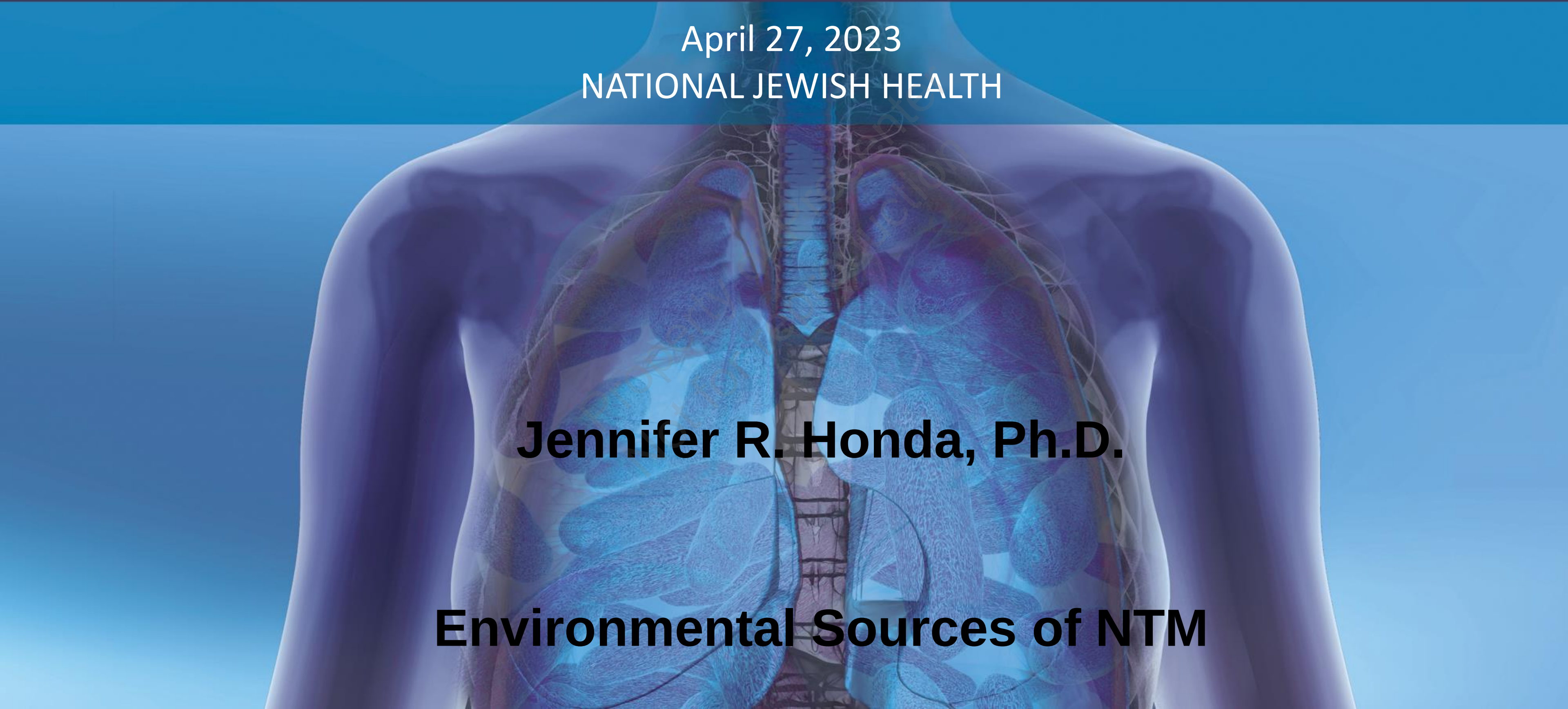
NTM Lecture Series for Providers



April 27, 2023

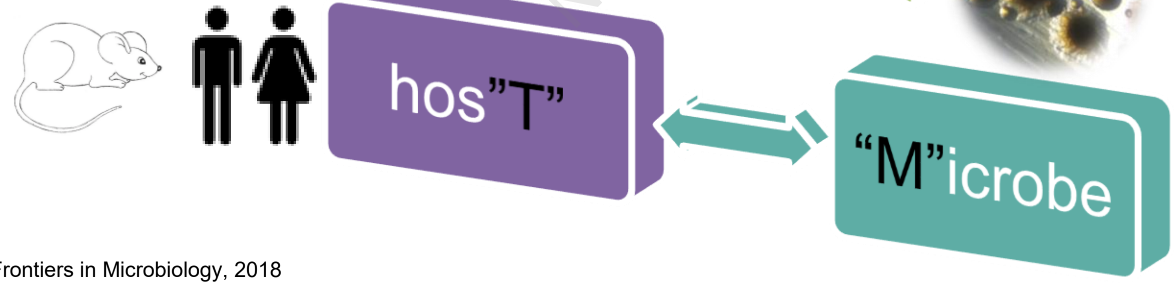
NATIONAL JEWISH HEALTH

Jennifer R. Honda, Ph.D.

Environmental Sources of NTM

No disclosures.

Honda Lab Research Program



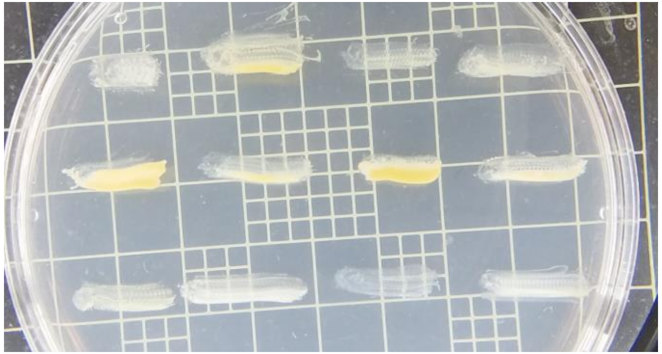
i.e., geography, water associated surface biofilms, soil, dust, animal reservoirs, climate, humidity, temperature, ash, etc…..

i.e., immune responses of macrophages and airway epithelial cells (humans, mice) to NTM infection

i.e., species of NTM matters, biofilm forming capacity, interaction with host and environment, pigment, virulence factors, etc…

Honda, *et al.*, Frontiers in Microbiology, 2018

What are *mycobacteria*?

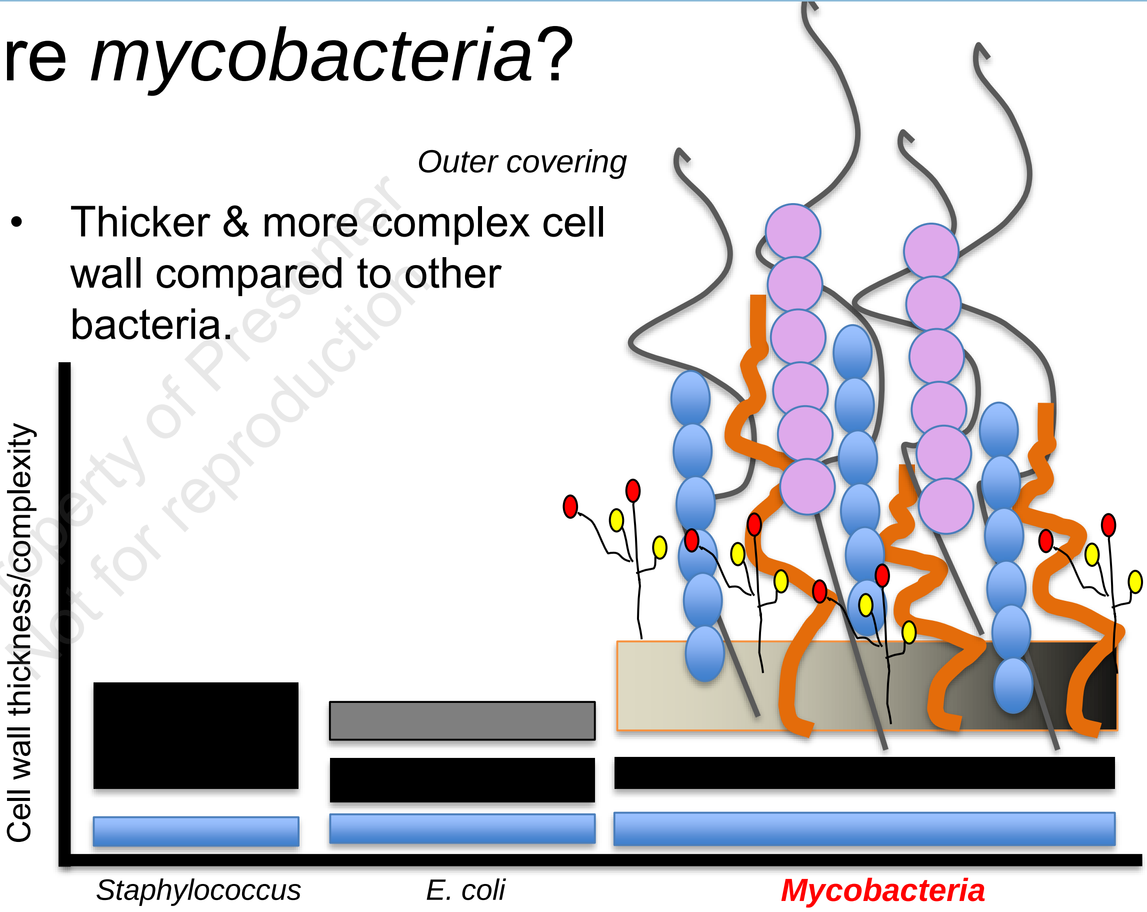


- > 190 different species
  http://www.bacterio.net/mycobacterium.html

Acid-fast bacteria

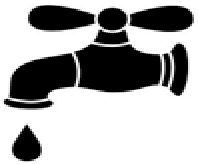
1) *Mycobacterium tuberculosis* (TB)
2) *Mycobacterium leprae* (leprosy)
3) **NTM.**

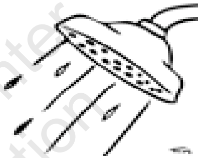
- Thicker & more complex cell wall compared to other bacteria.



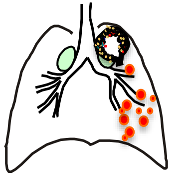
Do all mycobacteria cause lung disease?

Mycobacterium gordonae
M. gilvum
M. smegmatis

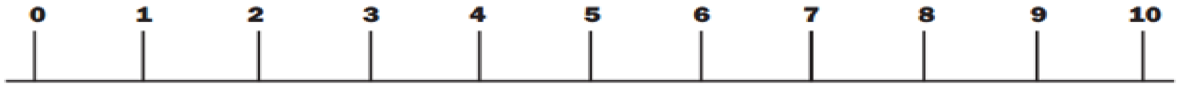




Mycobacterium abscessus
Mycobacterium avium complex (MAC)
(M. avium, M. intracellulare, M. chimaera)



Mycobacterium tuberculosis (TB)



Rarely causes lung disease

Causes true lung disease

NTM are one of two "flavors"

"***Rapid***-growing mycobacteria"
(RGM)

Visible growth is typically observed between 3-7 days.

Mycobacterium abscessus (MABS)
- *M. abscessus* subsp. *abscessus*
- *M. abscessus* subsp. *bolletii*
- *M. abscessus* subsp. *massilense*

"***Slow***-growing mycobacteria"
(SGM)

Visible growth is typically observed between 7-60 days.

