

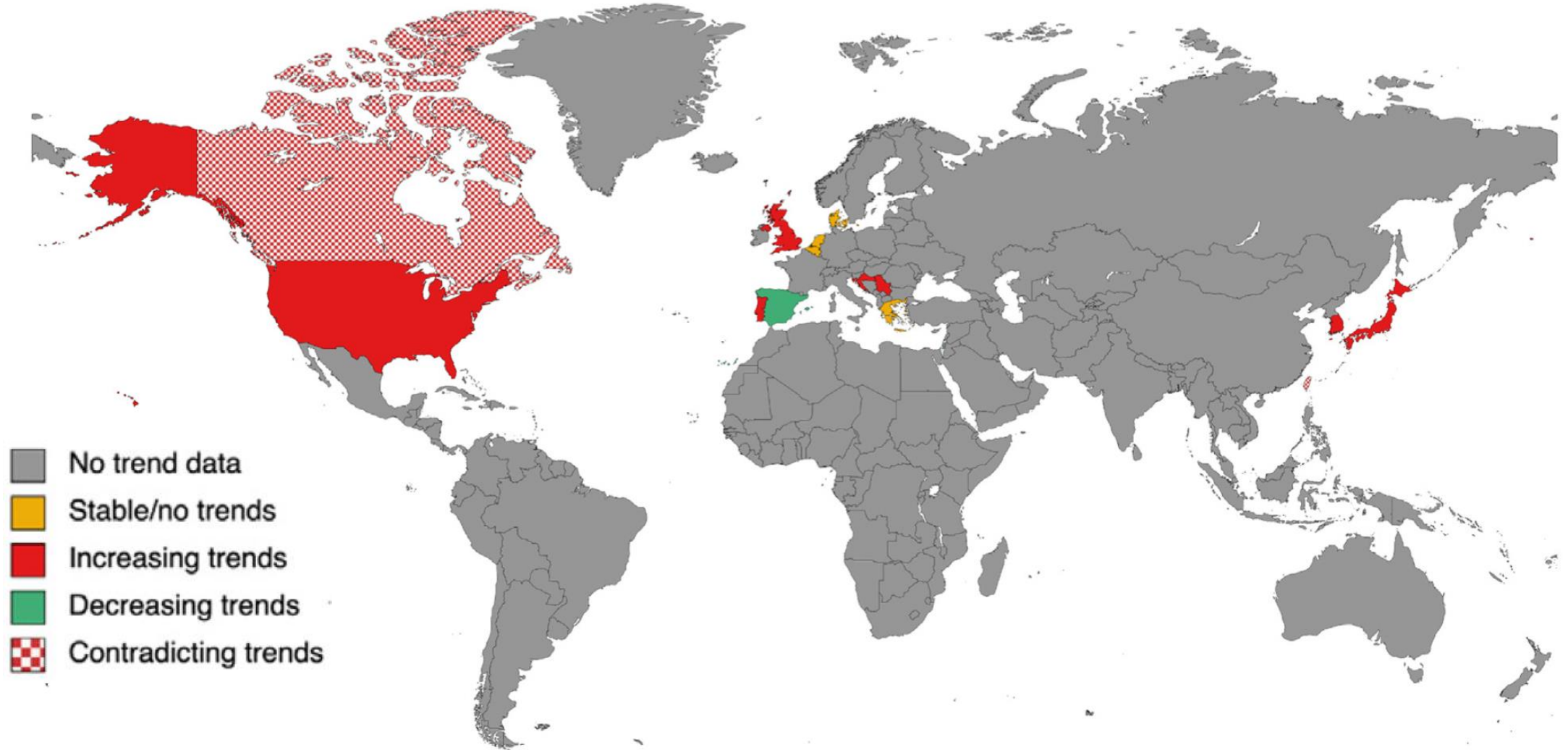
2023.10.28 제 3회 결핵 연구회 국제 심포지엄

Cross-transmission of Nontuberculous Mycobacteria among Patients with or without Cystic Fibrosis

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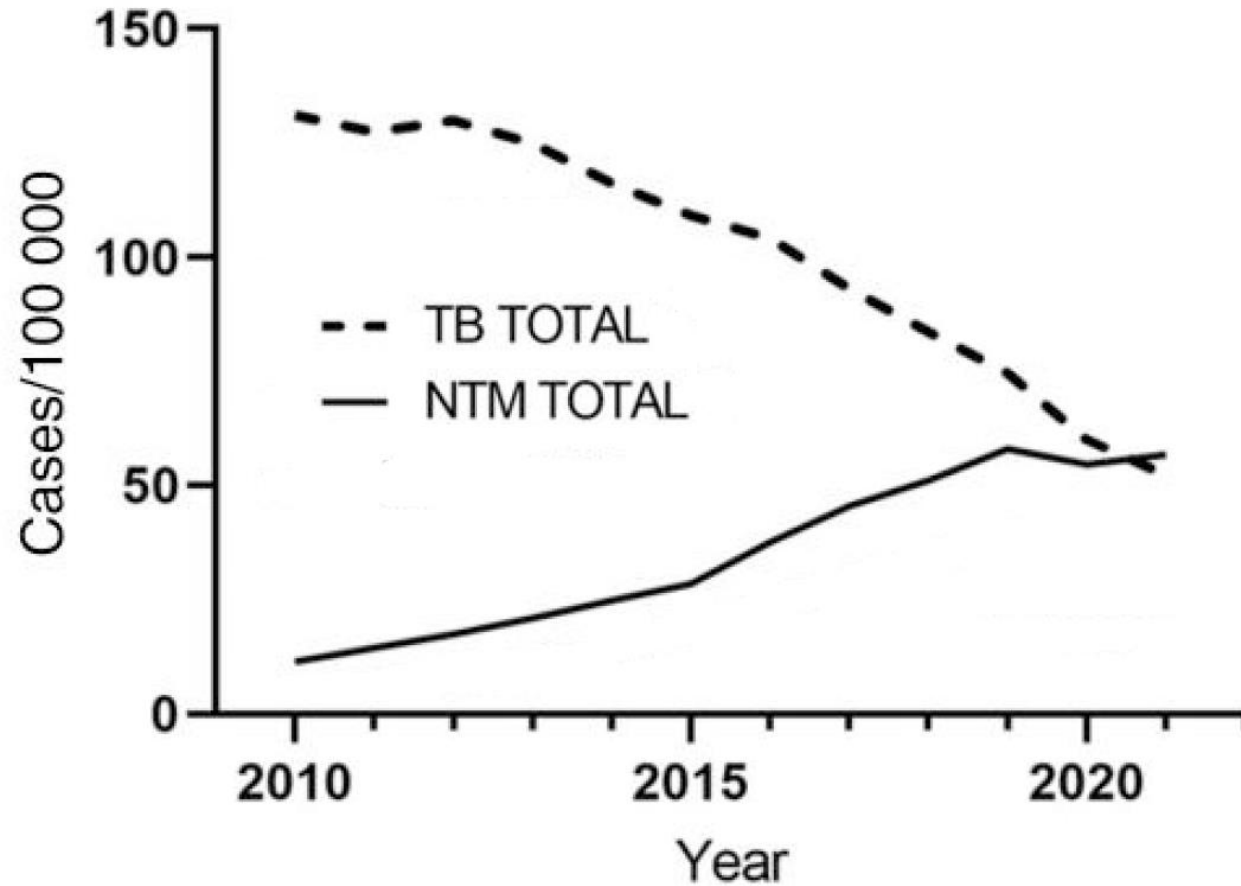
2000–2022, systematic review, 47 studies from 18 countries

The overall annual rate of change for NTM disease: **4.1% per 100,000 person/year**



Claims data of the Health Insurance Review and Assessment Service, South Korea (2010–2021)

ICD-10 code of NTM infections and tuberculosis



Prevalence in 2021

- **TB: 52.1 cases /100,000 population**
- **NTM: 56.7 cases /100,000 population**

Person-to-person transmission

as the possible cause of the increasing burden of NTM disease?

“There is no transmission between individuals;
therefore, there is no need to isolate patients infected with NTM.”

대부분의 NTM은 자연수와 토양 등 자연환경에 널리 분포하고 있으며, 병원성이 낮은 균이다. 사람과 사람 사
이에서의 전염은 없으며, 따라서 NTM에 감염된 환자를 격리할 필요는 없다.

2017 결핵진료지침 3판

(2017 Korean Guideline for Tuberculosis, 3rd edition)

Nontuberculous Mycobacterial Infections

Nontuberculous mycobacteria are pathogens found in the environment that can cause a variety of infections.

How Is Nontuberculous Mycobacterial Infection Acquired?

Nontuberculous mycobacteria are found in the environment, particularly in water and soil. Unlike with tuberculosis, these bacteria are generally acquired from exposure to the environment, not from infected people. However, among patients with cystic fibrosis, a specific nontuberculous mycobacterium may be transmitted to other patients with cystic fibrosis.

- 1. Transmission of *M. abscessus* complex among patients with cystic fibrosis**
- 2. Transmission of *M. abscessus* complex among patients with non-cystic fibrosis**
- 3. Cross-transmission of *M. avium* complex**
- 4. Phenomena inexplicable by transmission: environmental factors?**
- 5. Studies on NTM cross-transmission in South Korea**

The most common NTM species in South Korea

Mycobacterium avium complex (MAC)

M. avium

M. intracellulare

Mycobacterium abscessus complex (MABC)

M. abscessus subspecies *abscessus* (*M. abscessus*)

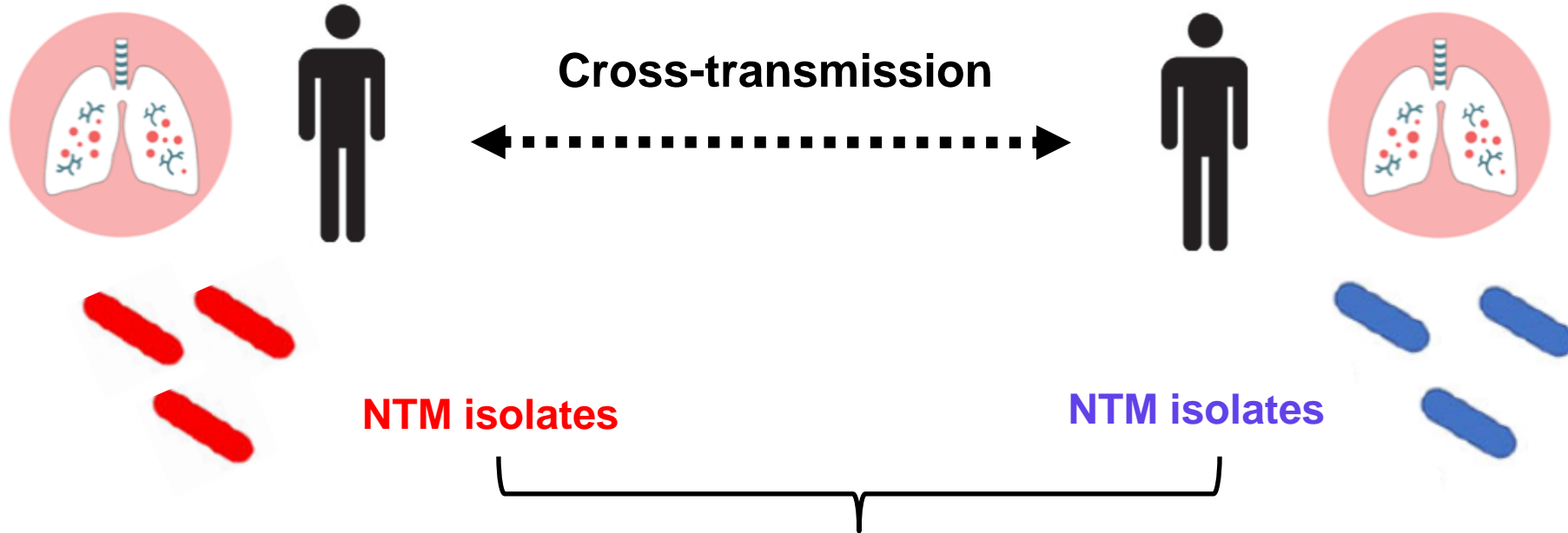
M. abscessus subspecies *massiliense* (*M. massiliense*)

M. abscessus subspecies *bolletii* (*M. bolletii*)

Mycobacterium kansasii

Patient **A** with NTM pulmonary disease

Patient **B** with NTM pulmonary disease



NTM isolates

NTM isolates

Genetic difference examination

→ Identical or near-identical strains

The genetic difference between patients' isolates

1) Pulsed-field gel electrophoresis

2) Whole genome sequencing (WGS), phylogenetic tree analysis

< 20–50 single nucleotide polymorphism (SNP)

“Cluster”

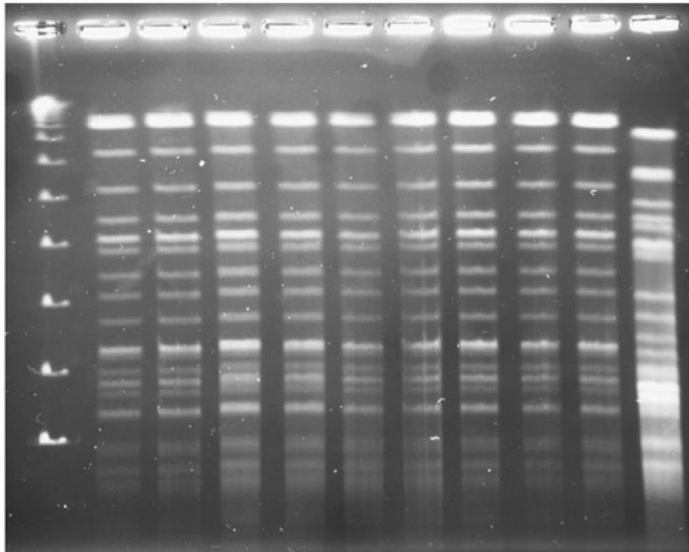
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Respiratory Outbreak of *Mycobacterium abscessus* Subspecies *massiliense* in a Lung Transplant and Cystic Fibrosis Center

