Impact of Routine Systematic Polymerase Chain Reaction Testing on Case Finding for Legionnaires’ Disease: A Pre–Post Comparison Study

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Background. Legionnaires’ disease cannot be clinically or radiographically distinguished from other causes of pneumonia, and specific tests are required to make the diagnosis. Currently, testing occurs erratically and, instead, clinicians rely on empiric treatment strategies and ignore public health implications of the diagnosis. We aimed to measure the increase in case detection of Legionnaires’ disease following the introduction of routine polymerase chain reaction (PCR) testing of respiratory specimens. PCR is the most sensitive diagnostic tool for Legionnaires’ disease.

Methods. In a quasi-experimental study in Christchurch, New Zealand, we compared the number of cases of Legionnaires’ disease requiring hospitalization diagnosed during a 2-year period before the introduction of a routine PCR testing strategy (November 2008–October 2010) with a similar period after the introduction (November 2010–October 2012). With this testing strategy, all respiratory specimens from hospitalized patients with pneumonia sent to the region’s sole tertiary-level laboratory were tested for Legionella by PCR, whether requested or not.

Results. During November 2008 to October 2010, there were 22 cases of Legionnaires’ disease compared with 92 during November 2010 to October 2012. Of 1834 samples tested since November 2010, 1 in 20 was positive, increasing to 1 in 9 during peak Legionella season (November to January). Increasing bacterial load was associated with increasing disease severity.

Conclusions. In our region, the burden of Legionnaires’ disease is much greater than was previously recognized. Routine PCR testing provides results within a clinically relevant time frame and enables improved characterization of the regional epidemiology of Legionnaires’ disease.

Keywords. Legionella; Legionnaires’ disease; pneumonia; PCR.
not being cost-efficient, although this has not been analyzed in detail.

Legionella species have long been recognized as a common cause of pneumonia in the Christchurch region of New Zealand [3, 4]. The local epidemiology is that of sporadic disease caused by a variety of Legionella species, with seasonal clusters of Legionella longbeachae pneumonia during spring and summer (September to March) associated with potting mix and compost exposure. Since the mid-1990s, all respiratory samples sent to Canterbury Health Laboratories, the region’s sole tertiary-level diagnostic laboratory, from hospitalized patients with pneumonia have been cultured for Legionella species. Motivated by the desire to enhance case finding and reduce turnaround time, we introduced a new testing strategy after October 2010 involving the systematic application of Legionella polymerase chain reaction (PCR), a more sensitive and faster testing method [2, 5], on all respiratory specimens from hospitalized patients with pneumonia. We are unaware of any other setting where such a testing strategy has been introduced into routine diagnostic use. Here we present the impact of this strategy on case finding for Legionnaires’ disease. We hypothesized that there would be a marked increase in detected cases of Legionnaires’ following the introduction of routine PCR testing.

METHODS

The study was a quasi-experimental pre–post comparison study comparing the numbers of cases of Legionnaires’ disease requiring hospitalization diagnosed during the 2 years before the introduction of the routine PCR strategy (November 2008 to October 2010) with the 2 years after the introduction (November 2010 to October 2012).

Setting

Christchurch, New Zealand (population approximately 350 000) is the second-largest city in New Zealand and has a temperate maritime climate. All patients with community-acquired pneumonia are admitted to one tertiary-level hospital system that serves the city and the surrounding health district (population >450 000). Canterbury Health Laboratories is the district’s sole tertiary-level diagnostic laboratory and performs all the Legionella diagnostic testing for the region.

Legionella Testing Strategy

Before the end of October 2010, all respiratory specimens (sputum, endotracheal aspirates, bronchoscopy specimens) sent to Canterbury Health Laboratories for which the clinical details included the words “pneumonia” or “consolidation,” or gave an indication that the patient was immunocompromised were cultured for Legionella species in addition to routine bacterial culture, whether specifically requested or not. No distinction was made between community-acquired and nosocomial pneumonia. Legionella urinary antigen tests, PCR, and serology were ordered at the discretion of attending clinicians during this time.

