Premise plumbing factors leading to mycobacterial growth in homes in the greater Philadelphia region

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Collaborators

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- Annie Pearce
- Joseph Falkingham III
- Amy Pruden
- Marc Edwards
Background

- Nontuberculous mycobacteria (NTM) are naturally occurring in water sources and are native to drinking water systems \(^1\)-\(^3\)
- NTM can cause pulmonary infections in healthy individuals and are difficult to treat \(^4\)
- *Mycobacterium avium* complex (MAC) accounts for most NTM related diseases in the US \(^1\)-\(^3\)
- NTM disease incidence had risen six fold (1.5 – 9 per 100,000) from 1997 – 2003 \(^5\)
- Recent estimates put NTM disease cases at 15 per 100,000 \(^1\)
NTM Characteristics

- Resistant to chlorine, chloramine and ozone \(^6\)
- Resistant to temperature - over 90% survive exposure to 50 °C (125 °F) for 60 mins or more \(^9\)
- Tolerate long periods of water stagnation \(^10\)
- Grow in low levels of oxygen \(^12\)
- Form biofilm on the insides of pipes \(^7,8\)
- Ideally adapted to growth in drinking water systems and premise plumbing \(^7,8\)
• Montgomery County NW of Philadelphia, PA has high incidence of NTM disease

• Lande et al. 2015 \(^{13}\) findings:
  • NTM present in source water and distribution system – 1-5 CFU/mL
  • NTM replicate in water heaters - >1,000 CFU/mL
  • Water heaters set at higher temperature were less likely to grow NTM
  • Elevating water heater temperature controlled growth of *M. avium* but at the risk of growth of *M. chelonae*
Objective

Determine premise plumbing factors leading to NTM growth in homes in greater Philadelphia region
Hypotheses

• Patient homes are more conducive to NTM growth
• Least used bathrooms are more conducive to NTM growth
• *Methylobacterium* spp. can control growth of *Mycobacterium avium* in the biofilm
Sampling Sites

13 Patient
- 11 Large
- 2 Small

9 Control
- 8 Large
- 1 Small
Data Collection per home

- Up to 10 Biofilm samples
- Water for qPCR
- ICP-MS for dissolved metals
- pH

- Total Chlorine and Temperature
  - Cold Stagnant
  - Hot Stagnant
  - Cold Flushed
  - Hot Flushed

- Ammonia and Nitrite
  - Cold Stagnant
  - Hot Stagnant
  - Cold Flushed
Data Collection per home

• Number of residents
• Plumbing materials
• Pipe and water heater sizing
• Fuel source
• Water heater model and make
• Insulation
• Time to hot water at fixture
• Age of the house
• Size of the house
Sampling Order

Small homes:
• Cold water first draw
• Hot water first draw
• Flushed hot
• Flushed cold
• Biofilm samples

Large homes:
• Cold water first draw (least)
• Hot water first draw (least)
• Flushed hot (least)
• Flushed cold (least)
• Cold water first draw (most)
• Hot water first draw (most)
• Flushed hot (most)
• Flushed cold (most)
• Biofilm samples
Key Takeaways

- In patient vs control homes, there were no differences in:
  - *Mycobacterium spp.* in biofilm
  - *Mycobacterium spp.* in water
  - *M. avium* in water

- Significantly more mycobacteria in biofilm cultures and water samples collected from least used showers compared to most used showers
  - Confirmed by other factors that co-vary with stagnation
    - Metals
    - Ammonia
    - Chlorine
    - Nitrite
Key Takeaways

- High counts (>100 CFU/swab) of *Methylobacterium* spp. was a significant indicator for lower *Mycobacterium* spp. counts in biofilms, and vice-versa.

- Factors affecting *M. avium* DNA (gc/mL) in water:
  - 16S rRNA, M. spp. (collinear)
  - Sampling characteristics – M/L, S/F (stagnation)
  - Size of the house (stagnation)
  - Age of the house
  - Recirculation systems
Total Chlorine, Ammonia and Nitrite
Copper and Zinc

Usage

L

M

Copper and Zinc (μg/L)

Stagnant Cold Flushed Stagnant Hot Flushed Stagnant Cold Flushed Stagnant Hot Flushed