Index patient: 22/M, cystic fibrosis

M. massiliense pulmonary disease (PD) for 7 years, 4+ sputum AFB smear-positive
Transition to cystic fibrosis center in Seattle

Eight months after the transition, four patients had newly infected *M. massiliense* PD



Pulsed-field gel electrophoresis of five isolates
→ **genetically indistinguishable**

Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study

Cystic fibrosis center at Papworth Hospital in the UK

168 consecutive isolates of MABC from 31 patients, 2007–2011

- *M. massiliense* (n = 15), *M. abscessus* (n = 13), *M. bolletii* (n = 2), coinfection (n = 1)

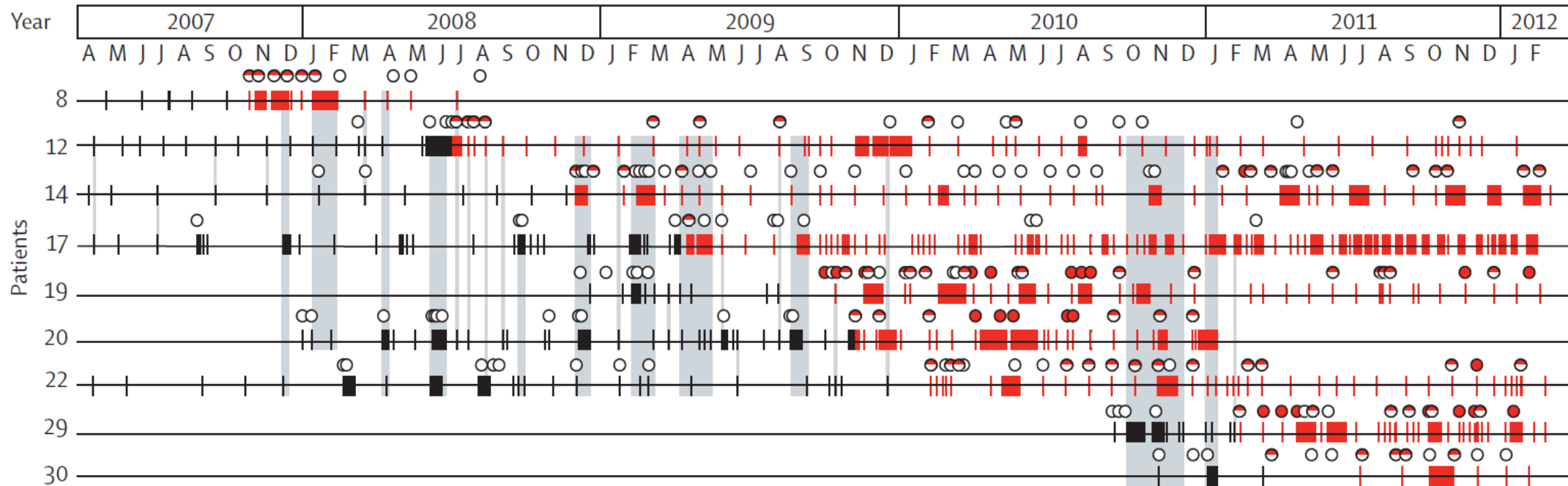
Whole genome sequencing, phylogenetic analysis

***M. massiliense* clusters** → cluster 1 (n = 9), cluster 2 (n = 2)

The patients infected with the transmitted *M. massiliense* clones
are not geographically grouped and do not share the same home water supply.

Extensive **environmental sampling** within the cystic fibrosis center, River, Pond
: **culture-negative** for mycobacteria

Epidemiological analysis for possible within-hospital transmission of NTM



All previously uninfected patients were present at the center at the same time as an infected individual on multiple occasions.

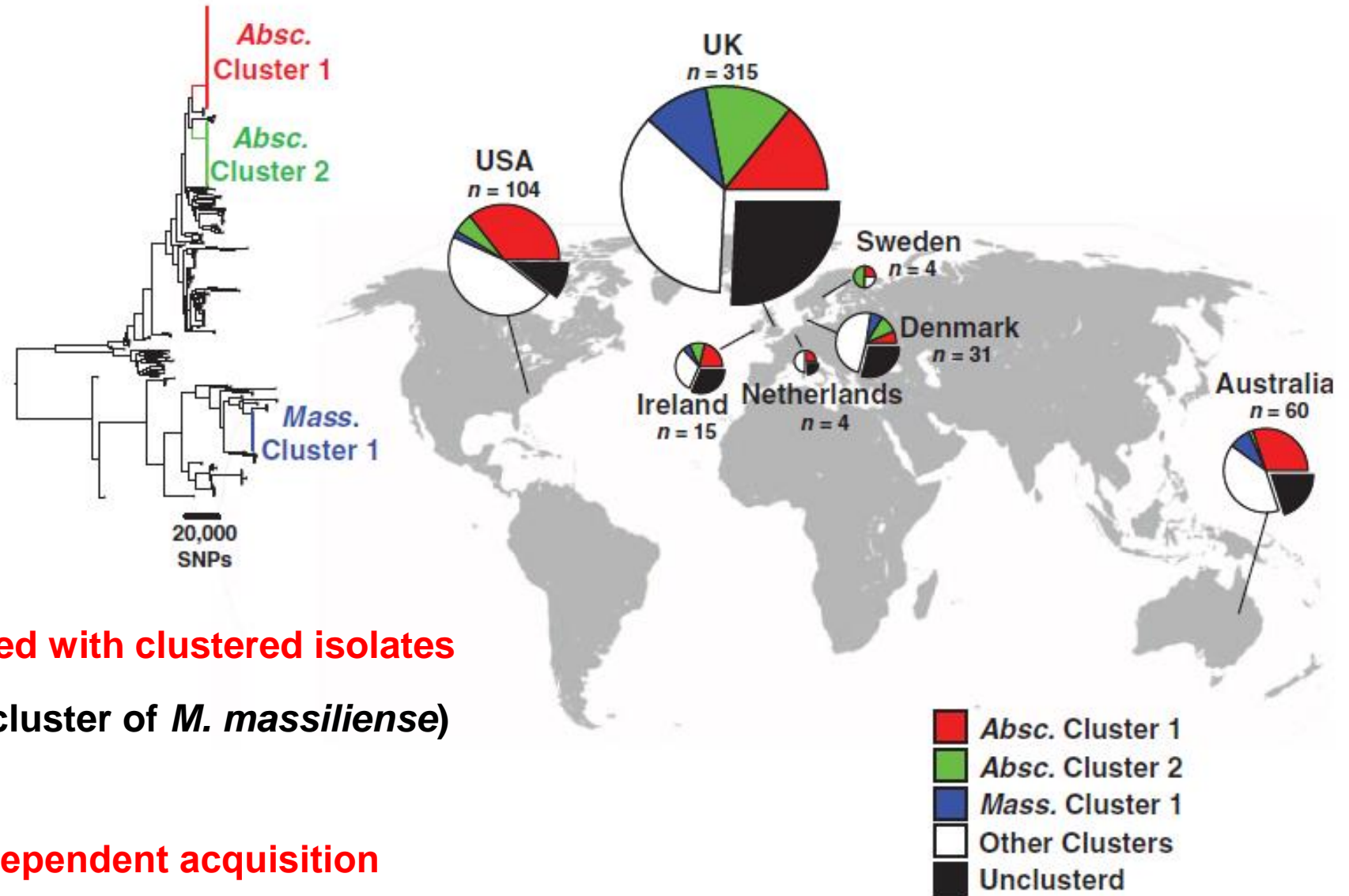
→ Clear opportunities for **within-hospital transmission**

Indirect transmission: fomite, inhalation of airborne droplet

UK, USA, Denmark,
Sweden, Netherlands, Australia

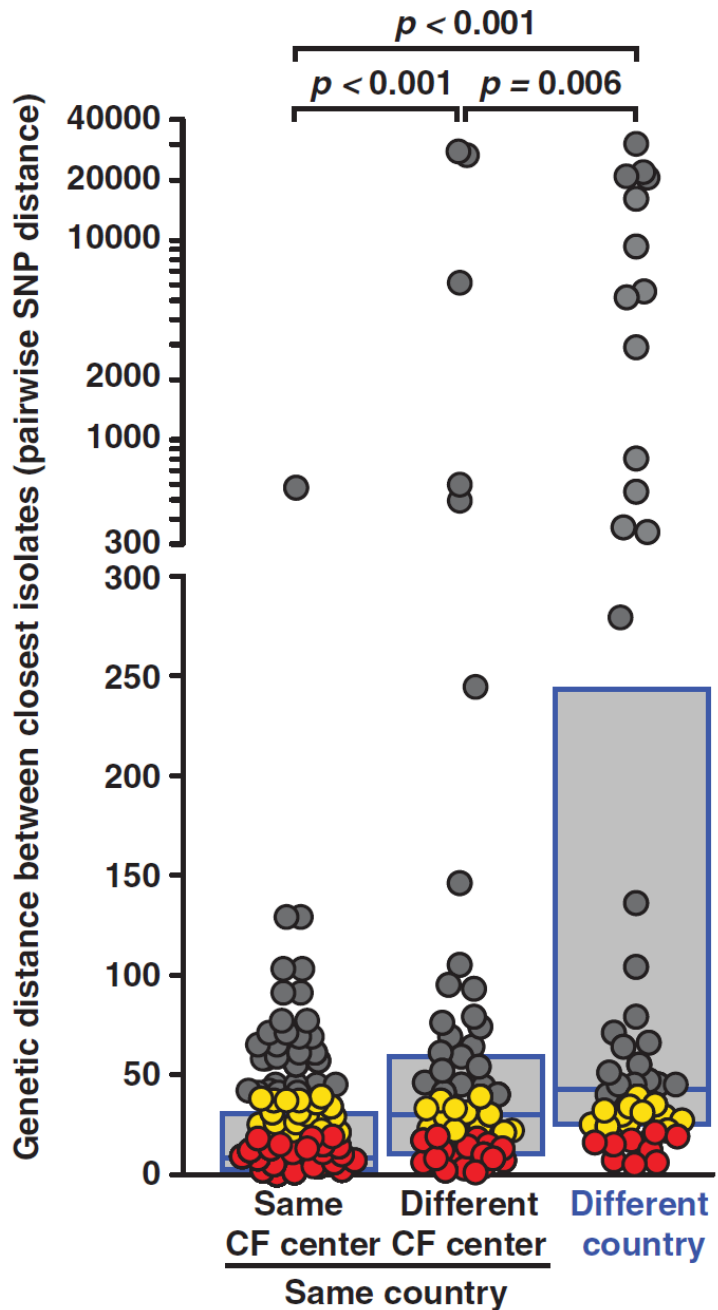
1080 MABC isolates,
517 patients with **cystic fibrosis**

- *M. abscessus* (n = 730)
- *M. massiliense* (n = 256)
- *M. bolletii* (n = 91)



Most patients (74%) were infected with clustered isolates
(2 clusters of *M. abscessus*, 1 cluster of *M. massiliense*)

**“Cross infection rather than independent acquisition
is the major source of infection for MABC.”**



Identical or near-identical isolates infecting groups
in different cystic fibrosis centers
and **across different countries.**

Fomite spread or generation of long-lived infectious aerosols
Human, zoonotic, or environmental vector of transmission

- >38 SNPs
- 20-38 SNPs
- <20 SNPs

Population Genomics of *Mycobacterium abscessus* from U.S. Cystic Fibrosis Care Centers

Ann Am Thorac Soc 2021;18:1960–1969

Methods: Whole-genome sequencing was performed on 558 *M. abscessus* isolates from 266 patients with CF attending 48 CF Care Centers in 28 U.S. states as part of a nationwide surveillance program. U.S. isolates were also compared with 64 isolate genomes from 13 previous studies to evaluate the prevalence of recently described dominant circulating clones.

Conclusions: Our study reveals the predominant genotypes of *M. abscessus* and frequency of shared strains between patients in U.S. CF Care Centers. Integrated epidemiological and environmental studies would help to explain the widespread presence of dominant clones in the United States, including the potential for broad distribution in the environment. Single site studies using systematic, evidence-based approaches will be needed to establish the contributions of health care–associated transmission versus shared environmental exposures.

Cystic fibrosis is an extremely rare disease in the Korean population.

