Epidemiology of legionellosis

June 25, 2012

Adriana Peci, Ellen Chan, Christine D’Souza, and Shilpa Raju
Outline

• Overview of *Legionella*
• Legionellosis in Ontario
• Laboratory testing for *Legionella*
• Exposure sources
• Risk factors
• Summary
What is *Legionella*?

- A gram negative bacteria, ubiquitous in the environment
- An intracellular bacteria that requires protozoa (amoebas) for survival and growth
- 52 species and 70 serogroups identified
- *L. pneumophila* sg. 1 accounts for over 60%, 90% and 70% of legionellosis cases in Ontario, USA, and Europe, respectively
What causes Legionella?

- *Legionella* species are implicated in two clinical syndromes grouped together as legionellosis: Pontiac Fever and Legionnaires’ disease
  - Both are reportable

- Different levels of virulence are associated with different serotypes; host immunity also affects disease severity.
Pontiac Fever

- Mild, self-limiting form of the disease
- Symptoms include fever, anorexia, malaise, lethargy and diarrhea
- Incubation period 5 hours to 3 days
- High attack rate (up to 95%) of those exposed during community outbreaks and no deaths were reported among cases manifesting Pontiac fever symptoms\(^2\)
Legionnaires’ Disease

• Frequent (unrecognized) cause of community-acquired pneumonia

• Second most common cause of pneumonia-related admission to ICU

• Incubation period of 2-10 days$^2$

• Low attack rate (up to 5%)$^2$

• High case fatality rate (5-40%); up to 50% in nosocomial outbreaks$^2$

• Complications include kidney/respiratory failure and septic shock
Factors that favour the growth of *Legionella*

- Warm temperatures
  - Can survive and multiply in water at temperatures of 25–45 °C for several hours and do not multiply below 20 °C
- Humidity
  - High humidity increases the likelihood of *Legionella* survival
- Stagnant water
  - Low water flow rates have been reported to also increase the growth of *Legionella* in water systems
- Biofilm helps the survival of *Legionella*
Mode of transmission

- Inhalation of contaminated aerosols (water droplets in the air)
  - Aerosol generating devices are potential sources of *Legionella* (e.g. cooling towers, air conditioners, etc.)
  - *Legionella* infections have been linked to sources at distances of up to 3.2 km
  - Respiratory therapy devices can harbor *Legionella*

- Not transmitted from person-to-person
Incidence of legionellosis in Ontario (2002-2011) as compared to Canada and Europe (2002-2008)

Source (Ontario data): Ontario Ministry of Health and Long-Term Care, integrated Public Health Information System (iPHIS) database, extracted by Public Health Ontario [2012/05/25]
Source (Ontario population): IntelliHEALTH Ontario, extracted by Public Health Ontario [2012/01/13]
Source (Canada): Public Health Agency of Canada, Canadian Notifiable Disease Section, Surveillance and Risk Assessment Division, Centre for Communicable Diseases and Infection Control
Source (Europe): European Working Group for Legionella Infections (EWGLI)
Legionellosis in Ontario, 2011

• In Ontario, 162 confirmed cases of legionellosis were reported in iPHIS in 2011

• The majority of the cases (71%, 115/162) were identified as Legionnaires’ disease based on the diagnosis of pneumonia

• Age and gender description of cases:
  • Male to female ratio is 2.7:1 (117:44)*
  • Average age is 62 years
  • Median age is 61 years (Range 28-91)

• Majority of the cases (65%, 106/162) were reported in Toronto, Peel, Hamilton and Durham health units

*One case had missing gender information
Incidence of legionellosis by age and sex: Ontario, 2011

Source: Ontario Ministry of Health and Long-Term Care, integrated Public Health Information System (iPHIS) database, extracted by Public Health Ontario [2012/05/25]
Source (Ontario population): IntelliHEALTH Ontario, extracted by Public Health Ontario [2012/01/13]
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Legionellosis by month: Ontario, 2007-2011

Source: Ontario Ministry of Health and Long-Term Care, integrated Public Health Information System (iPHIS) database, extracted by Public Health Ontario [2011/11/14] and [2012/05/25]
Possible reasons for recent increases in incidence in Ontario

- Changes in local environmental conditions
  - Higher summer temperatures leading to increased air conditioner use
  - Differences in rainfall and humidity compared to previous years
    - A study has found that hydrological changes in the local watershed may influence the risk of legionellosis
- Improved recognition and diagnosis of the disease by clinicians may be associated with increase in testing and consequently improve detection and reporting
  - Ease of use of noninvasive urine antigen detection method
Percentage of legionellosis cases by public health unit: Ontario, 2009-2011 (Top 5 in 2011)

Source: Ontario Ministry of Health and Long-Term Care, integrated Public Health Information System (iPHIS) database, extracted by Public Health Ontario [2012/05/25]
Incidence rates of legionellosis by public health unit: Ontario, 2009-2011 (Top 5 in 2011)

Source: Ontario Ministry of Health and Long-Term Care, integrated Public Health Information System (iPHIS) database, extracted by Public Health Ontario [2012/05/25]

Source (Ontario population): IntelliHEALTH Ontario, extracted by Public Health Ontario [2012/01/13]

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Percent positivity of *Legionella* cases by public health unit, PHOL, 2010-2011

<table>
<thead>
<tr>
<th>PUBLIC HEALTH UNIT</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
<td>Tested</td>
</tr>
<tr>
<td>ALGOMA DISTRICT</td>
<td>1</td>
<td>80</td>
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<tr>
<td>BRANT COUNTY</td>
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<tr>
<td>CHATHAM-KENT</td>
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<tr>
<td>DURHAM REGIONAL</td>
<td>8</td>
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<td>ELGIN-ST. THOMAS</td>
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<td>EASTERN ONTARIO</td>
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<td>HALIBURTON-KAWARTHA-PINE RIDGE DISTRICT</td>
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<td>HASTINGS AND PRINCE EDWARD COUNTIES</td>
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<td>LEEDS-GRENVILLE AND LANARK DISTRICT</td>
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<td>MIDDLESEX-LONDON</td>
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<td>137</td>
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<tr>
<td>NIAGARA REGIONAL AREA</td>
<td>3</td>
<td>86</td>
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*Source:* Public Health Ontario Laboratory [2012/05/30]
Percent positivity of *Legionella* cases by public health unit, PHOL, 2010-2011 (con’t)

<table>
<thead>
<tr>
<th>PUBLIC HEALTH UNIT</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
<td>Tested</td>
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<tr>
<td>NORTH BAY PARRY SOUND DISTRICT</td>
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<td>OXFORD COUNTY</td>
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<tr>
<td>PERTH DISTRICT</td>
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<tr>
<td>PEEL REGIONAL</td>
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<td>PORCUPINE</td>
<td>1</td>
<td>14</td>
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<td>PETERBOROUGH COUNTY-CITY</td>
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<td>54</td>
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<td>RENFREW COUNTY AND DISTRICT</td>
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<td>7</td>
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<td>SIMCOE MUSKOKA DISTRICT</td>
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<td>SUDBURY AND DISTRICT</td>
<td>3</td>
<td>52</td>
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<td>THUNDER BAY DISTRICT</td>
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<tr>
<td>TIMISKAMING</td>
<td>1</td>
<td>11</td>
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<tr>
<td>WATERLOO</td>
<td>3</td>
<td>148</td>
</tr>
<tr>
<td>WELLINGTON-DUFFERIN-GUELPH</td>
<td>2</td>
<td>89</td>
</tr>
<tr>
<td>WINDSOR-ESSEX COUNTY</td>
<td>2</td>
<td>51</td>
</tr>
<tr>
<td>YORK REGIONAL</td>
<td>5</td>
<td>364</td>
</tr>
<tr>
<td>OUT OF PROVINCE</td>
<td>5</td>
<td>506</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>122</strong></td>
<td><strong>5,319</strong></td>
</tr>
</tbody>
</table>

*Source: Public Health Ontario Laboratory [2012/05/30]*
Exposure sources reported in the literature

- Natural and artificial water sources:
  - Lakes, rivers and streams
  - Cooling towers
  - Spas, swimming pools and hot tubs
  - Decorative fountains
  - Showers
  - Nebulizers
  - Potting soil and compost

Source: Legionella and the prevention of legionellosis, WHO 2008; Available at: http://www.who.int/water_sanitation_health/emerging/legionella.pdf
Exposure sources among reported cases (iPHIS, 2011)

- Cases reported a variety of potential exposures in iPHIS, including but not limited to:
  - Institutions (e.g. long-term care homes)
  - Private homes: taps, showers and air conditioners
  - Misting equipment in grocery stores or on farms
  - Environmental water (e.g. streams, creeks, and lakes)
  - Construction sites
  - Travel outside of Canada

Source: Ontario Ministry of Health and Long-Term Care, integrated Public Health Information System (iPHIS) database, extracted by Public Health Ontario [2012/05/25]
Evidence about exposure sources (potting soil)

• In a soil survey performed in 1989 to 1990 in Australia: 8
  • 33 (73%) of 45 potting soil samples tested positive for *Legionella* and 26 (79%) of the 33 contained *L. longbeachae*.