Mycobacterium avium complex (MAC)
- *M. avium*
- *M. intracellulare*
- *M. chimaera*
- others

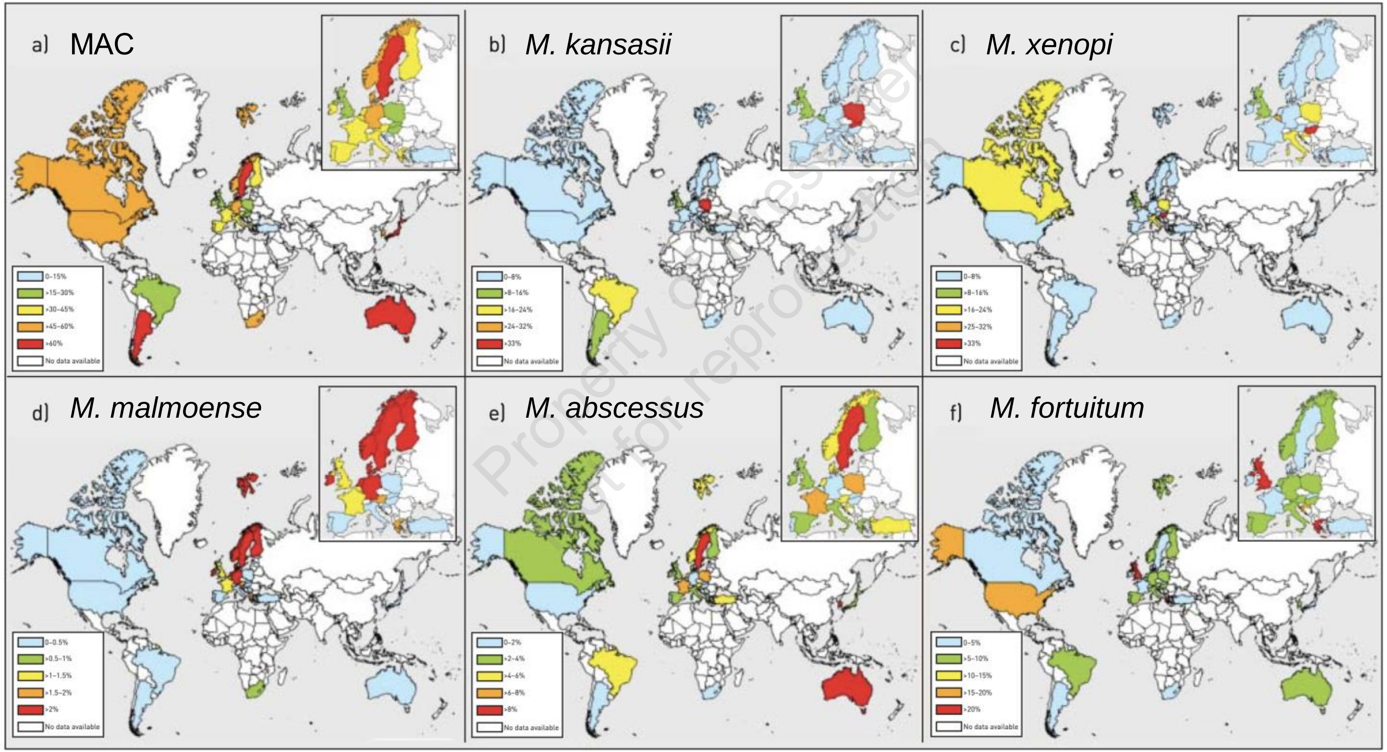
Learning Objectives

1). Become familiar with examples of environmental niches for NTM.

2). Recognize features that may promote NTM in the environment – showcasing Hawai'i.

3). Identify possible methods to reduce environmental NTM exposures.

Where you live may matter



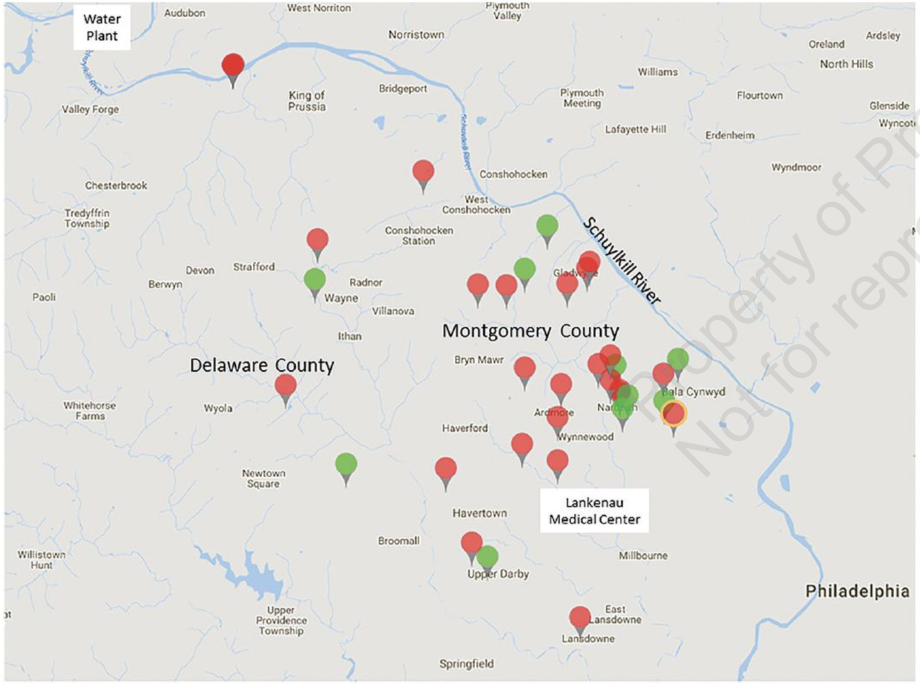
Hoefsloot, *et al.*, ERS, 2013

NTM identified from drinking water systems globally

Location:	% NTM recovery:	Species:	Reference:
Mexico	**16% (19/120)**	*M. mucogenicum* most common; ***M. avium*, no *M. abscessus***	Perez, *et al.*, BMC Res Notes, 2013
Greece	**22% (42/197)**	*M. chelonae* most common; **no M. *abscessus***	Tsintzou, et al., Water, Air, Soil Poll, 2000
USA	**33% (46/139)**	9 species, *M. mucogenicum* most common; **no M. *abscessus***	Covert, *et al.*, AEM 1999
Colombia	**50% (9/18)**	*M. mucogenicum* most common; **no M. *abscessus***	Dávalos, *et al.*, Env Res & Public Health, 2021
Argentina	**52% (64/124)**	*M. gordonae* most common	Oriani, *et al.*, Int J. Mycobacter, 2019
Australia	**62% (236/384)**	*M. gordonae* most common; ***M. abscessus* identified**	Thomson, *et al.*, BMC Microb, 2013
Paris, France	**72% (104/144)**	*M. gordonae* and *M. nonchromogenicum* most common; Potentially pathogenic, 16%; **no *M. abscessus*.**	Le Dantec, *et al.*, AEM, 2002

Which species of NTM is found, matters.

Genetically similar *M. avium* identified from participant lungs and households

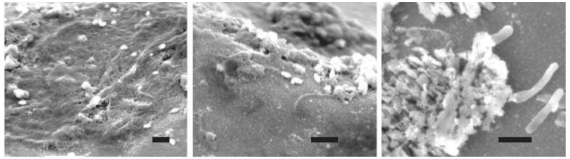


Matched Study (Pennsylvania)	
	% NTM recovery:
Recovery from non-participant households	81% (30/37)
Recovery from *M. avium* participant households	91% (19/21)
Of the *M. avium* participant households	52% (11/21) matched to environment.

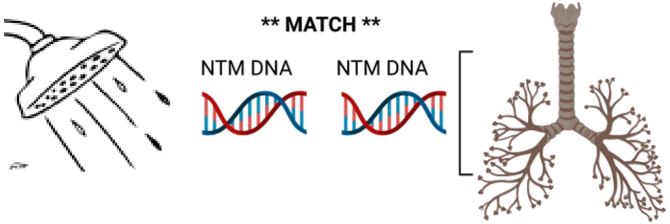
Lande, *et al.*, EID, 2019

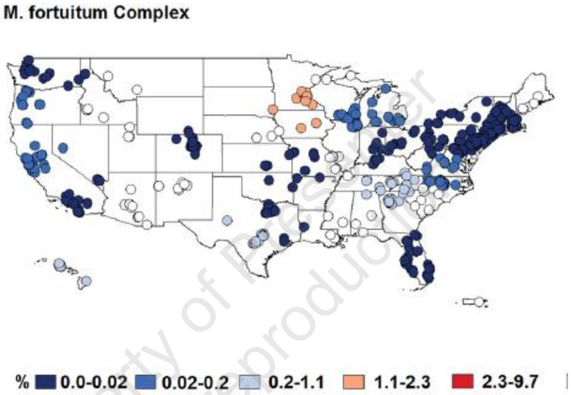
NTM and shower biofilms

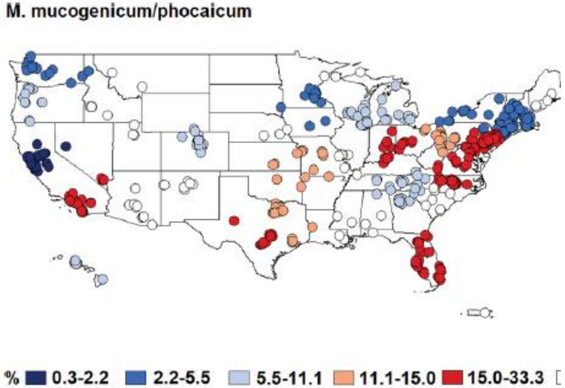
Showerhead biofilm

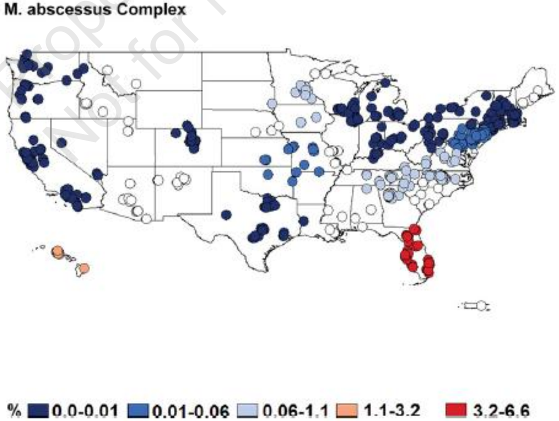


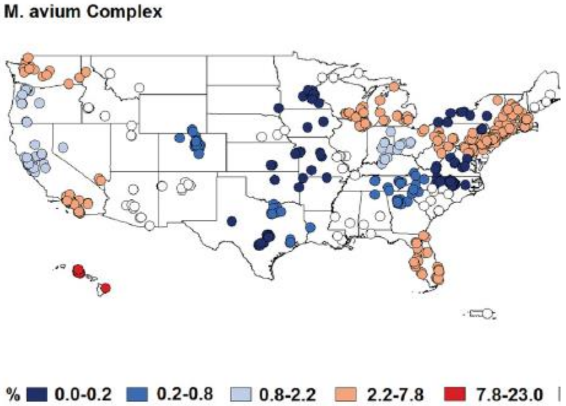
Showerhose biofilm











Feazel, *et al.,* PNAS 2009
Falkinham, *et al*., 2008, 2011
Proctor, *et al*., Water Research, 2018
Showerhead Microbiome Project (Gebert *et al*, mBio, 2018)

Other environmental niches for NTM

Drinking water distribution systems

Hot tubs

Showerheads

Sink faucets

Soil

Cigarette paper

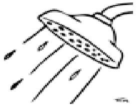
Footbaths

Natural surface water

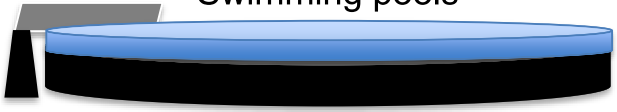
House dust

Aerators

Ice machines



Swimming pools



Bronchoscopes

Air Humidifiers

Galvanized or iron pipes

Chan, Breslawsky, NTMiR, 2019
Honda, *et al,* PLOS Neg Trop, 2015
De Groote, *et al,* AEM, 2006

Guimaraes, *et al,* Am J Infect Con, 2016
Martin, *et al,* Am Rev Resp Dis 1987
Briancesco, *et al,* Microchem J, 2014

Falkinham, *et al,* J Clin Micro, 1995
Falkinham, *et al,* Sem Resp Crit Care Med, 2013
Falkinham, *et al,* J Water Health, 2007

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Certain metals as NTM predictors – location dependent

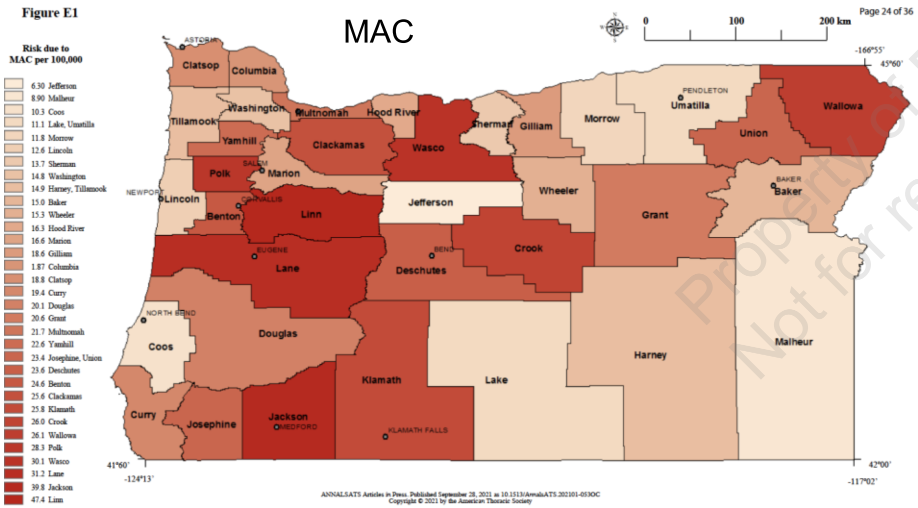
Oregon

As molybdenum increases, MAC infections increase by 45%.

