NTM Lecture Series for Providers

April 27-28, 2023
NATIONAL JEWISH HEALTH

National Jewish Health

Breathing Science is Life.

From Bench to Bedside:

Genomic Approaches to Investigate Nontuberculous Mycobacteria

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HALT NTM

Presenter disclosure

Funding from the CF Foundation.

Objectives

- Understand the role of whole genome sequencing in NTM analysis
- Recognize the utility of NTM outbreak investigations
- Identify resources for NTM whole genome sequencing

Background

- Nontuberculous mycobacteria (NTM) can be environmentally acquired from exposures to NTM-laden bioaerosols produced by soil and water
  - NTM colonize municipal water systems and have been identified in healthcare facilities
  - Clinically-relevant NTM have been cultured from environmental dust in homes of patients
- NTM lung disease is a risk for susceptible populations
  - Structural lung disease

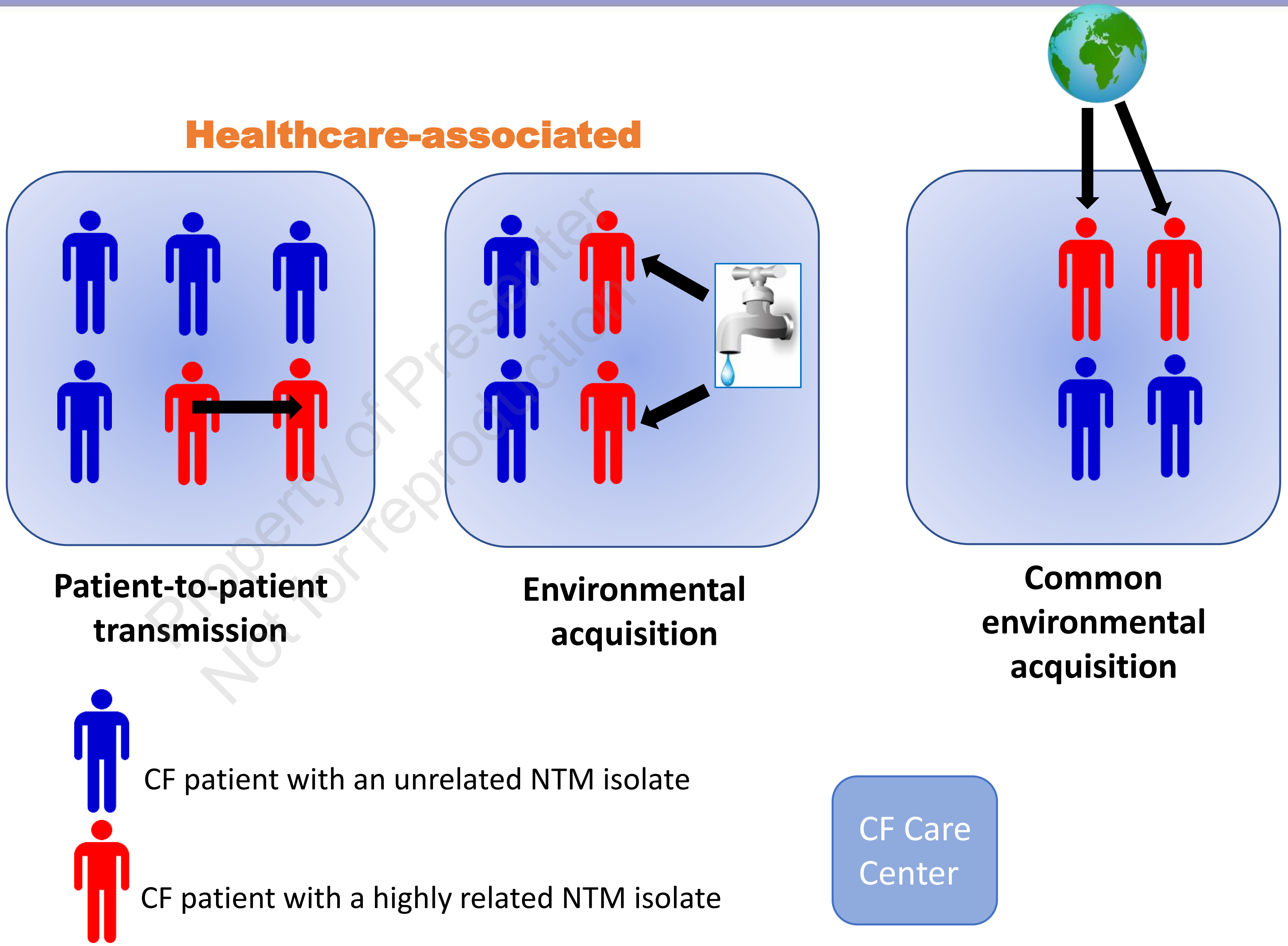
Primm et al., *Clin Microbiol Rev,* 2004
Falkinham, *Emerg Infet Dis* 2011
Honda et. al., *Front Microbiol,* 2018
Du Moulin et al., *JAMA,* 1988
Shin et al., *J Hosp Infect,* 2007

NTM and Cystic Fibrosis

- 10-14% of people with cystic fibrosis have had an NTM species isolated
- Recognized as one of the most challenging diseases to treat
    - Prolonged treatment courses
    - Significant toxicity
    - Often fails to clear infection
- Healthcare-associated transmission of NTM among people with cystic fibrosis has been investigated at CF Centers worldwide
- Modes of acquisition/transmission and exposure risks are poorly understood

Quist et al. Respir Res 2014
Floto et al. Thorax 2016
Cystic Fibrosis Foundation Annual Report 2019, 2020, 2021

Possible modes of NTM infection transmission



Healthcare-associated

Patient-to-patient transmission

Environmental acquisition

Common environmental acquisition

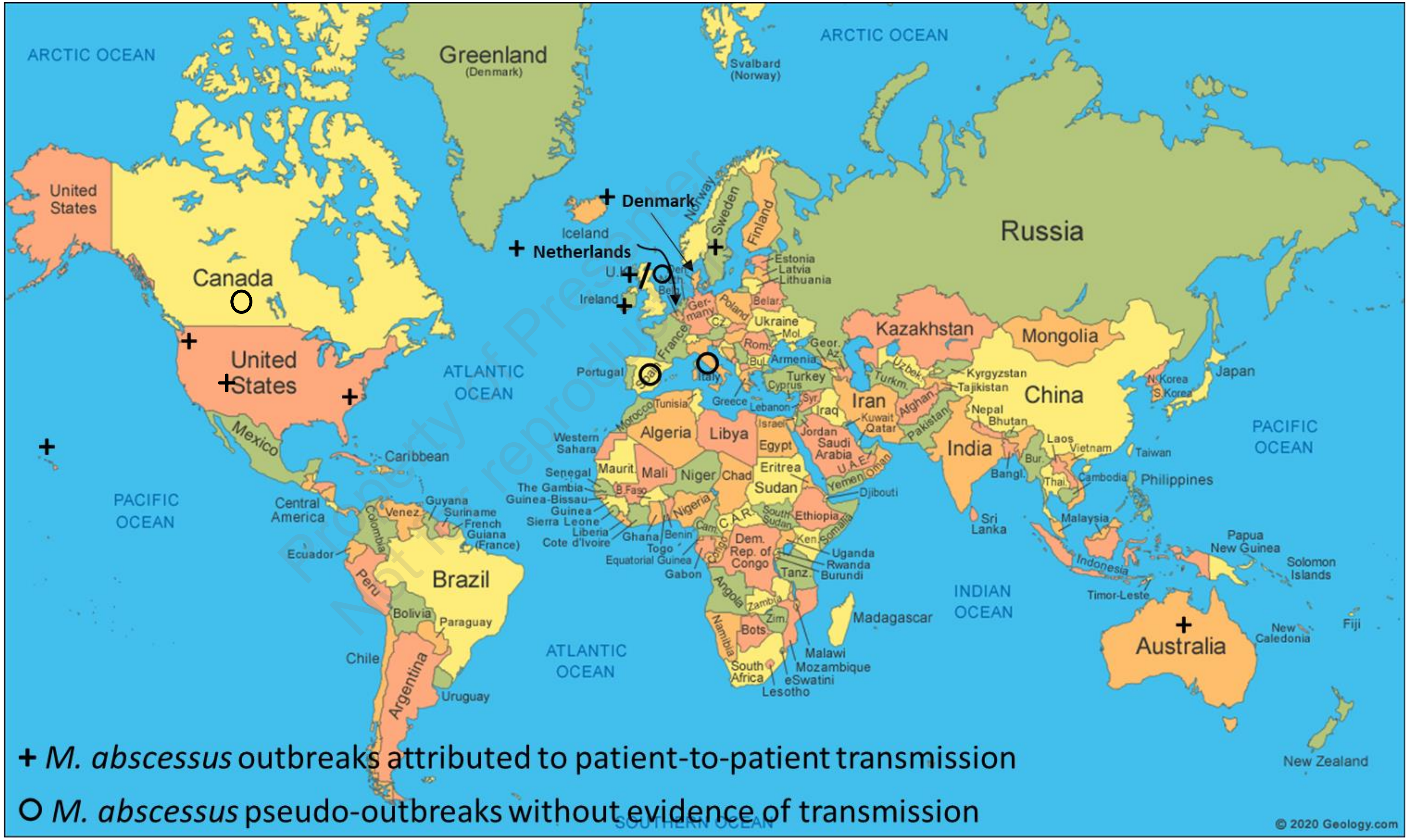
CF patient with an unrelated NTM isolate