• A case of *L. longbeachae* was reported from a patient’s sample who had been working in the garden prior to symptom onset in the Netherlands: 10
  • The same strain of patient’s isolate *L. longbeachae* was identified in the potting mix of patient’s garden. The genotype of both isolates was indistinguishable.
Evidence about exposure sources (store misting devices)

- In a case control study, 33 cases of Legionnaires' disease were identified among hospitalized patients with pneumonia in Louisiana:
  - Patient ages ranged from 36 to 88 years old
  - Cases were more likely than controls to report shopping at one grocery store in the 10 days before illness: Odds ratio (OR)=11.6; 95% Confidence Interval (CI)=2.4-108
  - Cases were also more likely to spend more than 30 minutes in the store (OR=8.6; CI=1.5-86.3) and to buy products located close to an ultrasonic mist machine
  - *Legionella pneumophila* sg 1, subtype 1,2,5,6, was identified in both patients’ respiratory samples and water in the reservoir of the mister.
Risk factors reported in the literature

- Age greater than 50 (IRR 9.54, 95% CI 8.41 to 10.81).
- Male more likely than females (IRR 1.57, 95% CI 1.41–1.76)
- Heavy alcohol consumption
- Smoker
- Underlying chronic conditions such as:
  - Cancer
  - Chronic obstructive pulmonary disease
  - End-stage renal disease
  - Diabetes
  - Receipt of immunosuppressant therapy (e.g. corticosteroid therapy)
## Risk factors among reported cases (iPHIS, 2011)

### Medical risk factors*** among confirmed cases of legionellosis, Ontario: 2011 (n=99)*

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Number of Cases*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic illness/underlying medical condition**</td>
<td>99</td>
</tr>
<tr>
<td>Immunocompromised</td>
<td>31</td>
</tr>
<tr>
<td>Reported use of respiratory therapy equipment</td>
<td>5</td>
</tr>
<tr>
<td>Other medical risk factors</td>
<td>24</td>
</tr>
<tr>
<td>Risk factor reported as unknown</td>
<td>14</td>
</tr>
</tbody>
</table>

* No total provided as cases may report greater than one risk factor. Medical risk factors were not reported for 41% (67/162) of cases.
** Includes cases reporting diabetes and chronic lung disease
*** The risk factors summarized are those provided under the heading ‘Medical Risk Factors’ under the ‘Risks’ tab in iPHIS.

*Source:* Ontario Ministry of Health and Long-Term Care, integrated Public Health Information System (iPHIS) database, extracted by Public Health Ontario [2012/05/25]
### Risk factors among reported cases, 2011 (cont’d)

**Behavioural risk factors** among confirmed cases of legionellosis, Ontario: 2011 (n=122)*

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Number of Cases*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker</td>
<td>72</td>
</tr>
<tr>
<td>Recent exposure to aerosolized water, e.g. water fountain, stream, lake, carwash, bath/shower</td>
<td>27</td>
</tr>
<tr>
<td>Gardening/disturbing soil</td>
<td>20</td>
</tr>
<tr>
<td>Alcohol abuse</td>
<td>14</td>
</tr>
<tr>
<td>Travel outside province in the last 2-10 days</td>
<td>14</td>
</tr>
<tr>
<td>Hot tub/spa user</td>
<td>9</td>
</tr>
<tr>
<td>Other behavioural risk factor</td>
<td>25</td>
</tr>
<tr>
<td>Risk factor reported as unknown</td>
<td>22</td>
</tr>
</tbody>
</table>

*No total provided as cases may report greater than one risk factor. Behavioral risk factors were not reported for 25% (40/162) of cases.

**The risk factors summarized are those provided under the heading ‘Behavioural Social Factors’ under the ‘Risks’ tab in iPHIS.**
Non-traditional at risk populations

• Some environmental working/living conditions might put someone non-typically vulnerable at increased risk for developing legionellosis
  • Construction workers
  • Car wash attendant
  • Truckers

2
11
12,13
Non-traditional at risk populations (cont’d)

- Legionellosis reported in a construction worker in London, UK was associated with concrete production:\n  - Warm water from a storage tank was used with a concrete batcher to help the chemical process during cold weather.
  - Both isolates from the patient and the water source taken from the storage tank were found to be *L. pneumophila* sg 1, indistinguishable from each other.
Severe outcomes: Ontario, 2011

- Hospitalization was reported for 79% of legionellosis cases (128/162) reported in iPHIS
- Fifteen cases (9%) died

![Graph showing number of tests per year for different methods.]

Source: Public Health Ontario Laboratory [2012/05/30]

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Testing methods and case identification

• Urine antigen detection is the most commonly used testing method

• Advantage: Samples can be easily obtained from patients

• Limitations:
  • Exclusively detects *L. pneumophila* sg.1 but not other *Legionella* serotypes
  • Urine samples cannot be cultured
    • Cannot link human case/s to environmental source/s for cluster detection or source identification

• Diagnosis using lower tract respiratory specimens is recommended
  • Better detection of disease caused by other serogroups or species of *Legionella*
  • Improved source identification $^2$ (can link human and environmental samples)
Summary

• *Legionella* can cause severe disease (Legionnaires’ disease), especially in at-risk populations

• Legionellosis follows a seasonal trend in Ontario with most endemic cases observed from July through to the end of October

• Overall, the number of cases reported has been increasing since 2009

• Improved awareness and more testing may partially account for the increase

• Lower respiratory tract specimens enables improved detection of *Legionella spp.* and the ability to link to environmental sources
Acknowledgments

Patrick Tang
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Jonathan Gubbay
Natasha Crowcroft

Ontario Public Health Units
Respiratory Legionella Department at PHOL-Toronto
Thank you!
2-Legionella and the prevention of legionellosis, WHO 2008; Available at: http://www.who.int/water_sanitation_health/emerging/legionella.pdf
Reference (con’t)


Legionella Environmental Aspects

June 25th, 2012

Ray Copes, MD, MSc
Chief, Environmental and Occupational Health
Legionella

- *Legionella* is ubiquitous in natural and man-made aquatic environments worldwide

- Incubation period for *Legionella* in human body is approximately 2-10 days (Heymann, 2004; WHO, 2007; Fraser, 2005)

- Multiple factors can influence the growth and aerosolization of *Legionella*

- *Legionella* isn’t ‘new’; yet our discovery of it is relatively recent
**Legionella**

- Mode of transmission - inhalation of aerosol, aspiration from oropharynx
- ‘Environmental disease’ in the sense that very few diseases are; although host factors are important in
- A disease of the ‘built environment’?
- Hot springs only natural source of infection?
- Relatively little info on infections in developing countries – underdiagnosis? Scant surveillance? Less water infrastructure and less disease?
Environmental Risk Factors

• Temperature one of the most important influences

• *Legionella* can survive and multiply between the temperatures 25-45°C *(WHO, 2007)*

• Optimal temperature between 32-42°C *(WHO, 2007)*

• Destroyed almost instantly at 70°C *(WHO, 2007)*

• Cooling towers, hot and cold-water systems, spa pools and humidifiers have similar temperature ranges
Environmental Risk Factors

• *Legionella* thrive in biofilms (WHO, 2007)

• Biofilms help *Legionella* survive under extreme conditions

• Biofilms are more likely to form:
  • in pipe systems with scale or corrosion
  • areas of low water flow and/or stagnation
Environmental Risk Factors

• Materials used within a system also a factor (WHO, 2007)
  • Rubber gaskets provide a rich nutrient base
  • Cross-linked polyethylene, PVC > Cu?

