Complete Genome Sequences of Six *Legionella pneumophila* Isolates from Two Collocated Outbreaks of Legionnaires’ Disease in 2005 and 2008 in Sarpsborg/Fredrikstad, Norway

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Here, we report the complete genome sequences of *Legionella pneumophila* isolates from two collocated outbreaks of Legionnaires’ disease in 2005 and 2008 in Sarpsborg/Fredrikstad, Norway. One clinical and two environmental isolates were sequenced from each outbreak. The genome of all six isolates consisted of a 3.36 Mb-chromosome, while the 2005 genomes featured an additional 68 kb-episome sharing high sequence similarity with the *L. pneumophila* Lens plasmid. All six genomes contained multiple mobile genetic elements including novel combinations of type-IVA secretion systems. A comparative genomics study will be launched to resolve the genetic relationship between the *L. pneumophila* isolates.

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**Legionella pneumophila** is an opportunistic bacterial pathogen capable of airborne transmission from contaminated freshwater systems to susceptible humans, resulting in a severe pneumonia known as Legionnaires’ disease (1). Sequence-based typing is the standard subtyping method for *L. pneumophila* (2), however, recent cost reductions and increased availability have enabled whole-genome sequencing-based subtyping (3).

Here, we report complete genome sequences of *L. pneumophila* isolates from two collocated outbreaks of Legionnaires’ disease in 2005 and 2008 in Sarpsborg/Fredrikstad, Norway (Table 1). One clinical and two environmental isolates were sequenced from each outbreak. Additional isolate information has been reported elsewhere (4–7).

Isolates were grown on buffered-charcoal-yeast-extract agar (72 h, 37°C). DNA was purified using Genomic-Tip 100/G (Qia- gen, Hilden, Germany). Sequencing was done with PacBio RSII (Menlo Park, CA) and Illumina MiSeq (San Diego, CA). RSII library was prepared using the 20 kb-protocol and size selection done with BluePippin (9 kb-cutoff). Sequencing was done using P6-C4 chemistry and one single-molecule real-time (SMRT) cell. MiSeq library (300 bp paired-end) was prepared with TruSeq PCR-free protocol. Approximately 90,000 RSII and 3,000,000 MiSeq reads were generated for each isolate. RSII reads were de novo-assembled with HGAP_v3.0. Minimus2 (AMOS_v3.1) was used for circularization and RS_Resequencing for mapping of RSII reads. MiSeq reads were mapped onto the final RSII assembly with Bionumerics_v7.6 (Applied Maths, Sint-Martens-Latem, Belgium). Annotation was done with NCBI PGAP_v3.3.

All genomes consisted of a 3.36 Mb-chromosome, while the 2005 genomes featured an additional 68 kb—episome showing high similarity (>97%) with a 39 kb-region of the 60 kb-episome of *L. pneumophila* Lens (Table 1). Average coverage was 284× (RSII) and 466× (MiSeq). All genomes showed conserved synthony with other *L. pneumophila* genomes and highest degree of similarity with Lens (>95%), in agreement with previous sequence-based typing (6). Average G+C content was 38.5% and number of protein-coding genes

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**Table 1** Isolate information and key genomic features of the *Legionella pneumophila* isolates subjected to whole-genome sequencing in this work

| Strain/isolate | Source | Country | Yr | SGb | STc | Genome size (bp) | Chromosome accession no. | Chromosome size (bp) | Episome accession no. | Episome size (bp) |
|---------------|--------|---------|----|-----|-----|------------------|------------------------|---------------------|---------------------|------------------|
| FFI102        | Clinical | Norway | 2005 | SG1 | ST15 | 3,431,790 | CP016868 | 3,363,654 | CP016869 | 68,145 |
| FFI103        | Environmental | Norway | 2005 | SG1 | ST15 | 3,431,761 | CP016870 | 3,363,616 | CP016871 | 68,145 |
| FFI104        | Clinical | Norway | 2008 | SG1 | ST462 | 3,362,494 | CP016874 | 3,363,658 | CP016875 | 68,146 |
| FFI105        | Environmental | Norway | 2008 | SG1 | ST462 | 3,363,998 | CP016873 | 3,363,998 | — | — |
| FFI347        | Environmental | Norway | 2008 | SG1 | ST462 | 3,362,463 | CP016876 | 3,362,463 | — | — |
| FFI337        | Environmental | Norway | 2008 | SG1 | ST462 | 3,362,463 | CP016876 | 3,362,463 | — | — |
| Lens*         | Clinical | France | 2004 | SG1 | ST15 | 3,405,519 | CR628337 | 3,345,687 | CR628387 | 59,832 |

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* The genome sequence of *L. pneumophila* Lens was obtained from GenBank and included as a reference because sequence-based typing had previously showed that the 2005 isolates had the same ST as Lens (ST15) while the 2008 isolates had a different ST (ST462) but were still closely related to Lens.

b * SG, serotype/serogroup.

c ST, sequence type.

d —, not applicable.
2,900, both comparable to Lens (38.4% and 2,932 genes, respectively) (8). _L. pneumophila_ often have a dynamic accessory genome consisting of mobile genetic elements, including integrative conjugative elements encoding type-IVA secretion systems (T4ASS), that may facilitate horizontal gene transfer, genome plasticity, and environmental adaption potential (3, 9). All genomes contained Dot/Icm type-IVB secretion system (T4BSS) and genomic island-associated T4ASS (GI-T4ASS). None of the genomes contained Lvh T4ASS, which is present in Lens (3), while all genomes contained Trb (P-type) T4ASS, which is absent in Lens. The 2008 genomes contained a Trb similar to the one in Corby/Alcoy, while the 2005 genomes contained a Trb similar to the one in Lorraine (3). Only the 2005 genomes contained Tra (F-type) T4ASS, which also is present in Lens. Tra was same as in Lens located on the episome in the 2005 genomes. All genomes contained additional virulence-associated elements including RtxA (10) and clustered regularly interspaced short palindromic repeats (CRISPR)-associated systems (Cas) (11). The RtxA in the 2005 genomes shared high similarity (>92%) with the one in Lens, while the RtxA in the 2008 genomes was more similar to the one in Corby/Alcoy.

Comparative genomics will be used to resolve the genetic relationship between the sequenced isolates. Efforts to increase the availability of _L. pneumophila_ genomes may serve as an important catalyst of advancements in this field.

**Accession number(s).** This whole-genome sequencing (WGS) project was deposited in GenBank under the accession numbers listed in Table 1.

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