One Patient, Multiple NTM Species: Noise or a Meaningful Signal

Jhun Byung Woo

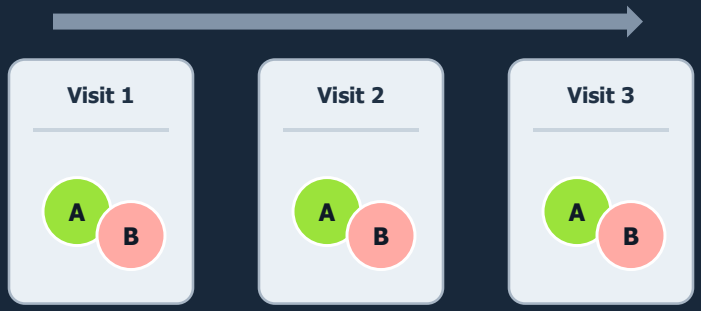
Associate Professor, Samsung Medical Center

Sungkyunkwan University School of Medicine, South Korea

One patient, multiple NTM species

SIMULTANEOUS ISOLATION

Species A and B detected **together** in the same sputum sample



A + B together in each sample

ALTERNATING ISOLATION

Different species detected in **separate** samples collected over time



A, then B, then A, then B in separate samples

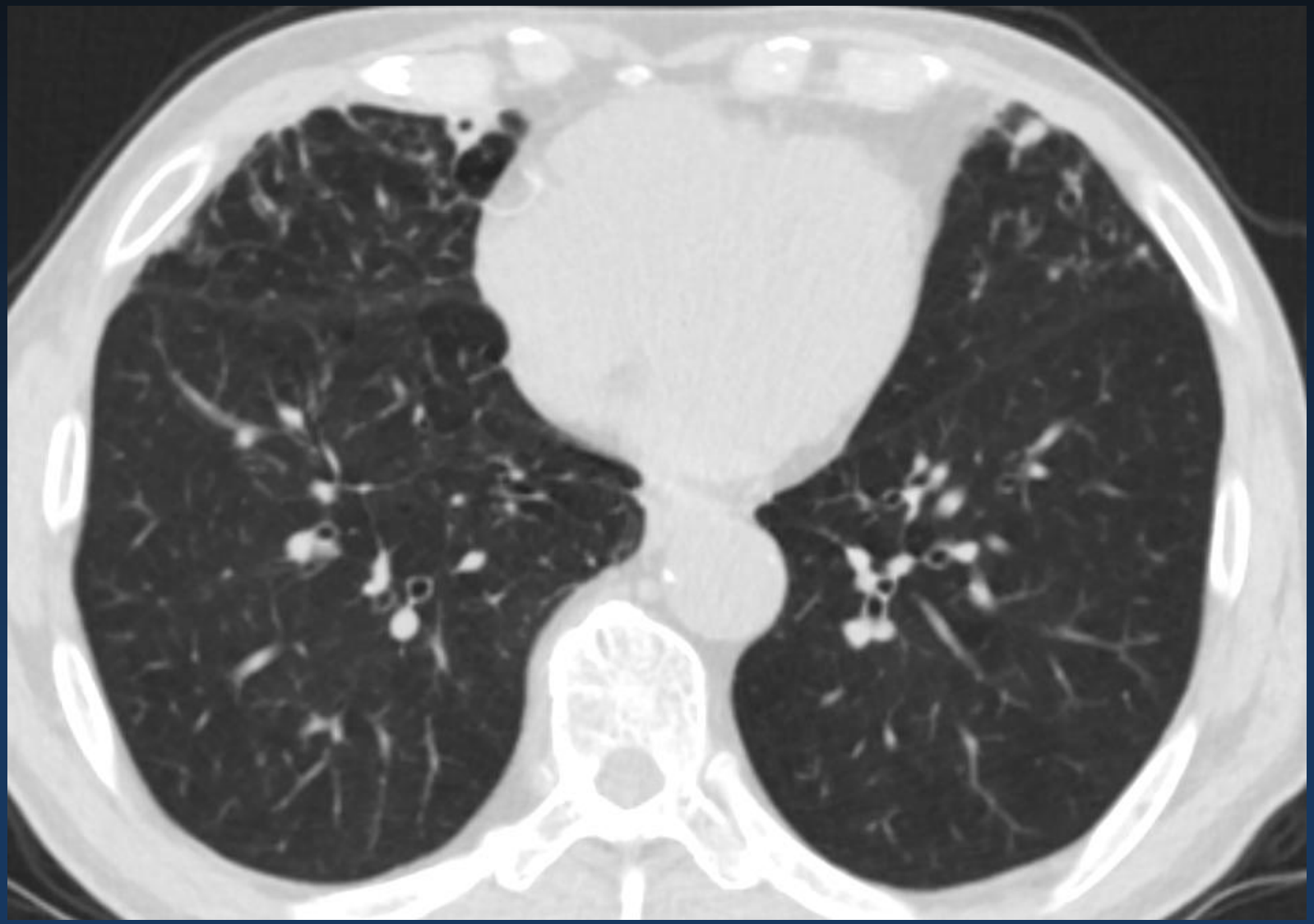
A Species A B Species B

Questions in Clinical Practice

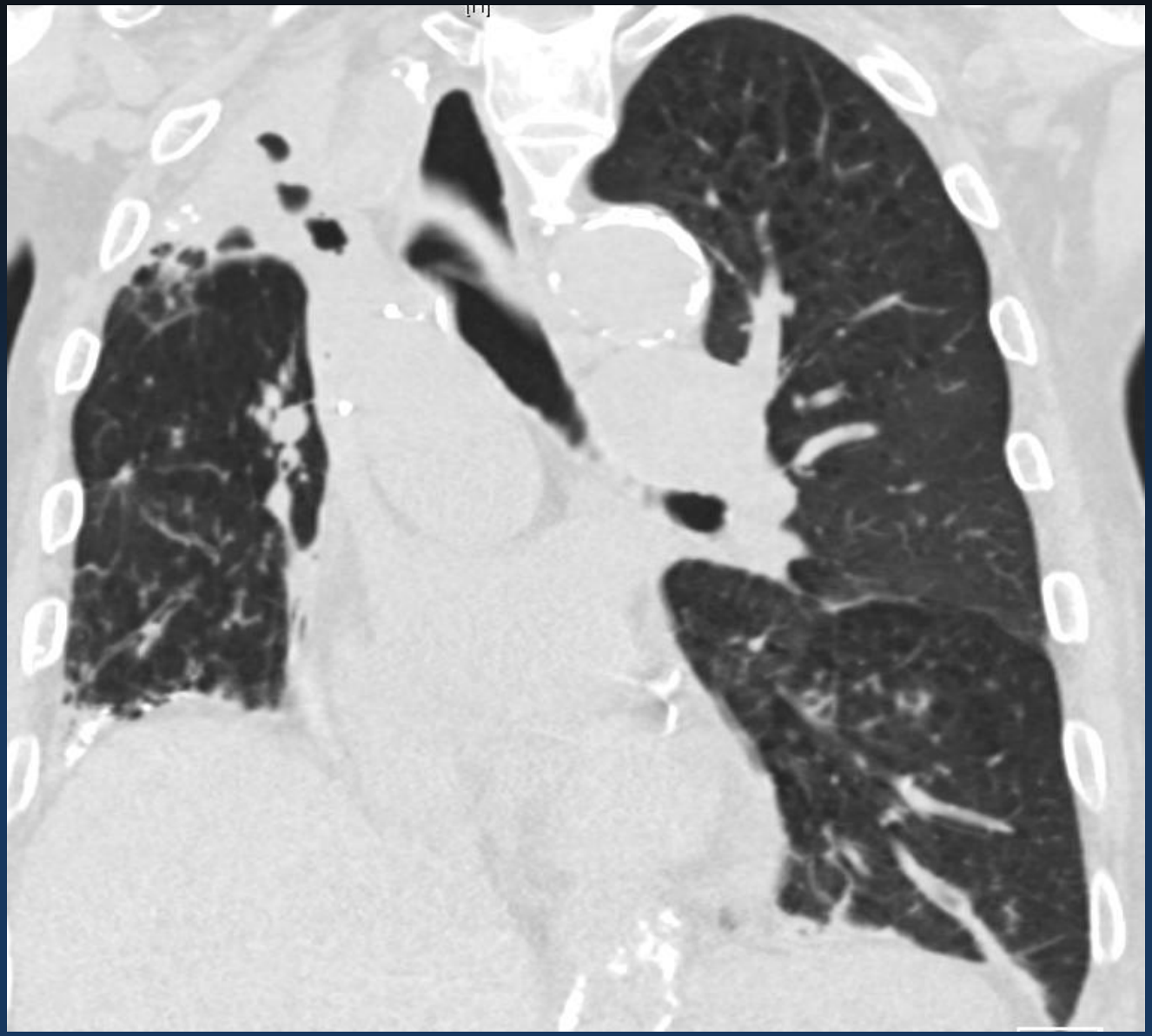
- Is mixed NTM infection real?
- Is mixed NTM infection more severe?
- Is mixed NTM infection associated with poorer outcomes?
- Can we safely adopt a watchful-waiting strategy?
- Which species should we treat—both or just one?

CASE

M. avium + M. intracellulare



M. abscessus + M. massiliense



M. avium + M. massiliense

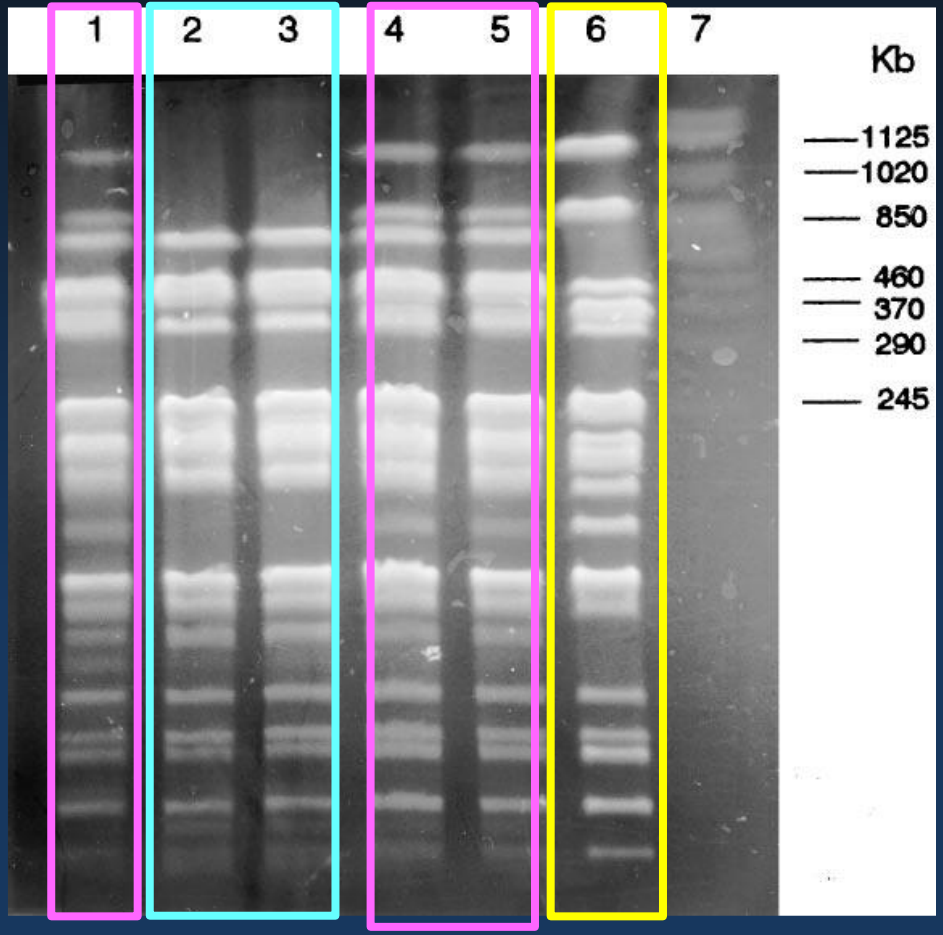


Definition and Pathophysiology ?

