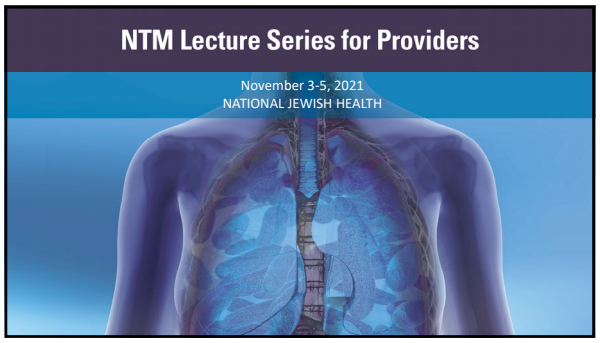

NTM Lecture Series for Providers

Genomic Approaches to Investigate
Nontuberculous Mycobacteria (NTM)

Presented by:
Michael Strong, Ph.D.
Associate Professor
Center for Genes, Environment, and Health
National Jewish Health

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Michael Strong, Ph.D. declares that he has no conflicts of interest.

Current Funding:
Cystic Fibrosis Foundation
National Science Foundation
State of Colorado, Bioscience Initiative

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Some Methods to Characterize Mycobacteria

- Culture on Selective Media
- Single Loci Gene Sequencing
- Pulsed-Field Gel Electrophoresis (PFGE)
- MALDI-TOF MS
- PCR and Hybridization Assays
- Whole Genome Sequencing

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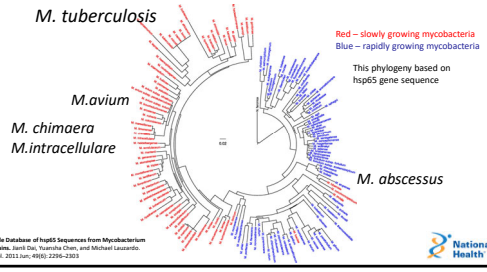
Phylogenetic Diversity of Nontuberculous Mycobacteria



Web-Accessible Database of hsp65 Sequences from Mycobacterium Reference Strains. Jianli Dai, Yuansha Chen, and Michael Lauzardo. J Clin Microbiol. 2011 Jun; 49(6): 2296–2303

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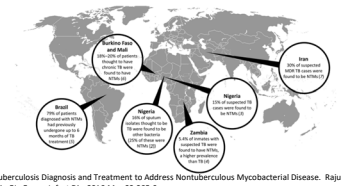
Suspected TB cases that are actually NTM



Leveraging Advances in Tuberculosis Diagnosis and Treatment to Address Nontuberculous Mycobacterial Disease. Raju RM, Raju SM, Zhao Y, Rubin EJ. Emerg Infect Dis. 2016 Mar;22:365-9

"NTM-associated disease is much more common than previously thought: more common than TB in the industrialized world and likely increasing in prevalence globally" *Emerg Infect Dis*. 2016 Mar;22:365-9

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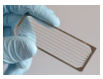
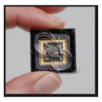
Next Generation Sequencing Platforms

Next Generation sequencing enables the rapid and robust sequencing of whole bacterial genomes.

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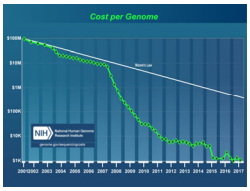
We can take advantage of the dramatic decrease in next-generation sequencing costs



Cost to Sequence Human Genome

http://www.genome.gov/sequencingcosts/

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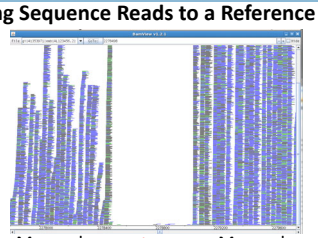
Mapping Sequence Reads to a Reference Genome



Mapped Reads | Deletion | Mapped Reads

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As we approach "affordable" bacterial genome sequencing, the challenge remains with streamlining high throughput workflows, analysis strategies, data archiving, and genomic data sharing.