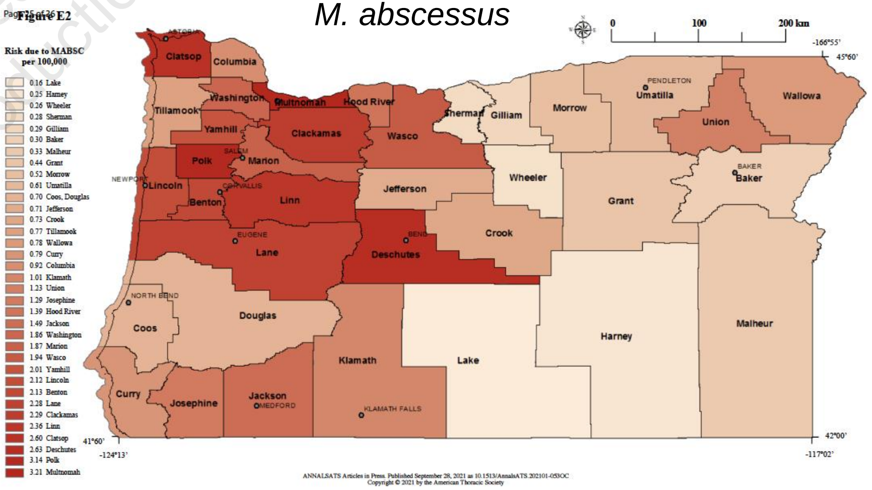
((Molybdenum associated with disease risk in Colorado)).

As vanadium increases, *M. abscessus* infections increase by 41%.

MAC



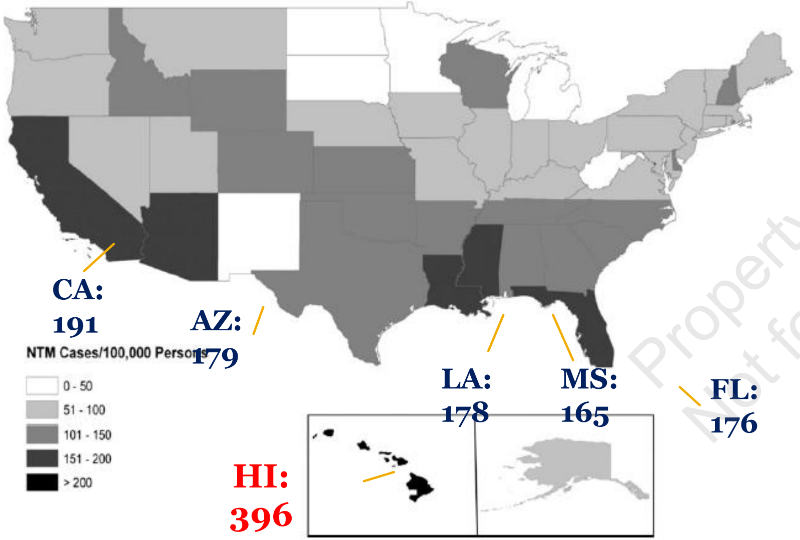
M. abscessus

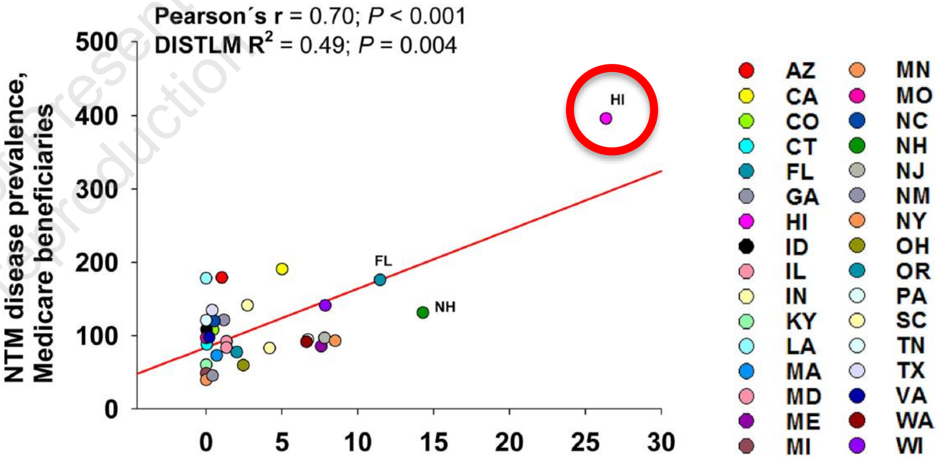


Lipner, *et al.*, Int J Env Res Pulb Health, 2020; Lipner, *et al*., Annals ATS, 2021

Hawai'i is a hot spot for NTM lung disease

Highest national prevalence - 396 cases/100,000 population among persons > 65 years-old (1997-2007).





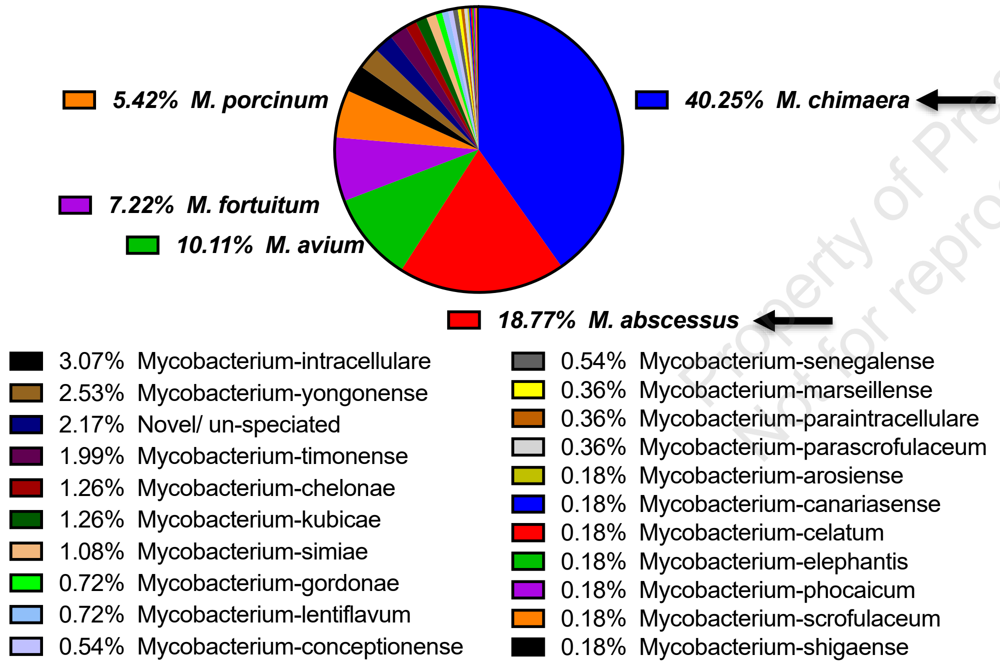
Hawai'i shows the highest, national age-adjusted mortality rates from NTM lung disease.

Adjemian, *et al.*, AJRCCM
Mirsaeidi, *et al.*, Plos One, 2014
Gebert *et al*, mBio, 2018
van Ingen *et al.*, Int J Syst Evol Micro, 2018
Adjemian, Honda, *et al.*, EID, 2017

Respiratory NTM isolate diversity

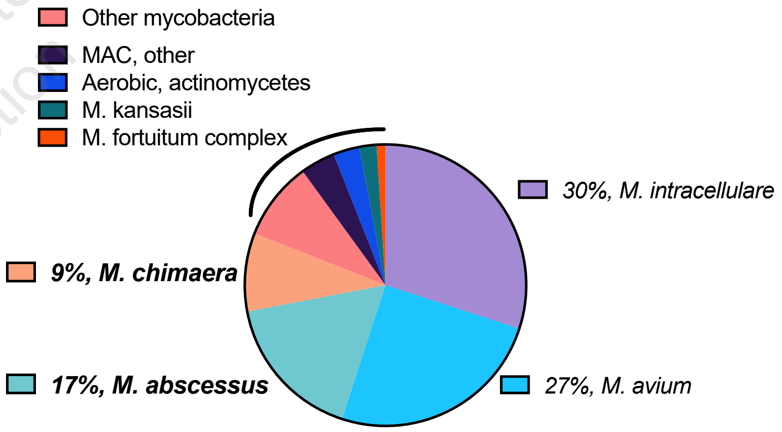
Diagnostic Laboratory Services, Hawai'i

Total: 554 clinical isolates



Unpublished

National Jewish Health

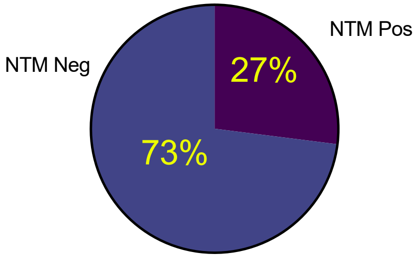


Total=100% (n=1,271 isolates)

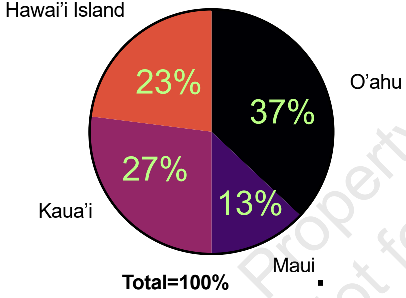
Acknowledgement: Dr. Reeti Khare; Director
NJH Mycobacterial Clinical Labs
Jan-Aug 2021

Large scale environmental NTM sampling, Hawai’i

All state (2,831 samples)



