



**Microbiome Approaches for
TB/NTM Diagnostics**

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Advances in the Science and Practice in Tuberculosis Control
Post-Graduate Course
24 February 2016

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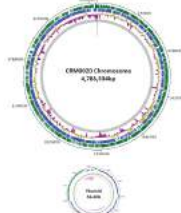
**Nontuberculous
Mycobacteria (NTM)
Research at NJH**

Division of Mycobacterial and Respiratory Infections
Adult CF Clinic
Mycobacteriology Lab
Advanced Diagnostics
Center for Genes, Environment & Health

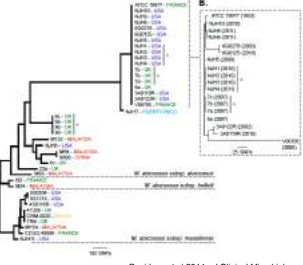
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Mycobacterium abscessus Genomics

Whole genome sequencing
M. abscessus subsp. *massiliense*
epidemic isolate from Rio de Janeiro, Brazil

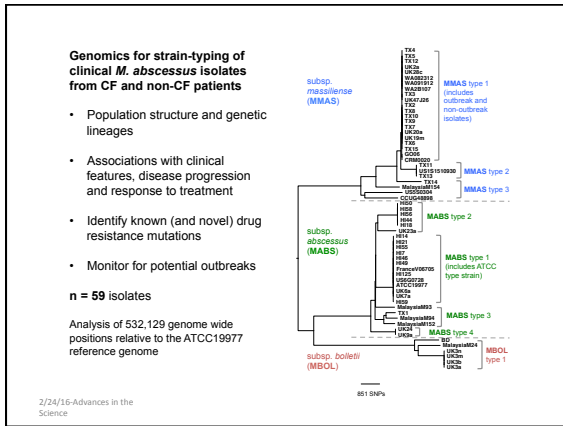


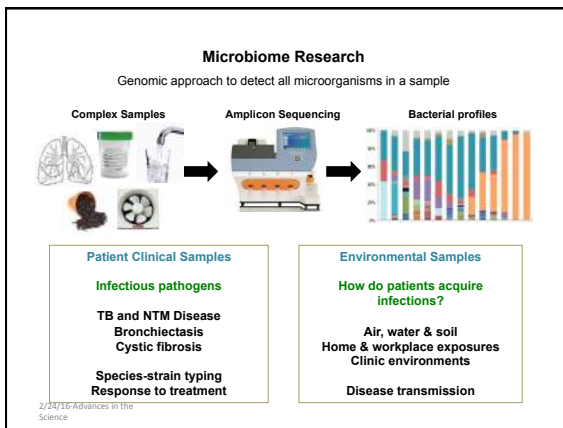
Phylogenomic comparisons
US clinical isolates vs. worldwide strains



Davidson et al 2013 - Genome Announcements
Davidson et al 2014 - J Clinical Microbiology

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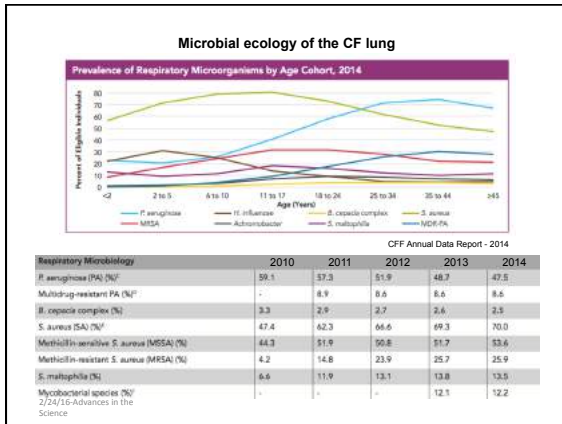


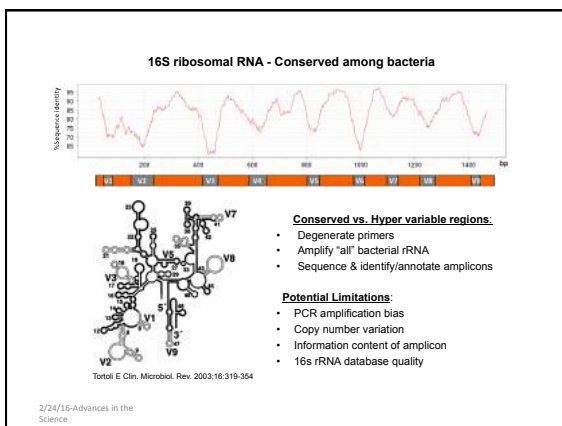
Lung microbiome study in sputum of Cystic Fibrosis (CF) patients

