ACCESS MICROBIOLOGY

CASE REPORT
Rafiei *et al.*, *Access Microbiology* 2020;2
DOI 10.1099/acmi.0.000113

MICROBIOLOGY SOCIETY

OPEN MICROBIOLOGY

Persistently positive culture of antimicrobial-susceptible *Legionella pneumophila* despite appropriate therapy

Nastaran Rafiei[1,*], Briony Shaw[2], Claire Dendle[1], Stephen Opat[2], Deborah Williamson[3] and Maryza Graham[1,4,5]

Abstract

Legionella pneumophila is a well-known cause of pneumonia that is infrequently cultured in clinical practice. We report a case of an immunocompromised patient with persistently positive *L. pneumophila* cultures from invasive respiratory samples despite prolonged treatment with appropriate antibiotics. *In vitro* testing showed that the isolate remained susceptible to ciprofloxacin and azithromycin.

INTRODUCTION

Legionella spp. are an important cause of pneumonia and are associated with significant morbidity and mortality. Immunocompromised individuals are at risk of both infection and poor outcomes. Diagnostic limitations mean that *Legionella* infection is likely under-recognized; this has important implications for treatment delay and prognosis. As *Legionella* spp. culture is not commonly attempted in clinical practice, antimicrobial susceptibility testing is rarely performed and little is known about the duration and significance of culture positivity.

CASE REPORT

A 60-year-old patient with a history of large B-cell lymphoma was admitted to hospital with fever. Since diagnosis in 2013, the patient had undergone multiple courses of chemotherapy and in 2017 had undergone autologous bone marrow transplantation. At the time of admission in February 2018, treatment consisted of radiation to a sacral lesion (single site relapse) with dexamethasone and idelalisib.

On presentation, the patient was febrile without localizing infective symptoms. White cell count was low (2.0×10^9 l^{-1}) and chest X-ray revealed consolidation of the left upper lobe. Treatment was initiated with piperacillin/tazobactam, but the patient deteriorated and became increasingly neutropenic (nadir $0.04\times10^9l^{-1}$) over the ensuing 5 days. The patient was transferred to the intensive care unit (ICU) and azithromycin was added on day 5. *Legionella pneumophila* DNA was detected on nasopharyngeal swab taken on day 7 [High-Plex, Respiratory Pathogens B 16-well PCR assay (catalogue number 20612, version 02), AusDiagnostics, Beaconsfield NSW, Australia] and urinary legionella antigen (Sofia Legionella fluorescent immunoassay, Quidel, San Diego, CA, USA) was detected on day 8 of admission. The patient initially stabilized and fevers abated within 2 days of commencing azithromycin. Neutrophil recovery occurred on day 9 and following administration of granulocyte colony stimulating factor, but the patient remained lymphopenic for the entirety of the admission and respiratory distress necessitated intubation on day 13.

L. pneumophila serogroup 1 was cultured from bronchial washings on days 13, 17 and 22 of admission. The organism was identified to species level by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with a score of >2.0 (MALDI Biotyper version 3.1, database MBT 7311 MSP library RUO, Bruker Daltonics Germany). Serotyping of the isolate was performed by Oxoid Legionella Latex test (Thermo Fisher Scientific Reference DR0801M).

Ciprofloxacin was substituted for azithromycin on day 15 due to clinical failure. Magnetic resonance imaging (MRI) of the

Received 17 August 2019; Accepted 12 February 2020; Published 02 March 2020
Author affiliations: [1]Monash Infectious Diseases, Monash Health, Clayton, Victoria, Australia; [2]Department of Haematology, Monash Health, Clayton, Victoria, Australia; [3]Microbiological Diagnostic Unit Public Health Laboratory, The Peter Doherty Institute for Infection and Immunity, Australia; [4]Department of Microbiology, Monash Health, Clayton, Victoria, Australia; [5]Faculty of Medicine Nursing and Health Sciences, Monash University, Melbourne, Australia.
***Correspondence:** Nastaran Rafiei, nastaran.rafiei@uqconnect.edu.au
Keywords: *Legionella pneumophila*; culture; immunocompromised host.
Abbreviations: AST, Antimicrobial susceptibility testing; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MIC, minimum inhibitory concentration; MRI, Magnetic resonance imaging; PCR, polymerase chain reaction.

Table 1. Susceptibility testing results of the *L. pneumophila* isolate cultured after patient had received 13 days of azithromycin

	Minimum inhibitory concentration (mg l^{-1})
Azithromycin	0.06
Ceftriaxone	0.5
Ciprofloxacin	0.5
Rifampicin	0.12
Tetracycline	32
Tigecycline	4

*McFarland, E-test (bioMérieux, Inc.) on BCYE agar (ThermoFisher Scientific).

brain showed an acute infarct in the right middle cerebral artery territory. Transesophageal echocardiogram on day 24 showed small echogenicities on the mitral and aortic valves, compatible with vegetations. Rifampicin was therefore added to ciprofloxacin on day 24.

E-test (bioMérieux, Inc., Durham, NC, USA) susceptibility testing at the reference laboratory on day 17 isolate showed that the isolate remained susceptible to azithromycin [minimum inhibitory concentration (MIC) 0.06 mg l^{-1}] according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria, as shown in Table 1. This isolate was grown from bronchoalveolar lavage that was performed after the patient had received 13 days of azithromycin. This specimen also grew *Aspergillus fumigatus*, further indicating the depth of the patient's immunosuppression.

The patient showed no signs of recovery after several days of dual therapy and passed away on day 29 of admission.

