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# Probable reinfection with *Legionella pneumophila* – A case report

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ABSTRACT

In Germany community-acquired Legionnaires' disease is usually caused by the species *Legionella pneumophila*. Recurrent cases of Legionnaires' disease are rarely reported and are due either to a second infection (reinfection) or a relapse of a previous case. We report a case of recurrent Legionnaires' disease in an 86-year-old female patient infected with *Legionella pneumophila* serogroup 1, monoclonal antibody-subtype Knoxville, sequence type unknown. Between the two disease incidents the patient had completely recovered. *Legionella pneumophila* was detected with the monoclonal antibody-subtype Knoxville, sequence type 182, in the drinking water of the patient's apartment. Exposure to contaminated drinking water was interrupted after the first incident exposure through the application of point-of-use water filters. The filters were later removed due to low water pressure, and the second illness occurred thereafter. It is unclear if immunological predisposition has contributed to this case of probable reinfection of Legionnaires' disease. Clinical, microbiological and epidemiological information combined suggest this is a case of reinfection of Legionnaires' disease. In cases of recurrent Legionnaires' disease complete collection of patient and water samples is necessary to differentiate relapse from reinfection cases, to implicate the source of infection and to gain more evidence for the role of immunological predisposition.

## 1. Introduction

*Legionella*, particularly the species *L. pneumophila (Lpn)*, can infect and sicken humans after inhalation or micro-aspiration of contaminated (aerosolized) water droplets. The disease generally manifests as pneumonia and is called Legionnaires' Disease (LD). Person-to-person transmission does not occur except in rare circumstances (Correia et al., 2016). *Legionella* bacteria can be found ubiquitously in aquatic environments (Luck and Steinert, 2006). Patients who experienced LD more than once in their lifetime are rarely reported (Dowling et al., 1983; Ko et al., 1996; Leverstein-van Hall et al., 1994; Morley et al., 1994; Sanders et al., 1980; Skrobot et al., 1986). We describe a case with recurrent LD that took place within the period of less than one year.

## 2. Case presentation

On 30 October 2016, we received the report of a case of LD in an 86-year-old female patient living in Berlin, Germany (Fig. 1). The disease began on 16 October 2016 and the patient was admitted to a hospital on 24 October 2016. Pneumonia was confirmed clinically by chest X-ray showing consolidation in the right upper lobe. According to German guidelines for the treatment of adult patients with community-acquired pneumonia (Ewig et al., 2016), *Legionella* infection was included in the differential diagnosis and was laboratory confirmed by urinary antigen test