After October 2010 this strategy was changed. PCR replaced culture as the routine diagnostic test applied, whether specifically requested or not, to all respiratory specimens with any of the following characteristics: (1) the clinical details included the words “pneumonia” or “consolidation,” (2) the clinical details indicated that the patient was immunocompromised, or (3) the patient had a urine sent for Legionella antigen testing. PCR was also performed if specifically requested. Only respiratory specimens that were PCR-positive were cultured. This change was made with support of clinical services and usually PCR was run daily during the working week. Legionella urinary antigen tests and serology continued to be ordered at the discretion of attending clinicians.

Diagnostic Tests

Respiratory specimens were digested as required using dithiothreitol (Sputosol, Oxoid, Cambridge, UK) in a 1:1 ratio to obtain a homogeneous solution for nucleic acid extraction. 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. The presence of Legionella DNA was detected using the primer and probes as described elsewhere [6], and confirmation of the PCR products that did not have melting curve data consistent with Legionella pneumophila was performed using real-time PCR [7] with a L. longbeachae probe (minor groove binder probe 5′- VIC – TATCATGCAAATA TGCCCGA-3′ NFQ (Life Technologies, Applied Biosystems). PCR inhibitor controls were used to validate negative PCR results.

Legionella culture was performed on buffered charcoal yeast extract-based agar, with and without modified Wadowski-Yee supplement, and incubated at 35°C for 7 days. The BinaxNOW Legionella Urinary Antigen Card (Alere, Scarborough, Maine) was used to detect L. pneumophila serogroup 1 antigen in urine. Sera were tested at Canterbury Health Laboratories and at the Legionella Reference Laboratory, Institute of Environmental Science and Research, Porirua, New Zealand, by the indirect immunofluorescent antibody test.

Clinical Information

Information on clinical outcome and disease severity (including the CURB-65 [confusion, urea, respiration, blood pressure, age ≥65] score [8]) were retrieved from clinical records.
Legionnaires’ Disease Case Definition
For this study, a case of Legionnaires’ disease was defined as any patient hospitalized with pneumonia who had a positive culture, PCR, or urinary antigen test for Legionella infection or had a ≥4-fold increase in reciprocal Legionella antibody titers. This differs from the confirmed case definition used in New Zealand for surveillance purposes, which only defines PCR-positive cases as “probable” cases [9].

Statistical Analysis
The main outcome measure was the number of cases of Legionnaires’ disease identified before and after the introduction of the new diagnostic testing strategy. The number of PCR tests performed since November 2010 was also recorded to calculate the proportion positive overall and by season. To assess whether specimen ordering patterns changed after the introduction of the new testing strategy, the proportion of respiratory specimens cultured for Legionella before November 2010 was compared with the proportion tested by PCR after this time. Continuous variables were compared using a 2-sided Wilcoxon rank-sum test, t test, or 1-way analysis of variance, as appropriate.

Ethical Approval
The Upper South Island B Ethics Committee reviewed the study protocol and indicated that formal ethical approval was not required for this study, which was regarded as an audit.

RESULTS
Over the 4-year study period, there were 114 cases of Legionnaires’ disease, 22 during the 2 years before the introduction of routine PCR testing and 92 during the 2 years after the introduction (Figure 1). All patients had community-acquired infection, the median age was 65 years (range, 25–90 years), and 78 (68%) were male. The majority of patients (95 [85%]) had infection with L. longbeachae, typically occurring in peaks during spring and summer. The remaining cases (14 [15%]) were caused by L. pneumophila, apart from 1 case of Legionella gormanii infection and 1 case for which the species is unknown.

Only 1 patient did not have a chest radiograph, but this individual had postmortem evidence of pneumonia. Of the other patients, only 3 did not have definite radiographic airspace opacity, although 1 had possible minor airspace opacity. All 3 of these patients were PCR-positive for Legionella, 1 was culture-positive, and 2 were on immunosuppressive drugs.