Polyclonal *Mycobacterium avium* Complex Infections in Patients with Nodular Bronchiectasis

RICHARD J. WALLACE, Jr., YANSHENG ZHANG, BARBARA A. BROWN, DAVID DAWSON, DAVID T. MURPHY, REBECCA WILSON, and DAVID E. GRIFFITH

- *Dra*I (restriction enzyme), Pulsed-Field Gel Electrophoresis patterns
- Six sputum cultures from one patient with MAC-PD



Lane 2, 3 = Pattern (A)

Lane 6 = Pattern (B)

Lane 1,4,5 = Pattern (A) + (B)

Polyconal MAC infection

Am J Respir Crit Care Med 1998;158(4):1235-44

Association between Polyclonal and Mixed Mycobacterial *Mycobacterium avium* Complex Infection and Environmental Exposure

Kohei Fujita[1], Yutaka Ito[1], Toyohiro Hirai[1], Takeshi Kubo[2], Koichi Maekawa[3], Kaori Togashi[2], Satoshi Ichiyama[4], and Michiaki Mishima[1]

- **Japan**, 120 MAC-PD patients , Variable number of tandem repeats
- Polyclonal MAC: different VNTR genotypes / Mixed MAC: different MAC species
- Factors associated with polyclonal and mixed mycobacterial infections

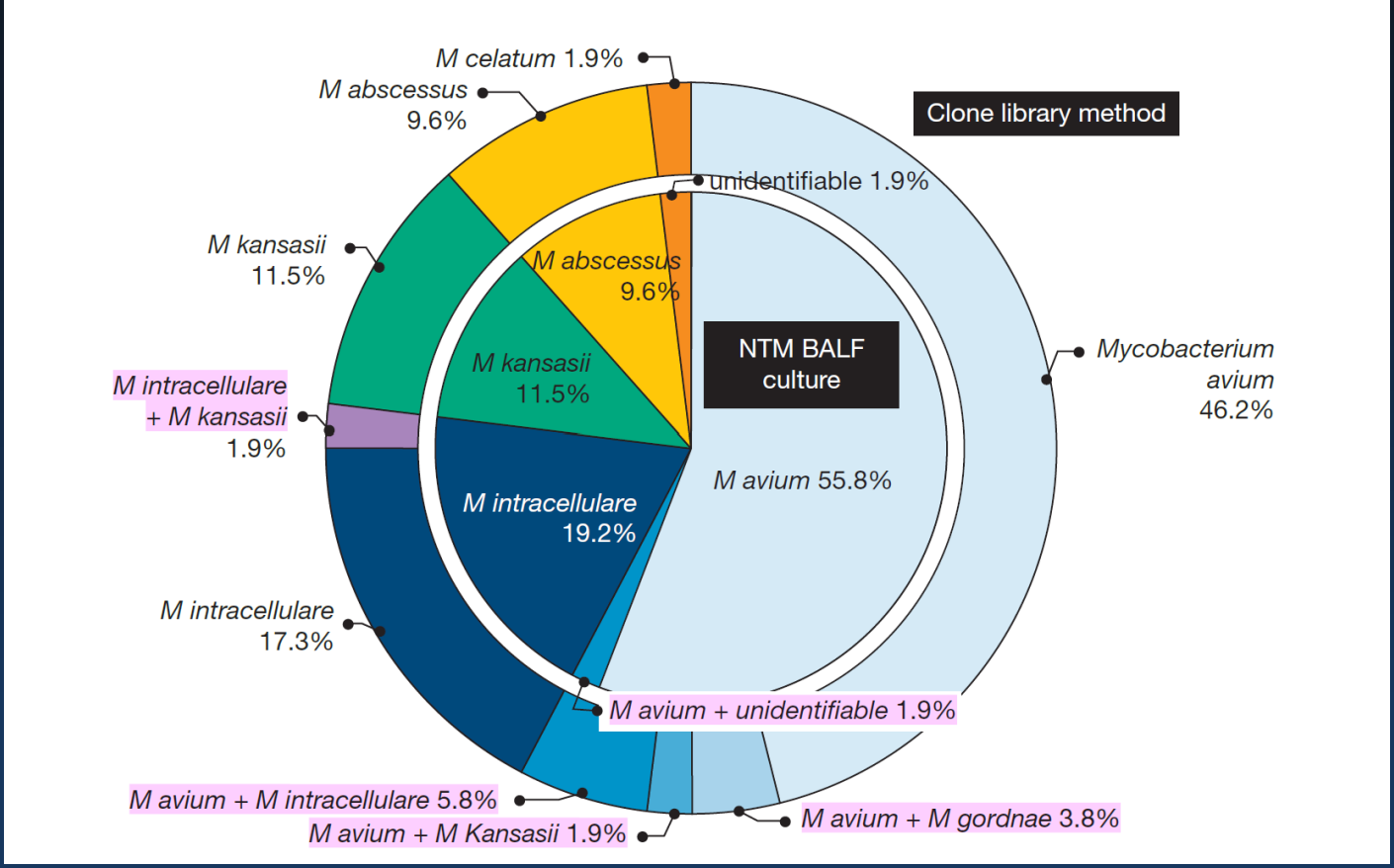
Variable	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Male sex	4.13 (1.73–9.81)	<0.01	2.94 (0.98–9.12)	0.054
History of asthma	15.40 (1.82–129.98)	0.013	11.56 (1.41–255.77)	0.021
No cavitation	2.90 (1.22–6.88)	0.014	2.50 (0.91–7.48)	0.075
High soil exposure	3.66 (1.19–11.70)	<0.01	4.31 (1.72–11.45)	<0.01
Shower use in a bathroom	3.73 (1.19–11.70)	0.018	4.57 (1.28–23.23)	0.018
Swimming in a pool	15.40 (1.82–129.98)	<0.01	9.69 (1.21–206.92)	0.032

Environmental exposure was associated with polyclonal and mixed mycobacterial MAC infection

Ann Am Thorac Soc 2014;11(1):45-53

NTM culture 'may' underestimate mixed NTM infection

- **Japan** 2010 and 2016, BALF from 120 patients with suspected NTM-PD
- Conventional culture + 16S rRNA gene clone library analysis



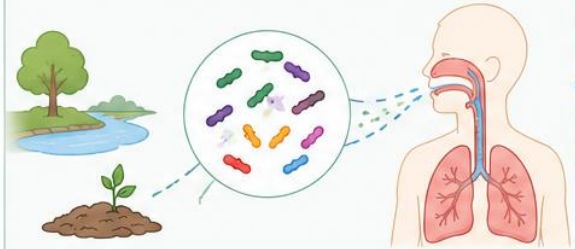
Clone library analysis revealed 7 patients with multiple NTM phylotypes

Chest 2020;158(6):2304-2313

How mixed NTM infection can occur ?

1. Polyclonal infection

At the same time,
multiple NTM species are
inhaled from the environment.

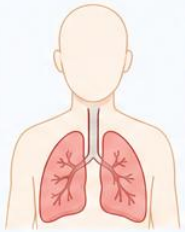


Different NTM species can
enter the lungs together.

2. Host susceptibility

When the host defense is not strong,
several species can survive,
establish, and persist.

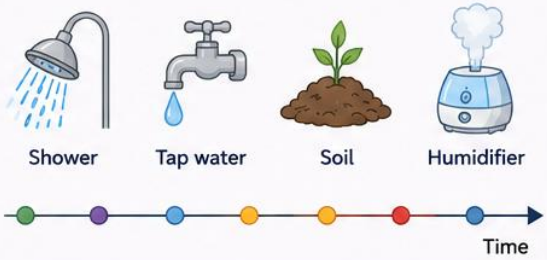
- Bronchiectasis
- Chronic lung disease (COPD)
- Prior TB
- Older age
- Immune problems
- Genetic factors



The environment in the lungs allows
multiple species to survive.

3. Repeated environmental exposure

Repeated exposure over time
can introduce new NTM species.



New species may be added
again and again.

Therefore, mixed NTM infection *can* occur.

Multiple NTM species may establish, persist,
and coexist in the lungs.

Over time...

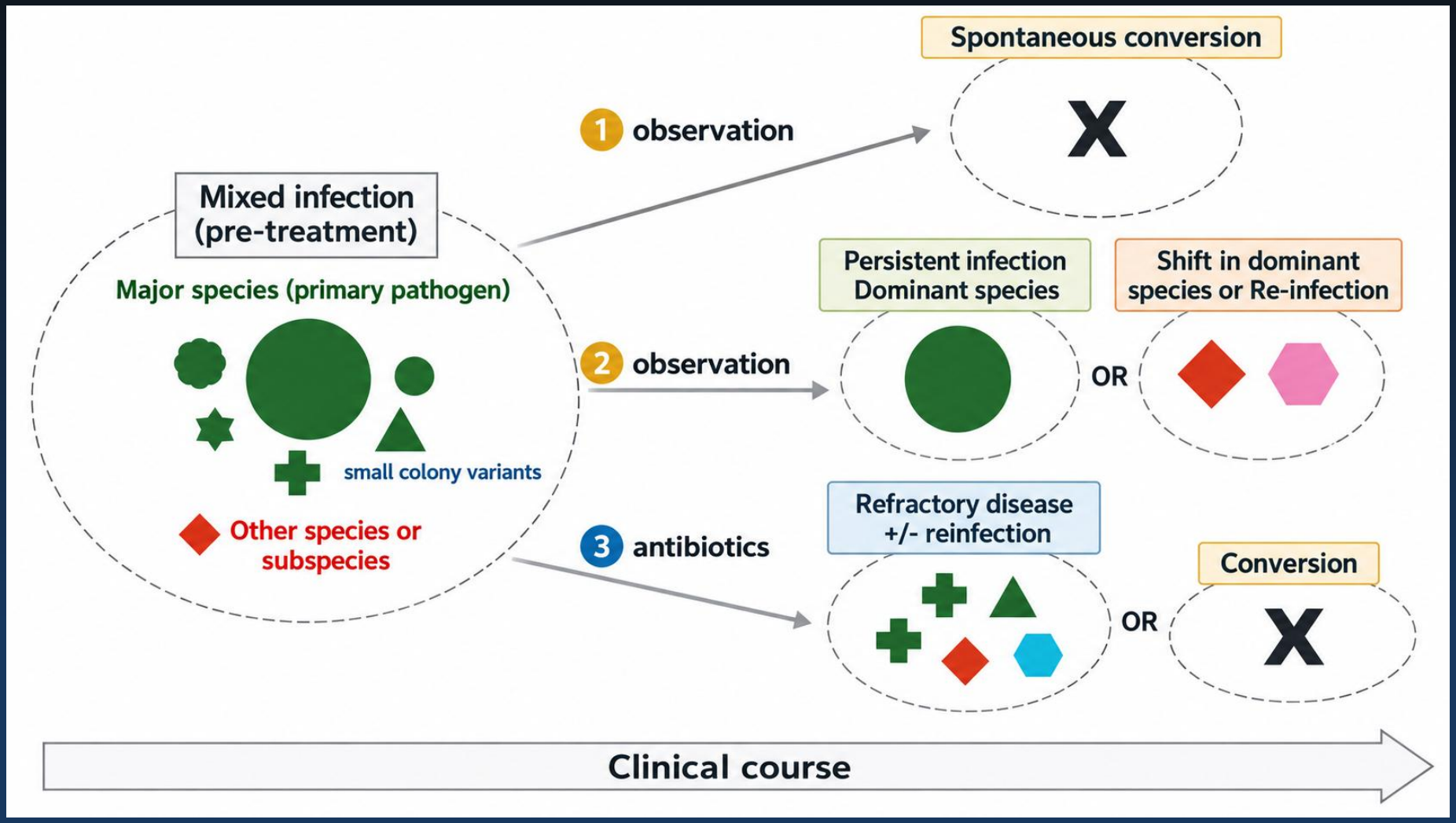


Possible consequences

- More difficult to treat
- More persistent infection
- Higher risk of poor outcomes

Because of polyclonal infection, host susceptibility, and repeated environmental exposure,
mixed NTM pulmonary disease is possible.

