Nontuberculous mycobacteria and the environment  
(insights from Hawai‘i)
## What’s the myco difference?

<table>
<thead>
<tr>
<th>Mycobacterium tuberculosis (M.tb)</th>
<th>Nontuberculous mycobacteria (NTM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical place of residence:</td>
<td>Ubiquitous environmental distribution</td>
</tr>
<tr>
<td>Lung, intracellular</td>
<td></td>
</tr>
<tr>
<td>Pathogenicity ruler:</td>
<td></td>
</tr>
<tr>
<td>Causes TRUE lung disease</td>
<td>Opportunistic pathogens</td>
</tr>
<tr>
<td>Overall available knowledge:</td>
<td>Rarely causes lung disease</td>
</tr>
<tr>
<td><img src="image" alt="Mycobacterium abscessus species" /></td>
<td><img src="image" alt="Mycobacterium avium complex (MAC)" /></td>
</tr>
<tr>
<td>M. tuberculosis</td>
<td>M. avium</td>
</tr>
<tr>
<td>M. gordonae</td>
<td>M. intracellulare</td>
</tr>
<tr>
<td>M. terrae</td>
<td>M. chimaera</td>
</tr>
<tr>
<td>M. gilvum</td>
<td>M. smegmatis</td>
</tr>
</tbody>
</table>

BSL-3 MICROBIOLOGICAL LABORATORY
NTM lung disease

- General population are constantly exposed, but infection is rare.
  - Most common of the “rare lung diseases.”
- General population: 4-7/100,000 persons
  - Elderly (>65yr) 15-47/100,000 persons
- Outbreaks of NTM have occurred.
- Treatment is inadequate, lengthy, and expensive.
- Person-to-person transmission is not known to occur, but may occur in patients with cystic fibrosis in close proximity to infected persons.
Why do we care about NTM lung disease?

• In the U.S., nearly 180,000 individuals are infected with NTM.
• Prevalence is increasing at >8.2% annually.


Major mycobacterial lipids
Tran, T. et al, Tuberc J, 2019; under review
Contributing host and environmental factors

HOST-RISK FACTORS

ANATOMIC
- Prior bronchiectasis
- Emphysema
- Pneumoconiosis
- Chronic aspiration
- Calcified chest adenopathy

IMMUNOLOGIC-GENETIC
- Cystic fibrosis, CFTR anomalies
- Alpha-1-antitrypsin anomalies
- Pulmonary alveolar proteinosis
- Partial deficiency in IFNg(?)
- TNFa antagonists*
- Inhaled corticosteroids
- Multigenic disorder
- Ciliary defect (MST1R)

ENVIRONMENTAL EXPOSURE
- Aerosolized water (hot tubs, showerheads)
- Aerosolized soil exposure
- Residence in Southeast U.S. and Hawai’i

HOST-susceptible phenotype of unknown cause
- Slender individuals with Marfanoid body habitus (scoliosis, pectus excavatum, mitral valve prolapse)

Completely normal hosts (size of inoculum matters?)

What are the most common NTM species identified in patient respiratory specimens at National Jewish Health?

Mycobacterial Clinical Labs
U.S. NTM isolates (7/2013-7/2015)

Of 3,096 NJH isolates, these are the top 7: (66%)

15.83%  *M. ABSCESSUS*
12.63%  *M. AVIUM*
9.01%  *M. FORTUITUM*
8.91%  *M. INTRACELLULARE*
8.85%  *M. MASSILIENSE*
5.46%  *M. CHELONAE*
5.33%  *M. CHIMAERA*

*Total=100%*
How are infections acquired?
Inhalation of NTM aerosolized from environmental sources

- Drinking water distribution systems
- Heater-cooler units
- Faucets, ice machines
- Animals
- Acidic, brown water swamps
- Hot tubs/spas
- Water tanks
- Soil
- Boreal forest soil and peats
- Showerheads
- Drinking water distribution systems
- Faucets, ice machines
- Animals
- Acidic, brown water swamps
- Hot tubs/spas
- Water tanks
- Soil
- Forest soil and peats
- Showerheads

Studies have reported NTM in a variety of different geographic locations. E.g., Asia, Europe, Africa, U.S.