Among Korean patients, **only 20 cases have been described until 2020, with the first case being diagnosed in 1988.**

1. Transmission of *M. abscessus* complex among patients with cystic fibrosis
- 2. Transmission of *M. abscessus* complex among patients with non-cystic fibrosis**
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5. Studies on NTM cross-transmission in South Korea

Epidemiology of *Mycobacterium abscessus* in England: an observational study

England, 2015–2019

Routinely collected MABC isolates, 2 Public Health England (PHE) laboratories

WGS as part of the routine clinical service

2297 MABC isolates from 906 patients

Sample type: sputum (87%), BAL (6%), non-respiratory sample (blood, skin biopsy, and others)

Respiratory diagnosis

- Cystic fibrosis (45%)
- Non-cystic fibrosis (33%, bronchiectasis, COPD, asthma, and others)
- No chronic respiratory diagnosis (22%)

	Patients with non-clustered isolates (n=342)	Patients with clustered isolates (n=519)
Diagnosis		
Bronchiectasis (n=146)	48 (32.9%)	98 (67.1%)
No chronic respiratory diagnosis (n=180)	89 (49.4%)	91 (50.6%)
Asthma (n=34)	14 (41.2%)	20 (58.8%)
Lung cancer (n=10)	1 (10.0%)	9 (90.0%)
Cystic fibrosis (n=400)	165 (41.2%)	235 (58.8%)
Chronic obstructive pulmonary disease (n=70)	21 (30.0%)	49 (70.0%)
Interstitial lung disease (n=21)	4 (19.0%)	17 (81.0%)

Cystic fibrosis patients

→ clustered isolates in 60%

Non-cystic fibrosis patients

→ clustered isolates in 63%

Potential Cross-Transmission of *Mycobacterium abscessus* among Non-Cystic Fibrosis Patients at a Tertiary Hospital in Japan

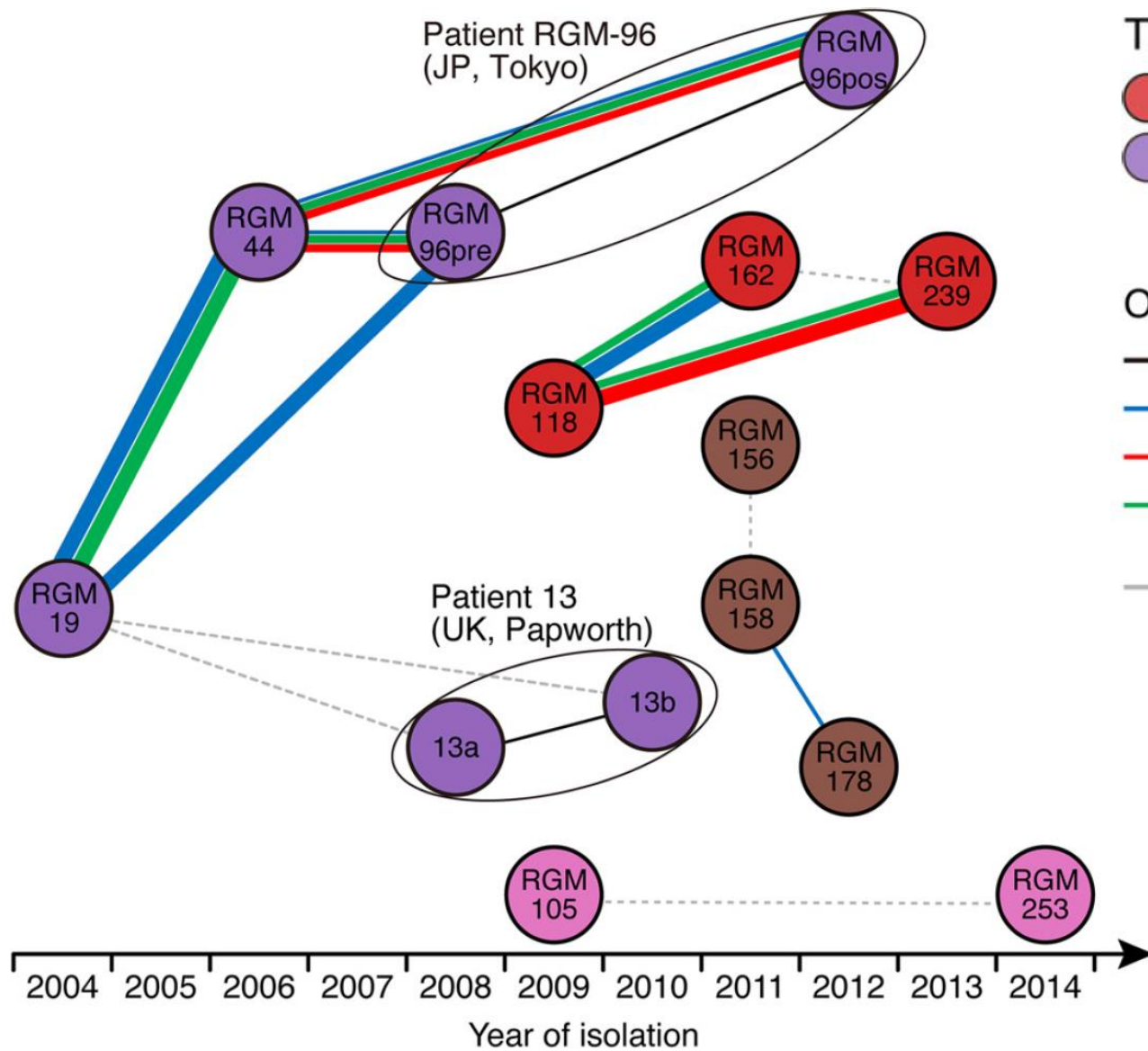
Fukujuji hospital, Tokyo, Japan

104 MABC isolates from **non-cystic fibrosis patients**, stored between 2004 and 2014

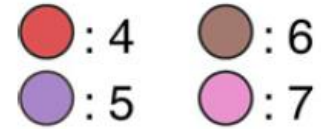
Variable number of tandem repeat (VNTR)

→ 25 isolates from 24 patients (2 *M. abscessus*, 23 *M. massiliense*)

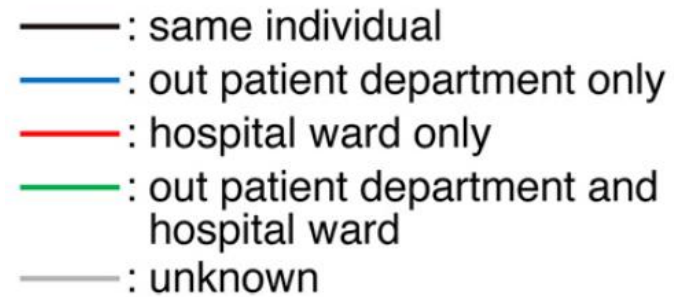
WGS: A total of 12 isolates belonged to 4 clusters



Transmissible cluster (TC)



Overlap between patients



Duration of overlap



**Suggesting the possibility of
MABC cross-transmission
among non-cystic fibrosis patients**

그것이 알고 싶다

SBS HD

시메이핑 마을 주민

저쪽 집이 무너졌다고 해서 구경하러 갔죠



MABC cross-transmission
among patients with **cystic fibrosis**

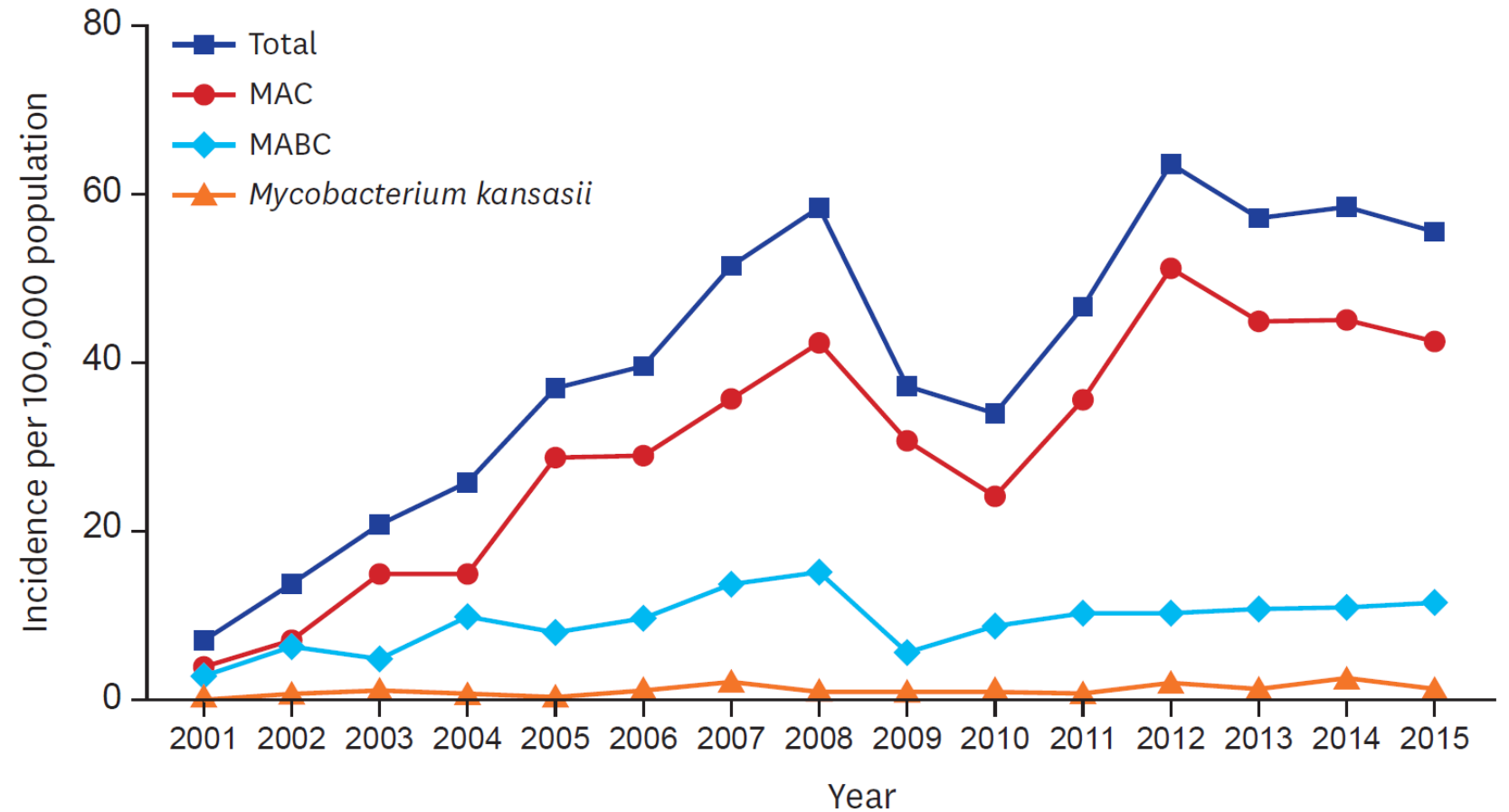


MABC cross-transmission
among patients with **non-cystic fibrosis**

2001–2015

Samsung Medical Center,
South Korea

Annual incidence of
NTM-PD caused by
MAC, MABC, and *M. kansasii*



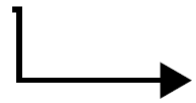
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Investigating Nontuberculous Mycobacteria Transmission at the Colorado Adult Cystic Fibrosis Program

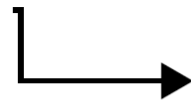
Am J Respir Crit Care Med 2022;205:1064–1074

*PwCF = People with Cystic Fibrosis

PwCF receiving care between 2012–2018, n = 507



NTM-positive between 2012–2018, n = 165



PwCF meeting WGS criteria = 108,
Excluded (did not meet WGS criteria) = 57



n = 80 (74%)

Colorado Adult
CF Center

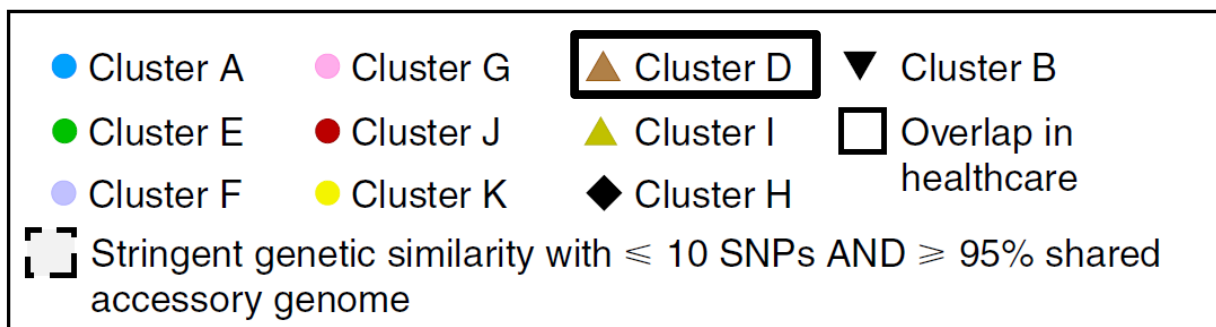
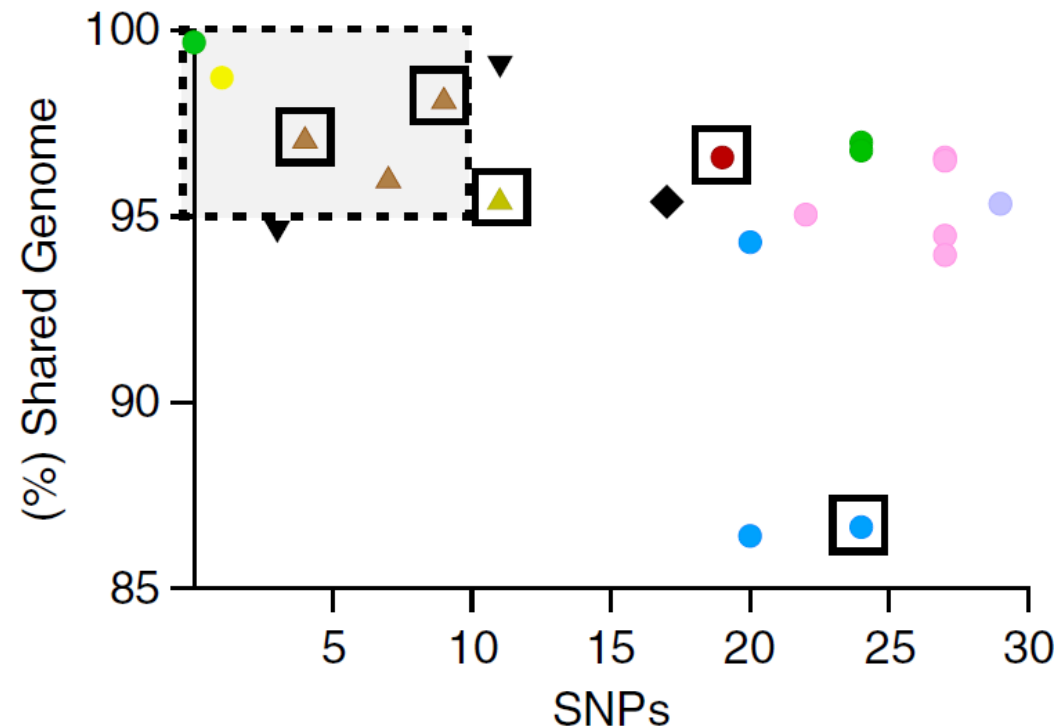
27 pwCF
(34%) identified in 11 NTM clusters