Clinical scenarios in mixed-species NTM-PD



Review of the Published Literature

Frequency of mixed-species NTM infection

Author, year	Center	Population	Definition	NTM ID	Mixed Rate
Zhang et al. 2019 BMC Infect Dis	Singapore	352 NTM-PD 30.1% (106/352)	≥2 species (simultaneous or sequential)	HPLC INNO-LiPA DNA probes	**30.1% (106/352)**
Zhang et al. 2020 Epidemiol Infect	Chongqing China	146 NTM ds. (97% NTM-PD)	≥2 species	PCR reverse dot-blot (21 species)	**7.5% (11/146)**
Kim KJ et al. 2023 TRD	Pusan South Korea	2,521 lab. specimen from 1,410 patients	≥2 species	AdvanSure Mycobacteria GenoBlot	**8.5% (61/718)**
Kim et al. 2024 JKMS	Severance Hospital South Korea	1,009 NTM-PD	≥2 species within 3 mo. (simultaneous or sequential)	rpoB/tuf PCR-RFLP +hsp65/rpoB seq.	**14.6% (147/1009)**

BMC Infect Dis 2019;19(1):436 *Epidemiol Infect 2020:149:e7*
Tuberc Respir Dis (Seoul) 2023;86(1):47-56 *J Korean Med Sci 2024;39(20):e167*

Case series on mixed NTM infection

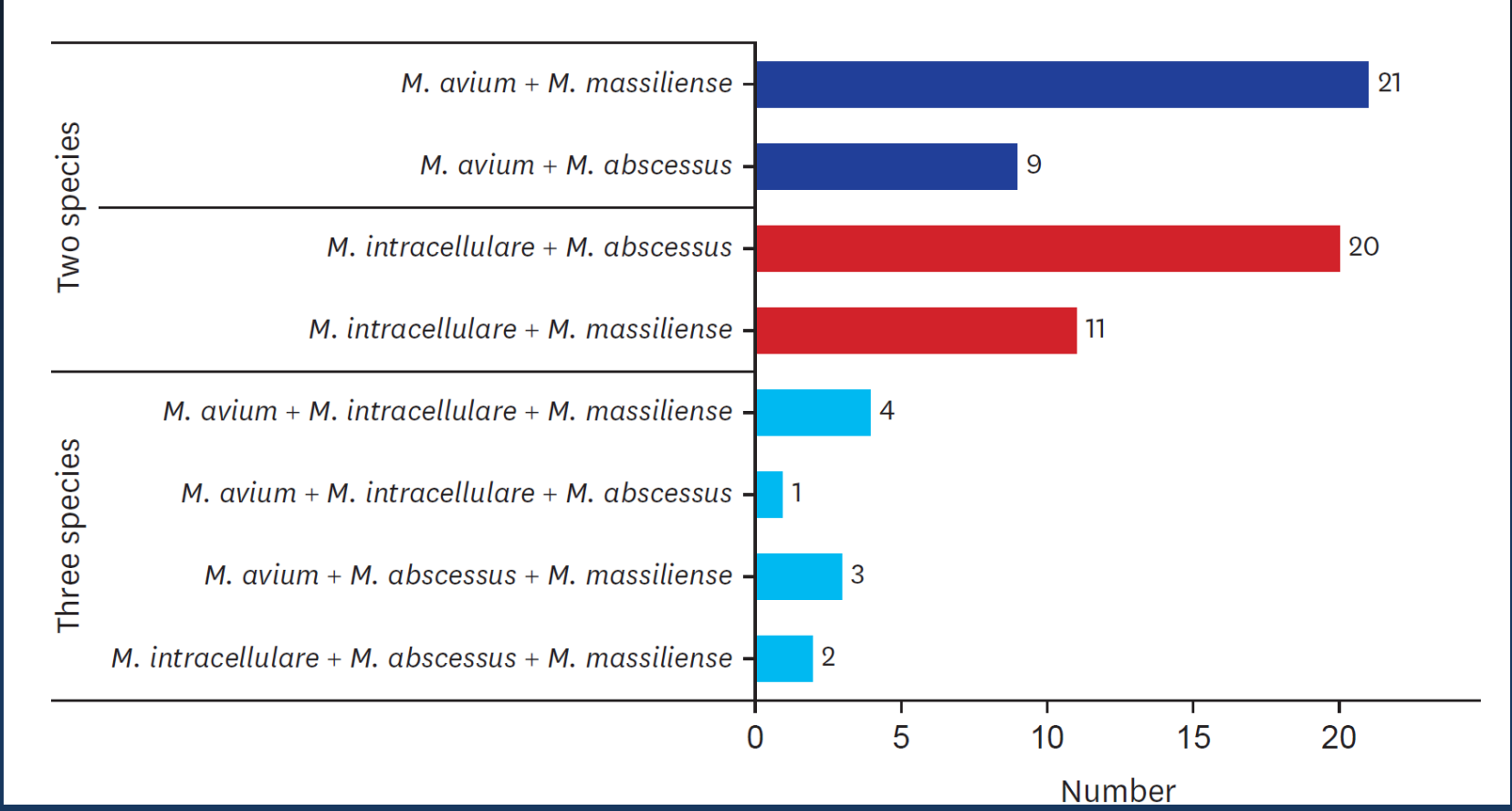
Study	Patients no.	Underlying	Common pattern	Treatment	Outcome
Lim et al., 2002-2008 SNUH, South Korea	33 NTM-PD	Prior TB 51 %	Transition to another NTM 70% Alternating isolation 27% Simultaneous isolation 3%	NA	NA
Asaoka et al., 2006-2015 Japan	55 Non-MAC PD/infection	COPD	Multiple non-MAC NTMs, or MAC +M. abscessus	Antibiotics 41%	NA
Hill & Wooten, 2022 USA, UCSD	1 Disseminated (lung & skin)	HIV, CD4 <10 cells	M. avium-intracellulare + M. kansasii	AZT EMB Rfb	sputum MAC (+) at 4 months
Chen et al., 2023 CHINA	1 Dialysis-Peritonitis	ESRD	M. smegmatis + M. abscessus + M. goodii	Moxifloxacin Cefotiam Amikacin	Temporary Improvement Death
Opperman et al., 2025 South Africa	1 MAC-PD with bacteremia	HIV, CD4 3 cells	M. kansasii + M. chelonae + M. avium	AZT EMB Rfb isoniazid	Temporary improvement, Death

Int J Infect Dis 2011;15(11):e795-8 *Intern Med 2021;60(20):3213-3219*
Open Forum Infect Dis 2022;9(10):ofac519 *BMC Nephrol 2023;24(1):95* *IJID Reg 2024:14:100502*

Common NTM Species Combinations

Common species combinations in mixed NTM

- **Severance Hospital**, South Korea, 147 (14.6%) of 1,009 NTM-PD patients
- Coinfection: 2 ≥ NTM species within 3 mo.
- Combinations of M. avium complex and (M. abscessus or M. massiliense) (N = 71)
- M. avium + M. massiliense = most common



J Korean Med Sci 2024;39(20):e167

Common species combinations in mixed NTM

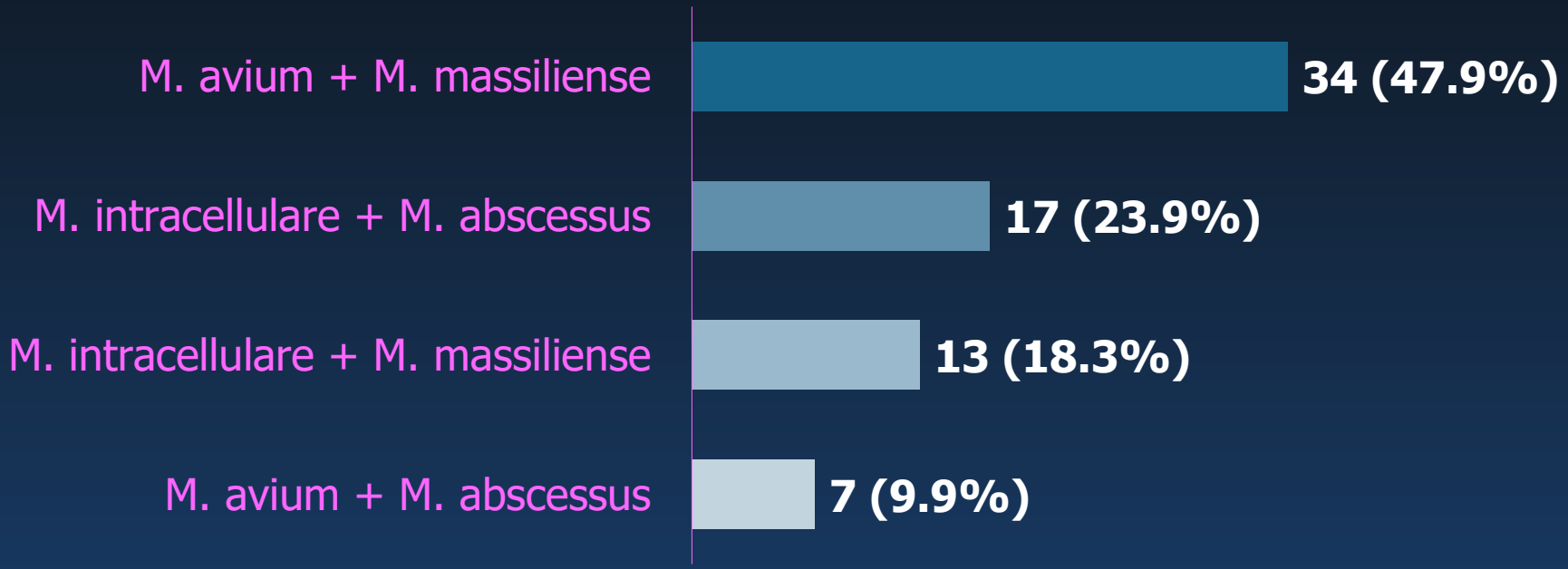
- **Pusan National University Yangsan Hospital**, South Korea, 2016–2020
- 2,521 NTM (+) specimens from 1,410 patients; species identified 718 isolates
- M. intracellulare + M. abscessus = most common

≥2 Species	61 (8.5)
M. intracellulare and M. abscessus subspecies *abscessus*	14 (1.8)
M. avium and *M. intracellulare*	10 (1.4)
M. avium and *M. abscessus* subspecies *abscessus*	5 (0.7)
M. avium and *M. abscessus* subspecies *massiliense*	5 (0.7)
M. intracellulare and *M. abscessus* subspecies *massiliense*	4 (0.6)
M. avium and *M. kansasii*	3 (0.4)
M. abscessus subspecies *abscessus* and *M. abscessus* subspecies *massiliense*	3 (0.4)
M. intracellulare and *M. fortuitum* complex	3 (0.4)
M. intracellulare and *M. kansasii*	2 (0.3)
M. marinum and *M. ulcerans*	2 (0.3)
M. intracellulare, M. abscessus subspecies *abscessus* and *M. abscessus* subspecies *massiliense*	2 (0.3)
M. avium, M. intracellulare and *M. abscessus* subspecies *massiliense*	2 (0.3)
M. avium and *M. fortuitum* complex	1 (0.1)
M. avium and *M. gordonae*	1 (0.1)
M. avium and *M. fortuitum* complex	1 (0.1)
M. flavescens and *M. fortuitum* complex	1 (0.1)
M. massiliense and *M. fortuitum* complex	1 (0.1)
M. intracellulare and *M. lentiflavum* or *M. genavense*	1 (0.1)
Unclassified (nontuberculous mycobacteria)	10 (1.4)

