Why do we care about the nasopharyngeal microbiota?

- Pneumonia – bacterial and influenza – is a leading cause of death in the United States and worldwide
  - 1.3 million child deaths annually (O’Brien, et al, Lancet 2009)
- We believe the upper respiratory tract flora informs, to a large extent, the microbiota of the lower respiratory tract (LRT) and is a precursor to LRT infections (e.g., pneumonia)
- Involved in maintenance and dissemination of pathogens across the population
- May also govern the acquisition of antibiotic resistance genes among bacteria from different genera
“The Big Four” in the nasopharynx

- *Haemophilus influenzae*
- *Streptococcus pneumoniae*
- *Staphylococcus aureus*
- *Moraxella catarrhalis*
- *Haemophilus influenzae*

List of members of the “normal” bacterial flora in the nose and oropharynx (partial)

- Staph epi
- Propionobacteria
- Staph aureus
- Streptococcus pneumoniae
- Strept pyogenes
- Neisseria spp (including meningitidis)
- Haemophilus influenzae
- Mycoplasma
- Corynebacterium diphtheriae (less common member of the normal flora after vaccination)

Our bodies are “colonized” with potentially pathogenic bacteria.
Unanswered questions

• How does bacterial colonization happen in the first place by potentially pathogenic bacteria
  • Host factors
• How do interspecies interactions alter bacterial composition (bacteria-bacteria, viral-bacteria)
• How do environmental factors alter the nasal flora?
  • Temperature
  • Humidity
  • Pollution
  • Cigarette smoke
  • Antibiotics
• How does microbe transition from colonizer to invader
Unanswered questions

- How does bacterial colonization happen in the first place by potentially pathogenic bacteria
  - Host factors
- How do interspecies interactions alter bacterial composition (bacteria-bacteria, viral-bacteria)
- How do environmental factors alter the nasal flora?
  - Temperature
  - Humidity
  - Pollution
  - Cigarette smoke
  - Antibiotics
- How does microbe transition from colonizer to invader
- How intranasal vaccine alters bacterial composition in the nose
  - Role of the host immune response
Nasopharyngeal microbiota

- The community is established in the first year after birth
- Varies throughout lifetime
- High inter-individual variability

Children (6 mos. to 6 years old in Philadelphia
- 69% African-American
- 88% completed PCV7 vaccine
- All had URI symptoms

**TABLE 1** Most frequent nasal swab OTUs\(^a\)

<table>
<thead>
<tr>
<th>OTU</th>
<th>Frequency (%)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unclassified <em>Moraxellaceae</em></td>
<td>19.00</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>17.86</td>
</tr>
<tr>
<td><em>Corynebacterium</em></td>
<td>7.04</td>
</tr>
<tr>
<td><em>Moraxella</em></td>
<td>6.46</td>
</tr>
<tr>
<td><em>Haemophilus</em></td>
<td>4.66</td>
</tr>
<tr>
<td>Unclassified <em>Pasteurellaceae</em></td>
<td>4.09</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>3.84</td>
</tr>
<tr>
<td><em>Acinetobacter</em></td>
<td>3.44</td>
</tr>
<tr>
<td><em>Dolosigranulum</em></td>
<td>3.21</td>
</tr>
<tr>
<td><em>Propionibacterium</em></td>
<td>3.13</td>
</tr>
<tr>
<td>Unclassified <em>Proteobacteria</em></td>
<td>2.59</td>
</tr>
<tr>
<td><em>Lactococcus</em></td>
<td>2.58</td>
</tr>
<tr>
<td><em>Neisseria</em></td>
<td>1.45</td>
</tr>
<tr>
<td><em>Actinomyces</em></td>
<td>1.24</td>
</tr>
<tr>
<td><em>Rothia</em></td>
<td>1.13</td>
</tr>
<tr>
<td><em>Veillonella</em></td>
<td>1.05</td>
</tr>
</tbody>
</table>

