Investigating * Legionella*: PCR Screening and Whole-Genome Sequencing

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Wadsworth Center
1976 Philadelphia Legionnaires' disease outbreak

The total number of cases reached 221, 34 had died.
Investigation of Nosocomial Legionellosis Using Restriction Enzyme Analysis by Pulsed-Field Gel Electrophoresis

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Comparison of Ribotyping and Restriction Enzyme Analysis Using Pulsed-Field Gel Electrophoresis for Distinguishing Legionella pneumophila Isolates Obtained during a Nosocomial Outbreak

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Received 16 September 1991/Accepted 14 March 1992
Reported Cases of Legionellosis by year
New York

Year

# Cases

0
1000

NYS Legionellosis
NYC Legionellosis
Total

Legionella Culture Archive

2945 archived (1982-2017)

- 1643 L. pneumophila 1
- 306 L. pneumophila 6
- 176 L. pneumophila 5
- 105 L. pneumophila 4
- 105 L. anisa
- 84 L. pneumophila 3
- 50 L. micdadei
- 42 L. pneumophila 12
- 27 L. pneumophila 4/5
- 24 L. feelei
- 20 L. bozemanii
- 133 Legionella species
- 77 L. pneumophila no serotype

*55 % Lp1
80 % Lp
PFGE Analysis

- 1990 implemented (27 yrs exp.)
- 1990-2003 Lab notebooks, reports
- 2003 Bionumerics Database 1477
  - 1477 entries
  - 415 patterns
Investigating Outbreaks (1982-2017)

• Total of 668 investigation testing requests
  – Average 19 requests per year
  – 128 total facilities/locations
  – Spanning 1 time testing to multiyear sampling
  – (2-34 years)
## Historic Outbreaks with Sources Identified

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
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</thead>
<tbody>
<tr>
<td>Hospital</td>
<td></td>
</tr>
<tr>
<td>Potable water</td>
<td>20</td>
</tr>
<tr>
<td>Potable water / Cooling tower</td>
<td>1</td>
</tr>
<tr>
<td>Cooling tower</td>
<td>2</td>
</tr>
<tr>
<td>Nursing home/assisted living</td>
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</tr>
<tr>
<td>Potable water</td>
<td>10</td>
</tr>
<tr>
<td>Cooling tower</td>
<td>3</td>
</tr>
<tr>
<td>Private home</td>
<td></td>
</tr>
<tr>
<td>Potable water</td>
<td>2</td>
</tr>
<tr>
<td>Hotel</td>
<td></td>
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<tr>
<td>Potable water</td>
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</tr>
<tr>
<td>Correctional Facility</td>
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</tr>
<tr>
<td>Potable water</td>
<td>2</td>
</tr>
<tr>
<td>Residential Complex</td>
<td></td>
</tr>
<tr>
<td>Potable water</td>
<td>2</td>
</tr>
<tr>
<td>Cooling Tower</td>
<td>1</td>
</tr>
<tr>
<td>School</td>
<td></td>
</tr>
<tr>
<td>Potable water</td>
<td>1</td>
</tr>
</tbody>
</table>

Cooling tower = 7, Potable water = 39

*36% success rate for determining source 46/128
Real-time PCR testing

- Started testing in 1998 (19 yrs exp.)
- Validations for clinical testing, NYS approvals
- Side by side testing comparisons

Screen samples, confirm identification of isolates and detect in clinical specimens

- *L. pneumophila* serogroup 1
- *L. pneumophila* (1-15)
- *Legionella* spp.
- Inhibition control

- *L. anisa*
- *L. micdadei*
Design and implementation of a protocol for the detection of *Legionella* in clinical and environmental samples

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Wadsworth Center, New York State Department of Health, Albany, NY 12208, USA

Received 22 February 2008; accepted 13 May 2008

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**Table 4**

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>PCR + (N/N)</th>
<th>Culture + (N/N)</th>
<th>PCR+/culture + (%)</th>
<th>PCR+/culture - (%)</th>
<th>PCR-/culture + (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td>100% (11/11)</td>
<td>18.2% (2/11)</td>
<td>18.2% (2/11)</td>
<td>81.8% (9/11)</td>
<td>0% (0/11)</td>
</tr>
<tr>
<td>Tissue</td>
<td>100% (4/4)</td>
<td>0% (0/4)</td>
<td>0% (0/4)</td>
<td>100% (4/4)</td>
<td>0% (0/4)</td>
</tr>
</tbody>
</table>

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**Table 5**

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>PCR + (N/N)</th>
<th>Culture + (N/N)</th>
<th>PCR+/culture + (%)</th>
<th>PCR+/culture - (%)</th>
<th>PCR-/culture + (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental</td>
<td>98.6% (138/140)</td>
<td>70.7% (99/140)</td>
<td>69.3% (97/140)</td>
<td>29.3% (41/140)</td>
<td>1.4% (2/140)</td>
</tr>
</tbody>
</table>
Legionella Outbreak Communication

Legionella Case Reports

Assessment of facility
Interaction with facility
Review of maintenance records
Sampling recommendations

Environmental Health

Legionella Case Reports

Interviews
Medical record reviews
Interaction with facility, physicians
Community or HAI
Request cultures, specimens