Tuberc Respir Dis (Seoul) 2023;86(1):47-56

Common species combinations in mixed NTM

Samsung Medical Center, 2010–2015, 71 patients with mixed NTM species



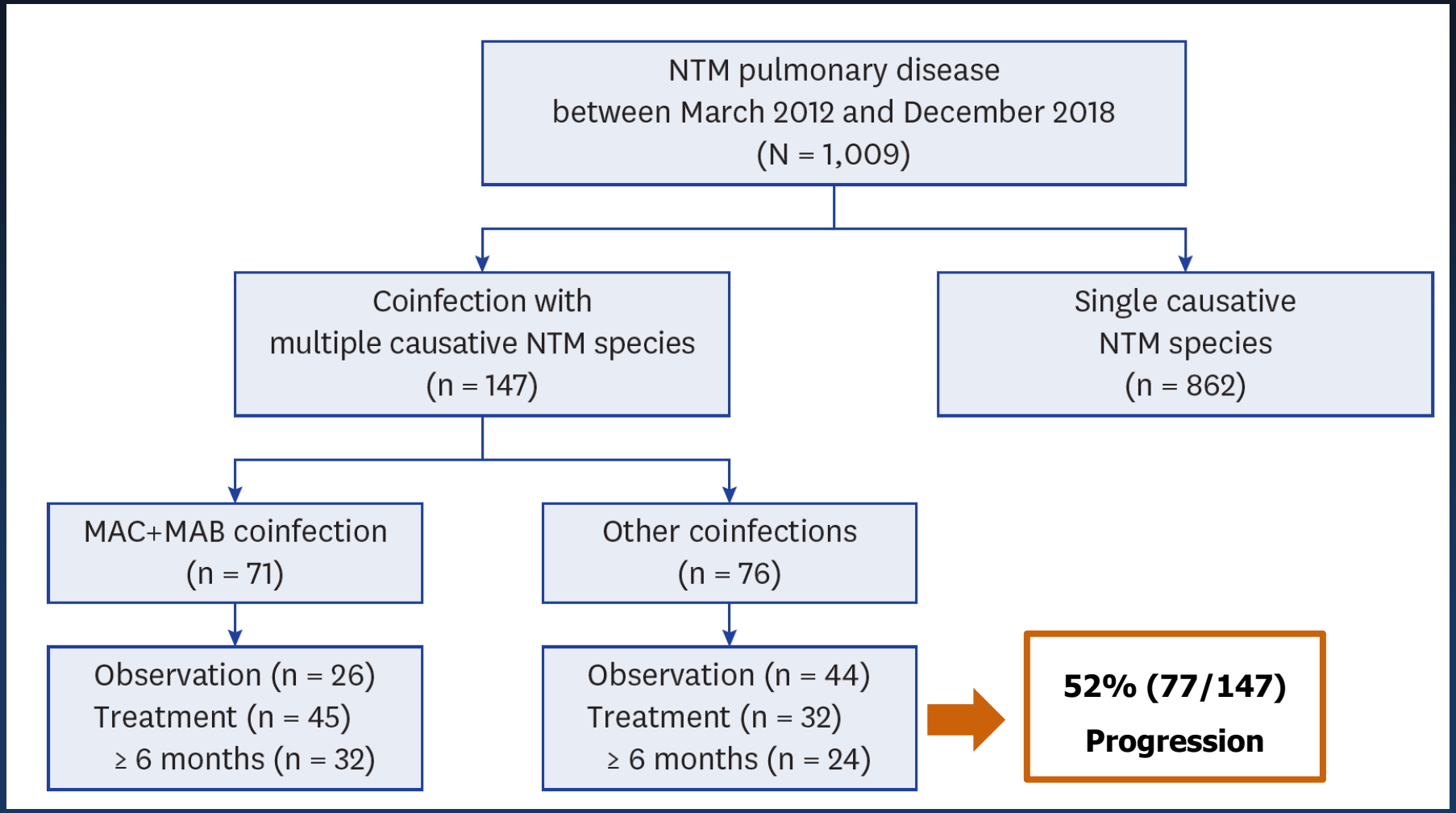
M. avium paired more often with M. massiliense, M. intracellulare with M. abscessus

Antimicrob Agents Chemother 2018;62(10):e01105-18

Prognosis or Severity ?

Treatment initiation rate in mixed-NTM-PD

- **Severance Hospital**, South Korea, 147 (14.6%) of 1,009 NTM-PD patients



J Korean Med Sci 2024;39(20):e167

Is mixed-species NTM-PD more severe ?

- **Severance Hospital**, South Korea, 147 (14.6%) of 1,009 NTM-PD patients
- MAC + MAB (M. abscessus/M. massiliense) versus Other NTMs

Characteristics	MAC+MAB (n = 71)	Other coinfections (n = 76)	*P* value
Age, yr	64.6 ± 10.8	64.9 ± 10.7	0.895
Sex, female	53 (74.6)	49 (64.5)	0.181
BMI, kg/m^2	20.2 ± 2.9	21.4 ± 3.2	0.020
Ever-smoker	10 (14.1)	17 (22.4)	0.182
Radiographic findings			0.430
Fibrocavitary	4 (5.6)	6 (7.9)	
NB with cavity	15 (21.1)	9 (11.8)	
NB without cavity	50 (70.4)	57 (75.0)	
Others[a]	2 (2.8)	4 (5.3)	
Positive AFB smear	12 (17.0)	10 (13.2)	0.543
Cavity present	21 (29.6)	15 (19.7)	0.166
No. of NTM ID	8 (5–11.5)	5 (3–7)	< 0.001
Symptoms			
Cough	32 (45.1)	32 (42.1)	0.717
Sputum	39 (54.9)	41 (54.0)	0.905
Hemoptysis	16 (22.5)	10 (13.2)	0.136
Dyspnea	4 (5.6)	7 (9.2)	0.410
Fever	3 (4.2)	6 (7.9)	0.354
Weight loss	3 (4.2)	2 (2.6)	0.594
General weakness	2 (2.8)	1 (1.3)	0.520
History of tuberculosis	30 (42.3)	29 (38.2)	0.613
History of NTM treatment	5 (7.0)	3 (3.9)	0.408
Comorbidity			
Hypertension	28 (39.4)	25 (32.9)	0.624
Diabetes mellitus	9 (12.7)	7 (9.2)	0.500
COPD	8 (11.3)	5 (6.6)	0.317
Asthma	5 (7.0)	1 (1.3)	0.454
Causative species			
Mycobacterium avium	38 (53.5)	48 (63.2)	0.236
M. intracellulare	38 (53.5)	52 (68.4)	0.064
M. abscessus	35 (49.2)	5 (6.6)	< 0.001
M. massiliense	40 (56.3)	6 (7.9)	< 0.001
M. kansasii	0	4 (5.3)	0.028
M. fortuitum	4 (5.6)	9 (11.8)	0.185
Others[b]	2 (2.8)	22 (28.9)	< 0.001
Treatment, ≥ 6 mon	32 (46.5)	24 (31.6)	0.043
Surgical resection	6 (8.5)	6 (7.9)	0.902
Follow-up duration, mon	63.0 (36.5–79.4)	40.0 (22.1–61.3)	0.002
All-cause mortality	4 (5.6)	8 (10.5)	0.279
Pulmonary cause	2 (2.8)	4 (5.3)	0.454

Lower BMI in MAC + MAB group

But, otherwise similar characteristics

J Korean Med Sci 2024;39(20):e167

Is lung involvement more extensive ?

- **Japan**, 120 MAC-PD patients, variable number of tandem repeats
- Polyclonal MAC: different VNTR genotypes / Mixed MAC: different MAC species

Variable	Patients with Monoclonal MAC Infections (*n* = 77)	Patients with Polyclonal and Mixed Mycobacterial MAC Infections (*n* = 42)	*P* Value
Radiographic pattern			
Nodular and bronchiectasis form	36 (46.8)	26 (61.9)	0.11
Cavitary form	16 (20.8)	5 (11.9)	0.32
Cavitary + nodular and bronchiectasis form	18 (23.4)	4 (9.5)	0.084
Unclassified form	7 (9.0)	7 (16.7)	0.22
HRCT findings			
Nodule	70 (90.9)	39 (92.9)	0.71
Bronchiectasis	69 (89.6)	35 (83.3)	0.32
Cavity	34 (44.2)	9 (21.4)	0.014
Consolidation	55 (71.4)	30 (71.4)	>0.99
Location			
Right upper lobe	48 (62.3)	26 (61.9)	0.96
Right middle lobe	68 (88.3)	36 (85.7)	0.68
Right lower lobe	51 (66.2)	25 (59.5)	0.54
Left upper lobe	35 (45.5)	19 (45.2)	0.98
Lingular	63 (81.8)	34 (81.0)	0.91
Left lower lobe	43 (55.8)	19 (45.2)	0.27
Thoracic abnormality			
Scoliosis	20 (25.6)	12 (28.6)	0.73
Pectus excavatum	9 (11.5)	6 (14.3)	0.66

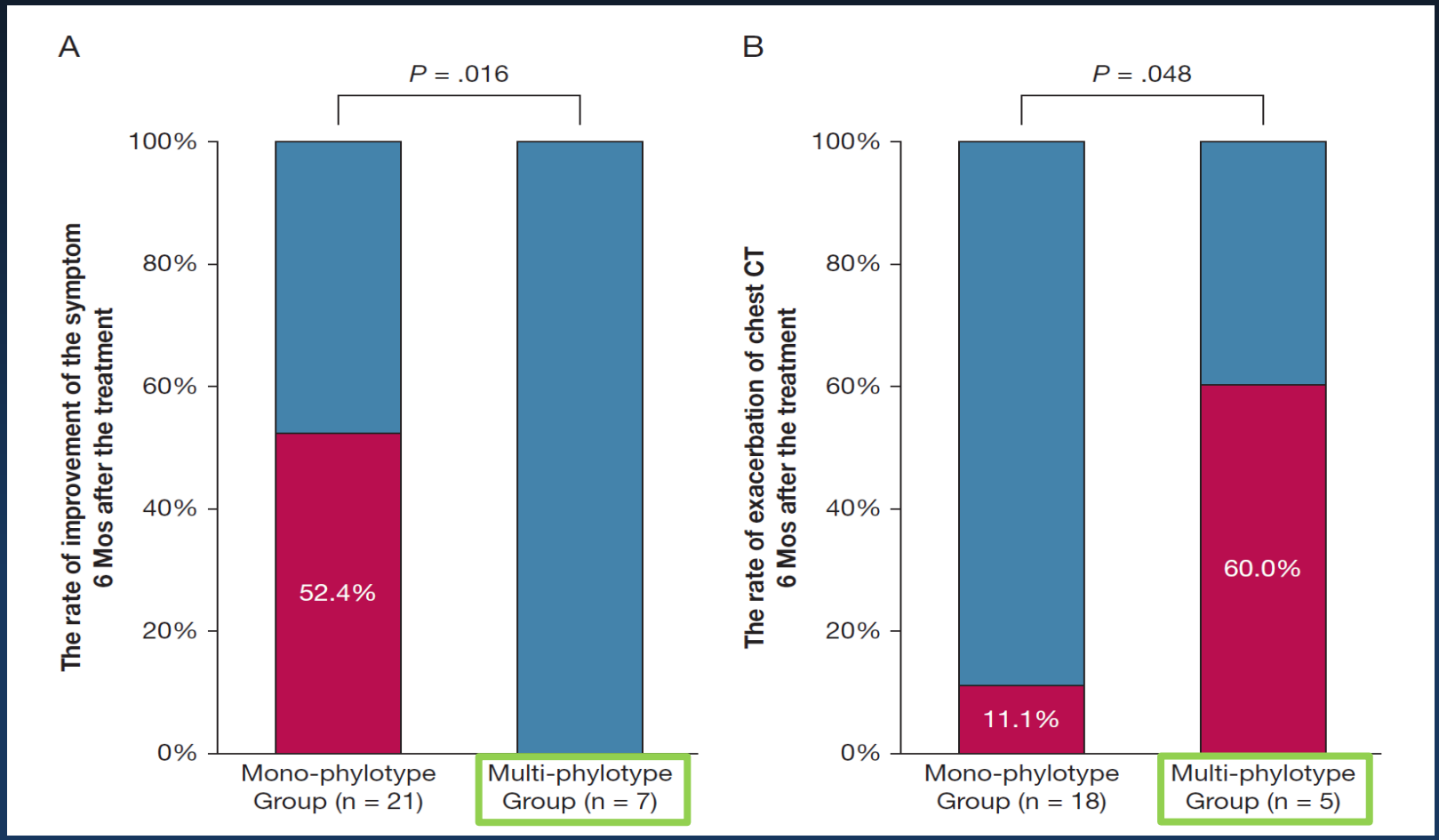
No significant difference

in the distribution of lung involvement or abnormalities

Ann Am Thorac Soc 2014;11(1):45-53

Less symptom improvement and more CT worsening?