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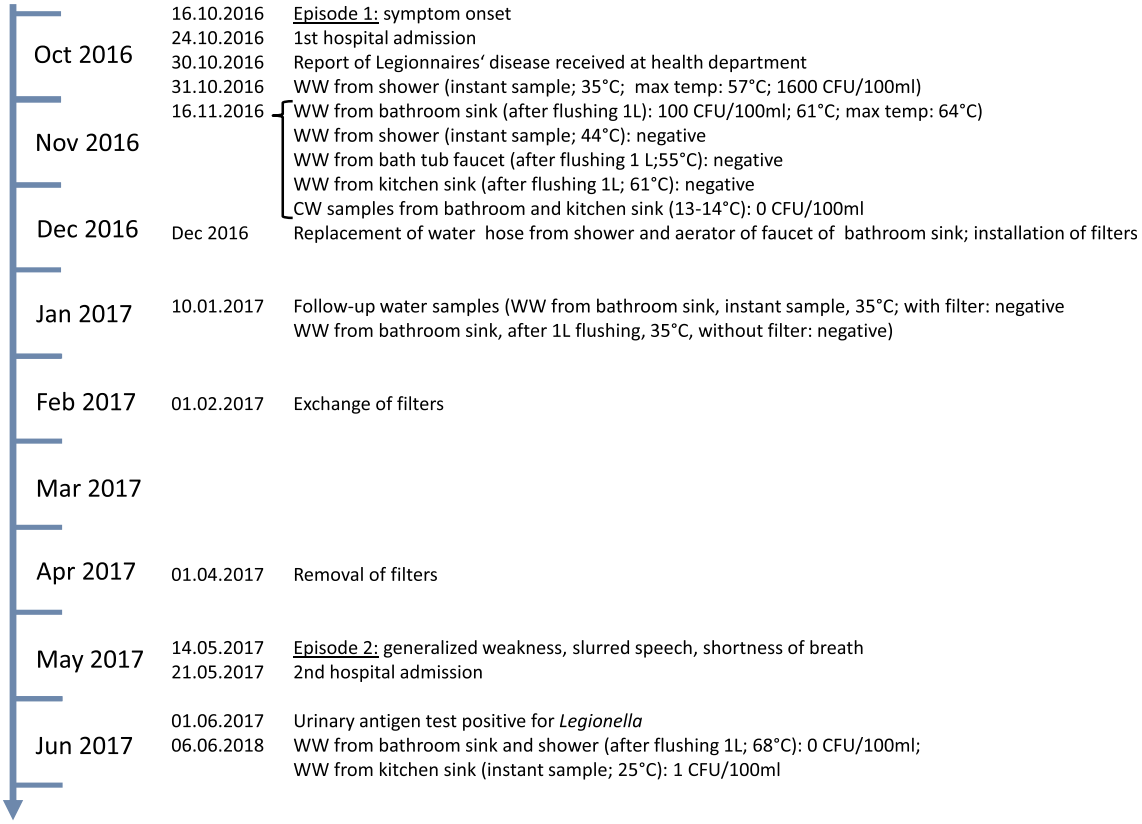


**Fig. 1.** Timeline of events and environmental results of case of reinfection of Legionnaires' disease; Berlin, Germany, 2016/2017. WW = warm water, CW = cold water, CFU = colony forming units.

("Sofia") (Quidel Website, 2017). Monoclonal antibody (mAb) typing was not done. No respiratory sample was taken, thus no sequence type is known. After 2 weeks' treatment with clarithromycin the patient's status had "significantly improved", a control chest X-ray was not done. Before the diagnosis of LD the patient was known to have suffered from diabetes, renal impairment, arterial hypertension and congestive heart failure. She did not take immunosuppressive medication. The patient mostly used a wheelchair to move around because of incomplete paraplegia due to lumbar spinal canal stenosis. There was neither a history of travel nor a hospital stay before disease onset. We inspected the patient's home and took water samples during the same appointment. A flow heater in the bathroom provided the apartment with warm water. On 31 October 2016 an instant sample of water was taken after opening the shower and yielded 1600 *Legionella* colony forming units (CFU) / 100 ml (tested according DIN EN ISO 11731-2) (International Organization for Standardization, 2008). The temperature of the water sample was 35 °C, the maximum water temperature at the same outlet was 57 °C. On 16 November 2016 a warm water sample taken from the faucet of the bathroom sink (after flushing 1 L) yielded 100 CFU / 100 ml (sample temperature 61 °C, maximum temperature 64 °C). On that occasion no *Legionella* was detectable in the warm water sample from the shower. Cold water samples from the bathroom and kitchen sink (temperature between 13 °C and 15 °C) were negative for *Legionella spp*. by culture. The isolates of the water samples were discarded without further subtyping. To prevent further exposure to *Legionella* the temperature was set to high values and the water hose of the shower as well as the aerator of the faucet of the bathroom sink were replaced. In addition filters were installed on the faucet and the shower of the patient's bathroom. During the follow-up inspection on 10 January 2017, control warm water samples from the bathroom sink both with and without filter attached were negative for *Legionella spp*. On 01 February 2017 filters were exchanged. Because the patient complained about low water flow from the faucet and shower, these filters were removed upon her request on 01 April 2017. Her daughter stated that her mother's condition had begun to deteriorate in April, and she had become weaker. Definitively, her general health began to decline on 14 May 2017 and continued to worsen. She began to suffer from generalized weakness, fatigue, slurred speech and shortness of breath. She had some dry cough. Within days her blood pressure also dropped, and systolic values ranged between 76 and 91. On 21 May 2017, the patient was admitted to hospital. A chest X-ray showed an infiltrate in the right upper lung with no indication of abscess or cavitation. A microbiological consultant considered the latest illness as a new infection rather than the persistence of a previous one. While a first urinary antigen test ("Sofia") (Quidel Website, 2017) conducted by the hospital's laboratory on 21 May 2017 was negative, a second test on 01 June 2017 confirmed the *Legionella* infection. Further subtyping showed that the urinary antigen was reactive with monoclonal antibodies confirming an infection with *Lpn* serogroup 1 (SG1), subtype Knoxville (Helbig et al., 2012). Only serum was available to test for immunological deficiencies which was tested for antibodies against IL-6, but returned negative. Other blood samples, such as EDTA- or heparinized blood, were not available. Due to progressive renal failure and septicemia only palliative care was initiated. The patient died on 09 June 2017.

