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Legionella pneumophila serogroup 1 infection associated with the use of an apartment building spa pool

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Abstract

Background

Legionnaires' disease is a notifiable condition in New South Wales (NSW), Australia; clinicians and laboratories are required to report the disease to NSW Health. We describe the investigation of a sporadic case associated with the use of a communal spa pool in the case's apartment building complex and the use of whole genome sequencing to examine relatedness between clinical and environmental *Legionella pneumophila* serogroup 1 (Lp1) strains.

Methods

In February 2018, a confirmed case of Lp1 infection was notified in a man in his 60s hospitalised with pneumonia. We asked the clinical team to obtain sputum in the event we found a potential source. The case described the use of the communal spa pool in his apartment building on two occasions during the putative exposure period. Environmental Health Officers from the Public Health Unit inspected the spa pool and found that the free chlorine level was well below the recommended concentration; a water sample was submitted for microbial analysis.

Results

Lp1 was grown from the case's sputum and microbial analysis of the spa water sample found Lp1 at a concentration of 20 CFU/mL. The human and environmental isolates were subjected to whole genome sequencing and found to be highly genomically related. There was no other plausible environmental source of legionella.

Conclusions

Whole genome sequencing of the clinical and environmental Lp1 isolates implicated a contaminated spa pool as the source of the case's exposure. This strongly supports the application of whole genome sequencing to the investigation of *single* cases of legionellosis. Communal spa pools in apartment buildings are not regulated in most Australian jurisdictions but must be considered to pose a potential legionella risk if improperly maintained.

Keywords: Legionella; legionnaires' disease; regulation; spa pools; whole genome sequencing

Introduction

Legionella species are ubiquitous in the moist natural and built environment. Infection by *Legionella pneumophila* serogroup 1 (Lp1) may lead to Legionnaires' disease, a potentially fatal form of atypical pneumonia associated with multi-organ damage. It generally results from inhalation of contaminated aerosols disseminated from cooling towers, baths, fountains, wastewater plant, humidifiers, ice or mist machines and a number of other sources.¹ A recent review of legionellosis associated with exposure to recreational waters identified public spa pools supplied with reticulated water as causes of legionellosis outbreaks, mostly due to Lp1.²

Whilst the focus of the regulation of air conditioning cooling towers and warm water systems in New South Wales (NSW) under the *Public Health Act 2010 (NSW)* is explicitly to mitigate the risk of legionellosis, the purpose of regulation of public spa pools and other public pools under the Act is to prevent transmission of infections generally. The disinfection and proper maintenance of spa pools is particularly important, as the higher water temperatures used reduces the effectiveness of chlorine and can be conducive to the propagation of legionellae and other pathogens. In addition, when in use, spa pools are likely to generate high volumes of aerosols, which can be easily inhaled by spa pool users or by people in the vicinity. However, spa pools located in residential premises, including apartment buildings, are not regulated in NSW, nor in most other Australian jurisdictions.

Legionnaires' disease is a notifiable condition in NSW under the *Public Health Act 2010*; clinicians are required to report to the local Public Health Unit all suspected cases and clinical laboratories are to notify detection of any *Legionella* species. Notification triggers a public health investigation to determine the source and mechanism of exposure to inform subsequent preventive interventions.

Various methods of genotyping of Lp1 have been employed to characterise isolates recovered from sporadic cases and outbreaks of legionellosis. The demonstration of similarity between bacteria cultured from environmental sources and clinical cases has been the most reliable approach to investigating transmission pathways. Gene sequencing based typing (SBT) methods were previously recommended to type Lp1 isolates in outbreak investigations.³ However, SBT discriminates among strains using only seven alleles within the genome; there are often problems identifying a type, given the high recombination rate of the *L pneumophila* genome. Therefore, the limited resolution of SBT has led to a wider application of whole genome sequencing (WGS) in the investigation of community outbreaks of legionellosis. WGS offers increased discrimination of outbreak isolates, as the whole genome is used in the analysis compared to the seven alleles in SBT.⁴⁻⁶ With WGS, further analysis can be performed such as Single Nucleotide Polymorphisms (SNP) based, core and pan-genome based comparisons of Lp1 isolates which can assist in deciphering and confirming transmission pathways during the investigation of complex outbreaks of legionellosis.⁷

This report describes the investigation of a case of Legionnaires' disease putatively associated with exposure to a spa pool within a residential apartment building complex and the use of WGS to examine relatedness between clinical and environmental Lp1 strains.

