Legionella sainthelensi pneumonia associated with aquatic traumatic injury in New South Wales, Australia

To the Editor,

Legionella species are important water-associated pathogens of public health interest and are classically associated with pneumonia in humans. In Australia, the most common species isolated from clinical samples are L. pneumophila and L. longbeachae. Legionella sainthelensi was first reported in 1980 from water near Mount St Helens, USA, and is a rarely isolated species which has been associated with severe pulmonary disease, as well as outbreaks.\(^2\)\(^-\)\(^5\) Distribution appears to be broad, with clinical isolates described from Reunion Island, New Zealand, and Canada, and environmental isolates from Portugal, China, USA and the Netherlands.\(^5\)\(^-\)\(^7\)

Here, we report the first described case of L. sainthelensi pneumonia in NSW, Australia, and the challenges in diagnosing infections caused by uncommon Legionella species using routine laboratory methods. Formal informed consent was obtained from the patient.

A 48-year-old woman was admitted to a tertiary trauma referral hospital following a fall onto submerged rocks. She sustained significant injuries including bilateral pneumothoraces, multiple fractures, and soft tissue lacerations, and required intubation on the scene. Her background history was significant for depression and chronic pain only. On day 9 of admission, she developed mixed respiratory failure, with worsening bilateral lung infiltrates on chest X-ray. Routine sputum culture from endotracheal aspirate isolated light growth of Haemophilus influenzae and Acinetobacter baumannii complex. Empirical intravenous piperacillin/tazobactam 4 g/0.5 g Q8H was commenced, but was later switched to cefepime 2 g Q8H to treat a presumed ventilator associated pneumonia. On day 12, a Gram-negative bacillus was isolated from the right upper lobe washings. This was presumptively identified as L. sainthelensi on matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectroscopy (Bruker Daltonik, Germany). No other significant pathogens were isolated.

A bronchoscopy evaluation and lavage was performed on day 14 due to progressive pulmonary disease and antibiotic treatment was escalated to intravenous meropenem 1 g Q8H. As legionella and nocardia culture was specified, the specimen was also inoculated onto buffered charcoal yeast (BCYE) agar, with and without selective antibiotics, and the specimen was also inoculated onto buffered charcoal yeast (BCYE) agar, with and without selective antibiotics. The isolate was referred to the state reference laboratory.

MIC of selected antimicrobial agents against both L. pneumophila ATCC35152 and L. sainthelensi were performed by gradient test strip (ETEST; bioMérieux, France), as per the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidance document on susceptibility testing of L. pneumophila.\(^9\) While no clinical breakpoints exist for L. sainthelensi, the tested MICs were below the tentative highest MIC for wild-type L. pneumophila isolates.

Legionella nucleic acid amplification testing (NAAT) was retrospectively performed on bronchial washing samples using an in-house PCR method on the Lightcycler II (Roche Diagnostics, Switzerland) targeting a genus-conserved 16S target and a L. pneumophila specific target. The genus specific target only was detected; melt-curve showed a similar profile to L. longbeachae (Fig. 1). The samples were also tested on the Atypical Pneumonia PCR 8-well assay (AusDiagnostics, Australia); neither L. pneumophila nor L. longbeachae were detected. Legionella immunofluorescence serology for L. longbeachae serogroup 1 and L. pneumophila serogroup 1 (Euroimmun, Germany) collected on days 45 and 51 were non-reactive. Unfortunately, no urine samples were available for legionella urinary antigen testing.

At the time of isolation of L. sainthelensi, our patient had received a total of 6 days of treatment with erythromycin and given her overall respiratory improvement, she was not prescribed further specific therapy. No other Legionella-active antimicrobials were administered during her admission.

ThermoFisher, USA). No agglutination was observed. The isolate also underwent 16S rRNA U1 and U3 polymerase chain reaction (PCR) and amplicon sequencing.\(^8\) A basic local alignment search tool against the NCBI GenBank database identified Legionella genus, but did not discriminate to species level.

The isolate underwent whole genome sequencing to confirm species level identification. Genomic DNA was extracted with a Qiagen kit, quality checked, and genomic libraries constructed using Nextera XT Library Preparation kit (Illumina, USA). Sequencing was performed on the Illumina NextSeq500 platform. The sequence data were first passed through an in-house quality control procedure, including assessment of read quality and contamination (Trimmomatic version 0.36, FastQC version 0.11.3 and Centrifuge version 1.0.4). The core-genome alignment was produced by Roary version 3.6.1. The trimmed reads were first assembled using SKEAS version 2.3.0, followed by genome annotation using Prokka version 1.13.3. This confirmed the genome identity as L. sainthelensi.

No known mutations which could confer in vitro resistance of bacteria to fluoroquinolones were identified by analysis of the gyrA, gyrB and parC genes in the QRDR regions of the L. sainthelensi genome. There was no evidence of macrolide resistance mutations in the reads of the 23s rRNA (either A2058G or C2611G) nor any mutations in rplD or rplV genes. Mutations in the lpeAB efflux pump promoter region, which have been associated with increased minimum inhibitory concentration (MIC) to macrolides in L. pneumophila, were not present as the L. sainthelensi genome appears to lack this operon.\(^9\)

Legionella sainthelensi pneumonia associated with aquatic traumatic injury in New South Wales, Australia, Print ISSN 0031-3025/Online ISSN 1465-3931 © 2023 Royal College of Pathologists of Australasia. Published by Elsevier B.V. All rights reserved.
Although several cases have been reported in New Zealand, Australia, and despite detailed history and public health investigation, diagnosis is often made later in the disease course. Achieving using MALDI-TOF but due to the slow growth rate, bronchoalveolar lavage. Identification can be accurately performed using MALDI-TOF but due to the slow growth rate, diagnosis is often made later in the disease course.