CF patient with a highly related NTM isolate

CF Care Center

Gross JE, et al., Investigating Nontuberculous Mycobacteria Transmission at the Colorado Adult Cystic Fibrosis Program. *Am J Respir Crit Care Med*. 2022

Distribution of *M. abscessus* outbreaks and pseudo-outbreaks within CF Care Centers



\+ *M. abscessus* outbreaks attributed to patient-to-patient transmission

O *M. abscessus* pseudo-outbreaks without evidence of transmission

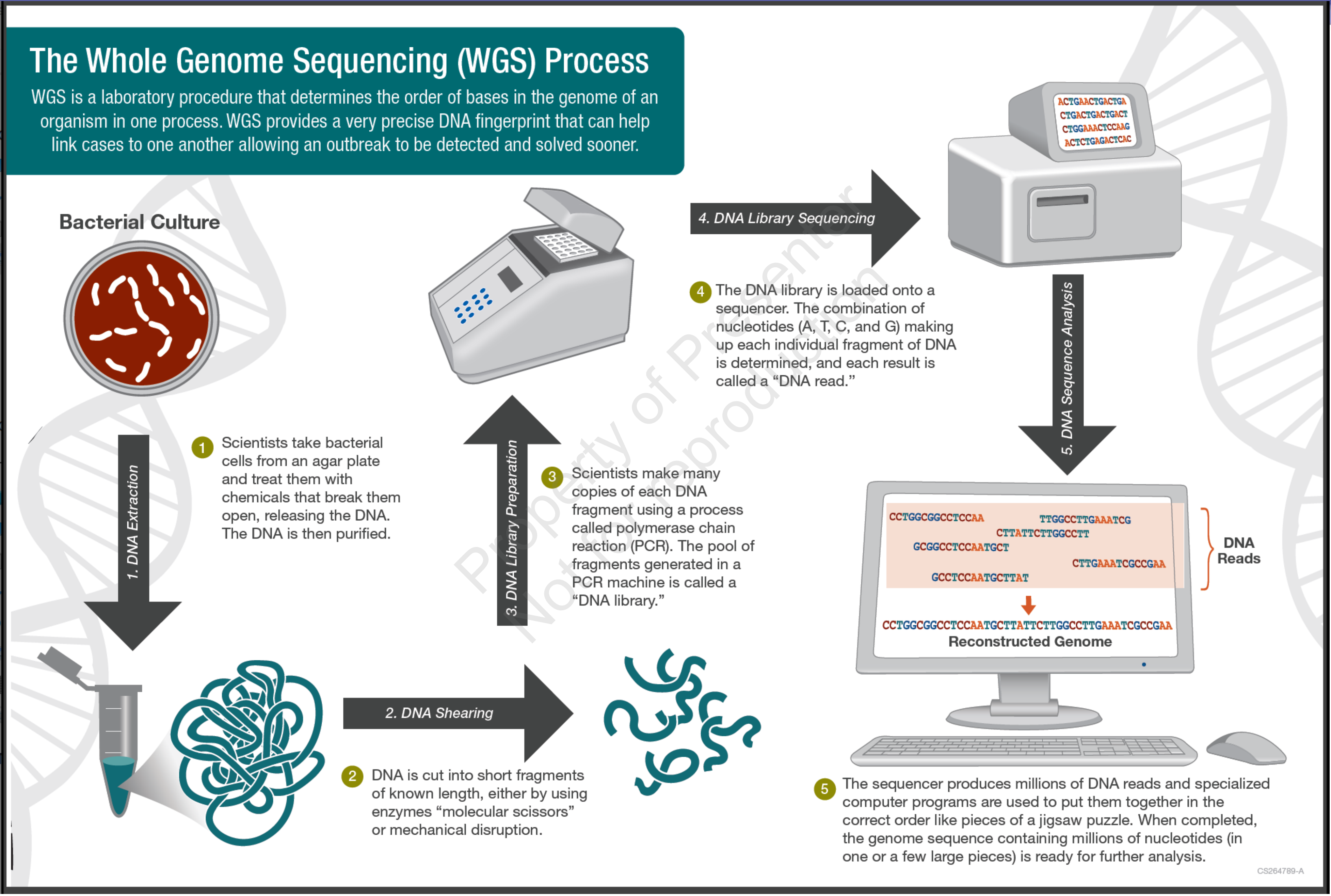
Whole genome sequencing

- The process of determining the DNA sequence of an organism’s genome
  - Unique DNA fingerprint
- Utilized in research and clinical care
  - Provides ability to pinpoint variations in the genetic code
  - Variations in the code are called single nucleotide polymorphisms (SNPs)
- SNP differences can be compared to determine relatedness of organisms
  - In genetic epidemiology SNPs are used to estimate transmission clusters
- Routine use in outbreak investigations
  - Foodborne disease outbreaks
  - Trends in foodborne infections and antimicrobial resistance

CDC image
https://www.cdc.gov/pulsenet/pathogens/wgs.htmlBi

The Whole Genome Sequencing (WGS) Process

WGS is a laboratory procedure that determines the order of bases in the genome of an organism in one process. WGS provides a very precise DNA fingerprint that can help link cases to one another allowing an outbreak to be detected and solved sooner.



① Scientists take bacterial cells from an agar plate and treat them with chemicals that break them open, releasing the DNA. The DNA is then purified.

② DNA is cut into short fragments of known length, either by using enzymes "molecular scissors" or mechanical disruption.

③ Scientists make many copies of each DNA fragment using a process called polymerase chain reaction (PCR). The pool of fragments generated in a PCR machine is called a "DNA library."

④ The DNA library is loaded onto a sequencer. The combination of nucleotides (A, T, C, and G) making up each individual fragment of DNA is determined, and each result is called a "DNA read."

⑤ The sequencer produces millions of DNA reads and specialized computer programs are used to put them together in the correct order like pieces of a jigsaw puzzle. When completed, the genome sequence containing millions of nucleotides (in one or a few large pieces) is ready for further analysis.