At the time the health department was notified of her second episode she could no longer be interviewed due to her worsened health status. We therefore contacted the daughter and visited her on 06 June 2017 in the household of her mother. The daughter had frequent contact with the case-patient and was thus asked to give information about possible exposures. We used a detailed questionnaire developed for a research study of sporadic, community-acquired LD cases (the LeTriWa study[1]), which

[1] The LeTriWa study is a collaborative research project of (1) the Robert Koch Institute, Berlin, Germany, (2) the German Environment Agency, Bad Elster, Germany and (3) the Institute for Medical Microbiology and Hygiene, Medical Faculty "Carl Gustav Carus", Technical University, Dresden, Germany, and funded by the German Federal Ministry of Health. One subproject attempts to

has been in use since December 2016. In the 2 weeks before symptom onset of the second episode, the patient was almost always at home and had left her house only three times, every time accompanied by her daughter: twice to go for a stroll, and once to go shopping at a nearby supermarket. She neither traveled, nor did she stay in a hospital or health care facility. As potential sources of infection we identified the faucet and shower in the bathroom as well as the faucet in the kitchen. The case-patient did not handle the soil in her flowerpots nor did she water the flowers. We could not identify any other likely sources of infection outside of her household. We took swabs and warm and cold water samples from water sources in the bathroom and kitchen. At that time, the maximum water temperature of the sink and shower in the bathroom was very high (68 °C). During the period when the case-patient was infected, the temperature was set at a "lower value" (but was unmeasured). Overall, 12 samples were taken. All of these were negative for *Legionella spp.* except one warm water sample from the faucet in the kitchen (25 °C) that yielded 1 CFU / 100 ml *Legionella*. The strain was identified as belonging to the mAb-subtype Knoxville, thus matching the patient's mAb-subtype. The sequence type was 182 which is a common sequence type in the region (Lück, 2011).