Of the 114 patients with Legionnaires’ disease, 57 had positive respiratory cultures for Legionella species (43 sputa, 12 endotracheal aspires, 1 bronchoalveolar lavage fluid, 1 postmortem specimen), 99 had positive respiratory specimen PCR results (82 sputa, 15 endotracheal aspires, 1 bronchoalveolar lavage fluid, 1 postmortem specimen), 16 had positive L. pneumophila serogroup 1 urinary antigen tests, and 14 had serological evidence of recent Legionella infection. Serology was only
ordered in a minority of cases but, for those tested, seroconversions were documented in all patients who were PCR-positive. The positive diagnostic test combinations are shown in Table 1. Of the 92 patients diagnosed since November 2010, 72 (78%) had urine tested by the BinaxNOW Streptococcus pneumoniae Antigen Card (Alere, Scarborough, Maine), and all tested negative. No patient had a positive blood culture.

A total of 1834 respiratory specimens were tested by PCR during the 2 years of routine testing (compared with 205 during the preceding 2 years), of which 87 (5%) were positive. During the Legionella season (September to March), 75 of 1073 specimens tested (7%) were positive, compared with 12 of 761 (2%) during April to August. During November to January (peak Legionella season), 56 of 509 specimens tested (11%) were positive. Some 1736 of 11 252 (15%) respiratory specimens tested (7%) were positive, compared with 12 of 761 (2%) during April to August. During November to January (peak Legionella season), 56 of 509 specimens tested (11%) were positive. Some 1736 of 11 252 (15%) respiratory specimens were cultured for Legionella species during the 2 years before November 2010, compared with 1834 of 14 381 (13%) respiratory specimens tested by PCR during the following 2 years.

Following the introduction of routine PCR testing, Legionella species was isolated from 39 of 84 (46%) PCR-positive respiratory specimens that were also cultured. Of the PCR-positive specimens, the mean cycle threshold (Ct) value was significantly lower (indicating a higher bacterial load) in those specimens that were culture-positive than those that were culture-negative (28 vs 34; P < .001; Figure 2). Legionella was cultured from all but 1 PCR-positive specimen with a Ct value of ≤28 (equivalent to greater than about 4000 colony-forming units/mL calculated by standard curve).

Overall, 37 patients (33%) were admitted to the intensive care unit and 12 (11%) died. There was evidence that the cases of Legionnaires’ disease detected after the intervention were, on average, less severe than those detected before the introduction of routine PCR testing (Table 2), although a statistically significant difference was only observed for length of hospital admission. In addition, there was an association between bacterial load and disease severity. The average Ct value was lower in patients admitted to the intensive care unit compared to those who were not (28 vs 33; P < .001), was lower in those who died compared to those who did not (26 vs 32; P < .001), and decreased with increasing CURB-65 score (34 for score 0, 32 for score 1, 30 for score 2, 28 for score 3 or 4; P = .003).

**DISCUSSION**

The major finding from this study is that the burden of Legionnaires’ disease in Christchurch is much greater than was previously recognized. Routine systematic testing of lower respiratory specimens by PCR was associated with a >4-fold increase in the number of detected cases of Legionnaires’ disease compared to routine culture. One in 9 specimens tested during peak Legionella season were positive. Routine PCR testing detected a greater proportion of patients with less severe disease and there was evidence that increasing bacterial load was associated with increasing disease severity.

PCR has been shown to be a reliable tool for diagnosing Legionnaires’ disease [2, 5]. The combination of high sensitivity, high specificity, rapid turnaround time, and the ability to detect all Legionella species makes PCR the preferred diagnostic for Legionnaires’ disease. Although Legionella DNA can be detected in nonrespiratory body fluids [10–13], the sensitivity is highest in respiratory specimens and these are the preferred specimen types if they are available. Although PCR is becoming more widely used for diagnosing Legionnaires’ disease, we are unaware of any other place globally where PCR has been introduced in such a systematic fashion into routine diagnostic use as we have done in Christchurch.

