Legionnaires’ Disease in South Africa, 2012–2014


During June 2012–September 2014, we tested patients with severe respiratory illness for Legionella spp. infection and conducted a retrospective epidemiologic investigation. Of 1,805 patients tested, Legionella was detected in samples of 21 (1.2%); most were adults who had HIV or tuberculosis infections and were inappropriately treated for Legionella.

Data are limited regarding prevalence of Legionella spp. bacteria that cause community-acquired pneumonia (CAP) in Africa (1), despite the high prevalence of HIV-infected adults in many African countries, including South Africa (2). Legionellosis is a notifiable disease in South Africa but is rarely reported. We sought to determine the prevalence of Legionella spp. infections in South Africa and describe epidemiologic characteristics of patients with Legionnaires’ disease (LD).

The Study
During June 2012–September 2014, we conducted a prospective, hospital-based, observational study as part of the severe respiratory illness (SRI) surveillance at 2 sites in South Africa: Klerksdorp-Tshepong Hospital Complex, Klerksdorp, North West Province; and Edendale Hospital, Pietermaritzburg, KwaZulu-Natal Province. A patient with SRI was defined as a person hospitalized with lower respiratory tract infection of any duration. We used a standardized questionnaire to collect demographic and clinical information. Nasopharyngeal specimens and induced sputum samples were tested for Legionella spp. infections by using a real-time PCR assay, as previously described (3). Specimens that were Legionella positive were also tested by real-time PCR assays to identify L. pneumophila and L. longbeachae. In addition, patients’ specimens were tested for other respiratory pathogens and for HIV. Of the 22 Legionella-positive patients, we could trace 17 with whom we conducted a retrospective epidemiologic investigation, which included interviews (detailed study methods in the online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/1/15-0972-Techapp.pdf).

During June 2012–September 2014, a total of 4,525 SRI patients were enrolled; induced sputum specimens, the recommended specimen type for Legionella spp. detection, were collected from 1,805 (40%). Of 1,803 patients with sputum specimens for which data were available, 885 (49%) were male, and 324 (18%) were children <5 years of age. HIV prevalence was 64% (1,025 of 1,594 patients with sputum specimens and known HIV status), and prevalence of active tuberculosis (TB) infection was 24% (421 of 1,758 patients with sputum specimens and known TB status). Of 1,720 patients with sputum specimens and known survival status, 142 (8%) patients died.

Among the 1,805 patients with sputum samples, 21 (1.2%, 95% CI 0.7%–1.7%) tested positive for Legionella spp. by real-time PCR. For 1 patient (designated E1 in the online Technical Appendix Table) from whom sputum could not be collected, Legionella spp. infection was detected in the nasopharyngeal specimen, so 22 patients with Legionella spp. infections were detected in total. Among the 21 patients whose sputum tested positive for Legionella spp. infections, median age was 40 years (range 19–59 years; Figure 1), and 11 (52%) were males.

A cluster of case-patients (15/21 [71%]) was observed during July–December 2012 (Figure 2), including all 6 from Edendale Hospital and 10 (10/16, 63%) from Klerksdorp-Tshepong Hospital Complex. These sites are geographically distant (≥600 km) from one another, so the respective clusters or outbreaks are unlikely to be related. We did not culture samples with Legionella spp. infection, so we were unable to perform strain typing to confirm whether the clusters were caused by related strains. The remaining 6 patients from Klerksdorp-Tshepong Hospital Complex appeared to have sporadic infections.

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Legionella patients resided in different areas or communities within the cities of Pietermaritzburg and Klerksdorp. Epidemiologic investigation revealed exposure to several potential sources of infection, such as waste management, air conditioners, plumbing, mining, and swimming pools; however, no common exposure could be identified, so environmental sampling and testing were not performed.

Fifteen (75%) of 20 Legionella patients with known HIV status were infected with HIV, and 9 (43%) of the 21 patients tested positive for TB at the admission during which Legionella infection was detected. HIV or TB infection, or both, was detected in 18 (90%) of 20 patients with known HIV and TB status. A history of active TB before the admission during which Legionella was detected was reported for 14 (82%) of 17 patients. For 17 Legionella spp.–infected patients for whom information was available, additional LD-associated factors included regular alcohol consumption (10 [59%]), cigarette smoking (9 [53%]), asthma (2 [12%]), and heart disease (2 [12%]).