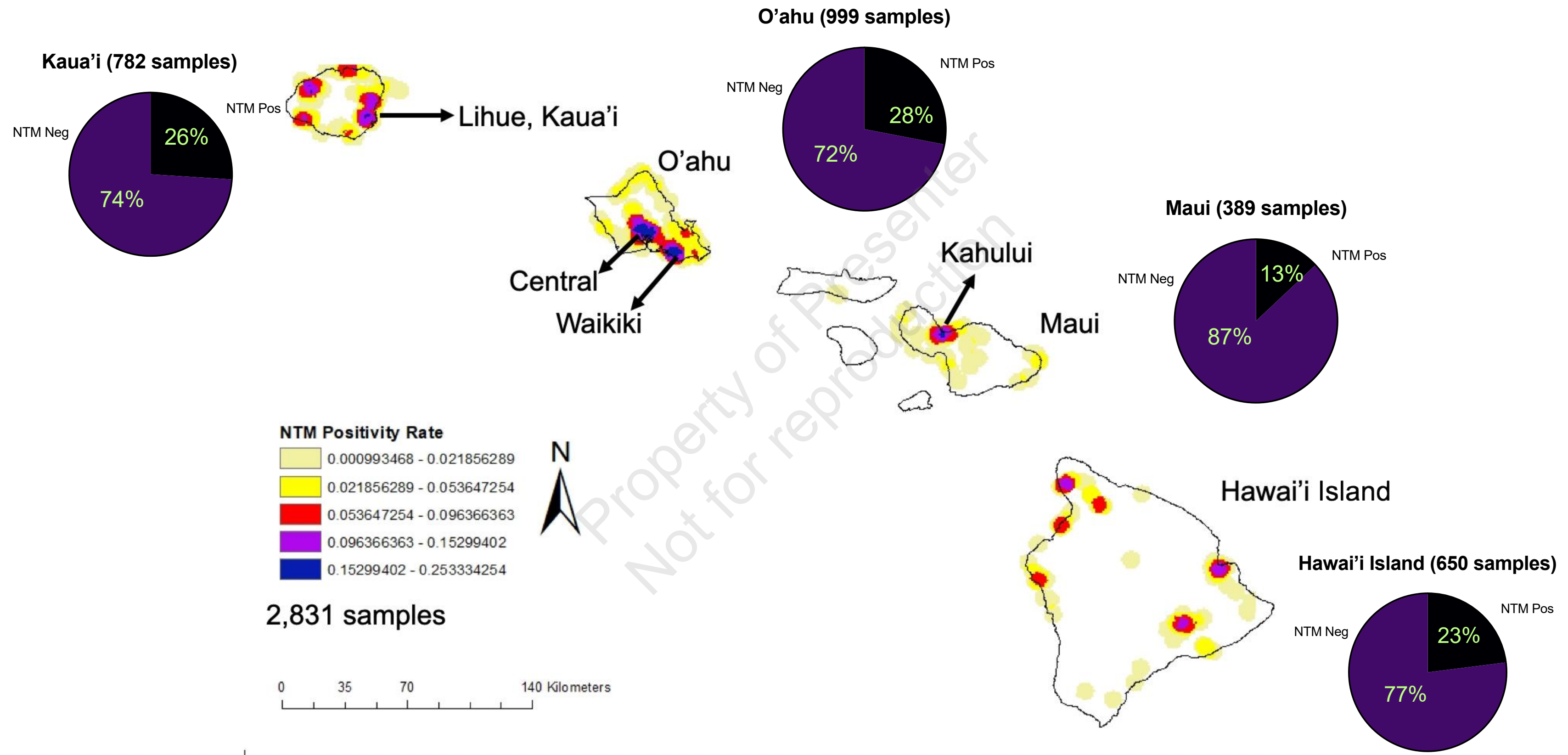
Island distribution
766 total NTM+ samples



Sample Type: NTM positive samples			
	Biofilm	***Soil***	***Dust***
Entire State	**458/1779 (26%)**	**251/728 (35%)**	**34/279 (12%)**
O’ahu	175/642 (27%)	79/213 **(37%)**	14/110 (13%)
Hawai’i Island	108/362 (30%)	59/230 (27%)	5/53 (9%)
Maui	50/265 (19%)	38/76 **(50%)**	9/48 (19%)
Kaua’i	123/499 (25%)	75/209 **(35%)**	6/68 (9%)

Similar to Falkinham 2011 US samples
28% (109/394)
(biofilm, soil, filters)

Unpublished
Falkinham, EID, 2011



Unpublished

Of

To

8.55%	Novel/ un-speciated
3.68%	Mycobacterium-gordonae
2.68%	Mycobacterium-mucogenicum
2.39%	Mycobacterium-avium
2.19%	Mycobacterium-iranicum
1.89%	Mycobacterium-gallinarum
1.69%	Mycobacterium-paragordonae
1.59%	Mycobacterium-peregrinum
1.49%	Mycobacterium-neoaurum
1.49%	Mycobacterium-phocaicum
1.39%	Mycobacterium-flavescens
1.39%	Mycobacterium-litorale
1.29%	Mycobacterium-fortuitum
1.29%	Mycobacterium-rhodesiae
1.19%	Mycobacterium-timonense
0.99%	Mycobacterium-septicum
0.89%	Mycobacterium-florentinum
0.89%	Mycobacterium-triplex
0.89%	Mycobacterium-stomatepiae
0.80%	Mycobacterium-conceptionense
0.80%	Mycobacterium-gilvum
0.80%	Mycobacterium-wolinskyi
0.70%	Mycobacterium-boenickei
0.70%	Mycobacterium-frederiksbergense
0.70%	Mycobacterium-intracellulare
0.70%	Mycobacterium-monacense
0.70%	Mycobacterium-moriokaense
0.70%	Mycobacterium-immunogenum
0.60%	Mycobacterium-grossiae
0.60%	Mycobacterium-vanbaalenii
0.50%	Mycobacterium-bacteremicum
0.40%	Mycobacterium-arceuilense
0.40%	Mycobacterium-aurum
0.40%	Mycobacterium-fluoranthenivorans
0.40%	Mycobacterium-marinum
0.40%	Mycobacterium-paraffinicum
0.40%	Mycobacterium-lentiflavum
0.40%	Mycobacterium-chubuense
0.30%	Mycobacterium-poriferae
0.30%	Mycobacterium-canariasense
0.30%	Mycobacterium-vaccae
0.30%	Mycobacterium-genavense
0.30%	Mycobacterium-marseillense
0.20%	Mycobacterium-alvei
0.20%	Mycobacterium-malmesburyense
0.20%	Mycobacterium-gadium
0.20%	Mycobacterium-franklinii
0.20%	Mycobacterium-smegmatis
0.20%	Mycobacterium-jacuzzii
0.20%	Mycobacterium-yongonense
0.20%	Mycobacterium-ratisbonense
0.20%	Mycobacterium-senegalense
0.20%	Mycobacterium-farcinogenes
0.20%	Mycobacterium-rutilum
0.20%	Mycobacterium-parmense
0.20%	Mycobacterium-llatzerense
0.20%	Mycobacterium-goodii
0.20%	Mycobacterium-barrassiae
0.10%	Mycobacterium-agri
0.10%	Mycobacterium-simiae
0.10%	Mycobacterium-longobardum
0.10%	Mycobacterium-mageritense
0.10%	Mycobacterium-parascrofulaceum
0.10%	Mycobacterium-psychrotolerans
0.10%	Mycobacterium-scrofulaceum
0.10%	Mycobacterium-indicus
0.10%	Mycobacterium-murale
0.10%	Mycobacterium-kansasii
0.10%	Mycobacterium-chitae
0.10%	Mycobacterium-asiaticum
0.10%	Mycobacterium-tokaiense
0.10%	Mycobacterium-arabiense
0.10%	Mycobacterium-colombiense
0.10%	Mycobacterium-insubricum
0.10%	Mycobacterium-sediminis
0.10%	Mycobacterium-arupense
0.10%	Mycobacterium-setense
0.10%	Mycobacterium-madagascariense
0.10%	Mycobacterium-phlei

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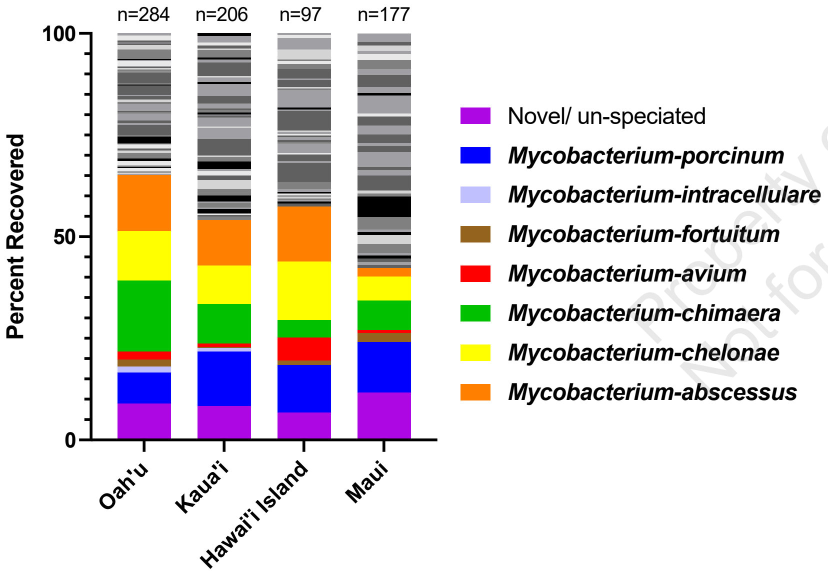
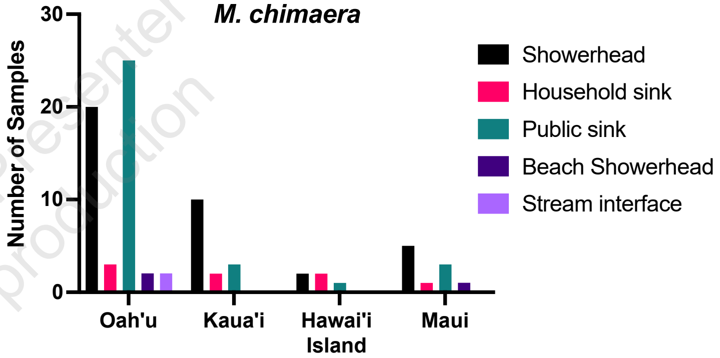
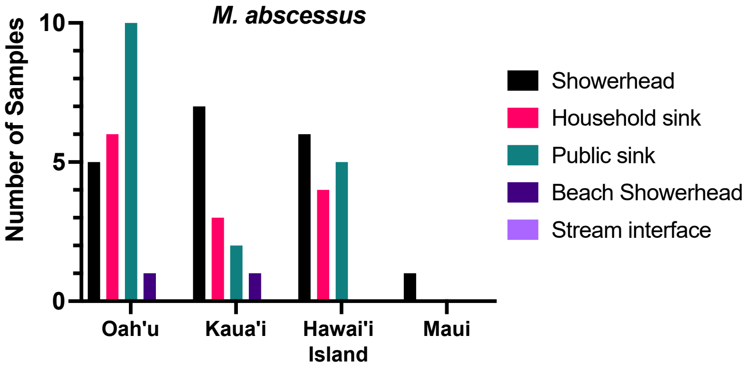
Unpublished

Environmental NTM distribution per island







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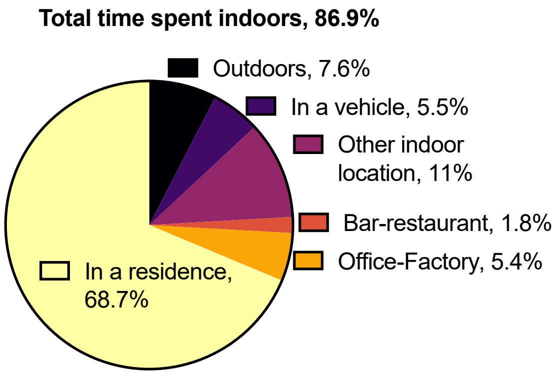
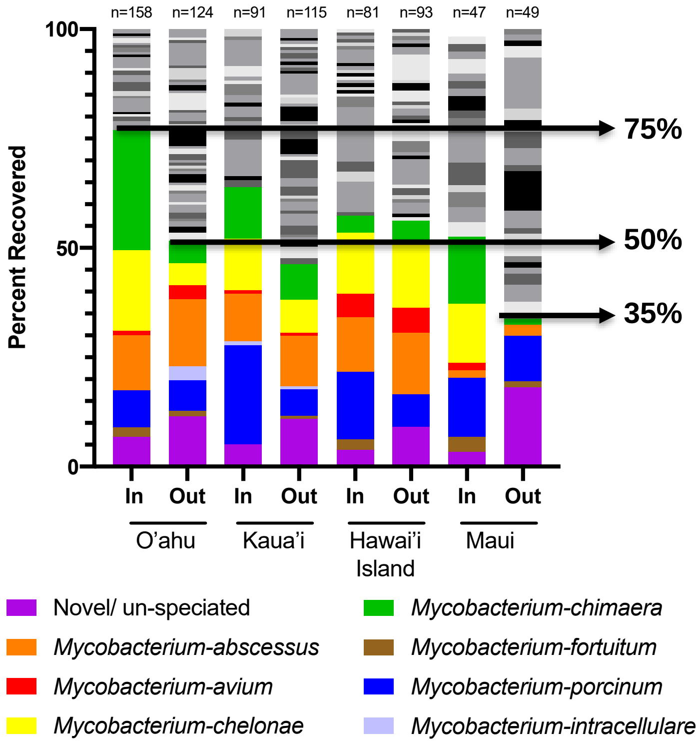
NTM in indoor vs outdoor Hawai'i environments



Nation, n = 9,196 (2010)

Adapted from:
Klepeis, *et al*, J Exp Analysis and Env Epi, 2010

Photo credit: J. Honda



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Are all showerhead biofilms created equal?