- Japan 2010 and 2016, BALF, 16S rRNA gene clone library analysis
- Mono-phylotype group (n = 45) Multi-phylotype group (n = 7)



CHEST 2020; 158(6):2304-2313

Outcomes of Mixed NTM-PD

Outcomes of case series on mixed-NTM

Study	Patients no.	Underlying	Common pattern	Treatment	Outcome
Lim et al., 2002-2008 SNUH, South Korea	33 NTM-PD	Prior TB 51 %	Transition to another NTM 70% Alternating isolation 27% Simultaneous isolation 3%	NA	NA
Asaoka et al., 2006-2015 Japan	55 Non-MAC PD/infection	COPD	Multiple non-MAC NTMs, or MAC +M. abscessus	Antibiotics 41%	NA
Hill & Wooten, 2022 USA, UCSD	1 Disseminated (lung & skin)	HIV, CD4 <10 cells	M. avium-intracellulare + M. kansasii	AZT EMB Rfb	sputum MAC (+) at 4 months
Chen et al., 2023 CHINA	1 Dialysis-Peritonitis	ESRD	M. smegmatis + M. abscessus + M. goodii	Moxifloxacin Cefotiam Amikacin	Temporary Improvement Death
Opperman et al., 2025 South Africa	1 MAC-PD with bacteremia	HIV, CD4 3 cells	M. kansasii + M. chelonae + M. avium	AZT EMB Rfb isoniazid	Temporary improvement, Death

Int J Infect Dis 2011;15(11):e795-8 *Intern Med 2021;60(20):3213-3219*
Open Forum Infect Dis 2022;9(10):ofac519 *BMC Nephrol 2023;24(1):95* *IJID Reg 2024:14:100502*

Treatment outcome in mixed NTM-PD

- **Severance Hospital**, South Korea, 147 (14.6%) of 1,009 NTM-PD patients
- Outcomes of MAC + MAB coinfection treated for ≥6 months (N = 31)
- Sputum culture conversion: 67.7%, Microbiological cure: 41.9%
- Major adverse drug reactions: 45.2%

Characteristics	Total (N = 31)	Treated for MAC (n = 14)	Treated for MAB (n = 4)	Treated for MAC and MAB (n = 13)	*P* value
No. of drugs	3 (3–5)	3 (3–3)	3 (3–4)	5 (5–5)	< 0.001
Representative regimen[a]		Macrolide + RFP + EMB	Macrolide + IV AMK + IV IPM or CFX	Macrolide + RFP + EMB + IV AMK + IV CFX	
Dose interval		Daily	Daily	Daily	
Regimen change[b]	3 (9.7)	0 (0.0)	0 (0.0)	3 (23.1)	0.039
Treatment duration, mon	18.2 (13.7–25.1)	15.8 (13.7–24.4)	17.8 (13.9–23.1)	21.9 (12.8–29.5)	0.845
IV drug duration, wk	11.0 (4.6–18.4)	12.9 (5.1–20.7)	19.5 (10.6–20.3)	10.1 (4.1–12.9)	0.511
Sputum culture conversion	21 (67.7)	9 (64.3)	4 (100.0)	8 (61.5)	0.331
Microbiological cure	13 (41.9)	6 (42.9)	2 (50.0)	5 (38.5)	0.916
Recurrence	6 (46.2)	4 (66.7)	1 (50.0)	1 (20.0)	> 0.999
Radiologic change					0.855
Worsened	2 (6.5)	1 (7.1)	0 (0)	1 (7.7)	
No change	10 (32.3)	5 (35.7)	2 (50.0)	3 (23.1)	
Improved	19 (61.3)	8 (57.1)	2 (50.0)	9 (69.2)	
Mortality	1 (3.2)	1 (7.1)[c]	0 (0)	0 (0)	0.534
Major adverse drug reaction	14 (45.2)	7 (50.0)	1 (25.0)	6 (46.2)	0.784

J Korean Med Sci 2024;39(20):e167

Treatment outcome in mixed NTM-PD

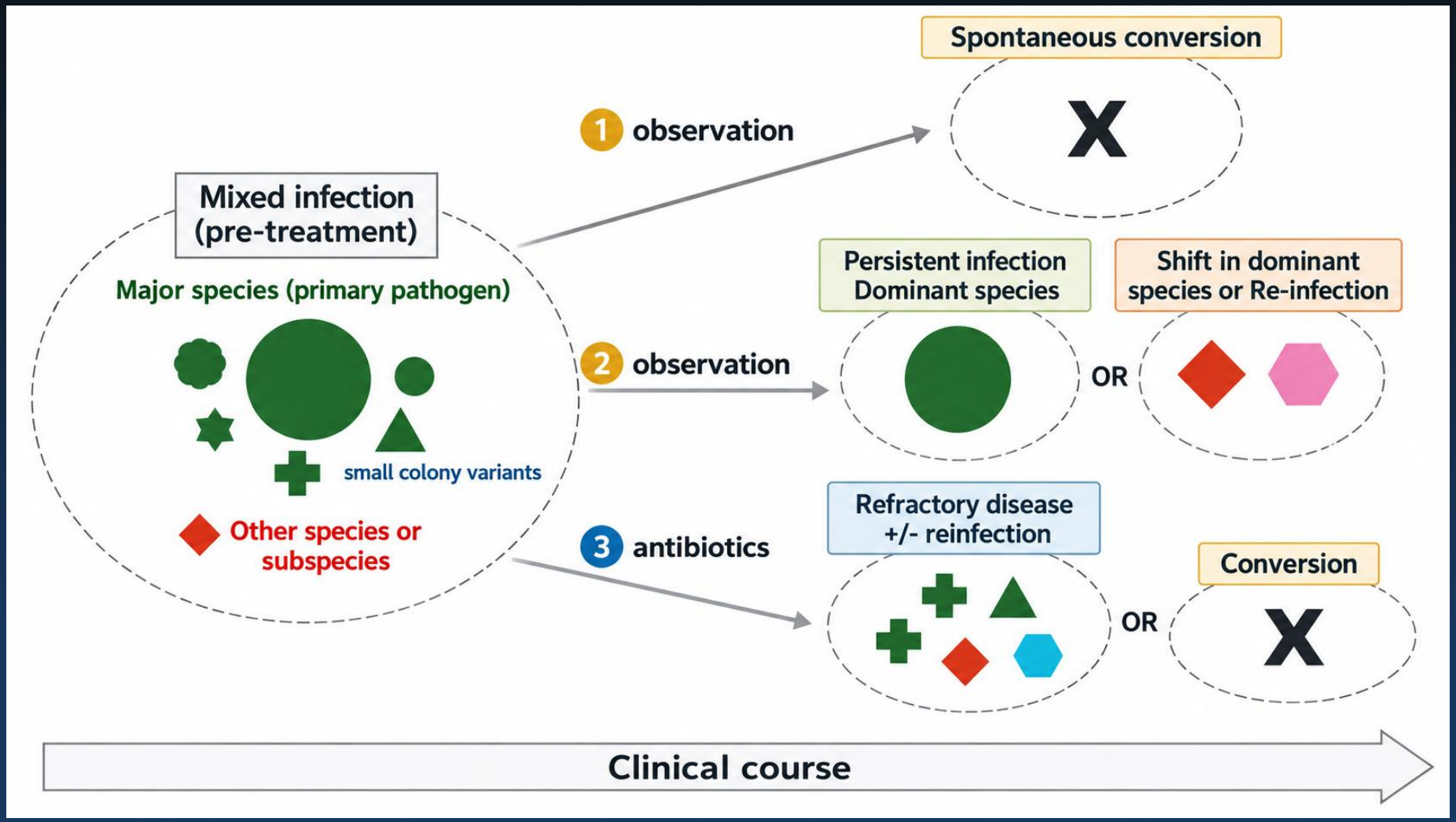
- **Samsung Medical Center**, South Korea: 71 MAC + MABC cases
- Outcomes at 12 months in patients treated >12 months (n = 43)
- Culture conversion of both pathogens: 51% (22/43)
- Recurrence after successful treatment: 48% (11/23)

Outcome at 12 months	Total (n = 43)	MAC + M. abscessus (n = 12)	MAC + M. massiliense (n = 31)	P - value
Both-conversion	22 (51%)	3 (25%)	19 (61%)	0.033
MAC culture conversion	31 (72%)	10 (83%)	21 (68%)	0.456
MABC culture conversion	30 (70%)	3 (25%)	27 (87%)	<0.001
Symptom improvement	39 (91%)	11 (92%)	28 (90%)	0.745†
CT improvement	39 (91%)	11 (92%)	28 (90%)	0.999†

Antimicrob Agents Chemother 2018;62(10):e01105-18

Recent Data

How should we interpret mixed-species NTM-PD?



How should we interpret mixed-species NTM-PD?

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1 Greater host susceptibility?

Could "more" impaired host immunity permit repeated co-infection by different NTM species?

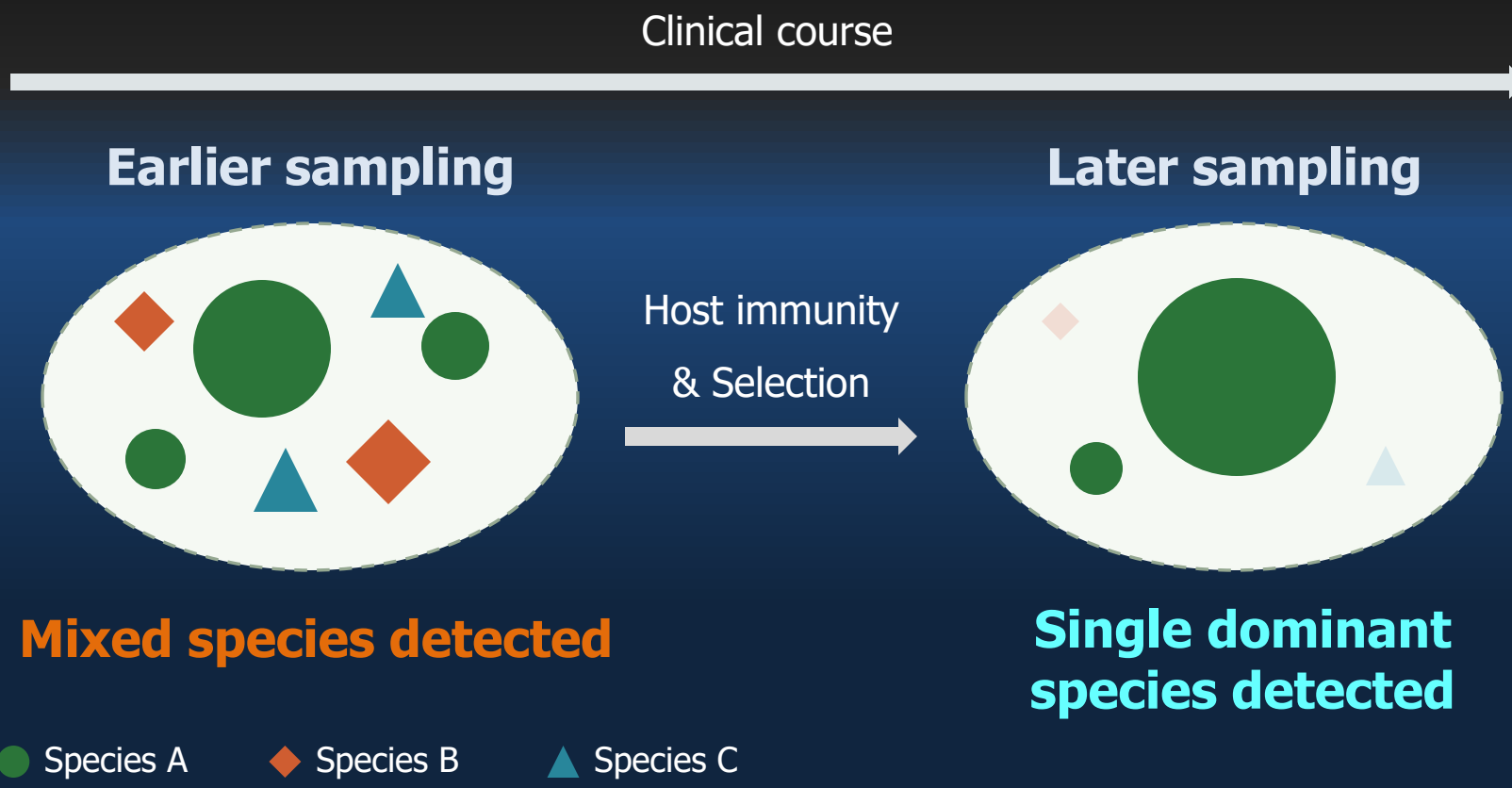
2 Selection from an initially mixed population?