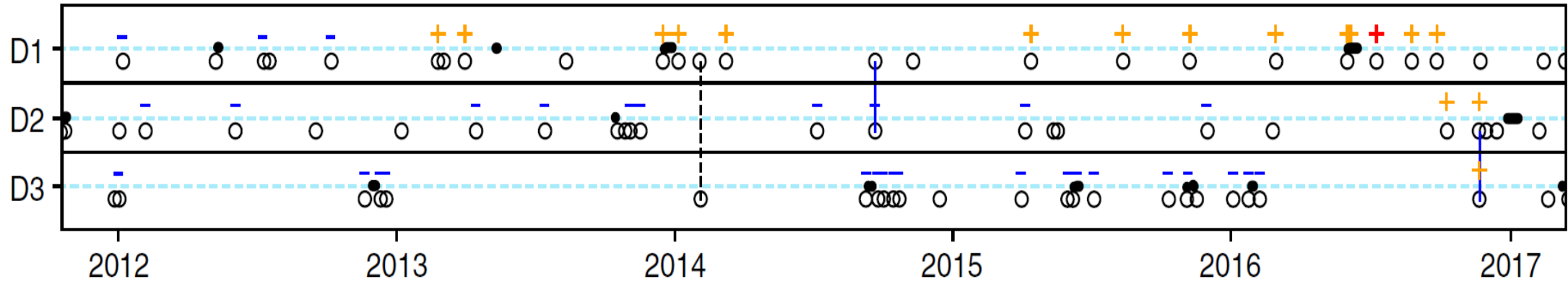
NTM clusters

- 6 *Mycobacterium abscessus* ssp. *abscessus*
- 2 *Mycobacterium Intracellulare*
- 2 *Mycobacterium avium*
- 1 *Mycobacterium abscessus* ssp. *massiliense*

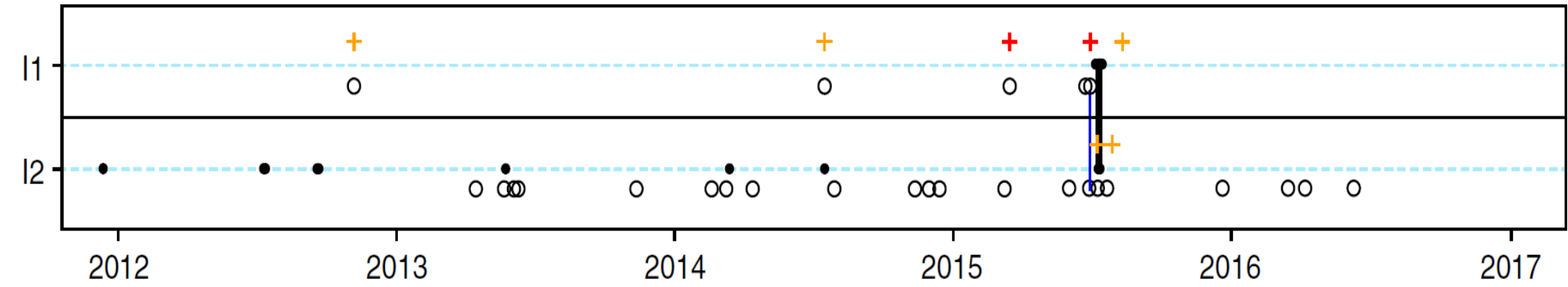
Integrated pan-genome analysis



M. avium



M. avium



Potential healthcare-associated **transmission of *M. avium***
among patients with cystic fibrosis

The proportion of clustered and non-clustered isolates in previous studies

Study	Species	Patients	Number	Clustered isolates	Non-clustered isolates
Lancet 2013	MABC	Cystic fibrosis patients	31 patients	35%	65%
Science 2016	MABC	Cystic fibrosis patients	517 patients	74%	26%
Lancet microbe 2021	MABC	Cystic fibrosis & non-cystic fibrosis patients	906 patients	60%–63%	37%–40%
Microbiol Spectr 2022	MABC	Non-cystic fibrosis patients	104 isolates	12%	88%
AJRCCM 2022	MABC & MAC	Cystic fibrosis patients	80 patients	34%	66%

**For some patients, there is evidence of healthcare-associated opportunity for transmission.
(overlapping encounters in the healthcare environment)**

However, genetically similar isolates were not noted in these patients.

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Cross-transmission Is Not the Source of New *Mycobacterium abscessus* Infections in a Multicenter Cohort of Cystic Fibrosis Patients

145 MABC isolates from 62 patients, Barcelona & London, 2000–2016

Single nucleotide variants by WGS

19 patients (31%): clustered isolates

1 pair of patients: epidemiological link to support cross-transmission

All other patients: direct transmission extremely unlikely

43 patients (69%): non-clustered unique isolates

**Acquired unique strains even after spending considerable time
on the same wards with other *M. abscessus*-positive patients**

Epidemiology of *Mycobacterium abscessus* in England: an observational study

UK, 2015–2019

Routinely collected MABC isolates, 2 Public Health England laboratories

WGS as part of the routine clinical service

2297 MABC isolates from 906 patients

- Cystic fibrosis (45%)
- Non-cystic fibrosis (33%, bronchiectasis, COPD, asthma, and others)
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Cystic fibrosis patients

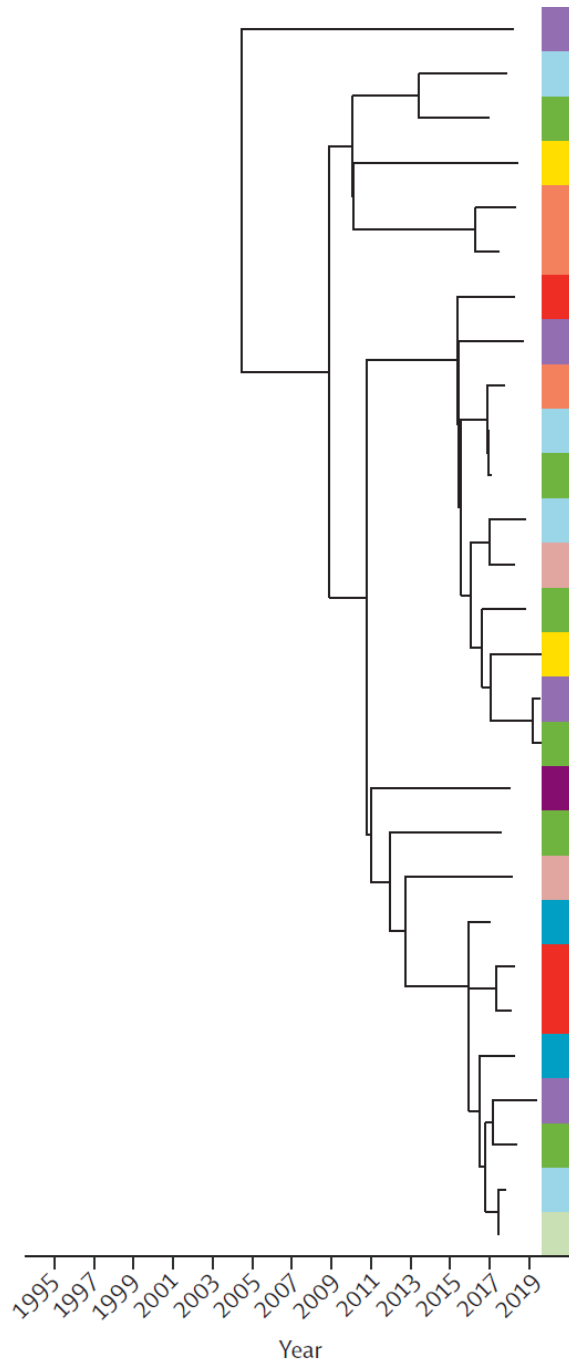
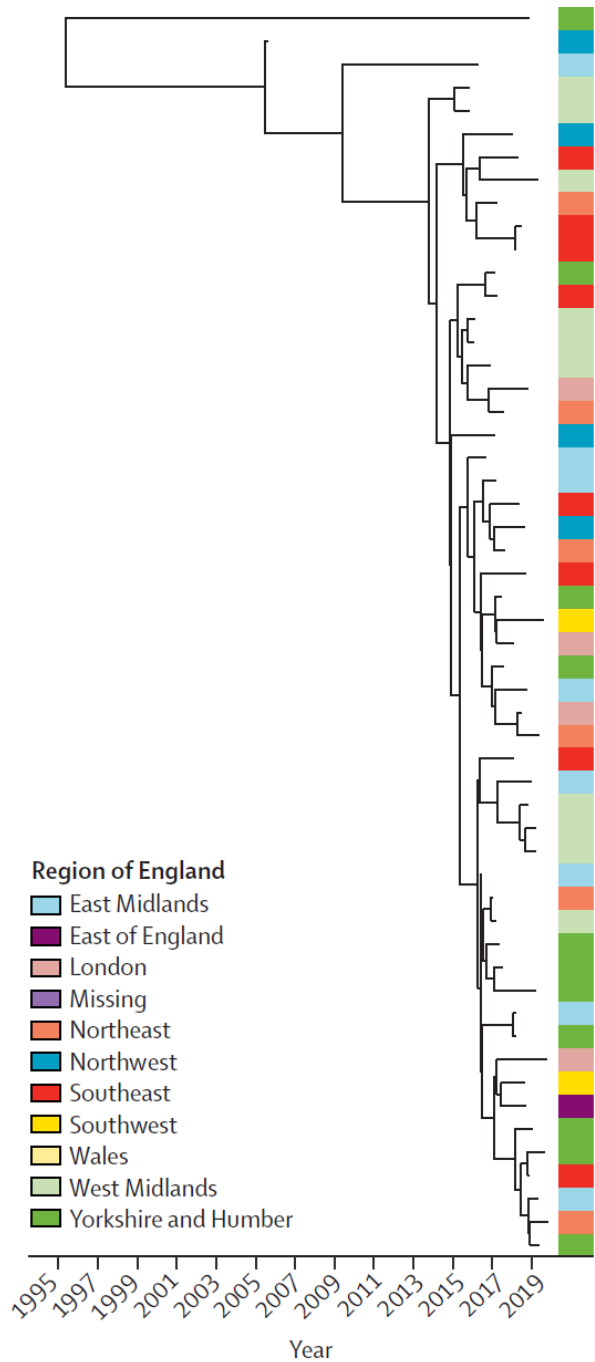
- clustered isolates in 60%

Non-cystic fibrosis patients

- clustered isolates in 63%

Only 13% had a **plausible epidemiological contact** by person-to-person transmission.