In our study, there were more cases of Legionnaires’ disease diagnosed following the introduction of routine PCR testing than during the preceding era of routine Legionella culture. We are unable to calculate precisely the increase in yield of PCR over culture because not all specimens were cultured for Legionella species since November 2010. As part of the business case for the new strategy, we replaced culture with PCR (rather than adding PCR in addition to culture) and only cultured

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**Table 1. Positive Diagnostic Test Result Combinations**

<table>
<thead>
<tr>
<th>Test Combination</th>
<th>November 2008–October 2010</th>
<th>November 2010–October 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture positive, PCR positive, urinary antigen negative or not tested</td>
<td>8</td>
<td>29</td>
</tr>
<tr>
<td>Culture positive, PCR not tested, urinary antigen negative or not tested</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Culture positive, PCR not tested, urinary antigen positive</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Culture positive, PCR positive, urinary antigen positive</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Culture negative, PCR positive, urinary antigen negative or not tested</td>
<td>3</td>
<td>45</td>
</tr>
<tr>
<td>Culture negative, PCR positive, urinary antigen positive</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Culture negative, PCR negative, urinary antigen positive</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Seroconversion only (none had respiratory specimens for testing)</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

**Total** 21 91

Abbreviation: PCR, polymerase chain reaction.

* One patient also had documented seroconversion to Legionella longbeachae.

* Nine patients also had documented seroconversion to L. longbeachae.
specimens that were PCR-positive. This approach was chosen in order for the change in testing strategy to be cost-neutral with respect to laboratory testing.

We are also confident about the specificity of our PCR results. We adhered to rigorous laboratory standards to prevent contamination and confirmed the species of positive results. In addition, other tests for Legionella were positive in about two-thirds of cases, no case had evidence of infection with another pneumonia pathogen such as S. pneumoniae, and the seasonal pattern of positive results followed the known spring/summer Legionella season. Of particular note, we did not see an increased number of cases during the peak pneumonia season in winter when the greatest numbers of respiratory specimens are received in the laboratory.

We questioned whether increased awareness of and enthusiasm for the local Legionella testing strategy by clinicians after the introduction of routine PCR would lead to improved recording of pneumonia in the clinical details of the specimen requisition form. However, this did not appear to happen given that the proportion of respiratory specimens cultured in the first 2 years of the study was similar to the proportion tested by PCR in the second 2 years.

We found evidence that routine PCR testing detects a greater proportion of Legionnaires’ patients with less severe disease. This is not an unexpected finding given that severe pneumonia was an indication for testing for Legionella infection in local guidelines prior to November 2010 and, therefore, severe cases were more likely to be detected. Our data also provide evidence that PCR is better at detecting lower bacterial loads than culture and that there is an association between bacterial load and disease severity. Consequently, PCR is a better test for milder cases of Legionnaires’ disease. Although there have been reports of PCR sensitivity increasing with increased Legionnaires’ disease severity [14], we are unaware of other data showing that bacterial load is associated with disease severity as has been demonstrated with pneumococcal pneumonia [15].

Despite a higher proportion of patients with less severe disease,

### Table 2. Measures of Disease Severity Among Patients With Legionnaires’ Disease

<table>
<thead>
<tr>
<th>Variable</th>
<th>November 2008–October 2010</th>
<th>November 2010–October 2012</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-hospital death</td>
<td>4/22 (18%)</td>
<td>8/92 (9%)</td>
<td>.19</td>
</tr>
<tr>
<td>Intensive care unit admission</td>
<td>10/22 (45%)</td>
<td>27/92 (29%)</td>
<td>.15</td>
</tr>
<tr>
<td>Median duration of admission, d</td>
<td>10.5</td>
<td>6</td>
<td>.01</td>
</tr>
<tr>
<td>CURB-65 score &gt;2</td>
<td>12/20 (60%)</td>
<td>36/85 (42%)</td>
<td>.15</td>
</tr>
</tbody>
</table>

**Figure 2.** Distribution of cycle threshold (Ct) values for polymerase chain reaction–positive specimens.
it should be highlighted that 29% of patients with Legionnaires’ disease still required intensive care unit admission and 9% died during the period of routine PCR testing.