Could most patients "initially" harbor multiple species or clones, with host immunity and the microbial environment favoring one population over time?

What do you think?

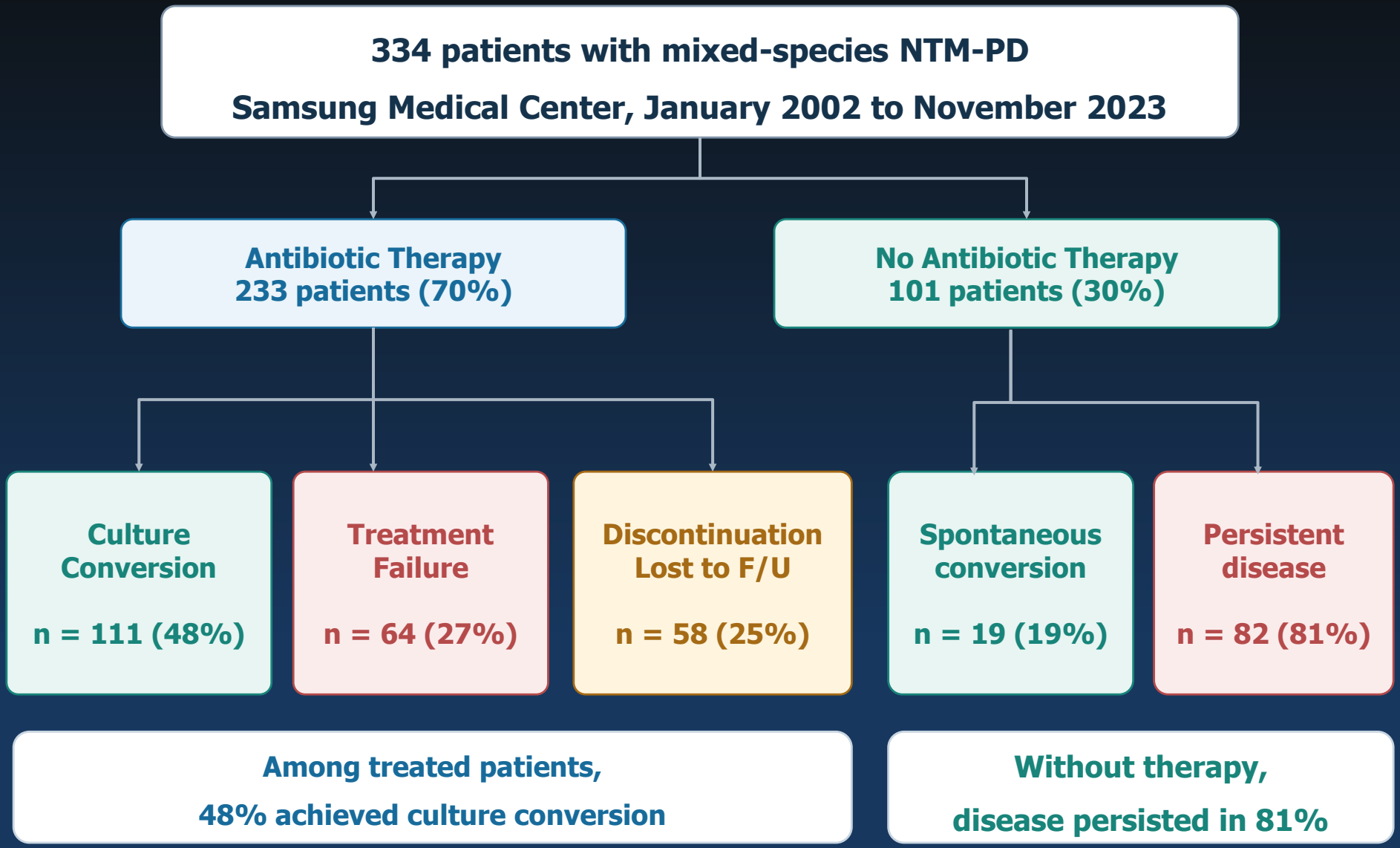
Could both mechanisms coexist?

Mixed or Single Species: Does the 'Timing' of Diagnosis Matter ?



The diagnostic label may depend on when we sample ?

Clinical Outcomes in Mixed-Species NTM-PD



Samsung Medical Center, unpublished data

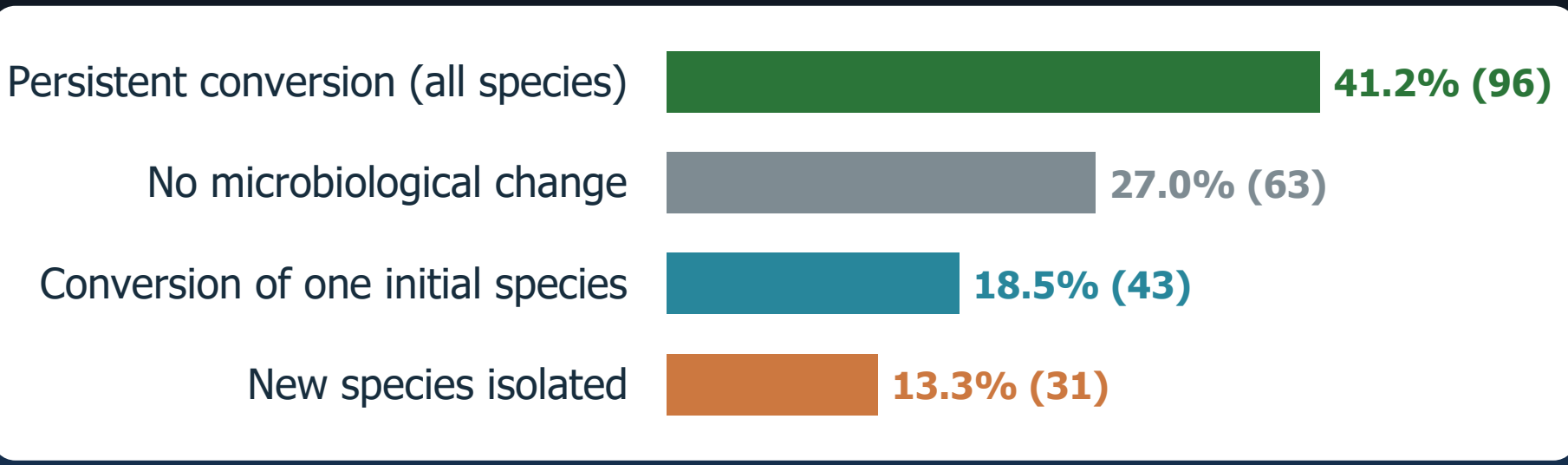
Course of untreated mixed NTM (n = 101)

No microbiological change: 45 patients (44.6%)

Microbiological course	n (%)	Species-level outcome	n
Persistent culture conversion (all species)	19 (18.8%)	All initial species remain culture-negative	19
Culture conversion of only one initial species	22 (21.8%)	Persistent *M. massiliense*	1
		Persistent MAC	16
		Persistent *M. abscessus*	5
New species isolated	15 (14.9%)	All initial species converted	-
		One initial species converted	6
		Initial species unchanged	9

Samsung Medical Center, unpublished data

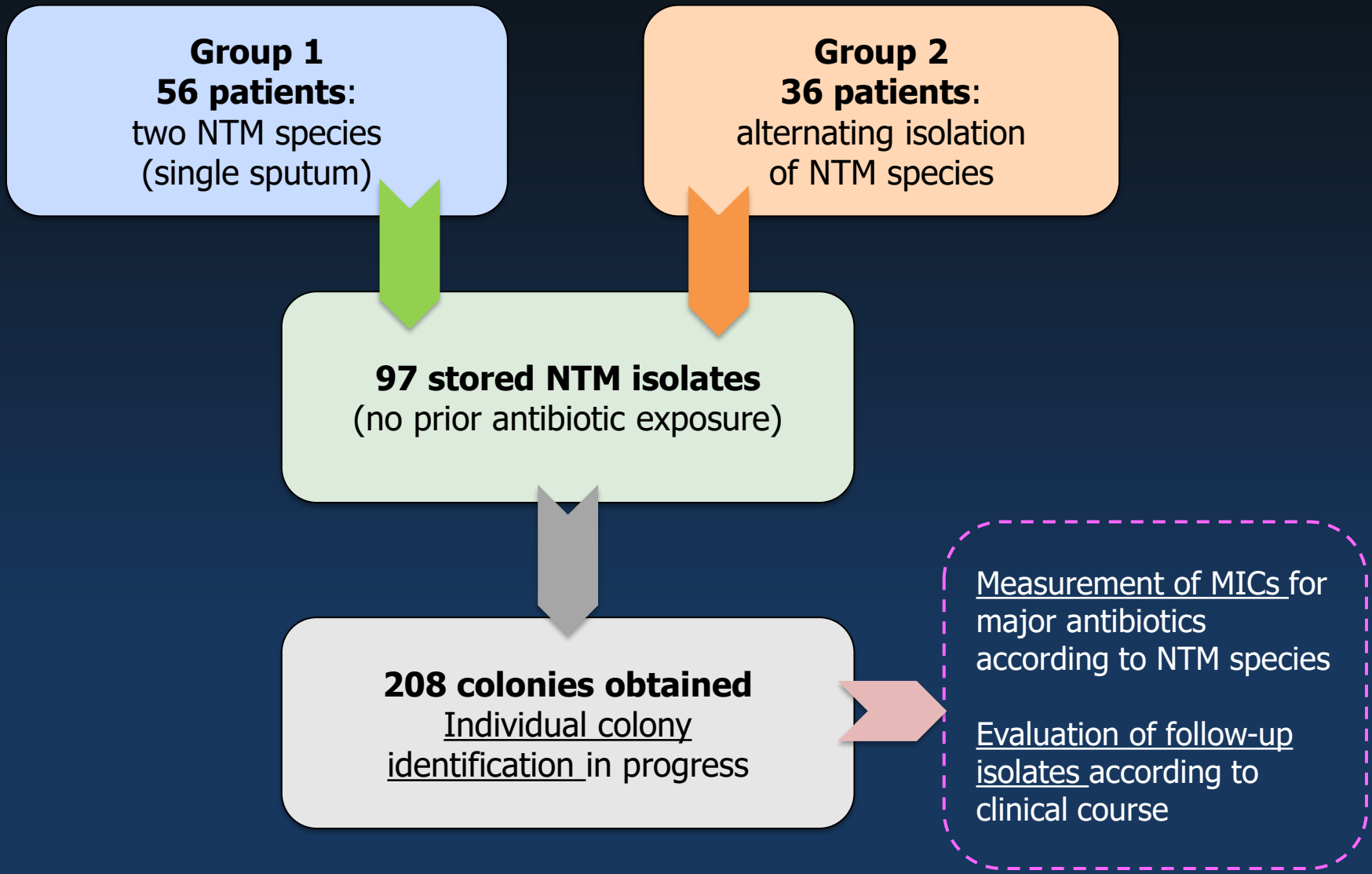
Treatment outcome, Mixed-Species NTM-PD (n = 233)