While the optimum therapy for *L. sainthelensi* pneumonia is unclear, the isolate had a low erythromycin MIC on susceptibility testing. Her remaining ICU stay was complicated by peritonitis and slow respiratory recovery from ARDS. She was discharged home after a prolonged inpatient admission including rehabilitation.

This case demonstrates the difficulty in establishing the diagnosis of uncommon *Legionella* species infection with current diagnostic techniques. Similar to other *Legionella* species, *L. sainthelensi* has a strong association with water. In our patient’s case, we hypothesise that acquisition occurred from water exposure during the initial trauma, although we were unable to definitively establish a link despite detailed history and public health investigation. *Legionella sainthelensi* has been reported only once before in Australia’s Notifiable Diseases Surveillance System, although several cases have been reported in New Zealand. It is possible that *L. sainthelensi* infection is underdiagnosed in Australia and worldwide, as usual techniques are largely biased towards *L. pneumophila* and *L. longbeachae*.

Table 1 Minimum inhibitory concentration (MIC) of selected antimicrobial agents against *Legionella pneumophila* ATCC35152 and *Legionella sainthelensi*, performed by gradient strip

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>L. pneumophila ATCC 35152 MIC (µg/mL)</th>
<th>L. sainthelensi MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>8</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Traditional diagnostic algorithms for *Legionella* pneumonia include urinary antigen testing, culture, convalescent serology, and NAAT. *L. sainthelensi* can be isolated after prolonged culture on selective media such as BCYE, although sensitivity of culture is reduced if the patient has received prior antibiotics. Not all respiratory samples are routinely cultured for *Legionella* species, and often optimal yield requires more invasive respiratory sampling such as bronchoalveolar lavage. Identification can be accurately achieved using MALDI-TOF but due to the slow growth rate, diagnosis is often made later in the disease course.

Historically, *Legionella* serology has been a cornerstone in the diagnosis of *L. pneumophila* and *L. longbeachae* infection as culture has suboptimal sensitivity, but serology is limited by the need of sampling acute and convalescent sera, with the diagnosis only being confirmed retrospectively. Our case demonstrated that *L. sainthelensi* infection did not induce antibodies that cross-react with of *L. pneumophila* and *L. longbeachae* antigens by immunofluorescence. In cases where *Legionella* infection is highly suspected but initial serology is non-reactive, further testing against additional antigens may assist with non-*L. pneumophila*, *L. longbeachae* infections. However, the kinetics of the antibody response to *L. sainthelensi* infection are also not well documented. It is unfortunate that urine samples were not tested for legionella urinary antigen contemporaneously, and that specimens were discarded before such testing could be added on retrospectively. Further studies to investigate the antigen and antibody response in *L. sainthelensi* infection should be considered.

Specific *L. pneumophila* and *L. longbeachae* NAAT targets did not show amplification on either the commercial AusDiagnostics Atypical Pneumonia PCR 8-well assay or the in-house assay. While the pan-*Legionella* target on the in-house PCR demonstrated expected amplification, melt-curve analysis of PCR product was indistinguishable from that of a control *L. longbeachae* strain (Fig. 1). We note melt-curve analysis is a crude method of typing and the probes used in this assay do not provide sufficient sequence heterogeneity to separate non-pneumophila species. 16S RNA sequencing was also unable to identify to species level due to high similarity of amplified sequence of *L. sainthelensi, L. longbeachae* and *L. oakridgensis* species. The genetic homology between *L. longbeachae* and *L. sainthelensi* make differentiation difficult and it is possible that previous cases in Australia have been misidentified as being caused by *L. longbeachae*.

Our case demonstrates the difficulty in diagnosis of *L. sainthelensi* pneumonia. Clinicians should consider other *Legionella* spp. as a potential cause of atypical pneumonia, especially in cases associated with recent water exposure. *Legionella* culture should be performed, ideally prior to antibiotic therapy, as routine molecular and serology methods may miss these infections. It is also important for clinicians to understand the targets included in diagnostic

Fig. 1 Melt curve analysis of in-house *Legionella* spp. polymerase chain reaction assay with fluorescence readout at 705 nm. Peak melt temperature for the patient’s samples were within 1°C of *Legionella longbeachae* control.

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platforms used in their laboratories and liaise closely with microbiologists if considering the diagnosis of uncommon pathogens. This case showed that the inclusion of a pan-Legionella 16S target as opposed to specific L. pneumophila or L. longbeachae targets in molecular platforms may rapidly and sensitively diagnose infections caused by other Legionella species. While L. sainthelensi is uncommonly isolated, it has the potential to cause both severe clinical disease and outbreaks and clinicians should be aware of its pathogenic potential. Further investigations are warranted to define the epidemiology, clinical manifestations, and optimal treatment of L. sainthelensi infection.

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