But NTM diversity has never been comprehensively characterized in a single, large geographic area.
Environmental NTM Biofilms

- U.S. showerheads are enriched for NTM (Feazel, 2009).
- Identical NTM fingerprints from the NTM isolates from patient lungs and their matched shower water isolates (Falkinham, 2008; 2011).
- Showerhead biofilms are the most frequently sampled environmental sources to describe the presence of environmental NTM globally.

Cut open shower hoses reveal diverse biofilms

Proctor, et al/ Water Research, 2018

Soto-Giron, AEM 2016
Showerhead Microbiome and NTM Lung Disease

M. avium Complex

Pearson’s $r = 0.70; P < 0.001$

$\text{DISTLM } R^2 = 0.49; P = 0.004$

Showerhead Microbiome Project (Gerbert et al, mBio, 2018)
Hawai‘i is a U.S. Hot Spot for NTM Lung Disease

- A 1979 survey of public health laboratories showed Hawai‘i had the highest *M. avium* complex isolation rate in the U.S. (Good, 1980).

- Hawai‘i has the highest prevalence - 396 cases/100,000 population among persons > 65 years-old (1997-2007). (Adjemian, AJRCCM, Vol 185, 2012)

- High-risk environments for NTM in particular geographic areas of Hawai‘i. (Adjemian, AJRCCM, Vol. 186, 2012)

- Hawai‘i also demonstrates the highest, national age-adjusted mortality rates from NTM-LD. (Mirsaeidi, 2014)
Current Lab Directions

**NTM lung disease**

- *i.e.*, environmental water biofilms, soil, animal reservoirs, climate, etc..

- **NTM lung disease**

  - *i.e.*, host genetics/immunity, behaviors
  - *i.e.*, species pathogenicity

---

*Honda, et al.* Frontiers in Microb, 2018
Epidemiology of NTM lung disease in Hawai‘i

- Retrospective study, EMR database query (2005-13)

- ~373,168 patient enrolled large healthcare system in Hawai‘i – 1/3 of the state’s population

- Period prevalence highest: 1) Oahu (153/100,000 cases); 2) Maui (91/100,000 cases), 3) Big Island (84/100,000 cases). No cases identified on other islands.

NTM species in respiratory specimens from Hawai‘i

n = 140 Oahu isolates (2018)

- 51.7% M. chimaera
- 18.1% M. massiliense
- 6.7% M. abscessus
- 4.7% M. fortuitum
- 3.4% M. intracellulare
- 2.7% M. avium
- 2.0% M. porcinum
- 2.0% M. yongonense
- 2.0% M. bolletii
- 2.0% M. phocaicum
- 2.0% M. arosiense
- 2.0% M. kubicae
- 2.0% M. parascrofulaceum
- 2.0% M. senegalense
- 2.0% M. simiae
First discoveries of NTM in Hawai‘i households, 2012

- 75 households samples
- 172 household plumbing biofilms and soil

Kauai (5/5; 100%)
Molokai (2/4; 50%)
Oahu (40/49; 82%)
Hawai‘i (1/4; 25%)

- No M. avium identified.
- M. intracellulare was very rare.

Honda, et al, PLOS Neg Trop Disease, 2016
Hawai’i 2018 environmental samples
~ 1,500 new samples

Analyses of the first 219 new samples

2012 Households

37% Household
63% Non household
NTM recovery by island, 2018

Kauai – **HH=83%** (5/6) vs Non-HH=21% (6/28)  

Maui – **HH=75%** (3/4) vs Non-HH=23% (5/22)  

Hawai‘i Island – **HH=73%** (8/11) vs Non-HH=92% (12/13)  

Oahu– **HH=90%** (10/11) vs Non-HH=32% (18/57)
Environmental species diversity

- **M. abscessus**
- **M. chelonae**
- **M. chimaera**
- **M. porcinum**
- **M. chimaera**
- **M. phocaicum**
- **M. fortuitum**
- **M. gilvum**
- **M. iranicum**
- **M. ... NTM**
- **M. avium**
- **M. chelonae**
- **M. chimaera**
- **M. fortuitum**
- **M. intracellulare**
- **M. porcinum**
- **M. chlei**
- **M. gilvum**
- **M. iranicum**
- **M. mucogenicum**
- **M. neoaurum**
- **M. phocaicum**
- **Novel NTM**

Proportion of NTM pos samples

- **Kauai**
- **Oahu**
- **Maui**
- **Hawai’i**

Number of species recovered

- **Hawai’i Island**
- **Maui**
- **Oahu**
- **Kauai**
Are all showerheads hot spots for NTM?

