NGS aspect to the investigation into a *Legionella* outbreak linked to a cooling tower

*What Dangers are Lurking in Your Water? NextGen Sequencing Applications for Waterborne Threats*
Legionella

- Fastidious (specialized media)
- Slow growing (3-10 days)
- >50 species
- 15 serogroups of *L. pneumophila* accounting for 90% of cases
- Implicated in community and hospital associated outbreaks
- Usual source of exposure is aerosol from water supply
Reported Cases of Legionellosis by year

Year

# Cases


800 700 600 500 400 300 200 100 0

NYS Legionellosis
NYC Legionellosis
Total
The total number of cases reached 221, and of those, 34 had died. At the time of the outbreak, epidemiological investigation protocols did not include active participation by both the laboratory specialists and investigators.
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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Because of the ubiquity of *Legionella* isolates in aquatic habitats, epidemiologic evaluation of *Legionella pneumophila* strains is important in the investigation and subsequent control of nosocomial outbreaks of legionellosis. In this study, ribotyping and restriction enzyme analysis by pulsed-field gel electrophoresis (PFGE) were used to compare isolates of *L. pneumophila* obtained from patients and the environment during a nosocomial outbreak with unrelated control strains. Restriction enzyme analysis by PFGE resolved 14 different patterns among the *L. pneumophila* serogroup 1 and *L. pneumophila* serogroup 6 isolates involved in the study. Two of the patterns were observed in the three *L. pneumophila* serogroup 6 isolates from patients with confirmed nosocomial infections and environmental isolates from the potable water supply, which was, therefore, believed to be the source of the patients’ infections. Three more patterns that were not present in isolates from patients with legionellosis were seen in isolates from the hospital environment, demonstrating the presence of multiple strains in the hospital environment. In the outbreak, one distinct pattern occurred among the *L. pneumophila* serogroup 1 isolates from patients with nosocomial infections, suggesting a common source; however, the source could not be determined. By comparison, ribotyping generated five patterns. However, some control strains of both *L. pneumophila* serogroups 1 and 6 possessed the same ribotypes as were present in the outbreak isolates. Both techniques were used successfully to subtype the isolates obtained during the investigation of the outbreak. Furthermore, restriction enzyme analysis by PFGE was useful for subdividing ribotypes and for distinguishing strains involved in the outbreak from epidemiologically unrelated strains.

*Legionella pneumophila* has been recognized as an important cause of nosocomial pneumonia, particularly among patients with community-acquired disease in New York State, except for New York City. Data of legionellosis and, likewise, requires subtyping systems that can discriminate between isolates of the two serogroups (e.g., by the PFGE technique) (June to September). Most of the cases reported
Wadsworth Center Legionella testing

- **Real-time PCR assay** (DNA detection-viable and nonviable)
  - screen samples, confirm identification of isolates and detect in clinical specimens (1999)
- **Culture**
  - BCYEα, BMPAα, DGVP
  - 5% sheep blood in brain heart infusion (1977)
- **DFA** (direct fluorescent antibody detection) (1980s)
- **PFGE** (pulsed-field gel electrophoresis) (1990s)
- **WGS** (whole genome sequencing) (2015)
WGS on Legionella

- Ongoing pilot project with the CDC Legionella laboratory-AMD funding - Spring 2015
- Bioinformatics pipeline for WGS SNP analysis
- Retrospectively WGS characterized 10 *Legionella pneumophila* SG1 outbreaks
Laboratory tests used at the Wadsworth Center

- **Real-time PCR assay** (DNA detection-viable and nonviable)
  - screen samples, confirm identification of isolates and detect in clinical specimens
- **Culture**
  - BCYEα, BMPAα, DGVP
  - 5% sheep blood in brain heart infusion
- **DFA** (direct fluorescent antibody detection)
- **PFGE** (pulsed-field gel electrophoresis)
- **WGS** (whole-genome sequencing)
Collaboration on NYC Legionella Investigations

- Started assisting NYC in January 2015
- South Bronx Requests- July 2015
  - Splitting samples
    - PCR Screen
    - Remediation culture
    - Culture
    - PFGE
    - WGS
WC received specimens almost every day for 3 weeks.

