The Airway Microbiome in Cystic Fibrosis

Overview

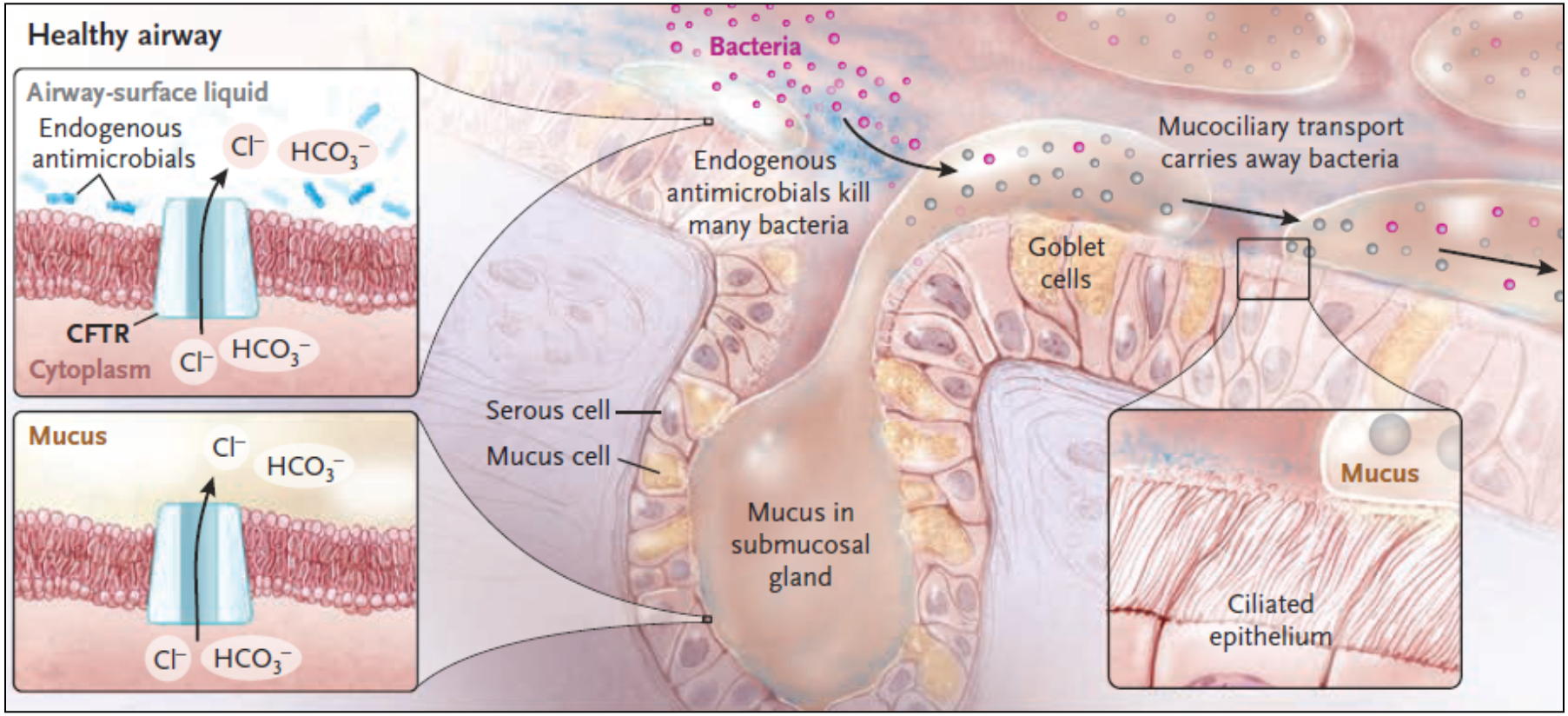
- Airway infections in cystic fibrosis (CF)
- Evaluating airway infections in CF
  - Sampling
  - Culture-based vs culture-independent methods
  - Gene sequencing techniques
  - Identifying and classifying taxa
- Characterizing the microbiome
  - Describing the microbiome: Richness, abundance, diversity, evenness
  - Measures of microbiome characteristics
- Characteristics of the healthy and CF airway microbiome
- Markers of worse clinical outcomes
- Response of the CF airway microbiome to antibiotic therapy

VERTEX

Airway Infections in CF

Lung Clearance in the Healthy, Non-CF Airway Is an Active Process

Lung clearance mechanisms include a combination of airway surface liquid (ASL), mucus secretion and transport, and antimicrobial action, which together prevent infection and contamination



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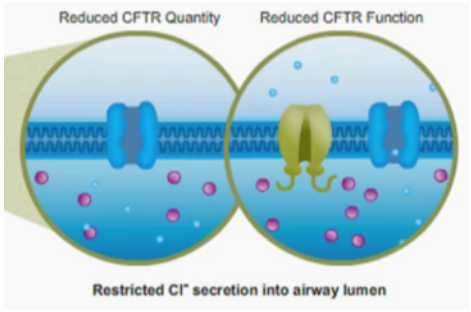
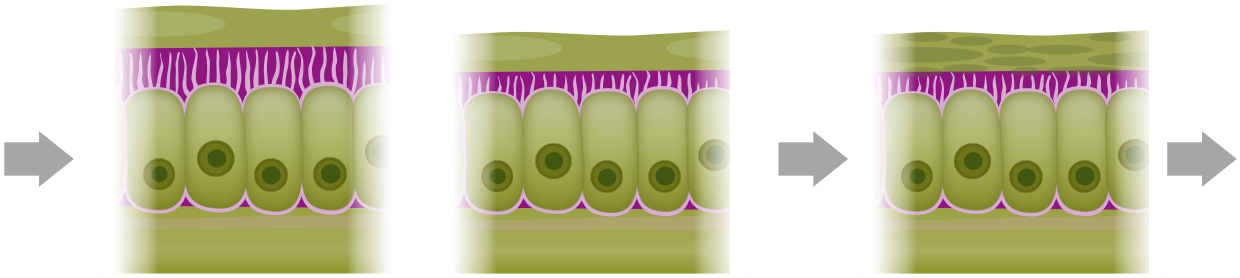
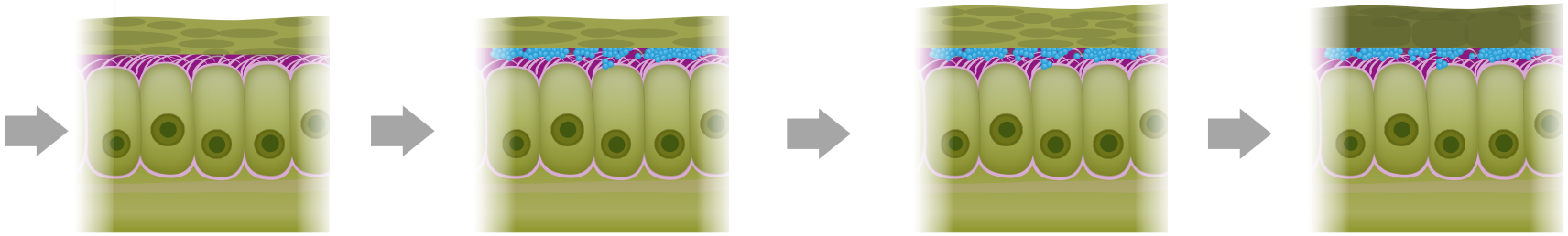
Impaired Cl^- Transport Leads to Depleted ASL and Failure of Mucus Clearance





Decreased Cl^- secretion → ASL depletion → Mucus concentration

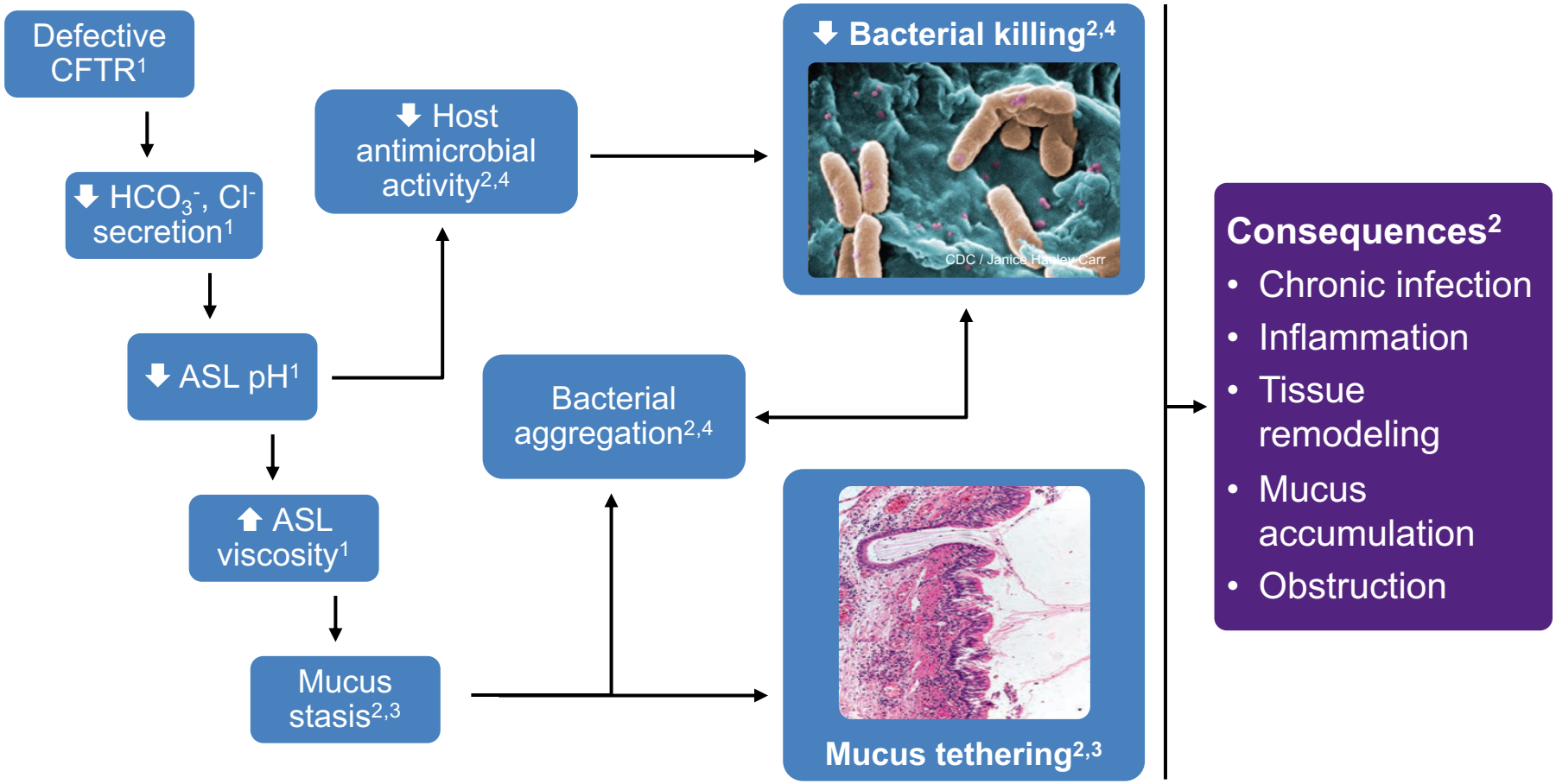


Collapse of PCL and cilia → Mucus clearance failure → Mucus thickening and adhesion → Mucus plugging

ASL, airway surface liquid; PCL, periciliary liquid.

Button B et al. *Cold Spring Harb Perspect Med*. 2013;3(8). pii: a009720. doi: 10.1101/cshperspect.a009720.

Defective CFTR Contributes to Increased Mucus Viscosity and Stasis, Mucus Tethering, and Bacterial Aggregation

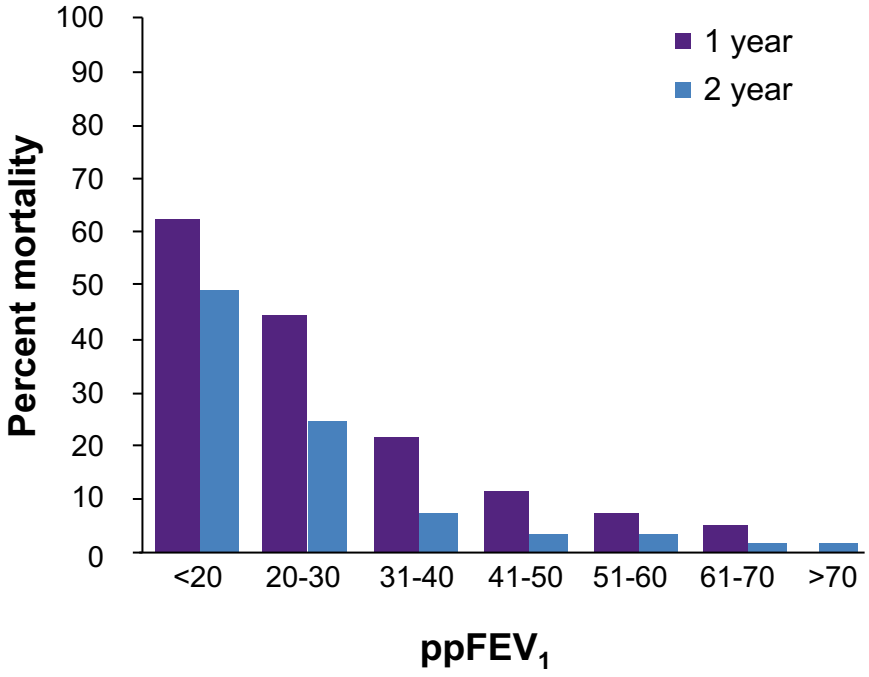


Bottom figure: From *The New England Journal of Medicine*, Stoltz DA, Meyerholz DK, Welsh MJ, Origins of cystic fibrosis lung disease, 372, 351-362. Copyright © 2015 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

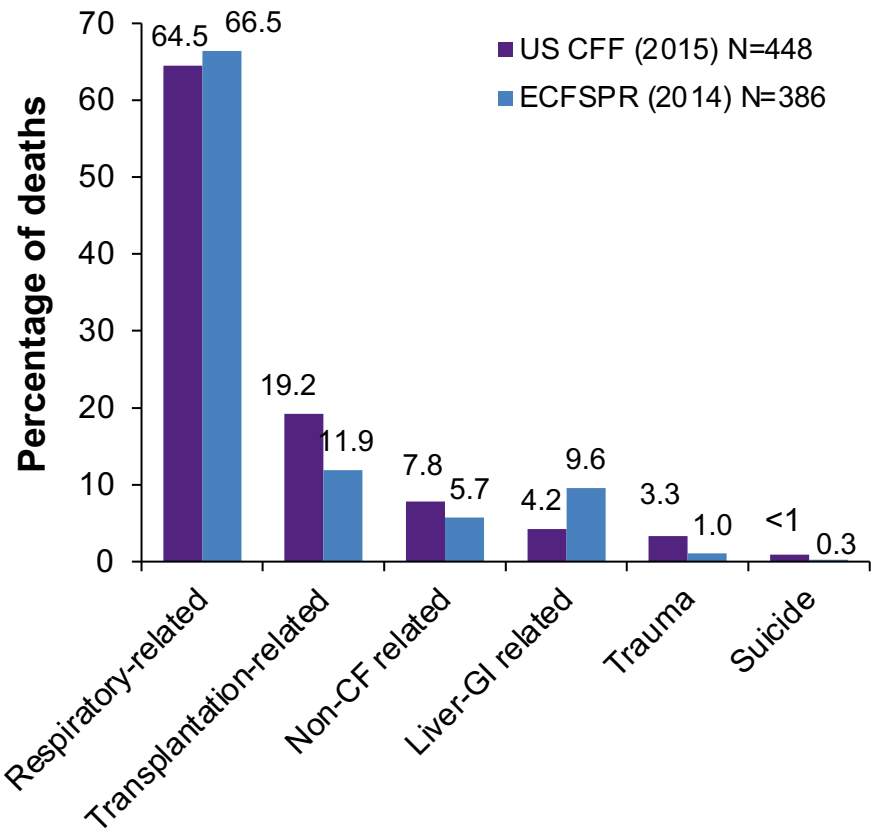
1. Tang XX et al. *J Clin Invest*. 2016;126(3):879-891. 2. Stoltz DA et al. *N Engl J Med*. 2015;372(4):351-362. 3. Hoegger MJ et al. *Science*. 2014;345(6198):818-822. 4. Staudinger BJ et al. *Am J Respir Crit Care Med*. 2014;189(7):812-824.