Healthcare-associated links in transmission of nontuberculous mycobacteria among people with cystic fibrosis (HALT NTM) study

HALT
NTM

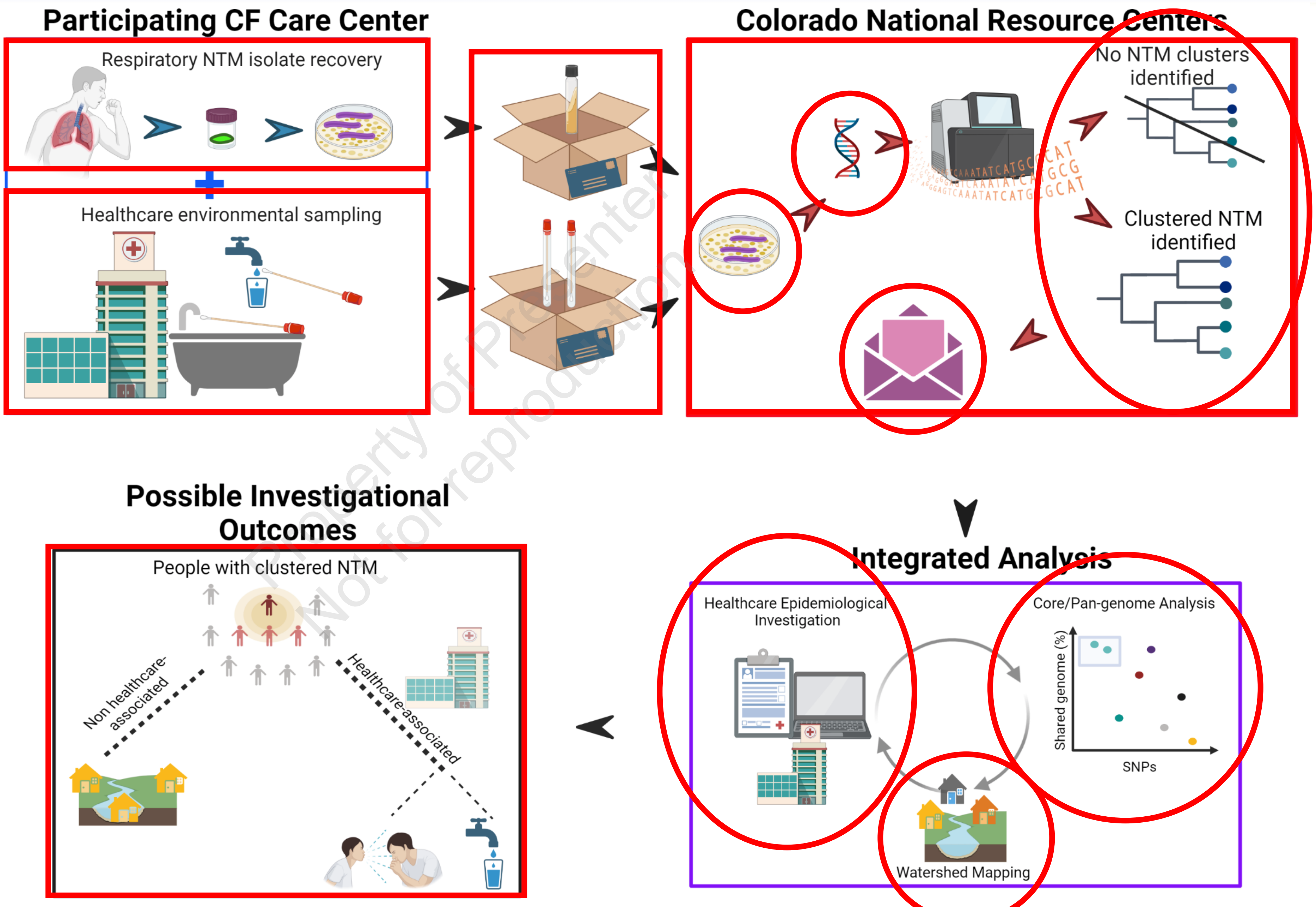
- Goal is to investigate potential NTM outbreaks in CF Care Centers
- Investigation of healthcare-associated transmission and/or acquisition of NTM

Objectives

To systematically investigate healthcare-associated transmission and/or acquisition of NTM

- To determine similarity among respiratory and environmental isolates
- To compare home residence watershed mapping among pwCF having genetically similar NTM isolates.

Overview of the HALT NTM study



Participating CF Care Center

Respiratory NTM isolate recovery

+

Healthcare environmental sampling

Colorado National Resource Centers

No NTM clusters identified

Clustered NTM identified

Integrated Analysis

Healthcare Epidemiological Investigation

Core/Pan-genome Analysis

Watershed Mapping

Possible Investigational Outcomes

People with clustered NTM

Non healthcare-associated

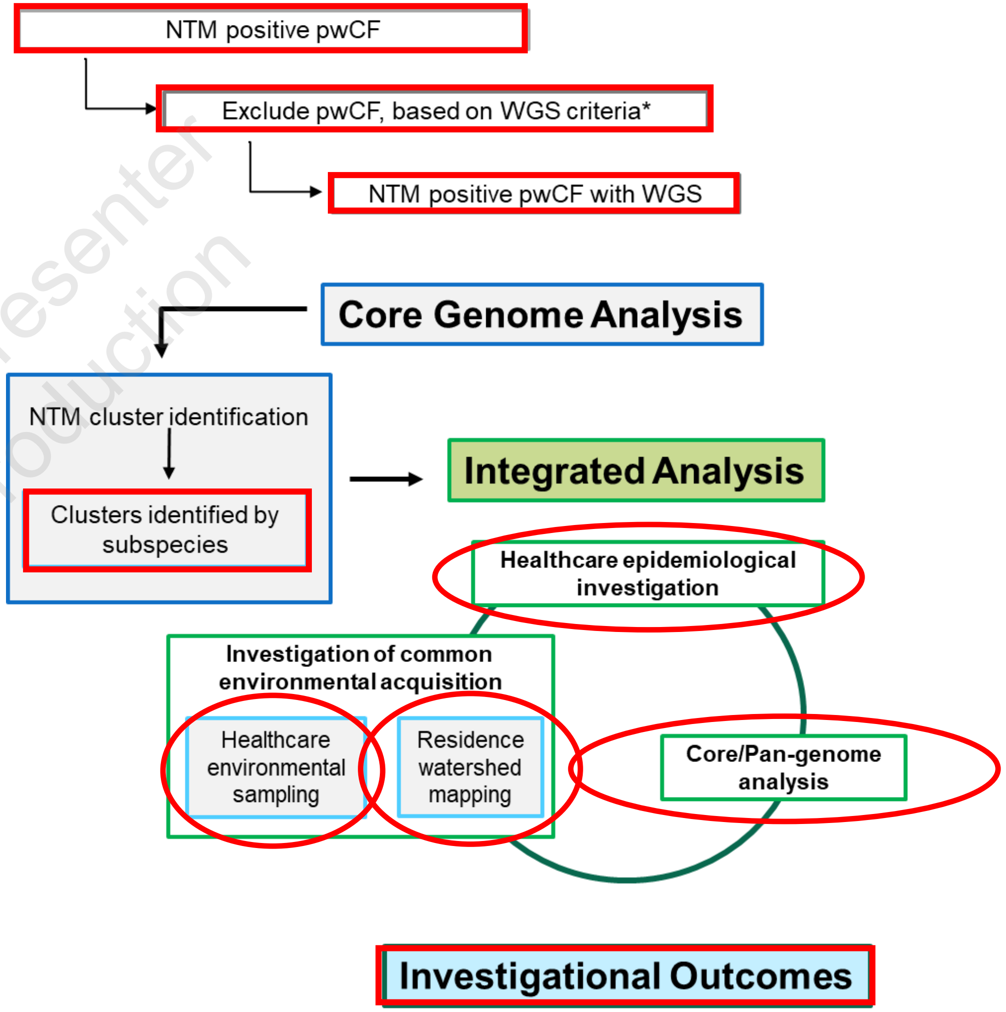
Healthcare-associated

Created in biorender.com

Methods

- Respiratory NTM isolates were collected from 4 Centers
- Whole genome sequencing (WGS) performed
- Analysis identified genetically similar clusters of NTM isolates from people who were cared for at a single Center.