Eighteen (86%) of 21 patients had symptoms >7 days before hospital admission, a delay possibly occurring because many patients were chronically ill (75% were HIV infected and ≥43% had TB). Median duration of hospitalization for Legionella patients was 4 days (range 1–35 days), and 1 (9%) patient was admitted to intensive care and survived the illness; 4 (20%) patients died. Antimicrobial drug treatment (in-hospital and discharge medication) was known for 21 patients and included amoxicillin/clavulanic acid (16 [76%]), anti-TB medications (15 [71%]), cotrimoxazole (7 [33%]), cefuroxime/ceftriaxone (5 [24%]), and erythromycin (5 [24%]).

Legionella spp. isolates were identified for 2 patients as L. pneumophila serogroup 1 and L. longbeachae. Species could not be determined for 19 patients because of low bacterial loads in their specimens. Of the 21 patients with Legionella-positive sputum specimens, co-infections were detected in 14 (67%). Co-infecting pathogens were Mycobacterium tuberculosis (9 [43%]), rhinovirus (6 [29%]), respiratory syncytial virus (2 [10%]), adenovirus (2 [10%]), Bordetella pertussis (1 [5%]), and Streptococcus pneumoniae (1 [5%]).

Legionella spp. detection rates in this study were similar to those described in other countries (4). However, age distribution tended toward younger adults, not the elderly, the population previously reported as most affected (4). Men and women were evenly distributed in our study, although a substantial male predominance is common for LD (2,4). Differences in age and gender distributions, compared with distributions in other studies, likely result from high HIV and TB prevalence among younger adults in our study population. LD is typically associated with summer because warm and wet conditions promote bacterial replication (2,4). Longer periods of surveillance are needed to establish seasonality of LD in South Africa.

Clinically, patients with LD in this study were likely to be HIV-infected, chronically ill persons with suspected or confirmed TB and were therefore usually treated for TB infection and discharged. HIV-induced immune suppression and lung damage because of biologic or chemical agents likely increased their susceptibility to Legionella infections. Cases of LD and TB occurring simultaneously have been previously described (5–7). Legionella infection in populations with HIV or TB co-infections may cause acute exacerbation of respiratory symptoms, prompting patients to seek hospital care.

In South Africa, treatment for CAP is usually penicillin or ampicillin for adults <65 years of age and amoxicillin/clavunate or cefuroxime for elderly or HIV-infected adults (8). However, treatment for LD should include a macrolide or fluoroquinolone (4). Only one fourth of Legionella patients in this study received appropriate treatment, likely because of clinical inability to distinguish LD from other forms of pneumonia and because clinicians rarely consider Legionella when they lack access to diagnostic testing and local prevalence data. This problem is further compounded by the high prevalence of HIV and TB in South Africa. Anti-TB treatment, which was
administered to more than two thirds of the Legionella patients, would have had therapeutic benefits; rifampin has been shown to have activity against Legionella spp. (9,10). However, suboptimal treatment of Legionella patients with co-infections likely contributed to a case-fatality ratio (20%) more than twice that for all SRI patients (8%) (4,11). Lack of appropriate treatment of patients with CAP in South Africa for atypical pathogens has been described ((12).

Conclusions
In South Africa, patients with LD often have chronic illness caused by co-infections such as HIV and TB at time of admission. Legionella infections in most patients were undiagnosed, and patients were suboptimally treated for TB or more typical causes of CAP. Increased awareness and improved diagnostic testing could result in earlier diagnosis, appropriate treatment, and improved outcomes for these patients. In addition to routine diagnostics, surveillance for LD should be performed on an ongoing basis for rapid identification and response to outbreaks.

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References

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Technical Appendix

Materials and Methods

Study Design

We conducted a prospective, hospital-based, observational study as part of Severe Respiratory Illness (SRI) surveillance from June 2012–September 2014 at 2 sites in South Africa: Klerksdorp-Tshepong Hospital Complex, Klerksdorp, North West Province; and Edendale Hospital, Pietermaritzburg, KwaZulu-Natal Province.

A case of SRI was defined as a person hospitalized with symptoms of any duration and meeting age-specific clinical inclusion criteria as follows: children from 2 days to <3 months of age with physician-diagnosed sepsis or lower respiratory tract infection (LRTI); children from 3 months to <5 years of age with physician-diagnosed LRTI; and patients ≥5 years of age who meet a modified World Health Organization case definition for SRI (1): 1) fever (>38°C) or reported fever; 2) cough or sore throat; and 3) shortness of breath or difficulty breathing, with or without clinical or radiographic findings of pneumonia. In addition, we enrolled patients with a clinical diagnosis of suspected tuberculosis (TB). Chest x-rays are not performed routinely on LRTI patients at the site hospitals, so radiologic confirmation of pneumonia could not be obtained. A case of Legionnaires’ disease was defined as a person hospitalized with SRI and positive results for Legionella spp. infection determined by PCR from an induced sputum or nasopharyngeal (NP) specimen. A standardized questionnaire was used to collect demographic and clinical information, such as age, sex, regular alcohol or cigarette use, mining exposure, underlying illness, prior antimicrobial drug use, duration of symptoms, length of hospitalization, in-hospital antimicrobial drug treatment, treatment with supplemental oxygen, admission to an intensive care unit, and outcome.
Specimen Collection

