

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

Katrine Hartung Hansen¹, Søren Uldum², Marc Stegger², Leif Percival Andersen¹

#EV0343

¹ Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark; ² Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark

CASE:

A 72-year old male patient, with a brain lymphoma died due to *Legionella pneumophila* infection 48 days after initial admission (Fig. 1).

- Initially admitted to the neurological dept. due to seizures.
- Relocated to the neurosurgical dept.: Normal lung stethoscopy, neutrophilic leukocytes and C-reactive protein (CRP).
- Diagnosed with brain lymphoma.
- Transferred to the dept. of haematology: A PET-CT revealed pneumonic infiltrations.
- Five days later the temperature and CRP increased, normal lung stethoscopy.
- Despite antibiotic treatment temperature and CRP continued to increase the following week.
- Relocation to the intensive care unit. Respiratory sample was tested positive for *L. pneumophila* by PCR.
- The following day the patient died due to multi-organ failure caused by the Legionella infection.

MATERIALS AND METHODS:

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.

COLLECTION OF WATER SAMPLES

- From taps located at the four departments (Fig.1) where the case-patient had been admitted.
- Determination of the concentration (CFU/L) of legionellae.

CHARACTERISATION OF *L. PNEUMOPHILA*

- Serogrouping
- Sequence based typing

WHOLE-GENOME SEQUENCING (WGS)

Performed on 16 *L. pneumophila* isolates belonging to serogroup 6 (ST728).

- The case-patient isolate
- Hospital WDS isolates (n=4)
- Retrospective isolates (1994 - 2010) from nosocomial patients (n=4) and hospital WDS (n=1)
- Epidemiological unrelated isolates (n=6)

BACKGROUND:

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

RESULTS:

CHARACTERISATION OF *L. PNEUMOPHILA*

- The clinical isolate, isolates from tap water from three of the four departments (Fig. 1, dept. 2,3 and 4) and the retrospective isolates from patients and WDS were all identified as *L. pneumophila* serogroup 6 (ST728).

WGS

Genome sequence comparisons revealed that:

- Isolates from the case-patient, hospital WDS and retrospective isolates clustered together (Fig. 2).
- These isolates 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.