Indoor household

Beach

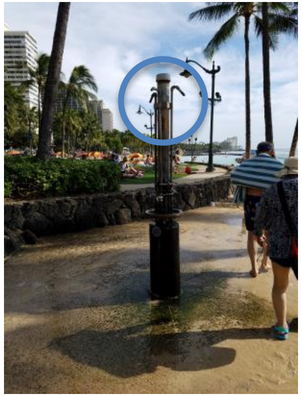
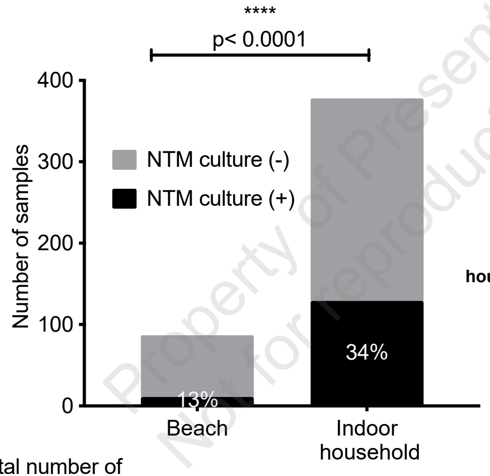
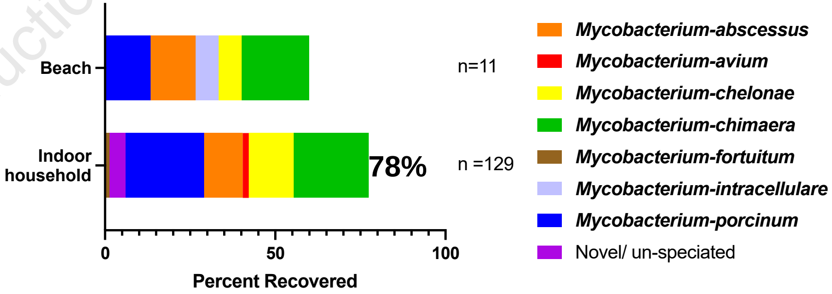


Photo credit: J. Honda



Total number of showerheads tested: n = 87 n = 378

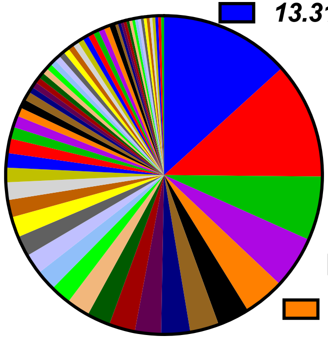


Are there unique environmental factors influencing NTM in the Hawai'i environment?

Unpublished

Soil minerals contribute to NTM in Hawai'i

Total: 251 soil samples



13.31% M. abscessus

11.83% Novel/ un-speciated

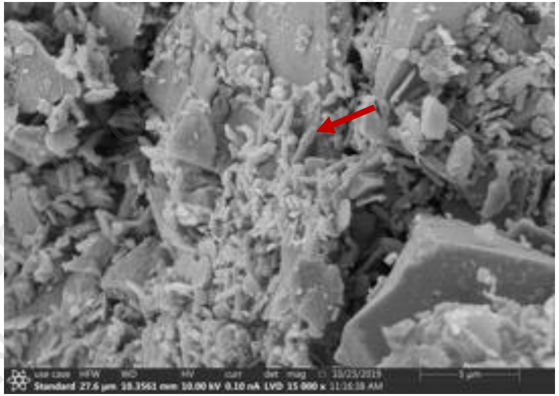
6.51% Mycobacterium-porcinum

5.33% Mycobacterium-gallinarum

4.14% Mycobacterium-chelonae

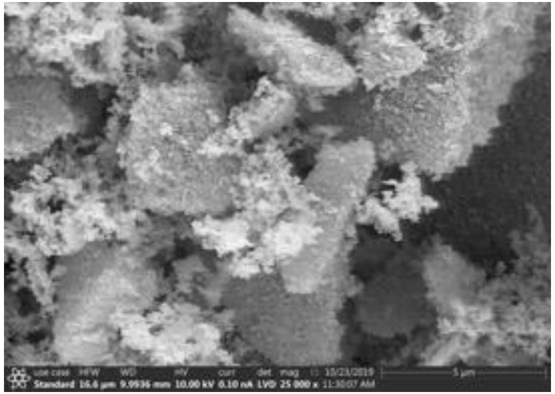
Iron minerals

More *M. abscessus* with *hematite*



Clay minerals

Less with *gibbsite*



<u>NTM probability highest in:</u>

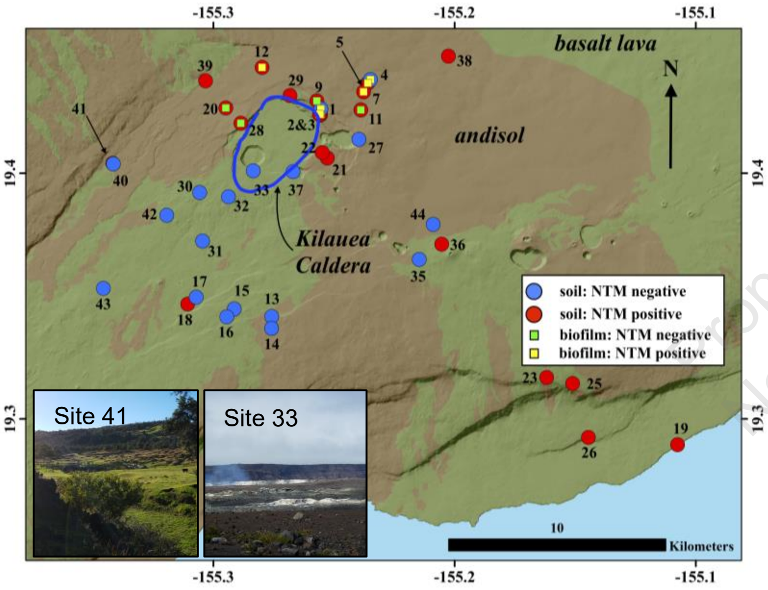
- Rich in ferric oxide.
- Expansive soils with a high-water balance.

Unpublished
Glickman and Virdi *et al.*, AEM, 2020

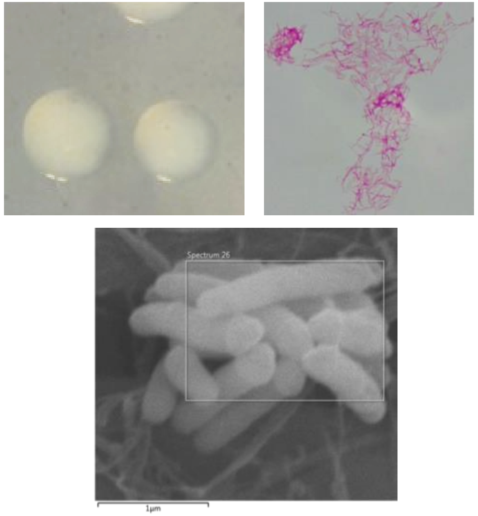
Kilauea Volcano, Hawai'i Island

https://www.usgs.gov/media/before-after/recent-changes-k-lauea-volcano-october-4-2021
Photo credit: https://www.usgs.gov/center-news/photo-and-video-chronology-k-lauea-may-29-2018

Do NTM inhabit volcanic environments?



Kilauea steam vent



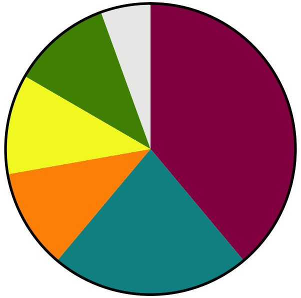
Taxa ID	BLAST ID
Mycobacterium abscessus	99.7%

Dawrs, *et al*., in preparation

Diversity of NTM in Kilauea water biofilms and soil

NTM at Kilauea		
Total sites sampled:	**No. NTM positive sites:**	**% NTM Positive:**
44	**26**	**59%**

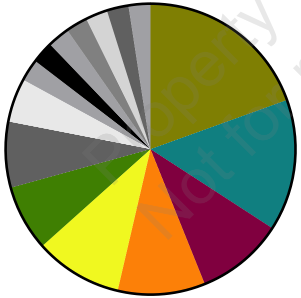
Water biofilms



- 38.9% *M. abscessus*
- 22.2% *M. chelonae*
- 11.1% *M. chimaera*
- 11.1% *M. avium*
- 11.1% Novel
- 5.6% *M. iranicum*

Total = 18 Isolates

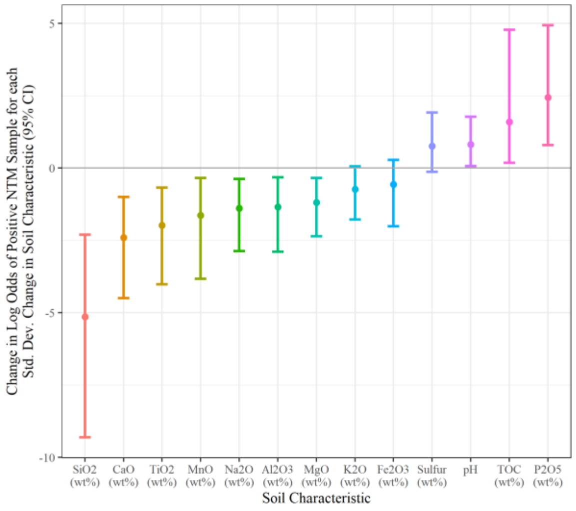
Soil



- 19.5% *M. triplex*
- 14.6% *M. chelonae*
- 9.8% *M. abscessus*
- 9.8% *M. avium*
- 9.8% Novel
- 7.8% *M. chimaera*
- 7.3% *M. timonense*
- 4.9% *M. genavense*
- 2.4% *M. florentinum*
- 2.4% *M. franklinii*
- 2.4% *M. gordonae*
- 2.4% *M. marseillense*
- 2.4% *M. paraffinicum*
- 2.4% *M. parascrofulaceum*
- 2.4% *M. parmense*

Total = 41 Isolates

phosphate



Dawrs, *et al.*, in preparation

Kilauea’s Most Recent Explosive Eruption

Jan. 19, 2018 [1]





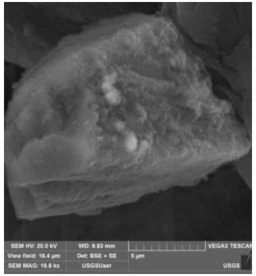
May 4, 2018 [2]

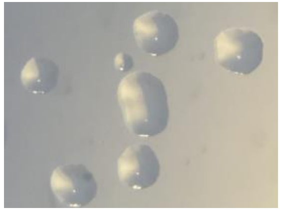
[1] Photo credit: J. Honda
[2] Photo credit: https://www.usgs.gov/center-news/photo-and-video-chronology-k-lauea-may-4-2018