Lung Disease Is the Strongest Predictor of Mortality in CF

Mortality rate by lung function[1]



Cause of death in patients with CF in US and EU registries[2,3]



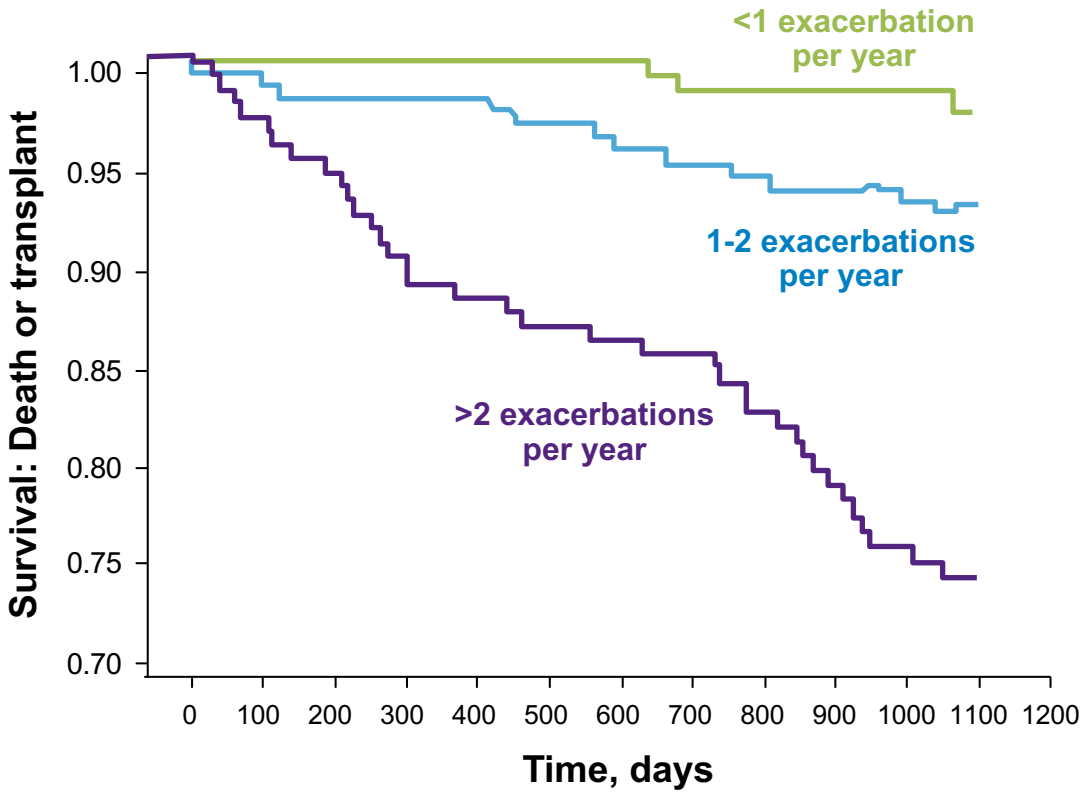
Left figure: From *The New England Journal of Medicine*, Kerem E et al, Prediction of mortality in patients with cystic fibrosis, 326, 1187-1191. Copyright © 1992 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

1. Kerem E et al. *N Engl J Med*. 1992;324(18):1187-1191. 2. CFF 2015 Annual Data Report, Bethesda, MD. © 2016. 3. Zolin A et al. ECFSPR Annual Report 2014. November 2016.

Pulmonary Exacerbations Have a Cumulative Effect on Loss of Lung Function and Risk of Death

- 1 to 2 exacerbations/year increases risk of death 3-fold[1]
  - ≥3 exacerbations/year increases risk of death 4.5-fold[1]
- >2 exacerbations/year leads to a >4-fold 3 year risk of death or lung transplant vs <1 exacerbation[2]
- Each acute pulmonary exacerbation within the year had an unexpectedly large, negative impact on 5-year survival equal to subtracting 12% from the measured FEV_1% value[3]

Pulmonary exacerbations[a] and survival[2]



Reproduced from *Thorax*, de Boer K et al, 66, 680-685, © 2011 with permission from BMJ Publishing Group Ltd.

[a]Pulmonary exacerbations requiring oral or IV antibiotics.

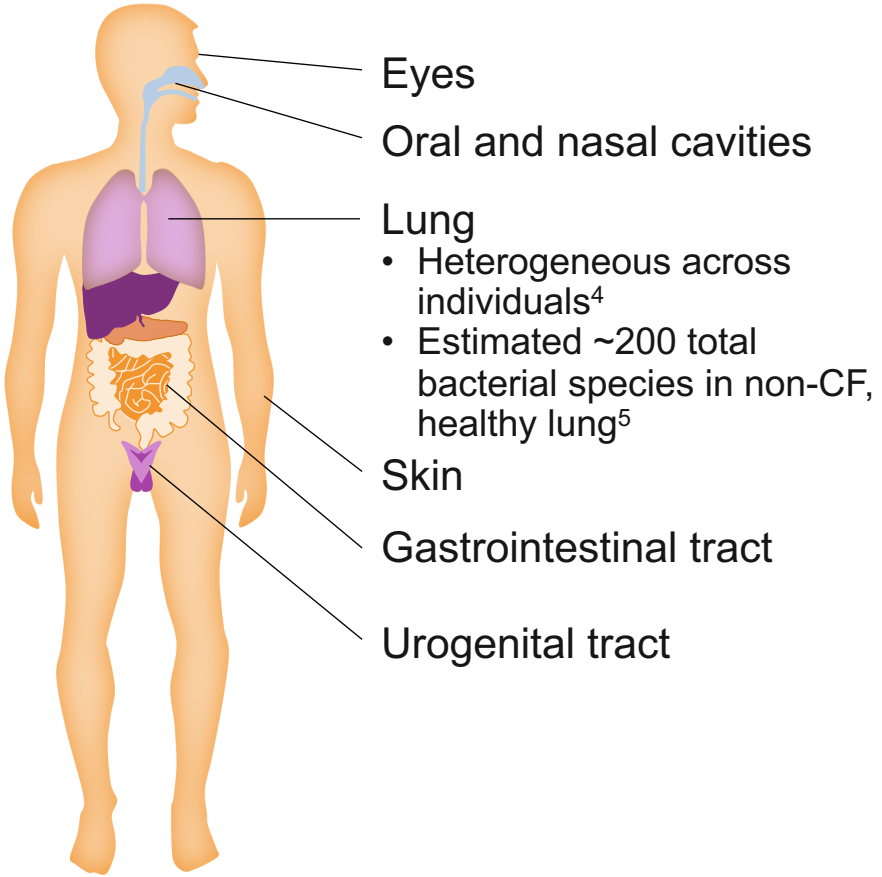
1. Stephenson AL et al. *Eur Respir J*. 2015;45(3):670-679. 2. De Boer K et al. *Thorax*. 2011;66(8):680-685. 3. Liou TG et al. *Am J Epidemiol.* 2001;153(4):345-352.

Evaluating Airway Infections in CF

The Microbiome

- The abundance and diversity of microbes on and within the human body is collectively referred to as the microbiome[1]
- Plays an important role in human health, especially digestion and immunity[2,3]
- Includes bacteria, fungi, and viruses[1,2]
  - Little data available on fungi and viruses

Locations of the human microbiome[2,3]



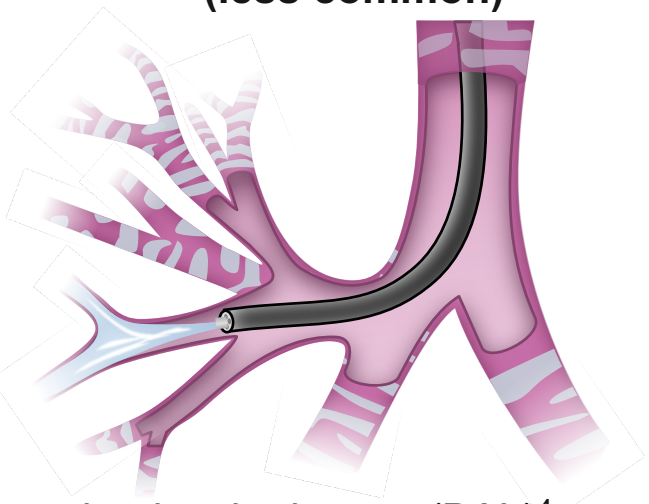
1. Dickson RP et al. *Expert Rev Respir Med.* 2013;7(3)245-257. 2. Lloyde-Price J et al. *Genome Med.* 2016;8(1):51. 3. The Human Microbiome Project Consortium. *Nature.* 2012;486:207-214. 4. Charson ES et al. *Am J Respir Crit Care Med.* 2011;184(8):957-963. 5. Dickson RP et al. *Ann Am Thorac Soc.* 2015;12(6):821-830.

Noninvasive and Invasive Methods Are Used to Sample the Airway Microbiome

Noninvasive
(most common)

- Cough swab[1]
  - Swab of posterior oropharynx area
  - Used in those who cannot expectorate sputum
- Expectorated sputum[1]
  - Requires sputum production in sufficient volume for analysis
  - Preferred method[2]
- Induced sputum[3]
  - Sputum produced by inhalation of nebulized saline for those who cannot expectorate

Invasive
(less common)



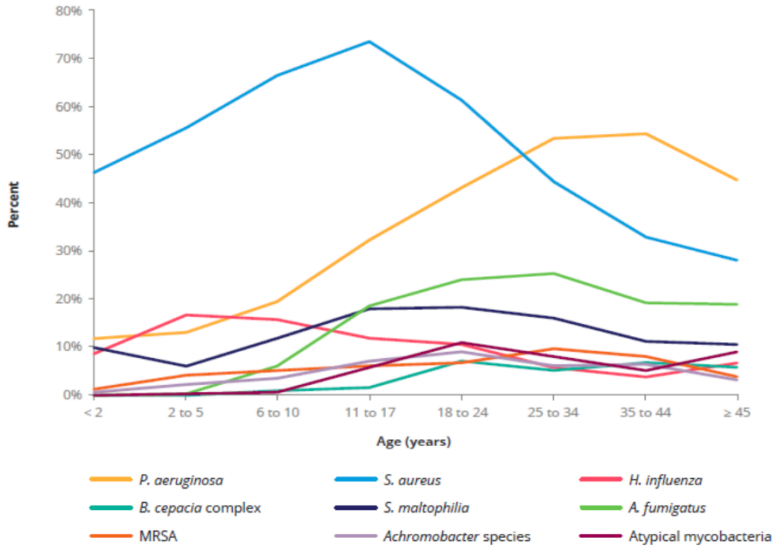
- Bronchoalveolar lavage (BAL)[4]
  - A bronchoscope is passed through the mouth or nose into the lungs
  - Saline is flushed into the lungs and collected

- Sputum samples with >20% squamous epithelial cells are considered inadequate because of contamination from saliva[3]

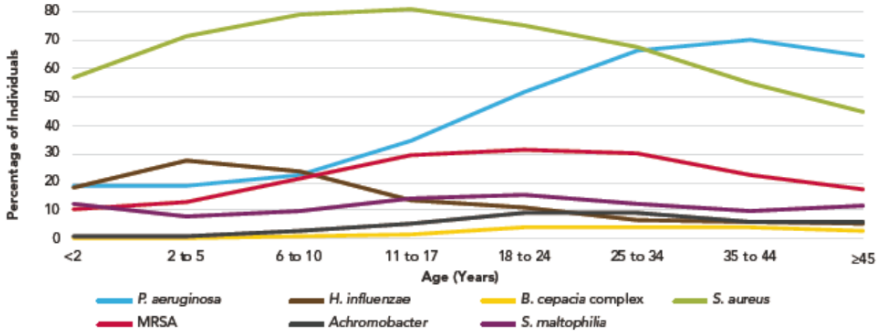
1. Seidler D et al. *PLoS One.* 2016;11(10):e0164232. 2. Gilligan PH et al. 2006. *Cumetech 43. Cystic Fibrosis Microbiology*. Coordinating ed., MD Appleman. ASM Press, Washington, DC. 3. Weiszhar Z, Horvath I. *Breathe.* 2013;9(4):301-306. 4. Baughman RP. *Sem Resp Crit Care Med.* 2007;28(5):475-485.

Pathogens Identified in Lung Infections Change as Patients Age

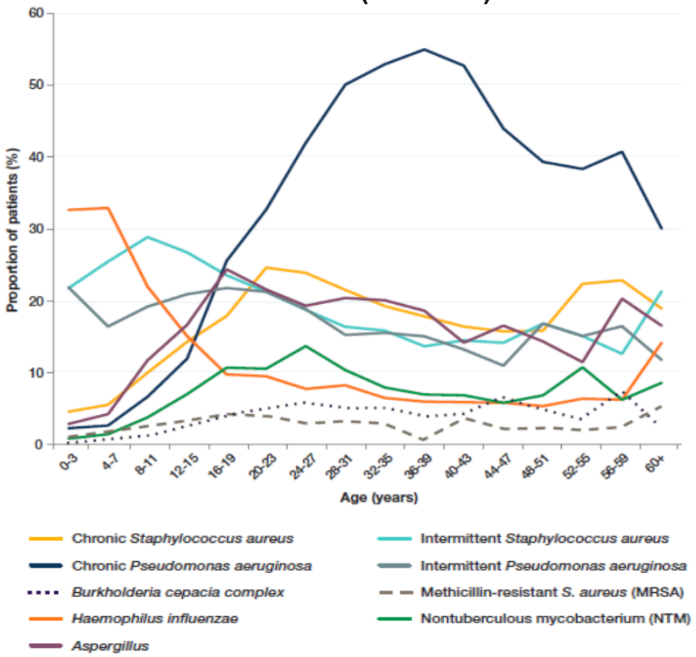
Age Prevalence of Respiratory Infections in Patients with CF (Canada Data)[1]



Age Prevalence of Respiratory Infections in Patients with CF (US Data)[2]



Age Prevalence of Respiratory Infections in Patients with CF (UK Data)[3]

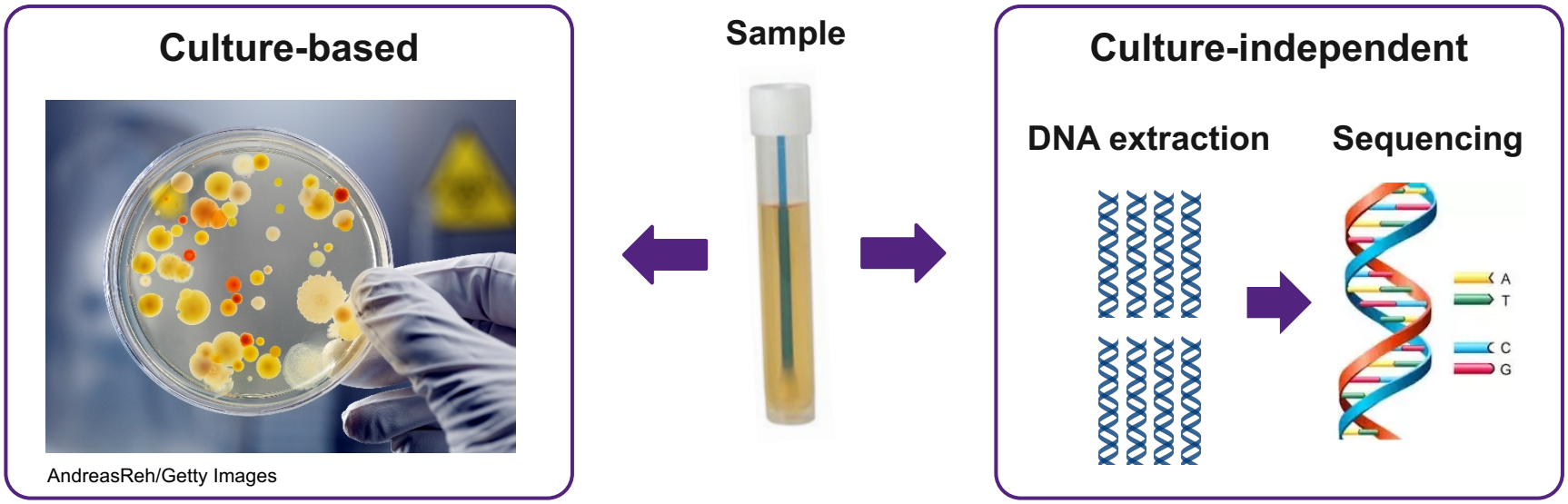


- *S. aureus* infection is more common in young patients,[1,2] and *P. aeruginosa* infection is more common in adults[1-3]
- Drug-resistant forms of some of these pathogens are also detected and increase in prevalence with age[1-3]

MRSA, methicillin-resistant *Staphylococcus aureus*

1. The Canadian Cystic Fibrosis Registry. 2018 Annual Report. 2. Cystic Fibrosis Foundation Patient Registry. 2018 Annual Report. Bethesda, MD. 3. UK Cystic Fibrosis Registry Annual Data Report 2018. Published August 2019.