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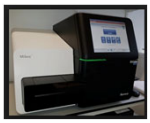
Sequence Analysis Pipeline

Bacterial Preparation
↓
Genomic DNA isolation
↓
Genomic Library Construction
↓
Next Generation Sequencing and Genomic Analysis
↓
Phylogenomic Comparisons
SNP and INDEL analysis
Plasmid Analysis
Phenotypic Implications



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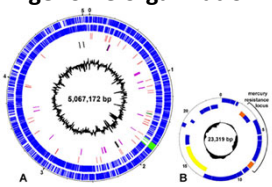
M. abscessus subspecies *abscessus* genome organization

Genome
4920 proteins



Plasmid
pMAB
22 proteins

Non mycobacterial virulence genes in the genome of the emerging pathogen Mycobacterium abscessus."
Ripoll F., Pasek S., Schenowitz C., Dossat C., Barbe V., Rottman M., Macheras E., Heym B., Herrmann J.L., Daffe M., Brosch R., Risler J.L., Gaillard J.L.
PLoS ONE 4:E5660-E5660(2009)

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Comparison of Mycobacterial Genomes

Table 1. General features of the *M. abscessus* genome and comparison with other *Mycobacterium* species.

Features	RGM				SGM			
	Mabs	**Msmeg**[a]	*M. gilvum*[a]	**Mvanba**[b]	**Mtb**[c]	*M. avium*[a]	*M. marinum*[d]	*M. ulcerans*[e]
Genome size, bp	5,067,172	6,988,209	5,619,607	6,491,865	4,411,532	5,475,491	6,636,827	5,631,606
G+C content, %	64.1	67	67	67	65.6	68	65	65
Protein coding, %	93	90	92	91	90.8	88	89	72
Proteins	4920	6716	5241	5979	3959	5120	5424	4160
tRNAs	47	47	47	50	45	45	46	45
rRNA operons	1	2	2	2	1	1	1	1
Prophage elements, no.	4[d]	NR	NR	NR	2	NR	10	2
IS, total no. of copies	5	112	NR	NR	54	NR	7	302

Non mycobacterial virulence genes in the genome of the emerging pathogen Mycobacterium abscessus."
Ripoll F., Pasek S., Schenowitz C., Dossat C., Barbe V., Rottman M., Macheras E., Heym B., Herrmann J.L., Daffe M., Brosch R., Risler J.L., Gaillard J.L.
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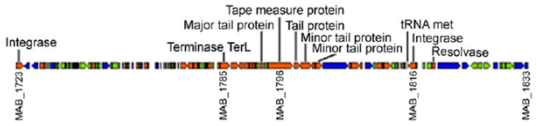
Integrated Prophage Elements in *M. abscessus*



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PLoS ONE 4:E5660-E5660(2009)

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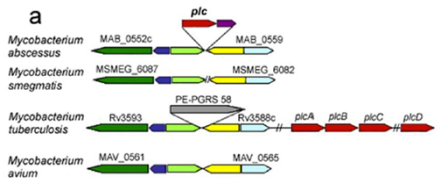
Genome organization in mycobacteria



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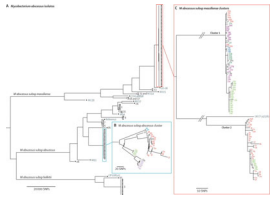
Potential NTM Transmission in CF clinics

Suggestive genomic evidence of person to person transmission of NTM



Whole-genome sequencing to identify transmission of Mycobacterium abscessus between patients with cystic fibrosis: a retrospective cohort study. Bryant JM, Grogono DM, Greaves D, Foweraker J, Roddick I, Inns T, Reacher M, Haworth CS, Curran MD, Harris SR, Peacock SJ, Parkhill J, Floto RA. Lancet. 2013 May 4;381(9877):1551-60. doi

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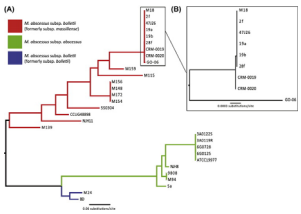
NTM Phylogenomics – based on whole genome sequence comparisons



