Dual infections with different *Legionella* strains

G. Wewalka[1], D. Schmid[1], T. G. Harrison[2], S. A. Uldum[3] and C. Lück[4] on behalf of the European Society of Clinical Microbiology Infectious Diseases Study Group for Legionella Infections (ESGLI)

1) *AGES, Institute for Medical Microbiology and Hygiene, Vienna, Austria,* 2) *Microbiology Service Division, HPA, London, UK,* 3) *Serum Statens Institute, Copenhagen, Denmark and* 4) *Institute of Medical Microbiology and Hygiene, Dresden, Germany*

Abstract

In 2010 a case of a dual infection with *Legionella pneumophila* serogroup (sg) 1 and sg 3 was identified by culture of a blood sample collected from a female Austrian patient with septic pneumonia. Subsequently all 35 European National Legionella Reference Laboratories were interviewed regarding the frequency of dual infections in legionellosis. The Reference Laboratories in Denmark, the UK and Germany reported the detection of another 14 cases of dual infections with different *Legionella* strains between 2002 and 2012. Among the 15 cases, there were four cases with different *Legionella* species, six cases with different *L. pneumophila* serogroups, and five cases of dual infections with *L. pneumophila* sg 1 with different MAb-types. The median age of the 15 cases was 56 years and the male to female ratio 1:1.14. Six of the 15 patients were receiving immunosuppressive treatment following organ transplantation (n = 3) or for underlying haematological and solid malignancies (n = 3). Five of the 15 cases died within 30 days following diagnosis. Efforts to detect dual infections with different *Legionella* strains will improve our ability to correctly elucidate the causative sources of infection and enhance our understanding of the epidemiology of Legionella infections.

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Corresponding author: G. Wewalka, Austrian National Reference Centre for Legionella Infections, Austrian Agency for Health and Food Safety, Institute for Medical Microbiology and Hygiene, Währinger Straße 25a, 1096 Vienna, Austria
E-mal: guenther.wewalka@ages.at

Introduction

Legionnaires' disease (LD) is characterized by pneumonia caused by *Legionella* spp. These intracellular bacteria, which live in water and other moist environments, multiply in amoebae. According to a German study [1], almost 4% of all cases of community-acquired pneumonia are caused by *Legionella* spp. It is estimated that about 20 000 cases of LD may occur in Germany every year but only about 500 cases are annually diagnosed and reported [2]. Apart from under-reporting, under-diagnosing of this disease also contributes to the underestimation of the true number of cases. From 2007–2010, 81.0 to 81.9% of the 23 834 cases of LD reported in Europe to the European Working Group for Legionella Infection (EWGLI) and from 2009 on to the European Legionnaires' Disease Surveillance Network (ELDSNet) were diagnosed by urinary antigen detection [3–5] .

According to a review article from the Lancet, multiple-strain infections have been shown in more than 50 human pathogens [6]. The real frequency of multiple-strain infections might be underestimated in many cases because of technical limitations of detection. For LD it is not possible to identify cases of dual infections with different strains using only urinary antigen detection tests. Usually isolation of *Legionella* by culture is required to identify cases with more than one *Legionella* strain. A combination of culture and PCR/urinary antigen detection can reveal information on a dual infection with different *Legionella* species (e.g. case 15). Among the European cases notified in 2007 to 2010 only 8.8 to 10.3% were diagnosed by culture. In cases confirmed by culture only one single strain of *Legionella* is generally identified [3–5].

We report on an Austrian case of an infection caused by two different *Legionella* strains. Recognition of this case raised the question of how common are infections with more than one *Legionella* strain.