Methods

Epidemiological investigation

In February 2018, the Public Health Unit was notified of a man in his 60s hospitalised with a history of high fever, lethargy, cough and shortness of breath, in whom a diagnosis of Legionnaires' disease was confirmed by the detection of urinary Lp1 antigen. He had no specific risk factors for legionellosis apart from his age. The clinical team was asked to arrange collection of sputum samples for legionella

culture in the event that an environmental isolate could also be obtained. Sputum specimens were processed using standard microbiological techniques, and Lp1 was isolated; the isolate was forwarded to the Centre for Infectious Diseases and Microbiology Laboratory Services, NSW Health Pathology, for confirmatory testing and genotyping.

The case was interviewed by a public health nurse using a standard questionnaire. The case's movements during the ten-day exposure period prior to illness onset were documented, in order to map these against potential *Legionella* sources. The questionnaire asks specifically about exposures to pools, spas (including home spas), water parks, water fountains or sprinklers among other water sources. The case reported use of a communal spa pool in his apartment complex on two occasions, eight and five days prior to his illness onset.

Environmental health investigation

Two days after the case was notified, Environmental Health Officers from the Public Health Unit went to the complex where the case owned an apartment. The complex has an air conditioning cooling tower which was inspected, and from which a water sample was collected for microbiological analysis. In keeping with Australian legionellosis investigation guidelines, samples of reticulated water (including hot water from the shower) were not collected.

The communal recreational facilities included a heated spa pool, which had water chemistry routinely conducted for pH and chlorine levels once per week. Water samples had never been taken from the spa pool for microbial analysis (and were not legally required).

The building manager was told of the reason for the visit and granted permission for the officers

Figure 1: Implicated heated fibreglass spa pool



to inspect and take samples from the spa pool (Figure 1). At the time of inspection the spa pool was open but there were no bathers. During the inspection, chemical testing of the spa pool water was carried out using a recently calibrated Pooltest 9 Photometer (Palintest Australia Pacific, Peakhurst, NSW, Australia) with a DPD1 tablet for free residual chlorine; a DPD1 mixed with a DPD2 tablet to assess the total chlorine; and a phenol red tablet to assess pH.

Once the chemical tests were completed, a water sample was collected for the purpose of microbiological analysis, using aseptic technique, in a 250mL sterile container which had a trace of thiosulphate to neutralise chlorine.

The cooling tower and spa pool microbial water samples were placed into a foam cooler containing an ice freezing brick and delivered to the Forensic and Analytical Science Service Legionella Reference Laboratory.

Culture of *Legionella* from water samples

Both water samples were tested as soon as possible on receipt. Quantitative analysis for *Legionella* was performed in accordance with Australian Standard AS4276.1:2021 *Water Microbiology*. The spread plate technique was used, in which 0.1 mL aliquots of sample were inoculated onto both agar plates with non-selective culture (buffered charcoal, yeast extract: BCYE), and agar plates with a selective culture (BCYE containing the antibiotics glycine, vancomycin hydrochloride, polymyxin B sulphate and cycloheximide: GVPC). After incubation at 37 °C for 5 to 7 days, plates were examined at 7–40× total magnification using a stereomicroscope. Colonies expressing characteristic *Legionella* morphology were tallied. A representative number of presumptive *Legionella* colonies, of each morphological type, were streaked onto BCYE and tryptone soya plus sheep blood agar (TSSBA) plates. *Legionella* isolates were defined as those which grew on BCYE but not on TSSBA. *Legionella* isolates were further identified serologically as Lp1, using the Legionella Latex Test (Oxoid,

Thermo Fisher Scientific, North Ryde, NSW). A report was issued as Lp1 colony forming units (CFU) per millilitre based on the highest final confirmed colony count; that is, one colony from a 0.1 ml aliquot equated to 10 CFU/mL. Samples with zero colonies on a 0.1 mL plate were reported as < 10 CFU/mL. Pure colonies of Lp1 were forwarded to the Centre for Infectious Diseases and Microbiology – Public Health for genotyping.