Phylogenomics of Brazilian epidemic isolates of Mycobacterium abscessus subsp. bolletii reveals relationships of global outbreak strains. Davidson RM, Hasan NA, de Moura VC, Duarte RS, Jackson M, Strong M. Infection Genetics and Evolution Sep 18;20C:292-297. 2013

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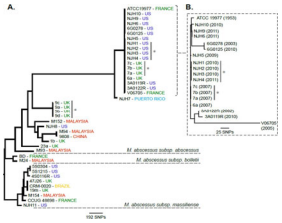

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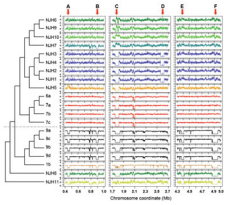
Genomic Comparisons



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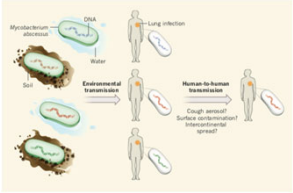
Where do NTM infections originate?
NTM Sequencing For Surveillance



Microbiology: Bacterial transmission tactics. Michael Strong and Rebecca M. Davidson. *Nature*, 543: 495-496 (2017).

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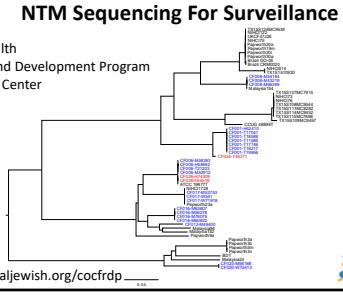

https://www.nationaljewish.org/cocfrdp

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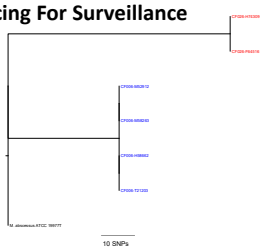

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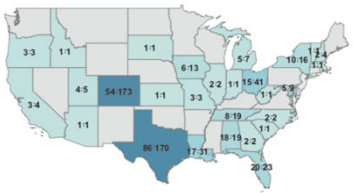
NTM Sequencing For Surveillance – *M. abscessus*



Population Genomics of Mycobacterium abscessus from United States Cystic Fibrosis Care Centers. Davidson RM, Hasan NA, Epperson LE, Benoit JB, Kammlade SM, Levin AR, Calado de Moura V, Hunkins J, Weakly N, Beagle S, Sagel SD, Martiniano SL, Salfinger M, Daley CL, Nick JA, Strong M. *Ann Am Thorac Soc.* 2021 .

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NTM Sequencing For Surveillance – *M. abscessus*

***M. abscessus* subspecies**

Category (n)	subsp. *abscessus* (MAB)	subsp. *massiliense* (MMAS)	subsp. *bolletii* (MBOL)	Total
Isolates	440	107	11	558
Patients with 1 isolate	121	40	4	158
Patients with ≥ 2 isolates	83	24	3	108
Total Patients*	204	64	7	266

*Total does not sum to 266 due to patients who cultured multiple subspecies

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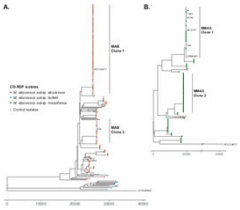

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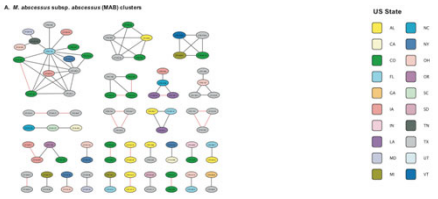
NTM Sequencing For Surveillance – *M. abscessus*



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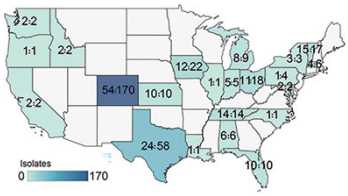
NTM Sequencing For Surveillance – M. avium Complex



Population Genomics and Inference of Mycobacterium avium Complex Clusters in Cystic Fibrosis Care Centers, United States Nabeeh A. Hasan, Rebecca M. Davidson, L. Elaine Epperson, Sara M. Kammlade, Sean Beagle, Adrah R. Levin, Vinicius Calado de Moura, Joshua J. Hunkins, Natalia Weakly, Scott D. Sagel, Stacey L. Martiniano, Max Salfinger, Charles L. Daley, Jerry A. Nick, and Michael Strong. *Emerging Infectious Diseases* 27:11 (2021).

