL). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were 635 U/L (normal range, 13–33 U/L) and 150 U/L (8–42 U/L), respectively. Renal function was slightly deteriorated (blood urea nitrogen [BUN] value, 28 mg/dL; normal range, 0–20 mg/dL). In addition, chest radiography showed consolidation with an air bronchogram on the bilateral lung. Collectively, the clinical data suggested bacterial pneumonia, complicated by abnormal liver function and low-grade renal failure.

He was given the standard treatment for bacterial pneumonia, including the provision of oxygen (5 L/min) and the administration of the antibiotics ciprofloxacin (600 mg/day, days 1 to 24) and sulfamethoxazole/trimethoprim (800 mg/day, days 2 to 12). The lung lesion showed improvement from hospital day 4 onwards. Aspirated sputum samples were

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collected and examined by bacterial culture using WYO α agar (Eiken Chemical Co., Ltd, Tokyo, Japan). *Legionella pneumophila* antigen was detected in a urine sample using an immunochromatographic assay (Duopath *Legionella*; Merck KGaA, Darmstadt, Germany) and the bacterium was isolated from the patient's sputum. A diagnosis of *Legionella pneumonia* was therefore confirmed.

Epidemiological data regarding the patient's visit to the hot spring and the subsequent detection and isolation of *L. pneumophila* led the patient's physician to suspect the site of the infection was contaminated water at the hot spring facility. The physician filed with Gunma Prefectural Maebashi Health Center a surveillance report of *L. pneumophila* infection possibly linked to a hot spring.

To confirm the source of *L. pneumophila*, we collected water samples from the relevant hot spring and examined the sample using GVPC agar (bioMérieux, Marcy l'Etoile, France). *L. pneumophila* was detected in the water sample. The isolates of *L. pneumophila* from the patient and hot spring water were identified as serogroup (SG) 1. Using polymerase chain reaction (PCR), we genotyped these isolates as previously described (3,4). In addition, the PCR products, or amplicons, were examined by agarose gel electrophoresis and the isolates from the patient and hot spring water were genotyped as *L. pneumophila* (Fig. 1). We then performed pulsed-field gel electrophoresis (PFGE) with endonuclease *Sfi*I, as previously described (5). PFGE band patterns between isolates taken from the patient and the hot spring water were conclusively matched (Fig. 2), and the isolates were genotyped as *L. pneumophila* (SG1). On the basis of these data, the hot spring operators were deemed in violation of the Public Bath House Law (Issue 7, Item 1) and the Director of the Gunma Prefectural Maebashi Health Center ordered the bath house to close for 2 weeks.

L. pneumophila is the causative agent of *Legionella pneumonia* and Pontiac fever. This pathogen parasitizes amoeba

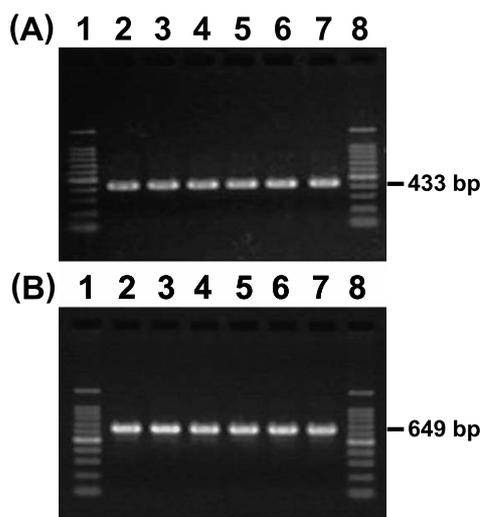


Fig. 1. Agarose gel electrophoresis of the PCR products. (A) Genus *Legionella* 16S rRNA gene 433 bp. (B) *L. pneumophila* macrophage infectivity potentiator gene 649 bp. Amplicons were electrophoresed on a 1.5% agarose gel. Lanes 1 and 8, Marker (100-bp DNA Ladder); Lanes 2, 3, and 4, amplicons derived from isolates of hot spring water; Lanes 5 and 6, amplicons derived from the patient; Lane 7, amplicons derived from ATCC 33152 strain used as a standard.

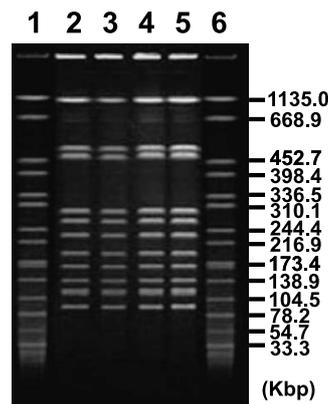


Fig. 2. Photographs of PFGE band patterns of isolates using an endonuclease *Sfi*I cleaved genomic DNAs. Lanes 1 and 6, Molecular size marker; Lanes 2 and 3, PFGE band patterns of isolates derived from hot spring water; Lanes 4 and 5, PFGE band patterns of isolates derived from the patient.

(*Acanthamoeba castellanii*), and it is thought that hot spring water and cooling-tower water provide favorable conditions for the propagation of the amoeba and the pathogen (6). *Legionella pneumonia* may, therefore, be caused by the inhalation of water aerosols contaminated with *L. pneumophila* (7). In Japan, the majority of *Legionella pneumonia* cases are caused by hot spring water contaminated with *L. pneumophila*. Consequently, most Japanese hot spring facilities are now equipped with an engineered closed-water circulation system. When the disinfection of the circulating hot spring water is inadequate, carrier amoebas and *L. pneumophila* may propagate and disseminate.

The case reported here should serve as an important reminder of the risk posed by public water systems as well as of the need for hot spring water facilities to disinfect against *L. pneumophila* and to operate closed water circulation systems to guard against this life-threatening pathogen.

This article appeared in the Infectious Agents Surveillance Report (IASR), vol. 29, 193–194, 2008 in Japanese.

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