NP, induced sputum, and whole blood samples were collected. NP specimens, including combined NP and oropharyngeal swabs (FLOQSwabs, Copan Diagnostics, Murrieta, CA, USA) for patients ≥5 years of age or NP aspirates for children <5 years of age were placed in universal transport medium (Copan Diagnostics) and transported at 4°C to the National Institute for Communicable Diseases, Johannesburg, South Africa. As of July 2013, sputum samples were stored at −20°C and transported on dry ice.

Detection of Legionella spp.

Sputum samples were digested with dithiothreitol (Roche Diagnostics, Mannheim, Germany). Total nucleic acids were extracted from 200 µL of sample by using the MagNA Pure 96 instrument (Roche) and MagNA Pure 96 DNA and Viral NA SV kit (Roche). Nucleic acids were eluted into 100 µL of elution buffer and stored at −20°C.

NP and induced sputum samples were tested for Legionella spp. by using a multiplex real-time PCR assay that also detected Mycoplasma pneumoniae, Chlamydia pneumoniae, and the human RNaseP gene (2). Twenty-five µL reactions were performed by using PerfeCTa Multiplex qPCR Supermix (Quanta Biosciences, Gaithersburg, MD, USA), 6.5 µL DNA, and primers and probes, as previously described (2). Samples with a Ct-value >45 were recorded as negative. PCR was performed by using an Applied Biosystems 7500 Fast real-time PCR instrument (Thermo-Fisher Scientific, Waltham, MA).

Legionella spp. Identification

Legionella-positive specimens were further tested in 2 real-time PCR assays. The first assay confirmed Legionella spp. and identified L. pneumophila and L. pneumophila serogroup 1 (3). The second assay identified L. longbeachae. The reaction mix comprised of 12.5 µL of PerfeCTa Multiplex qPCR Supermix (Quanta Biosciences), 5 µL DNA, 25 µmol/L primers (LLB-F –TGGTTTTCGAAATCATCAGTATGC, LLB-R-CTGTCTAAAACACTTCTCTCCCGATA) and 5 µM probe (Quasar670-TTTAATTTAGTCCACCAGCAAGGTGGC-BHQ3) made to a final volume of 25 µL. Cycling conditions were as follows: 1 cycle of 95°C for 10 min, followed by 50 cycles of 95°C for 15 s and 60°C for 1 min.

TB Testing

TB testing was conducted at the site laboratory; additional testing was done at the NICD. Sputum samples were tested for Mycobacterium tuberculosis by smear microscopy,
culture or PCR. Tests were performed as follows: smear microscopy by using fluorescence auramine staining for acid-fast bacilli; liquid media by using BD Bactec MGIT 960 (Beckton, Dickinson, Franklin Lakes, NJ, USA) for culture; and TB PCR by using the Xpert MTB/RIF system (Cepheid, Sunnyvale, CA, USA). Positive cultures were identified as *M. tuberculosis* complex by using Ziehl-Neelsen staining and antigen testing.

**Detection of Other Respiratory Pathogens**

SRI patients were tested for additional respiratory pathogens. NP specimens were tested for specific respiratory viruses (i.e., influenza, adenovirus, enterovirus, rhinovirus, human metapneumovirus, respiratory syncytial virus, and parainfluenza types 1–3) by multiplex real-time PCR (4). NP and induced sputum specimens were tested for *Bordetella pertussis* (5), and blood specimens were tested for *Streptococcus pneumoniae* (6) and *Haemophilus influenzae* (7).

**Determination of HIV Status**

HIV results were obtained from a combination of 2 sources: patient clinical records when available and for consenting patients, a blind, linked dried-blood spot tested at NICD. For patients for whom both results were available, the NICD result was used. Testing included HIV ELISA testing for patients $\geq$18 months of age and PCR testing for children <18 months of age.

**Epidemiologic Case Investigation**

An epidemiologic investigation was conducted retrospectively for *Legionella*-positive patients who could be traced and included collection of additional information from the patient or a close relative for a deceased patient. Additional information included potential *Legionella* exposure, past medical history, factors associated with disease, and discharge medication.

