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Short report

Legionellosis acquired through a dental unit: a case study

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SUMMARY

In 2012, an elderly immunocompromised man died from legionellosis at a hospital in Uppsala, Sweden. The patient had visited a dental ward at the hospital during the incubation period. *Legionella* spp. at a concentration of 2000 colony-forming units/L were isolated from the cupfiller outlet providing water for oral rinsing. Isolates from the patient and the dental unit were *Legionella pneumophila* serogroup 1, subgroup Knoxville and ST9. Pulsed-field gel electrophoresis and whole-genome sequencing strongly suggested that the isolates were of common origin. This report presents one of few documented cases of legionellosis acquired through a dental unit.

Introduction

Legionellosis is a potentially fatal respiratory disease. Dental waterlines have been shown to be one of many possible sources of legionella infection. However, a direct link between the dental unit and patients is rarely shown.[1,2]

In December 2012, an elderly immunocompromised man was diagnosed with legionellosis at Uppsala University Hospital (UUH) in Uppsala, Sweden, and subsequently died. Epidemiological investigation showed that, during his hospital admission the patient had visited a dental ward at the hospital during the incubation period (i.e. nine days before symptoms were observed). The visit had been a routine dental check-up. The infection control unit took several water samples from the ward the patient had been admitted to and from the dental ward. The objective was to identify and stop the route of transmission by establishing a link between the patient and possible sources of *Legionella* spp. in order to take the correct measures. The purpose was also to investigate whole-genome sequencing (WGS) as a tool, and to compare it with gold standard typing techniques in contact tracing investigations.

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Methods

Clinical diagnosis

The patient was diagnosed with urine antigen (Binax NOW Legionella Urinary Antigen Test, Alere, Galway, Ireland) and by bronchoscopy with subsequent analysis of sputum by real-time polymerase chain reaction (PCR) based on *rnpB* gene,[3] and cultivation on buffered charcoal yeast extract medium with L-cysteine (BCYE, bioMérieux, Marcy l'Etoile, France). All samples were analysed at the clinical laboratory at UUH.

Environmental sampling and culture conditions

Two potential sources of infection were investigated by the infection control staff at the Department of Clinical Microbiology and Infection Control at UUH. Water samples (500 mL) were collected without preflushing the taps, and were analysed for *Legionella* spp. according to SS-EN ISO 11731-2:2008. Samples were collected from the shower and from taps for drinking and handwashing in the room that the patient had occupied prior to infection (N=5). All showers on this ward had point-of-use filters, and samples were taken before and after removal of the filters. Samples collected from the dental ward were taken from the cupfiller outlet on the spittoon providing water for oral rinsing, and from regular taps used for drinking and handwashing (N=39). All samples were analysed at the clinical laboratory at UUH.

The bacterial isolate from the patient and one of the isolates from the cupfiller outlet used for rinsing at the dental ward were sent to the Public Health Agency of Sweden where the isolates were cultivated on BCYE (bioMérieux) at 36°C for two days before typing.

Serotyping and immunological subgrouping

Serotyping of the isolates was performed by a latex agglutination test (Dryspot, Oxoid, Basingstoke, UK). Immunological subgrouping was performed using the Dresden panel of monoclonal antibodies.[4]

Epidemiological typing using sequence-based typing and pulsed-field gel electrophoresis

The genetic profile of the isolate from the patient (enumerated as SBT220) was compared with the environmental isolate from the dental unit (SBT219) using the sequence-based typing (SBT) scheme for *Legionella pneumophila* developed by the European Working Group for Legionella Infections.[5] For each isolate, a distinct allelic profile and a sequence type (ST) was assigned through the European SBT database.[5]

The isolate from the patient and the isolate from the dental unit were analysed by pulsed-field gel electrophoresis (PFGE) at the clinical laboratory at UUH using a method described previously.[6]

Analysis by whole-genome sequencing

The single isolates were also analysed using WGS. Barcoded libraries were prepared for the Ion Torrent 400 (Thermo Fisher Scientific, Waltham, USA) base pair chemistry using Library Builder (Thermo Fisher Scientific, Waltham, USA). The resulting libraries were pooled and size selected using Pippin Prep (Saga Science, Beverly, USA). The libraries were sequenced using Ion Torrent PGM (Thermo Fisher Scientific, Waltham, USA). All data analyses were performed using CLC Genomics Workbench v8.0 (QIAGEN, Aarhus, Denmark). Draft genomes were created using de-novo assembly. Each set of raw sequence reads was mapped back to the corresponding draft genome, and genome quality was evaluated using total size, contig count, N50 contig length, and depth and uniformity of coverage. Each set of raw reads was mapped to each other's draft genome and variants were called (frequency >90%, coverage >10x). The number of SNPs called was used as a measure of genetic distance between each pair of samples. Recombination events were filtered by clustering SNPs within 500 bp from each other together as a single event. Large-scale insertions and deletions were analysed by inspecting the mappings in CLC Genomics Workbench v8.0 (QIAGEN).

In the WGS analysis, three other previously typed (SBT) genomes within ST42 were analysed in the same run as controls; two samples with common origin (clinical isolate enumerated SBT115 and environmental isolate SBT116) and one unrelated sample with the same sequence type as the other two samples (SBT113) (Table I).

Table I
Legionella spp. isolates included in the whole-genome sequencing analysis. Isolates enumerated SBT219 and SBT220 originated from the patient and the dental unit in the present case, isolates SBT115 and SBT116 were included as reference isolates sharing an epidemiological link, and SBT113 was an external reference isolate

Sample number	Source	ST	Case
SBT113	Reference	42	Unrelated
SBT115	Clinical isolate	42	Control case
SBT116	Environmental isolate	42	Control case
SBT219	Clinical isolate	9	Dental case
SBT220	Environmental isolate	9	Dental case

Results

Clinical diagnosis

The patient was diagnosed with pulmonary legionellosis by a positive urine antigen test, and by PCR and cultivation of sputum acquired from bronchoscopy.

Contact tracing and environmental sampling

Water samples from the taps in the patient's room were positive for *Legionella* spp. Showers with point-of-use filters were negative distal to filters but positive proximal to filters. Samples proximal to filters contained *L.* non-*pneumophila* at concentrations of 1000–2000 colony-forming units (cfu)/L. The cold water sample from the tap for handwashing was positive for *L.* non-*pneumophila* at a concentration of 150 cfu/L. Six out of 39 samples from the dental ward were positive for *L.* non-*pneumophila* in low numbers (<100 cfu/L). The sample from the cupfiller outlet of the dental unit was positive for *L. pneumophila* serogroup 1 at a concentration of 2000 cfu/L.