• Non-touch faucets were more likely to be positive for *Legionella* (WHO, 2007)
Routine sampling

• Mixed evidence on benefit of routine water sampling

• Some argue little correlation between human health risk and test results (WHO, 2007)

• Need to distinguish between the community and health care facilities, case fatality rate higher in hospital acquired infection

• Some suggest that need to conduct routine water sampling is unclear unless it is an area with transplant patients or immunosuppressed patients (O’Neill & Humphreys, 2005)
• Others argue for routine water sampling with treatment if more then 30% of samples in a facility test positive (ACDH, 1997)
  • One positive result does not necessarily mean an increase in the likelihood of infection
  • Some literature has shown a relationship between colonization of hospital ware supplies and hospital-acquired Legionella (Stout et al., 2007)
Controversy

- US CDC and Allegheny County Dept Health /Pittsburgh researchers
- Should surveillance in health care facilities be directed to detecting illness or proactive monitoring of potable water supply and corrective action if > 30% of distal samples are positive.
- Veterans Administration hospitals have adopted algorithm that includes water sampling.
Recommendations on environmental sampling when clinical illness occurs

• Ontario Infectious disease protocol recommends that environmental sampling be reserved for disease clusters or outbreaks (MOHLTC, 2009)

• Other guidelines suggest that sampling be performed in circumstances even when a single case is found
  • E.g. Single hospital-acquired case (New Zealand Ministry of Health, 2011)
Evidence on criteria for sampling

- Number and type of samples taken determined on individual system basis

- Research suggests a need for multiple water samples (WHO, 2007)
  - Can see variation in legionella sample results from same source around the same time
    - Bacteria are not continuously shed
    - False negative results are possible
Institutionally acquired vs. Community acquired

• Reasonable to distinguish

• Case fatality rate

• Probability of common source of exposure for other individuals at higher risk of infection with *Legionella*

• Size of building and its water supply as a risk factor?
Control of *Legionella*

- Maintenance of temperatures outside the 20–50°C temperature range can help reduce risk from *Legionella*
  - Regulations designed to reduce the risk of scalding may require that hot water temperatures be kept below 50°C

- Biofilm prevention is an important control measure against proliferation of *Legionella*
  - Biofilms are difficult to remove once established

- Eliminating stagnant water in unused piping systems (“dead ends”)

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Disinfection Measures

• **Copper-Silver ionization** (Lin, 2011; WHO, 2007; Zhang, 2009)
  • The most well studied and effective control method for legionella in hospital water systems
  • Easily installed and maintained
  • Hospitals report cooper-silver ion resistant *L. pneumophila* a few years after installation of the system

• **Monochloramine** (Lin, 2011)
  • Effective against biofilm-associated *Legionella* in model plumbing systems
  • Provides a stable residual that penetrates biofilms
  • Has a wider working pH range than copper-silver ionization and chlorine
  • No long-term studies
Disinfection Measures

• Ultraviolet (UV) radiation system (Lin, 2011)
  • Point-of-entry
  • No chemicals are added to the drinking water
  • Effectiveness against *Legionella* within the biofilm is very limited
  • Does not provide a residual effect

• Ozone (Blanc, 2005; EPA, 2001)
  • Instantaneously inactivates *Legionella*
  • Short half-life and decomposes quickly back to oxygen
  • Does not provide a residual effect
  • Point of application
Disinfection Measures

- **Hyperchlorination** (Lin, 2011)
  - Could have inadequate penetration of the agent into biofilms in piping
  - Persistence of the organisms after treatment
  - Corrosion of the water distribution system
  - Creation of potentially harmful by-products in the water

- **Chlorine dioxide** (Lin, 2011; Zhang, 2009)
  - Penetration into biofilms is superior to chlorine
  - Biocidal action is maintained over a wider range of pH than chlorine and copper-silver ionization
  - Challenge to maintain effective residual
  - Prolonged duration is necessary
  - Corrosion of the water distribution system
  - Creation of potentially harmful by-products in the water
**Legionella** and water systems

- Analogy with indoor mould? Cyanobacterial blooms?
- May be tempting to draw analogy with *E. coli* in drinking water but this is likely misleading
- Need for ‘ecosystem’ approach?
- Control vs eradication
Implications

- Is there a need to review surveillance for *Legionella* and approach to investigation of *Legionella* infections in Ontario in light of current evidence and practices elsewhere?
- Should focus be on ‘high risk environments’, ‘high risk populations’ or a combination?
- Risk of infection or risk of fatal outcome?
- Improved surveillance by itself achieves little, unless coupled to decision and action.
Resources

Thanks to: Amira Aker, Emily Peterson
Questions?

ray.copes@oahpp.ca
Environmental Laboratory Testing for *Legionella* spp.

Vanessa G. Allen, MD FRCPC

June 25, 2012

Legionella Workshop: Testing, Control and More
Environmental Testing for *Legionella spp.*: Main Challenges

- Lack of uniform standards for acceptable levels of environmental *Legionella spp.*
  - Legionellosis is secondary to environmental exposure
  - *Legionella spp.* are ubiquitous in the environment
Environmental Testing for *Legionella* spp.: Main Challenges

• Focus on link between human illness and environmental contamination via molecular typing
  • Multiple exposures to water
  • Need for culture to do clinical typing
    • Clinical testing is predominantly achieved through urinary antigen testing
Overview of Environmental Testing for *Legionella* spp.

- When to perform environmental testing for *Legionella* spp.
- Sampling techniques
- Environmental testing methods
- Interpretation of results
Why and When to Perform Environmental Testing for *Legionella* spp.
Environmental *Legionella spp.* Testing as a Tool for Outbreak Investigation: Flower Show 1999

![Bar chart showing dates of onset of illness in 186 cases of Legionnaires' disease, February 16–March 18, 1999.](chart.png)
Water Testing Sites During the Investigation
Environmental *Legionella* spp. Testing as a Tool for Outbreak Investigation

- 145 environmental samples from 12 sites
  - 3 positive: 2 whirlpools and the sprinkler system
Routine Environmental *Legionella* spp. Testing

• Aim is prevention
  • Eradication of *Legionella* spp. in high risk environments
    • Hospitals
    • Long term care facilities
    • Hotels
  • Potential disadvantages
    • Cost
    • Side effects of treatment (eg scalding)
  • No guidelines for routine testing in North America
Mandatory Testing as Part of Water Safety Plan in France

<table>
<thead>
<tr>
<th>Box 6.1 Example of limit values for <em>Legionella</em> concentrations and microbiological indicators in water used in health-care settings in France</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Limit values</strong></td>
</tr>
<tr>
<td>For patients with classical individual risk factors such as the elderly, those with alcoholism or tobacco addiction:</td>
</tr>
<tr>
<td>• target level</td>
</tr>
<tr>
<td>• alert level</td>
</tr>
<tr>
<td>• maximum level</td>
</tr>
<tr>
<td>For high-risk patients, such as those with severe immunodepression, transplantation, corticotherapy with an equivalent dose of 0.5 mg/kg per day prednisolone for 30 days or more, or 5 mg/kg per day for 5 days or more:</td>
</tr>
<tr>
<td>• target level</td>
</tr>
<tr>
<td>• alert level</td>
</tr>
</tbody>
</table>

Wide Variability of Guidelines for Environmental Standards for *Legionella*

**OSHA Technical Manual**

**Action 1:** Prompt cleaning and/or biocide treatment of the system.

**Action 2:** Immediate cleaning and/or biocide treatment. Take prompt steps to prevent employee exposure.

**Table III:7-1. Colony forming units (CFU) of *Legionella* per milliliter**

<table>
<thead>
<tr>
<th>Action</th>
<th>Cooling tower</th>
<th>Domestic water</th>
<th>Humidifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1,000</td>
<td>100</td>
<td>10</td>
</tr>
</tbody>
</table>

