Enhanced Detection of Legionnaires’ Disease by PCR testing Induced Sputum and Throat Swabs

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Take Home Message: Testing induced sputum samples from those who cannot produce sputum increases diagnosis of Legionnaires’ disease.
To the Editor,

Legionnaires’ disease, particularly that caused by non-pneumophila species is notoriously underdiagnosed.[1,2] We recently found a four-fold increase in case detection of Legionnaires’ disease through a laboratory-initiated strategy of systematic PCR testing for Legionella species of all lower respiratory specimens from patients with pneumonia or immune compromised status.[3] This strategy relies on the availability of lower respiratory specimens and the recording of relevant clinical information on laboratory requisition forms by clinicians. We recognized that this strategy will miss testing patients who could not expectorate sputum and when inadequate clinical information is written on laboratory requisition forms. To address this diagnostic gap we enhanced case detection by actively identifying patients with community-acquired pneumonia and by collecting induced sputum from those unable to expectorate voluntarily. In addition, we evaluated throat swabs as an alternative specimen for PCR testing.

From October 2012 to March 2013 patients admitted to hospital in Christchurch, New Zealand with community acquired pneumonia and aged ≥18 years, were recruited. For logistic reasons, recruitment occurred on weekdays only. The study period was chosen to coincide with peak Legionnaires’ disease activity in Christchurch.[3] Patients were excluded if the pneumonia was hospital-acquired or was associated with bronchial obstruction, bronchiectasis or tuberculosis. Patients were not eligible for sputum induction if they required high flow oxygen or assisted ventilation at enrolment.

Ethical approval was obtained from the New Zealand Northern A Ethics Committee. Informed consent was obtained from the patient or their next of kin with separate informed consent for induced sputum collection.
Legionnaire’s disease was defined by a positive sputum PCR, sputum culture or urinary antigen test result in a patient with pneumonia. Community-acquired pneumonia was defined as an acute illness with clinical features of pneumonia and radiographic pulmonary shadowing that is at least segmental or present in one lobe and is neither pre-existing or because of some other known cause.

Sputum induction was performed on the day it was requested, using ultrasonically nebulised 7% hypertonic saline over 20 minutes via a DeVilbiss Ultraneb (DeVilbiss Healthcare, Somerset, Pennsylvania, USA). Throat swabs were collected using nylon flocked swabs (Floqswabs, Copan flock technologies, Brescia, Italy) and placed in universal transport media. Sputum and throat swabs were tested for Legionella species using PCR. The isolation of DNA from clinical specimens was performed using the SPRI-TE (Beckman Coulter, Auckland, New Zealand) nucleic acid extractor and gDNA Extraction Kit as recommended by the manufacturer. Legionella DNA was detected using real-time PCR,[2] confirmation of the PCR products that did not have melting curve data consistent with Legionella pneumophila was performed using a real-time PCR with a different gene target.[3] This confirmatory PCR had both genus and Legionella longbeachae specific probes. PCR inhibitor controls were used to validate negative PCR results.[3-5] Urine samples were tested for Legionella pneumophila serogroup 1 and Streptococcus pneumoniae using the BinaxNOW Legionella Urinary Antigen Card and BinaxNOW S. pneumoniae Antigen Card (Alere, Scarborough, Maine), respectively. Blood cultures were taken at the request of attending physicians.

Over the study period, 145 patients were eligible with 114 enrolled. Twenty six patients did not consent and four who were initially enrolled were excluded when the diagnosis of
malignancy (one), tuberculosis (two), and hospital-acquired pneumonia (one) was made. One was excluded because pneumonia was not the primary reason for admission.

The median age of those included was 69 years (range 18-101 years), with 67 (59%) female. There were 22(19%) cases of Legionnaires’ disease: 16 cases caused by *Legionella longbeachae*, three caused by *L. pneumophila*, and three non-typable *Legionella* species. Of these, 21 were detected by sputum PCR, eight had positive sputum cultures (all PCR positive), and three had positive urinary antigen tests (two also detected with sputum). The three non-typable *Legionella* species were all culture negative, urinary antigen negative and of insufficient copy number to type. Of the 114 patients, 17 (15%) had positive urinary antigen tests for *S. pneumoniae* and one (1%) other patient had a positive blood culture for *S. pneumoniae*.