Genotyping and sequencing of clinical and environmental isolates

Lp1 isolates were subjected to WGS in order to verify or refute the hypothesis that they could be related. All isolates were sequenced on the NextSeq 500 platform (Illumina) as described previously.⁷ Single nucleotide polymorphisms (SNPs) were identified using Snippy v3.2ⁱ by mapping reads against curated *L. pneumophila* reference genome strain Philadelphia (GenBank accession NC002942) and a SNP phylogeny constructed using PhyML. Genomes were assembled using SPAdes 3.9.0,⁸ and the *L. pneumophila* sequence type was determined by uploading identified alleles to the database and obtaining SBT if the latter existed in the database of 2,793 SBT types.³ To provide context for the case isolates, genomes of epidemiologically unrelated Lp1 isolates were included in the phylogenetic analysis and are listed in Table 1.

This investigation was conducted under the authority of the *Public Health Act 2010 (NSW)* and hence institutional ethics approval for its conduct was not required.

Results

Documentation kept by the complex's building manager revealed that the spa pool temperature was maintained at 37–38 °C. The spa pool was being dosed manually with chlorine, and the single record of water chemistry carried out during the case's putative exposure period showed a very low free chlorine concentration

ⁱ <https://github.com/tseemann/snippy>.

Table 1: List of *L. pneumophila* isolates and their sequence type (SBT)

Strain number	Isolate type	SBT
BE21	Environmental (present investigation)	Non-typeable
BC11	Clinical (present investigation)	Non-typeable
BC10	Clinical	42
BE1	Environmental	211
BC1	Clinical	211
RC1	Clinical	211
RE6	Environmental	211
BC8	Clinical	211
BE3	Environmental	Non-typeable
Reference 1	Clinical	—
Reference 2015	Clinical	Non-typeable
BE5	Environmental	1
BE20	Environmental	284
BE12	Environmental	1
Reference 2013	Clinical	762
GC2	Clinical	Non-typeable

of 0.46 mg/L (NSW requirement at least 2.0 mg/L). At the time of inspection, the pH was 7.6 (NSW requirement pH 7.0–7.8) while there was a low free chlorine concentration of 1.05 mg/L (requirement at least 2.0 mg/L).

