Short Communication

Characteristics of *Legionella pneumophila* Serogroup 2 Strains by Colony Morphology

Michio Koide*, Makoto Furugen, Shusaku Haranaga, Futoshi Higa, Masao Tateyama, Nobuhisa Yamane1 and Jiro Fujita

Department of Medicine and Therapeutics, Control and Prevention of Infectious Diseases (First Department of Internal Medicine) and 1Department of Clinical Laboratories, Faculty of Medicine, University of the Ryukyus, Okinawa 903-0215, Japan

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**SUMMARY**: The isolation rate of *Legionella pneumophila* serogroup 2 from clinical samples is low. In 2007, we encountered the second case known to occur in Japan. As the *L. pneumophila* serogroup 2 type strain, the Kobe strain isolated in 1988, and the Okinawa strain isolated from the present patient could not be differentiated using the usual biochemical and serological tests, we tried to achieve differentiation by observing colony morphology. In Oxoid BCYEα medium, colonies of the Kobe strain developed multiple protuberances on the surface, but these did not develop on the other two strains. In Becton-Dickinson BCYEα medium, colonies of the Okinawa strain had several outgrowths from the margin, but the type strain and the Kobe strain did not have any outgrowths. The Okinawa strain isolated from the present case showed intermediate characteristics between the type strain and the Kobe strain in the appearance of colony morphology. It may be useful to conduct an investigation of this rare serogroup.

The genus *Legionella* comprises 52 species, almost half of which have been recovered from clinical samples. Of these, *Legionella pneumophila* has been responsible for over three-fourths of *Legionella* infections, with *L. pneumophila* serogroup 1 responsible for over two-thirds of *L. pneumophila* infections (1–4). However, the isolation rate for *L. pneumophila* serogroup 2 from clinical samples has been low compared to the rate for *L. pneumophila* serogroups 3, 4, 5, and 6 as well as *L. pneumophila* serogroup 1 in the United States, Europe, and Japan (1–4). In 1988, we experienced the first case of *L. pneumophila* serogroup 2 pneumonia in Japan from which the bacteria was able to be isolated (5,6). A long time after that first case, the second case in Japan occurred in Okinawa in 2007 (7). These two *L. pneumophila* serogroup 2 cases showed the typical clinical features of *Legionella* pneumonia and seemed to have no significant difference from pneumonia caused by other serogroups of *L. pneumophila*. These two strains and the *L. pneumophila* serogroup 2 type strain Togus-1 (ATCC33154) could not be differentiated using the usual biochemical and serological tests. However, investigation of this rare serogroup was considered to be important. Therefore, we tried to compare the colony morphology characteristics of these two *L. pneumophila* serogroup 2 strains as well as the *L. pneumophila* serogroup 2 type strain.

To observe colony morphology of the strains, we poured each 0.1-ml volume of the 500–50 cfu/ml concentrations of *L. pneumophila* serogroup 2 strains onto Oxoid BCYEα agar (Oxoid, Hampshire, UK) and BD BCYEα agar (Becton-Dickinson, Sparks, Md., USA) for duplication. The preparations were incubated at 35°C for 18 days so as to observe colony morphology. Plates that grew 8–20 colonies were chosen for measurement of the colony diameters.

In Oxoid BCYEα medium, the type strain and the Okinawa strain made visible colonies on the 3rd day, and the Kobe strain made visible colonies on the 4th day. During the following incubation period, the Kobe strain colonies developed multiple protuberances on the surfaces, but the other two strains did not (Fig. 1). The final appearance of the type strain and the Okinawa strain colonies was smooth, circular, and slightly convex, while the Kobe strain had irregular margins and was slightly convex. The final size of the colonies after 18 days of incubation was almost the same at 6-mm diameter.

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* Corresponding author: Mailing address: Department of Medicine and Therapeutics, Control and Prevention of Infectious Diseases (First Department of Internal Medicine), Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara-cho, Okinawa 903-0215, Japan. Tel: +81-98-895-1144, Fax: +81-98-895-1414, E-mail: koide-mi@med.u-ryukyu.ac.jp
for the three strains. Consequently, the Kobe strain could be differentiated from the other two strains (Table 1).