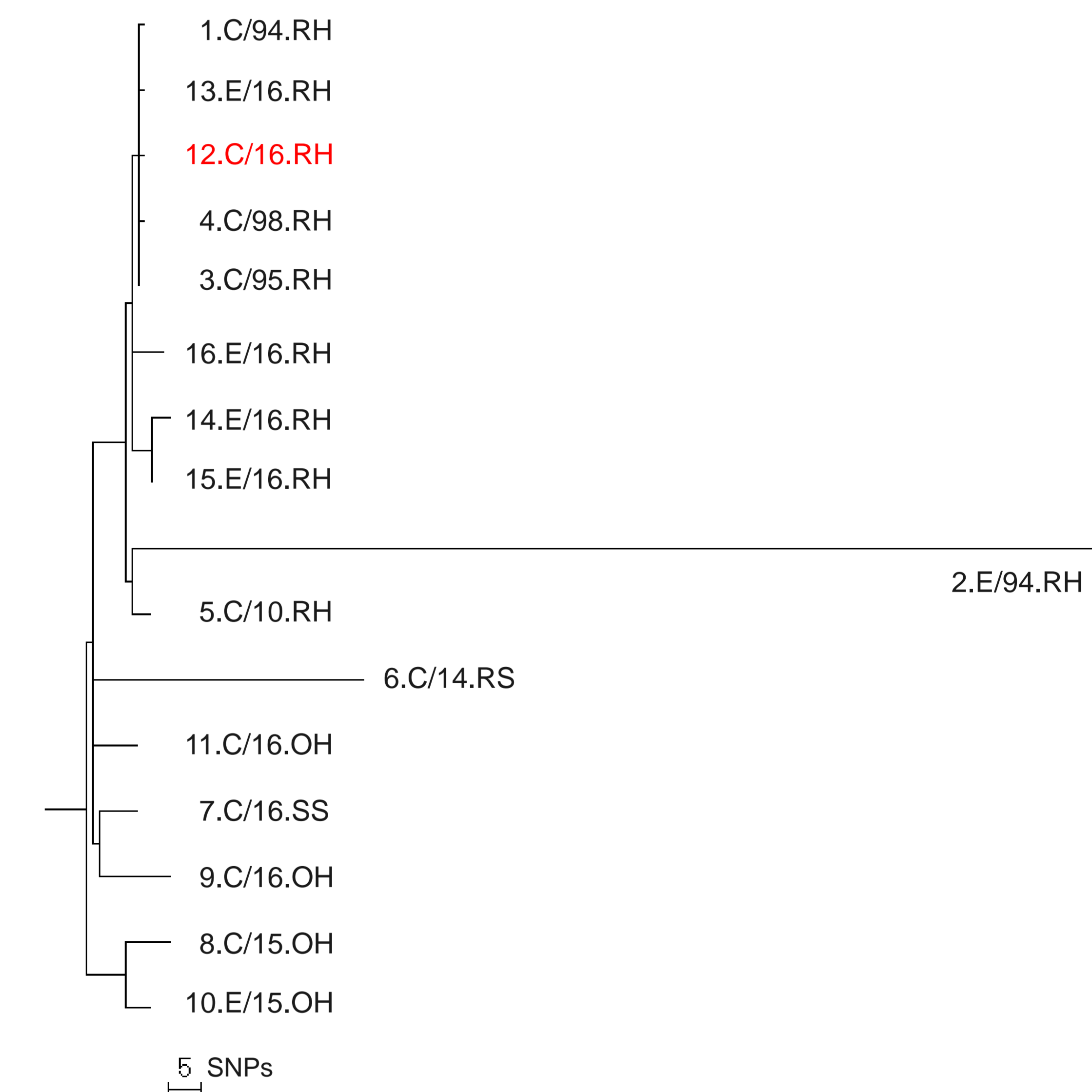


Figure 2. Phylogenetic tree of *L. pneumophila* serogroup 6 ST728 isolates.

A scaffold genome of the epidemiological unrelated isolate 6.C/14.RS was used as a reference. SNP analysis based on 92% of the core genome. Isolates are labelled with the isolate number, origin, isolation year and hospital name and the isolate from the case-patient is coloured in red. C, clinical isolate; E, environmental isolate.

CONCLUSIONS:

Nosocomial transmission of *L. pneumophila* is a likely source for infection due to the combination of:

- the radiologic confirmation of disease
- the onset of disease for the case-patient
- the results of the WGS analysis

Transmission most likely happened through tap water at the neurosurgical dept. (Fig. 1, dept. 2).

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.

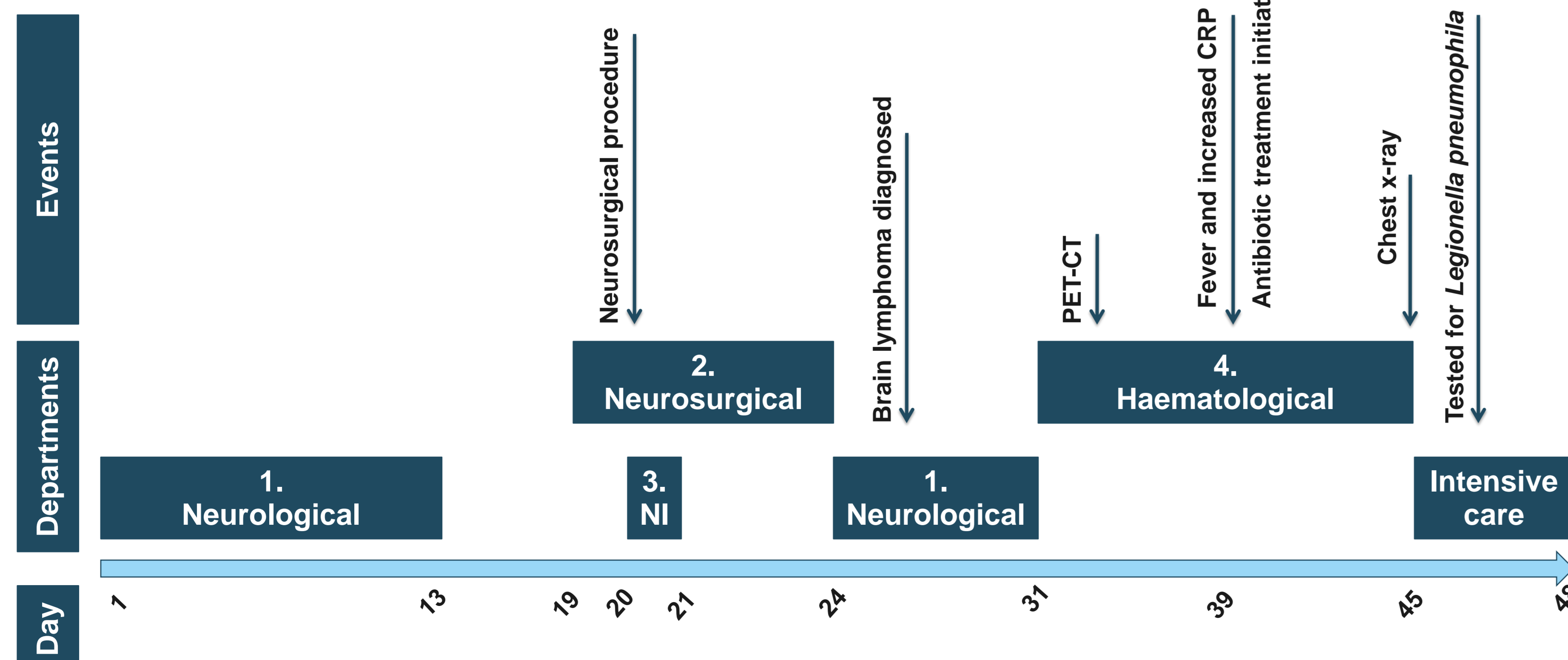


Figure 1. Overview of the patient case. NI, neurointensive care.