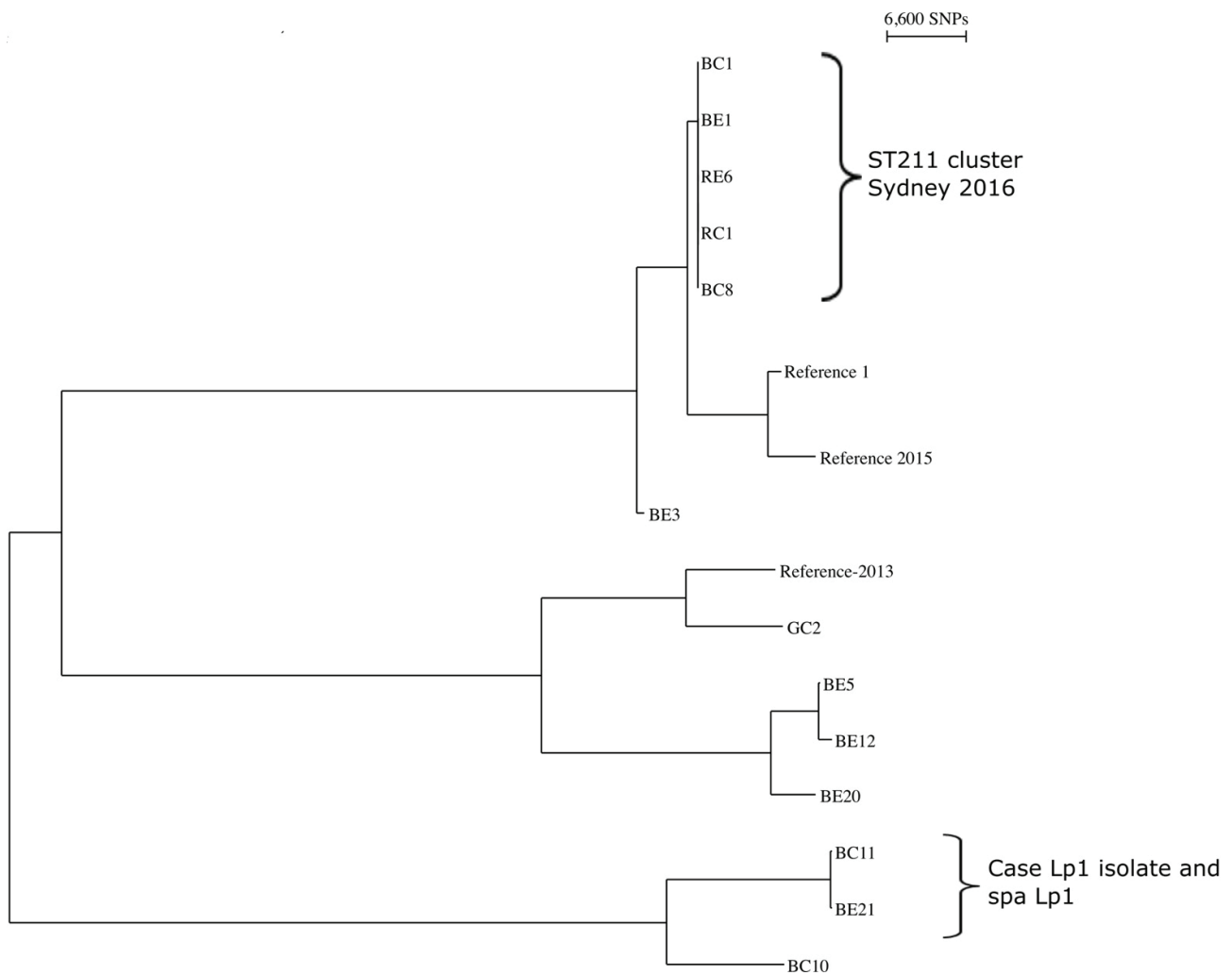
As the free chlorine levels were low and an investigation for Legionnaires' disease was being undertaken, the building manager voluntarily closed the spa pool after the inspection. The following day the building manager engaged a swimming and spa pool consultant to drain and clean the pool and an automatic dosing system was subsequently installed to ensure that adequate free chlorine concentrations were maintained. The spa pool was closed to users for some time whilst these improvements were undertaken. Inspection of the water cooling tower for the building's air conditioning system determined that it was well maintained.

Using the standard quantitative cultural microbiological techniques described above,

the sample of cooling tower water was found to have < 10 CFU/mL of total *Legionella*, whilst the spa pool water sample was found to contain 20 CFU/mL of Lp1. This result was relayed to the building manager at the apartment complex.

The isolates from the case and from the spa pool were found to be highly genomically related (Figure 2), with the distance between genomes only two SNPs, and they were distinct from other Lp1 strains previously investigated and reported in association with community outbreaks of Lp1 in Sydney (Table 1). Of note, these isolates were not typeable using the SBT scheme. Given the one case and one environmental sample, it is important to note that background prevalence of this strain in the environment is unknown.

Figure 2: Maximum likelihood single nucleotide polymorphism (SNP) phylogenetic analysis of 16 isolates of *L. pneumophila* serogroup 1



Discussion

This combined environmental health and microbiological investigation using whole genome sequencing showed a very high degree of relatedness between the clinical and environmental isolates, supporting the conclusion that the communal spa pool was the source of the case's Lp1 exposure. The building's water cooling tower was negative for *Legionella* and so was not likely to be the source. Although overseas studies have found legionellae to be widely distributed in reticulated and other water supplies,⁹⁻¹¹ and present in domestic hot water¹² and showers,¹³ the latter are a rarely identified source due in part to the relatively high temperatures at which hot water is delivered. Only a single Australian report of Legionnaires' disease

acquired from domestic hot water has been published, in that case due to Lp serogroup 6,¹⁴ and hence Australian legionellosis investigation guidelines do not advise collection of samples of reticulated drinking or shower water. For this reason, we did not seek access to the case's apartment to collect water samples.