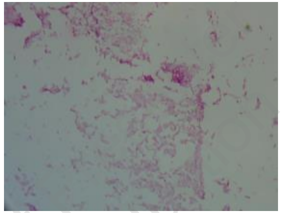
Kilauea Volcanic Ash Harbors Viable NTM

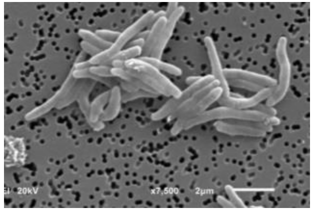
David Damby, PhD

Ash SEM

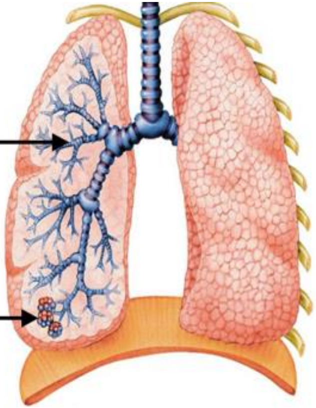








rpoB **Taxa ID**	**BLAST ID**
Mycobacterium avium	99.2%

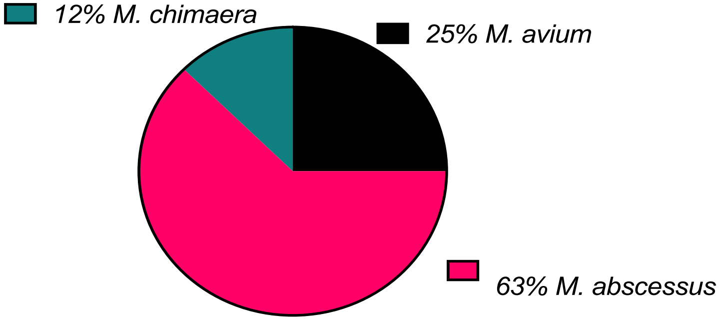


< 32 µm
"Fine" ash

< 5 µm
"Respirable" ash
can reach the alveoli

8 NTM-like colonies were isolated

Kilauea ash, May 2018

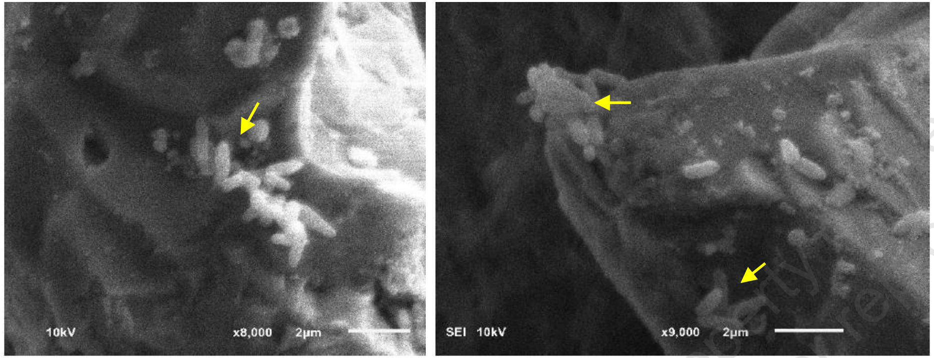


Total = 8 Isolates

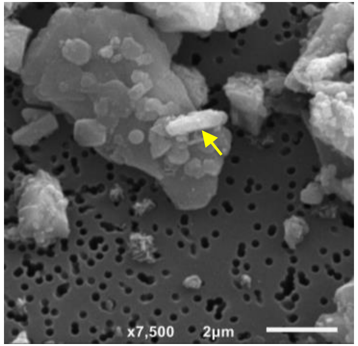
Dawrs, *et al.*, in preparation

NTM adheres to Kilauea ash

***M. abscessus* + ash**

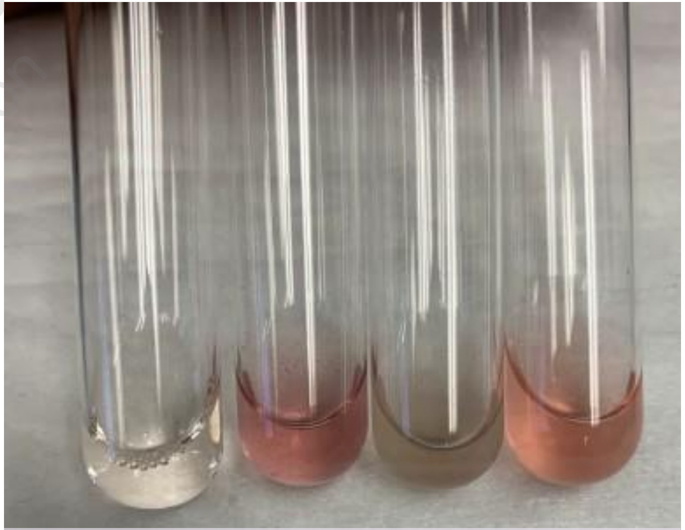


***M. chelonae* + ash**



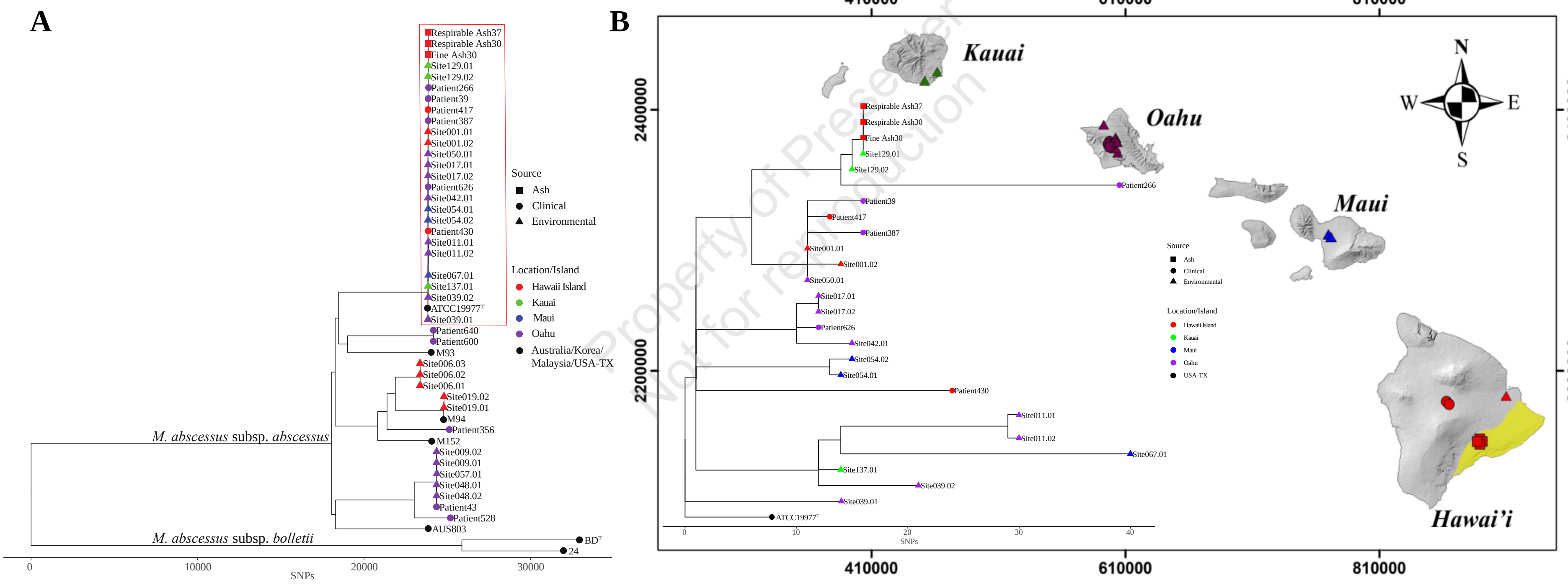
Dawrs, *et al*., in preparation

Viability Assay



NTM are viable in the presence of ash.

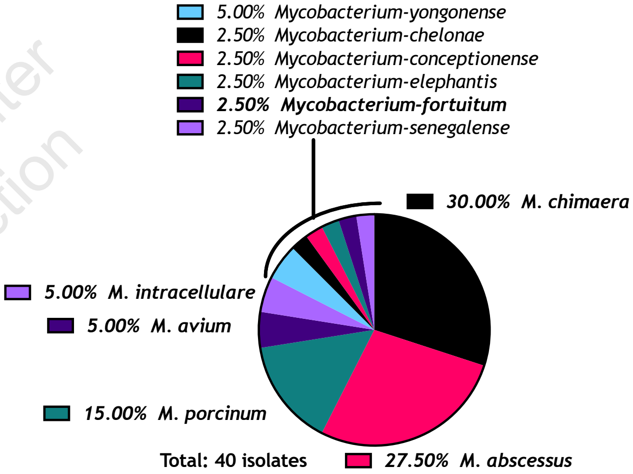
Kilauea ash as a possible fomite for NTM transmission



Dawrs, *et al.*, in preparation

NTM in Hawai'i – A Matched Study

	Cases (n=35)	Controls (n=28)	p-value
Gender			
Female	18 (51.4%)	15 (53.6%)	1
Male	17 (48.6%)	13 (46.4%)	1
Age			
40-50	21 (60.0%)	14 (50.0%)	0.59
51-60	2 (5.7%)	1 (3.6%)	1
61-70	4 (11.4%)	4 (14.3%)	1
71-80	7 (20%)	7 (25%)	0.89
81-90	1 (2.9%)	2 (7.1%)	0.58
Race/Ethnicity			
Asian	13 (37.1%)	4 (14.3%)	0.08
Native Hawaiian and Pacific Islander	2 (5.7%)	2 (7.1%)	1
White	15 (42.9%)	12 (42.9%)	1
Other	5 (14.3%)	10 (35.7%)	0.09

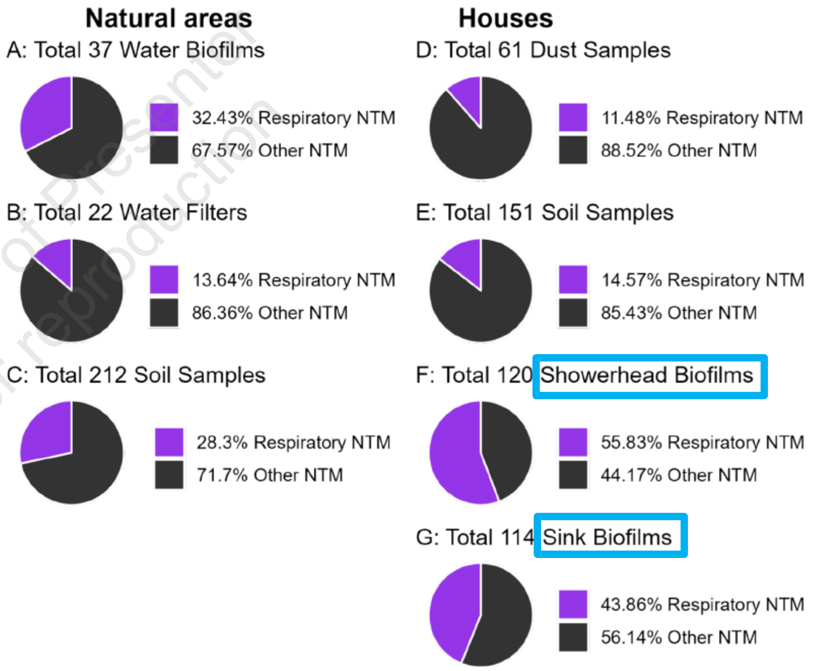


	Kitchen Faucet	Showerhead	Soil
M. abscessus	5/5 (100%)	0/7 (0%)	N/A
M. chimaera	0/7 (0%)	4/4 (100%)	0/2 (0%)