Persistent conversion (all species): among common combinations

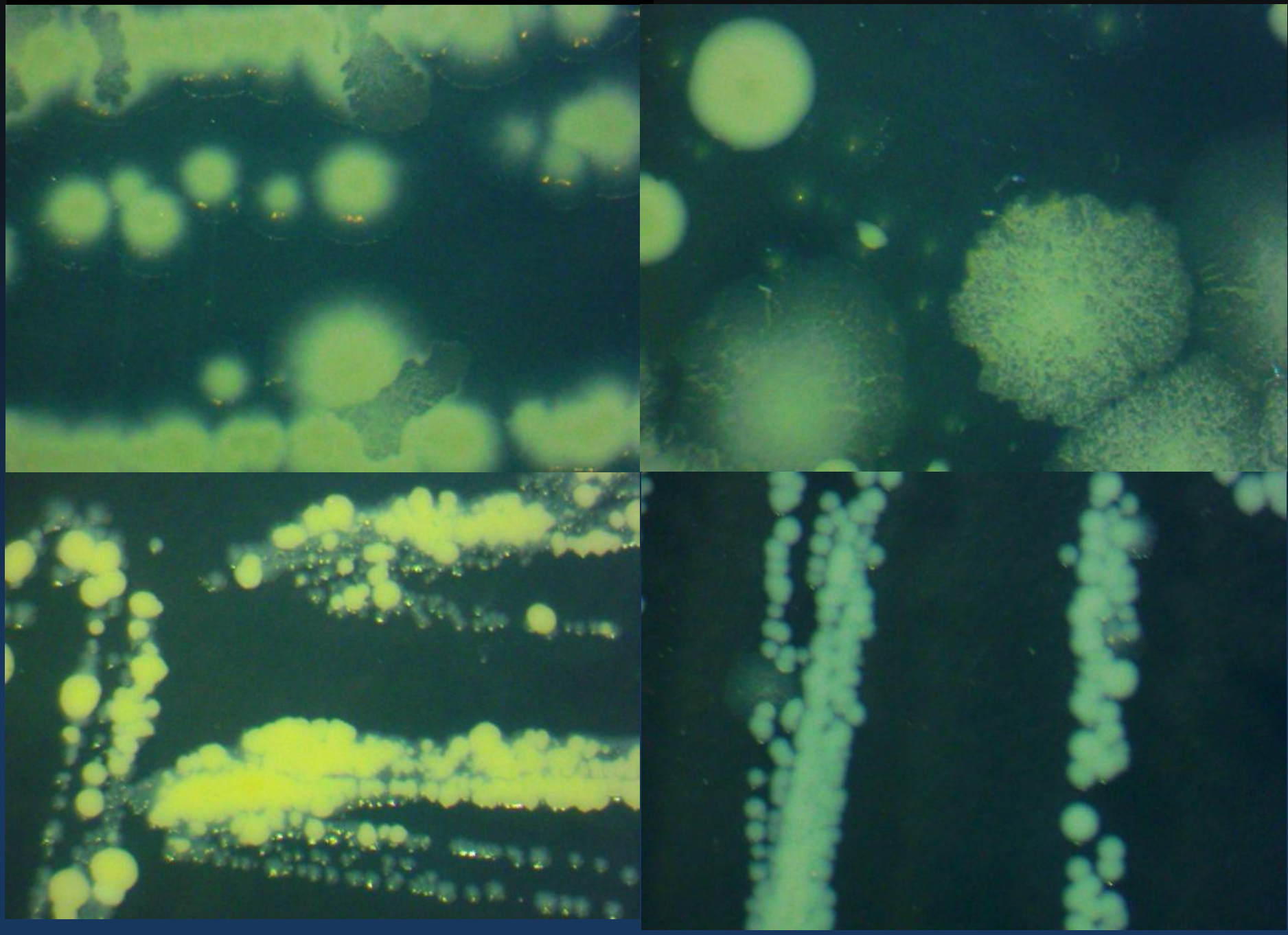
M. massiliense + MAC	M. avium + M. intracellulare	M. abscessus + MAC
52.3%	**35.9%**	**29.5%**
45/86 patients	23/64 patients	18/61 patients

Current progress, clinical NTM isolates

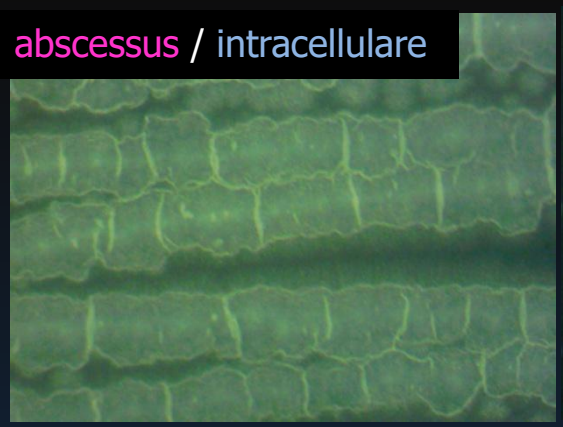


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Study patient no. 7, 10, 39, 51, with mixed infection



abscessus / intracellulare



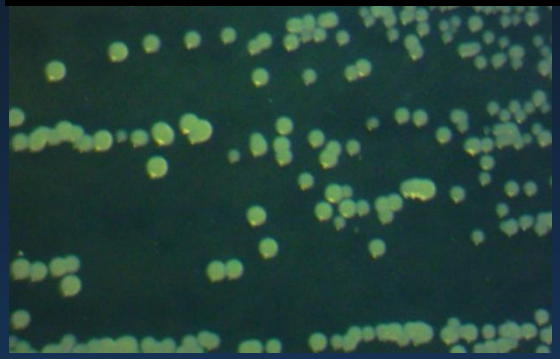


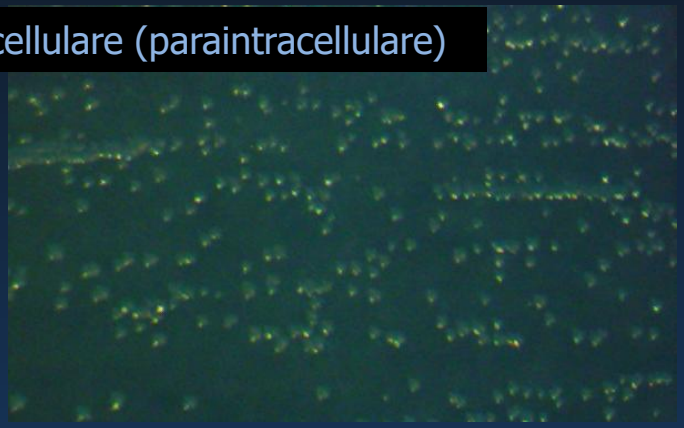
Single colonies isolation

NTN species identification

Samsung Medical Center, unpublished data

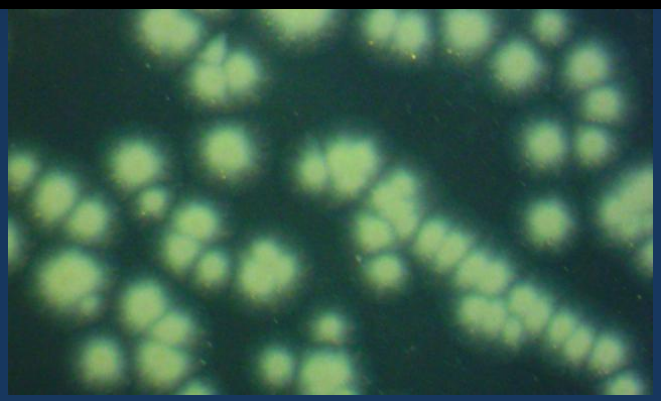
avium (ssp. hominissuis) / intracellulare (paraintracellulare)

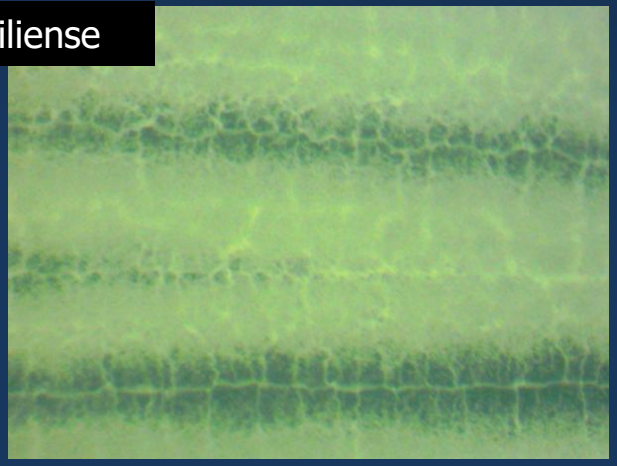




intracellulare (paraintracellulare) / intracellulare (ssp. yongonense) / massiliense

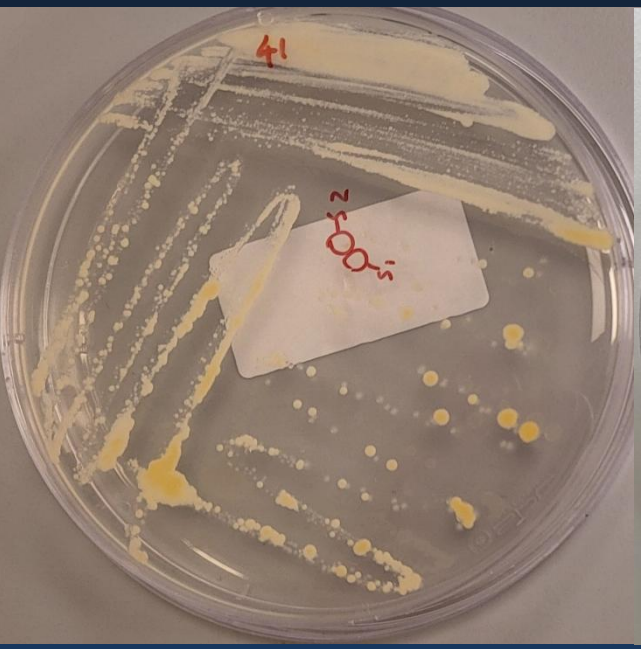
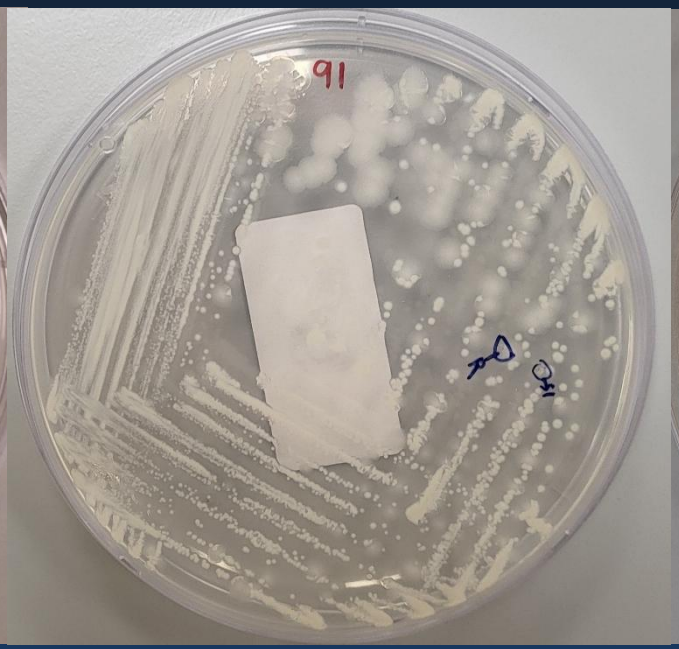






1. Most smooth-type SGM/RGM colonies grow to a limited size and maintain clear boundaries without merging.

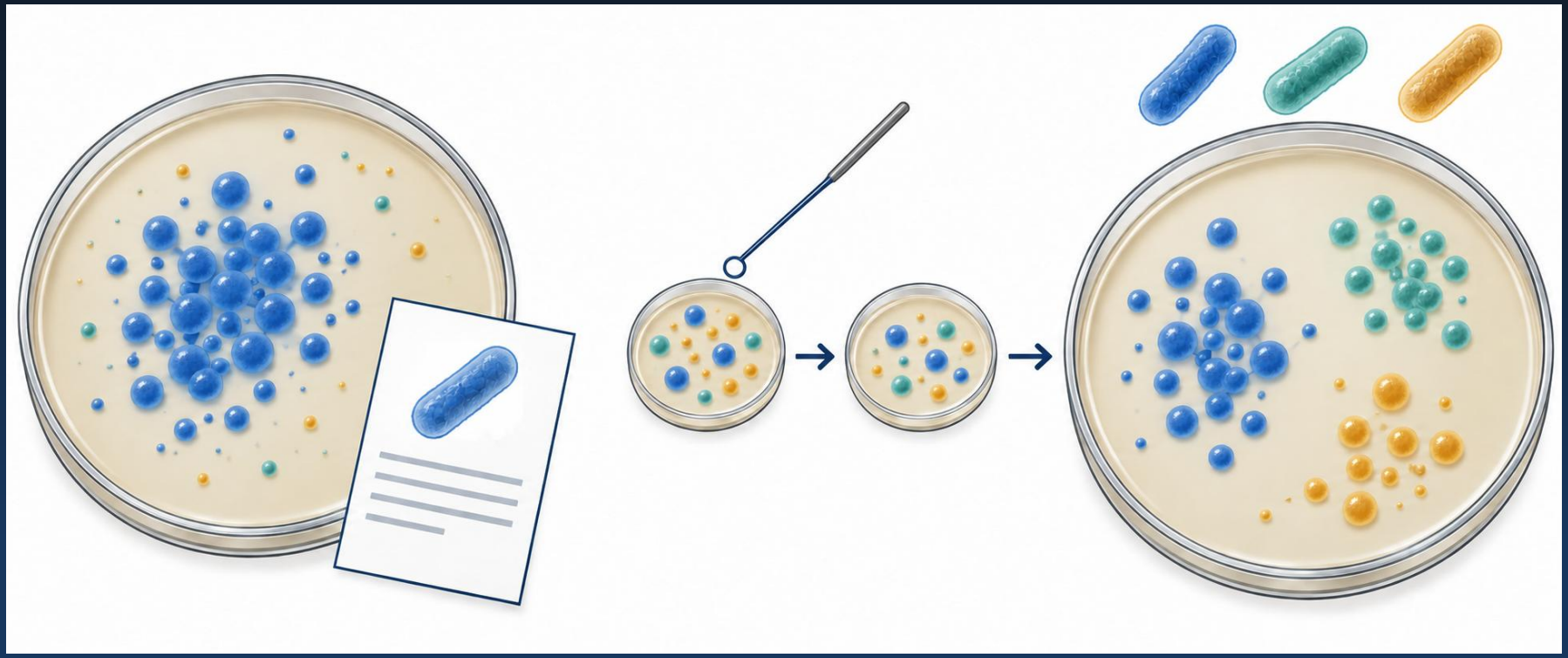
2. Rough-type RGM colonies spread aggressively without clear boundaries and can cover the agar surface (making it difficult to detect late-appearing SGM colonies).