DISCUSSION

Legionella spp. are a well-described cause of both community- and hospital-acquired pneumonia [1]. Often referred to as a cause of 'atypical' pneumonia, there are several clinical features (such as hyponatraemia, gastrointestinal symptoms and transaminitis) that are suggestive of legionellosis, but diagnosis cannot be made on clinical findings alone and the presence of lobar consolidation does not exclude the diagnosis [2]. Disease is associated with significant mortality and delay in initiation of appropriate antibiotics is associated with increased mortality [3].

Timely microbiological diagnosis is thus crucial and numerous diagnostic modalities exist. These include serology, urinary legionella antigen assays, polymerase chain reaction (PCR) and culture isolation. All have considerable limitations. Serological diagnosis requires convalescent serology at a minimum of 2 weeks after onset of symptoms but seroconversion is often delayed for months. Most urinary legionella antigen assays only detect *L. pneumophila* serotype 1, which is problematic in Australia, where *Legionella longbeachae* accounts for a significant proportion of disease. Many patients may not be able to produce adequate respiratory samples for PCR or culture techniques. In a study comparing different laboratory techniques, the sensitivity of culture was low at 48% [4].

Culture isolation is also necessary for antimicrobial susceptibility testing (AST), however, due to technical issues and issues of interpretation, AST is not routinely performed. EUCAST has recently published an updated guideline on AST of *L. pneumophila* with the recommendation that testing be used for resistance detection rather than for guidance of treatment [5]. The correlation between *in vitro* results and clinical outcome is uncertain.

To date, there have been few cases of persistent culture positivity of *Legionella* spp. despite appropriate treatment. O'Reilly *et al*. described an immunocompromised patient with an occult pulmonary abscess and persistently positive cultures over a 30-day period despite appropriate therapy [6]. Glaser *et al*. described a 70-year-old immunocompetent patient with pneumonia who had positive cultures over 58 days with no nidus of infection [7]. Both patients were cured of their infections.

There have been few reports of resistance as a cause of clinical failure. A study of 98 consecutive clinical isolates of *L. pneumophila* from France demonstrated low MICs to erythromycin, rifampicin and ciprofloxacin. Despite this, the mortality rate in this patient group was 27%, which suggests that antimicrobial resistance is not the main cause of clinical failure in *Legionella* infection [8]. In 2014, Bruin *et al*. described the first clinical case of a potentially ciprofloxacin-resistant *L. pneumophila* isolate (MIC 2 mg l^{-1}) in a patient who had received ciprofloxacin therapy for 4 days and was cured with sequential therapy with clarithromycin [9].

The poor outcome in our patient can probably be attributed to her greatly immunocompromised state and possibly the delay in initiating appropriate therapy. Both are known to be risk factors for worse outcome [3]. O'Reilly *et al*. suggest searching for a nidus of infection in patients with persistently positive *Legionella* cultures [6]. It is possible although not proven that our patient had endocarditis and may have benefited from combination antibiotics, but there is a paucity of randomized trials to determine optimal treatment. Our case report suggests that further studies are required to determine the relative importance of combination antimicrobial therapy, source control by drainage of collections and immune modulation in the management of severe or refractory *Legionella* infections.

With a move towards molecular diagnostics, we hope that there will be more patients that have reflex cultures performed specifically for isolation of *Legionella*. This will enable sequencing, AST and potentially comparison of different strains, and thereby enrich our understanding of *Legionella* infections. Furthermore, it may help shed light on the clinical and microbiological significance of persistently positive cultures.

Funding information
This work received no specific grant from any funding agency.

Conflicts of interest
The authors declare that there are no conflicts of interest

Ethical statement
The authors have no ethical conflicts to disclose.

References

1. Phin N, Parry-Ford F, Harrison T, Stagg HR, Zhang N *et al*. Epidemiology and clinical management of Legionnaires' disease. *Lancet Infect Dis* 2014;14:1011–1021.
2. Kümpers P, Tiede A, Kirschner P, Girke J, Ganser A *et al*. Legionnaires' disease in immunocompromised patients: a case report of *Legionella longbeachae* pneumonia and review of the literature. *J Med Microbiol* 2008;57:384–387.
3. Heath CH, Grove DI, Looke DF. Delay in appropriate therapy of *Legionella pneumonia* associated with increased mortality. *Eur J Clin Microbiol Infect Dis* 1996;15:286–290.
4. Lindsay DSJ, Abraham WH, Findlay W, Christie P, Johnston F *et al*. Laboratory diagnosis of Legionnaires' disease due to *Legionella pneumophila* serogroup 1: comparison of phenotypic and genotypic methods. *J Med Microbiol* 2004;53:183–187.
5. The European Committee on Antimicrobial Susceptibility Testing. Antimicrobial susceptibility testing of Legionella pneumophila [Internet]. [Accessed 26 July 2018]. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Legionella_guidance_document_20171208.pdf 2018.
6. O'Reilly KMA, Urban MA, Barriero T, Betts RF, Trawick DR. Persistent culture-positive *Legionella* infection in an immunocompromised host. *Clin Infect Dis* 2005;40:e87–e89.
7. Gläser S, Weitzel T, Schiller R, Suttorp N, Lück PC. Persistent culture-positive *Legionella* infection in an immunocompetent adult. *Clin Infect Dis* 2005;41:765–766.
8. Onody C, Matsiota-Bernard P, Nauciel C. Lack of resistance to erythromycin, rifampicin and ciprofloxacin in 98 clinical isolates of *Legionella pneumophila*. *J Antimicrob Chemother* 1997;39:815–816.
9. Bruin JP, Koshkolda T, IJzerman EPF, Lück C, Diederen BMW *et al*. Isolation of ciprofloxacin-resistant *Legionella pneumophila* in a patient with severe pneumonia. *J Antimicrob Chemother* 2014;69:2869–2871.