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Short Communication

Evaluation of *Legionella pneumophila* SGUT Serotypes Isolated from Bath Water Using a Multiplex-PCR Serotyping Assay

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ABSTRACT: *Legionella pneumophila*, the primary causative agent of Legionnaires' disease, is classified into at least 15 serogroups (SGs). Before genotyping, serotyping is first performed to limit the sources of *L. pneumophila* infections that caused an outbreak. In addition to conventional assays using monoclonal or polyclonal antisera, serotyping using multiplex polymerase chain reaction (M-PCR) was recently developed for *L. pneumophila*. In this study, we applied the M-PCR system to 41 strains that remained to be SGUT (untypable) by slide agglutination tests among the 220 *L. pneumophila* strains isolated from bath water in Kobe City during 2016–2020, to determine SG-genotypes (SGg) by PCR amplification of the specific target gene of the SGs. Among the 41 SGUT strains, SGg4/10/14 was the most predominant (24/41, 58.5%), followed by SGg1 (7/41, 17.1%). Seven strains, except for the strains determined as SGg1, were identified as belonging to a single SGg by M-PCR serotyping (SGg5 [3/41, 7.3%], SGg8 [3/41, 7.3%], and SGg7 [1/41, 2.4%]). Furthermore, we found that the seven strains identified as SGg1 harbored particular genotypes. In conclusion, the M-PCR serotyping assay will be helpful for investigating the distribution of *L. pneumophila* in environmental and clinical settings.

Legionnaires' disease (LD) outbreaks are caused by *Legionella* contamination of aquatic environments. In Japan, several outbreaks associated with bath facilities have been reported (1–3). *Legionella pneumophila*, the main causative pathogen of LD, is classified into at least 15 serogroups (SGs) (4). In Japan, cases of *L. pneumophila* SG1 infection are the most common (5). Before genotyping, serotyping is the primary assay used to identify sources of *L. pneumophila* infections that caused an outbreak. Serotyping assays using monoclonal or polyclonal antisera have been extensively used in epidemiological studies on *L. pneumophila* for several years (6,7). Recently, a multiplex polymerase chain reaction (M-PCR) serotyping assay was developed (8). In this study, we performed M-PCR serotyping to characterize *L. pneumophila* SGUT (untypable) isolated from bath water in Kobe City, Japan.

We isolated 220 *L. pneumophila* strains from 74 bath facilities using routine environmental monitoring from July 2016 to October 2020. The serogroups of all the strains were confirmed via slide agglutination tests using commercially available monovalent polyclonal antisera (Denka Company Limited, Tokyo, Japan). Among the 220 *L. pneumophila* strains, SG1 was the most frequently isolated, accounting for 30.0% (66/220), followed by SGUT (41/220, 18.6%), SG6 (30/220, 13.6%), and SG5 (26/220, 11.8%) (Table 1). SG6 and SG5 are predominantly isolated from bath water (9). Moreover, *L. pneumophila* SGUT strains were isolated from 24 bath facilities over different years. Therefore, we investigated *L. pneumophila* SGUT SGs

Table 1. Distribution of serogroups of 220 *Legionella pneumophila* strains isolated from bath water during 2016–2020

Serogroup (SG)	Number of strains (%)
SG1	66 (30.0)
SGUT	41 (18.6)
SG6	30 (13.6)
SG5	26 (11.8)
SG9	18 (8.2)
SG10	10 (4.5)
SG3	9 (4.1)
SG11	9 (4.1)
SG8	5 (2.3)
SG2	3 (1.4)
SG12	2 (0.9)
SG7	1 (0.5)

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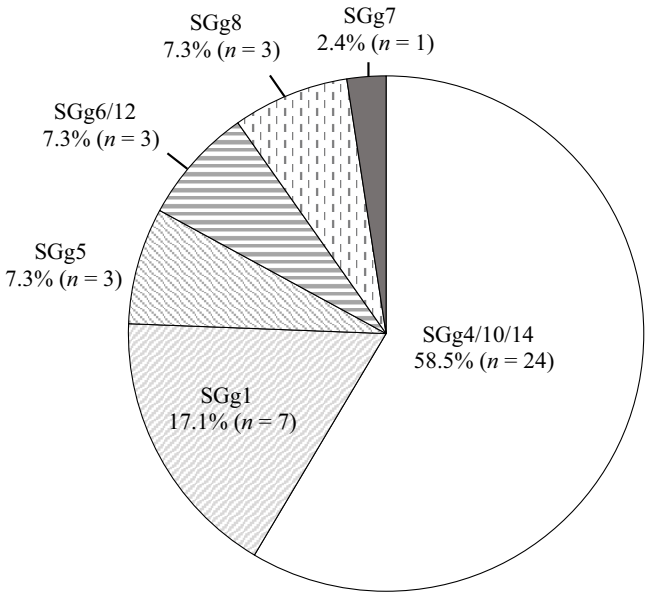

Fig. 1. Distribution of serogroup genotypes (SGg) in 41 *Legionella pneumophila* SGUT (untypable) based on a multiplex polymerase chain reaction (M-PCR) serotyping assay.

