Letter to the Editor

# A Case Note on Legionnaires' Disease Caused by Serogroup 1, Sequence Type ST496 in Singapore

**Dear Editor,**

*Legionella pneumophila* has previously been found to be endemic in Singapore.[1-3] Local environmental isolates have been studied and characterised,[2-4] but speciation of local clinical isolates from patients with Legionnaires' disease has, to our knowledge, not been reported. As a result, the link between environmental and disease-causing *Legionella* isolates in Singapore remains elusive. Herein, we described a case of Legionnaires' disease in Singapore, and the serogroup, allelic profile and sequence type of the clinical isolates recovered.

A 61-year-old local Chinese male was admitted to the intensive care unit (ICU) of Ng Teng Fong General Hospital for acute hypoxemic respiratory failure. He was a shipboard repair engineer, and had comorbidities of ischaemic heart disease, hypertension, dyslipidaemia and chronic obstructive pulmonary disease. He had first developed symptoms of acute breathlessness, productive cough and fever while at sea, and was initially treated by a shipboard physician with a course of oral antibiotics.

The patient lived in a naturally ventilated apartment equipped with an instantaneous water heater system—there was no hot water storage tank. He stayed aboard cruise ships for work a few days each time. He did not repair pipes or water tanks. His family and colleagues were reported to be well.

The patient subsequently presented to our hospital upon disembarkation after 3 days of illness as his condition had deteriorated. He was dyspnoeic and septic on admission. Clinical examination revealed absence of breath sounds over the left lung base. The blood pressure was 93/60 mmHg, pulse was 110 beats per minute, temperature was 38.7ºC, respiratory rate was 26 breaths per minute and peripheral oxygen saturation ($SpO_2$) was 84% on room air and 95% on non-rebreather mask. A chest radiograph revealed dense left lower zone consolidation with air bronchogram, and a diagnosis of severe pneumonia was made. Intravenous amoxicillin-clavulanate, ceftazidime and azithromycin therapy was initiated as per hospital guidelines for severe community-acquired pneumonia, and the patient was admitted to the ICU.

Investigations revealed leucocytosis of 13.95 x 109/L (reference range: 3.37-11.03 x 109/L), serum creatinine of 155 µmol/L (reference range: 64-104 µmol/L), serum lactate of 1.0 mmol/L (reference range: 0.0-1.8 mmol/L), and type 1 respiratory failure on arterial blood gas ($PaO_2$ 71.8 mmHg, $PaCO_2$ 32.3 mmHg, $FiO_2$ of 1.00). The patient was intubated, and treated with bronchodilators, intravenous steroid, and paralysis for severe bronchospasm.

Urine *Legionella* antigen testing (Alere BinaxNOW *Legionella* urinary antigen card) returned positive on the second day of hospitalisation. Antibiotics were rationalised to a week of azithromycin monotherapy. The patient improved rapidly thereafter, was successfully extubated on the third day, and was discharged after 7 days of hospitalisation.

Two endotracheal tube (ETT) aspirate samples were obtained and sent to the Environmental Health Institute's laboratory for isolation of *Legionella pneumophila*, and for sequence-based typing.[4,5] Both samples were directly plated on glycine, vancomycin, polymyxin B, cicloheximide (GVPC) and buffered charcoal yeast extract, alpha-ketoglutarate containing L-cysteine (L-cysteine-BCYEα) agars (Oxoid, Thermo Fisher Scientific), and incubated at 36 ± 1ºC for up to 10 days. The plates were observed daily and colonies were observed 3 days after inoculation. Purified polymerase chain reaction (PCR) products were sequenced by capillary electrophoresis using Applied Biosystems® 3730/3730xl deoxyribonucleic acid (DNA) Analyzer and BigDye Terminator v3.1 (Axil Scientific, Singapore). Latex agglutination test (*Legionella* latex test, Oxoid) revealed that the *L. pneumophila* isolates from the 2 endotracheal aspirates samples belonged to serogroup 1, and sequencing identified them as belonging to sequence type (ST) 496.

*L. pneumophila* serogroup 1 (Lp1) has been reported to be the most common (61-88%) serogroup responsible for Legionnaires' disease.[6,7] In this case study, we successfully used sequence-based typing—the gold standard method for subtyping *L. pneumophila*—to characterise and sequence type local clinical Lp1 isolates to ST496. ST496 was previously isolated from clinical samples in the Netherlands (2003) and environmental sampling of spa pools in Singapore (2006).[4,8] Our findings demonstrate that ST496 is also associated with sporadic human cases in Singapore.

In Singapore, suspected cases of Legionnaires' disease are usually tested only with urinary antigen test specific for Lp1, without further attempts to isolate *L. pneumophila*.[2,9]

Thus, the diversity of *L. pneumophila* strains among the Lp1 population is not characterised, preventing comparison of clinical and environmental isolates for outbreak investigations and epidemiological research by public health professionals. Sequence typing of these clinical isolates has provided additional information on the genetic profile of local Lp1 strains, which would ultimately facilitate better understanding of the local molecular epidemiology and ecology of *L. pneumophila*. Our study highlights the importance of obtaining microbiological culture and isolation of *L. pneumophila* (besides urinary antigen testing) in suspected clinical cases so as to aid matching with environmental and other clinical isolates in the future.

***Acknowledgements***

*The authors would like to thank the following: Associate Professor Ng Lee Ching (Director of the Environmental Health Institute; Adjunct Associate Professor, School of Biological Science, Nanyang Technological University) for her support of this study and proof-reading the manuscript; Ms Lalitha Kurupatham (Senior Assistant Director of Policy & Control Branch, Communicable Diseases Division; Head, National Public Health Unit) for notifying the Environmental Health Institute (National Environmental Agency) of this case; and Dr Nitin Mahobia (Consultant Microbiologist, Department of Laboratory Medicine, Ng Teng Fong General Hospital) for facilitating the collection of specimens for testing at the Environmental Health Institute laboratory.*

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