**Statistical Analysis**

As sputum specimens are the optimal specimen type for *Legionella* spp. detection, analyses were performed for patients who had a sputum sample collected. Detection rate was defined as the number of *Legionella* case-patients among all SRI case-patients with sputum samples collected and tested.
Ethical Approval

The protocol was approved by the Universities of the Witwatersrand (M081042) and KwaZulu-Natal (BF157/08). The U.S. Centers for Disease Control and Prevention deemed the study a non-research, surveillance activity.

References

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   http://dx.doi.org/10.1016/j.diagmicrobio.2010.11.014


   http://dx.doi.org/10.1128/JCM.02498-06

   http://dx.doi.org/10.1128/JCM.00102-07
**Technical Appendix Table.** Demographic, epidemiologic, and clinical characteristics of patients positive for *Legionella*, South Africa, June 2012–September 2014 (N = 22)*

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Hospital, admission date</th>
<th>Sex, age, y</th>
<th>Symptom duration, d†</th>
<th>Potential exposure to contaminated water source</th>
<th>HIV</th>
<th>TB‡</th>
<th>Risk factors§</th>
<th>Hospital duration, d (treatment¶)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1#</td>
<td>Edendale, 28 Jun 2012</td>
<td>M, 56</td>
<td>4</td>
<td>Worked/lived at waste management compound, compost exposure</td>
<td>Unk</td>
<td>Unk</td>
<td>Sput unav</td>
<td>Heart disease, alcohol</td>
<td>35 (TB, amoxicillin-clavunate)</td>
</tr>
<tr>
<td>E2**</td>
<td>Edendale, 3 Jul 2012</td>
<td>M, 52</td>
<td>Unk</td>
<td>Unk</td>
<td>Unk</td>
<td>Unk</td>
<td>Sput –; treated for TB in past year</td>
<td>None</td>
<td>6 (Amoxicillin-clavunate)</td>
</tr>
<tr>
<td>E3**</td>
<td>Edendale, 9 Jul 2012</td>
<td>F, 55</td>
<td>19</td>
<td>Unk</td>
<td>+</td>
<td>None</td>
<td>None</td>
<td>3 (Amoxicillin-clavunate)</td>
<td>DIED</td>
</tr>
<tr>
<td>E5**</td>
<td>Edendale, 6 Nov 2012</td>
<td>F, 41</td>
<td>66</td>
<td>Unk</td>
<td>+</td>
<td>Sput –</td>
<td>None</td>
<td>(Cefuroxime)</td>
<td>Survived</td>
</tr>
<tr>
<td>E6</td>
<td>Edendale, 13 Nov 2012</td>
<td>F, 32</td>
<td>8</td>
<td>Cleaner at hospital/air conditioner maintenance</td>
<td>+</td>
<td>Sput +</td>
<td>None</td>
<td>7 (TB, amoxicillin-clavunate, cefuroxime)</td>
<td>Survived</td>
</tr>
<tr>
<td>T1**</td>
<td>Tshepong, 3 Jul 2012</td>
<td>F, 34</td>
<td>30</td>
<td>Unk</td>
<td>+</td>
<td>Sput +</td>
<td>None</td>
<td>1 (HIV, TB, amoxicillin-clavunate, cotrimoxazole)</td>
<td>Died out of hospital</td>
</tr>
<tr>
<td>T2**</td>
<td>Tshepong, 4 Jul 2012</td>
<td>F, 46</td>
<td>30</td>
<td>Unk</td>
<td>+</td>
<td>Sput –</td>
<td>None</td>
<td>5 (TB)</td>
<td>Survived</td>
</tr>
<tr>
<td>T3</td>
<td>Tshepong, 10 Jul 2012</td>
<td>F, 32</td>
<td>61</td>
<td>None known</td>
<td>+</td>
<td>Sput +</td>
<td>Heart disease</td>
<td>2 (HIV, TB, ceftriaxone, cefuroxime)</td>
<td>Survived</td>
</tr>
<tr>
<td>T4</td>
<td>Tshepong, 30 Jul 2012</td>
<td>M, 44</td>
<td>1</td>
<td>Gardening/plumbing work, compost exposure</td>
<td>–</td>
<td>Sput –</td>
<td>Current smoker, alcohol</td>
<td>2 (TB, erythromycin)</td>
<td>Survived</td>
</tr>
<tr>
<td>T5</td>
<td>Tshepong, 14 Aug 2012</td>
<td>M, 34</td>
<td>10</td>
<td>Mining, gardening, renovation work</td>
<td>+</td>
<td>Sput +</td>
<td>Asthma, former smoker, alcohol, Alcohol</td>