Flow diagram of an Individual Center Investigation



NTM positive pwCF

Exclude pwCF, based on WGS criteria*

NTM positive pwCF with WGS

Core Genome Analysis

NTM cluster identification

Clusters identified by subspecies

Integrated Analysis

Healthcare epidemiological investigation

Investigation of common environmental acquisition

Healthcare environmental sampling

Residence watershed mapping

Core/Pan-genome analysis

Investigational Outcomes

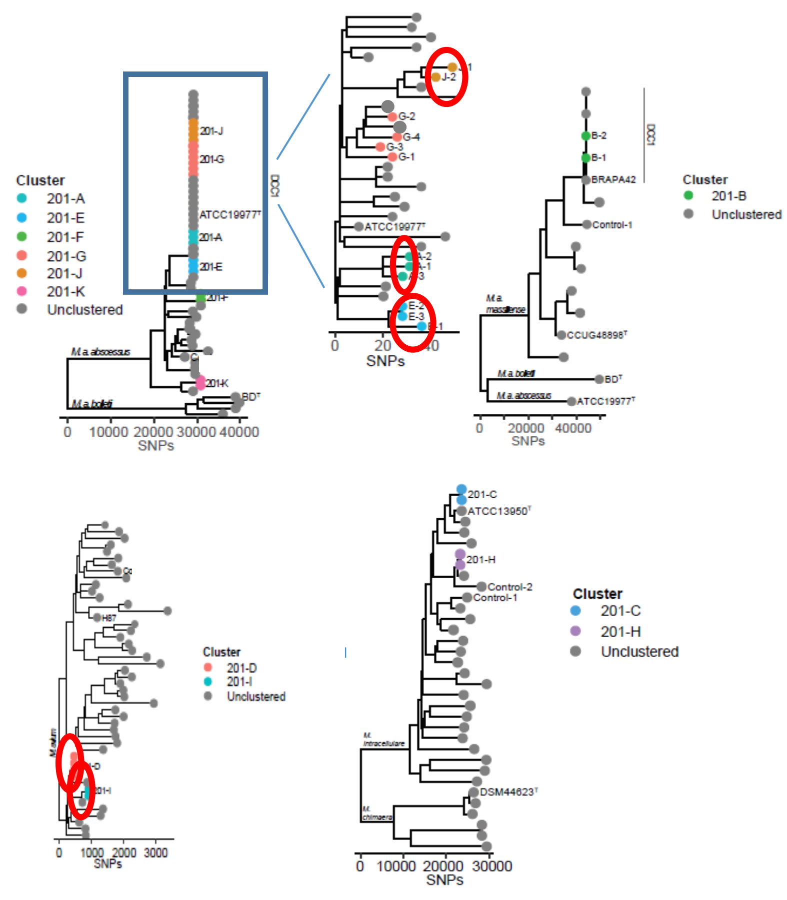
Gross, JE *et al. PLOS One,* 2021
Gross, JE *et al. AJRCCM,* 2022

HALT NTM Study Investigation	CO Adult Program	Center 2	Center 3	Center 4
General Characteristics				
Total of pwCF seen at the center during the extraction range, n	507	61	326	292
NTM positivity with in pwCF during the extraction range, n (%)	165 (19%)	23 (38%)	126 (38.5%)	65 (22.2%)
PwCF meeting WGS criteria, n (%)	108 (65.4%)	23 (100%)	54 (42.8%)	57 (87.7%)
pwCF not meeting WGS criteria	57	0	0	8 (12%)
PwCF with WGS, n (%)	80 (74%)	23 (100%)	54 (100%)	57 (100%)
Core Genome Result				
Total pwCF with WGS available and identified in a cluster, n (%)	27 (34%)	11 (47.8%)	23 (42.6%)	15 (26.3%)
Total of NTM clusters identified	11	2	7	4
NTM subspecies identified (n, NTM ID)	*6 M. abscessus ssp. abscessus* *2 M. intracellulare* *2 M.avium* *1 M.abscessus ssp. massiliense*	*1 M. chimaera* *1 M. avium*	*4 M. abscessus ssp. abscessus* *3 M.abscessus ssp. massiliense*	*2 M. abscessus ssp. abscessus* *2 M.abscessus ssp. massiliense*

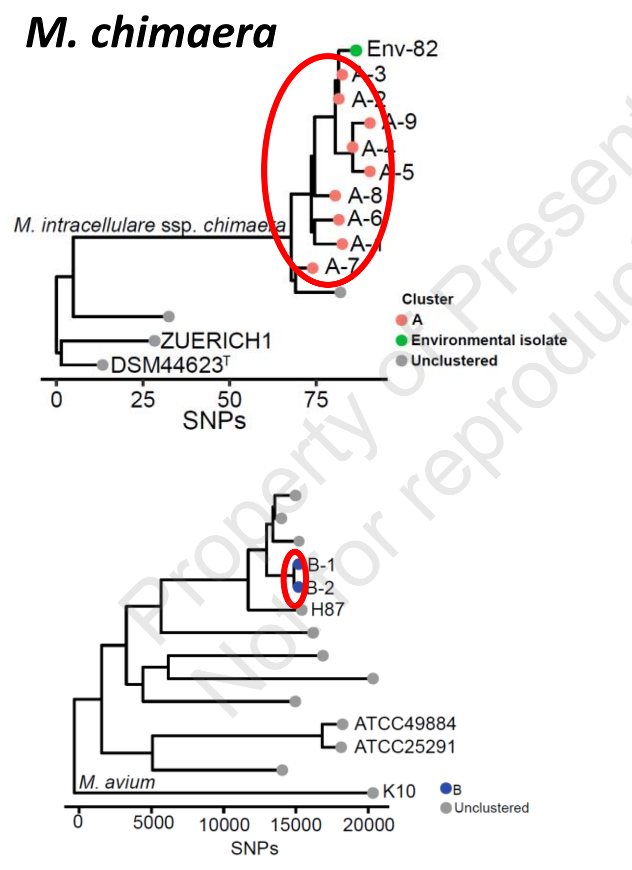
M. avium

M. abscessus ssp. abscessus

M. abscessus ssp. massilliense



CO Adult Program



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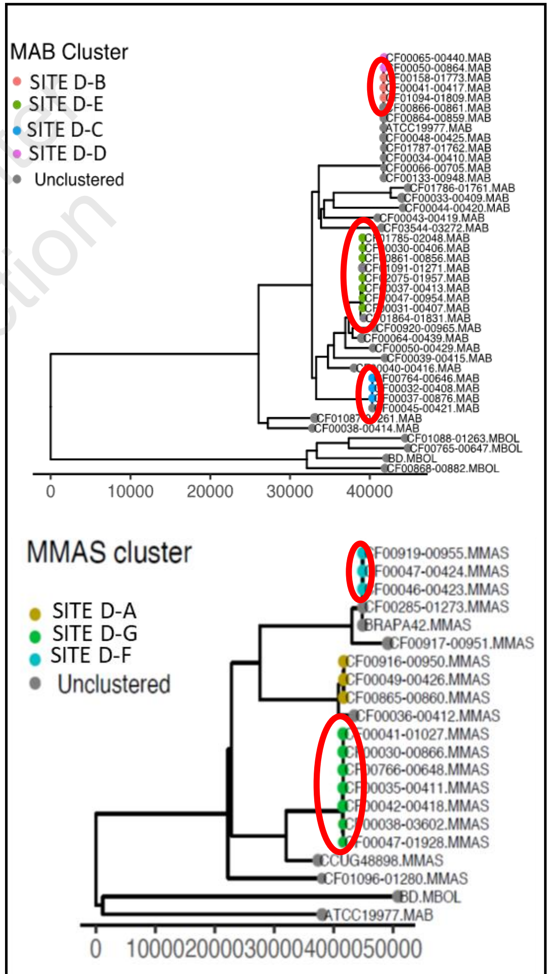