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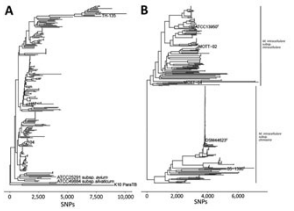
NTM Sequencing For Surveillance – M. avium Complex



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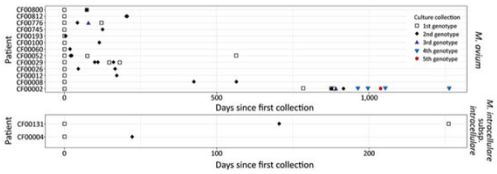

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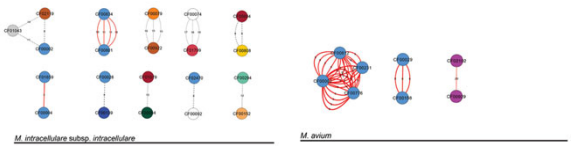

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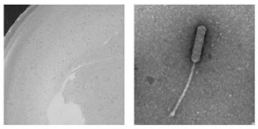
What can we learn about Mycobacteriophage using NTM genomics?

***Mycobacterium* phage Corndog**



Cluster O : Lytic Phage

Graham Hatfull, PhD and colleagues – The Actinobacteriophage Database

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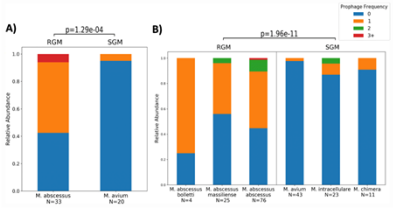
Genomic Analyses Reveals Prophage in NTM



Characterization of integrated prophages within diverse species of clinical nontuberculous mycobacteria. Cody Glickman, Sara M. Kammlade, Nabeeh A. Hasan, L. Elaine Epperson, Rebecca M. Davidson, Michael Strong. *Virology Journal* 17:124 (2020).

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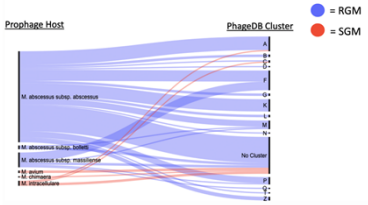
Genomic Analyses Reveals Prophage in NTM



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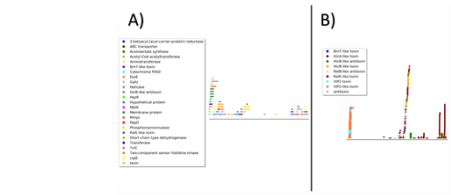
Genomic Analyses Reveals Prophage Mediated Virulence Proteins



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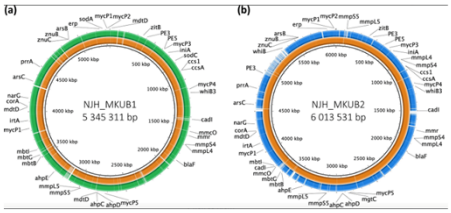
Genome Analysis Enables Genome Wide Comparisons



Intraspecies plasmid and genomic variation of Mycobacterium kubicae revealed by the complete genome sequences of two clinical isolates. Jo Hendrix, L. Elaine Epperson, David Durbin, Jennifer R. Honda, Michael Strong. *Microbial Genomics* (2020).