Culture-Based and Cultured-Independent Methods Are Used to Identify Microbes Within the Microbiome



- Conventional culture-dependent methods identify individual pathogens[1,2]
- Newer gene sequence techniques characterize the identity and relative abundance of all of the bacterial species present[2]

1. Gilligan PH et al. 2006. *Cumetech 43. Cystic Fibrosis Microbiology*. Coordinating ed., MD Appleman. ASM Press, Washington, DC. 2. Rogers GB et al. *Thorax*. 2015;70(1):74-81.

Culture-Based Methods Identify Organisms Based on Characteristics and Viability in Culture

- Organisms are identified by[1]
  - Morphology, color, size
  - Cell wall composition
  - Secreted products
  - Conditions required for growth
- Limited to species that can be cultured[2]
- Multiple growth conditions can expand the number of species collected[2]

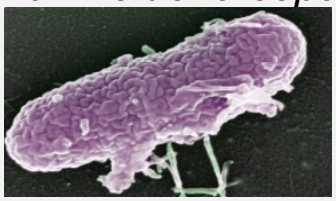
Representative bacterial identification using selective culture media[3,4]

Pseudomonas aeruginosa

MacConkey agar
Chocolate agar
Cetrimide agar

35-37◦C in air,12-72 h

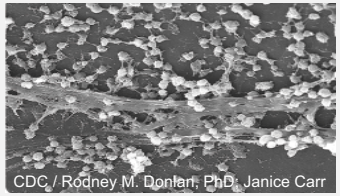
Burkholderia cepacia complex



PC medium
BCSA
MAST

35-37◦C in air, 5 days

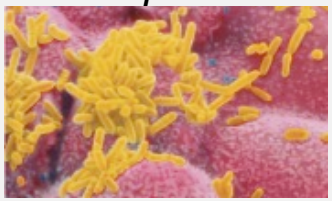
Staphylococcus aureus



MSA
CHROMagar

35-37◦C in air, 12-24 h

Haemophilus influenzae



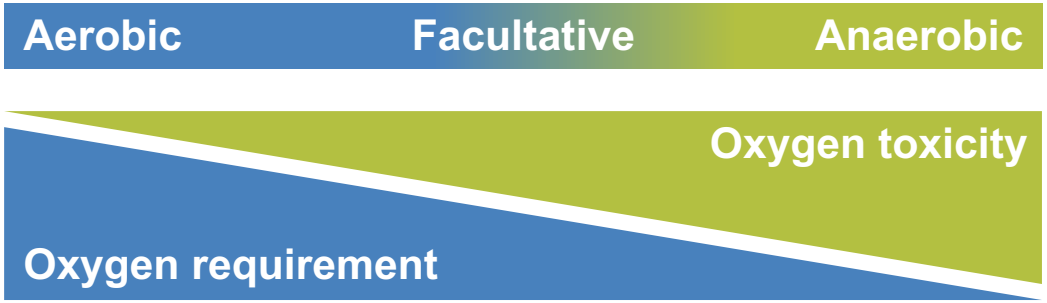
Chocolate agar
Horse blood agar

35-37◦C in 5% CO_2, 12-24 h

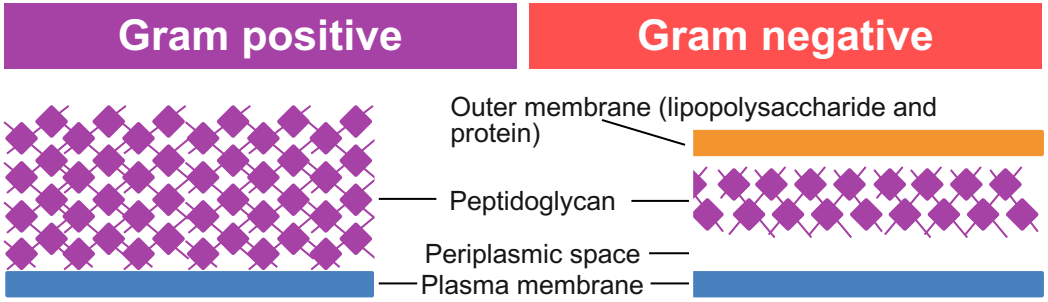
1. Bisen PS. Microbial taxonomy. In: *Microbes in Practice.* IK International, New Delhi; 2014. 2. Hiergeist A et al. *ILAR J.* 2015;56(2):228-240. 3. UK CF Trust Microbiology Laboratory Standards Working Group. 2010. 4. Gilligan PH et al. 2006. *Cumetech 43. Cystic Fibrosis Microbiology*. Coordinating ed., MD Appleman. ASM Press, Washington, DC.

Major Subtypes of Bacteria Are Classified Based on Physical and Physiological Characteristics

Classification by oxygen utilization[1]



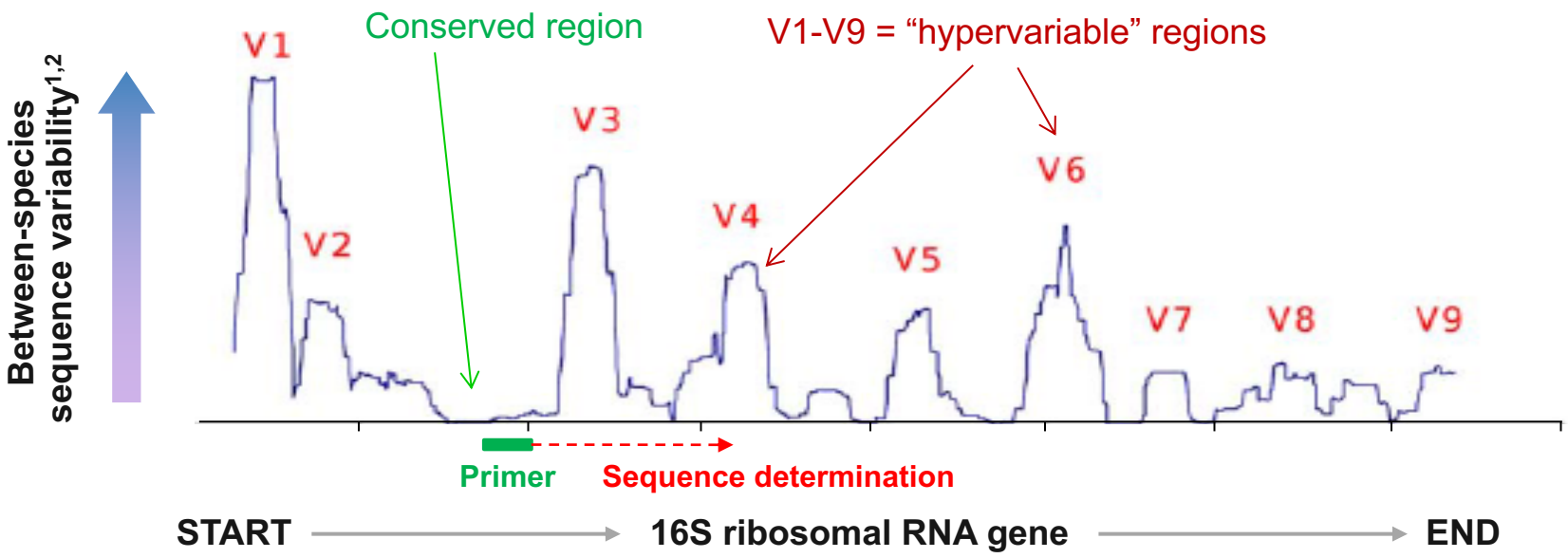
Classification by outer membrane structure[2]



1. Hentges DL. *Med Microbiol*. 1996, 4th edition, Chapter 17. 2. Silhavy TJ et al. *Cold Spring Harb Perspect Biol.* 2010;2(5):a000414.

Sequencing Often Relies on the 16S Ribosomal RNA Gene Sequence to Identify Microbiome Members

16S ribosomal RNA gene sequencing is the "gold standard" to identify microbiome members[1]

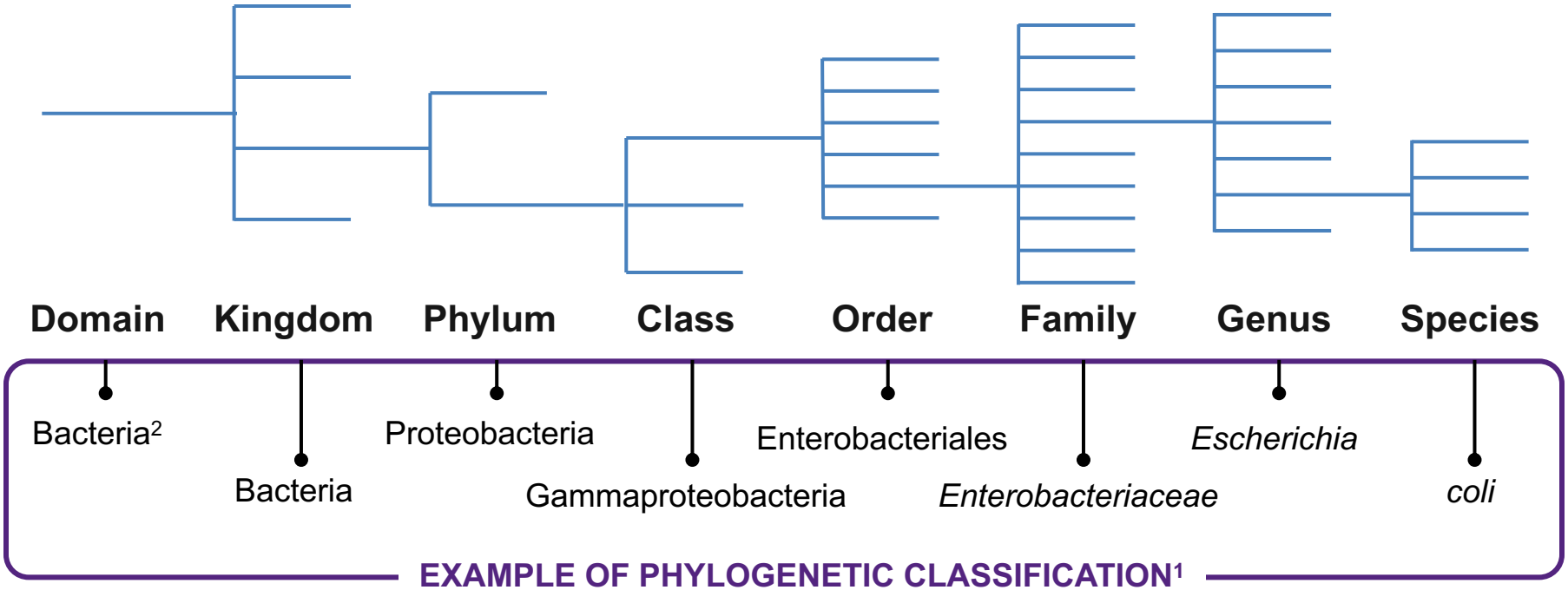


Adapted from Bodilis J et al. *PLoS One*. 2012;7(4):e35647.

- Primers that bind to conserved regions of the 16S rRNA gene are used to sequence flanking hypervariable regions that discriminate between taxa using high-throughput sequencing techniques[1]

1. Hiergeist A et al. *ILAR J.* 2015;56(2):228-240. 2. Bodilis J et al. *PLoS One*. 2012;7(4):e35647.

Operational Taxonomic Units Are Used to Categorize Bacteria Based on Genetic Similarity



EXAMPLE OF PHYLOGENETIC CLASSIFICATION[1]

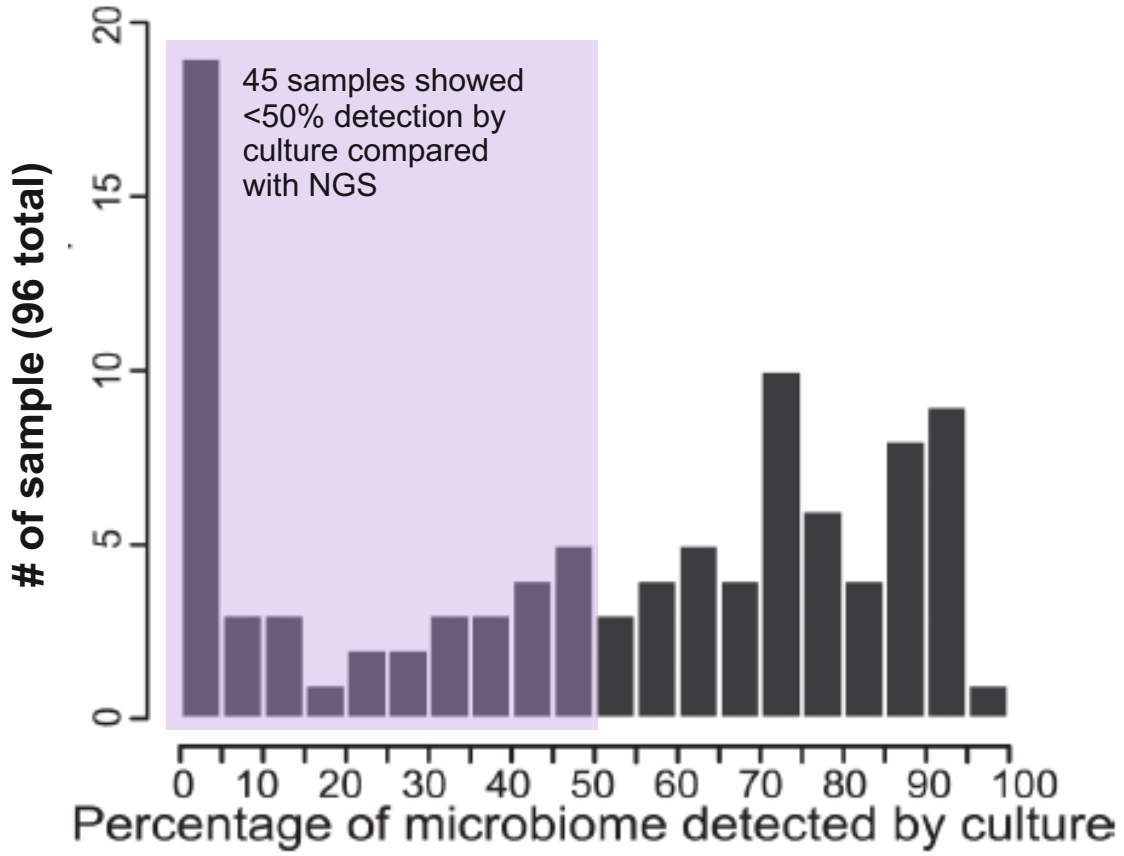
- Operational taxonomic unit (OTU) classification is based on gene sequence similarity to known sequences in a reference database[3]
- ≥97% sequence identity, using 16S rRNA gene sequencing, is often used to identify a "species"[3,4]

1. Retrieved April 2020, from the Integrated Taxonomic Information System (ITIS) (http://www.itis.gov). 2. Woese CR, et al. Proc Natl Acad Sci. 1990;87:4576-4579.
3. Rogers GB et al. *Thorax*. 2015;70(1):74-81. 4. Janda JM, Abbott SL. *J Clin Microbiol*. 2007;45(9):2761-2764.