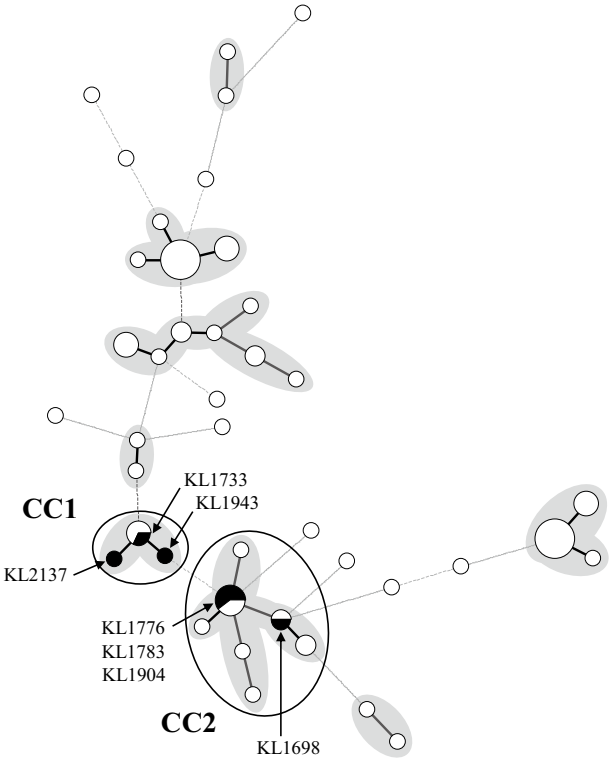

Fig. 2. Minimum spanning tree of all 73 *Legionella pneumophila* serogroup 1 (SG1) strains included the seven strains identified as SGg1 using M-PCR isolated from bath water based on multiple-locus variable number tandem repeat analysis (MLVA). The clonal complexes (CCs) that were generated with single-locus and double-locus variants are indicated by the shaded backgrounds. Seven strains identified as SGg1 using M-PCR are shown in black. Strains KL1733, KL1943, and KL2137 belonged to CC1, whereas KL1783, KL1698, KL1904, and KL1776 belonged to CC2. The size of the circle is proportional to the number of strains sharing a particular MLVA type. The length of the lines is proportional to the number of different loci. Solid and dashed lines indicate single- or double-locus and three-locus or higher variants, respectively.

to determine their distribution in bath water using the M-PCR serotyping assay.

The 41 *L. pneumophila* SGUT strains were classified into six SG-genotypes (SGg) (Fig. 1). SGg4/10/14 was the predominant serogroup (24/41, 58.5%) followed by SGg1 (7/41, 17.1%). Because SG4/10/14 primers were designed to detect these serogroups according to the ORFs shared by SG4, SG5, SG7, SG8, SG9, SG10, SG13, and SG14 (8), some strains identified as SGg4/10/14 using M-PCR may belong to other serotypes. All strains, except the three strains belonging to SGg6/12 and 24 strains to SGg4/10/14 that were not agglutinated by any antiserum, were classified as a single SGg using M-PCR serotyping. Although the phenotypes and genotypes of the antigens were not identical, we divided all strains into distinct PCR serotypes. In contrast, all seven strains identified as SGg1 using M-PCR were identified as SG1 using the Oxoid Legionella latex test (Kanto Chemical Co., Inc., Tokyo, Japan). According to the manual, the Oxoid Legionella latex test uses unheated bacterial antigens, whereas the manual for slide agglutination tests recommends the use of prepared heat-killed bacterial antigens. Slide agglutination tests were performed on seven strains using unheated bacterial antigens, and all strains agglutinated to the SG1 antiserum. Therefore, these results suggest that the reactivity of the antiserum changes owing to differences in the prepared bacterial antigens.

Because *L. pneumophila* SG1 is the most frequent cause of LD associated with bath water in Japan (10), it should be appropriately identified using serotyping. To clarify the genetic relatedness of the seven strains identified as SGg1 using M-PCR with the 66 *L. pneumophila* SG1 strains aggregated with the SG1 antiserum, their genotypes were investigated using multiple-locus variable number tandem repeat analysis (MLVA) and sequence-based typing. The MLVA was conducted as previously reported (11) and a minimum spanning tree based on MLVA was constructed using Bionumerics ver.7.5 (Applied Maths, Sint-Martens-Latem, Belgium). Sequence-based typing was performed according to the protocol of the European Working Group for *Legionella* infections (EWGLI) using seven genes (*flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA*, and *neuA*) as described previously (12,13). The minimum spanning tree had eight clonal complexes (CCs) generated with single- and double-locus variants (Fig. 2). The minimum spanning tree showed that the seven strains identified as SGg1 by M-PCR were mainly divided into two groups (Fig. 2). Strains KL1733, KL1943, and KL2137 belonged to CC1, whereas KL1783, KL1698, KL1904, and KL1776 belonged to CC2. Three strains belonging to CC1 were classified with a novel sequence type (ST) (allelic profile:6, 6, 15, 3, 21, 7, 11), whereas four strains belonging to CC2 were assigned to ST128 (allelic profile:7, 6, 17, 3, 14, 11, 11, KL1783) and novel STs (allelic profile of KL1698: 23, 6, 17, 3, 14, 11, 11; allelic profile of KL1904: 7, 12, 17, 3, 14, 11, 11; and allelic profile of KL1776: 7, 10, 17, 3, 14, –1, 11). KL1776 harbored a new allele number with a

one-base difference from allele number 10 of *proA*. *L. pneumophila* SG1 strains assigned to these particular genotypes may have difficulty reacting with antiserum SG1 because their lipopolysaccharide biosynthetic loci differ from in part of those of other SG1 strains.

In summary, we revealed the distribution of *L. pneumophila* SGUT serotypes isolated from bath water using an M-PCR serotyping assay. Given that the M-PCR serotyping assay was helpful in identifying the SGUT serotype using antiserum, this assay may aid in investigating the distribution of *L. pneumophila* in environmental and clinical settings.

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Conflict of interest None to declare.

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