Journal of Hospital Infection 96 (2017) 392–395

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

Short report

Whole-genome sequencing for identification of the source in hospital-acquired Legionnaires' disease

A.M. Rosendahl Madsen [a,*], A. Holm [a], T.G. Jensen [a], E. Knudsen [a], H. Lundgaard [a], M.N. Skov [a], S.A. Uldum [b], M. Kemp [a]

[a] Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark
[b] Statens Serum Institute, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 18 January 2017
Accepted 24 April 2017
Available online 27 April 2017

Keywords:
Legionella
Nosocomial infection
Whole-genome sequencing

SUMMARY

Acquisition of Legionnaires' disease is a serious complication of hospitalization. Rapid determination of whether or not the infection is caused by strains of *Legionella pneumophila* in the hospital environment is crucial to avoid further cases. This study investigated the use of whole-genome sequencing to identify the source of infection in hospital-acquired Legionnaires' disease. Phylogenetic analyses showed close relatedness between one patient isolate and a strain found in hospital water, confirming suspicion of nosocomial infection. It was found that whole-genome sequencing can be a useful tool in the investigation of hospital-acquired Legionnaires' disease.

Introduction

Legionella pneumophila is a bacterium found in fresh water and other moist environments, including in the water supply in houses and hospitals. *L. pneumophila* can be transmitted to humans by inhalation of contaminated droplets, causing severe pneumonia (Legionnaires' disease) after an incubation period of two to 10 days [1]. Diabetes, old age, male sex, lung disease, immunosuppression and recent surgery are among the recognized risk factors. Nosocomial Legionnaires' disease is a rare but serious complication of hospital treatment, with a case-fatality rate of 15–30% [1]. Besides tap water and water in air-conditioning devices, treatment devices such as humidifying nebulizers and decorative fountains may serve as sources of infection in hospitals. It is of utmost importance to identify cases and sources of hospital-acquired *L. pneumophila* infection, as outbreaks may result from continued release of the bacterium [2].

Approximately 120–160 laboratory-confirmed cases of Legionnaires' disease are registered in Denmark every year. Five to ten percent of these are registered as nosocomial cases. Real-time polymerase chain reaction on respiratory samples and legionella urine antigen test are used for diagnosis at the study institution. In positive cases, culture of *L. pneumophila* from the respiratory sample is subsequently attempted.

Isolates are serotyped by a panel of monoclonal antibodies into serogroups (SGs) and subgroups if applicable, and genotyped by sequence-based typing (SBT), a variant of multi-locus sequence typing (MLST) with seven loci. Both are well-established methods used for surveillance and epidemiological typing of Legionnaires' disease [3,4]. Whole-genome sequencing (WGS) offers a unique opportunity to determine

* Corresponding author. Address: J.B. Winsloewsvej 21.2, 5000 Odense C, Denmark. Tel.: +45 65414797 or +45 22563134.
E-mail address: Anne.Rosendahl@rsyd.dk (A.M. Rosendahl Madsen).

http://dx.doi.org/10.1016/j.jhin.2017.04.020

relatedness of bacteria, and has been used previously to study outbreaks of various bacterial infections including Legionnaires' disease [5,6]. The aim of this study was to test the use of WGS in the investigation of nosocomial Legionnaires' disease. The draft genome of a strain of *L. pneumophila* isolated from a patient with suspected nosocomial Legionnaires' disease was compared with genomes of isolates from other patients and water sources in the hospital in order to identify the source of infection.

Methods

Bacterial isolates

Isolates of *L. pneumophila* were obtained by culture from patient samples or filtered water samples on modified Wadowsky Yee Oxoid agar at 35 °C (MWY-O agar, Statens Serum Institut, Copenhagen, Denmark). Species identity was confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry. Isolates were stored at −80 °C.