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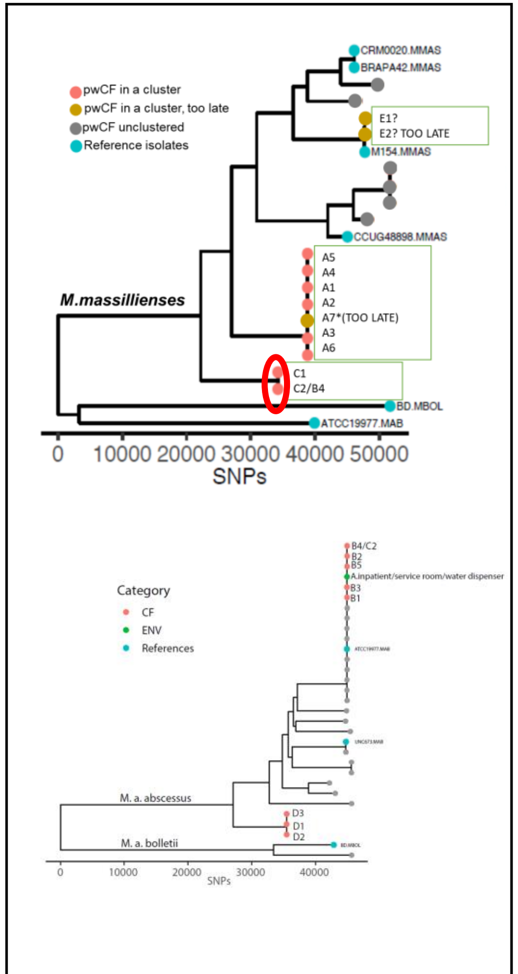

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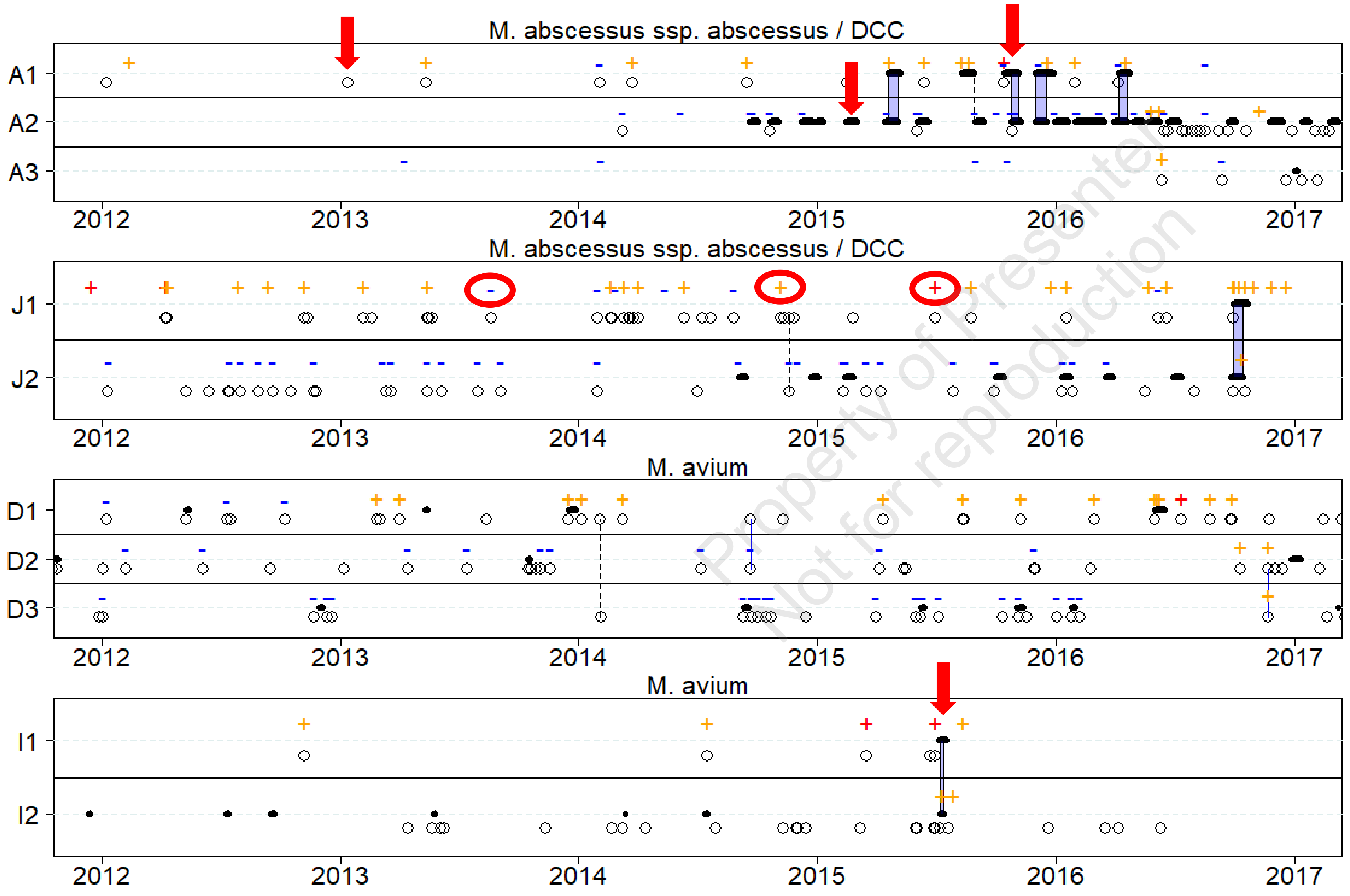
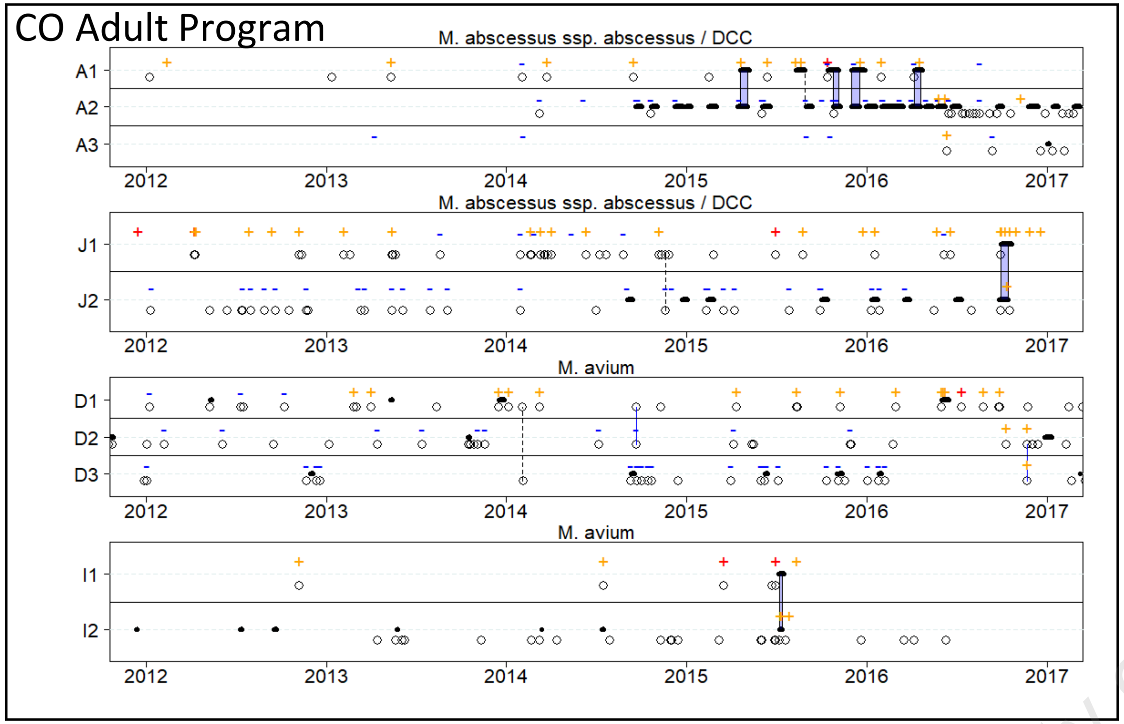
CO Adult Program