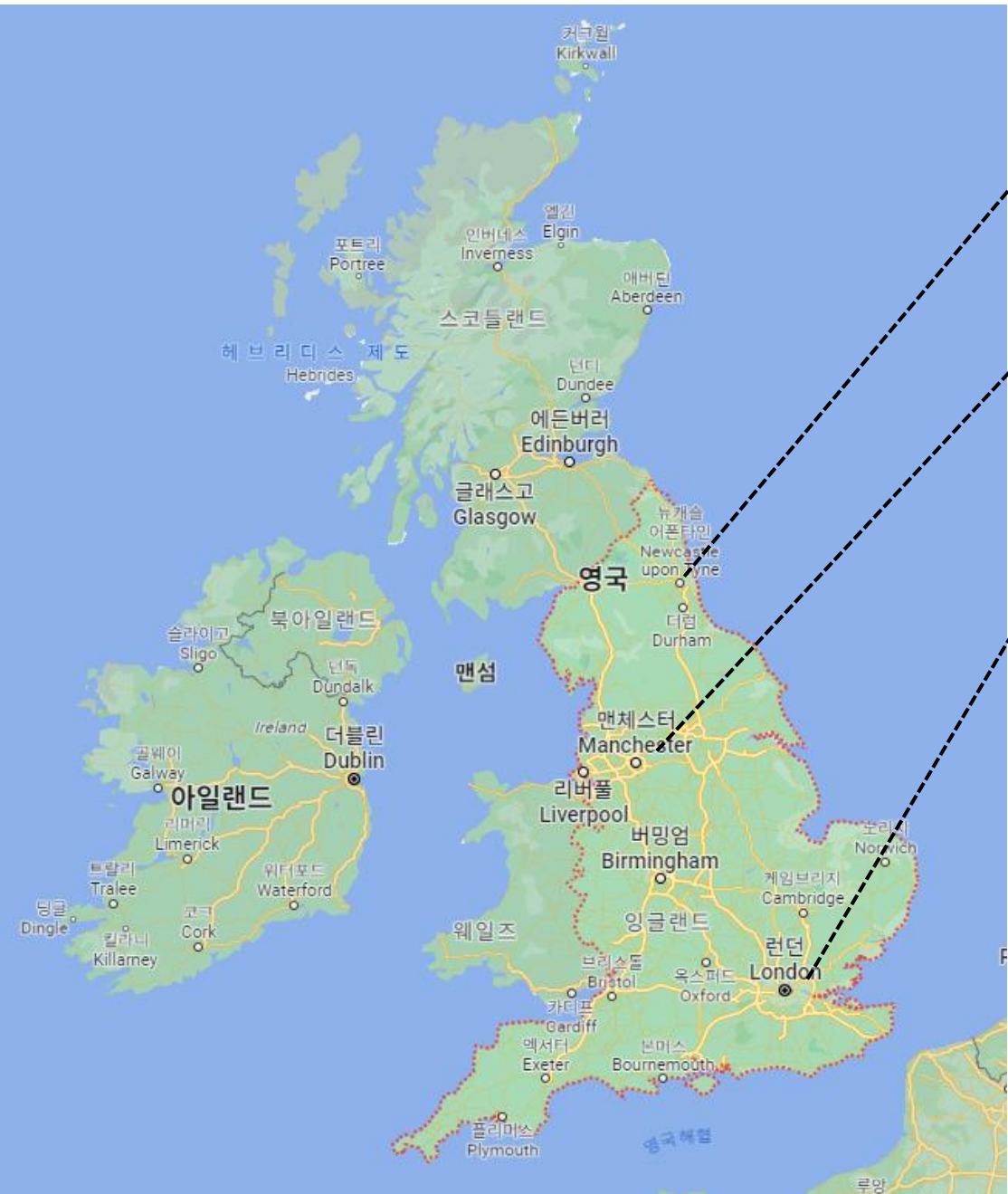
A vast majority of patients have no identifiable epidemiological links.



96% had available postcodes
and linked to one of nine geostatistical regions
in England.

Geographical distribution of MABC isolates
in the **two largest clusters**

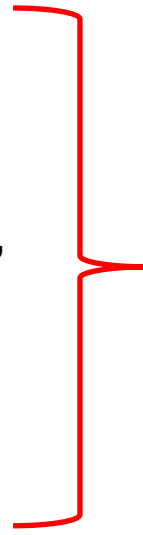
**Widely disseminated genetically
near-identical clones
over the widespread geographical area.**



Patient with COPD,
M. abscessus pulmonary disease

Patient without a respiratory disease,
M. abscessus skin abscess

Patient with cystic fibrosis,
M. abscessus pulmonary disease

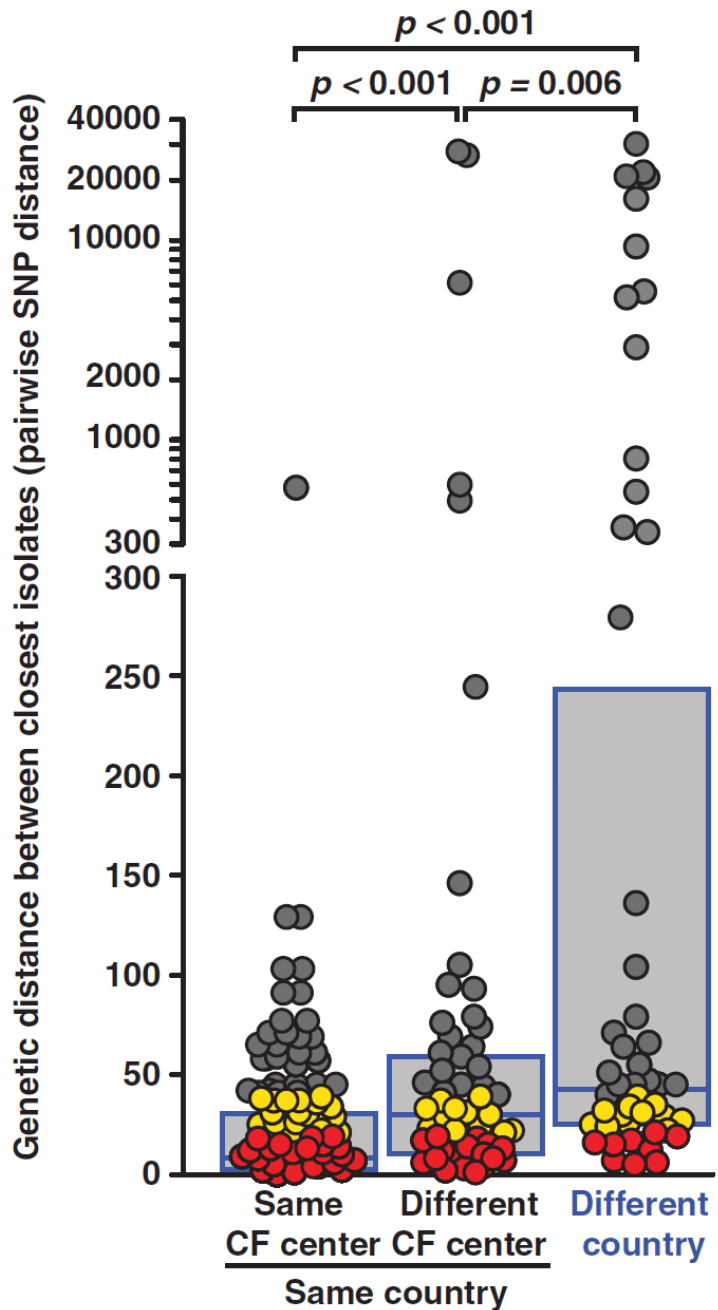


Identical strains

Geographically separate regions
Patients do not access the same center
Unlikely to share a social connection

→ No epidemiological link

How do these patients transmit the infection, even indirectly, between each other?



Identical or near-identical isolates infecting groups in different cystic fibrosis centers and across different countries.

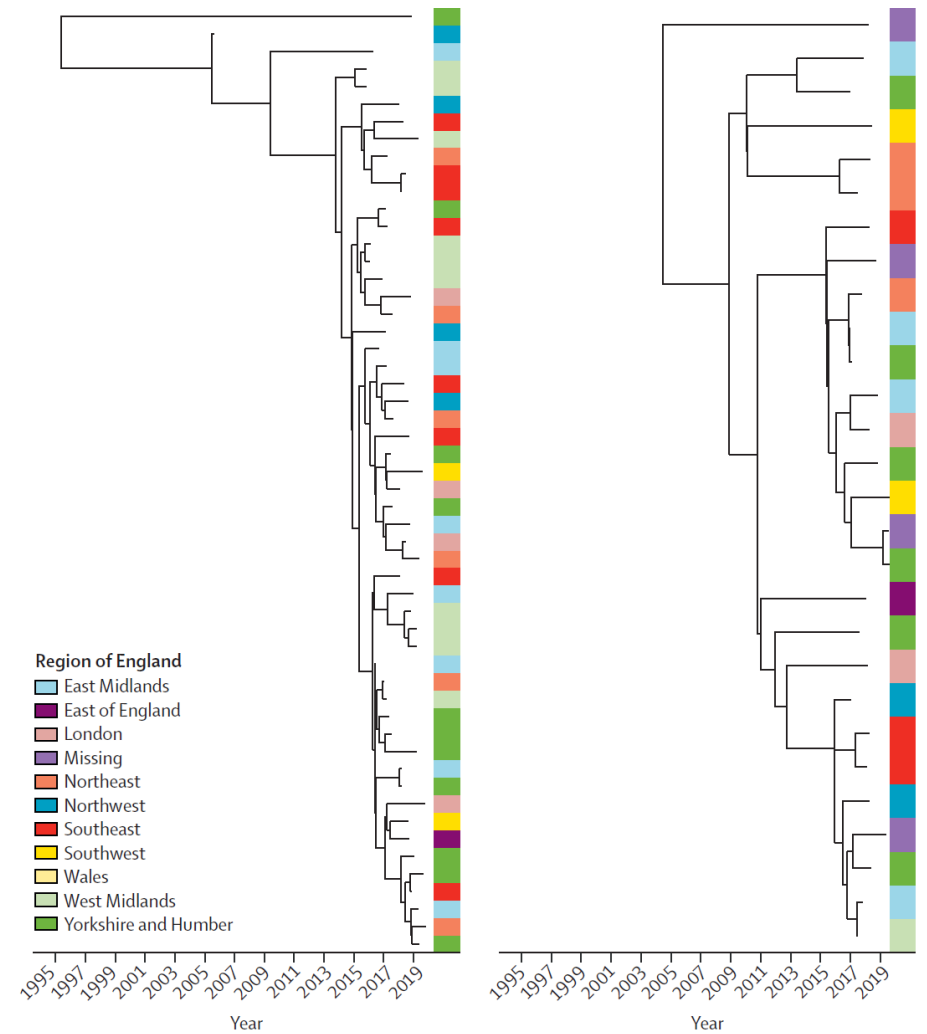
Fomite spread or generation of long-lived infectious aerosols

Human, zoonotic, or environmental vector of transmission

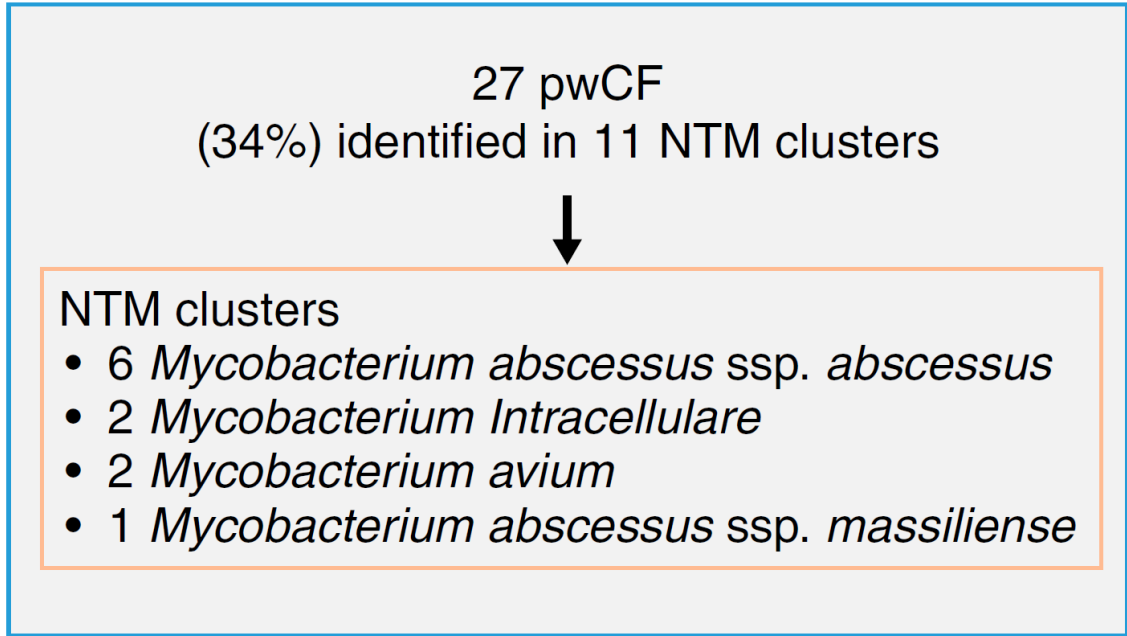
→ Only analyzed isolates from patients with cystic fibrosis