Restricted to testing when clinical cases identified

- Occupational Safety & Health Administration, 1999
  [http://www.osha.gov/dts/osta/otm/otm_iii/otm_iii_7.html#app_iii:7_4](http://www.osha.gov/dts/osta/otm/otm_iii/otm_iii_7.html#app_iii:7_4)
Collecting Environmental Samples for *Legionella* spp. Testing
Principles of Environmental Sampling for *Legionella* *spp.*

- Water system knowledge +/- engineer
- Epidemiologically related to case/outbreak
  - May consider use of a hypothesis generating questionnaire
    Eg http://www.cdc.gov/legionella/files/hypothesis-generating-questionnaire.pdf
- Conditions that favour the growth of *Legionella*
  - Stagnation
  - Temperatures between 20° and 50° C
  - pH between 5.0 and 8.5
  - Sediment (biofilm)
  - Micro-organisms which supply essential nutrients for growth of Legionella or harbor the organism
- Sites with potential for aerosolization
- Safety of person collecting samples

Conditions adapted from: Occupational Safety & Health Administration 1999
http://www.osha.gov/dts/osta/otm/otm_iii/otm_iii_7.html#app_iii:7_4
Potential Sites of Collection (eg hospital)

- **Potable water system**
  - incoming water main
  - water softener
  - holding tanks, cisterns
  - water heater tanks (at the inflows and outflows)

- **Potable water outlets**
  - faucets or taps
  - showers

- **Humidifiers**
  - bubblers for oxygen
  - water used for respiratory therapy equipment

- **Cooling tower, evaporative condenser**
  - basin (i.e., area under the tower for collection of cooled water)
  - sump (i.e., section of basin from which cooled water returns to heat source)
  - heat sources (e.g., chillers)

- **Other sources**
  - decorative fountains
  - irrigation equipment
  - fire sprinkler system (if recently used)
  - whirlpools, spas
**Legionella Sampling during Outbreak Investigation**

### Table 16: Legionella – Environmental Sample Collection and Transportation Instructions

**Legionella Investigations – Water Sampling**

<table>
<thead>
<tr>
<th>Collection of Samples</th>
<th>Laboratory Requisitions:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Water samples can be collected in standard water bacteriology bottles distributed by the PHL.</td>
<td>For liquid samples collected in water bottles, use either of the following requisitions:</td>
</tr>
<tr>
<td>- One litre of water should be collected for potable water. Five standard water bottles filled to the fill line will satisfy this requirement. For non potable waters, a single bottle of 200mL is sufficient.</td>
<td>- Bacteriological Analysis of Water – Multiple Sample Requisition for Official Agencies</td>
</tr>
<tr>
<td>- Taps or showers should not be allowed to run before sampling. Turn water on and allow the water to run slowly into sample bottles.</td>
<td>- Bacteriological Analysis of Water – Single Sample Requisition for Official Agencies</td>
</tr>
<tr>
<td>- Use of Personal Protective Equipment (PPE) should be guided by public health unit internal health and safety guidelines.</td>
<td></td>
</tr>
</tbody>
</table>

From The Ontario Public Health Inspector’s Guide
Methods for Environmental Testing for

*Legionella spp.*
Methods for Environmental Testing of *Legionella*

- **Culture**
  - The “gold standard”
  - Standardized methods available
  - Relatively insensitive 500 CFU/ L
- **Requires**
  - source of iron
  - Essential amino acid, L-cysteine
- May be overgrown by environmental bacteria
- Prolonged method (up to 14 days)
- Enables molecular typing

CDC Procedures for Recovery of *Legionella* from the Environment 2005
Methods for Environmental Testing of Legionella

• Polymerase chain reaction (PCR)
  • More sensitive than culture in most instances
  • Can detect viable non-culturable organisms
  • Cannot distinguish between killed and live organisms

• Targets
  • Ribosomal RNA (rRNA)
  • Gene coding for heat-shock protein (dnaJ)
  • RNA polymerase gene (rpoB)
  • Macrophage infectivity potentiator gene (mip)

• May be able to be used to rule out potential sources

Viable but Non-culturable (VBNC) Legionella spp.

Interpretation of Environmental Testing for *Legionella* spp.
Interpretation of Results of Environmental Testing for *Legionella spp.*

- No threshold concentration of *Legionella spp.* in water source has been established for human risk

- However, the following results may be helpful to guide an investigation of Legionella infection
  - Suggestive of potential source:
    - Presence of Legionella spp. in an epidemiologically linked source
    - Number of samples that are positive
    - Similar species or molecular typing pattern to clinical samples
  - Not supportive of a source:
    - Negative PCR of environmental samples
Conclusions

• Environmental testing can help to support case/ outbreak investigation
  • Less evidence for testing as a preventative measure for risk reduction

• Sampling techniques require knowledge of water system
  • And search for sites that are favorable for the growth of *Legionella spp.*

• Changes in testing may lead to new tools for outbreak investigation
  • But difficult to interpret for routine surveillance

• Interpretation requires multidisciplinary approach
  • Including cultures from clinical specimens
Thank you... Any questions?
Clinical Testing for Legionella

Legionella Workshop, June 25, 2012

Jonathan Gubbay, Medical Microbiologist
Clinical Testing for Legionella – Talk Overview

• Discuss importance of clinical testing in diagnosis of Legionellosis.

• Review specimen collection, storage and transport.

• Summarize current laboratory testing methods
  • Urinary antigen testing
  • Direct fluorescent antibody
  • Legionella culture
  • Serology
  • PCR

• Review of legionella molecular typing – Cyril Guyard
Importance of Clinical Testing

- 0.5% to 5% of adults hospitalized for pneumonia have Legionnaire’s Disease.
- LD can’t be readily distinguished other forms of community-acquired pneumonia by clinical, radiological, or nonspecific lab studies.
  - Several attempts at developing a clinical scoring system to distinguish LD failed.
- Promptly treated LD can be cured in 95% to 99% of otherwise healthy persons.
  - Overall fatality rate is 12%
- Untreated LD results in 15% death in previously healthy, 75% of severely immunocompromised.
- Outbreak investigations rely on obtaining clinical culture isolates.
Specimen Collection: Culture specimens

- Culture of available sputum, bronchoscopy/BAL specimens, lung biopsy, pleural fluid should be routine for diagnosis – other sites tested if involved in disease process.
  - Expectorated sputum, pleural fluid - low sensitivity
  - Highest yield from lung tissue >BAL >sputum.

- Less common sources: pleural fluid, blood

- Other sites tested if specifically involved (e.g. liver, myocardium bone marrow).

- Sputum microscopic scoring criteria cannot be used to screen sputums for culture.
Specimen Collection: Culture/PCR specimens

• Respiratory tract specimens should be collected in sterile containers.

• Transportation and storage should be at 2-8C if more than several hour delay to plating.

• Very long term storage best at -70C.
  • May reduce bacterial concentration.
Specimen Collection: Urine for Antigen Detection

- Collect in a sterile container.
- Transport to laboratory at 2-8°C.
  - Freeze at 20°C if > 14 days transit (may reduce sensitivity)
- Binax Now Legionella Urinary Antigen Kit Insert:

**SPECIMEN COLLECTION**

Urine specimens should be collected in standard containers. The samples can be stored at room temperature (59-86°F, 15-30°C) if assayed within 24 hours of collection. Alternatively, specimens may be stored at 2-8°C for up to 14 days or at -10°C to -20°C for longer periods before testing. Boric acid may be used as a preservative.

When necessary, urine specimens should be shipped in leakproof containers at 2-8°C or frozen.
Specimen Collection: Blood for serum antibody detection.

- Collect in standard tubes, transport to lab at room temperature.
  - Clotted unseparated blood can be stored for several days at room temperature without affecting test performance.
  - Advise store/transport at 2C-8C.
- Long term storage at -20C.
Urine Antigen Detection

- LD due to \textit{L. pneumophila} serogroup 1 can be diagnosed by bacterial antigenuria.
  - Several immunoassays for detection of bacterial antigenuria – immunochromatographic card assay most convenient.
  - Assays target Pontiac/MAB2/MAB3-1 monoclonal subtype of serogroup 1.

