

Supplementary Appendix

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2. Author contributions

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4. Methods

Culture, serogrouping and molecular typing. Respiratory specimens were directly inoculated onto buffered charcoal yeast extract (BCYE) medium (Biokar diagnostics) and onto a semiselective BCYE-based medium supplemented with cefamandole, polymyxin B and anisomycin (BMPA medium; Oxoid).¹⁻³ Water specimens were inoculated onto a semiselective BCYE-based medium supplemented with glycine, vancomycin, polymyxin B and cycloheximide (GVPC medium). Cultures were incubated at 36+/-1°C in an aerobic humidified atmosphere and inspected every 2 days for up to 14 days. Suspected *Legionella* colonies (those showing the characteristic cut-glass appearance under a stereoscopic magnifier) were sub-cultured to BCYE plates before proceeding with species and serogroup identification by latex agglutination (*Legionella pneumophilla* Latex Test Kit, Oxoid). Genotypic characterization of *L. pneumophila* isolates was performed by sequence-based typing (SBT), an epidemiological typing scheme for clinical and environmental isolates developed by members of the European Working Group for *Legionella* Infections (EWGLI). SBT protocols are available at the EWGLI Sequence-Based Typing Database webpage at http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php.

Whole-genome sequencing. High-quality genomic DNA samples from pure bacterial cultures of both clinical isolates were used to prepare Nextera XT Illumina libraries that were subjected to paired-end sequencing (2x150 bp) on a MiSeq system (Illumina Inc., San Diego, CA, USA), according to the manufacturer's instructions. Samples from AFTL and MFTL were prepared and run in different weeks in order to discard the possibility of any cross contamination during the WGS procedure. Illumina reads were subjected to quality improvement and further assembled using Velvet version 1.2.10.⁴ The process was optimized using the VelvetOptimiser script version 2.2.5. The obtained mean depth of coverage was 138-fold and 156-fold for PtVF66/2014 and PtVF89/2014 isolates, respectively. Both draft sequences (scaffold/contigs) were aligned using the progressive algorithm of Mauve software (version 2.3.1)⁵ and a core-alignment was extracted by keeping and concatenating regions where genomes aligned over at least 500 bp. We were able to use more than 99.8% of each draft sequence to study the genetic relatedness of both *L. pneumophila* clinical isolates. Exclusion of false positive polymorphisms was thoroughly performed by the visual inspection of the assembled reads in Tablet 1.14.04.10.⁶ Reads were submitted to the Sequence Read Archive (SRA) database and are available under the accession numbers SRP056096 (for the isolate PtVF66/2014) and SRP056100 (for the isolate PtVF89/2014).

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