- >38 SNPs
- 20-38 SNPs
- <20 SNPs

Diagnosis	Patients with non-clustered isolates (n=342)	Patients with clustered isolates (n=519)
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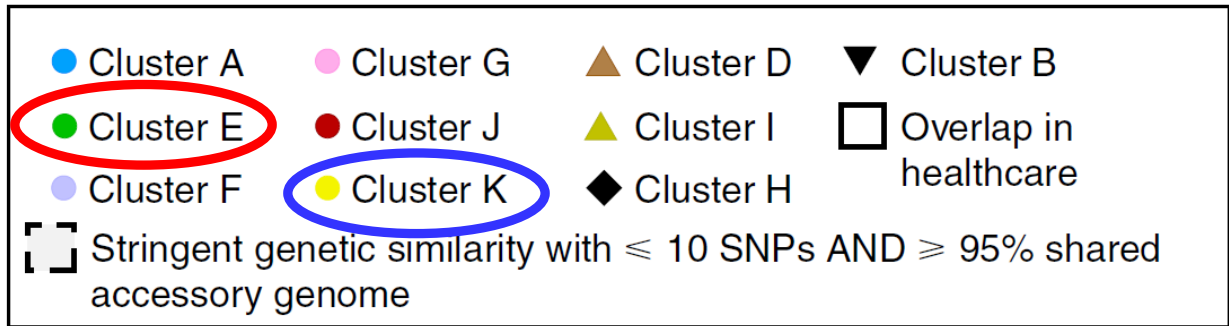
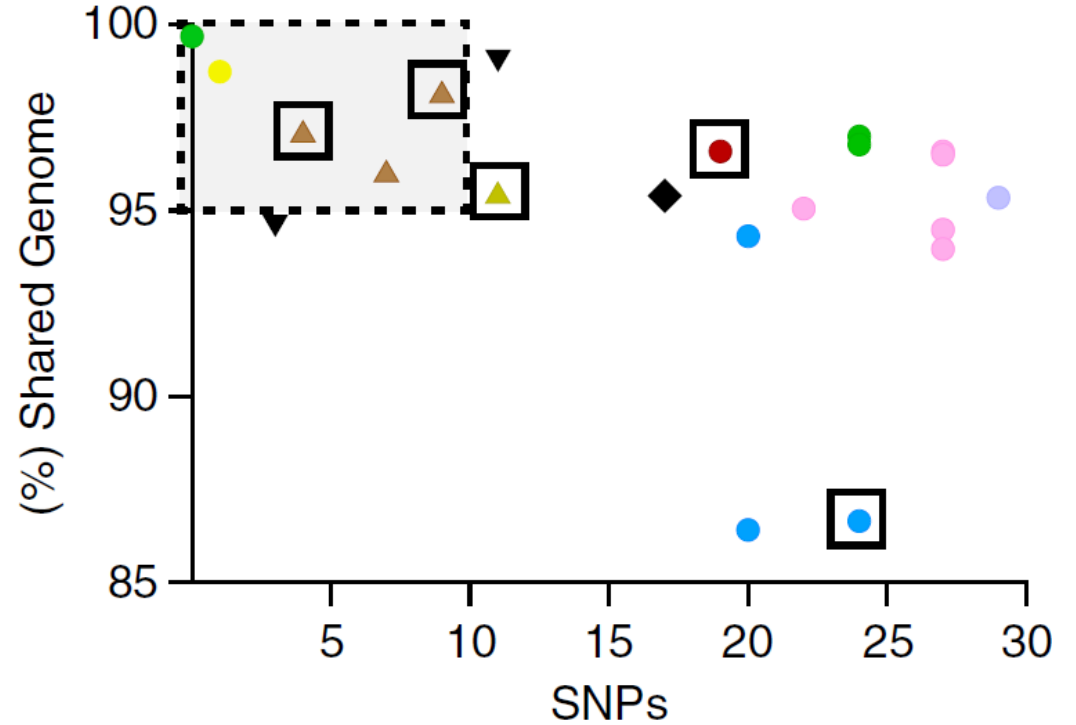
Genomic clusters of MABC are widely geographically dispersed and shared across all patient groups.



Cluster E, Cluster K: *M. abscessus*

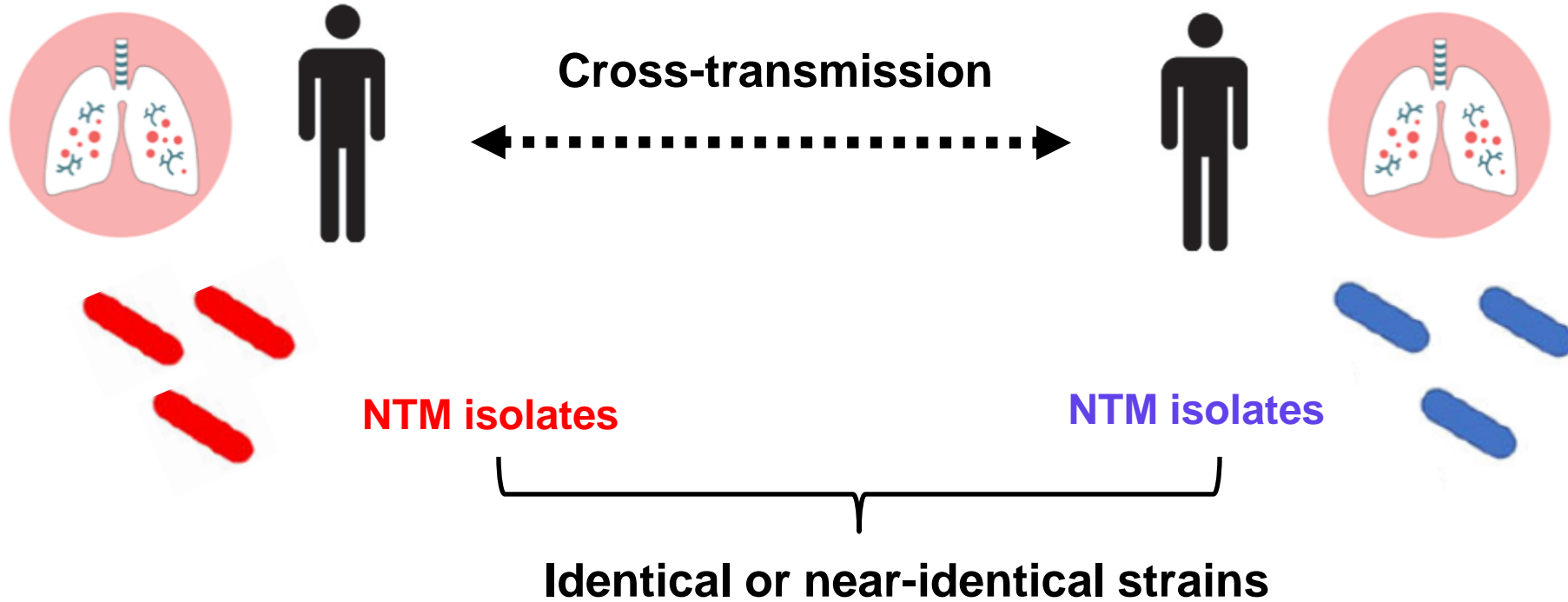
→ no evidence of healthcare-associated opportunity for transmission

Integrated pan-genome analysis

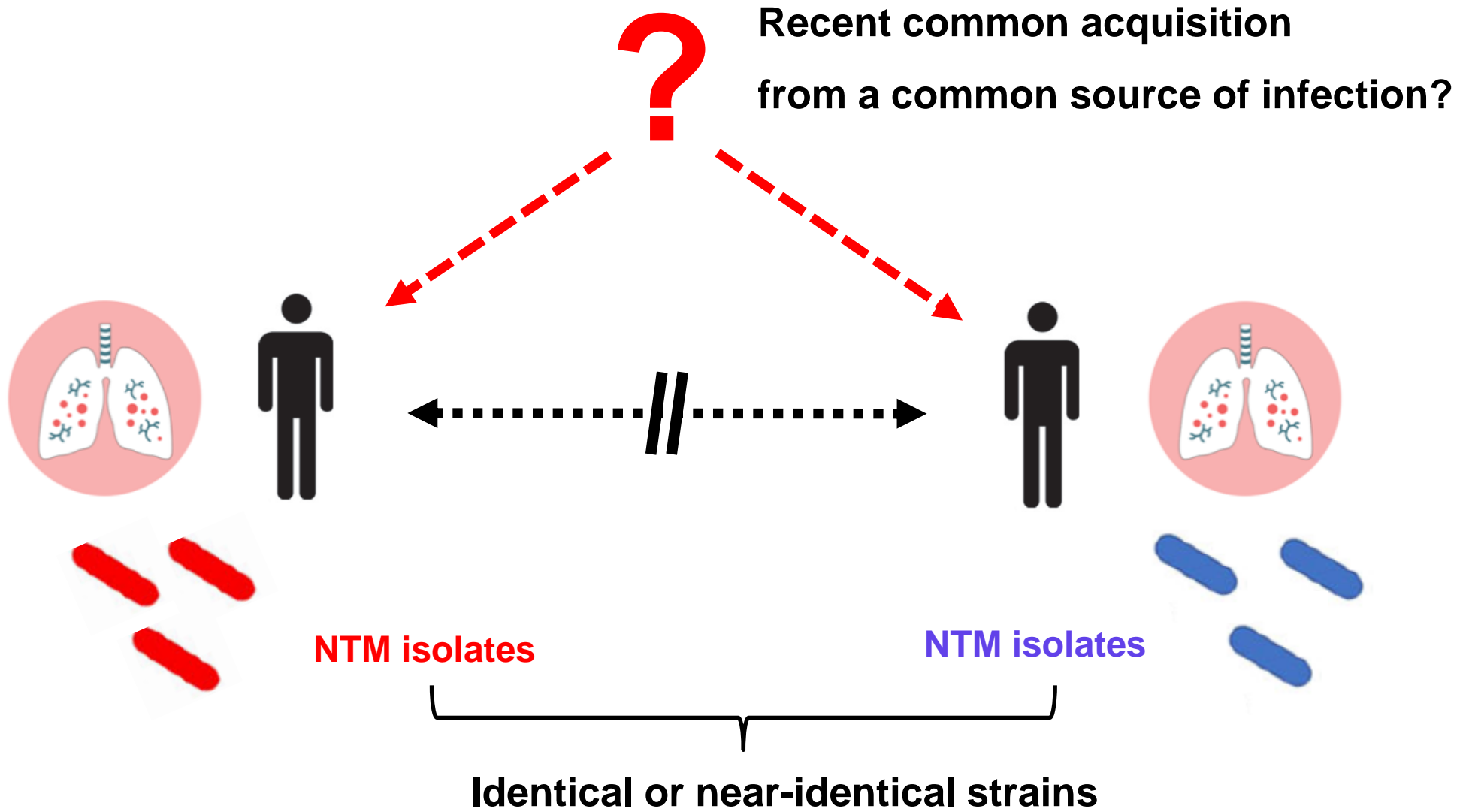


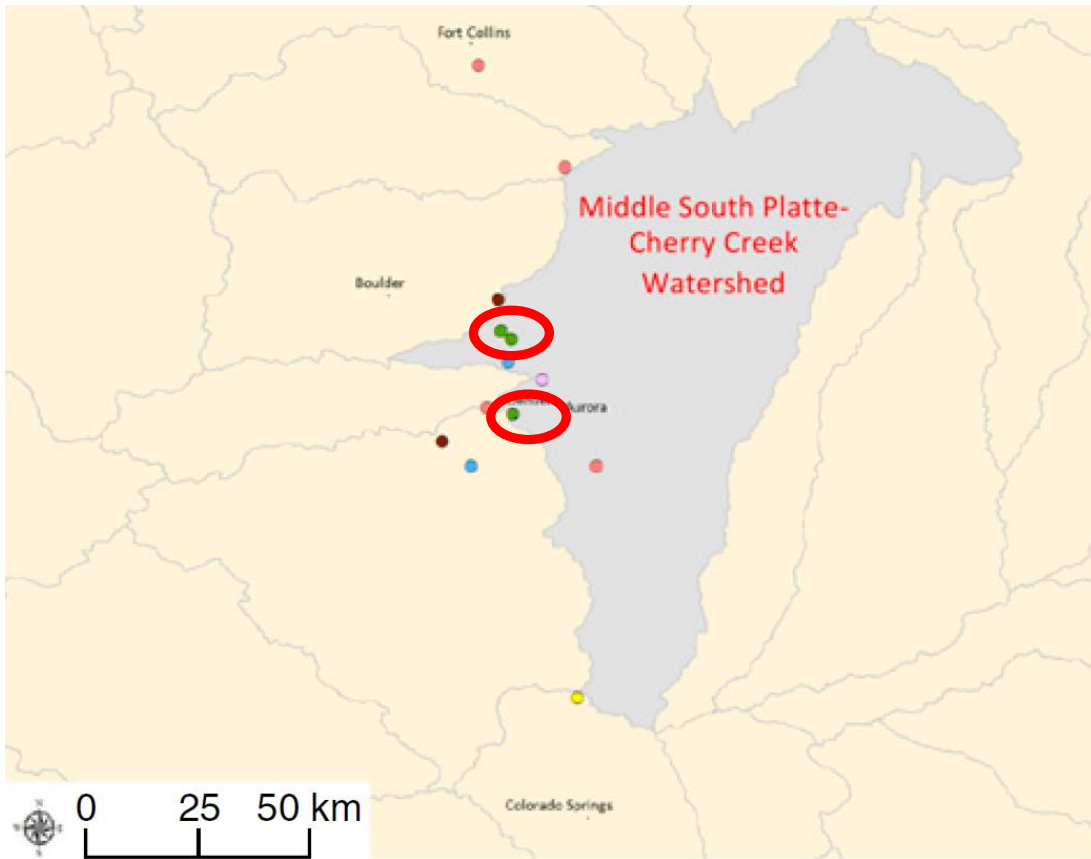
Patient **A** with NTM pulmonary disease

Patient **B** with NTM pulmonary disease

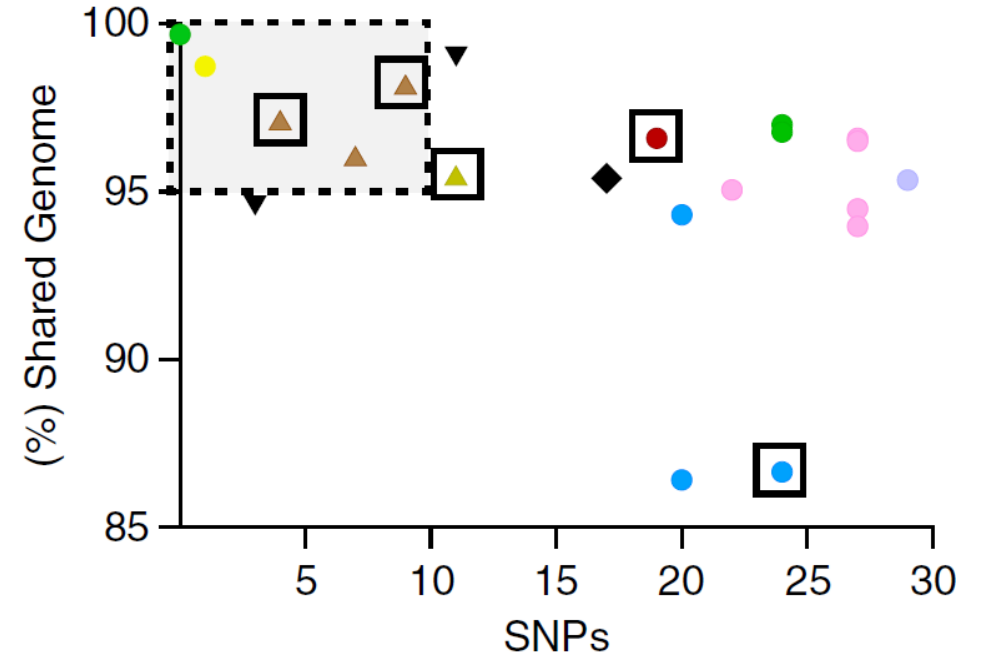


“The presence of genetically similar isolates is **insufficient to demonstrate healthcare-associated NTM transmission.”**