- ICTs made by at least 3 companies – 2 FDA cleared (Binax NOW assay and SA Scientific, San Antonio, TX).

- One non-FDA cleared kit (Biotest, Dreieich, Germany) designed to detect non-serogroup 1 \textit{L. pneumophila}.

- ICTs may be less sensitive than microtube-based immunoassays.
Binax NOW Legionella Urinary Antigen Detection Test
NOTE: For convenience, the swab shaft has been scored and may be snapped off after closing the device. Avoid dislodging the swab from the well when doing so.
Urine Antigen Detection – Immunoassay Test

Performance

• Performance depends on:
  • pretest probability of L. pneumophila serogroup 1, Pontiac subtype (causes 90% of community acquired Legionnaire’s disease).
  • disease severity

• Detects 90-95% with LD in ICU/ventilated

• Detect 60%-70% of L. pneumophila serogroup 1 Pontiac monoclonal subtype epidemic infections.
  • 90% of sporadic pneumonia due to same subtype.

• 50% of outpatients with mild disease caused by Pontiac subtype infections.

• 40% of hospitalized with other L pneumophila serogroup 1 subtypes

• 5-40% hospitalized with infections caused by other L. pneumophila serogroups and non-pneumophila Legionella species.
Urine Antigen Detection – Immunoassay Test Performance

• May be negative during day 1 of illness
  • Severe cases likely to be positive on presentation.
  • Repeat testing on day 2-3 of illness may detect small number who were test-negative initially.

• Therapy for suspected Legionella infection should not be ceased based on a negative urine antigen test.
Urine Antigen Detection – Immunoassay Test Performance

• Specificity very high – 99 to 99.9%

• False positive tests:
  • Rheumatoid factors
  • Freeze-thawing
  • Excessive urinary sediment

• Patients with extensive Legionnaire’s disease may excrete urinary antigen for weeks to months post recovery.
Binax Urinary Antigen Detection – Immunoassay Test Interpretation

<table>
<thead>
<tr>
<th>Result</th>
<th>Recommended Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Presumptive positive for <em>L. pneumophila</em> serogroup 1 antigen in urine, suggesting current or past infection.</td>
</tr>
<tr>
<td>Negative</td>
<td>Presumptive negative for <em>L. pneumophila</em> serogroup 1 antigen in urine, suggesting no recent or current infection. Infection due to <em>Legionella</em> cannot be ruled out since other serogroups and species may cause disease, antigen may not be present in urine in early infection, and the level of antigen present in the urine may be below the detection limit of the test.</td>
</tr>
</tbody>
</table>

Positive urine antigen is sufficient laboratory evidence for case confirmation (Ontario, CDC, EWGLI)
Immunofluorescent microscopy

• Most sensitive/specific method for direct detection in tissues and sputum.
• Requires expertise by microscopist.
• Test sensitivity low compared to other methods.
• Now rarely used for direct examination.
• Satisfies laboratory criteria for “probable case” of Legionellosis in Ontario.
• Recently replaced by PCR at PHOL.
Histopathology – staining of embedded tissues

LEGIONELLA PNEUMOPHILA
silver-positive bacteria (black)

macrophages
Legionella Culture

- Gold-standard for diagnosis of Legionellosis
  - Sensitivity 90% in severe disease, 20% in mild disease.

- Amino acids, rather than carbohydrates used as energy source

- *Legionellaceae* dependent on L-cysteine for growth

- Optimal growth with iron, at 35C to 37C, narrow pH range of 6.7-6.9.

- Growth of some species enhanced in 2 to 5% CO2.

- Growth of *L. pneumophila* enhanced by addition of α-ketoglutarate to media via unknown, non-nutritive mechanism.
Culture media used to grow *Legionella* species.

### TABLE 2  Composition and selectivity of media used to grow *Legionella* spp. from clinical and environmental specimens

<table>
<thead>
<tr>
<th>Medium</th>
<th>Synonym</th>
<th>Selective agents</th>
<th>Main use</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCYEα</td>
<td>CYE</td>
<td>None</td>
<td>Clinical, culture maintenance</td>
<td>None</td>
</tr>
<tr>
<td>BMPA</td>
<td>PAC</td>
<td>Cefamandole, polymyxin B, antifungal</td>
<td>Clinical, environmental</td>
<td>Normal respiratory microbiota, 3+; enterics, 3+; yeasts, 3+; molds, 1+; <em>Legionella</em> spp., 1+ to 4+</td>
</tr>
<tr>
<td>PAV</td>
<td>VAP</td>
<td>Vancomycin, polymyxin B, antifungal</td>
<td>Clinical, environmental</td>
<td>Normal respiratory microbiota, 2+; enterics, 2+; yeasts, 3+; molds, 1+; <em>Legionella</em> spp., 1+</td>
</tr>
<tr>
<td>MWY</td>
<td>VGP</td>
<td>Vancomycin, polymyxin B, antifungal, glycine</td>
<td>Environmental</td>
<td>Normal respiratory microbiota, 2+; enterics, 2+; yeasts, 3+; molds, 1+; environmental bacteria, 2+; <em>Legionella</em> spp., 1+</td>
</tr>
<tr>
<td>CCVC</td>
<td></td>
<td>Cephalothin, polymyxin E, vancomycin, cycloheximide</td>
<td>Environmental</td>
<td>Normal respiratory microbiota, 2+; enterics, 3+; yeasts, 2+; molds, 2+; <em>Legionella</em> spp., 1+ to 4+</td>
</tr>
<tr>
<td>BCYEα-l</td>
<td></td>
<td>None (made without L-cysteine)</td>
<td>Organism identification</td>
<td><em>Legionella</em> spp., 4+ (no growth of <em>Legionella</em> spp. on this medium)</td>
</tr>
</tbody>
</table>

*Antifungal, either amikacin or natamycin antifungal compounds; normal respiratory microbiota, normal upper respiratory tract bacteria.*

*Selectivity scale range 0 to 4+: 0, does not inhibit these organisms; 1+, slight inhibition, allows about 75% growth; 2+, allows about 25 to 50% growth; 3+, allows about 10% growth; 4+, allows less than 1% growth.

PHOL sets up BCYEα, PAV, and blood agar
Legionella Cultures

- Colonies begin to appear on day 3 of incubation.
  - Rarely appear later than 5 days.
  - Some very rarely isolated Legionella require up to 14 days incubation.
  - May see growth on day 2 of heavily infected autopsy lung.

- Once L-cysteine dependence confirmed, further identification by serotyping
  - Immunofluorescence or agglutination methods.
  - Identification of *Legionella spp.* other than *L. pneumophila* and *L. pneumophila* serogroup 1 to species or serogrouping level difficult due to cross reactivities.
Legionella Cultures: Isolate identification

• Molecular identification of Legionella spp. has replaced identification techniques in many research/specialty laboratories:
  • Increased availability of inexpensive DNA sequencing.
  • 16rRNA or mip gene sequences mostly used.
    • HPA in UK has set up a mip gene database.
  • rpoB gene, 16S-23S ribosomal spacer PCR (does not require DNA sequencing).
Legionella Cultures: Isolate identification

- Molecular methods can’t be used to accurately serogroup *L. pneumophila*.
  - Sequencing *dnaJ* gene can distinguish some, but not all *L. pneumophila* serogroups.
  - Can’t distinguish some different serotypes that have similar genotypes.
Antibody Detection

• Indirect immunofluorescent assay is gold standard.
  • Test for total antibodies (some persons infected make IgM, IgG or IgA only).

• Insensitive, of low specificity unless paired acute and convalescent sera tested.
  • Best used for epidemiologic investigations.

• Optimal yield if convalescent sera collected 4, 6 and 12 weeks after onset.
  • 50% seroconvert at 2 weeks, 80%-90% by 4 weeks, remaining over additional 2 to 8 weeks.
  • Only 75% with culture-proven Legionnaire’s disease will seroconvert; higher in epidemics.
Antibody Detection

- Specificity affected by test methodology, including type of antigen used, fixation method.
  - Few commercially available serologic tests of optimal specificity/sensitivity.
- Only seroconversion to L. pneumophila serogroup 1 considered of high enough specificity for clinical use by many experts.
  - Antibodies to other serogroups and species have low specificity, not recommended by some experts.