Next-Generation Sequencing (NGS)Techniques Detect Microbes Not Identified by Culture-Based Analysis

Comparison of NGS with culture analysis

- From 96 samples*, 76 genera were found by NGS compared with 18 by culture

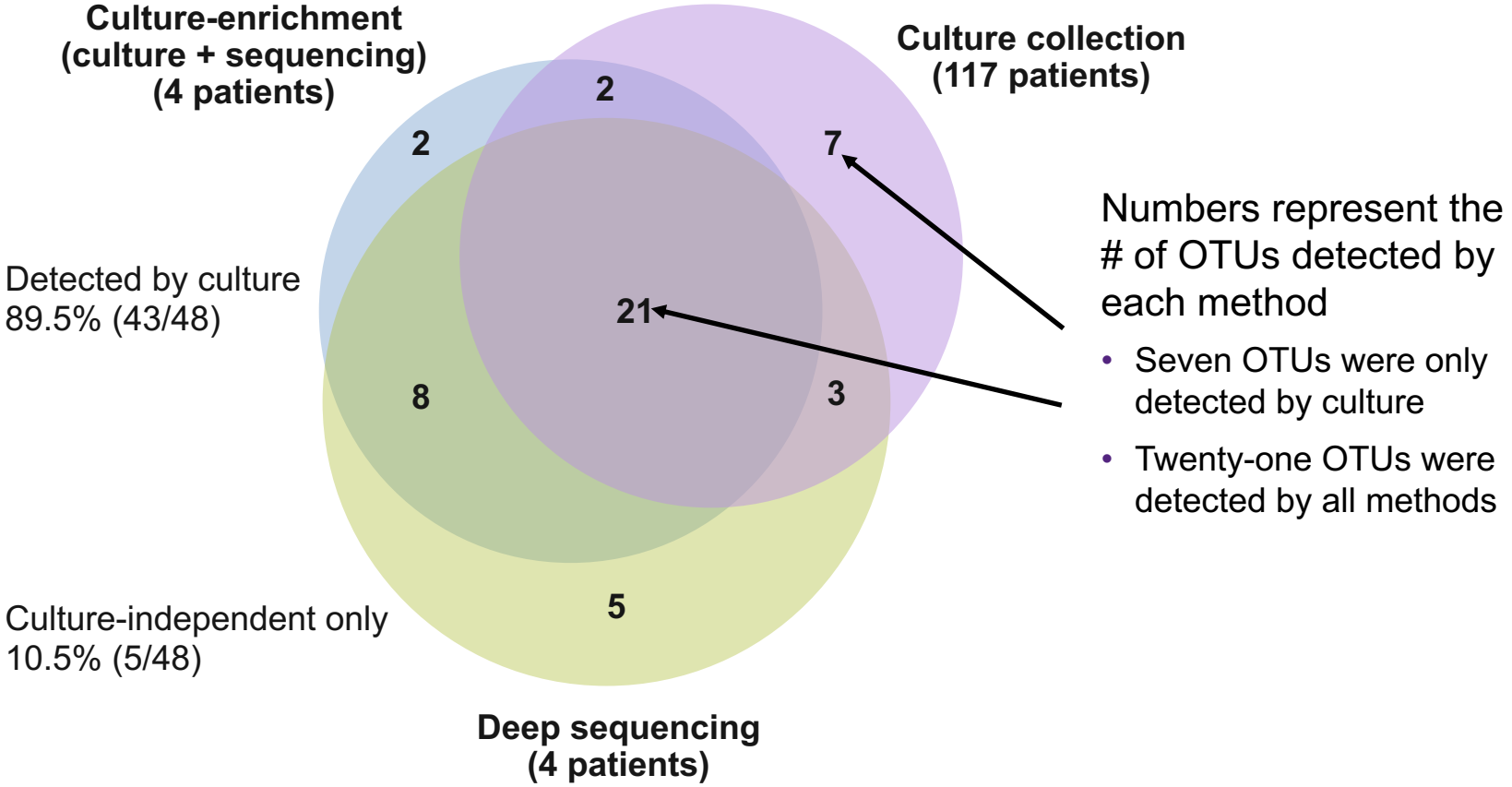


Adapted from Boutin S et al. *PLoS One.* 2015;10(1):e0116029.

*Ninety-eight samples (sputum=32, nasal=36, throat=30) from 20 patients with CF were analyzed by NGS and culture.
Boutin S et al. *PLoS One.* 2015;10(1):e0116029.

VERTEX

Enhanced Culture Techniques Plus Next-Generation Sequencing Enhances Sensitivity



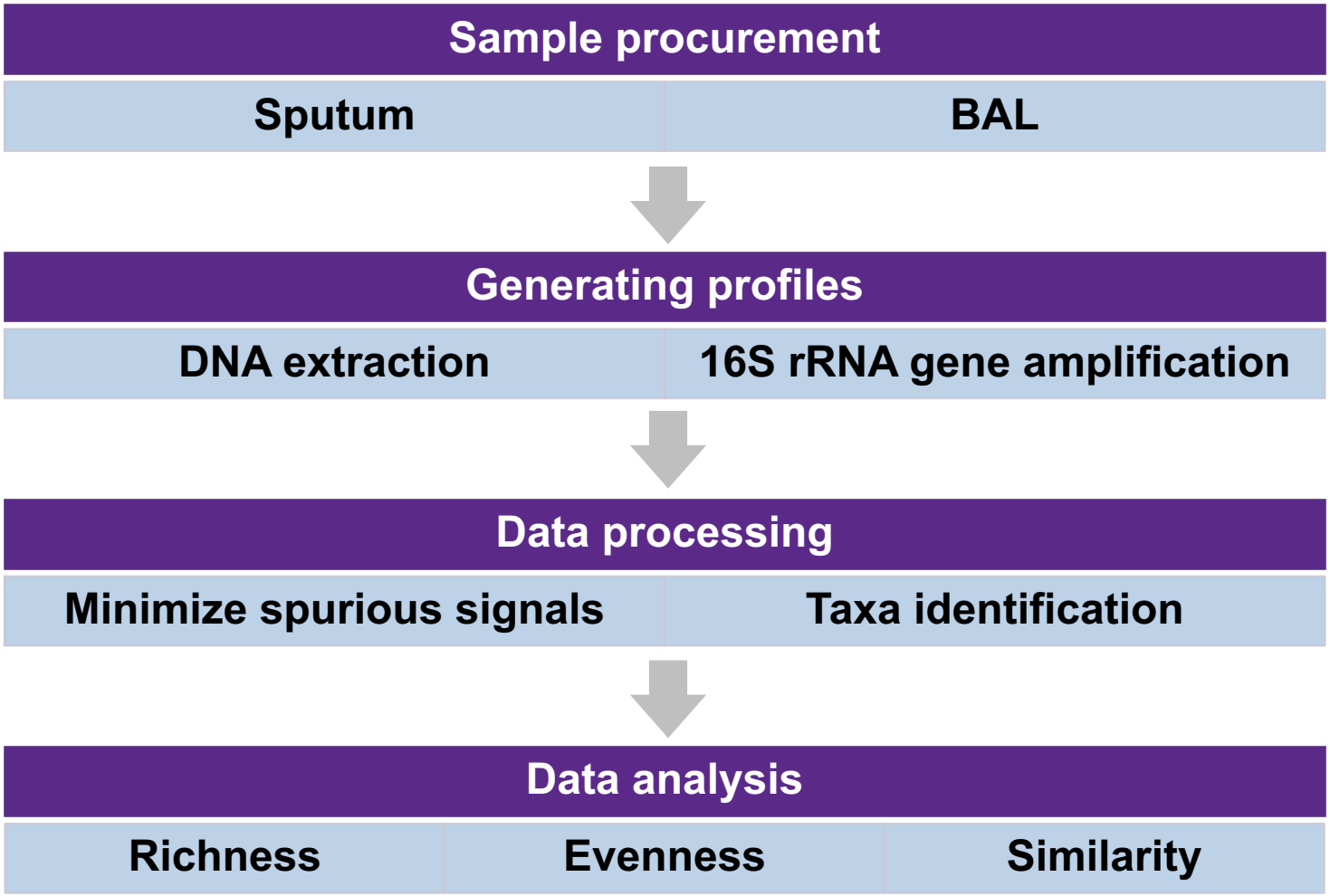
Adapted from Sibley CD et al. *PLoS One.* 2011;6(7):e22702.

OTU, operational taxonomic unit.
Sibley CD et al. *PLoS One.* 2011;6(7):e22702.

Characterizing the Microbiome

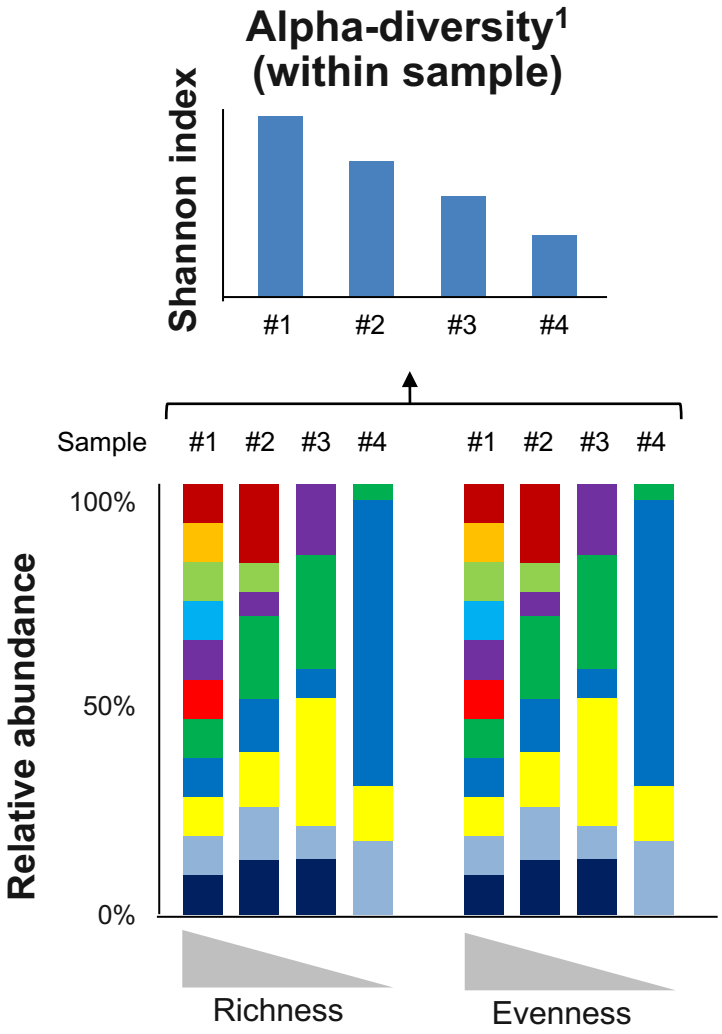
A number of measures are used to describe the complex microbial community within a sample or to compare samples

Newer Gene Sequencing Techniques Identify and Classify Organisms Based on Their DNA



Rogers GB et al. *Thorax*. 2015;70(1):74-81.

Multiple Measures Are Used to Describe the Microbiome: Within-Sample Characteristics



Diversity[1]: The number of distinct types of organisms (taxa)

Alpha diversity[1]: Diversity within samples

- **Relative abundance:** The percent composition of organisms (taxa)
- **Richness:** The number of organisms (taxa) in a sample
- **Evenness:** The degree to which each organism (taxon) is of equal abundance

Alpha-diversity is commonly calculated using the Shannon index or the Simpson index[1]

Range for the Shannon index[2]

0 – only 1 organism (taxon)

High values for many organisms (taxa) of low relative abundance

Range for the Simpson index[2]

0 – organisms (taxa) are equally abundant

1 – 1 organism (taxon) dominates

1. Morgan XC, Huttenhower C. *PLoS Comp Bio*. 2012;8(12):e1002808. 2. Kim BR, et al. *J Microbiol Biotechnol*. 2017 Oct 14. doi: 10.4014/jmb.1709.09027.

Multiple Measures Are Used to Describe the Microbiome: Between-Sample Comparisons

Diversity: The number of distinct types of organisms (taxa)[1]

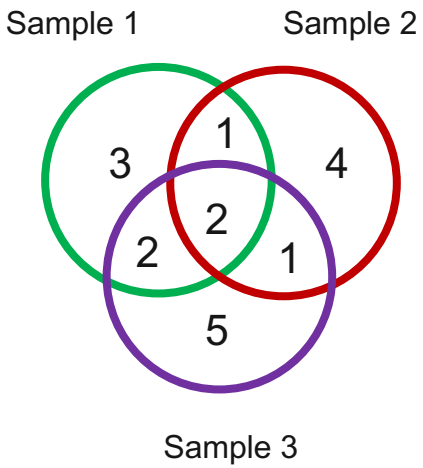
Beta-diversity: The degree to which different samples are similar or dissimilar[1]

Beta-diversity can be determined using Bray-Curtis dissimilarity[1]

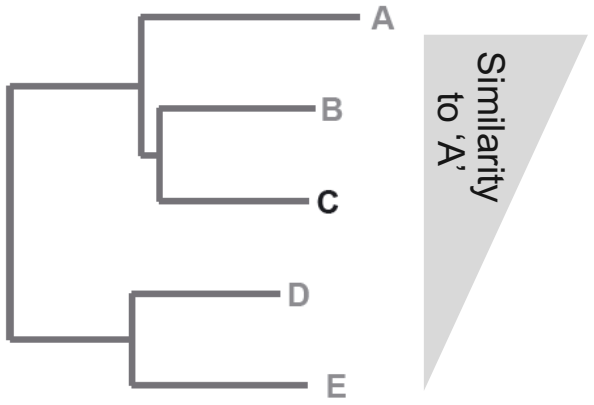
Identifies groups of samples that have similar microbial communities

Phylogenetic diversity[2]: The phylogenetic distance between pairs of members in a community

Beta diversity[1] (between samples)



Phylogenetic diversity[2]



Example: Net relatedness index (NRI)

1. Morgan XC, Huttenhower C. *PLoS Comp Bio.* 2012;8(12):e1002808. 2. Herrera-Alsina L, Villegas-Patraca R. *Ecol Evol* 2014;4(7):968-976.