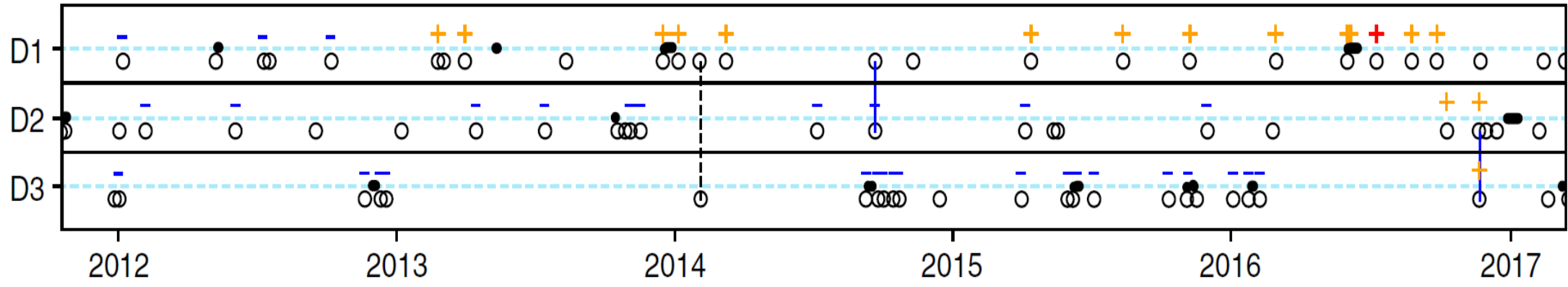




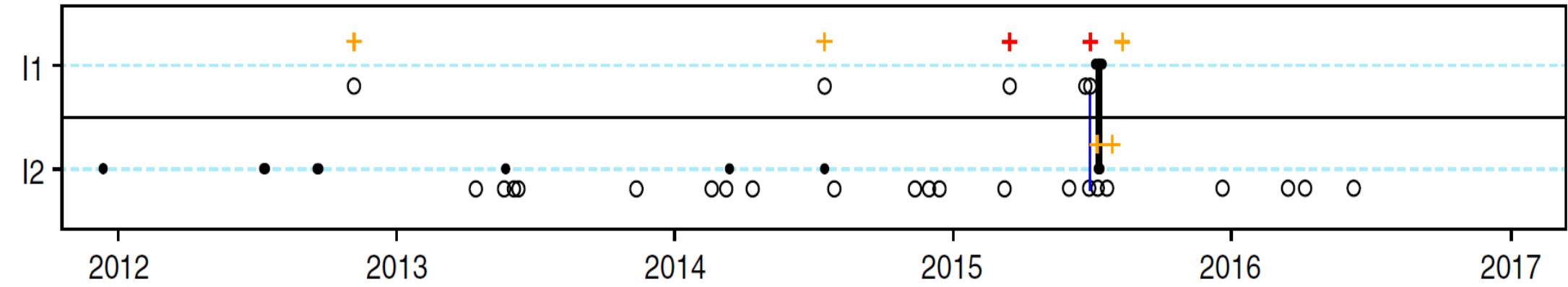
All three patients in *M. abscessus* cluster E lived within the same watershed.



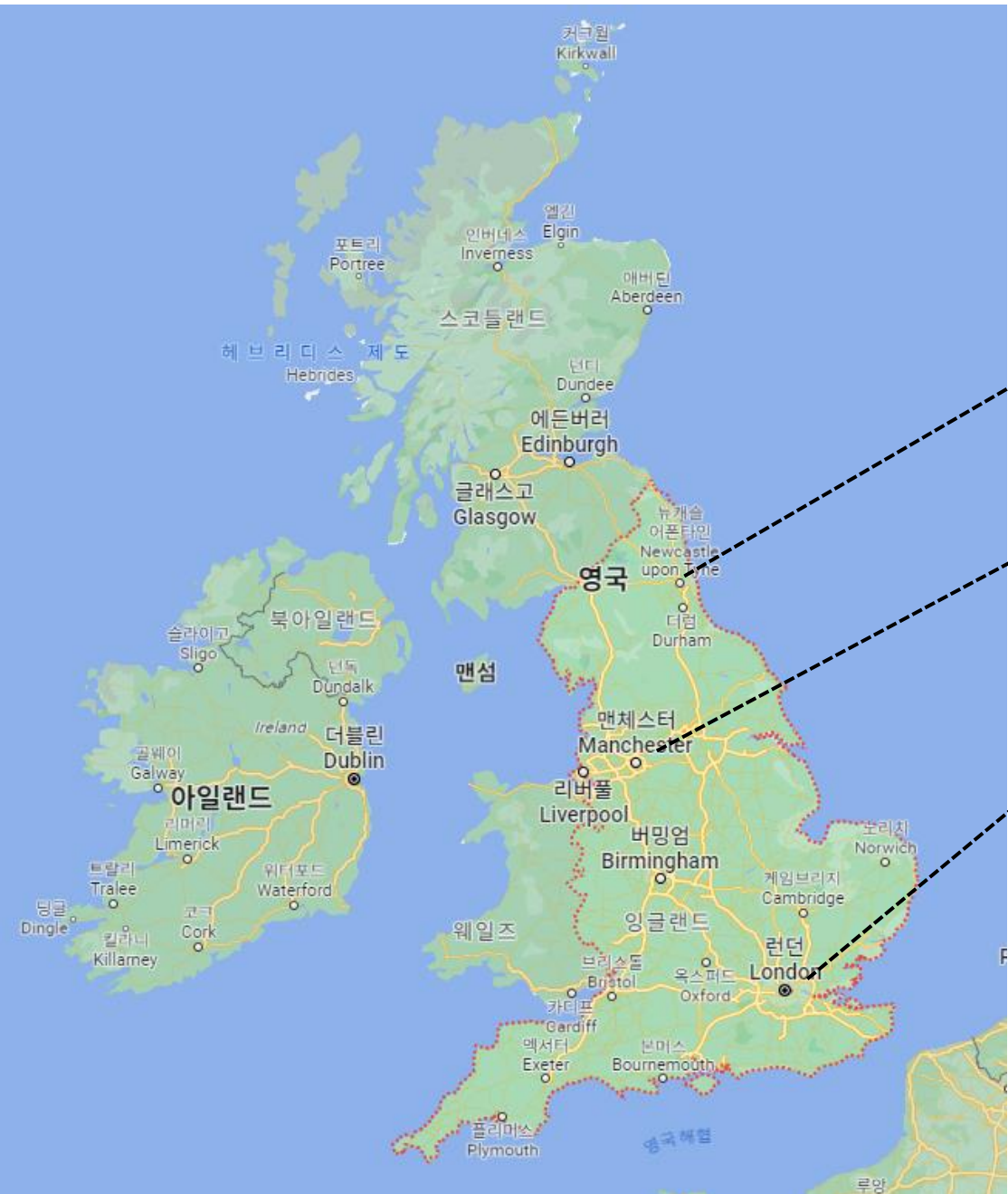
M. avium



M. avium



***M. avium* clusters did not demonstrate shared home of residence watershed.**



Patient with COPD,
M. abscessus pulmonary disease

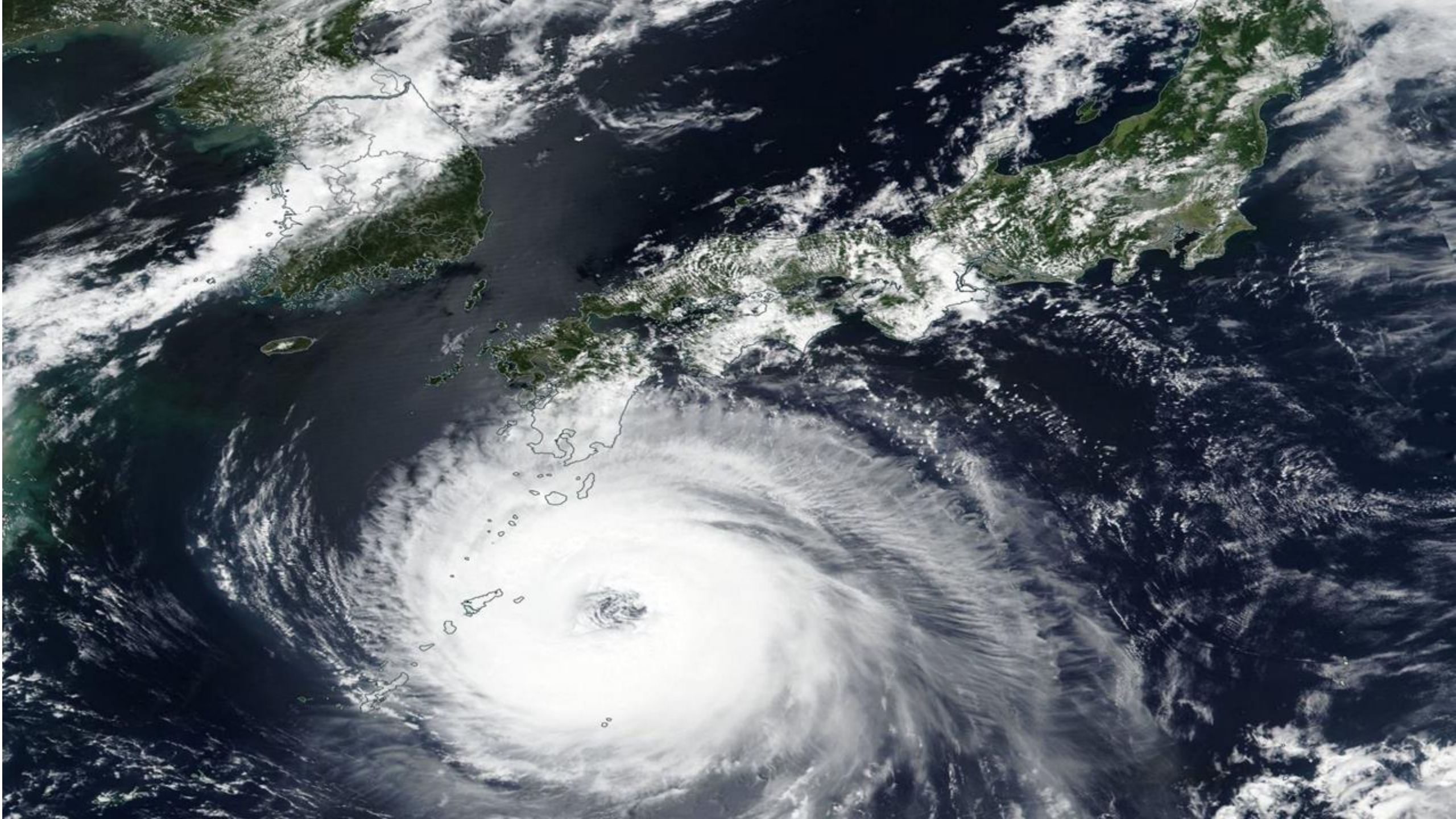
Patient without a respiratory disease,
M. abscessus skin abscess