Serology Interpretation at PHOL

- A four-fold rise in titre to greater or equal to 1:128 is indicative of recent infection
- Single or Standing titre of 1:128 is of doubtful significance, please send follow-up
- A standing or single titre of equal or greater than 1:256 is considered as presumptive evidence of infection
Molecular Detection of Legionella spp.

- Nucleic acid detection of Legionella spp. in sputum, urine and blood used in research and reference laboratories.
  - Most target macrophage infectivity potentiator (mip) gene to detect L. pneumophila.
  - Legionella spp. Most commonly detected using rRNA target, usually 16S; 23S also used.
  - At least 3 commercial assays.

- Studies show equivalent or superior sensitivity to culture.

- Sensitivities for:
  - LRT secretions: 80 to 100%
  - Serum: 30 to 50%
  - Urine: 50 to 90%
Molecular Detection of Legionella spp.

  - Reflects predominance of *L. pneumophila* serogroup 1, Pontiac subgroup in community acquired disease.

- Contamination of almost any molecular reagent with Legionella spp. nucleic acid a concern (commonly found in water).
  - False positive PCR tests for Legionella spp. Attributed to contaminated commercially produced “pure” water and nucleic acid extraction columns.
  - Molecular detection results in a case being “probable” (Ontario, Europe) or “suspected” (CDC) Legionellosis.
Legionella – Change in testing methodology to Real-Time PCR Testing

To Health Care Providers:

Effective May 28, 2012, a new PCR test for Legionella will be used for lower respiratory tract specimens. This PCR test will replace the current Direct Fluorescent Antibody (DFA) assay, and routine culture isolation which will be set up only for PCR positive specimens. The real-time PCR has 2 targets, one that detects all Legionella species, and another that detects L pneumophila.

Done on lower respiratory tract specimens – BAL, lung tissue; collect in sterile container
Molecular Detection of Legionella spp.

Dual detection of *Legionella pneumophila* and *Legionella* species by real-time PCR targeting the 23S-5S rRNA gene spacer region

G. Yang, R. Benson, T. Pelish, E. Brown, J. M. Winchell and B. Fields

Respiratory Diseases Branch, Division of Bacterial Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

*Clin Microbiol Infect* 2010; 16: 255–261

1 set of primers targeting 23S-5S rRNA spacer region
2 probes – *Legionella* species, *L. pneumophila*
Detected all 50 species tested against at CDC
Verified at PHOL (C. Guyard) using 95 positive samples (82 *L. pneumophila*, 13 non-LP).
Limit of detection 1 genome copy per reaction.
Legionellae identified at PHOL May 1978 to Dec 2010

- Total number of cases (all Legionellae) from May 1978 to Dec 2010 was 1804
- 974 identified to species by culture or IFA seroconversion or urine Binax ICT
Distribution of *Legionella* sp. In Ontario  
May 1976 to 2010

<table>
<thead>
<tr>
<th>Legionella species</th>
<th># cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP1</td>
<td>599</td>
<td>58.7%</td>
</tr>
<tr>
<td>LP6</td>
<td>62</td>
<td>6.1%</td>
</tr>
<tr>
<td>SAINTHELENSII 1</td>
<td>43</td>
<td>4.2%</td>
</tr>
<tr>
<td>MICDADEI</td>
<td>38</td>
<td>3.7%</td>
</tr>
<tr>
<td>MACEACHERNII</td>
<td>37</td>
<td>3.6%</td>
</tr>
<tr>
<td>LP8</td>
<td>24</td>
<td>2.4%</td>
</tr>
</tbody>
</table>

*First case reports of this species*
### Distribution of *Legionella* sp. Causing Clinical Infections In Ontario

<table>
<thead>
<tr>
<th>Legionella species</th>
<th># cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOZEMANII 1</td>
<td>24</td>
<td>2.4%</td>
</tr>
<tr>
<td>DUMOFFII</td>
<td>17</td>
<td>1.7%</td>
</tr>
<tr>
<td>LONGBEACHAE 1</td>
<td>18</td>
<td>1.8%</td>
</tr>
<tr>
<td>LP3</td>
<td>14</td>
<td>1.4%</td>
</tr>
<tr>
<td>OAKRIDGENSIS</td>
<td>14</td>
<td>1.4%</td>
</tr>
<tr>
<td>LP4</td>
<td>13</td>
<td>1.3%</td>
</tr>
</tbody>
</table>
Distribution of *Legionellae* sp. Causing Clinical Infections In Ontario

<table>
<thead>
<tr>
<th>Species/serogroup</th>
<th>No. of Cases</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. feelii</em></td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>L. santicrucis</strong></td>
<td>5</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>L. parisensis</strong></td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td><em>L. cherrii/steigerwaltii</em></td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td><em>L. wadsworthii</em></td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td><em>L. bozemanii</em></td>
<td>2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*These isolates were first discovered in Ontario

**These isolates are the first clinical cases reported worldwide (previously found environmentally in other countries)*
Positive Legionella specimens by year

![Graph showing the number of positive Legionella specimens by year, with data points for IFA, D/C, and ELISA tests.](image)
Acknowledgements

• Respiratory Bacteriology Laboratory
• Shirley Brown, Patrick Tang and other technical staff.
Molecular Epidemiology and outbreak investigations of legionellosis in Ontario

Dr. Cyril Guyard

✓ University of Toronto, Department of Laboratory Medicine and Pathobiology

✓ Mount Sinai Hospital, Department of Microbiology

✓ Public Health Ontario
Questions once one or several case(s) are detected:

- Are we facing an outbreak?
- Is there a common source of infection?
- How to assess if the suspected source is the “real” common source of infection?
- Are the taken water sanitations measures adapted to the problems?
- How to prevent the emergence of additional cases in timely manner?
- What is a “safe” concentration of *Legionella* in water systems (lack of guidelines)?

Challenges:

- *Legionella* bacteria are ubiquitous in man-made water systems.
- Economical impacts: Hotels, vacation resorts, cruise ships,…
- Need for a method to accurately match clinical isolates with environmental strains.
What is the source of infection?

Isolated cases

Source(s) of the infection?

Source 1

Source 2

Source 3

Match

+  

Timely intervention to prevent further infections
Are we facing an outbreak? Is there a common source?

Suspected outbreak

Source(s) of the outbreak?

Freshwater sources (80%)

Source 1

Source 2

Source 3

Match

Yes or No

Timely intervention to prevent further infections
Why do we need molecular typing data?

Isolated cases

Source(s) of the infection?

Suspected outbreak

Source(s) of the outbreak?

suspected sources

Source 1

Source 2

Source 3

Necessary to obtain subtyping data.
Sequence Based Typing = SBT

What is SBT?

- Comparative analysis using nucleotide sequences of multiple loci encoding housekeeping genes (under stabilising pressure) and genes under diversifying pressure (e.g., virulence genes).

- **Genes under diversifying pressure**: flaA = Flagellum subunit, mip = Macrophage infectivity potentiator, momps = major outer-membrane protein, pilE = type IV pilin and proA = zinc metalloprotease.

- **Housekeeping genes**: asd = Aspartate-b-semialdehyde dehydrogenase and neuA = N-Acylneuraminate cytidyl transferase.
Advantages of Sequence Based Typing.

• Robust, reliable.

• Reproducible.

• Portable: Allows comparison between different laboratories (ex: travel associated outbreak).

• Allow comparison over a long period of time (retrospective analysis).

• Sharable: For Legionella a regularly implemented web database is available.
SBT Typing Principle

• Sequencing of 7 genes:
  \( asd, flaA, mip, momps, pilE, proA \) and \( neuA \).

• Predetermined order of loci.

• Each allele is assigned a specific number
  \( ST25 = 4,7,11,3,11,12,1 \).