**Cooling Tower**

**Potable Water**

**NYC**

**NYS**

**Building Owners**

**Isolates**

**Autopsy**

**Specimens**

**Major Events in South Bronx Legionnaires' Disease Outbreak**

- **DOHMH investigation starts**: Jul 20
- **DOHMH disease detectives investigate cases**: Jul 21-27
- **Cooling towers suspected; DOHMH begins sampling**: Jul 28
- **Hotel tests positive; DOHMH orders disinfection**: Jul 29
- **First Commissioner's Order issued; Hotel tested**: Jul 30
- **Hotel completes disinfection**: Aug 01
- **Last case in South Bronx cluster becomes sick**: Aug 03
- **Citywide cooling tower legislation introduced**: Aug 10
- **Commissioner orders all cooling towers in NYC to be disinfected within 14 days**: Aug 06
- **Mayor signs cooling tower legislation**: Aug 18
- **Hotel confirmed as source of outbreak**: Aug 20

**Hotel = Hotel H**

- 31 cases
- 44 cases
- 65 cases, 1 death
- 81 cases, 7 deaths
- 100 cases, 10 deaths
- 138 cases, 16 deaths

90% cases hospitalized
Environmental Source Identification- NYC

- Locate nearby cooling towers
- Owners not required to register cooling towers
- City administrative data
- Satellite imagery
- Reports from outside NYC
Contaminated Cooling Towers

Five buildings have been identified as the potential source of the Legionnaires' disease outbreak in the South Bronx.

- Possible sources of Legionnaires' outbreak
- Additional sites found with legionella bacteria
- Locations of people with Legionnaires'

Source: New York Mayor's Office
By The New York Times
Cooling Towers

- Large community outbreaks
- Heat from air conditioning or industrial process into water that may contain *Legionella*
- Fans transfer heat from pool to air, creating a fine mist
- Leads to bioaerosol dispersion (cases up to 12 km away)
Cooling tower sampling, receipt at lab, accessioning and water sample processing

327 cooling tower and potable water samples from 97 locations
DNA Extraction and In-house Developed Real-time PCR Testing to Screen Samples
## PCR Screening Results

**Available in <24 hours**

<table>
<thead>
<tr>
<th>NYS IDR#</th>
<th>Facility Name</th>
<th>Date Collected</th>
<th>Sample No.</th>
<th>Sample Type</th>
<th>Source Description</th>
<th>Real-time PCR Result (H2O)</th>
<th>Comments / mip PCR CT</th>
<th>Estimate of Legionella bacteria /ml (live or dead)</th>
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<td>7/29/15</td>
<td>51</td>
<td>cold water</td>
<td>Cooling tower -end</td>
<td>Positive for <em>Legionella pneumophilia</em> serogroup 1 DNA</td>
<td>28/29 all</td>
<td>Approx 2000 CFU/ml</td>
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<td>Cooling tower-intake</td>
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<td>Can’t rule out serogroup 1 as that target is less sensitive</td>
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<tr>
<td></td>
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<td>Positive for <em>Legionella pneumophilia</em> DNA</td>
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<td>Approx &lt;20 CFU/ml</td>
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<td>Cooling Tower-return</td>
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<td>Cooling Tower-makeup</td>
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<td>Hospital, L</td>
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<td>91</td>
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<td>Cooling Tower (Indoor) Basın makeup</td>
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<td>swab East Cell S side (CT)</td>
<td>Positive for <em>Legionella</em> species DNA</td>
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</tbody>
</table>
Legionella Culture and Confirmatory Testing for PCR-positive Samples

- Acid washing
- Heat treatment
- 4 culture plates set up

Cooling tower culture
Pulsed-field Gel Electrophoresis (PFGE)

Sequence-based Typing (SBT)