Spa pools are a recognised source of *Legionella* exposure.^{2,15,16} Recent reviews of legionellosis related to spa pools supplied by reticulated water found strongest evidence for causation in relation to *public* spa pools.^{2,17} Sporadic cases and small outbreaks were also associated with *private* spa pools; but strong evidence of causation, based on matching of clinical and water isolates using sequence based typing, was often missing.²

High water temperatures in spa pools, combined with low water volumes compared to heavy bather loads, can result in low disinfectant levels which allow bacterial multiplication. Inadequate water treatment and/or inadequate free disinfectant levels were identified in most spa pools associated with cases of legionellosis.¹⁷ The spa pool associated with our case was being manually dosed with chlorine rather than using an automated dosing system, and we measured the free chlorine level as very low compared to the requirement of at least 2.0 mg/L under the NSW *Public Health Regulation 2012* and compared to a minimum of 3.0 mg/L in both the United States of America (USA)¹⁸ and European¹⁹ standards.

The legionella count in the spa pool in our investigation was 20 CFU/mL, much lower than the counts generally found in outbreaks associated with contaminated cooling towers. However, exposure to spa pool waters is much more intense and likely more prolonged than putative cooling tower exposures, and a recent quantitative microbial risk assessment found that risk of legionellosis cannot be excluded in the presence of even very low concentrations of *L. pneumophila* in spa pool water.²⁰

WGS showed that the isolates from the case and from the spa pool were highly genomically related. Whilst we describe a single case, the recent review of outbreaks associated with spa pools identified a number of events where multiple cases occurred over extended periods, suggesting long term failure of disinfection conditions.² We are of the opinion that recognition of this sporadic case and our intervention may have prevented further cases of Legionnaires' disease among users of this communal spa pool.

The added value of WGS in this investigation is highlighted by the fact that the isolates were not typable using the SBT scheme. While the 7-allele gene SBT method has been used for outbreak investigations in the past,³ the limited resolution of SBT has encouraged a wider application of WGS. WGS is universally acknowledged to be a powerful tool that can

provide high-resolution information on strain type, probable transmission pathways, outbreak origin and antibiotic resistance, for a growing number of community outbreaks of bacterial infections both in Australia⁷ and internationally.^{21,22} For example, the application of WGS in the USA as a public health surveillance tool for foodborne outbreaks caused by *Listeria monocytogenes* greatly increased the number of detected and solved outbreaks.²³ The WGS approach relies on the availability of culture, but high-resolution and standardised sequencing data can now be used as the basis for more accurate cluster assignment without the reliance on the 7-allele SBT scheme given the latter method's limited resolution and utility.

Conclusion

This appears to be the first report of a single case of legionellosis linked through the use of WGS molecular technology to exposure to a contaminated spa pool. The exposure is believed to have occurred despite the comparatively low Lp1 concentration in the spa pool water of 20 CFU/mL. This finding also supports the value of investigating single notifications with a small number of potential sources present (such as a communal spa pool), and it demonstrates the importance of the collection of clinical samples for Lp1 culture in order to have isolates which can then be subjected to WGS.

Spa pools have operating conditions that facilitate the growth of pathogens, including legionellae, such as: the high temperature of the circulating water; the presence of aerosol-generating jets; and a small volume of water with a potential high bather load. However, in NSW, communal spa pools in apartment complexes are not defined as public spa pools and are therefore exempt from the operational requirements set by the *Public Health Regulation 2012*. Throughout Australia, the regulation of public spa pools is highly variable; specific legislation exists in NSW, South Australia, Tasmania, Victoria, and Western Australia, but of these, communal spa pools in apartment buildings are regulated only in South Australia under

the *South Australian Public Health (General) Regulations 2013* and in Western Australia under the *Health (Aquatic Facilities) Regulations 2007*. As communal spa pools installed in multi-dwelling complexes that are poorly disinfected are a potential *Legionella* source, we believe that building managers should be made aware of the need for their proper maintenance.

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