Methods

The question of whether to ascertain information on further infections due to two (or more) different *Legionella* strains in Europe was put to the 35 European National Legionella Reference Laboratories, through the European Working Group for Legionella Infections (EWGLI), renamed as the ESCMID Study Group for Legionella Infections (ESGLI) in September 2012. These enquiries, to which all the Reference Laboratories responded, revealed 14 further cases. Information on such cases was provided by laboratories in Denmark (six cases), Germany (four cases) the UK (four cases). Data for the cases with dual *Legionella* infections on age, sex, underlying disease, categorized as severe (including status after organ transplantation and treatment for malignant disease) or moderate (including acute and chronic respiratory disease, chronic heart disease and status after surgical intervention), category of exposure (nosocomial, travel associated or community acquired), causative *Legionella* strains, source of infection and outcome were collected and analysed. The isolates of *L. pneumophila* sg 1 were serotyped by use of the monoclonal antibody panel (MAb-types) described by Helbig *et al.* [7]. All isolates were genotyped by sequence-based typing (SBT) of seven genes according to Gaia *et al.* [8] and Ratzow *et al.* [9]. Species of *Legionella* other than *pneumophila* were confirmed by mip gene sequencing [10] or MALDI-TOF (matrix-assisted-laser-desorption-deionization mass spectrometry) [11]. We also performed a review of the literature of the past 10 years focusing on dual infections with different *Legionella* strains in terms of multiple *Legionella* strain infection.

Results

Case series

Case 1 from Austria, 2010: On 24 July a 70-year-old female was admitted to hospital C with signs and symptoms of pneumonia. The patient was diagnosed with Legionnaires' disease (LD) by a positive Legionella urinary antigen test on 25 July. The patient died on 26 July. The underlying disease, a bronchial carcinoma, had been known since 2009. The patient received 11 cycles of intravenous cytostatic therapy, followed by oral administration, but had developed brain metastasis and also suffered from moderate stage chronic obstructive pulmonary disease. The onset of LD was on 22 July. On day 1 and day 2 prior to LD onset (20–21 July), the patient was in hospital B for a γ-knife intervention; on day 2 to day 6 prior to onset (16–20 July), the patient stayed at home; and on day 6 to day 16 (6–16 July) the patient was in hospital A. In order to locate the source of infection, efforts were made to gain a post-mortem isolate. An autopsy was denied for religious reasons and bronchial secretion taken prior to the patient's death was not available. Blood cultures obtained pre-mortem on 25 July, which had given negative results by routine culture methods, were still available and sent to the Austrian National Reference Laboratory for Legionella Infections. Volumes of 0.1 and 0.5 mL from the bottles (Becton Dickinson, Heidelberg, Germany) were directly plated onto glycin-polymyxin B-vancomycin-cycloheximide (GVPC) agar and buffered charcoal yeast extract (BCYE) agar. *Legionella pneumophila* serogroup (sg) 1 and sg 3 were detected by latex agglutination of several single colonies followed by tests with monoclonal antibodies (MAb). Based on the colony counts of the plates resulting in 10 colony-forming units per mL blood culture fluid and considering a dilution of 1:4, a concentration of about 40 *Legionella* cells per mL blood was estimated.

Water samples were taken from locations to which the patient had been exposed within the 10 days prior to disease onset and were examined for *Legionella* according to ISO 11731-2. Table 1 shows the water sampling and the results of the water investigation. *Legionella* was detected in low concentrations in the water system of hospital A, hospital B and the patient's home. MAb-typing and genotyping by SBT (Tables 1 and 3) proved that the patient acquired both the strain of *L. pneumophila* sg 1 and sg 3 from the water system of hospital A.

Case 2 from Denmark, 2002: a 47-year-old male who had a kidney transplant, had onset of disease approximately 1 month

TABLE 1. Results of environmental sampling related to the Austrian case of dual infection with different *Legionella* strains (case 1)

Date of sampling	Sites of sampling	*Legionella* strain isolated	Concentration
29/07	Hospital A, patient room, faucet (mixed water)	*L. pneumophila* sg 1, MAb-type Bellingham, ST 81	1 CFU/100 mL
29/07	Hospital A, patient room, shower (mixed water)	*L. pneumophila* sg 3, ST 93	7 CFU/100 mL
29/07	Hospital B, patient room, shower (mixed water)	*L. pneumophila* sg 1. MAb-type OLDA, ST 442	1 CFU/100 mL
04/08	Patient home, bathroom and kitchen (mixed water)	*L. pneumophila* sg 10	1–220 CFU/100 mL

CFU, colony forming units.