\(^a\) Frequency of ≥1%.
\(^b\) Percentage of total sequences per nasal microbial community, i.e., per child.
Nasal microbiota composition

Danish Twin Registry study: (2015)

- Adults in Denmark
- Median nasal bacterial density \( \approx 4 \times 10^6 \) 16S rRNA copies per nasal swab (range \( 6.7 \times 10^5 \) to \( 2.1 \times 10^9 \) copies)
  - Women had less than half the nasal density of men (2.97 vs. \( 7.94 \times 10^6 \) copies)
- Most ubiquitous bacterial taxa are:
  - Corynebacterium (88.2%)
  - Propionibacterium acnes (83.7%)
  - Staphylococcus epidermidis (90.4%)

*Liu et al, Science Advances, 2015*
Nasal microbiota composition

Danish Twin Registry study: (2015)

Important themes

- The microbiome of a particular site is a community, where the number of pathogens are kept in check.
- The inhabitants of individual communities can look very different from persons to person or from body niche body niche, but the communities function similarly in the healthy state.
- When that community is perturbed in such a way that you have elimination of the normal inhabitants, you have proliferation of the bad actors, and perhaps even the emergence of newly acquired pathogens.
Effects of pneumococcal vaccine

• Nasopharyngeal colonization precedes bacterial pneumonia and otitis media
  • Invasive diseases (septicemia, meningitis)
  • Children often carry multiple serotypes
• Several *S. pneumoniae* vaccines in clinical use
  • Pneumococcal polysaccharide-based (PPS)
    • Pneumovax – 23 polysaccharide
  • Pneumococcal conjugate vaccines
    • PCV7, PCV10, PCV13 (Prevnar)
• PCV programs have been successful in decreasing incidence of pneumococcal diseases... but eliminating the strain-specific serotypes in vaccine is followed by emergence of non-vaccine serotypes in the population
  • New clones become more evident
  • Capsule switching
Table 2
Effects of pneumococcal vaccination on nasopharyngeal carriage rates of *S. aureus*, *H. influenzae*, and *M. catarrhalis* in children.