Legend

- ○ Clinic visit
- ● Hospitalization day
- ┆ Within 24 hour clinic overlap
- │ Within 12 hour clinic overlap
- ▯ Hospitalization overlap
- + NTM smear and culture positive
- + NTM smear negative and culture positive
- - NTM culture negative
- + NTM smear positive and culture positive-Species level
- + NTM smear negative and culture positive-Species level

CO Adult Program



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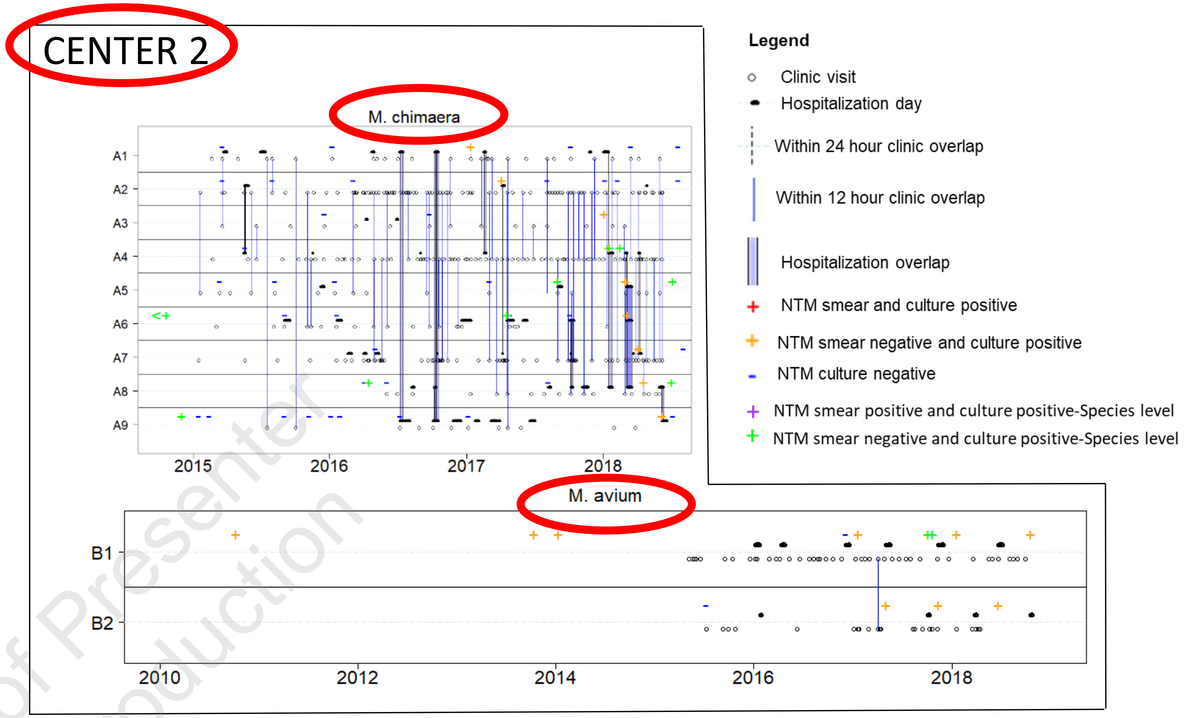

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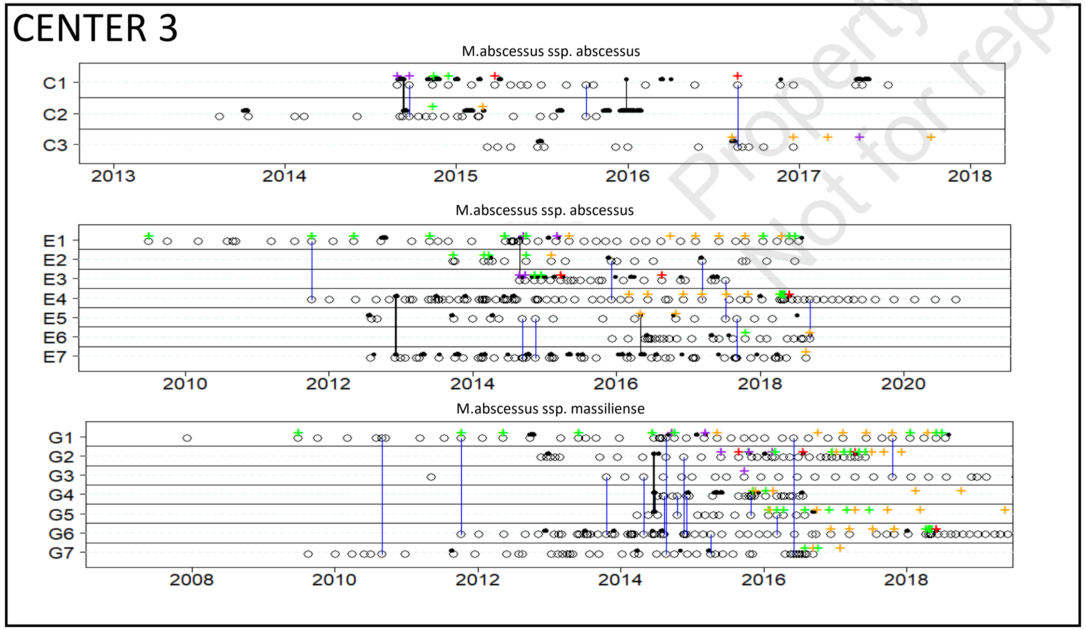
CENTER 3