Unpublished

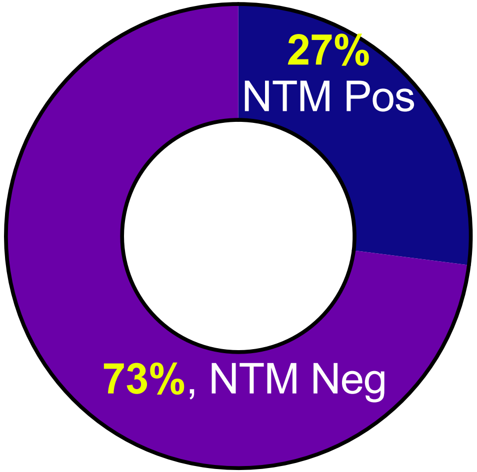
Possible significance of NTM in Hawai’i

Ranking	*Top Environmental:*	*Top Respiratory:*
1	***M. abscessus***	***M. chimaera***
2	***M. chelonae***	***M. abscessus***
3	***M. chimaera***	***M. avium***
4	***M. porcinum***	***M. fortuitum***
5	***Novel/un-speciated***	***M. porcinum***
6	***M. gordonae***	***M. intracellulare***
7	***M. mucogenicum***	***M. yongonense***
8	***M. avium***	***Novel/un-speciated***
9	***M. iranicum***	***M. timonense***
10	***M. gallinarum***	***M. chelonae***



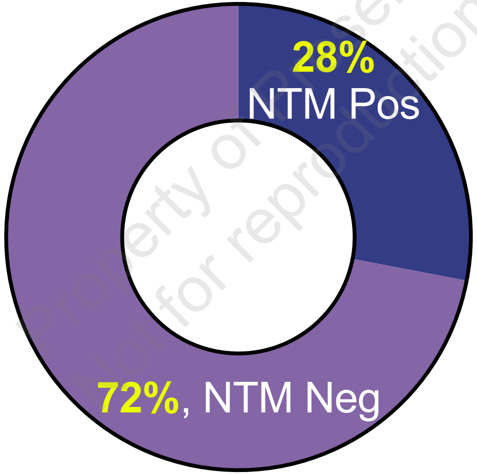
NTM may not be as “ubiquitous” as presumed

Hawai’i



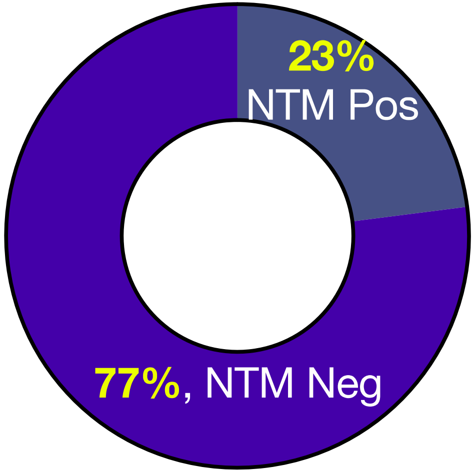
n = 2,831 samples

Falkinham, USA



n = 394 samples

non-Hawai’i, USA



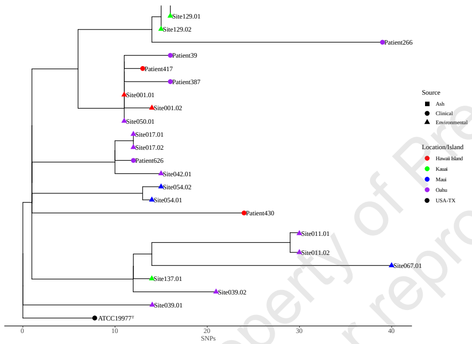
n = 525 samples

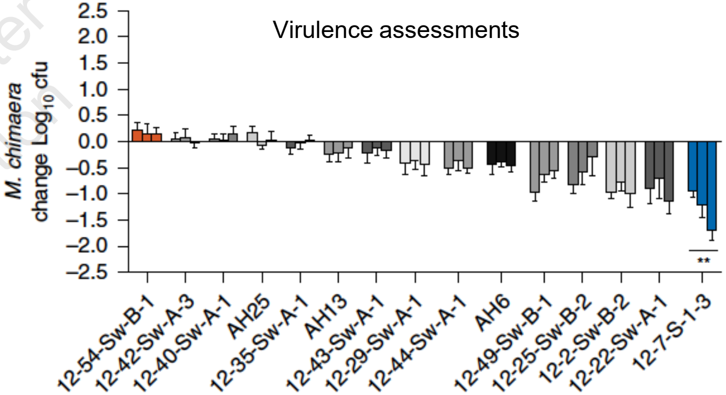
Unpublished
Falkinham, et al., EID, 2011

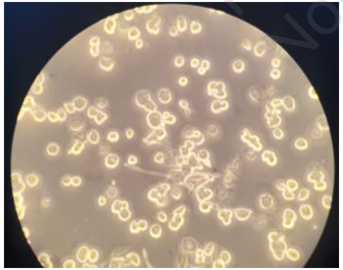
Testing new hypothesis – ongoing work

Biorepository:	
	No. of ENV Isolates:
M. chimaera	154
M. abscessus	182
M, chelonae	153
M. porcinum	183
M. avium	27
M. intracellulare	11
M. fortuitum	20
Other NTM isolates	733
Total:	**1,463**

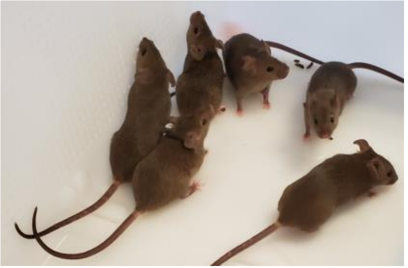
Apply whole genome sequencing information

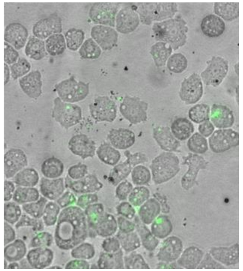





Macrophages


ALI, lung organoids

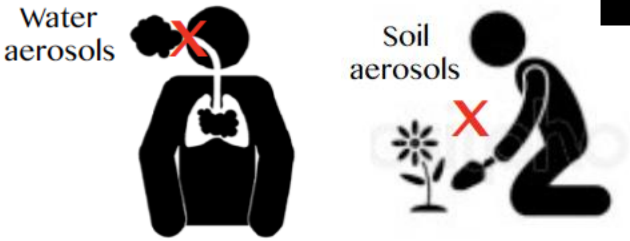

Mice


Amoebae

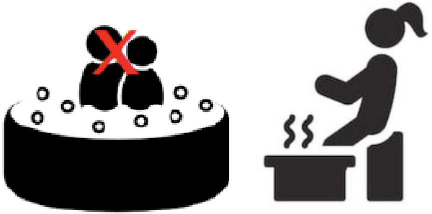
Honda, *et al.*, AJRCMB, 2019
Unpublished

Proposed methods to reduce exposures

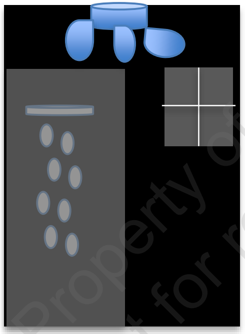
Avoid aerosol generating activities



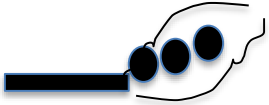
Avoid bubbling water (*i.e.*, hot tubs, nail foot baths, humidifiers)



Use fans or open windows in shower area



Avoid smoking



Replace or clean showerheads regularly
Avoid fine mist showerheads

preferred

NOT recommended

Flush water heater frequently
Increase hot water heater temperature

Avoid using tap water for nasal irrigations

Falkinham, *et al,* Ann Am Thor Soc, 2013
Chan, Breslawsky, NTMiR, 2019

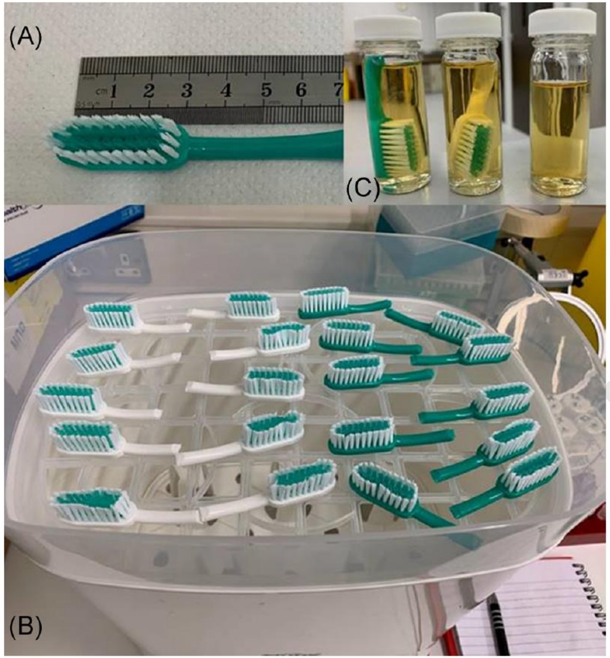
Learning Objectives

1). Become familiar with examples of environmental niches for NTM.

2). Recognize features that may promote NTM in the environment – showcasing Hawai'i.

3). Identify possible methods to reduce environmental NTM exposures.

Steam disinfection of toothbrushes reduces NTM



M. abscessus subsp. *abscessus* (n=2)
M. abscessus subsp. *bolletii* (n=2)
M. abscessus subsp. *massiliense* (n=2)

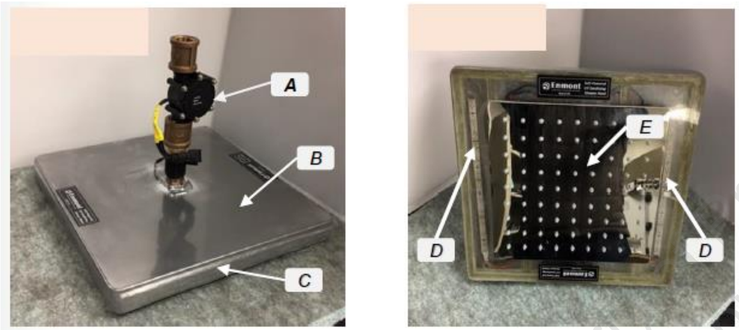
None of the 80 isolates examined survived at 91℃ for 4 min. [1]

** Boiling water for 10-15min reduces NTM. [2]

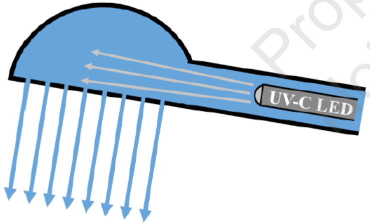
[1] Millar, *et al*., Pediatric Pulmonology, 2020
[2] Falkinham, WhiteJ, 2013

UV disinfection to reduce NTM in freshwater

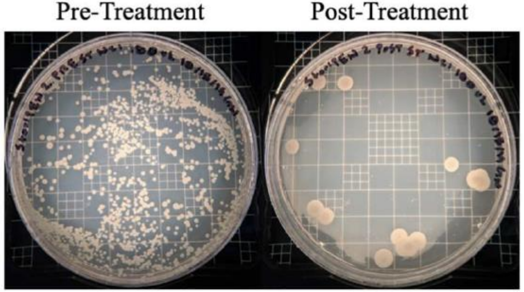
UV-LED adapted showerhead reduces *Legionella* (EPA)