<table>
<thead>
<tr>
<th>Study [ref]</th>
<th>Study description</th>
<th>Ages examined</th>
<th><em>S. aureus</em></th>
<th><em>H. influenzae</em></th>
<th><em>M. catarrhalis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Madhi et al. 2007 [45]</td>
<td>Randomised controlled trial of PCV9 in South Africa</td>
<td>Mean age 5.64 years (5.3 years after third dose of vaccine)</td>
<td>No differences in carriage between PCV9 and placebo groups</td>
<td>No differences in carriage between PCV9 and placebo groups</td>
<td>ND</td>
</tr>
<tr>
<td>Prymula et al. 2009 [69]</td>
<td>Randomised controlled trial of PCV11 in Czech Republic and Slovakia</td>
<td>6, 12–15, 13–16, 15–18, 19–22, and 24–27 months</td>
<td>ND</td>
<td>Lower carriage in the PCV11 group (10%) compared to control (18%) at 15–18 months; no longer significant when molecular assays differentiating NTHi and <em>H. haemolyticus</em> applied</td>
<td>ND</td>
</tr>
<tr>
<td>Lee et al. 2009 [33]</td>
<td>Prospective observational study in two time periods following PCV7 introduction (2–3 and 5–6 years post-PCV7) in the United States</td>
<td>Mean age 2.7 years</td>
<td>Carriage rate remained stable at 14% in both time periods examined</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>van Gils et al. 2011 [65,66]</td>
<td>Randomised controlled trial of PCV7 in the Netherlands</td>
<td>6 weeks and 6, 12, 18, and 24 months</td>
<td>Higher carriage in the 2 + 1 dose group (10%) compared to unvaccinated controls (5%) at 12 months</td>
<td>No differences between vaccinated children and unvaccinated controls</td>
<td>No differences between vaccinated children and unvaccinated controls (5%) at 12 months</td>
</tr>
<tr>
<td>Prymula et al. 2011 [70]</td>
<td>Randomised controlled trial of PCV10 in Czech Republic</td>
<td>12–15, 13–16, 15–18, 19–22, and 24–27 months</td>
<td>ND</td>
<td>Lower carriage of NTHi (differentiated from <em>H. haemolyticus</em>) in the PCV10 group (10%) compared to unvaccinated controls (16%) at 24–27 months</td>
<td>ND</td>
</tr>
<tr>
<td>Dunne et al. 2012 [59]</td>
<td>Randomised controlled trial of PCV7 with or without 23 valent polysaccharide booster (23vPPS) in Fiji</td>
<td>17 months</td>
<td>ND</td>
<td>No differences in carriage between PCV7 (with or without 23vPPS) and unvaccinated controls</td>
<td>No differences in carriage between PCV7 (with or without 23vPPS) and unvaccinated controls</td>
</tr>
<tr>
<td>Ho et al. 2012 [64]</td>
<td>Cross-sectional study in Hong Kong</td>
<td>Mean age 3.9 years</td>
<td>No difference in carriage between PCV7 vaccinated and unvaccinated children</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Dukers-Muijres et al. 2012 [32]</td>
<td>Cross-sectional study in the Netherlands</td>
<td>6 weeks to 4 years</td>
<td>No difference in carriage between PCV7 vaccinated and unvaccinated children</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Spijkerman et al. 2012 [63]</td>
<td>Cross-sectional study in two time periods following PCV7 introduction (3 and 4–5 years post-PCV7) compared to pre-PCV7 data in the Netherlands</td>
<td>11–12 months and 24 months</td>
<td>Higher carriage in both post-PCV7 time periods (9% and 14%) compared to pre-PCV7 (9%) at 11–12 months</td>
<td>Higher carriage in both post-PCV7 time periods at 11–12 months (68% and 65% post-PCV7 compared to 46% pre-PCV7) and at 24 months (73% and 76% post-PCV7 compared to 52% pre-PCV7)</td>
<td>Higher carriage 4–5 years post-PCV7 (80%) compared to pre-PCV7 (50%) at 24 months</td>
</tr>
</tbody>
</table>

Note: Only statistically significant differences are reported. ND = not determined.
Conclusions from a number of epidemiological studies:
- Widespread use of PCV has coincided with increased incidence of MRSA infections
- Carriage of *S. aureus* has been shown to increase or not change following introduction of PCV
  - $\text{H}_2\text{O}_2$ produced by *S. pneumoniae* kills *S. aureus*?
  - No study shows significant association between *S. pneumoniae* and *S. aureus* carriage
- *S. pneumoniae* carriage does appear to be positively associated with *H. influenzae* carriage and *Moraxella catarrhalis* in most studies
  - Serotype specific
  - PCV vaccine study in Netherlands: (Spijkerman *Plos One* 2012)
    - Vaccine strains of Sp decreased; increase in non-vaccine strains
    - *H. influenzae* prevalence increased

*S. aureus* carriage does appear to be positively associated with *H. influenzae* carriage and *Moraxella catarrhalis* in most studies

Serotype specific
- PCV vaccine study in Netherlands: (Spijkerman *Plos One* 2012)
  - Vaccine strains of Sp decreased; increase in non-vaccine strains
  - *H. influenzae* prevalence increased

*Dunne et al, Vaccine* (2013)
Changes in the nasopharyngeal microbiome after PHiD-CV in Kenyan toddlers

Table 2. Relative abundance of common nasopharyngeal bacterial 16S rRNA sequence types