• For a new allele, 0 is assigned and a new allele will be added to a database.
SBT typing protocol

1. DNA extractor
2. Purified DNA
3. PCR
4. Electrophoresis
5. DNA sequencer
6. Web database
7. Sequence type
8. Quality assessment

Lp1

4, 3, 1, 1, 7, 12, 7, 6
Data mining using EWGLI web-database

Legionella pneumophila Sequence-Based Typing

SBT Allelic Profile Database Query Form

Please click here for brief instructions, or this icon leads to a field. Alternatively, email Dr. Norman Fry.

Check this box if you would like to view only your submissions that match the search criteria.

Please enter either a Sequence Type:

or

Select one or more allele numbers from the drop-down menus to retrieve data on similar allele positions from the database.

Select "?" if you want any previously identified allele variant to match that locus.

You may search for entries using whole or partial strain designations.

Enter a search term:

- Remove only sequences from the EUL culture collection?
- Remove only sequences that are not part of the EUL culture collection?

Would you like to specify a serogroup?

Check this box if you would like to exclude your chosen serogroup from the results.

You may view results from specific countries by checking one or more boxes below.

Leaving all boxes blank will return results from all countries.

Retrieval profiles

Reset form fields


Retrieve data from previously reported isolates matching the Sequence Type of the query

Queries based on:
- Sequence type
- Allele combination
- Serogroup
- Location (Countries)
**Data mining using EWGLI Web-database**

*Legionella pneumophila Sequence-Based Typing*

Click on an **EUL number** in the “Identifier” column to display more detail on that entry. Click on a column heading to sort the rows of the table by the contents of that column. Results for allelic profile: 1,4,3,1,1,1

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Strain</th>
<th>Sequence type</th>
<th>Serogroup</th>
<th>mAb subgroup</th>
<th>Isolation date</th>
<th>Source</th>
<th>Town</th>
<th>Region</th>
<th>Country</th>
<th>Investigation context</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUL00001</td>
<td>IBS-002</td>
<td>1</td>
<td>1</td>
<td>Philadelphia</td>
<td>1998-02-01</td>
<td>Clinical</td>
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_result page of the queries provides: Sequence type (ST), serogroup, Isolation date, source, town, country and investigation context._
Examples of epidemiological investigation.

➢ **Problematic:** - In 2007, 67 specimens from the same Public Health Unit.

➢ **Diagnosis:** - Five cases of Legionellosis over 2 months.

➢ **Question:** Outbreak or isolated cases?

Ontario Public Health laboratories obtained 3 clinical isolates.

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Sequence based typing suggests that these clinical cases are unrelated.
Retrospective Outbreak investigations: tracking the environmental source

- Clinical isolates (n=7): ST222
- Environmental isolates (n=9): ST222 100%

- Clinical isolates (n=2): ST226
- Environmental isolates (n=7): ST226
  ST1: 14%, ST59: 14% 72%

- Clinical isolates (n=1): ST1
- Environmental isolates (n=17): ST1
  24% new ST 76%

- Clinical isolates (n=1): ST154
- Environmental isolates (n=6): ST154

72 to 100% of correlation between ST# from clinical and environmental isolates.

Legionella workshop, June 25, 2012

PublicHealthOntario.ca
Use of SBT to study the Molecular Evolution of Pathogenic Bacteria.

• Why?
  • Detect the emergence of a new population of Legionella.
  • To ensure adequacy of our diagnostic methods (mutations).
  • For the early identification of hypervirulent strains.
  • To understand how bacteria are constantly evolving adapting to their environment. (*Tijet et al., Emerging Infectious Diseases, 2010*)

Prevention of infectious diseases
Acknowledgments

Funding sources.

- Ministry of Health and Long-term Care.
- Public Health Ontario.
Legionella
Occupational Health and Safety Issues

Leon Genesove MD FRCPC
Chief Physician
June 25, 2012

Ministry of Labour
Notes

- This presentation has been prepared to assist workplace parties in understanding their obligations under the Occupational Health and Safety Act (OHSA) and the regulations. It is not intended to replace the OHSA or the regulations and reference should always be made to the official version of the legislation.

- It is the responsibility of the workplace parties to ensure compliance with the legislation. This presentation does not constitute legal advice. If you require assistance with respect to the interpretation of the legislation and its potential application in specific circumstances, please contact your legal counsel.
Learning Objectives

- Review of Legionella
- Recognize potential sources
- Learn about cooling towers and prevention and control measures for cooling towers
- Review Occupational Health and Safety Regulations
- MOL Expectations of Employer
- Gain awareness of Guidelines:
  - ASHRAE, CSA, CDC, MOL
What is Legionella?

- Bacteria found in natural freshwater sources, moist soil and man-made water systems
- Grows best in slime, sediment, or biofilms
- Grows inside single cell organisms e.g. amoebae, protozoa & slime mold
- Resistant to chlorination levels in domestic water systems
- Usual low levels of Legionella found in water systems not associated with disease
- May cause potentially fatal pneumonia and Pontiac Fever
What are the Conditions for Bacterial Growth?

Conditions that promote the growth of Legionellae bacteria in water systems include:

- Hot temperatures (20-450C; optimal 35-450C)
- Stagnation (>3 days or used <once per week)
- Sediment, rust, scale, sludge (as nutrient source)
- Slime or common water organisms (which provide nutrients and protect Legionellae)
- Cold water systems in which temperature is not maintained below 200C
How is Legionella transmitted?

- Increase in Legionella growth in water systems or air handling systems with cooling towers under certain conditions
- Risk when droplets/aerosols created by spraying, splashing, misting, or bubbling of air through contaminated water
- Infection is caused by inhalation of water droplets or aerosols deep into lungs
- No person to person transmission
- Infection also possibly by aspiration of contaminated water (hospitalized patients)
What are Sources of Legionellae?

Legionellae bacteria have been isolated from or outbreaks have been associated with:

- Water mist from cooling towers or evaporative condensers
- Humidifiers and grocery produce misters
- Hot and cold potable water distribution systems
- Hot tubs, spa baths and decorative fountains
- Non-potable water cooling systems
What are cooling towers?

- Function like a heat exchanger
- Used to cool water and dissipate unwanted heat to the atmosphere through water evaporation.
- Provide cooled water for air-conditioning, manufacturing and electric power generation.
- Water diffused in droplets into an airflow to maximize contact between the water and the air moved through the tower by fan.
Closed Circuit Cooling Tower or Evaporative Condenser
Cooling Towers – Conditions for Legionella Growth & Transmission

- Typical water temperature in cooling towers – 29 to 35 °C
- Effective air scrubbers removing and accumulating organic material and other debris and evaporation concentrates material – serve as food source
- Large wet surface areas on which biofilms can form
- Production and blow out of water droplets
Ministry of Labour Role

- MOL coordinates with public health where there is joint jurisdiction (i.e. workers are involved as well as the public)
- MOL investigates to ensure employer takes appropriate precautions to protect workers and prevent a recurrence
MOL’s Expectations of Employer

- Compliance with the OHSA and Regulations
- Worker training/education and protection
- JHSC involvement
- Implementation of regular preventive maintenance, routine maintenance and emergency maintenance procedures based on ASHRAE, CSA, CDC and/or other acceptable guidelines
What should employers do?