Household showerhead biofilms

- 53% (23/43) NTM positive

Beach showerhead biofilms

- 20% (10/50) NTM positive
M. avium discovered in Hawai‘i streams

- M. avium absent from the 2012 Hawai‘i study
- 2018 - 75% (6/8) samples collected from Oahu streams were NTM positive.

Oahu stream biofilms

<table>
<thead>
<tr>
<th>NTM Species</th>
<th>Number of Species Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. avium</td>
<td>0</td>
</tr>
<tr>
<td>M. chelonae</td>
<td>3</td>
</tr>
<tr>
<td>M. chimaera</td>
<td>2</td>
</tr>
<tr>
<td>M. intracellulare</td>
<td>1</td>
</tr>
<tr>
<td>Novel NTM</td>
<td>1</td>
</tr>
</tbody>
</table>

Total: 4 species recovered.
A study within the study

What is a Hawai‘i specific factor that could impact NTM lung disease?

Kilauea Volcano, 1-17-18

Kilauea Volcano's lower E. Rift zone on 5-19-18, ~8:18 AM, HST.

Kilauea, Halema'uma'u, 5-9-18

Bottom images courtesy of USGS
Kilauea’s potential association with NTM lung disease?

- Volcanic ash - tiny jagged pieces of rock and glass.
- Abrasive ash particles can be inhaled into the lungs.
- Spread over broad areas by wind.
- Ashfall can enter water systems and soil.

- **Direct:** NTM laden ash can be inhaled into the lungs resulting in infection.

- **Indirect:** Volcanic soils/water provide a niche that foster growth of NTM already in the environment.
NTM and the Kilauea volcano

Before eruption - 1/17/18-1/19/18

39 soil/ash/cinder samples collected
7 households sampled

>>>vegetation
< vegetation

Tamar Elias
HVO, USGS
Volcano soil composition and NTM

More $P_2O_5$ is associated with higher odds sample will contain NTM.
Non-NTM and NTM recovered from Kilauea, Jan. 2018

Non-NTM

Total=58

- 31.03% *Bacillus tropicus*
- 8.62% *Rhodococcus equi*
- 8.62% *Rhodococcus hoagii*

NTM

Total=52

- 19.23% *M. chelonae*
- 9.62% *M. triplex*
- 9.62% *M. abscessus*
- 7.69% *M. timonense*
- 7.69% *M. avium*
- 3.85% novel
- 7.69% *M. genavense*

24/35 unique soil sample location sites
Kilauea steam vent

*Kilauea*M. abscessus

Respirable ash fraction

Fine ash fraction

Kilauea ash recovered on 5-28-19

SEM

Kilauea *M. abscessus* binds to Kilauea ash.

x 9,000 magnification

x 2,500 magnification
Is NTM detectable in the ash samples?

A) Respirable Ash

B) Fine Ash

- M. avium
- M. abscessus
What has Hawai‘i taught us about environmental NTM?

• High recovery of NTM from households compared to non-household sources.
  o Household showerhead biofilms contain significant more NTM than beach.

• Clinically relevant *M. abscessus* and *M. chimaera* are found on all islands tested.

• NTM recovery varies by island: Less NTM on Kauai and Maui; more NTM on Oahu and Hawai‘i Island.

• Hawai‘i Island shows high diversity of NTM and novel species.

• *M. avium* can be recovered from Hawai‘i streams and the Kilauea Volcano.

• Kilauea and its ash maybe a new risk factor for NTM lung disease in Hawai‘i.
Acknowledgements

Funding: National Science Foundation Ecology, Evolution and Infectious Disease (#1743587), Padosi Foundation, Shoot for the Cure Foundation

Honda Lab
Ravleen Virdi
Stephanie Dawrs
Grant Norton

National Jewish Health
Denver, CO
Edward D. Chan, MD
Michael Strong, PhD
Nabeeth A. Hasan, PhD
L. Elaine Epperson, PhD
James Crooks, PhD
Cody M. Glickman

NOAA
National Centers for Environmental Information, Honolulu, HI
John Marra, PhD

ERT, Inc., at NOAA
National Centers for Environmental Information, Asheville, NC
Michael Kruk