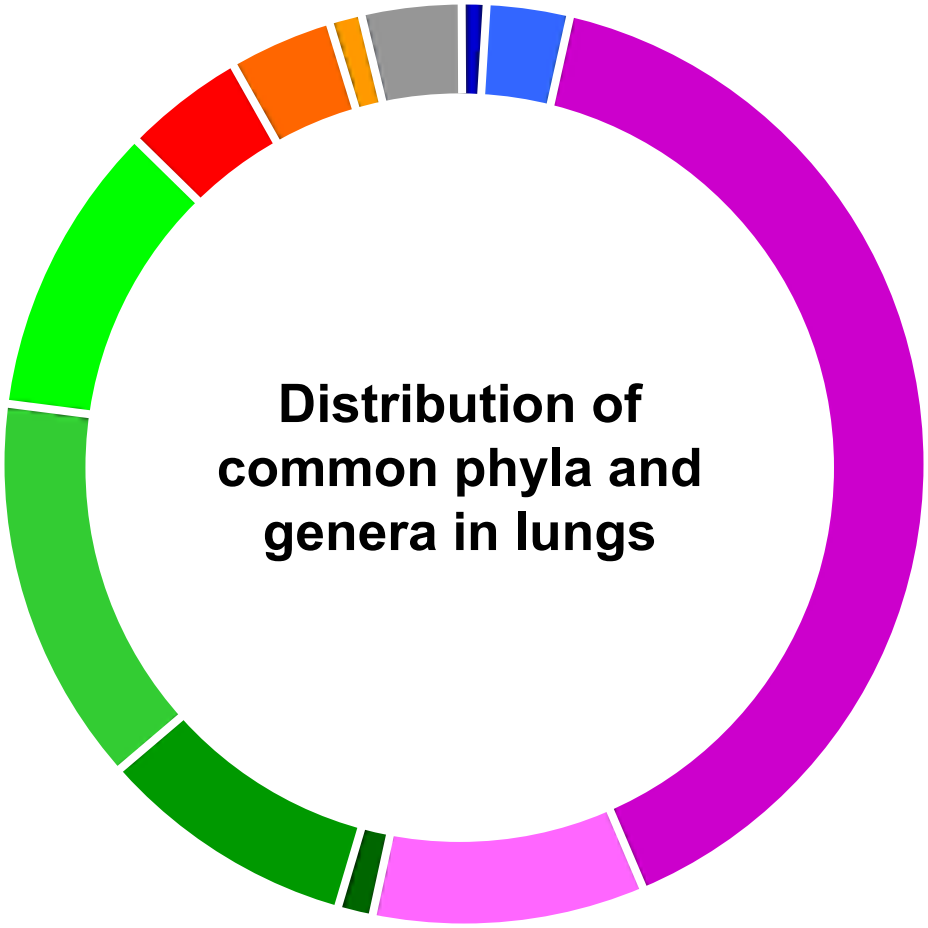
Sample Analysis: Culture-Based vs Next-Generation Sequencing (NGS) of Operational Taxonomic Units

Culture	NGS
Detects organisms based on viability in culture[1]	Detects the presence of OTU DNA from viable AND nonviable organisms[1]
<20% of bacterial taxa can be cultured in traditional defined media[2]	Detects both culturable and nonculturable members of the microbiome[2]
Slow-growing or low-abundant organisms may be out-competed and not identified[2]	Deep sampling and high-throughput sequencing can identify all members of a microbiome[2]
Enhanced techniques and “culturomics” allow identification and culturing of a broad variety of bacteria[2-4]	Depth of taxonomic level classification is limited by the length of the sequence read, database bias, and other artifacts[5]
Allows testing of co-culture effects (eg, protection) and antibiotic susceptibility[3,6]	16S rRNA sequencing does not detect intra-species variations[2]
Culture of CF samples is labor intensive[7]	Sequencing amplifies contaminating DNA from equipment, reagents, staff, requiring corrective measures[8]

1. Dickson. *Expert Rev Respir Med.* 2013;7(3):245-257. 2. Hiergeist A et al. *ILAR J.* 2015;56(2):228-240. 3. Tunney MM et al. *Am J Respir Crit Care Med.* 2008;177(9):995-1001. 4. Sibley CD et al. *J Med Microbiol.* 2010;59(Pt 5):534-540. 5. Poretsky R et al. *PLoS One.* 2014;9(4):e93827. 6. Sherrard LJ et al. *Int J Antimicrob Agents.* 2016;47(2):140-145. 7. Gilligan PH et al. 2006. *Cumetech 43. Cystic Fibrosis Microbiology*. Coordinating ed., MD Appleman. ASM Press, Washington, DC. 8. Millar BC, et al. *J Clin Microbiol.* 2002;40(5):1575–1580.

Overview of the Healthy, Non-CF and CF Airway Microbiome

Composition of the Microbiome in Non-CF, Healthy Lungs



- Actinobacteria/*Corynebacterium*
- Other Actinobacteria
- Bacteroidetes/*Prevotella*
- Other Bacteroidetes
- Firmicutes/*Staphylococcus*
- Firmicutes/*Streptococcus*
- Firmicutes/*Veillonella*
- Other Firmicutes
- Proteobacteria/*Haemophilus*
- Proteobacteria/*Neisseria*
- Other Proteobacteria
- Fusobacteria/*Fusobacterium*

Hilty M et al. *PLoS One.* 2010;5(1):e8578.

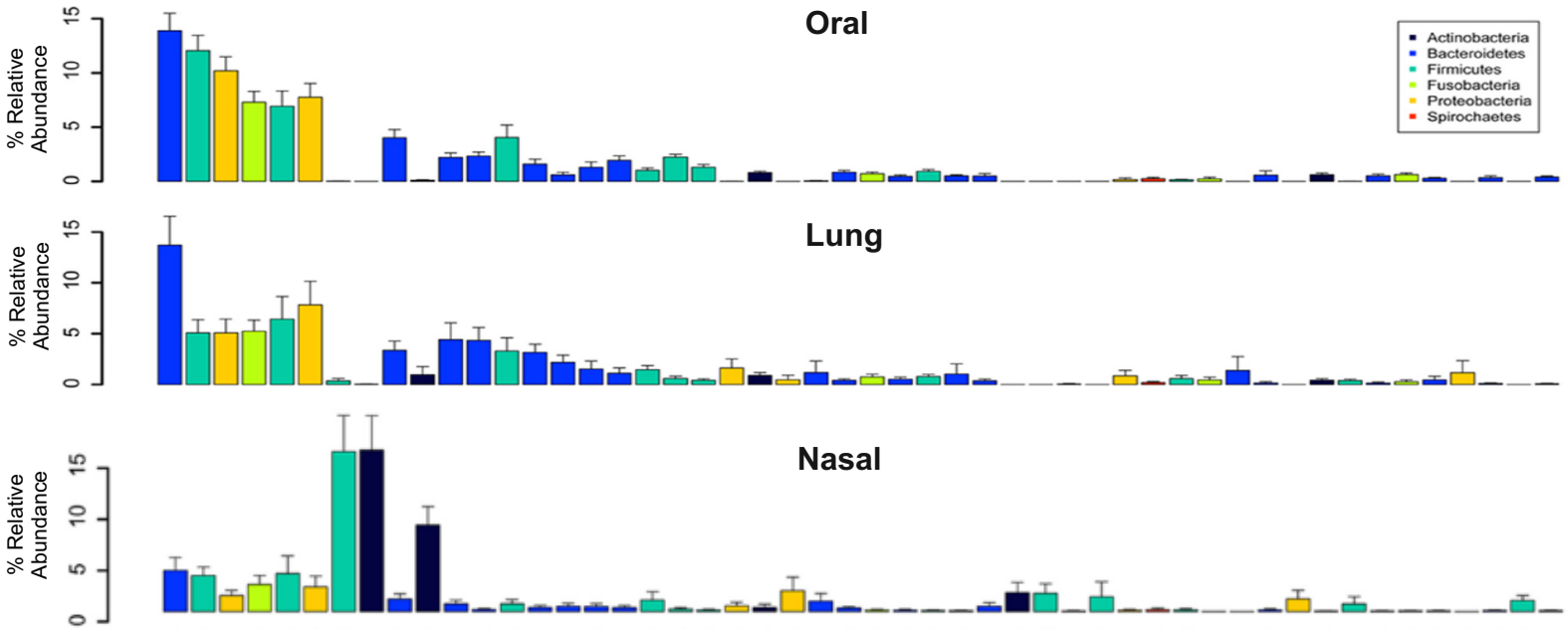
The Lung Microbiome in Healthy Individuals Is Determined by Three Factors

Immigration[1]
Primarily microaspiration of the oral microbiome

Growth conditions[1]
Nutrients, oxygen, pH, temperature, local microbial competition, and host interactions

Elimination[1]
Cough
Mucociliary clearance
Immune defenses

Oral and lung microbiomes share significant membership that differs from the nasal microbiome[2]

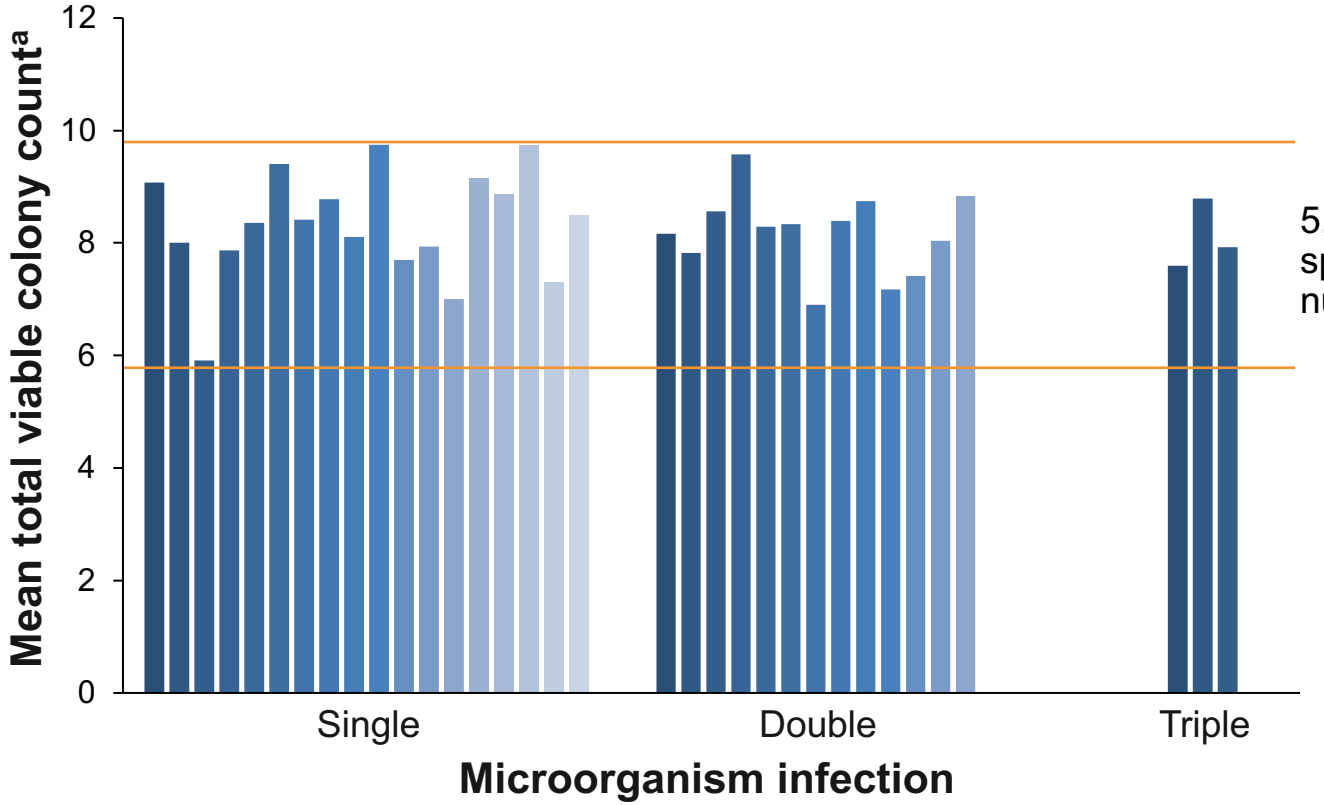


Reprinted from Bassis CM et al. *MBio*. 2015;6(2):e00037.

1. Dickson RP, Huffnagle GB. *PLoS Pathogens.* 2015;11(7):e1004923. 2. Bassis CM et al. *MBio.* 2015;6(2):e00037.

Quantification Shows Large Bacterial Load in the Sputum of Adult Patients With CF

Total quantitative microbiological counts of co-infecting/colonizing taxa microflora in sputum of adult patients with CF using culture-based analysis

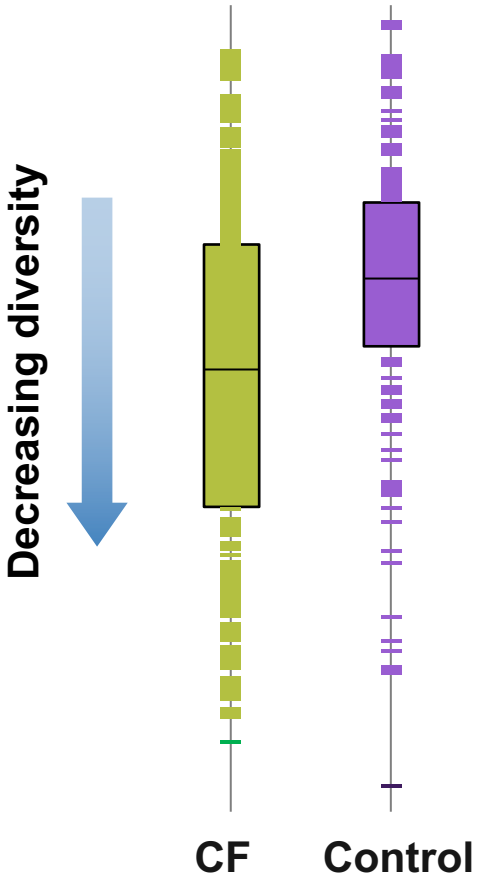


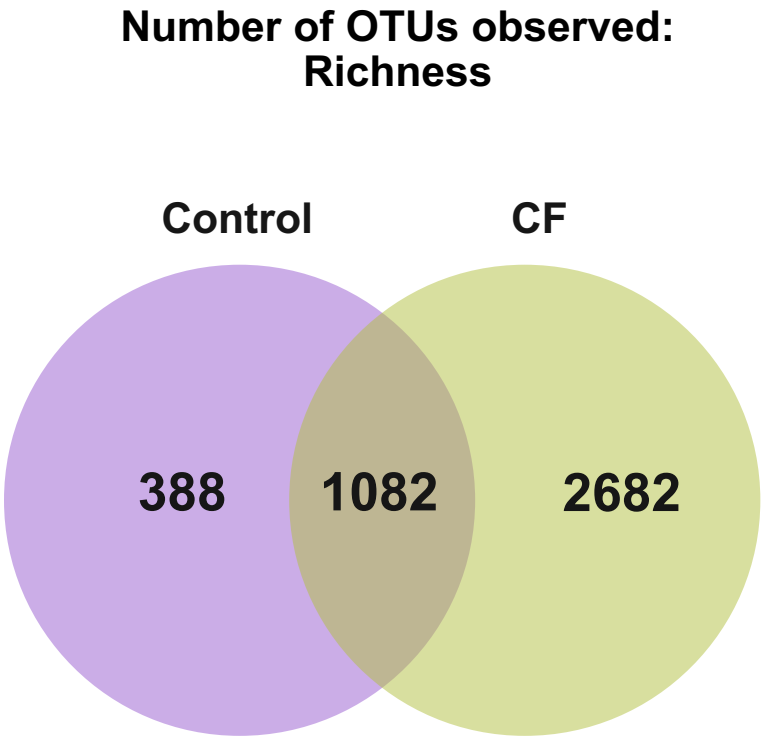
5.9 to 9.7 $\log_{10}$ cfu/g sputum, regardless of number of infecting species

[a]Fresh sputum from 34 adult patients with CF was analyzed for microflora using Columbia agar base (Oxoid CM331), and total viable colony counts were expressed as $\log_{10}$ colony-forming units per gram (cfu/g) of original sputum.

Moore JE et al. *Br J Biomed Sci*. 2005;62(4):1-4.

Microbiome of Patients With CF Have Lower Diversity and Higher Richness Compared With Healthy Controls





Figures adapted from Li et al. *PLoS One,* 2016.

Diversity: The number of distinct types of organisms (taxa); Richness: the number of organisms (taxa) in a sample.
Li J et al. *PLoS One.* 2016;11(10):e0164510.