Patient with cystic fibrosis,
M. abscessus pulmonary disease

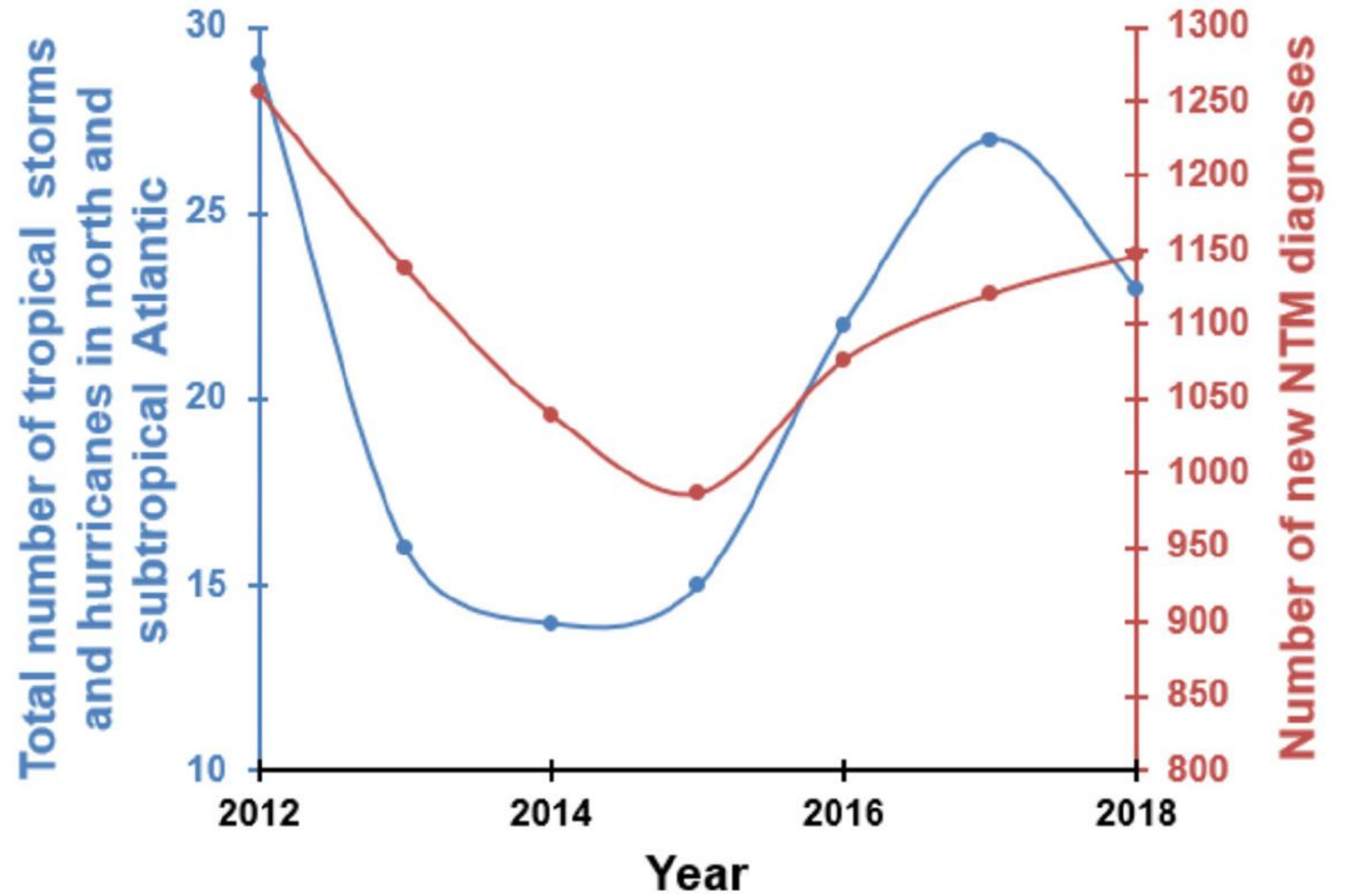
Identical strains

A significant reservoir of **healthy and asymptomatic carriers**
in the general population?

National dissemination via a widely distributed environmental vector,
possibly **water-associated** exposure?



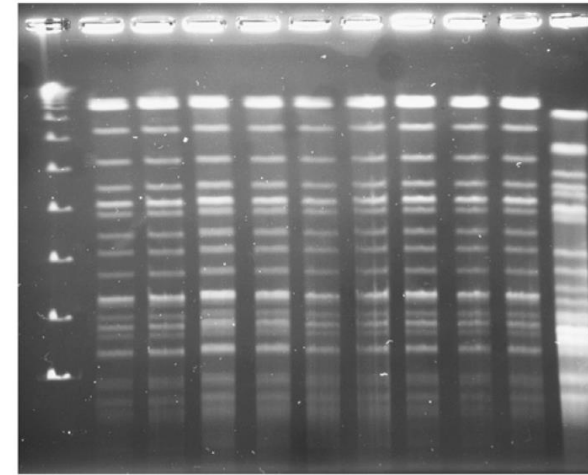
Tropical storms and hurricanes in Florida, United States and the number of new cases of NTM infections between 2012 and 2018



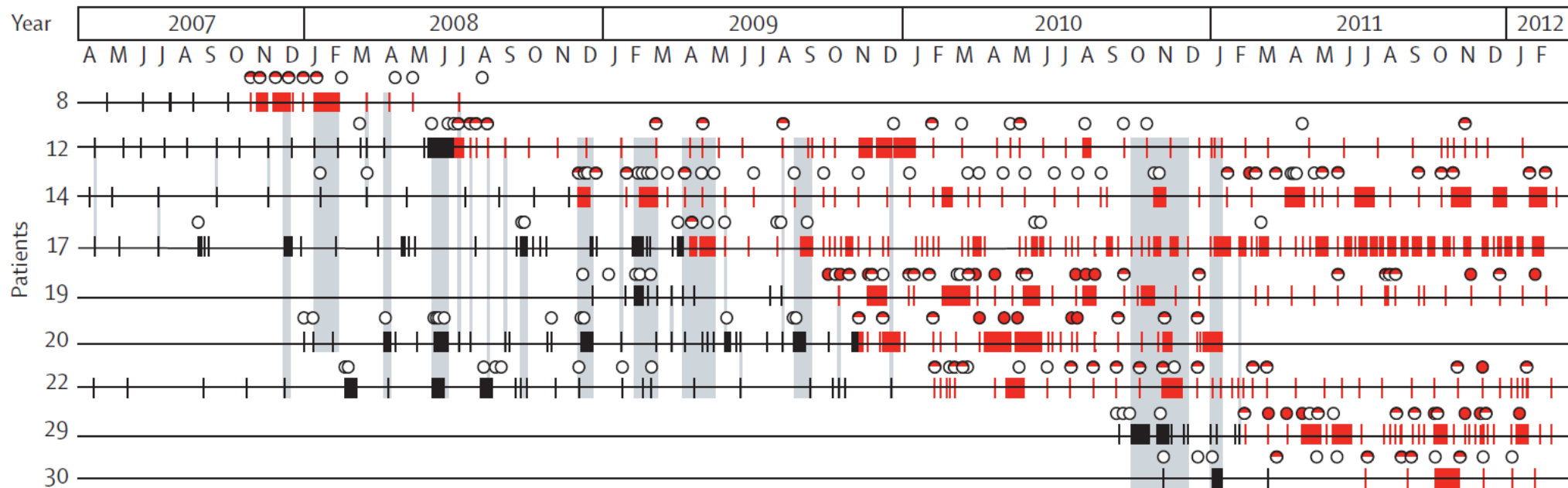
Summary

Healthcare-associated cross-transmission

The outbreak of *M. massiliense* pulmonary disease in cystic fibrosis center in Seattle.



Am J Respir Crit Care Med 2012;185:231–232



Lancet 2013;381:1551–1560

Summary

Healthcare-associated cross-transmission

- In patients with **cystic fibrosis**
 - Evidence for transmission of MABC and MAC
- In patients with **non-cystic fibrosis**
 - Evidence for transmission of MABC, but the number of literature is limited.

Potentially possible, plausible only if they have epidemiological links.

An uncommon and rare phenomenon

No evidence of cross-transmission even with long-term exposure

→ Low risk of person-to-person transmission

Most patients with similar isolates have no identifiable epidemiologic links

→ Unknown potential locally or nationally distributed vectors

*“The oldest and strongest emotion of mankind is fear,
and the oldest and strongest kind of fear is fear of the unknown.”*

H.P. Lovecraft (1890–1937)

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5. **Studies on NTM cross-transmission in South Korea**

Whole genome sequencing of Nontuberculous Mycobacterium (NTM) isolates from sputum specimens of co-habiting patients with NTM pulmonary disease and NTM isolates from their environment

BMC genomics 2020;21:322

Table 1 Characteristics of the three patient pairs in this study

	Age /Relationship	Sources	NTM Collection Date	NTM Species by conventional method
Patient A	81 /Mother	Sputum	5 June 2017	<i>M. avium</i>
Patient B	51 /Daughter	Sputum	19 July 2017	<i>M. avium</i>
Patient C	77 /Husband	Sputum	13 April 2017	<i>M. intracellulare</i>
Patient D	71 /Wife	Sputum	3 May 2017	<i>M. intracellulare</i>

No patient pair showed

near-identical NTM isolates

Three pairs of patients with NTM-PD who had been co-habiting for at least 15 years

“Study of subspecies proportion and antibiotic resistance mechanism between *Mycobacterium abscessus* complex from pulmonary disease patients : a prospective multicenter study”

The late Prof. **Wong-Jung Koh**, Samsung Medical Center

6 university hospitals in South Korea

- Gachon University Gil Medical Center
- Pusan National University Hospital
- Samsung Medical Center
- Seoul National University Hospital
- Asan Medical Center
- Soon Chun Hyang University Medical Center

Samsung Medical Center

366 MABC clinical strains,
of which a DST was requested to
the Korean Institute of Tuberculosis in 2017

DNA extraction, 316 MABC isolates
(207 *M. abscessus*, 109 *M. massiliense*)

Korean Institute of Tuberculosis

A WGS library was constructed for 302 strains,
as of September 8, 2023

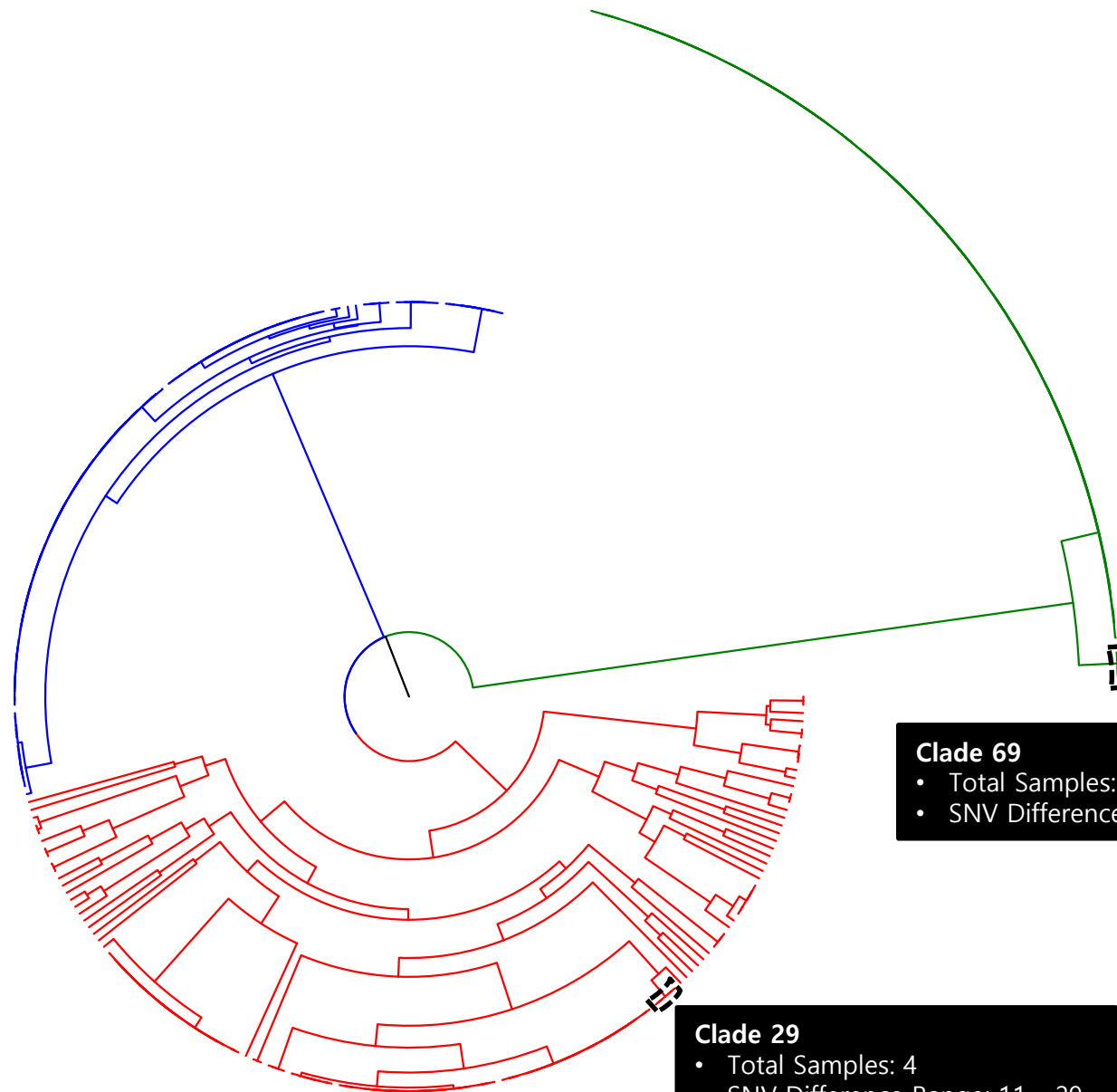
WGS analysis (Illumina Miseq, Miseq reagent)

**Graduate School of Public Health,
Seoul National University**

Phylogenetic analysis

Analysis Pipeline

- **Samples**
 - 302 NTM samples from Korean
- **Reference genome**
 - *Mycobacteroides abscessus* ATCC 19977
- **Detecting Single-Nucleotide Variant (SNV)**
 - **Trimming low-quality sequences:** trimmomatic (v 0.39)
 - **Mapping reads:** BWA-MEM (v 0.7.17-r1188)
 - **Calling variants:** bcftools (v 1.16)
 - **Filtering variants:** bcftools (v 1.16)
- **Phylogenetic Analysis**
 - **Tool:** BEAST (Bayesian Markov chain Monte Carlo analysis of genetic sequences)
 - **Molecular clock model:** log-normal relaxed and strict clock model for 10,000,000 states
 - **Tree prior model:** Yule Process with a random starting tree



Clade 69
• Total Samples: 3
• SNV Difference Range: 15 - 20

Clade 29
• Total Samples: 4
• SNV Difference Range: 11 - 29

Thank you for your attention.