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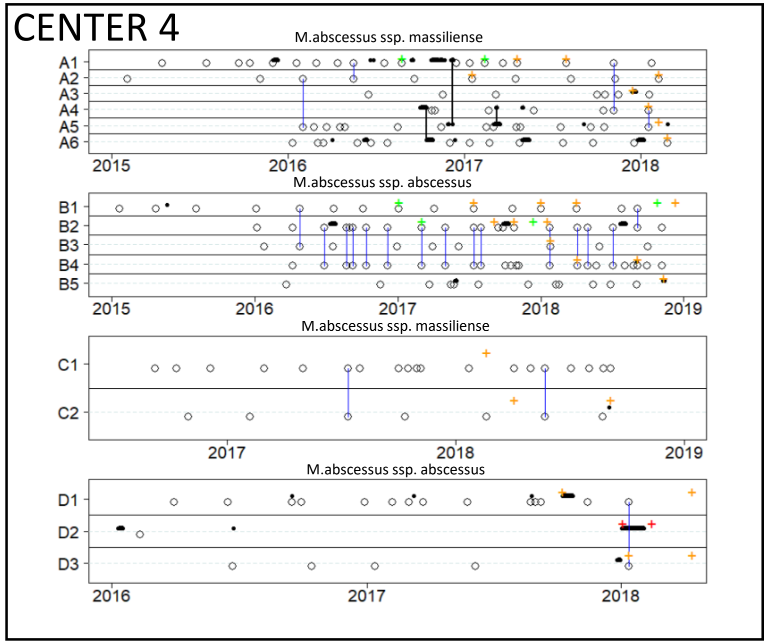
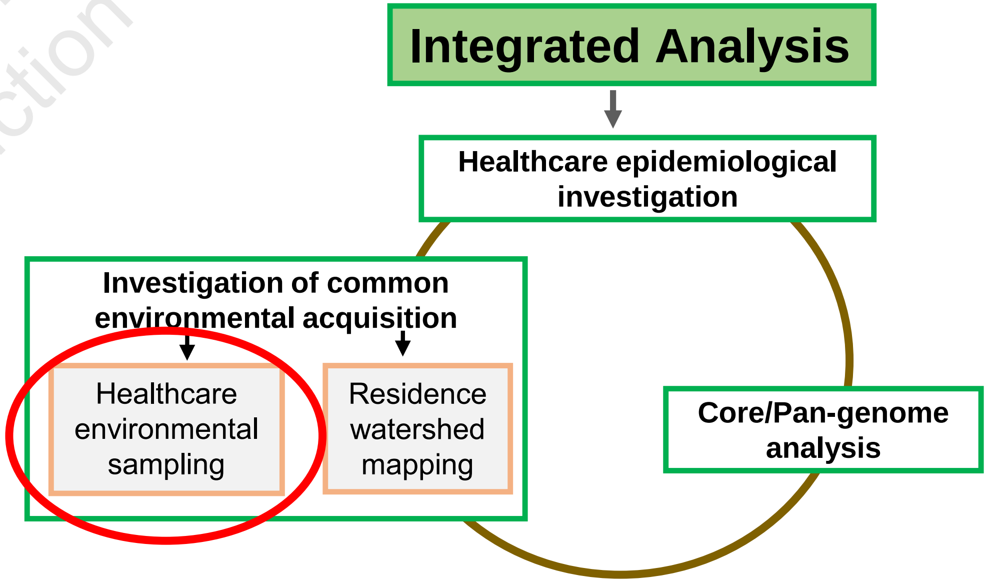


Investigation of common environmental acquisition



Integrated Analysis

Healthcare epidemiological investigation

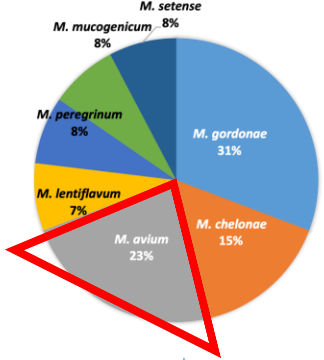
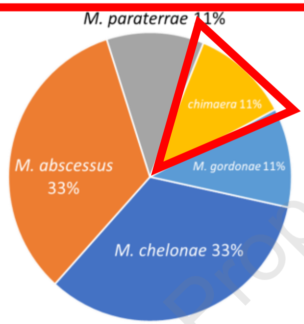
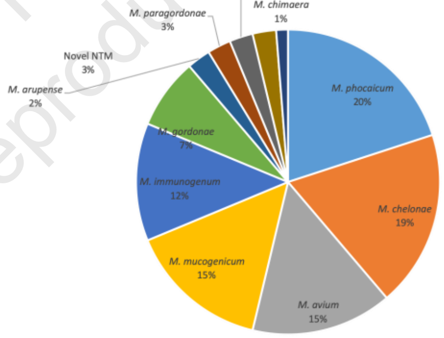
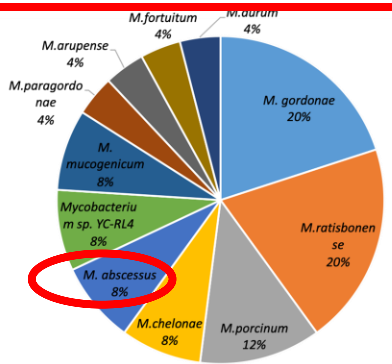
Investigation of common environmental acquisition