Thirteen isolates (P1–P13) of *L. pneumophila* from patients diagnosed with Legionnaires' disease in the study hospital and 12 isolates (W1–W12) from hospital tap water obtained over a six-year period (2009–2014) were included. P3 was from a patient who was likely to have contracted the infection during hospitalization. He was admitted to hospital because of a hip fracture and stayed in hospital for non-infectious complications. He developed respiratory symptoms and fever after 13 days of hospitalization, and was diagnosed with Legionnaires' disease three days later by real-time polymerase chain reaction on a sputum sample. Subsequently, *L. pneumophila* was cultured from the sample. Eight water samples were collected from different water sources in the ward two weeks after detection of *L. pneumophila* in the patient. *L. pneumophila* was cultured from six of the water samples (W1, W2, W7, W8, W9 and W10). W1 was isolated from the shower in a bathroom on the ward; W2 from the tap in the room in which the patient stayed; W7 from the shower in another bathroom on the ward and W8 from the tap in the same bathroom; W9 from the tap in the kitchen; and W10 from the tap in the first bathroom. The remaining isolates, both environmental and patient isolates, were epidemiologically unrelated to this case and were included to create a basis for comparison. P7 was from a previous case where nosocomial infection was suspected but could not be confirmed. The patient was hospitalized in a different department approximately four years before the present case (P3).

Serotyping and SBT were performed at the national reference laboratory at Statens Serum Institut, Copenhagen, Denmark. SG was determined for all isolates. SBT was undertaken for all patient isolates and for environmental isolates if there was a match to a patient isolate at SG/subgroup level.

Genome sequencing and characterization

Stored isolates of *L. pneumophila* were grown overnight on MWY-O agar. DNA from single colonies was extracted by DNeasy Tissue Kit (Qiagen, Valencia, CA, USA). Libraries were constructed using Nextera XT DNA sample preparation kits (Illumina, San Diego, CA, USA) according to the standard protocol, and sequenced using the Illumina Miseq platform with 150-bp paired-end reads. On average, a coverage of 58x was obtained. Sequence reads were directly uploaded to BaseSpace (www.basespace.illumina.com), where genomes were assembled de novo using the SPAdes 3.0 app. Assembled genomes were compared in the online tool CSI phylogeny 1.1 (http://www.genomicepidemiology.org/). The published genome sequence of *L. pneumophila* strain Paris chromosome (GenBank 67228) was used as reference. Trees were constructed and visualized in FigTree 1.4.2. (http://tree.bio.ed.ac.uk/software/figtree/). To obtain better sequencing coverage, P3 and four closely related isolates (P4, P7, P12 and W8) were resequenced. For each isolate, the forward reads from the two sequencing runs were merged into one fastq file, as were the reverse reads. The merged files were uploaded to CSI phylogeny 1.1 and analysed again using default settings.

Results

A phylogenetic tree including all the patient and environmental isolates of *L. pneumophila* is shown in Figure 1. For each isolate, the SG is noted, including the subgroup for SG 1 isolates, and the sequence type (ST) where it was known.

The isolates were genetically diverse and represent a variety of SGs and STs. P3 showed a close relationship to W8, which was isolated from tap water in one of the bathrooms used by the patient during hospitalization. P3 and W8 were clustered together with P7 and P12. All these isolates were SG 1, subgroup Oxford/Olda and ST 1.

P7 was from a patient with suspected nosocomial Legionnaires' disease, although this was epidemiologically unrelated to P3. *L. pneumophila* was found in some of the water samples from the ward on which the patient (P7) stayed (W3, W4 and W6), but all were SG 10. Nosocomial infection could not be confirmed, and the phylogenetic analysis confirmed that W3, W4 and W6 were unrelated to P7 (Figure 1). P12 was from a patient who was admitted to hospital one year before the case (P3), and nosocomial infection was not suspected. P7 and P12 were isolated three years apart from patients admitted to different hospital departments.

Figure 2 shows a close-up of the cluster containing P3 in Figure 1; P3, P7, P12 and W8. P4 is also included to create some perspective. The distance matrix shows the number of single nucleotide variation (SNV) differences in pairwise comparison. P3 and W8 are very closely related with only two SNV differences. This, in combination with the exposure of the patient to water from the source of W8 and an incubation period suggesting acquisition during hospitalization, strongly suggests nosocomial infection from tap water in this room. The table within Figure 2 also illustrates that although P3, P7 and P12 are all SG 1, Oxford/Olda and ST 1, they are not identical, but closely related.

Discussion

Suspicion of nosocomial Legionnaires' disease should be assessed promptly to identify the source of infection and prevent further transmission. Several typing methods have been used to allow comparison of isolates from patients and possible sources of infection. At the present time, SBT is the gold-standard method for typing of *L. pneumophila*, although an extended MLST scheme with 50 genes has recently been proposed to be more discriminative and yield better epidemiological concordance [4]. For many bacterial species, data from