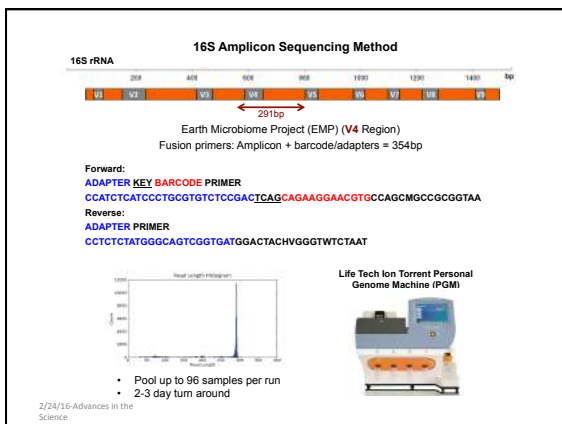
Goals of the project:

- Develop and optimize molecular and data analysis methods for 16s amplicon microbiome sequencing
- Compare the microbiome (molecular) approach to culture results:
 - Pseudomonas aeruginosa*
 - Staphylococcus aureus*
 - Achromobacter xylosoxidans*
 - Stenotrophomonas maltophilia*
 - Mycobacterium sp. (NTM)*
- Better understand lung ecology and how antibiotics may affect competition and cooperation among microbial species:
 - patterns that define treatment success/failure
 - undesirable selection of pathogens?

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Data Analysis Methods: QIIME Workflow

PREPROCESSING

1. Trim reads for length and quality
2. Reformat and rename reads
FASTX and Perl

OTU PICKING PIPELINE

3. Cluster reads into OTUs –
97% ID - UCLUST
4. Align representative sequences
5. Assign taxonomy
Greengenes 16S DB

DOWNSTREAM ANALYSES

6. Normalization
equal # reads per sample
Units = 16s read counts,
relative abundance
7. Taxonomy summary at phylum,
class, order, family, genus and
species levels

OTU = Operational taxonomic unit
a unique 16s sequence cluster

Positive Controls

Mock Community 1:
equal mass of 6 DNA samples (species):
Pseudomonas aeruginosa
Haemophilus influenzae
Staphylococcus aureus
Klebsiella pneumoniae
Mycobacterium tuberculosis
Mycobacterium abscessus

Mock II:
1% Mock 1 + 99% *Homo sapiens*

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Clinical features of CF Patients in Microbiome Study

n = 32 patients

Continuous Variables		Average	Std Dev
Age		29.75	8.56
No. of hospitalizations - last 2 years		3.56	3.14
Age of diagnosis (months)		50.47	95.78
FEV1 at sputum collection		56.19	27.7

(Range = 0-399)

Categorical Variables		F	M
Gender		15	17

FEV ₁ - 3 classes	Mild (>=70)			Moderate (40-69)			Severe (<=40)		
	F	M	Total	F	M	Total	F	M	Total
	8	14	22	14	10	24	10	4	14

CFTR Genotype	Homozygous del508		Het. del508		Other	
	F	M	F	M	F	M
	19	11	11	2	2	2

Mycobacterium (NTM) culture	Positive in past 2 yrs		Negative for past 2 yrs	
	F	M	F	M
	14	18	14	18

Laboratory Methods

- Custom DNA isolation from 100ul of raw sputum
- PCR amplification of V4 region of 16s rRNA
- 400bp amplicon sequencing on the Ion Torrent PGM

Analysis Methods

- Trim reads for quality and length (>=275bp)
- Analyze with QIIME and Greengenes 16s DB
- Normalize to 10,000 sequence reads per sample
- Exclude rare taxa: any OTU <1% of total reads in all samples
- Genus level analysis

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Microbiome profiles of 32 patients

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
<i>P. aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. maltophilia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. xylosoxidans</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
<i>Mycobacterium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Myc Last 2 yrs	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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 **Acknowledgements**

National Jewish Health

Center for Genes, Environment & Health	Colorado State University
Elaine Epperson	Mary Jackson
Michael Strong	Vivianus Calado Nogueira de Moura
Nabeeth Hasan	Mary Ann De Groot
Benjamin Garcia	Universidade Federal do Rio de Janeiro
Eve Farias-Hesson	Rafael Duarte
NJH Adult CF Clinic	Funding
Jerry Nick	NTM Center of Excellence - NJH
Ken Malcolm	Amon G. Carter Foundation
Katie Poch	Zucker Foundation
Division of Mycobacterial and Respiratory Infections	Boettcher Grant
Charles Daley	Colorado Bioscience Discovery Grant
Adrah Levin	
Mycobacteriology Lab	
Max Saffinger	
Jamie Marola	
Sarah Totten	
Molecular Diagnostics (AdX)	
Paul Reynolds	

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