Healthcare environmental sampling

Residence watershed mapping

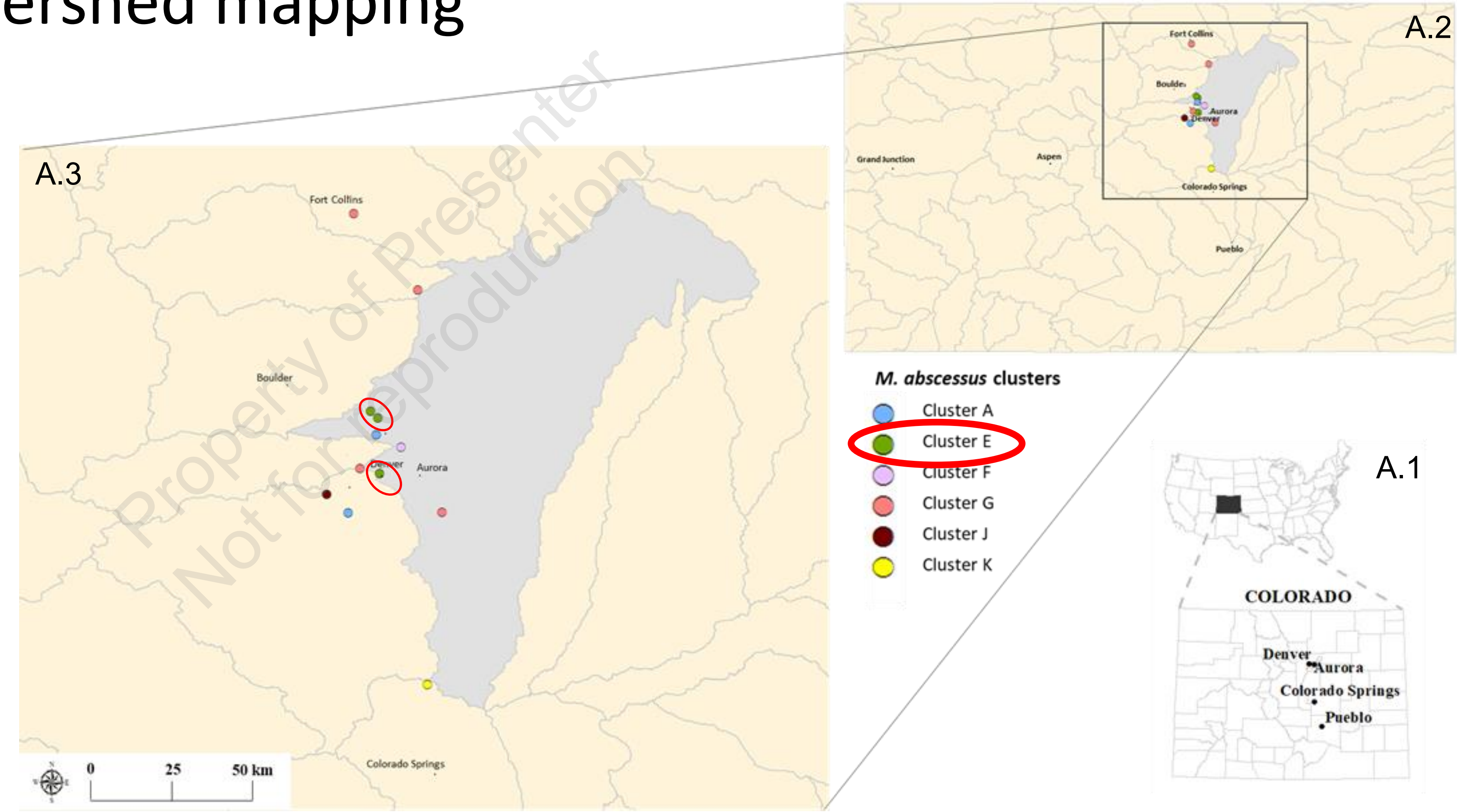
Core/Pan-genome analysis

Healthcare environmental sampling

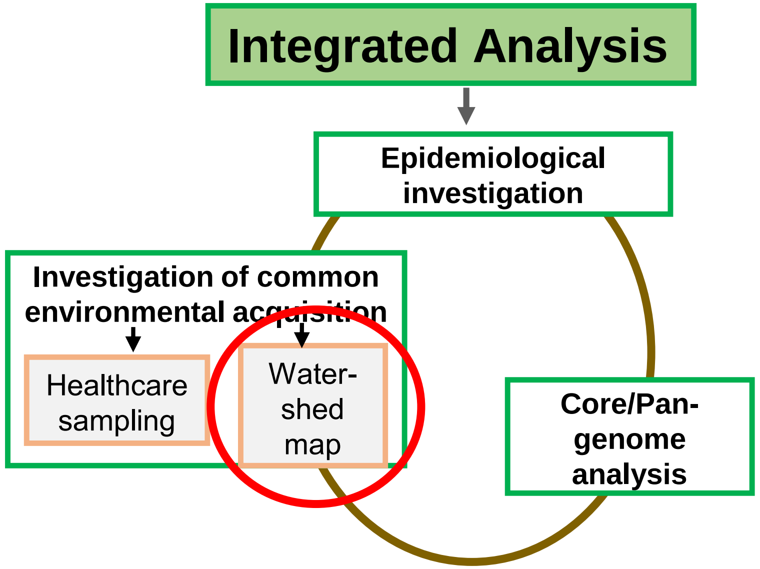
Center 1	**Center 2**	**Center 3**	**Center 4**
161 samples/swabs collected	132 samples/swabs collected	297 samples/swabs collected	137 samples/swabs collected
11 swabs presented growth of ≥ 1 NTM species (6.8%)	8 swabs presented growth of ≥ 1 NTM species (6%)	61 swabs presented growth of ≥ 1 NTM species (21%)	17 swabs presented growth of ≥ 1 NTM species (12%)
7 NTM species identified in the healthcare environment	5 NTM species identified in the healthcare environment	11 NTM species identified in the healthcare environment	11 NTM species identified in the healthcare environment
			
No similarity found between environmental and respiratory NTM isolates	Genetic similarity found between environmental and respiratory *MCHIM* among 8/9 pwCF in a cluster	No similarity found between environmental and respiratory NTM isolates	Possible similarity between environmental and respiratory *MABS*, further investigation in progress

Investigation of common environmental acquisition: Home residence watershed mapping

State of Colorado Watershed Boundaries



M. abscessus clusters
- 🔵 Cluster A
- 🟢 Cluster E
- 🟣 Cluster F
- 🔴 Cluster G
- 🟤 Cluster J
- 🟡 Cluster K



Integrated Analysis

Epidemiological investigation

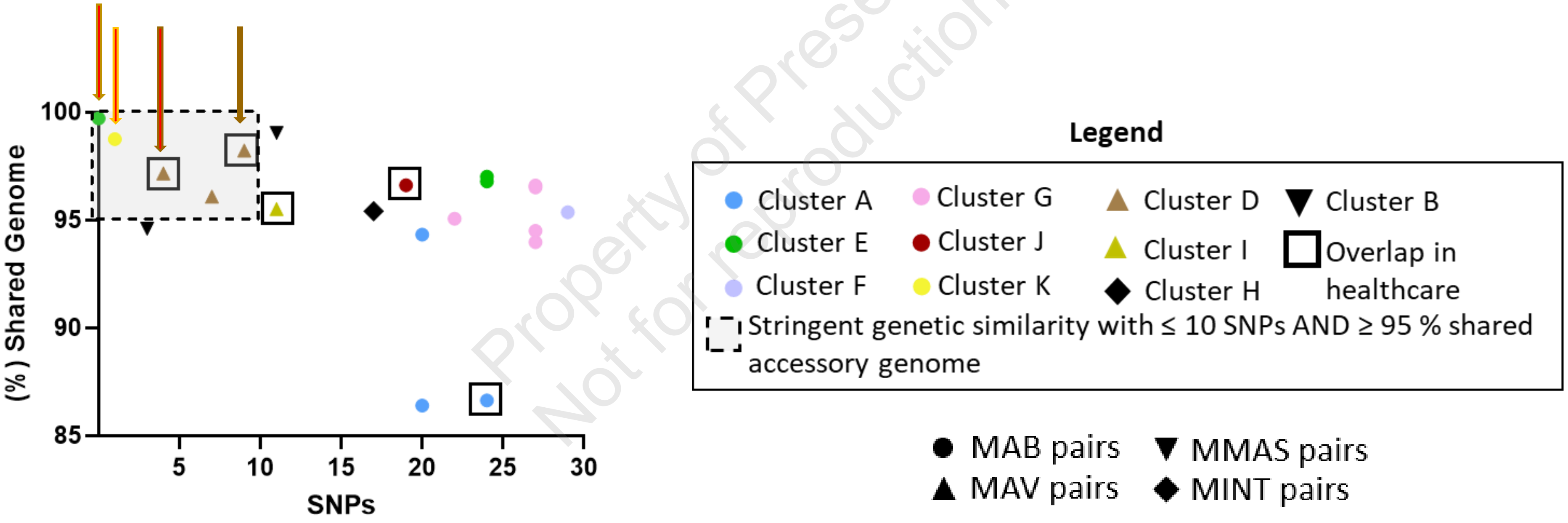
Investigation of common environmental acquisition

Healthcare sampling

Water-shed map

Core/Pan-genome analysis

Core genome SNP differences versus % shared accessory genome of the same isolate pairs



Investigation outcomes

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Investigational Outcomes				
Overlapping encounters in the healthcare environment	4/11 clusters	2/2 clusters	3/7 clusters	4/4 clusters
Clustered subjects living in the same watershed	1 *M. abscessus cluster*	None	None	None
Number of clinically relevant NTM in the healthcare environment	2	4	4	4
Environmental vs. respiratory isolate comparison	No similarity	8/9 subjects with respiratory *M.chimaera* genetically similar to healthcare isolate	No similarity	1 respiratory *M. abscessus* isolate genetically similar to healthcare isolate
Conclusions: Healthcare-associated transmission or acquisition	Rare *M.abscessus* transmission Likely *M. avium* transmission	No transmission Likely *chimaera* acquisition	Transmission and acquisition unlikely	Transmission unlikely Acquisition possible

Gross, JE *et al. AJRCCM,* 2022
*Gross JE, et al., *Ann Am Thoracic Soc*. 2023

HALT NTM Study

- Provides a framework to standardize outbreak investigations
- Improve understanding of frequency and nature of healthcare-associated NTM transmission among people with CF
- May identify where NTM acquisition might be at a higher probability
- Each Center investigation is unique
- Evidence of healthcare-associated
    - Patient-to-patient transmission
    - Environmental acquisition

Prospective HALT NTM study

- Prospective concurrent parallel CF Center investigations are underway
  - 6 centers enrolled
  - Serial collection of respiratory and environmental NTM isolates
- Open for enrollment
  - ClinicalTrials.gov NCT05686837
  - Date of registry January 6, 2023

Summary

- Whole genome sequencing
  - NTM fingerprint
  - Compare NTM isolates to determine degree of relatedness
  - Ideal for NTM outbreak investigations
- Standardized epidemiologic investigations
  - Improved understanding of source(s) healthcare-associated outbreaks
  - Guide outbreak prevention and mitigation
  - Inform infection prevention & control guidelines

Acknowledgements

HALT NTM

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Breathing Science is Life.


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NTM whole genome sequencing


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Watershed analysis

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MEDICAL CENTER

UT SOUTHWESTERN
MEDICAL CENTER

Ascension

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Thank you!

Questions?

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