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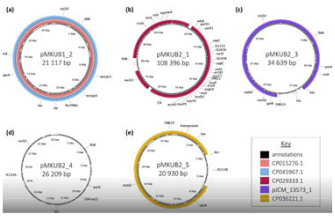
Genome Analysis Reveals Plasmid Diversity in NTM



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Genomic Encoded Features Pertinent to Drug Resistance

Table 1. Details of theoretical hybridization profiles of the DNA strip GenoType NTM-DR

Gene	Sequence	Hybridization profile	Associated phenotypic susceptibility pattern
erm(41)	presence of erm(41)	SP9 + SP10	intrinsic resistance to clarithromycin and azithromycin
erm(41)	large deletion	SP9 and no SP10	intrinsic susceptibility to clarithromycin and azithromycin
erm(41)	c28	erm(41) + erm(41) c28	intrinsic susceptibility to clarithromycin and azithromycin
erm(41)	t28	erm(41) + erm(41) t28	intrinsic resistance to clarithromycin and azithromycin except if SP9 present and no SP10 (indicates the erm(41) deletion observed in *M. abscessus* subsp. *massiliense*)
rrl	WT	rrl WT	intrinsic susceptibility to clarithromycin and azithromycin for *M. avium* complex and *M. chelonae*; for *M. abscessus*, the susceptibility depends on the erm(41) bands
rrl	a2058c	rrl MUT1	acquired resistance to clarithromycin and azithromycin
rrl	a2058g	rrl MUT2	acquired resistance to clarithromycin and azithromycin
rrl	a2058t	no rrl WT only	acquired resistance to clarithromycin and azithromycin
rrl	a2059c	rrl MUT3	acquired resistance to clarithromycin and azithromycin
rrl	a2059g	rrl MUT4	acquired resistance to clarithromycin and azithromycin
rrl	a2059t	no rrl WT only	acquired resistance to clarithromycin and azithromycin
rrs	WT	WT	intrinsic susceptibility to amikacin or tobramycin depending on species identification
rrs	g1408g	rrs MUT1	acquired resistance to amikacin and tobramycin[a]
rrs	t1406c	no rrs WT only	acquired resistance to amikacin and tobramycin[a]
rrs	c1409t	no rrs WT only	acquired resistance to amikacin and tobramycin[a]

[a]Amikacin for *M. avium* complex and *M. abscessus*, tobramycin for *M. chelonae*.

Evaluation of the new GenoType NTM-DR kit for the molecular detection of antimicrobial resistance in non-tuberculous mycobacteria Faiza Mougari, Jade Loiseau, Nicolas Veziris, Christine Bernard, Béatrice Bercot, Wladimir Sougakoff, Vincent Jarlier, Laurent Raskine, Emmanuelle Cambau, on behalf of the French National Reference Center for Mycobacteria Author Notes *Journal of Antimicrobial Chemotherapy*, Volume 72, Issue 6, June 2017, Pages 1669–1677

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Phenotypic Characterization of NTM strains

TABLE 3 Drug susceptibility of NIH *M. abscessus* clinical isolates to a 20-drug panel, reported as MICs

Drug	MIC (μg/ml)										No. of resistant isolates/total no. of isolates[a]
	NIH1	NIH4	NIH5	NIH6	NIH7	NIH8	NIH9	NIH10	NIH11		
Amikacin	16	≤8	≤8	≤8	>64	32	16	≤8	32		1/9
Kanamycin	16	≤8	≤8	≤8	>64	32	16	≤8	16		1/9
Tobramycin	16	16	8	8	>16	16	8	16	>16		6/9
Gentamicin	>16	8	4	8	>16	>16	16	8	>16		5/9
Imipenem	16	>16	>16	8	8	8	16	>16	>16		6/9
Cefoxitin	64	64	64	32	64	64	32	64	64		9/9
Ceftriaxone	>64	>64	>64	>64	>64	>64	>64	>64	>64		9/9
Cefotaxime	>64	>64	>64	>64	>64	>64	>64	>64	>64		9/9
Cefepime	32	>32	>32	>32	>32	32	>32	>32	>32		9/9
Clarithromycin	0.5	0.5	1	2	1	1	1	>32	0.5		1/9
Azithromycin	≤16	≤16	32	32	32	32	≤16	>256	≤16		1/9
Amoxicillin/clavulanic acid	>32/16	>32/16	>32/16	>32/16	>32/16	>32/16	>32/16	>32/16	>32/16		9/9
Ciprofloxacin	8	>8	8	4	>8	>8	8	>8	8		9/9
Moxifloxacin	>4	>4	>4	>4	>4	>4	>4	>4	>4		9/9
Trimethoprim/sulfa	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76		9/9
Doxycycline	16	>16	>16	>16	>16	>16	>16	>16	>16		9/9
Minocycline	8	>8	>8	>8	>8	>8	>8	>8	>8		9/9
Tigecycline	1	0.5	≤0.25	2	1	1	0.5	2	1		0/9
Linezolid	>16	16	>16	16	>16	>16	16	>16	>16		9/9