HAI Epi

Regional Epi

Environmental Health

Discussing testing volumes, types
Preparing – ordering media, labelling
Testing timelines
Shipping, Testing info

Lab

BCDC Epi
Can whole-genome sequencing (WGS) provide additional resolution to outbreak investigations?
Ongoing pilot project with the CDC Legionella Lab AMD funding-Spring 2015

Genomic Resolution of Outbreak-Associated *Legionella pneumophila* Serogroup 1 Isolates from New York State


Respiratory Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; Wadsworth Center, New York State Department of Health, Albany, New York, USA

**ABSTRACT**

A total of 30 *Legionella pneumophila* serogroup 1 isolates representing 10 separate legionellosis outbreaks (WGS) that occurred in New York State between 2004 and 2012 were selected for evaluation of WGS approaches for molecular subtyping of this organism. Clinical and environmental isolates were initially examined by pulsed-field gel electrophoresis (PFGE). Sequence-based WGS data yielding complete sequence types (ST) for isolates representing 8 out of the 10 outbreaks. Two STs from separate outbreaks sharing the same ST also contained the fewest differences in core morphisms (SNPs) and the greatest proportion of identical allele sequences in a whole-genome (wgMLST) scheme. Both core SNP and wgMLST analyses distinguished isolates from separate outbreaks sharing indistinguishable PFGE profiles. Isolates from a hospital-associated outbreak shared indistinguishable PFGE profiles but displayed differences in their genome sequences, suggesting environmental sources. Finally, the *rtx* gene demonstrated differences in the repeat region sequence between different outbreaks, suggesting that variation in this gene may be useful for targeted molecular *pneumophila*. This study demonstrates the utility of various genome sequence analysis approaches for environmental source attribution studies while furthering the understanding of *Legionella* ecology.
Results

Maximum parsimony tree, ref seq based on 24,424 Core SNP matrix

- 2004-2006
- 2009
- 2006-2007
- 2011-2012

418 SNPs
NYS Facility Investigation

- 4 patients diagnosed in Aug/Sept 2015
- Water restrictions and remediation occurred
- November 2015 facility letter final culture
- January 2015 5\textsuperscript{th} patient diagnosed
  - No evidence that the patient spent any time in areas of facility where \textit{Legionella pneumophila} serogroup 4 (Lp4) has been recovered from potable water and no distinct water exposure could be identified.
- Patient’s Lp4 isolate was indistinguishable by PFGE from other patients at facility and two environmental cultures from the potable water system

\[\text{WGS initiated}\]
PFGE and serotyping were performed on 4 patient isolates and 3 environmental samples.

<table>
<thead>
<tr>
<th>Isolate Identifier</th>
<th>Serogroup</th>
<th>NYS Number</th>
<th>PFGE Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A (AB)</td>
<td>4</td>
<td>IDR1500008823</td>
<td>LpnS13297</td>
</tr>
<tr>
<td>Patient B (AS)</td>
<td>4</td>
<td>IDR1500062697</td>
<td>LpnS13297</td>
</tr>
<tr>
<td>Patient C (ER)</td>
<td>4</td>
<td>IDR1500064419</td>
<td>LpnS13297</td>
</tr>
<tr>
<td>Patient D (TL)</td>
<td>4</td>
<td>IDR1600003296</td>
<td>LpnS13297</td>
</tr>
<tr>
<td>Hospital A – Room D-4110</td>
<td>4</td>
<td>IDR1500065605</td>
<td>LpnS13297</td>
</tr>
<tr>
<td>Hospital B – Room D-3126</td>
<td>4</td>
<td>IDR1500065606</td>
<td>LpnS13297</td>
</tr>
<tr>
<td>Hospital C – Ice Machine East D-31</td>
<td>4</td>
<td>IDR1500065607</td>
<td>LpnS13297</td>
</tr>
</tbody>
</table>

All isolates were determined to be a serogroup 4*

PFGE results indicated the isolates were all the same strain.
• The 5th patient was actually a closer match to Patient B, Patient A, and environmental sources than Patient C was.
• All patient and environmental samples in this investigation were **1-13 SNPs** different when compared to the 5th patient and **>23,000 SNPs** different from any other of the >320 Legionella we have sequenced to date.
The Challenge of interpretation of SNP differences

- 1 SNP difference is meaningful
- <10 SNPs, <5 SNPs indicate the same source?
- Heterogeneity in sources
- Reference genome- when is this critical for interpretation of an outbreak?
Legionella Persistence in Facilities

 Facility 1

- *Legionella micdadei*
- 2600 bed Hospital in Metropolitan NY
- 10 clinical samples, 9 environmental samples, 1 unknown sample

![PFGE Fingerprint Designation]

- 0-5 SNPs from each other
- 0-3 SNPs from each other
- 20 SNPs different
Legionella Whole-genome Sequencing

South Bronx

454 Legionella to date
WGS activities

• Outbreak Investigations
• Facility Persistence
• CT Heterogeneity
• Serogroup switching
• Primary specimens
• CDC AMD wgMLST
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NYSDOH

NYS CEH/NYC CEH

CDC Pneumonia Response and Surveillance Laboratory (NCIRD/DBD/RDB)-
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Jeffrey W. Mercante, Jonas M. Winchell
Wadsworth Center New York State Department of Health

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