In BD BCYE* medium, the type strain and the Okinawa strain made visible colonies on the 3rd day, and the Kobe strain made visible colonies on the 4th day. During the following incubation period, colonies of the Okinawa strain had several outgrowths from their margins, but the type strain and the Kobe strain did not have any outgrowths. Colony appearance and size were different for each. The type strain was smooth, mucoid, and flat, and had a convex center. The Okinawa strain had outgrowths from the margin and was non-mucoid and flat. The Kobe strain was smooth, mucoid, and convex. The final average size of each colony (18 days incubation) was 9 mm (range 7-11 mm) for the type strain, 7 mm (range 6-8 mm) for the Okinawa strain, and 8 mm (range 6-10 mm) for the Kobe strain. Thus the Okinawa strain was distinguishable from the other two strains in these respects.

Consequently, by using both Oxoid and BD BCYE* mediums, colonies of the three strains could be distinguished from one another. The reason why colony morphology of the Kobe strain resembles the serogroup 2 type strain more than the Okinawa strain in BD medium remains unknown. The yield from colonies on Oxoid agar (agar no. 1) had a greater count according to results obtained by Edelstein et al., but contrary to their findings, the size of the colonies in our study was larger on BD agar (formerly Difco bacto agar) (8). In our previous report, the Kobe strain began to develop protuberances on the colony surface at the 5th day of incubation, but in the present study, protuberances did not develop until the 11th day (6). This was probably due to either the change of the medium component (e.g., agar washed with distilled water) or the phenotypic change of bacterial characteristics during the long period of storage (9).

With random amplified polymorphic DNA analysis, the Okinawa strain resembled the type strain Togus-1, and the Kobe strain differed from the type strain (data not shown). Consequently, a slight DNA rearrangement may have occurred in some genes of the Okinawa strain, and a significant DNA rearrangement may have occurred in the same gene of the Kobe strain.

The phenomenon of papillated colony morphology on agar grown Legionella was first reported for Legionella micdadei by Pascule et al. in 1980 (10). L. micdadei colonies grown on CYE agar, isolated from the patient's lung biopsy, developed multiple protuberances (papillae) on the surfaces and developed several outgrowths from the margins after 10 days of incubation. Following our report on the L. pneumophila serogroup 2 in 1995, Downes et al. reported resembling colonies (satellites) of human oral anaerobes which they designated as Shuttleworthia satelles in 2002 (11). They speculated that satellite colonies of S. satelles might occur in a spontaneous chromosomal rearrangement responsible for the change in colony morphology, which may also disrupt genes encoding sugar-fermentation enzymes. They suspected that chromosomal rearrangement of S. satelles might be DNA duplication located in some genes, as reported in Pseudomonas tolaasii (12). It is unknown whether chromosomal rearrangement occurred in the L. pneumophila serogroup 2 Kobe strain, but it may be useful to conduct an investigation along with the Okinawa strain, since the Okinawa strain showed intermediate characteristics between those of the type strain and the Kobe strain.

In conclusion, it may be of value to investigate this rare serogroup. The Okinawa strain isolated from the present case showed intermediate characteristics in the appearance of colony morphology between the type strain Togus-1 and the Kobe strain.

**REFERENCES**


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**Table 1. Colony characteristics of Legionella pneumophila strains**

<table>
<thead>
<tr>
<th>Colony characteristic</th>
<th>Agar plate used</th>
<th>Type strain</th>
<th>Okinawa strain</th>
<th>Kobe strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days for colony become visible</td>
<td>Oxoid</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>BD</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Days for colony become atypical</td>
<td>Oxoid</td>
<td>No</td>
<td>No</td>
<td>Yes, 11</td>
</tr>
<tr>
<td>BD</td>
<td>No</td>
<td>Yes, 10</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Colony morphology</td>
<td>Oxoid</td>
<td>typical, circular, low convex</td>
<td>typical, circular, low convex</td>
<td>irregular margin, develop papilla on surface</td>
</tr>
<tr>
<td>BD</td>
<td>smooth, mucoid, flat, convex center</td>
<td>non-mucoid, flat, outgrowths from margins</td>
<td>smooth, mucoid, convex</td>
<td></td>
</tr>
<tr>
<td>Final colony diameter, 18 days</td>
<td>Oxoid</td>
<td>6 mm</td>
<td>6 mm</td>
<td>6 mm</td>
</tr>
<tr>
<td>BD</td>
<td>7-11 mm (9 mm)*</td>
<td>6 - 8 mm (7 mm)*</td>
<td>6 - 10 mm (8 mm)*</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers of parenthesis represent average.

BD, Becton-Dickinson; No, not atypical.