There are several limitations to our study. We were reliant on obtaining respiratory specimens for testing. As many as half of patients with Legionnaires’ disease may not produce sputum [16–19], so we will have missed cases from patients who could not expectorate. We were also reliant on the recording of clinical information on specimen requisition forms by clinicians. Undoubtedly, respiratory specimens from many patients with pneumonia were not tested for Legionella simply because no clinical details were provided. The majority of Legionnaires’ disease cases in this study were caused by L. longbeachae. It is uncertain whether a routine testing strategy would result in the same increase in case detection in regions where other Legionella species predominate, although there is no reason to expect any difference and we found a similar increase in case detection among non–L. longbeachae species as well. With the quasi-experimental study design, it is possible that temporal changes other than the new testing strategy might have influenced the increased case detection. Of particular note were the earthquakes centered around Christchurch from September 2010. It is possible that the major disturbances arising from this seismic activity may have altered the local ecology of Legionella. However, we have now observed the same magnitude of Legionnaires’ disease cases in 3 spring/summer seasons since the first earthquake, including during the most recent 2012/2013 season outside the period of the present study. There was no evidence of changes in potting mix or compost use or major climatic changes during the study period that could account for the dramatic increase in case detection of Legionnaires’ disease.

The practical implications of our findings are several-fold. Routine PCR testing provides a simple tool to improve case detection within a time frame that can influence clinical management. The use of diagnostic tests in community-acquired pneumonia is controversial, although guidelines support the use of tests that have a relatively high yield and a positive influence on clinical management, especially in severe disease [20, 21]. On this basis, Legionella PCR testing on sputum specimens and pneumococcal urinary antigen testing are recommended for any adult admitted with community-acquired pneumonia in our region. The routine PCR testing strategy also provides a more accurate surveillance tool for monitoring Legionnaires’ disease activity and to better characterize regional epidemiology of this disease. At present, notification data are reliant on inconsistent and erratic testing strategies that markedly underestimate the incidence of Legionnaires’ disease. By applying a systematic testing strategy such as ours, it is possible to obtain a more accurate picture of disease burden that can be compared across regions. Local Legionella testing strategies can also be informed by defining seasonal peak periods of activity, enabling enhanced testing during these times. The increase in case detection can also provide larger sample sizes to enable research on Legionnaires’ disease, including clinical trials. The inability to recruit sufficient numbers of patients has been a major obstacle to such studies in the past. The findings of this study have already prompted considerable local interest in the identification and implementation of more effective preventative strategies for Legionnaires’ disease.

Historically, the major barriers to Legionella PCR testing have been the technical expertise required to run the tests and cost. With the availability of improved techniques and commercial assays, the former is now not a barrier. Indeed, many laboratories would now find it easier to adopt PCR than Legionella culture, for which expertise is generally waning. The relative cost of PCR is also decreasing such that it is being widely introduced into diagnostic laboratories as a routine and cost-efficient testing method.

In our region, routine systematic PCR testing quadrupled the detection of Legionnaires’ disease when compared to routine culture and provided results within a clinically relevant timeframe. Most places do not routinely culture respiratory specimens for Legionella. Therefore, the increase in case detection by introducing a routine PCR testing strategy such as ours is likely to be even greater in other regions where Legionella testing only occurs on an ad hoc basis. Further research is needed to determine whether case detection can be further enhanced by making greater efforts to obtain respiratory samples from pneumonia patients who are unable to expectorate (eg, by obtaining induced sputum). The role of bacterial load as a prognostic marker also needs to be explored.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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