Trace Back to the “Source” — The Collaborative Effort to Detect the Source of a Legionella Outbreak in Minnesota

David Boxrud, Enterics Unit Supervisor, Acting Sequencing and Bioinformatics Unit Supervisor, Minnesota Department of Health
Confirmed Legionnaires’ Disease Cases
Minnesota, 2004 – 2016

Number of Cases

0 20 40 60 80 100 120
17 34 26 30 25 27 36 28 51 50 58 51 115
Challenges with Legionnaires’ Outbreak Detection

• The most common diagnostic method- *Legionella* urinary antigen test
  • culture-independent diagnostic method
  • rarely are there isolates available to subtype to see if cases may be related

• Cooling tower cases can be dispersed in a community
Hopkins Outbreak: Timeline

• Mid-August: Case 1, symptom onset August 4, reported to MDH. Case was a Hopkins, MN resident who traveled for work

• September 7-8, three cases reported to MDH with symptom onset the last week of August. 2 cases were Hopkins residents, 1 case worked in Hopkins

• September 9, 5th case reported, Hopkins resident with onset the last week of August

• Cases did not share a common exposure history
Hopkins Outbreak: September 9
News Release
September 9, 2016

Contact Information

Legionnaires’ disease investigation underway

The Minnesota Department of Health is working with Hennepin County Public Health officials to investigate a cluster of five confirmed cases of Legionnaires’ disease in people who live or work in Hopkins, Minn.

The people became ill between Aug. 4 and Sept. 1. Three are currently hospitalized, and two others were hospitalized and have recovered. The patients are all over the age of 50.

MDH and Hennepin County are still investigating the source of this outbreak. Legionnaires’ disease is spread by inhaling the fine spray (aerosols) from water sources containing Legionella bacteria. It is not spread person to person and you cannot get it by consuming water. Outbreaks of Legionnaires’ disease have been linked to cooling towers (air-conditioning units for large buildings), cooling misters, decorative fountains or plumbing systems.

Minnesota typically sees 50 to 60 cases of Legionellosis each year. More than 60 cases have been reported in the state so far this year, mirroring a national increase in cases in 2016. No other clusters have been reported in Minnesota this year.

Most people exposed to Legionella don’t develop Legionnaires’ disease. People over the age of 50, smokers, or those with certain medical conditions, including weakened immune systems, chronic lung disease or other chronic health conditions, are at increased risk for Legionnaires’ disease. Symptoms of Legionnaires’ disease include muscle aches, chills, shortness of breath,
Summary of 23 Outbreak Cases

- 23 cases from Aug 4-Sep 22
- Age range: 29 – 97 years (median age 59 years)
- Male: 17/24 (71%)
- Hospitalized: 18 (75%)-1 death.
  - 6 (35%) required mechanical ventilation.
- Serious long-term sequelae for cases included stroke and kidney failure.
- All cases were positive by urine antigen test; four also had positive cultures of respiratory samples
- All lived, worked, or spent time in Hopkins
Lab Testing

• Identify and characterize cases
• Identify environmental sources
• Compare clinical and environmental isolates
Lab Testing

- Clinical cases-culture
- Environmental-molecular (New York-Wadsworth)
  - Environmental-culture (MDH)
- Clinical/environmental-subtyping
  - PFGE
  - WGS
Water Testing-New York

- Multiplex PCR
  - *Legionella* species
  - *L. pneumo* 1
  - *L. pneumo* 1-15
- Negative - testing complete
- Positive - perform culture
Culture of Water at MDH

Water Spin

BCYE GPAV

Acid Washed

BCYE GPAV

25 ml

Inc. 2 weeks at 37C

Read at 24 hr, 72 hr, every other day, neg at 14 day

Suspicious colonies-small, purplish, 72 hrs for visible growth
Summary of Environmental Testing

• 16 Environmental sources were investigated

• 1 grocery store mister: tested negative (initial cases reported exposure)