- Identify and assess the risk of bacterial growth in all water and ventilation systems
- Develop a written preventive maintenance program, with appropriate control measures
- Develop non-emergency and emergency start up and shut down procedures
- Train workers who are maintaining, operating, or inspecting air handling and water systems in measures and procedures (including precautions and PPE)
- Monitor and record effectiveness of control program on scheduled basis (e.g. measure water temperature, check biocide levels, etc.)
Precautions while Inspecting and Collecting Water Samples during an Outbreak

- Appropriate training and supervision of public health inspectors
- Wear appropriate fit tested respirator (N95 or higher) while inspecting and collecting water samples near an aerosol generating water source if a significant potential exists for exposure to high concentrations of contaminated aerosols
  - For example, operating spray humidifier, water mister, fountain, or shower, in a facility where there is a confirmed outbreak.
- Shut down cooling towers, spray humidifiers, etc. before inspecting where there is a confirmed outbreak
- Gloves and protective clothing if possible contact with biofilm, contaminated water or other hazards
Legislation and Guidelines

- Occupational Health and Safety Act
- Regulation for Health Care and Residential Facilities
- Proposed ANSI/ASHRAE Standard, CSA Standards, Health Canada, CDC,
**Occupational Health and Safety Act Employers’ Responsibilities**

- S. 25(1)(b) – shall ensure that equipment, materials and protective devices are maintained in good condition (i.e. maintain water and ventilation systems to prevent Legionellae growth)

- S. 25(2)(a) – shall provide information, instruction and supervision to protect workers (e.g. workers involved in preventive maintenance or operation of water and ventilation systems)

- S. 25(2)(h) – shall take all reasonable precautions for the protection of workers (i.e. identify, assess and implement control measures to prevent Legionellae growth in water and ventilation systems)

- S. 52(2) – Employer must report occupational illnesses to MOL and JHSC, in writing within 4 days
Regulation for Health Care and Residential Facilities (HCRF Reg.) Employers’ Responsibilities

- S. 8 – in consultation with JHSC, shall develop, establish and put into effect measures and procedures to protect the health and safety of workers
- S. 9(1) 4. – shall reduce to writing measures and procedures for the health and safety of workers to control infections from Legionella
- S. 9(1) 1. – shall reduce to writing measures and procedures to safely inspect, clean and maintain water and ventilation systems
- S. 9(1) 12. – shall reduce to writing measures and procedures for the use, wearing and care of all PPE
- S. 9(4) – in consultation with JHSC, shall develop and provide training programs on the measures and procedures
HCRF Reg. – PPE Employers’ Responsibilities

- S. 10(1) – shall ensure that workers who are required to wear or use any protective clothing, equipment or device (e.g. to maintain ventilation systems) are trained on its care, use and limitations before wearing or using it.

- S. 10(2) – shall ensure that the protective equipment is properly used, maintained, inspected, stored, and is a proper fit (e.g. appropriate size, fit testing for respiratory protection, etc.)
HCRF Reg. – Ventilation Employers’ Responsibilities

- S. 19(2) – the mechanical ventilation system shall be inspected every 6 months to ensure it is in good condition
- S. 19(3) – shall be inspected by a qualified person
- S. 19(4) – qualified person to file inspection report and provide copy to JHCS
- S. 19(5) – shall be serviced and maintained in good condition as recommended by manufacturer or by qualified person as per inspection report
Guidelines for Health Care Facilities

- CDC Guidelines for Preventing Health-Care-Associated Pneumonia (2003)
CSA Standard CAN/CSA-Z317.2-10
Special Requirements for HVAC Systems in Health Care

- 4.1 Special HVAC requirements in health care result from the need to protect occupants from infectious diseases; fire and smoke; hazards created by specialized equipment and processes; failure or improper operation of HVAC systems and hazardous environmental contaminants

- 5.5 Infection Control – HVAC systems shall be designed, installed, operated and maintained to assist in protection of occupants from infection via airborne transmission (organisms transmitted through the air are of concern e.g. Legionella)
Construction and Renovation in Health Care Facilities


- Submit notice of project to MOL for construction or major renovation
Other Relevant Standards

- Ontario Building Code
  - Ventilation and Plumbing Requirements

Resources

- Toronto Public Health Fact Sheet

- Canadian Centre for Occupational Health and Safety
  [http://www.ccohs.ca/oshanswers/diseases/legion.html](http://www.ccohs.ca/oshanswers/diseases/legion.html)

- U.S. Department of Labour--Occupational Safety and Health Administration

- Association of Water Technologies (AWT) 2003
  [www.awt.org Legionella: An Update and Statement by AWT](http://www.awt.org)

- Centers for Disease Control and Prevention (CDC)
  [http://www.cdc.gov/legionella/index.htm](http://www.cdc.gov/legionella/index.htm)

- Control of Legionellosis-Health and Safety Executive, United Kingdom

- WHO Legionella and Prevention of Legionellosis
Questions?

Thank you
Legionella: Issues in Case and Outbreak Management
June 25, 2012
M. Baird
Overview

• Legionellosis in Hamilton
• Case Management: What’s Involved?
• Case Management: What are the issues?
• Community Outbreak Management: What’s Involved?
• Community Outbreak Management: What are the Issues?
• Conclusion and Next Steps
The Hamilton Perspective

• The annual number of cases of Legionellosis in Hamilton increased.

• Seasonality with respect to when cases appear.

• Clusters of cases occurring outside of usual timeframe.
These figures are subject to change due to case follow-up procedures.

**Source:** Ontario Ministry of Health and Long-Term Care integrated Public Health Information System database. **Data Extracted:** 2012-06-11

**Prepared by:** City of Hamilton Public Health Services
2006: Why we are Where We are Today?

• Outbreak in 2006 linked to specific downtown area
• Cooling towers within exposure area investigated
• 2006 outbreak investigation received attention from local media
Surveillance Definition

• **3.1 Confirmed Case**
  – Laboratory confirmation of infection with clinically compatible signs and symptoms:
    • • Isolation of *Legionella* spp. or detection of the antigen from appropriate clinical specimens (e.g., lung tissue, pleural fluid, sputum)
    • OR
    • • A significant (i.e., fourfold or greater) rise in *Legionella* spp. total antibody titre between acute and convalescent sera
    • OR
    • • Single specimen or standing total antibody titre ≥ 1:256 against *Legionella* spp.
    • OR
    • • Demonstration of *L. pneumophila* serogroup 1 antigen in urine

• **3.2 Probable Case**
  – Clinically compatible signs and symptoms with:
    • Demonstration of *Legionella* spp. DNA by nucleic acid amplification test (NAT), such as PCR
    • Detection of specific *Legionella* antigen or staining of the organism in respiratory secretions, lung tissue, or pleural fluid by direct fluorescent antibody (DFA) staining, Immunohistochemistry (IHC), or other similar method
Case Management

• All reported Legionellosis cases are investigated
• All clients interviewed with detailed tool with objective of identifying possible exposures
• Interview tool includes collection of information such as:
  – Exposure to hot tubs
  – Exposure to decorative fountains/sprays
  – Travel
  – Grocery stores
• All exposures considered and environmental sampling possible
Case Management: What are the Issues?

• Challenging to determine possible exposures in community settings.
• Often it isn’t possible to interview case and the next of kin is not always able to offer an accurate account of activity.
• If source is a community source such as a Cooling Tower how do we determine point of exposure? Look at travel patterns?
Community Outbreak Management: What do we do?

- Hamilton conducts extensive investigation into clusters of Legionellosis cases, including but not limited to:
  - Detailed case interviews
  - Mapping client travel patterns
  - Community environmental sampling (splash pads, misters, small scale irrigation systems)
  - Mapping and review of cooling towers within geographic area of exposure sites; sampling of cooling towers and maintenance review

- Medical and media advisory issued
Outbreak Management: What are the Issues

- How do you know you have an outbreak?
- Challenging to apply usual outbreak definitions (person/place/time)
  - What is the time frame?
  - Possibly ongoing but intermittent exposures
  - Not necessary to have an overlap in exposure window…or is it?
- How do you establish links between cases?
- Multitude of possible community exposures; what do you sample?
- Urine antigen test does not allow for genetic relationships to be made among cases
What Have We Learned in Hamilton?

- The value of sampling within a private dwelling is limited.
- Source of community acquired Legionellosis in Hamilton is likely cooling towers…and we’ve learned a lot about cooling towers!
- Weather patterns appear to play a role in when we experience cases of Legionellosis.
- We have been unsuccessful to date in identifying a specific common exposure to explain seasonal clusters/outbreaks in Hamilton.
Have we had success?

• Locally we have a Cooling Tower Bylaw; emphasis on proper ongoing maintenance and registration.
• Significant surveillance data
• Developed an educational brochure for hot tub owners that is distributed at local supply stores
• Well established outbreak management team.
Next Steps?

• Considering how to conduct surveillance on exposure information.
• We need to better understand whether we are seeing seasonal increases in sporadic, unrelated cases or true outbreaks linked to a common source.
• We would appreciate sharing the experiences and learning of other health units with respect to investigation of community acquired Legionellosis.