Virginia Tech
Blacksburg, VA
Joe Falkingham, III, PhD
Myra Williams

Volcano Science Center
USGS
Menlo Park, CA
David E. Damby, PhD

Edward D. Chan, MD
Michael Strong, PhD
Nabeeth A. Hasan, PhD
L. Elaine Epperson, PhD
James Crooks, PhD
Cody M. Glickman

Hawai’i Volcano Observatory & USGS: Volcano, HI
Tamar Elias
Morgan Nasholds
Shauna Bladt
Gail Ferguson
Mike Pearson
Howard Hoshide
Tina Neal

NOAA
National Centers for Environmental Information, Honolulu, HI
John Marra, PhD

ERT, Inc., at NOAA
National Centers for Environmental Information, Asheville, NC
Michael Kruk

Volcano Science Center
USGS
Menlo Park, CA
David E. Damby, PhD

Edward D. Chan, MD
Michael Strong, PhD
Nabeeth A. Hasan, PhD
L. Elaine Epperson, PhD
James Crooks, PhD
Cody M. Glickman

Hawai’i Volcano Observatory & USGS: Volcano, HI
Tamar Elias
Morgan Nasholds
Shauna Bladt
Gail Ferguson
Mike Pearson
Howard Hoshide
Tina Neal

NIH: Bethesda, MD
Jennifer Adjemian, PhD
(to 2018)
Becky Prevots, PhD

U of Colorado, Boulder, CO:
Noah Fierer, PhD
Matt Gerbert

Kaiser Permanente Center for Health Research
Honolulu, HI:
Stacey Honda, MD
Vanessa Simiola, PhD
Emmalyn Pilande
Janet Myers, MD
Yihe Dida, PhD
Tim Frankland

Diagnostic Laboratory Services: Aiea, HI
Matt Bankowski, PhD
(to 2017)
Terrie Koyamatsu

Brigham Young University
Department of Geological Sciences
Provo, UT
Steve Nelson, PhD
Schuyler Robinson
Kevin Rey

Honda Lab
Ravleen Virdi
Stephanie Dawrs
Grant Norton

National Jewish Health
Denver, CO
Edward D. Chan, MD
Michael Strong, PhD
Nabeeth A. Hasan, PhD
L. Elaine Epperson, PhD
James Crooks, PhD
Cody M. Glickman

NOAA
National Centers for Environmental Information, Honolulu, HI
John Marra, PhD

ERT, Inc., at NOAA
National Centers for Environmental Information, Asheville, NC
Michael Kruk

Virginia Tech
Blacksburg, VA
Joe Falkingham, III, PhD
Myra Williams

Volcano Science Center
USGS
Menlo Park, CA
David E. Damby, PhD

Edward D. Chan, MD
Michael Strong, PhD
Nabeeth A. Hasan, PhD
L. Elaine Epperson, PhD
James Crooks, PhD
Cody M. Glickman

Hawai’i Volcano Observatory & USGS: Volcano, HI
Tamar Elias
Morgan Nasholds
Shauna Bladt
Gail Ferguson
Mike Pearson
Howard Hoshide
Tina Neal

NIH: Bethesda, MD
Jennifer Adjemian, PhD
(to 2018)
Becky Prevots, PhD

U of Colorado, Boulder, CO:
Noah Fierer, PhD
Matt Gerbert

Kaiser Permanente Center for Health Research
Honolulu, HI:
Stacey Honda, MD
Vanessa Simiola, PhD
Emmalyn Pilande
Janet Myers, MD
Yihe Dida, PhD
Tim Frankland

Diagnostic Laboratory Services: Aiea, HI
Matt Bankowski, PhD
(to 2017)
Terrie Koyamatsu

Brigham Young University
Department of Geological Sciences
Provo, UT
Steve Nelson, PhD
Schuyler Robinson
Kevin Rey
Soil compounds that may affect NTM growth

Enhances NTM Growth

Reduces NTM Growth

Main ore of iron.

Main mineral form of aluminum hydroxide.
Kauai – HH=83% (5/6) vs Non-HH=21% (6/28)  
Oahu – HH=90% (10/11) vs Non-HH=32% (18/57)  
Maui – HH=75% (3/4) vs Non-HH=23% (5/22)  
Hawai‘i Island – HH=73% (8/11) vs Non-HH=92% (12/13)