65Cu

66Zn
<table>
<thead>
<tr>
<th>Location</th>
<th>Biofilm (n)</th>
<th>Biofilm</th>
<th>Sample</th>
<th>qPCR (n)</th>
<th>M. spp.</th>
<th>M. avium</th>
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<tbody>
<tr>
<td>Patient</td>
<td>11</td>
<td>Cold</td>
<td>Flush</td>
<td>10</td>
<td>9 (90%)</td>
<td>4 (40%)</td>
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<tr>
<td></td>
<td>24 ± 59</td>
<td>Hot</td>
<td>Stag</td>
<td>11</td>
<td>10 (91%)</td>
<td>5 (45%)</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td>172 ± 279</td>
<td>1 ± 1</td>
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<td></td>
<td></td>
<td></td>
<td>10 (91%)</td>
<td>5 (45%)</td>
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<td>72 ± 122</td>
<td>17 ± 45</td>
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<td>10 (91%)</td>
<td>8 (73%)</td>
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<td>335 ± 421</td>
<td>76 ± 158</td>
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<tr>
<td>Least</td>
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<td>Cold</td>
<td>Flush</td>
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<td>9 (100%)</td>
<td>4 (44%)</td>
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<td>42 ± 50</td>
<td>9 ± 27</td>
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<td>Stag</td>
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<td>6 (67%)</td>
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<td>859 ± 1539</td>
<td>23 ± 67</td>
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<tr>
<td>Least</td>
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<td>9 (100%)</td>
<td>6 (67%)</td>
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<td>124 ± 155</td>
<td>39 ± 76</td>
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<td>7 (78%)</td>
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<td>5024 ± 9511</td>
<td>823 ± 1621</td>
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<tr>
<td>Control</td>
<td>5</td>
<td>Cold</td>
<td>Flush</td>
<td>5</td>
<td>5 (100%)</td>
<td>2 (40%)</td>
</tr>
<tr>
<td></td>
<td>0 ± 0</td>
<td>Hot</td>
<td>Stag</td>
<td>5</td>
<td>5 (100%)</td>
<td>2 (40%)</td>
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<td>109 ± 105</td>
<td>8 ± 17</td>
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<td>5 (100%)</td>
<td>2 (40%)</td>
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<td>94 ± 156</td>
<td>2 ± 5</td>
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<td>Least</td>
<td>4</td>
<td>Cold</td>
<td>Stag</td>
<td>4</td>
<td>4 (100%)</td>
<td>1 (25%)</td>
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<tr>
<td></td>
<td>2 (50%)</td>
<td>Hot</td>
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<td>59 ± 93</td>
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<td>4 (100%)</td>
<td>3 (75%)</td>
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<td>80 ± 87</td>
<td>1 ± 1</td>
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<td>4 (100%)</td>
<td>4 (100%)</td>
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<td>5 ± 6</td>
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<td>196 ± 133</td>
<td>11 ± 15</td>
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Mycobacterium spp. (Patient vs Control)
Mycobacterium spp.
**Mycobacterium avium (Patient vs Control)**

<table>
<thead>
<tr>
<th>Patient/Control</th>
<th>C</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>log (Mycobacterium spp. [gc/mL])</th>
<th>Stagnant</th>
<th>Flushed</th>
<th>Stagnant</th>
<th>Flushed</th>
<th>Stagnant</th>
<th>Flushed</th>
<th>Stagnant</th>
<th>Flushed</th>
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<tbody>
<tr>
<td>Cold</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hot</td>
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</tr>
</tbody>
</table>

21
Mycobacterium avium
Mycobacterium spp. Vs Methylobacterium spp.

Statistically significant relationship between Methylobacterium spp. (+/-) and Mycobacterium spp. Kruskal-Wallis Test, p=0.002
Dunn’s Test – Among Factors

<table>
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<th>A</th>
<th>B</th>
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<tbody>
<tr>
<td></td>
<td>B</td>
<td>C</td>
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<tr>
<td>A</td>
<td>0</td>
<td>0.0009</td>
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<tr>
<td>B</td>
<td>1</td>
<td>0.369</td>
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<tr>
<td>C</td>
<td>0.0009</td>
<td>0.0369</td>
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</table>
Mycobacterium spp. and Mycobacterium avium
Factors affecting *M. avium* at the tap

- Most/Least - stagnation
- Stagnant/Flush – stagnation
- 16S rRNA – collinear
- *Mycobacterium* spp. – collinear
- Area of house - stagnation
- Floors - stagnation
- Year built in
- Recirculation
Future Work

- 16S amplicon sequencing on samples to determine microbial community makeup
- Follow-up sampling to study impact of raising water heater temperature as a control strategy
References