Samsung Medical Center, unpublished data

Mixed NTM species revealed by subculture

Routine identification **Repeated subculture** **Multiple NTM species**



Single species reported
(routine lab. report)

Multiple species detected
within the **same original isolate**

Samsung Medical Center, unpublished data

Shotgun Metagenome Sequencing

- NTM-selective culture media-

Storage of NTM clinical isolate



■ **Sputum collection**

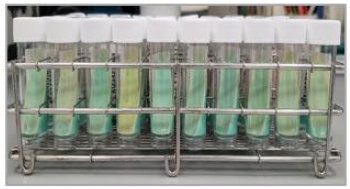




■ **BACTEC MGIT(5 ml) 960 system**

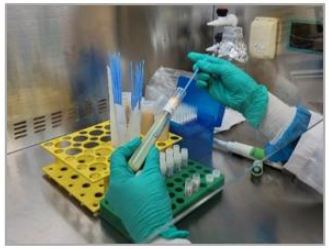
- 1ml : AFB stain & TB PCR
- 1ml : NTM ID
- 2ml : NTM DST
- 1ml : Sub-culture in Ogawa media

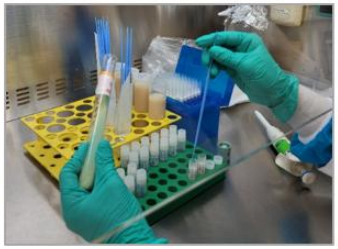


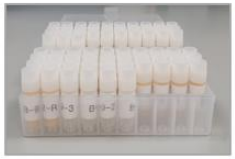




■ **Sub-culture in 3% Ogawa media**









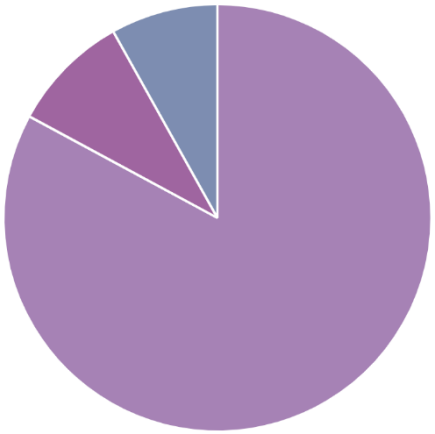
■ **Stock of NTM culture**

- Scrap NTM whole culture by loop
- Storage in cryotube at -80°C

Samsung Medical Center, unpublished data

Shotgun Metagenome Sequencing

- NTM-selective culture media-



Mycobacteroides abscessus subsp. abscessus

Mycobacteroides abscessus subsp. bolletii

Mycobacteroides abscessus subsp. massiliense

Species

Show 10 entries Search:

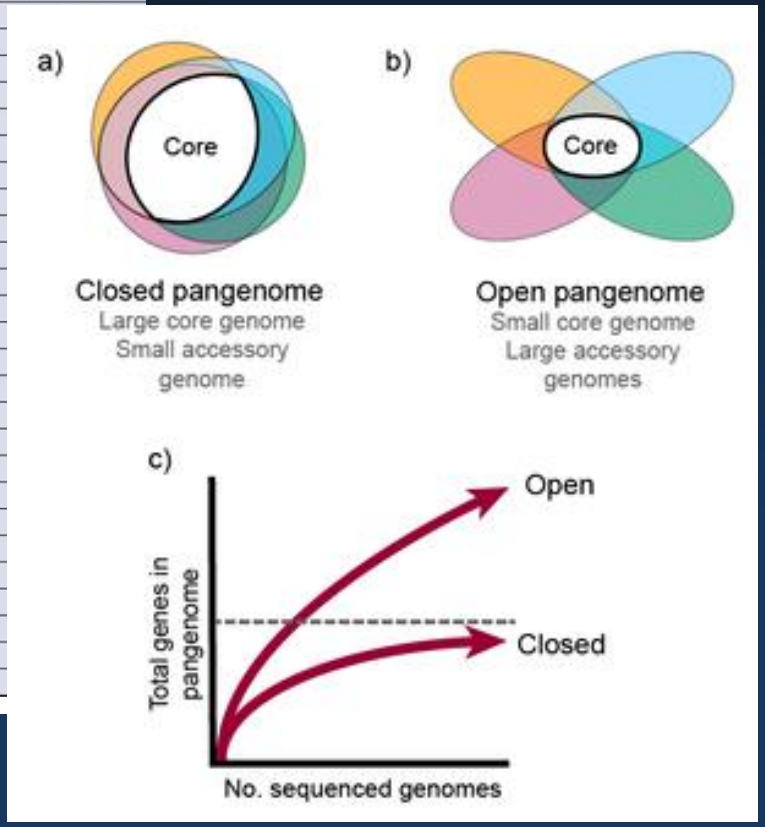
Taxon name	Count	Proportion(%)
Mycobacteroides abscessus subsp. abscessus	1,451,827	82.8816
Mycobacteroides abscessus subsp. bolletii	157,797	9.0151
Mycobacteroides abscessus subsp. massiliense	140,511	8.1034

Samsung Medical Center, unpublished data

Table 1. General information about bacterial core genes

A total of 148 genes collected from UBCG1, bac120, and bcgTree are shown. Their hidden Markov model (HMM) profiles, functions, presence, and si
3,508 complete genome sequences. There are two values for the ratios; when trusted cutoff is used and when 10E-5 (the expectation value; E-value) is u
in the core gene sets is marked by O and X.

	Gene	HMM*	Length	COG†	COG category§	Function	Presence ratio (%) Trusted cutoff is used (10E-5 E-value is used)	Single-copy ratio (%) Trusted cutoff is used (10E-5 E-value is used)
1	*rplE*	PF00281.18	57	COG0094	J	50S ribosomal protein L5	99.91 (99.91)	99.71 (99.09)
2	*rpsH*	PF00410.18	127	COG0096	J	30S ribosomal protein S8	99.86 (99.86)	99.66 (97.63)
3	*rplB*	TIGR01171	275	COG0090	J	50S ribosomal protein L2	99.77 (99.94)	99.63 (99.03)
4	*rpsB*	TIGR01011	225	COG0052	J	30S ribosomal protein S2	99.83 (99.97)	99.49 (96.44)
5	*rpsI*	PF00380.18	120	COG0103	J	30S ribosomal protein S9	99.8 (99.8)	99.46 (98.03)
6	*rplC*	TIGR03625	202	COG0087	J	50S ribosomal protein L3	99.46 (99.97)	99.34 (98.92)
7	*rplN*	TIGR01067	122	COG0093	J	50S ribosomal protein L14	99.46 (99.63)	99.32 (98.77)
8	*rpsL*	TIGR00981	124	COG0048	J	30S ribosomal protein S12	99.4 (99.43)	99.17 (98.8)
9	*rplW*	PF00276.19	86	COG0089	J	50S ribosomal protein L23	99.32 (99.37)	99.14 (98.35)
10	*rpsP*	TIGR00002	78	COG0228	J	30S ribosomal protein S16	99.32 (99.63)	99.14 (99)
11	*rplJ*	PF00466.19	100	COG0244	J	50S ribosomal protein L10	99.26 (99.26)	99.09 (97.55)
12	*rpsJ*	TIGR01049	99	COG0051	J	30S ribosomal protein S10	99.29 (99.86)	99.09 (99.54)
13	*rpsC*	TIGR01009	212	COG0092	J	30S ribosomal protein S3	99.09 (99.91)	
14	*rpsO*	TIGR00952	86	COG0184	J	30S ribosomal protein S15	99.2 (99.69)	
15	*rplM*	TIGR01066	141	COG0102	J	50S ribosomal protein L13	99.06 (99.69)	
16	*rplS*	TIGR01024	114	COG0335	J	50S ribosomal protein L19	98.95 (99.57)	
17	*rpsE*	TIGR01021	156	COG0098	J	30S ribosomal protein S5	98.86 (99.83)	
18	*tsf*	TIGR00116	293	COG0264	J	Elongation factor Ts	98.83 (99.52)	
19	*rpmA*	TIGR00062	83	COG0211	J	50S ribosomal protein L27	98.83 (99.49)	
20	*cgtA*	TIGR02729	329	COG0536	DL	GTPase ObgE/CgtA	98.77 (99.57)	
21	*infB*	TIGR00487	587	COG0532	J	Translation initiation factor IF-2	98.72 (99.89)	
22	*rplL*	TIGR00855	125	COG0222	J	50S ribosomal protein L7/L12	98.83 (99.34)	
23	*rplQ*	TIGR00059	112	COG0203	J	50S ribosomal protein L17	98.6 (99.57)	
24	*rplF*	TIGR03654	175	COG0097	J	50S ribosomal protein L6	98.6 (99.77)	
25	*rplO*	TIGR01071	144	COG0200	J	50S ribosomal protein L15	98.55 (99.6)	
26	*rpoA*	TIGR02027	298	COG0202	K	DNA-directed RNA polymerase subunit alpha	99.06 (100)	
27	*rplV*	TIGR01044	103	COG0091	J	50S ribosomal protein L22	98.55 (99.26)	
28	*rplU*	TIGR00061	101	COG0261	J	50S ribosomal protein L21	98.52 (98.8)	
29	*rpsM*	TIGR03631	113	COG0099	J	30S ribosomal protein S13	98.49 (99.52)	
30	*rpmC*	TIGR00012	56	COG0255	J	50S ribosomal protein L29	98.46 (98.69)	
31	*rplI*	TIGR00158	148	COG0359	J	50S ribosomal protein L9	98.43 (98.86)	
32	*rplX*	TIGR01079	104	COG0198	J	50S ribosomal protein L24	98.43 (99)	
33	*ksgA*	TIGR00755	256	COG0030	J	Ribosomal RNA small subunit methyltransferase A	98.35 (99.37)	
34	*rpsQ*	TIGR03635	72	COG0186	J	30S ribosomal protein S17	98.29 (99.69)	
35	*rpsG*	TIGR01029	154	COG0049	J	30S ribosomal protein S7	98.26 (99.91)	
36	*rpsS*	TIGR01050	92	COG0185	J	30S ribosomal protein S19	98.15 (99.63)	
37	*rplK*	TIGR01632	140	COG0080	J	50S ribosomal protein L11	98.29 (99.71)	
38	*rpoB*	TIGR02013	1238	COG0085	K	DNA-directed RNA polymerase subunit beta	98.35 (99.69)	



Questions in Clinical Practice

- Is mixed NTM infection real?
- Is mixed NTM infection more severe?
- Is mixed NTM infection associated with poorer outcomes?
- Can we safely adopt a watchful-waiting strategy?
- Which species should we treat—both or just one?

Thank you