Lung Bacteria in CF Can Be "Core" or "Satellite" Depending on Prevalence in and Across Patients With CF

Predominant "core" bacterial taxa in sputum samples from 14 patients with CF

Family	Taxon Name	O_2 Utilization	Oral Bacterim
Porphyromonadaceae	*Porphyromonas catoniae*	Anaerobe	Yes
Prevotellaceae	*Prevotella melaninogenica*	Anaerobe	Yes
Prevotellaceae	*Prevotella oris*	Anaerobe	Yes
Prevotellaceae	*Prevotella salivae*	Anaerobe	Yes
Prevotellaceae	*Prevotella tannerae*	Anaerobe	Yes
Prevotellaceae	*Prevotella veroralis*	Anaerobe	Yes
Streptococcaceae	*Streptococcus mitis/pneumoniae*	Aerobe	Yes
Streptococcaceae	*Streptococcus parasanguis*	Aerobe	Yes
Lachnospiraceae	*Catonella morbi*	Anaerobe	Yes
Veillonellaceae	*Veillonella atypica*	Anaerobe	Yes
Veillonellaceae	*Veillonella dispar*	Anaerobe	Yes
Veillonellaceae	*Veillonella parvula*	Anaerobe	Yes
Neisseriaceae	*Neisseria cinerea*	Aerobe	Yes
Neisseriaceae	*Neisseria flava/sicca/mucosa/pharyngis*	Aerobe	Yes
Pseudomonadaceae	*Pseudomonas aeruginosa*	Aerobe	No

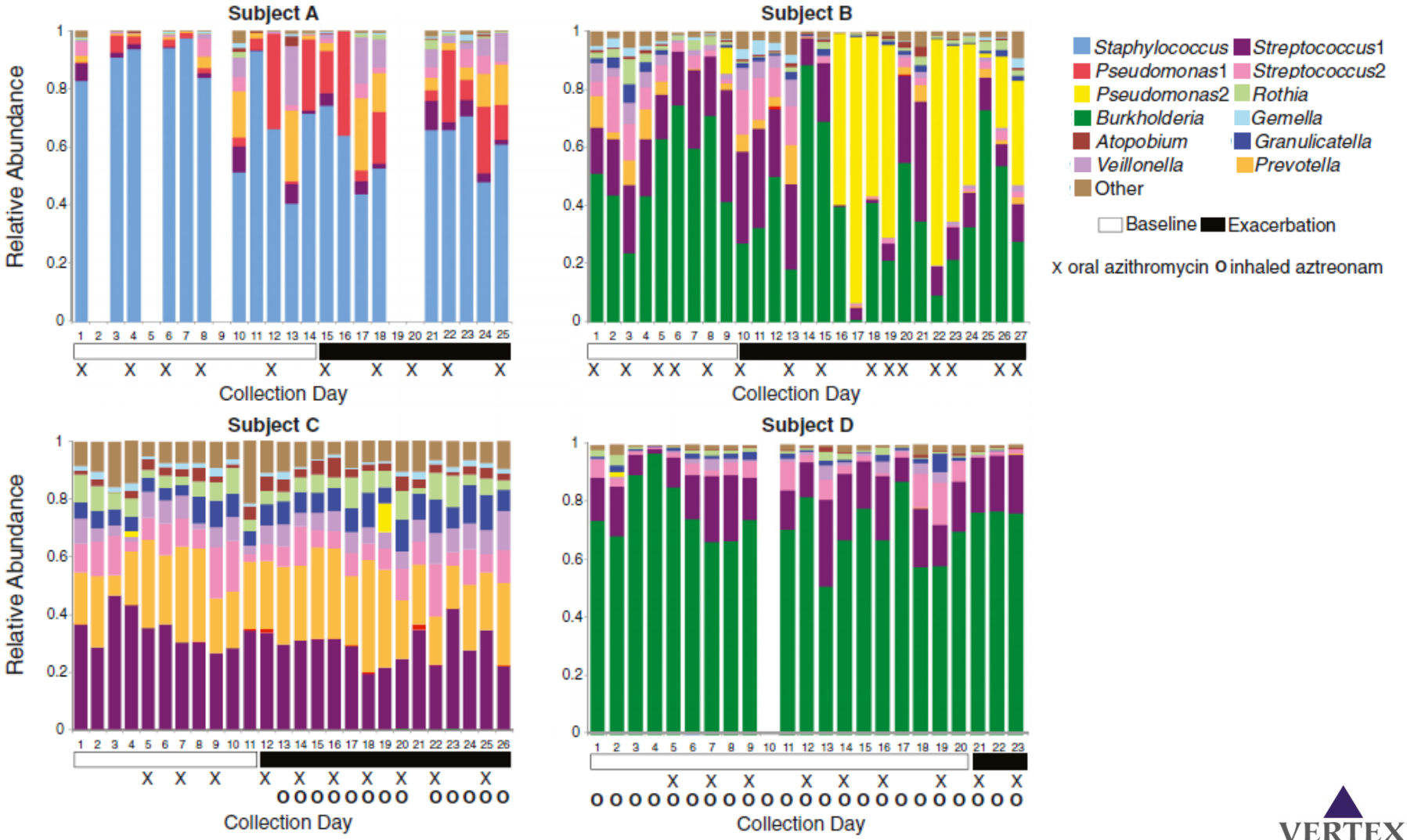
- *P. aeruginosa* accounted for 70.6% of the total abundance and was present in 13/14 patients
- The less predominant "satellite" group, while comprised of 67 different bacterial taxa, only accounted for 11.1% of the total abundance, and was found only in a subset of patients

van der Gast CJ et al. *ISME J.* 2011;5780-5791.

VERTEX

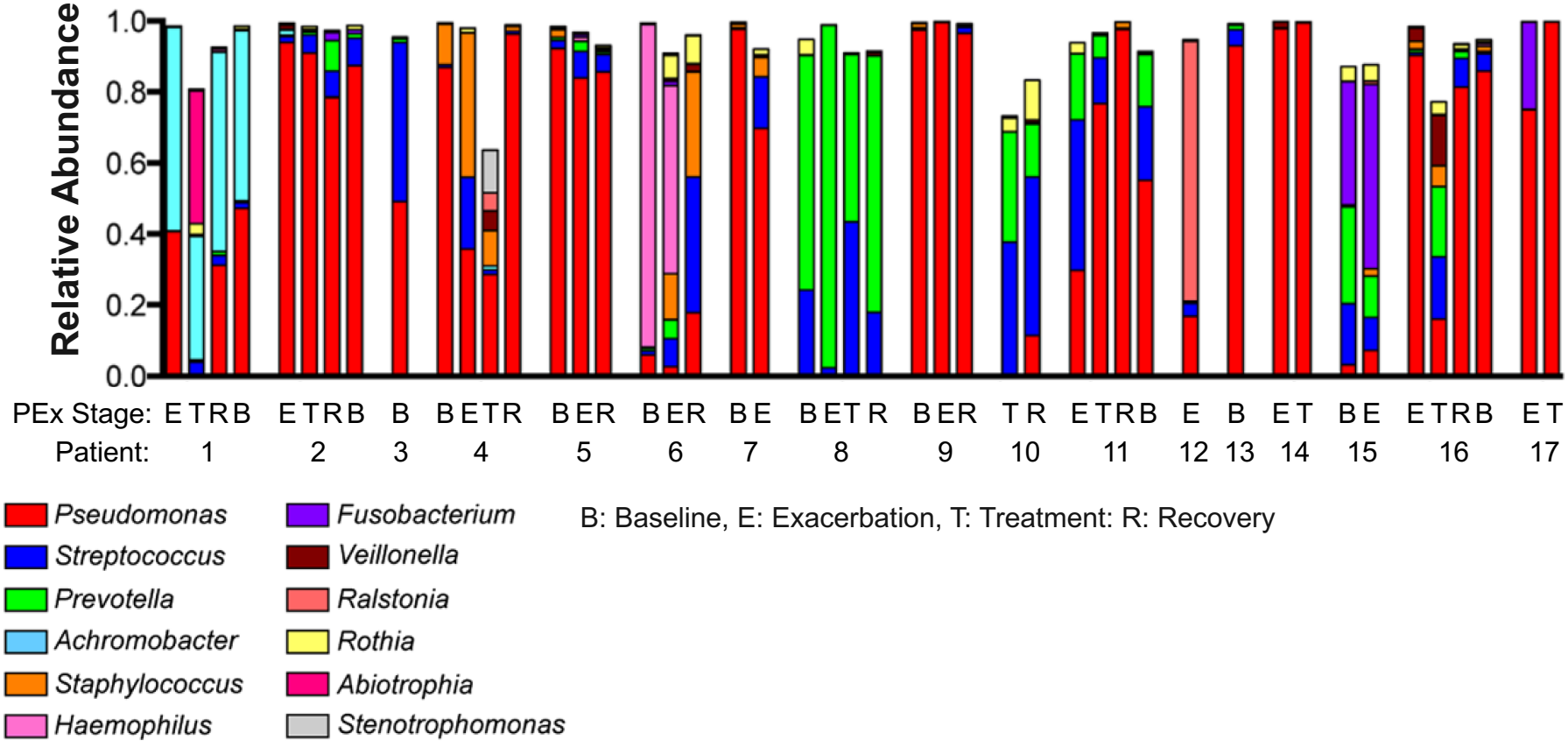
Microbiome Composition Is Unique to Each Individual With CF

Relative Abundance of Top Operational Taxonomic Unit (OTUs) in Daily Samples



Carmody LA et al. *Microbiome*. 2015;3:12. doi: 10.1186/s40168-015-0074-9.

Individuals' Microbiome Compositions Are Unique Across Clinical Stage



- The microbiome of each individual at exacerbation is more similar to the same individual's microbiome when stable than to other individuals' microbiomes at exacerbation

Price EK et al. *Microbiome.* 2013;1:27.

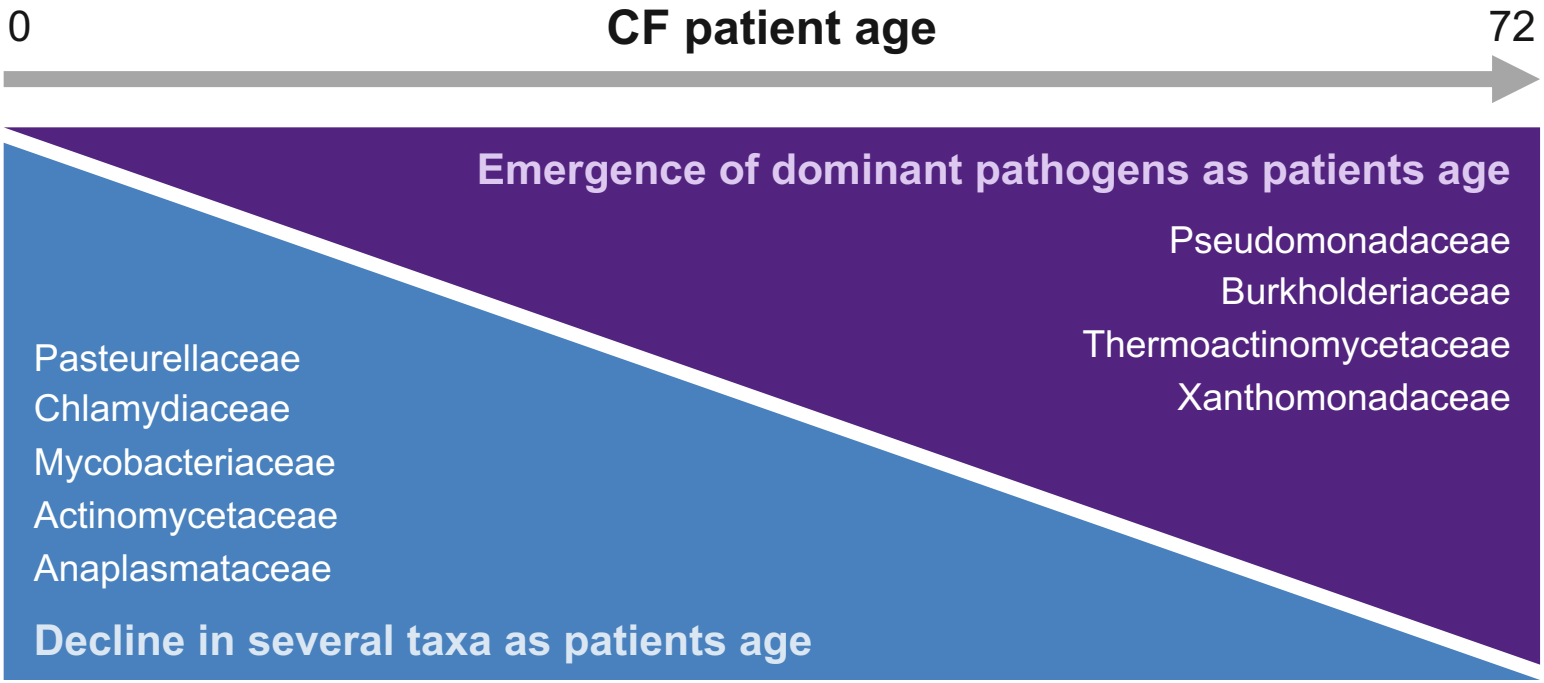
Markers of Worse Clinical Outcomes

Certain Changes in the CF Airway Microbiome Are Associated With Worse Clinical Outcomes

⬇ Microbial Diversity	Chronic pathogen colonization	Host-microbe interactions	Microbe-microbe interactions*
• Decreased lung function[1]	• Decreased lung function[1] • Increased inflammation[2] • Increased mortality[3]	• Increased inflammation[2]	• Worse lung function[4,5] • More frequent PEx[4] • Increased rate of lung function decline[5,6]

1. Cox MJ et al. *PLoS One*. 2010;5(6):e11044. 2. Lund-Palau H et al. *Exp Rev Respir Med*. 2016;10(6):685-697. 3. Courtney JM, et al. *Pediatr Pulmonol*. 2007; 42:525 532. 4. Limoli DH et al. *Eur J Clin Microbiol Infect Dis*. 2016;35(6):947-953. 5. Folescu TW et al. *BMC Pulm Med.* 2015;15:158. doi: 10.1186/s12890-015-014. 6. Maliniak ML, et al. J Cyst Fibros. 2016;15:350-356.