<table>
<thead>
<tr>
<th>Clinical Isolates</th>
<th>PCRplex</th>
<th>Mbs</th>
<th>fiaA</th>
<th>pilE</th>
<th>ads</th>
<th>mip</th>
<th>morpS</th>
<th>proA</th>
<th>seuA</th>
<th>ST</th>
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<td>NYPH Well Co.</td>
<td>12/26/14</td>
<td>Lp1</td>
<td>1.2*</td>
<td>1</td>
<td>4</td>
<td>3</td>
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<td>Montefiore</td>
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<td>10</td>
<td>19</td>
<td>28</td>
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<td>4</td>
<td>11</td>
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<td>Montefiore</td>
<td>5/30/15</td>
<td>Lp1</td>
<td>1.2*</td>
<td>7</td>
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<td>17</td>
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<th>Environmental Isolates</th>
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<th>Mbs</th>
<th>fiaA</th>
<th>pilE</th>
<th>ads</th>
<th>mip</th>
<th>morpS</th>
<th>proA</th>
<th>seuA</th>
<th>ST</th>
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</table>

NYC and WC

CDC
Whole-genome Sequencing
All Legionella isolates underwent Illuminia Miseq sequencing, were mapped to a reference genome, SNP calling software.

SNP calling with Samtools:
Min. Depth of 20x, 95% of the reads in agreements.
Whole-genome Sequencing

• 80 samples (clinical and environmental) were sequenced by WGS over a period of 15 days (Aug. 11th to Aug. 26th)

• Philadelphia 1 is 3.4 Mb but on average only ~ 3.01 Mb of the genome (88%) can be assessed with confidence

• Assessment of other reference genomes and analysis
However, historic isolates and East Bronx investigation complicate the story.

2 clinical and 1 environmental samples from 2011/2012 NY Nursing Care Center East Bronx patients.

August NYS College Staff Housing Cooling Tower

East Bronx patient?

1100 SNPs

0-6 SNPs
Pulsed-field Gel Electrophoresis (PFGE)

Both PFGE and SBT worked well in this outbreak, however, they could not discriminate these closely related strains.

**Sequence-based Typing (SBT)**

<table>
<thead>
<tr>
<th>Clinical Isolates</th>
<th>Date of Culture</th>
<th>PCRplex</th>
<th>Mba</th>
<th>flaA</th>
<th>pilE</th>
<th>sad</th>
<th>mpo</th>
<th>mompS</th>
<th>proA</th>
<th>seuA</th>
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NYC and WC

CDC
A second Cooling Tower detected later in the outbreak shared the same PFGE pattern

- **Shelter P**
  - \( n = 33 \)

- **Hotel H**
  - \( n = 56 \)

- Both clusters highly unlikely by chance alone
Persistent endemic strain in NYC?

Minimum Spanning Tree
Using Closed Genome South Bronx Strain

South Bronx outbreak cluster (2015)

41 Clinical and 5 Environmental Samples

Persistent endemic strain in NYC?
What if we missed other strains in the sampling and culture?

- Cooling tower samples stored at 4°C (4-5 months)
- Perform culture, LPSG1 detection
- Send all isolates to PFGE and WGS
Cooling Tower Legionella heterogeneity?

- Re-processed 46 cooling tower samples

- 170 isolates of *Legionella*
  - 124 *L. pneumophila* SG1

- 18 PFGE patterns detected
  - Shared patterns
    - LpnS13315 (EC, CP)
    - LpnS13317 (EC, LH, CP, TSC)
  - Outbreak pattern: LpnS13203
  - Shelter

- 72 Submitted for WGS

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of PFGE Patterns</th>
<th>Unique Patterns</th>
<th>Shared Patterns</th>
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<tr>
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</table>
Cooling Tower Legionella heterogeneity?

Heterogeneity was observed.

South Bronx outbreak strain was not found in a different cooling tower.
Come visit our Posters

P-032
A Collaborative Laboratory Response to Legionnaires’ Disease in New York City

Presenter: Scott Hughes, New York City Department of Health & Mental Hygiene, New York, NY, Phone: 212.447.6121, Email: shughes@health.nyc.gov

P-020
A Comprehensive Testing Approach to Detect and Characterize Legionella pneumophila serogroup 1 in Autopsy Specimens During a Large New York City Outbreak
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Liz Nazarian

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NYSDOH
NYS CEH

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Isaac Benowitz, MD