• 1 decorative fountain: shut down, unlikely to be the source due to location and enclosed area
• 14 cooling tower sites
  • 7 remediated without testing
    • 1 inaccessible for testing
    • 2 just outside of 1 mile radius
    • 4 identified after remediated and late in the outbreak (10/7)
  • 7 tested
    • 3 cooling towers were positive for Lp1
      • 1 cooling tower had matching PFGE to clinical isolates
    • 2 positive for Lp1 with a different PFGE strain
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>LPS Type</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2016014994-3</td>
<td>LPS1</td>
<td>Human</td>
</tr>
<tr>
<td>M2016015185-6</td>
<td>LPS1</td>
<td>Enviro. Water CS-01A-01G</td>
</tr>
<tr>
<td>M2016015186-5</td>
<td>LPS1</td>
<td>Enviro. Water CS-02A-02G</td>
</tr>
<tr>
<td>M2016015187-3</td>
<td>LPS1</td>
<td>Enviro. Swab CS-01</td>
</tr>
<tr>
<td>M2016015188-4</td>
<td>LPS1</td>
<td>Enviro. Swab CS-02</td>
</tr>
<tr>
<td>M2016014548-1</td>
<td>LPS1*</td>
<td>Human</td>
</tr>
<tr>
<td>M2016014532-3</td>
<td>LPS2*</td>
<td>Enviro. Water City Hall</td>
</tr>
</tbody>
</table>
WGS during Hopkins Outbreak

- Highly important organism/outbreak
- Critical to identify the source
- PFGE does not always produce enough discrimination/specificity
  - *Salmonella* Enteritidis (Taylor et. al., JCM, 2015)
  - Legionella (Raphael et. al., Appl Env Micro, 2016)
WGS of *Legionella*

- Contact Brian Raphael (CDC)
- Sequencing conditions and analysis
Whole Genome Sequencing

- Legionella isolates-BCYE plates
- **Extraction**-Qiagen QIAamp DNA Blood Mini Kit-Qiagen QIAcube (RNase A treated prior to extraction)
- **Quantification**-Qubit HS dsDNA Kit
- **Sequencing** wet lab-Illumina MiSeq (Nextera XT Sample Prep kit, 500 cycle V2 Standard Reagent Kit)
- FastQ files transferred to CDC by FTP
- hqSNP and wgMLST analysis
- Turnaround time for analysis: Usually only a few hours
### wgMLST Results

<table>
<thead>
<tr>
<th>Outbreak</th>
<th>Sample_ID</th>
<th>Serogroup</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN-16</td>
<td>M2016014532-3</td>
<td>Lp1</td>
<td>Environmental</td>
</tr>
<tr>
<td>Reference</td>
<td>Philadelphia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MN-16</td>
<td>C2016014069</td>
<td>Lp1</td>
<td>Clinical</td>
</tr>
<tr>
<td>MN-16</td>
<td>C2016014870</td>
<td>Lp1</td>
<td>Clinical</td>
</tr>
<tr>
<td>MN-16</td>
<td>M2016014548-1</td>
<td>Lp1</td>
<td>Clinical</td>
</tr>
<tr>
<td>MN-16</td>
<td>M2016014994-3</td>
<td></td>
<td>Clinical</td>
</tr>
<tr>
<td>MN-16</td>
<td>M2016015185-6</td>
<td></td>
<td>Environmental</td>
</tr>
<tr>
<td>MN-16</td>
<td>M2016015185-5</td>
<td></td>
<td>Environmental</td>
</tr>
<tr>
<td>MN-16</td>
<td>M2016015187-3</td>
<td></td>
<td>Environmental</td>
</tr>
<tr>
<td>MN-16</td>
<td>M2016015188-4</td>
<td></td>
<td>Environmental</td>
</tr>
</tbody>
</table>
Hopkins Outbreak, Lessons Learned

• Difficulty in identifying cooling towers
• Difficulty conducting environmental sampling
  • Sampling materials generally not stocked
  • Difficult to access locations, industrial equipment
• Laboratory issues
  • Overgrowth of non-Legionella spp.
  • Legionella spp. are slow-growing, PCR was useful for epidemiologic investigation
  • PFGE and WGS useful for source identification
    • PFGE faster
    • WGS definitive
• Collaboration crucial to effective response
  • Epi, NY, CDC
Acknowledgements

• MDH
  – Angie Taylor, Sean Wang, Ellen Laine, Paula Snippes, PFGE lab, outbreak investigation staff (lab and epi)

• CDC
  – Brian Raphael, NGS Legionella group

• New York State Department of Health