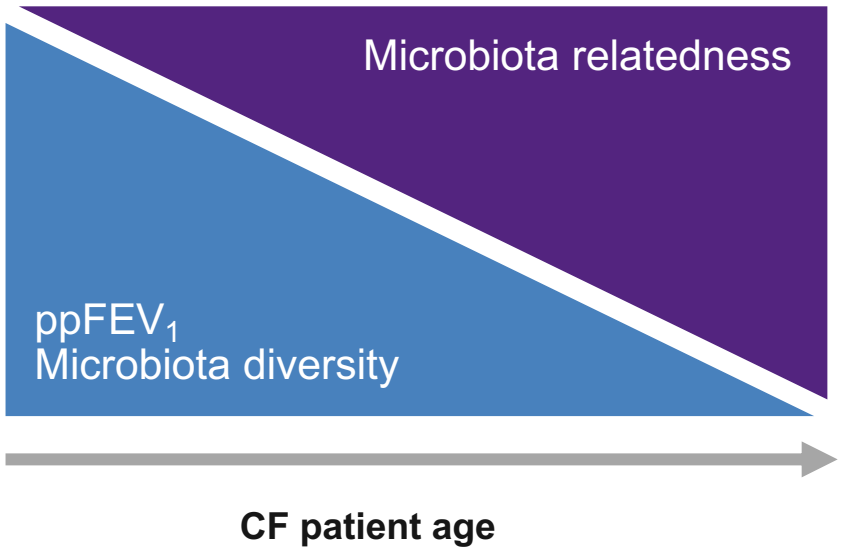
Domination of the Microbiome With Specific Pathogens Occurs Over Time as Patients With CF Age



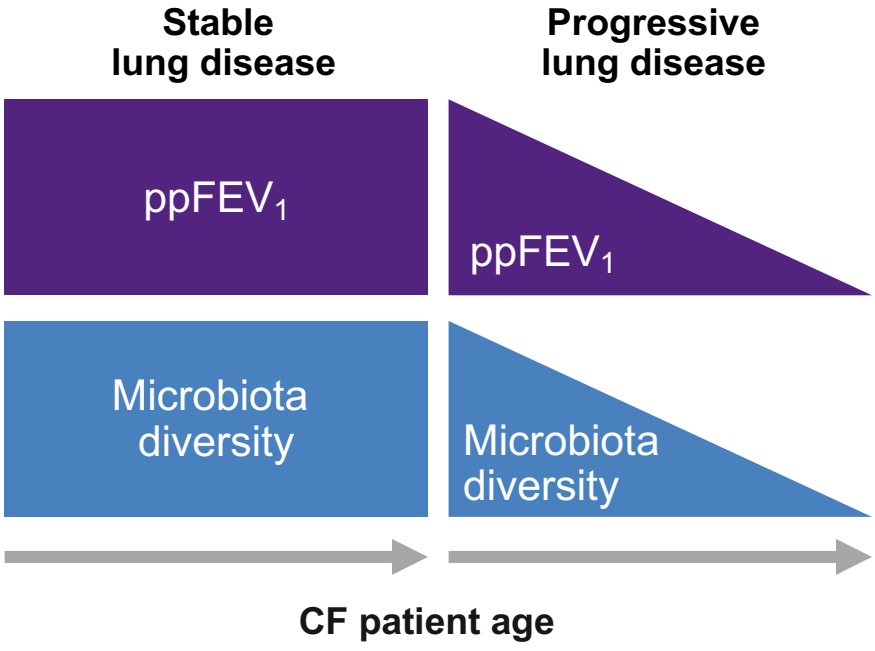
Cox MJ et al. *PLoS One*. 2010;5(6):e11044.

Decreasing Microbial Diversity With CF Patient Age Is Associated With Declining Lung Function

Overall changes with age in patients with CF[1]



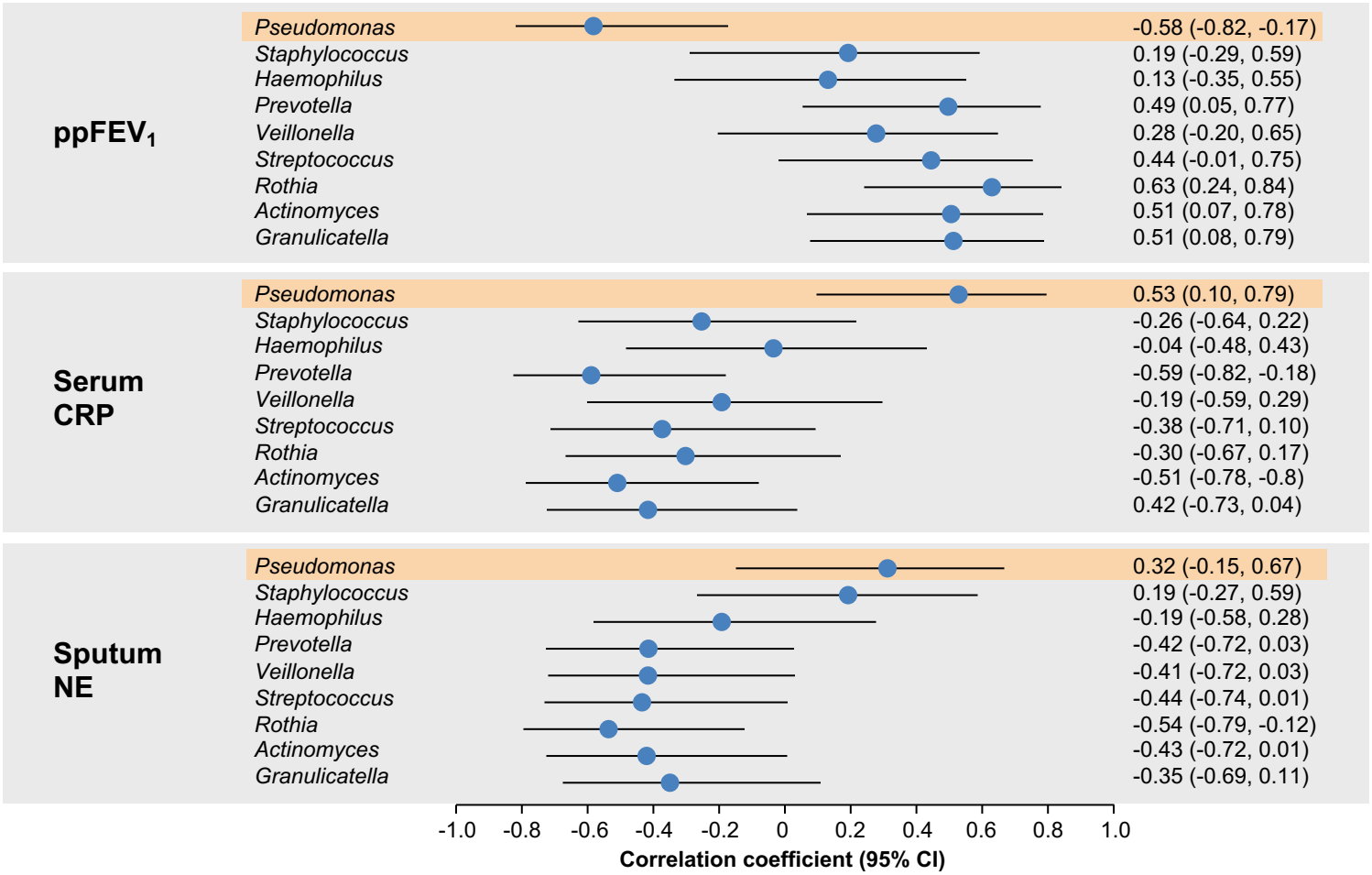
Changes with age in patients with CF by lung disease phenotype[2]



1. Cox MJ et al. *PLoS One.* 2010;5(6):e11044. 2. Zhao J et al. *Proc Natl Acad Sci U S A.* 2012;109(15):5809-5814.

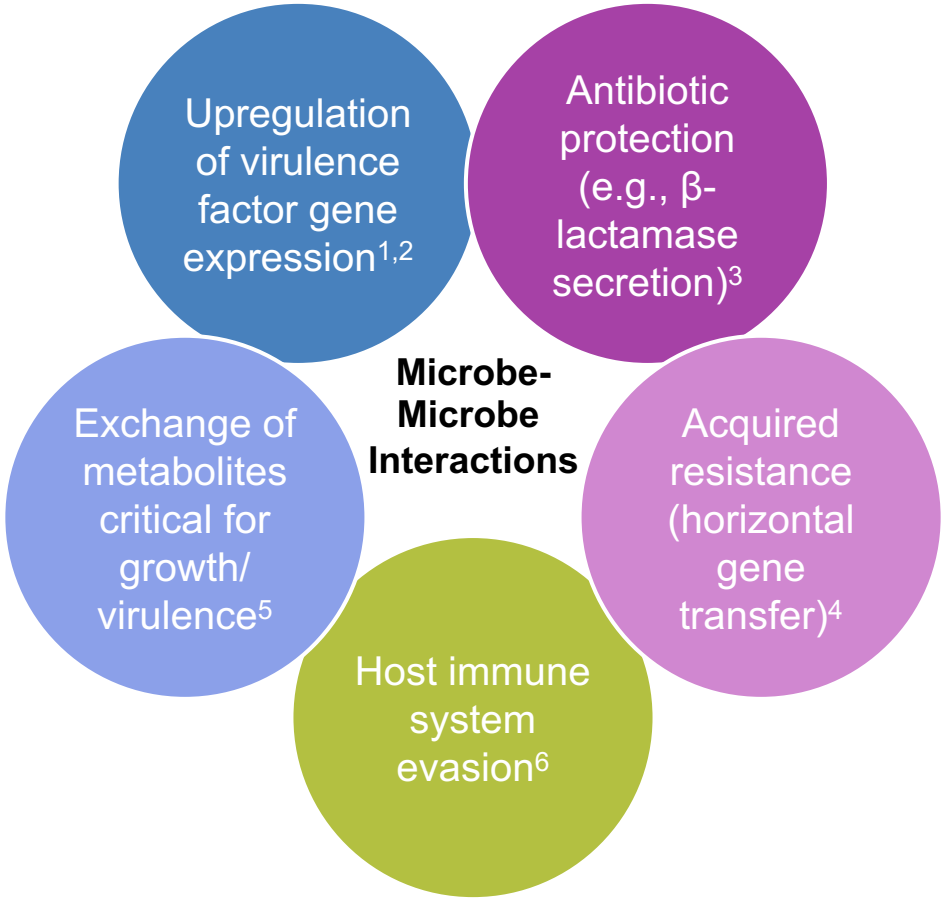
Higher *P. aeruginosa* Relative Abundance Correlates With Lower $ppFEV_1$ and Increased Inflammation During PEx

Relation between relative abundance of genera, lung function, and inflammatory biomarkers at early treatment of PEx



CRP, C-reactive protein; NE, neutrophil elastase.
Zemanick ET al. *PLoS One.* 2013;8(4):e62917.

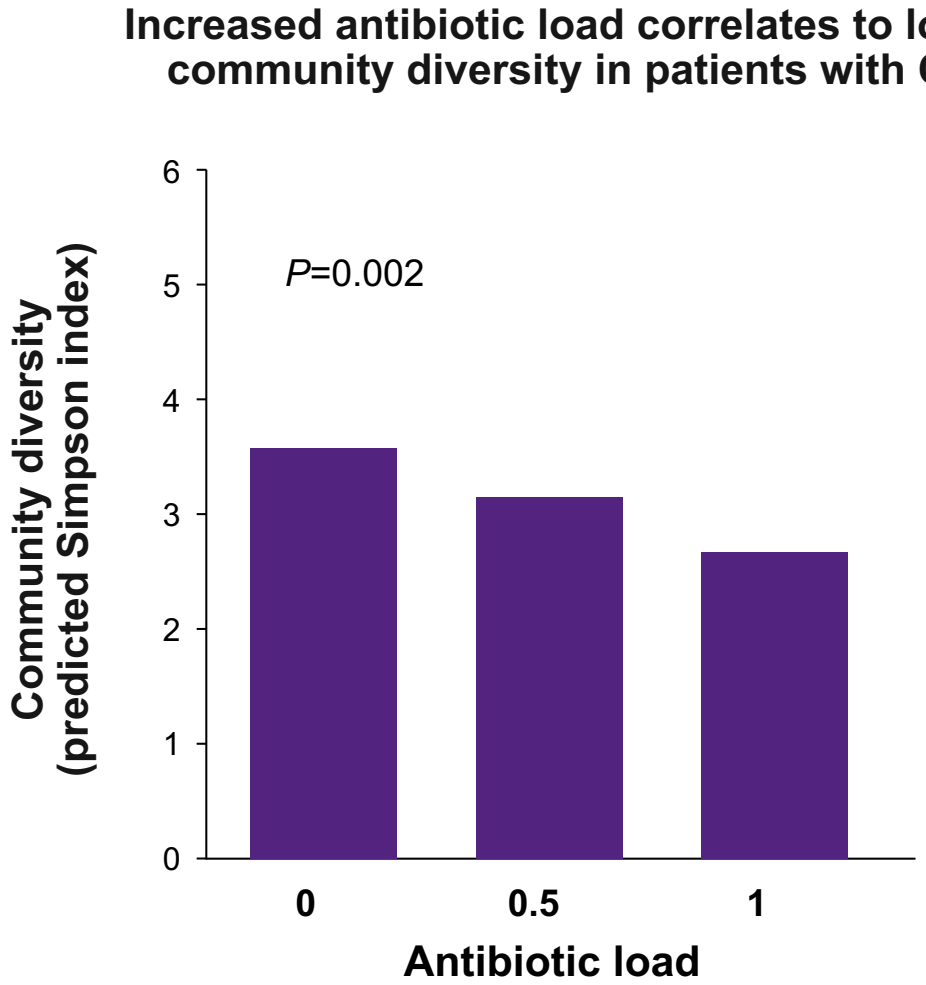
Types of Microbe-Microbe Interactions That Could Worsen Outcomes During Co-Infection in CF



1. Sibley CD et al. *PLoS One*. 2008;4(10):e1000184. 2. Duan K et al. *Mol Microbiol*. 2003;50(5):1477-1491. 3. Sherrard LJ et al. *Int J Antimicrob Agents*. 2016;47(2):140 145. 4. Munita JM, Arias CA. *Microbiol Spectr*. 2016;4(2). doi:10.1128/microbiolspec.VMBF-0016-2015. 5. Hammer ND et al. *Cell Host Microbe*. 2014;16(4):531-537. 6. Armbruster CR et al. *MBio*. 2016;7(3):e00538-16. doi:10.1128/mBio.00538-16.

The Response of the CF Lung Microbiome to Antibiotic Therapy

Antibiotics Are Associated With Declining Airway Microbial Diversity

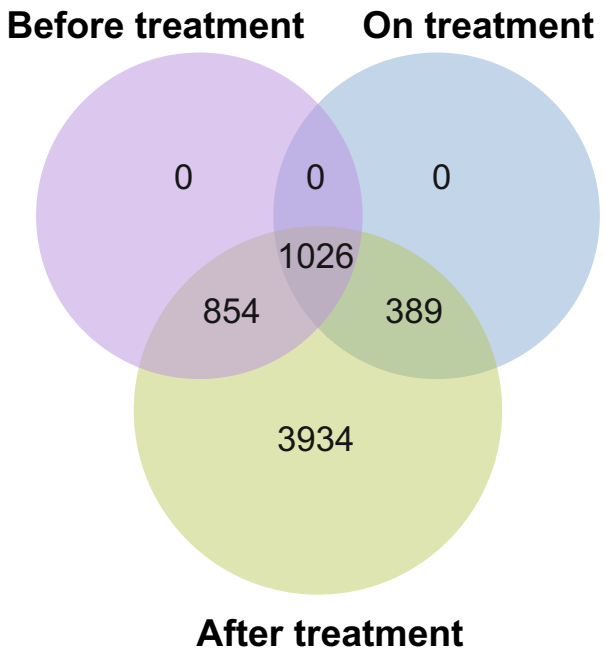


Antibiotic load consisted of three components

- Dosing duration
- Timing of administration relative to sample collection
- Antibiotic type and route of administration

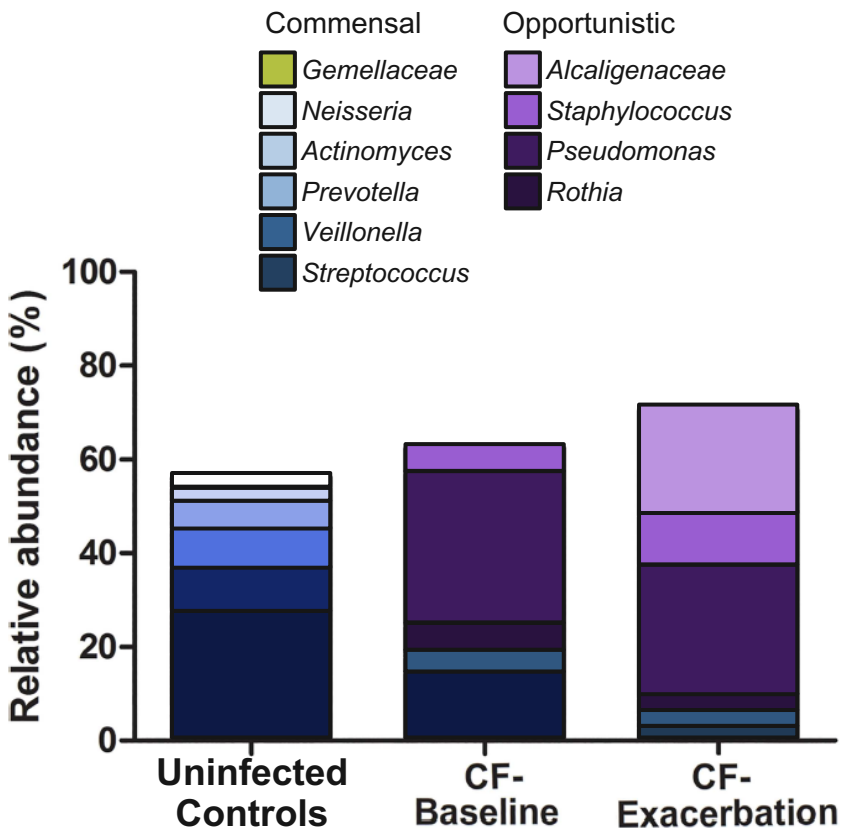
Zhao J et al. *Sci Rep.* 2014;4:4345. doi: 10.1038/srep04345.

