The Microbiome of the Healthy and Diseased Lung:  
*Is there role for prebiotics or probiotics?*

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The Airway Microbiome

- The upper airways are home to a diverse microbiota.
  - high carriage rates of pathogens
  - reservoir for antibiotic resistance/virulence genes
  - the interface with the environment

- Respiratory infections account for more morbidity and mortality in both the developed and developing world than diarrheal disease

- Exacerbation of chronic airways diseases are the most common reason for emergency room visits in the developed world.
The Upper Respiratory Tract Microbiome

Human Microbiome Project (HMP)
- define “healthy microbiome profiles at multiple body sites

Respiratory Infections

HMP Adults

*Age information not reported for three cases.
The Upper Respiratory Tract Microbiome Shifts and Loses Spatial Distinctions and Complexity in Healthy Elderly

A. PCoA of HMP and HNC using Weighted UniFrac

HMP Adult Nose

HMP Adult Throat

Participant Group
- HMP (Adult)
- NHC (Elderly)

Swab Biogeography
- Red: Anterior nares
- Blue: Oropharynx

HMP = human microbiome Project data
(242 healthy adults, 18 to 40 yrs, http://hmpdacc.org)

F. Whelan (2014)
The Upper Respiratory Tract Microbiome Shifts and Loses Spatial Distinctions and Complexity in Healthy Elderly

F. Whelan (2014)
OP/NP swabs from healthy children and parent

The OP swabs form a tight cluster indicating similar microbial communities in all samples (children and their parents).

The NP swabs are distinct from the OP swabs but are more dispersed and some but not all children cluster with the adults.

J. Stearns et al 2015
The dominant groups of bacteria separated the two major groups of young children and adult nasopharyngeal microbiomes.
The dominant groups of bacteria separated the two major groups of young children and adult nasopharyngeal microbiomes.

This is consistent with a maturation of the NP microbiome to adult-like between age 1-3 yrs.
The total bacterial burden decreases as in the nasopharynx decreases as the microbiome matures.

This data is by culture but true also by qPCR.

Anecdotally it appears that it may drop even further in the elderly - which will test in our next study.
The maturation and aging of the healthy URT microbiome

- Throats 1-5 year olds and their parents are the same.

- The noses of young kids mature to an adult microbiota in 1-3 yrs of age – this coincides with maturation of the gut microbiome and immune system.

- The bacterial burden of the nasal cavity decreases as the microbiome matures.  
  \[\text{Stearns et al 2015}\]

- The spatial distinction between the nose and throat of the elderly is lost.  
  \[\text{Whelan et al 2014}\]

- The elderly microbiome is not temporally stable and strongly influenced by co-morbidities and immune status.  
  \[\text{Verschoor et al in prep.}\]
NP swabs for children 3 and 12 month and during an acute respiratory infections (ARI)

Clinical Microbiology (qPCR)

For *S. pneumoniae*

Carriage < $10^4$ genomes/ml

Infection > $10^4$ genomes/ml

Microbiome Profiling –
e.g. *Moraxella*, dominant bacterial taxa in both healthy and acute infections samples.

Often hard to tell when a pathogen is behaving a pathogen or a commensal

PJ Subbarao et al CHILD
The Airway Microbiome

The upper airways are home to a diverse microbiota.

The lower airways are thought to be effectively sterile.

• We breathe in ~about 100,000 bacteria/day.

• A transient or stable lung microbiome in healthy individuals?
  
  Morris et al AJRCCM (2013), Segal et al. Microbiome (2013)

• Any immune compromise sets the lung up for rapid colonization/infection, and constant re-inoculation in chronic airway disease.
In chronic airway disease, it is now well established that the lower airways are *colonized* by a polymicrobial community.
Severe Asthma samples characterized by high neutrophil and low eosinophil. (Param Nair)

“Culture negative” by clinical microbiology.

* The CF, asthma and healthy samples all cluster into distinct communities.

An increase in anaerobes a signature of disease?
The top 10 most common genera recovered from the lower airways in chronic disease (CF, asthma, COPD...).

*Streptococcus*
*Prevotella*
*Veillonella*
*Rothia*
*Actinomyces*
*Gemella*
*Granulicatella*
*Fusobacterium*
*Neisseria*
*Atopobium*

This group of organisms are present in all lower airway disease and seem to be particularly well adapted to growth in the lungs.
Acute Airway Infections
e.g. Etiology of community-acquired pneumonia

“Defining the etiology of pneumonia is a messy business.”

The Modern Quest for the “Holy Grail” of Pneumonia Etiology
Seema Jain, and Andrew T. Pavia
Clinical Infectious Diseases 2016
Acute Airway Infections
e.g. Etiology of community-acquired pneumonia


“...A total of 700 patients with etiologic evaluation were included: 276 hospitalized and 424 ambulatory patients. We were able to define the aetiology of pneumonia in 55.7% (390/700). “

Clin Inf Dis. 2010:50 (15 January) Etiology of Community-Acquired Pneumonia: Increased Microbiological Yield with New Diagnostic Methods

“A bacterial etiology was found for 106 patients (58%), 9 of whom had more than 1 bacterial species identified (Table 2). The most frequently detected bacteria were S. pneumoniae (70 patients [38%]), M. pneumoniae (15 patients [8%]), H. influenzae (9 patients [5%]), and M. catarrhalis (7 patients [4%]). A viral pathogen was identified for 53 (29%) of the 184 patients.”

