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Low genetic diversity of *Legionella pneumophila* serogroup 6 ST728 isolates from nosocomial cases during 20 years

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Background: *Legionella pneumophila* is responsible for Legionnaires' disease; a severe pneumonia with high mortality caused by inhalation of aerosolized *Legionella* bacteria. *Legionella* occurs naturally in aqueous environments and as a contaminant of water systems e.g. in hospitals.

Material/methods: A 72-year old male patient, with a brain lymphoma died due to *L. pneumophila* infection. Epidemiological investigations suggested that the water distribution system (WDS) of the hospital was linked to the case, as previous *L. pneumophila* cases with identical sequence type (ST) had been associated with the hospital. Water samples were collected from taps located at the four departments where the case-patient had been admitted. The concentration (cfu/L) of legionellae was determined and *L. pneumophila* isolates were characterised by serogrouping and sequence based typing. Whole-genome sequencing (WGS) was performed on 16 *L. pneumophila* isolates belonging to serogroup 6 ST728 and included the case-patient isolate, hospital WDS isolates (n=4), and retrospective isolates collected between 1994 and 2010 from nosocomial patients (n=4) and the hospital WDS (n=1) together with epidemiological unrelated ST728 isolates (n=6).

Results: Lung stethoscopy, neutrophilic leukocytes and C-reactive protein (CRP) were normal during the stay in in the Neurosurgical department. Two weeks later the patient was admitted to the department of haematology where a PET-CT revealed pneumonic infiltrations. Five days later the

temperature and CRP increased but lung stethoscopy was normal. Despite antibiotic treatment, temperature and CRP continued to increase during the next week and treatment was changed. The patient was relocated to the intensive care unit where the patient died due to multi-organ failure caused by the Legionella infection diagnosed 48 days after initial admission. The clinical isolate was confirmed as *L. pneumophila* serogroup 6 ST728. Isolates from tap water from three of the four departments and retrospective isolates from patients and WDS were identified as *L. pneumophila* serogroup 6, ST728. Genome sequence comparisons revealed that these isolates clustered together and only differed by two to eight single nucleotide polymorphisms (SNPs) except one isolate from water collected in 1994 which differed by > 150 SNPs probably due to a single recombination event. Isolates from patients collected in 1994, 1995 and 1998 and the case-patient differed by only one to two SNPs while they differed by 14 to 56 SNPs to the epidemiological unrelated ST728 isolates.

Conclusions: The radiologic confirmation of disease and the onset of disease for the case-patient combined with the results of the WGS analysis indicate nosocomial transmission of *L. pneumophila* as a likely source for infection. Presence of only one to two SNPs between patient isolates collected over a twenty-year-period indicates that *L. pneumophila* serogroup 6 ST728 is dormant in the biofilm of the WDS of the hospital.