Antibiotic Treatment Causes Limited Effects on the Dominant Opportunistic Bacteria But Significant Effects on the Commensal Bacteria



- Of the 3394 distinct taxonomies after antibiotic treatment for PEx
  - 1.7% (58/3394) were shared with healthy controls
  - 66.7% (2263/3394) were shared with CF baseline

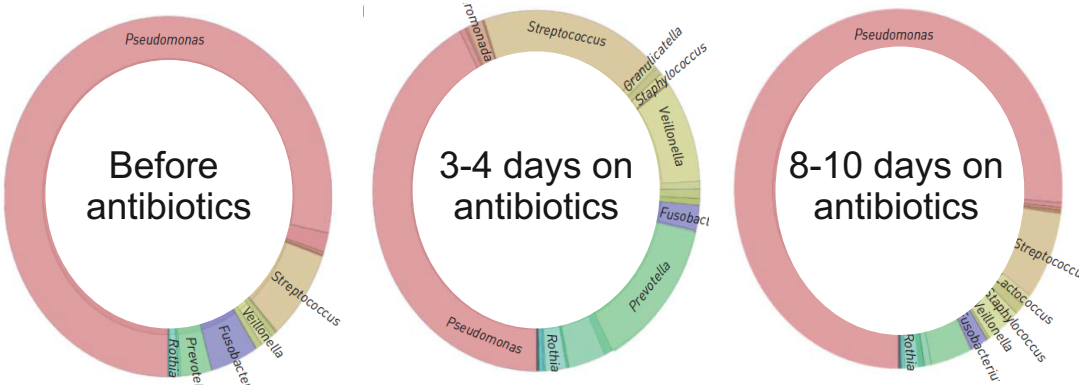
Rank Abundance of Dominant Bacteria in the Microbiota of Healthy Individuals and CF Patients



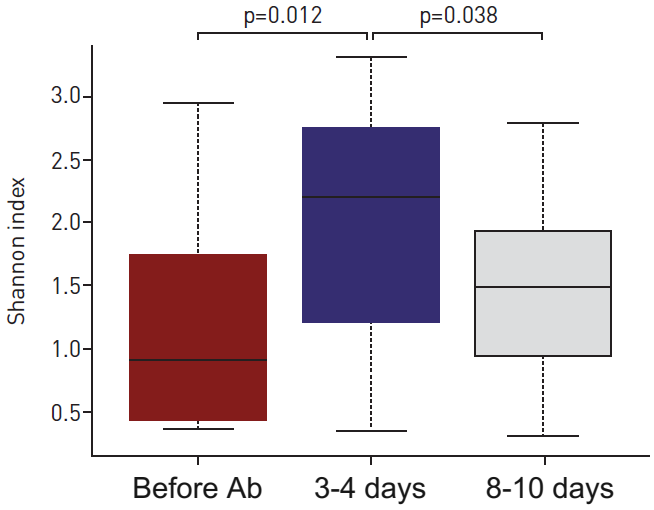
Li J et al. *PLoS One.* 2016;11(10):e0164510.

Changes in CF Airway Microbiome in Response to IV Antibiotics Are Transient

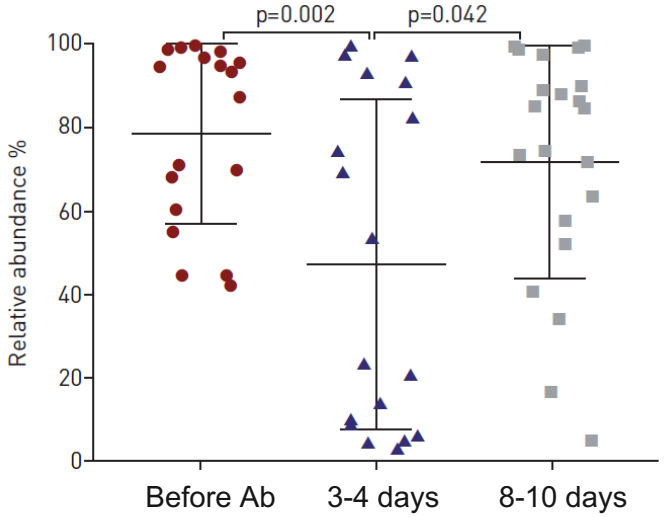
Changes in CF sputum microbiota during PEx



Changes in community diversity during PEx



Changes in relative abundance of *Pseudomonas* during PEx

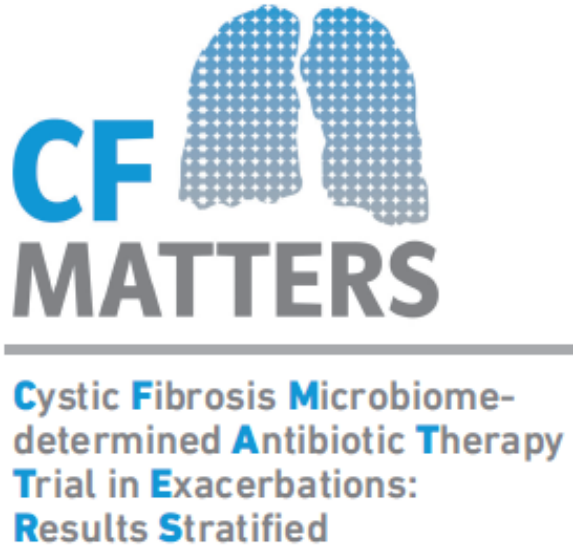


Ab, antibiotics.

 European Respiratory Journal Oct 2014, 44 (4) 922-930; DOI: 10.1183/09031936.00203013

Future Directions

State of the Art CF Microbiome Personalized Medicine Study[1,2]



Each patient with CF has a microbiome sputum analysis conducted

Standard treatment (ceftazidime + tobramycin)

Standard treatment + third antibiotic based on microbiome analysis

Primary outcome: Percentage change in recovery (post-exacerbation) FEV_1

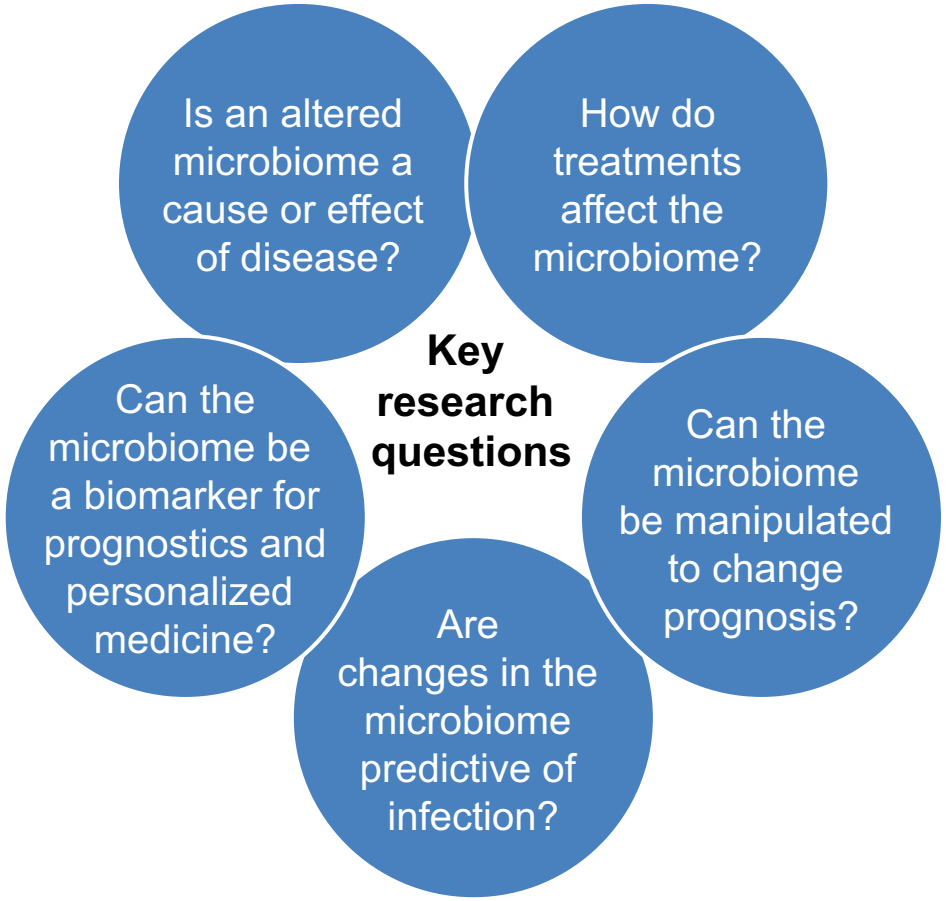
The goal is to reduce antibiotic usage and optimize dosage and duration strategies. Therapies are tailored to each individual patient's needs, resulting in fewer adverse effects and reducing the emergence of drug resistance

1. Clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/NCT02526004. Accessed April, 2020. 2. CF Matters. Cystic Fibrosis Microbiome-Determined Antimicrobial Therapy Trial in Exacerbations: Results Stratified. https://www.cfmatters.eu. Accessed April, 2020.

Challenges in Integrating Microbiome Analysis Into Routine Clinical Management

Standardizing sampling and analysis

Sample procurement	• Sputum • BAL
Generating profiles	• DNA extraction • 16S rRNA gene amplification
Data processing	• Minimize spurious signals • Taxa identification
Data analysis	• Richness • Evenness • Similarity



Rogers GB et al. *Thorax*. 2015;70(1):74-81.

Summary

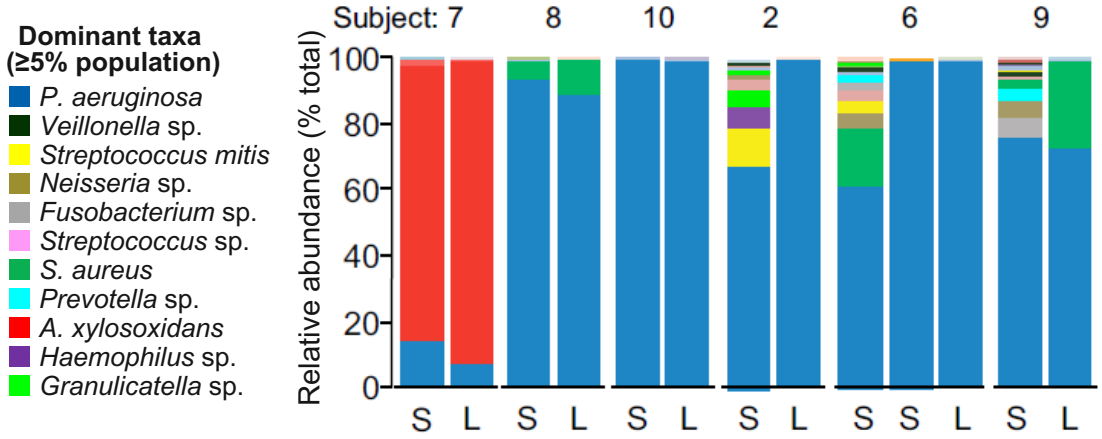
- The defect in CFTR leads to acidic pH in the ASL, contributing to mucus abnormalities, defects in mucociliary clearance, inflammation, and lung infection[1,2]
- Characterization of the airway microbiome can be done using culture-dependent and culture-independent methods to identify pathogens[3]
- There is significant heterogeneity of the airway microbiome across individuals regardless of lung health status[4]
- Patients with CF have lung microbiomes with lower diversity compared with healthy non-CF individuals[5]
- Lung microbiome diversity in patients with CF declines with age, and *P. aeruginosa* becomes the dominant species in older patients[6]
- Microbe-host interactions contribute to inflammation and worsen lung function[7]
- Microbe-microbe interactions affect clinical outcomes in patients with CF[8-10]
- The microbiome may serve as a reservoir of antibiotic resistance[5]
- Microbiome studies may lead to better prognostics and therapeutics[11]

1. Tang XX et al. *J Clin Invest*. 2016;126(3):879-891. 2. Stoltz DA, et al. *N Engl J Med*. 2015; 372:351-62. 3. Sibley CD et al. *PLoS One*. 2011;6(7):e22702. 4. Price EK et al. *Microbiome*. 2013;1:27. 5. Li J et al. *PLoS One*. 2016;11(10):e0164510. 6. Cox MJ et al. *PLoS One*. 2010;5(6):e11044. 7. Zemanick ET al. *PLoS One*. 2013;8(4):e62917. 8. Limoli DH et al. *Eur J Clin Microbiol Infect Dis*. 2016;35(6):947-953. 9. Folescu TW et al. *BMC Pulm Med*. 2015;15:158. doi: 10.1186/s12890-015-014. 10. Hudson VL et al. *J Pediatr*. 1993;122(6):854-860. 11. Clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/NCT02526004. Accessed December 15, 2017

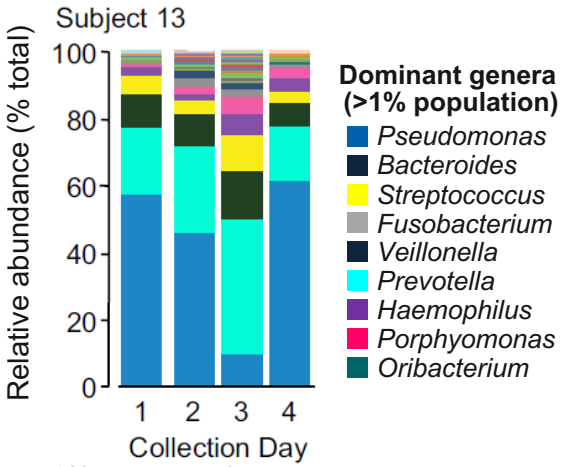
Backup

Interpretation Challenges in Microbiome Studies Arise From Sampling Sites and Temporal Variability

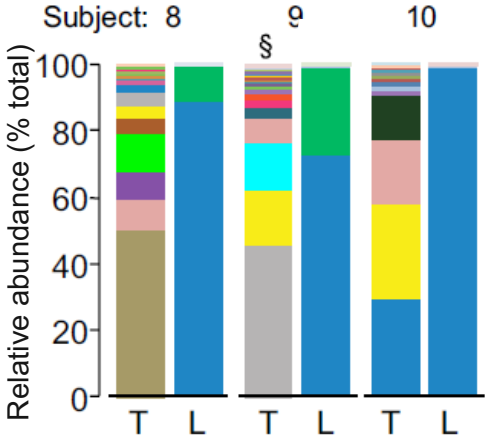
Sputum (S) samples identify the dominant organism but can overestimate diversity



Sputum samples show day-to-day variation



Throat (T) samples poorly reflect lung (L) microbiota



- Throat, sputum, and lung samples are subject to variable oropharyngeal contamination
- In a study comparing samples taken directly from lung tissue (after transplant) to sputum samples; 3 of 7 sputum samples contained an array of atypical organisms

Goddard AF et al. *Proc Natl Acad Sci U S A*. 2012;109(34):13769-13774.