(this study threw all their best new diagnostics at these patients)
Respiratory infections

30-60% of bacterial pneumonias are “culture negative”

20% of asthma exacerbations are “culture negative” but the great majority of these are “culture positive” but resolved with antibiotic therapy

COPD exacerbations are often “culture negative” but resolved with antibiotic therapy

Sepsis can be “culture negative” (in this case really negative!)

Conventional pathogens do not appear to account for a large fraction of bacteria associated respiratory disease.

Even when a pathogen is identified, it may not be the most abundant organism and other organisms may contribute to disease phenotype.
Like chronic airway infections, many acute respiratory infections are likely polymicrobial.

.. and the OF and other organisms we don’t recover in clin micro could be important in acute infections as well.
β-Hemolysis of Red Blood Cells is the “calling card” of Most Pathogenic Streptococcus

- 0.3% of “commensal” Streptococcus isolates were β-hemolytic on CBA (Sheep Blood) (n= 300)
- 56% were β-hemolytic on CBA (Human Blood)
- Gemella species 0/96 on sheep vs 86/96 on human blood
- We underestimate the pathogenic potential of oropharyngeal microbiota (OF)
The difference between commensal and pathogen is not always clear – more a gradient than a sharp distinction

Commensal

Pathogen

*Streptococcus pneumonia*

*Staphylococcus aureus*

*Haemophilus influenzae*

*Moraxella catheralis*

...

*Asymptomatic carriage of respiratory pathogens*

Pathobiont - a microbe normally consider a commensal but can turn pathogenic under specific conditions

*More like “resident pathogens”*
The position on this spectrum can be shifted in different strains of the same species

Quim Madrenas et al (Peres et al 2015)

- Both TLR2 dependent, but thru distinct pathways
- Pro-inflammatory p38 dependent (and requires internalization)
- Anti-inflammatory PI3-Akt dependent
- Different strains vary in their ability to activate these two pathways
The *Streptococcus* Milleri/Anginosus Form a Distinct Phylogenetic Group of the Streptococci

*SMG are responsible for more invasive disease than Group A and Group B Streptococcus combined.*

Strain and Donor Heterogeneity in Host Response

• we were unable to distinguish respiratory tract isolates from invasive isolates based on bacterial phenotypes and virulence factor production (Ginwis et al J Clin Micro 2010)

• These organisms are heterogeneous with respect to microbiology (phenotype and genotype variability)

• 36 strains (12 of each species) recovered from a variety of samples (complete genome sequences)

• Human response to SMG by profiling cytokine responses in peripheral blood mononuclear cells (PBMCs)
The SMG fall into 3 groups

The invasive isolates were enriched in the high and intermediate immunogenicity groups

Kaiser et al. 2014
As expected there is significant donor variability. Each donor has a distinct profile but you can see Donor A and B for example do seem to make much IL17A, unlike Donor C.

These are individual donor phenotypes, however there are also modulated by the strain.

Kaiser et al 2014
As expected there is significant donor variability. Each donor has a distinct profile but you can see Donor A and B for example do seem to make much IL17A, unlike Donor C. These are individual donor phenotypes, however there are also modulated by the strain. Here is an example of a strain that induces IL-17 in Donor A and B but does not activate Donor C.
VAP and the ICU

PROSPECT Study – Probiotics to reduce infection rates in ICU

The normal biogeography of the microbiome is severely compromised in the ICU even prior to VAP. *(none of the 45 patients had a normal looking microbiome)*

Baseline samples before intervention and low VAP numbers (still blinded)
Probiotic QC for the PROSPECT Clinical Trial

Each Site in the trial (n=6) would send a capsule of the probiotic for each lot #

Quantitative culture for Lactobacillus, check for consistent colony morphology (occasional testing by RAPD PCR)

Plating on other media for other organisms (including checking outside of capsule)

Although viable organisms dropped with time, CFU/ml was above the minimal CFU/ml at the expiry data

True at all enrolled sites (4 Canadian, 2 US)

Minimal effort but valuable both for reviewers and study!
Probiotic QC for the PROSPECT Clinical Trial

One Adverse Event from PROPSECT
*Lactobacillus* positive blood culture!

We obtained strain from clinical and art.line (recovered one organism by culture- not *Lactobacillus*)

Blood culture isolate was probiotic strain

No subsequent blood cultures were positive

Art-line was not contaminated (no Lactobacillus biofilm!)

Conclusion- skin contamination.

We did all the testing in two days
The Progression of the Microbiome with Age

**Opportunities for Pre/Pro-Biotic Interventions**

**Birth**
- Period of instability, co-evolution of the microbiome and the immune system

**1-3 Years**
- Relatively Stable Microbiome

**Adolescence**
- Resilient – able to rebound from perturbations (infection, other illness, antibiotics, dietary changes...)

**Adulthood**

**Elderly Adults**
- Period of declining stability, loss of resilience, low level inflammation, *de-evolution of the microbiome and the immune system*

Interventions to Altered the Microbiome
(probiotics, prebiotics, dietary changes)
Take Home Messages

• The changing microbiome with age likely contributes to susceptibility and severity of respiratory infections.

• Some respiratory pathogens are missed and *culture negative infections* account for a significant burden of disease.

• Pathogenic potential of commensal microbiota underappreciated.

• Strain level heterogeneity (and individual specific host response) needs to be considered when mining the human microbiome for *beneficial* organisms.

• Opportunities for pre-/pro-biotic interventions to reduce respiratory infections and promote healthy aging in the respiratory tract.