[a] Determination of isolates with resistant phenotypes was based on previously defined MIC thresholds specific to each drug.

Genome sequencing of *Mycobacterium abscessus* isolates from patients in the United States and comparisons to globally diverse clinical strains. Rebecca M. Davidson, Nabeeh A. Hasan, Paul R. Reynolds, Sarah Totten, Benjamin Garcia, Adrah Levin, Preveen Ramamoorthy, Leonid Heifets, Charles L. Daley, and Michael Strong. *Journal of Clinical Microbiology*, 52:3573-3582 (2014).

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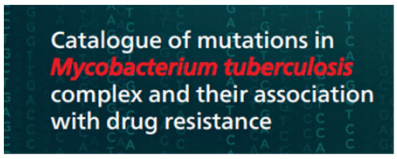
In *M. tuberculosis* – very strong genotype to phenotype correlations in Drug Resistance



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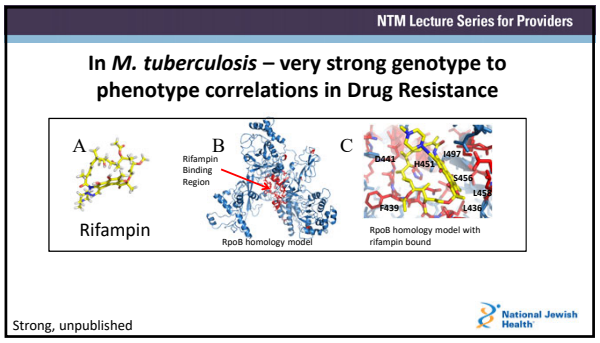

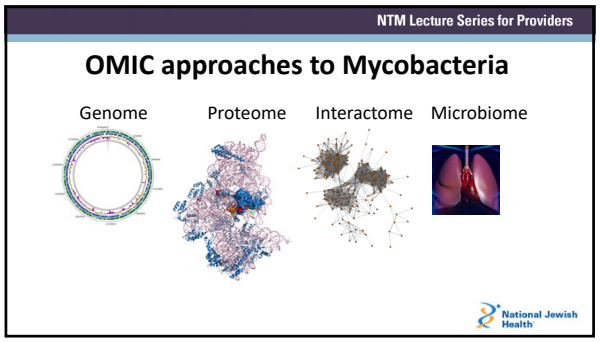

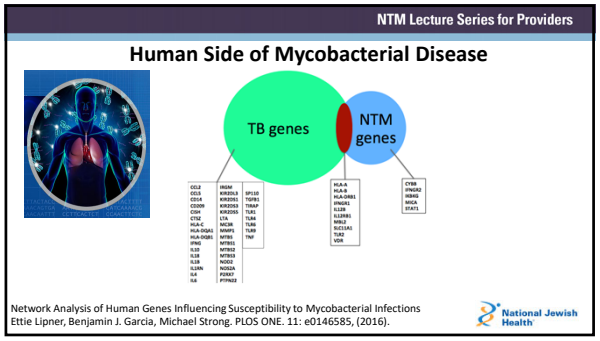

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Summary

We now sequence 100s of mycobacterial genomes per year.

We have expanded our understanding of the diversity of clinically important NTM species through genomic comparisons.

Phylogenomic characterization facilitates outbreak analysis, putative transmission analysis, and diversity investigations.

We welcome new projects pertinent to public health, patient health, and scientific advancement.

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Thanks to:
Nabeeh Hasan PhD
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Rebecca Davidson PhD
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Charles L. Daley MD
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