A summary of the yield of diagnostic specimens is presented in table 1. Of the 114 cases, 46 (40%) were unable to spontaneously expectorate sputum. Induced sputum was requested for 31 patients and 15 did not give consent or were ineligible. Of the 31 who consented to have sputum induced, 10 (34%) produced an expectorated sputum sample with nursing or physiotherapist assistance (four were positive for *Legionella*), 19 underwent sputum induction and 2 were discharged prior to the procedure. Of the 19 who underwent sputum induction, all produced sputum without adverse events and four tested positive for *Legionella*. Therefore, eight of 22 cases (36%) of Legionnaires’ disease would not have been detected without active specimen collection. Of the PCR-positive sputum specimens, 11/21 (52%) had >10 squamous epithelial cells per low power field and 12/21 (57%) had <25 leucocytes per low power field, criteria often used to indicate poor quality specimens.
Throat swabs were performed on all 114 patients and were positive in only three (3%), all of whom also had positive sputum specimens.

The identification of *Legionella sp.* altered the treatment in 16 (73%). In five this was inclusion of an agent active against *Legionella* and in 11 there was a rationalisation of antimicrobials.

Table 1. Summary of microbiological results.

<table>
<thead>
<tr>
<th>Diagnostic Specimen</th>
<th>Legionella PCR positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expectorated sputum</td>
<td>14/32</td>
</tr>
<tr>
<td>Expectorated sputum obtained with nursing or physiotherapist assistance</td>
<td>4/10</td>
</tr>
<tr>
<td>Induced sputum</td>
<td>4/19</td>
</tr>
<tr>
<td>Throat swab</td>
<td>3/114</td>
</tr>
</tbody>
</table>

We found that active specimen collection increased the case detection of Legionnaire’s disease in our region by at least one third over and above PCR testing of routine specimens. During spring and summer in our region, Legionnaires’ disease accounted for about one fifth of all cases of adult community-acquired pneumonia admitted to hospital, and was more common than confirmed pneumococcal pneumonia.
The diagnostic performance of *Legionella* PCR has been reported previously, but in brief it is highly sensitive and specific, with a rapid turn-around time and disease severity correlates with bacterial load.[3,6]

Sputum is the preferred diagnostic sample for *Legionella* PCR testing.[2] However, over 40% of pneumonia patients in our study were unable to spontaneously expectorate sputum. We managed to obtain a sputum sample through extra assistance or induction in only about two thirds of these cases but, in doing so, still increased the case detection of Legionnaires’ disease by 36%. The most common reason for not obtaining sputum was ineligibility for sputum induction. We were cautious in selecting patients for sputum induction to avoid adverse reactions and it was universally well-tolerated. The technique of induced sputum has been shown to be safe by others and could be more widely applied.[7]

It has been reported that throat swabs may be useful for diagnosing the cause of lower respiratory tract infections by PCR.[8-10] In our study, a throat swab was positive in a minority of cases and offered no advantage over sputum samples.

The relatively small sample size and study period over only one spring/summer are important limitations of our study. Additionally, the conclusions are only valid in regions with a high incidence of non-pneumophila Legionnaires’ disease such as Australasia.

In conclusion, active collection of sputum samples for PCR testing, including by induction, in patients with community-acquired pneumonia enhances the case detection of Legionnaires’ disease, and is recommended in high-prevalence regions. Throat swabs offer no advantage over sputum samples, but further work is needed to assess their potential role in situations when lower respiratory samples cannot be obtained.
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**Author contributions**

Study concept and design: MJM, SS, PG, RGP, TPA, KB, SAY, ADP, MJE, AMW, STC, DRM.

Acquisition of data: MJM, SS, A-MC, KB, PG, RGP, TPA, KB, SAY, STC, DRM. Analysis and interpretation of data: MJM, SS, STC, DRM. Drafting of the manuscript: MJM, DRM. Critical revision of the manuscript for important intellectual content: MJM, SS, A-MC, KB, PG, RGP, TPA, KB, SAY, ADP, MJE, AMW, STC, DRM. Administrative, technical, or material support: MJM, SS, RGP, TPA, KB, SAY, ADP, MJE, AMW, STC, DRM. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**Potential conflicts of interest**

We declare that we have no conflicts of interest.
References