## 3. Discussion

We describe a case of LD with two discrete illness incidents separated by a time interval of seven months. In general recurrent illness of LD may occur because a previous infection flares up again (relapse) (Dowling et al., 1983; Morley et al., 1994; Sanders et al., 1980) or the patient experiences a second, independent infection (reinfection) (Ko et al., 1996; Leverstein-van Hall et al., 1994; Skrobot et al., 1986). Deciding whether a patient had a relapse or reinfection is often not straightforward. Relapse usually develops within 1 week after completion of therapy (Leverstein-van Hall et al., 1994), but may arise up to 4 months later (Morley et al., 1994). Predisposing factors are severe immunosuppression (Dowling et al., 1983; Morley et al., 1994; Sanders et al., 1980) and relapse has been attributed to monotherapy with erythromycin (Leverstein-van Hall et al., 1994). In contrast, reinfections can occur at any time after the first disease episode. Another aspect is the clinical presentation. In relapse, one would expect a rather uninterrupted course of illness, and an infiltrate at the same location (perhaps with signs of abscess formation or cavitation) (Gump et al., 1979), perhaps increasing after a period of improvement. In reinfection one would instead expect two separate episodes of infection interrupted by a period of complete recovery. In addition, infiltrates at different sites of the lungs would favor reinfection. Molecular genetic methods may be helpful, too. For example, identification of different sequence types in the patient would suggest reinfection, while the identification of the same sequence type in both infections warrants individual risk assessment. In this case no such information was available from the patient's strain. Nevertheless, while microbiological details may aid in differentiating relapse from reinfection it is often the clinical and/or epidemiological details that provide crucial pieces of information. For example, in a published case of probable reinfection a 50-year-old immunocompromised man was diagnosed with LD confirmed by culturing *Lpn* SG1 from sputum (Leverstein-van Hall et al., 1994). He was homebound for many weeks before the onset of his illness. After treatment with erythromycin for 3 weeks he recovered. *Lpn* SG1 was also cultivated from water samples from his apartment. Plumbing changes were made and the temperature of the warm water was adjusted to > 59 °C. Subsequent cultivation did not yield any *Legionella*. Three months after the first illness history repeated itself: the patient suffered from pneumonia again, having been homebound in the preceding weeks, and cultures from water samples again yielded *Lpn* SG1. Moreover, pulsed field gel electrophoresis of the isolates from both illness incidents as well as from the water samples taken after both illness incidents yielded restriction patterns that were indistinguishable or had only one band difference. Also in the case described in this report, circumstantial evidence suggests that the patient was infected two times independently from each other, and there are a number of parallels to the case reported by Leverstein-van Hall (Leverstein-van Hall et al., 1994). After the first pneumonic illness and appropriate antibiotic therapy she recovered. Similar to the case described by Leverstein-van Hall she was homebound, *Legionella spp.* grew in water samples from her apartment, and the exposure to *Legionella* was temporarily stopped, in this case by installation of point-of-use filters. She was likely exposed to *Legionella* again after removal of the filters. The identification of mAb-subtype Knoxville is indicative as this is a subtype of the monoclonal group 3/1 (Dresden mAb-type panel). MAb 3/1 positive strains cause approximately 80–90 % of LD in Germany, whereas it is only found in about 10–15 % of isolates grown from random (case-independent) water samples (Lück, 2011). Although the patient's infiltrates were twice in the upper right lung (indicating a relapse) the lack of an abscess or cavitation on X-ray, the long interval between the two episodes, as well as the full recovery after the first incident suggests a reinfection. The assumption of reinfection is further supported by the fact that the drinking water in the patient's home contained *Legionella* around the first period of illness, was free of *Legionella* in between and, after removal of the filter and after the second illness of the patient, contained a *Legionella* strain that is known to be infectious to humans (mAb type Knoxville, ST182). The patient's strain was also mAb-type Knoxville.

Although the water temperature at the time of taking water samples after the second episode was high this does not mean that the temperature was that high during the time when the infection occurred. It is possible that the change of temperature to a higher level after the second episode has killed or moved *Legionellae* that were present to a dormant stage (viable but not culturable) which led to a very low *Legionella* concentration. The one positive sample – even with a very low *Legionella* concentration – enabled us to identify a strain that is known to be virulent and matched the patient's strain on mAb-level.

The preventive measures taken after the first episode included replacement of the water hose of the shower, installation of filters and setting the temperature to high levels. In addition, one could have also considered testing or replacing the thermostatic mixer. Should the thermostatic mixer have been contaminated it might have facilitated reexposure of the patient after removal of the filters, particularly if the temperature had also been lowered to a more "*Legionella*-friendly" level.