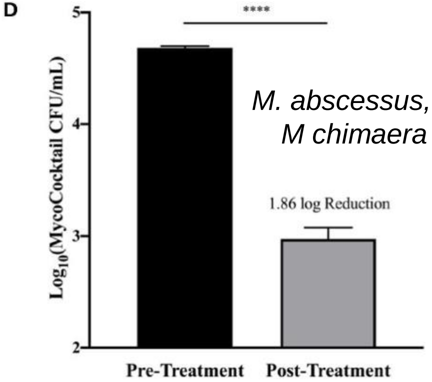


Top View   Bottom View



UV Water Bottles reduce NTM from drinking water

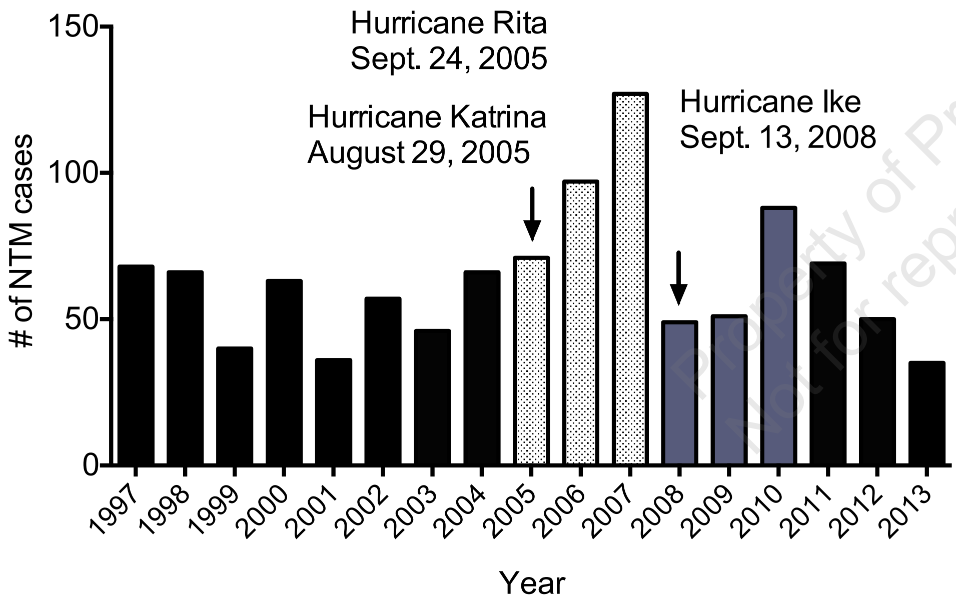




M. abscessus, M. avium, M chimaera cocktail

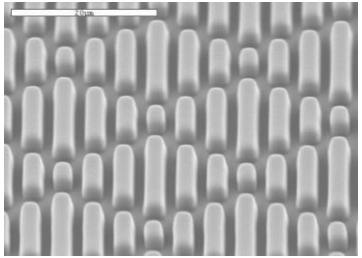
Norton, *et al.*, Frontiers in Public Health, 2020
https://www.epa.gov/sciencematters/uv-led-showerhead-invention-reduces-risk-legionella-contamination
Hessling, *et al.*, Hosp Pract Res, 2018

Reduce exposure to aerosol generating events



Honda, *et al,* Chest, 2015
Image: https://www.usgs.gov/special-topic/fire
Image: https://volcanoes.usgs.gov/volcanic_ash/ash_gas.html

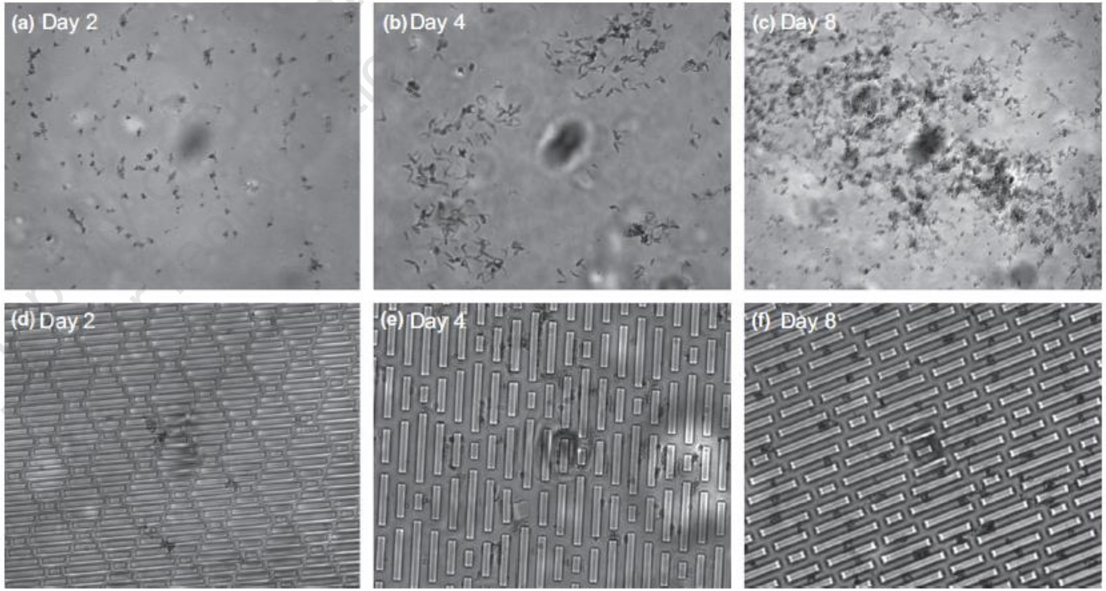
Novel applications to reduce surface NTM adherence



M. abscessus

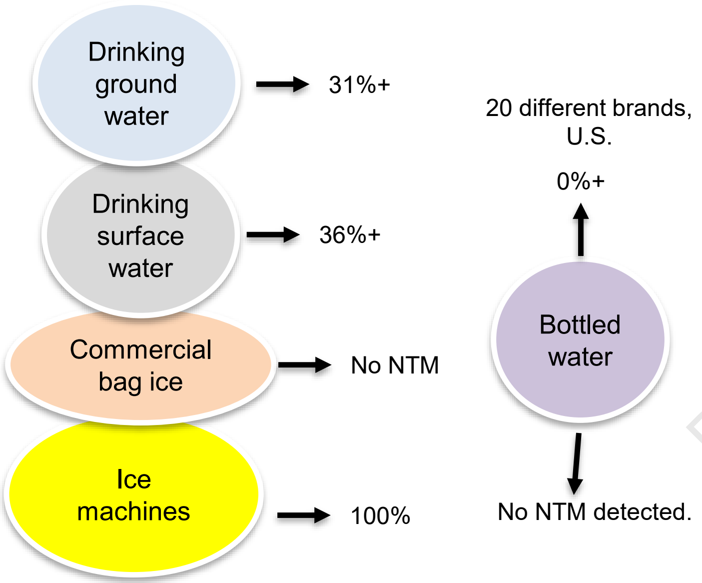
Sharklet(-)

Sharklet(+)



Kim *et al*., FEMS Microb Letters, 2014

Expand Drinking Water Awareness



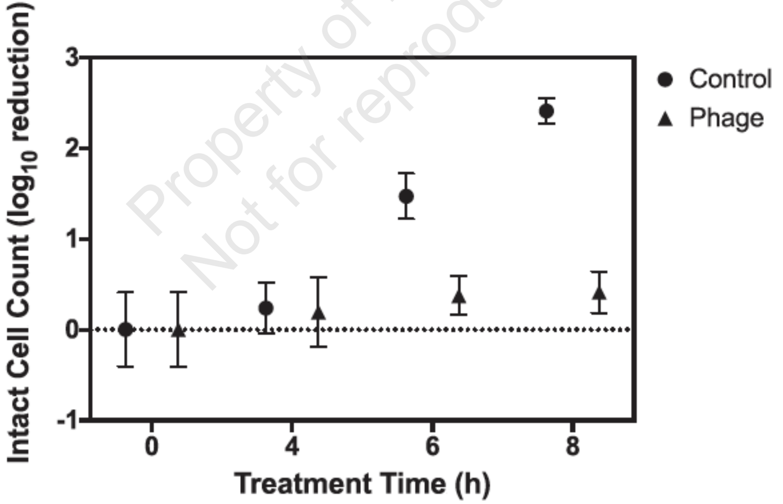
Bottled Water (Honda Lab, 2021):

Water Tested:	Type of water (Source = U.S.A.) unless noted:	Characteristic:	Results:
1	Bottled Water, Brand 1	Natural spring water	None
2	Bottled Water, Brand 2	Purified water	None
3	Bottled Water, Brand 3	Natural spring water	None
4	Bottled Water, Brand 4	Water from snow	None
5	Bottled Water, Brand 5	Volcanic rock filtered water	*Mycobacterium neoaurum* *Mycobacterium phocaicum*
6	Bottled Water, Brand 6	Volcanic rock filtered water	None
7	Bottled Water, Brand (non-U.S.A)	Volcanic rock filtered water	None
8	Distilled water	Commercially available	None
9	Sink faucet 1	Municipal water, Colorado	*Mycobacterium abscessus*
10	Sink faucet 2	Municipal water, Colorado	None
11	Sink faucet 3	Municipal water, Colorado	None
12	Water fountain	Municipal water, Colorado	None
13	Wall mounted water bottle filling station	Municipal water, Colorado	None

Holtzman, *et al*, J Food Protect 1997
Covert, *et al*, AEM, 1999
Totaro, *et al*, J Water Health, 2018
https://www.bottledwater.org/public/CCL4%20Microbes%20of%20Interest%20in%20Drinking%20Water_0.pdf0
Honda Lab, unpublished.

Phages as possible sanitizers to prevent NTM in the built environment

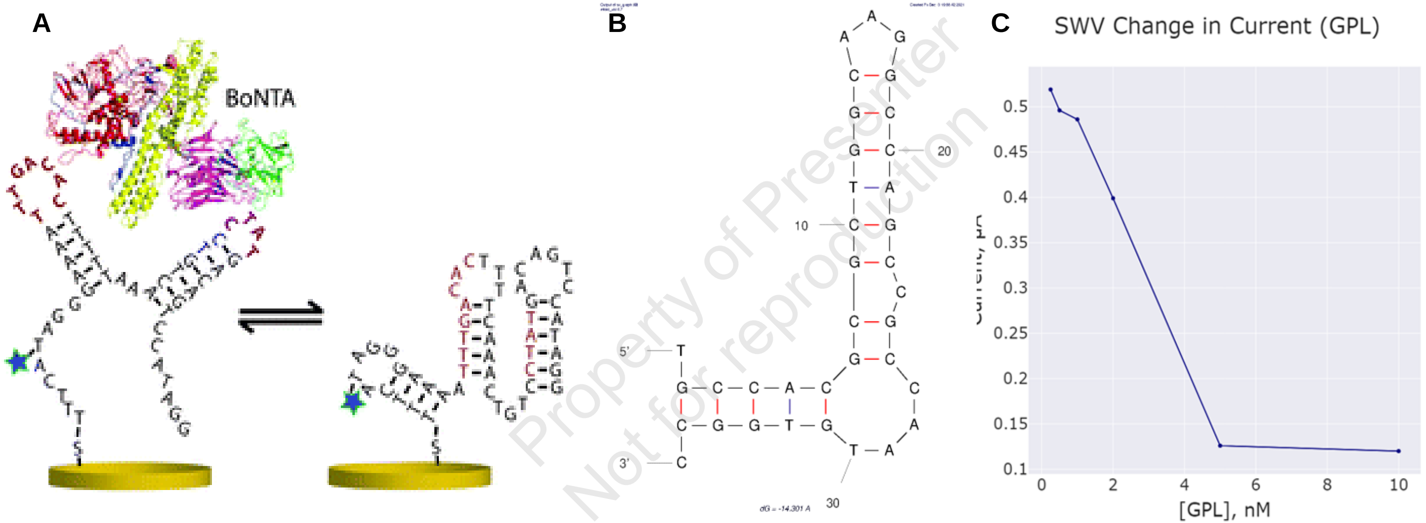
Bacteriophage Treatment before Chemical Disinfection Can Enhance Removal of Plastic-Surface-Associated *Pseudomonas aeruginosa*



Stachler *et al.*, AEM, 2021

Developing new point of use DNA biosensors to detect environmental NTM

(Andrew Bonham, PhD, Metro State University)



Electrochemical DNA-based biosensor for GPL. A) Schematic of the general biosensor approach, wherein a DNA aptamer selected to bind to a target, in this example Botulinum neurotoxin A (BoNTA), is attached to a gold electrode with a redox-active tag (blue star). Binding of the target changes DNA conformation and generates a specific, reproducible change in electrical current. **B)** SELEX-evolved DNA aptamer specific to GPL. **C)** Preliminary graph of electrical current response change when tested against purified GPL.

Poch, *et al.*, Rowdy Scholar, 2020

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