<td>1 (HIV, erythromycin, amoxicillin-clavunate)</td>
<td>Survived</td>
</tr>
<tr>
<td>T6</td>
<td>Tshepong, 16 Aug 2012</td>
<td>F, 19</td>
<td>13</td>
<td>None known</td>
<td>–</td>
<td>Sput +</td>
<td>None</td>
<td>3 (TB, amoxicillin-clavunate)</td>
<td>Survived</td>
</tr>
<tr>
<td>T7</td>
<td>Tshepong, 20 Sep 2012</td>
<td>F, 50</td>
<td>Unk</td>
<td>None known</td>
<td>+</td>
<td>Sput –</td>
<td>Asthma, current smoker (snuff), alcohol, Alcohol</td>
<td>5 (HIV, TB, cotrimoxazole, amoxicillin-clavunate)</td>
<td>Survived</td>
</tr>
<tr>
<td>T9</td>
<td>Tshepong, 5 Nov 2012</td>
<td>F, 39</td>
<td>188</td>
<td>None known</td>
<td>+</td>
<td>Sput –</td>
<td>Current smoker (snuff)</td>
<td>11 (HIV, TB, cotrimoxazole)</td>
<td>Survived</td>
</tr>
<tr>
<td>T10</td>
<td>Tshepong, 6 Dec 2012</td>
<td>M, 55</td>
<td>21</td>
<td>None known</td>
<td>+</td>
<td>Sput –</td>
<td>Former smoker</td>
<td>1 (TB, amoxicillin-clavunate, cotrimoxazole, erythromycin)</td>
<td>Survived</td>
</tr>
<tr>
<td>T11</td>
<td>Tshepong, 6 May 2013</td>
<td>F, 52</td>
<td>22</td>
<td>Hospital admission 22 Apr 2013–1 May 2013</td>
<td>+</td>
<td>Sput –</td>
<td>None</td>
<td>3 (HIV, cotrimoxazole, amoxicillin-clavunate, erythromycin)</td>
<td>Died</td>
</tr>
<tr>
<td>T13</td>
<td>Tshepong, 11 Jun 2013</td>
<td>M, 42</td>
<td>10</td>
<td>Building and cleaning of dams/swimming pools</td>
<td>+</td>
<td>Sput +</td>
<td>None</td>
<td>Unk (HIV, TB, amoxicillin-clavunate, cotrimoxazole)</td>
<td>Survived</td>
</tr>
<tr>
<td>Case no.</td>
<td>Hospital, admission date</td>
<td>Sex, age, y</td>
<td>Symptom duration, d†</td>
<td>Potential exposure to contaminated water source</td>
<td>HIV</td>
<td>TB‡</td>
<td>Risk factors§</td>
<td>Hospital duration, d (treatment¶)</td>
<td>Outcome</td>
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<tr>
<td>----------</td>
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</tr>
<tr>
<td>T15</td>
<td>Tshepong, 5 Dec 2013</td>
<td>M, 38</td>
<td>3</td>
<td>None known</td>
<td>+</td>
<td>Sput –</td>
<td>Current smoker, alcohol</td>
<td>4 (TB, cotrimoxazole, amoxicillin-clavunate)</td>
<td>Survived</td>
</tr>
<tr>
<td>T16</td>
<td>Tshepong, 16 Apr 2014</td>
<td>M, 38</td>
<td>13</td>
<td>Mining exposure</td>
<td>+</td>
<td>Sput –</td>
<td>Former smoker</td>
<td>27 (HIV, erythromycin)</td>
<td>Survived</td>
</tr>
</tbody>
</table>

*None known, patient or family member did not report exposure to any water source listed on questionnaire; TB, Mycobacterium tuberculosis infection; Unk, unknown: information could not be obtained because interviews could not be conducted; +, positive; –, negative; unav, unavailable; Sput, sputum.
†Symptom duration includes time from symptom onset until hospital admission
‡TB status was determined by sputum test with positive (Sput +) or negative (Sput –) results. Sputum test was unavailable for patient E1.
§Risk factors assessed were asthma, lung disease, kidney disease, heart disease, liver disease, diabetes, cancer, emphysema, former/current cigarette smoking, and current alcohol consumption.
¶Includes in-hospital and discharge treatment, mostly for HIV or TB infection or both.
#Legionella spp. identified with nasopharyngeal specimen.
**Retrospective epidemiologic investigation not possible because patient died or patient or family member could not be traced.