It is unclear why cases of reinfection with *Legionella* are reported so infrequently. It is possible that a number of reinfections do occur, but are not diagnosed both times. Also, it cannot be ruled out that immunocompetent patients develop a certain immunity which may dampen the course of a second infection or prevent it altogether. Alternatively it is possible that only a small subgroup of patients has a certain, specific type of immune deficiency that predisposes to *Legionella* infections, such as the deficiency of a certain type of toll-like receptors (Bhan et al., 2008; Newton et al., 2007). To uncover and understand the role of these types of immunodeficiency, sporadic cases like this one can be used, for example, in a genetic case-control design (Berrington and Hawn, 2013). Household controls would be ideal as they would be exposed in a very similar way as were cases. Here, we have been unable to get the necessary patient samples before the death of the patient. To test for toll-like receptors and IL-1-receptor one would need to take heparinized blood. However, only a serum sample was still available, and this tested negative for antibodies against cytokines.

## 4. Conclusion

We describe a fatal case of Legionnaires' disease due to *Lpn* SG1, mAb-subtype Knoxville. Infection likely occurred twice through the

(*footnote continued*)
elucidate the sources of infection of sporadic community-acquired cases of LD as well as risk factors for cases of community-acquired LD associated with drinking water.

patient's household drinking water. If recurrent cases of LD arise in the future as much information as possible should be collected, including water samples and patient samples to elucidate if reinfection or relapse occurred, which source caused the infection and if there are specific immune deficiencies present that may predispose to infection with LD.

## Consent

Written informed consent was obtained from the patient's next of kin for publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

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## Declaration of interest

None.

## Ethical declaration

Ethics approval: Investigations were conducted in the framework of the legal requirement to identify the source of infection in cases of Legionnaires' disease (Infection Protection Act https://www.rki.de/DE/Content/Infekt/IfSG/Belehrungsbogen/belehrungsbogen_lebensmittel_englisch.pdf?_blob=publicationFile). After the second episode additional investigations were conducted within the "LeTriWa study" where ethics approval was granted from the Ethics Committee of the Charité University Hospital, registered under EA1/303/15.

## CRediT authorship contribution statement

**Udo Buchholz:** Methodology, Conceptualization, Funding acquisition, Investigation, Formal analysis, Supervision, Writing – original draft. **Franziska Reber:** Investigation, Project administration, Supervision, Writing – review & editing. **Ann-Sophie Lehfeld:** Formal analysis, Validation, Data curation, Writing – review & editing. **Bonita Brodhun:** Conceptualization, Methodology, Writing – review & editing. **Walter Haas:** Methodology, Conceptualization, Resources, Funding acquisition, Supervision, Writing – review & editing. **Benedikt Schaefer:** Methodology, Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Fabian Stemmler:** Investigation, Project administration, Supervision, Writing – review & editing. **Christina Otto:** Project administration, Resources, Supervision, Writing – review & editing. **Corinna Gagell:** Investigation, Project administration, Supervision, Validation, Writing – review & editing. **Christian Lück:** Methodology, Conceptualization, Resources, Funding acquisition, Investigation, Supervision, Validation, Writing – review & editing. **Ronny Gamradt:** Investigation, Resources, Writing – review & editing. **Maxi Heinig:** Investigation, Resources, Writing – review & editing. **Christian Meisel:** Investigation, Resources, Writing – review & editing. **Uwe Kölsch:** Investigation, Resources, Writing – review & editing. **Martin Eisenblätter:** Investigation, Resources, Writing – review & editing. **Heiko J. Jahn:** Methodology, Investigation, Project administration, Supervision, Writing – review & editing.

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## List of abbreviations

| | |
|---|---|
| CFU | colony forming units |
| IL-6 | interleukin 6 |
| LD | Legionnaires' Disease |
| LeTriWa | Legionellen im Trinkwasser (*Legionella* in drinking water) (acronym for the LeTriWa project) |
| *Lpn* | *Legionella pneumophila* |
| mAb | monoclonal antibody |
| SG | serogroup |

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