<table>
<thead>
<tr>
<th>Taxa</th>
<th>All Subjects</th>
<th>PHiD-CV Group (N = 25)(^a)</th>
<th>Control Group (N = 29)(^a)</th>
<th>Day 180-Day0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 0</td>
<td>Day 180</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 180</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 180</td>
<td>Day 180</td>
<td>Comparison (p-value)(^b)</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>56.9% (33.7–70.6)</td>
<td>58.6% (31.4–70.2)</td>
<td>61.7% (46.2–78.3)</td>
<td>53.8% (36.1–70.6)</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>1.6% (0–9.8)</td>
<td>1.6% (0–7.9)</td>
<td>1.0% (0–4.9)</td>
<td>2.0% (0–13.8)</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>12.3% (3.7–24.5)</td>
<td>15.7% (3.4–28)</td>
<td>12% (1–24.6)</td>
<td>9.2% (3.7–18.8)</td>
</tr>
<tr>
<td>Moraxella nonliquefaciens</td>
<td>2.1% (0.6–10)</td>
<td>2.5% (1.2–9.5)</td>
<td>4.0% (0.8–14)</td>
<td>1.4% (0.3–10.2)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>25.9% (15–46.8)</td>
<td>20.1% (11.8–44.8)</td>
<td>18.2% (8.6–46.6)</td>
<td>26.6% (19.9–46.9)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>4.4% (0.2–25.4)</td>
<td>4.0% (0.3–32.3)</td>
<td>10.3% (0.4–37.7)</td>
<td>4.9% (0–21.1)</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>7.8% (1.8–21.6)</td>
<td>8.5% (1.5–15.8)</td>
<td>5.1% (0.9–9.2)</td>
<td>6.9% (2.3–22.1)</td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>5.6% (1.7–19.8)</td>
<td>8.5% (0.9–15.4)</td>
<td>3.8% (0.8–7.7)</td>
<td>5.2% (2–21.1)</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>0.4% (0.1–3.8)</td>
<td>0.7% (0.2–4.1)</td>
<td>1.0% (0–4.2)</td>
<td>0.3% (0.1–2.4)</td>
</tr>
<tr>
<td>Other Phyla</td>
<td>0% (0–0.2)</td>
<td>0% (0–0.3)</td>
<td>0.1% (0–0.2)</td>
<td>0.1% (0–0.2)</td>
</tr>
</tbody>
</table>

Changes in microbiome following viral vaccine

• Has not been examined
• Study was conceived as a means of interrogating whether type I interferons were an important mechanism for post-viral bacterial pneumonias
• Used LAIV nasal vaccine as means of stimulating the host antiviral immune response
Effects of influenza vaccine

- Healthy adult volunteers between ages 18-65 in Los Angeles
  - Non-smokers, no chronic medical conditions
- Sampled nasal swabs+nasal wash at baseline, 2 weeks, and 6 weeks after live attenuated influenza vaccine (intranasal LAIV) or saline nasal spray (control)
- Examined changes in the microbiome by 16S sequencing
- Concurrently obtained nasal epithelial brushings for host transcriptome analysis (microarray) to determine immune responses
<table>
<thead>
<tr>
<th>Phylum</th>
<th>Controls</th>
<th>LAIV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Visit 1 (%)</td>
<td>Visit 2 (%)</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>37.46</td>
<td>38.94</td>
</tr>
<tr>
<td><em>Corynebacterium</em></td>
<td>24.89</td>
<td>25.75</td>
</tr>
<tr>
<td><em>Propionibacterium</em></td>
<td>10.29</td>
<td>11.09</td>
</tr>
<tr>
<td><em>Actinomyces</em></td>
<td>1.41</td>
<td>1.62</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>32.18</td>
<td>25.56</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>1.11</td>
<td>2.14</td>
</tr>
<tr>
<td><em>Bacilli Class</em></td>
<td>2.67</td>
<td>2.10</td>
</tr>
<tr>
<td><em>Bacillales</em></td>
<td>1.87</td>
<td>1.37</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>23.91</td>
<td>30.29</td>
</tr>
<tr>
<td><em>Moraxella</em></td>
<td>11.66</td>
<td>22.16</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>7.59</td>
<td>3.12</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em></td>
<td>0.92</td>
<td>1.20</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>1.40</td>
<td>0.85</td>
</tr>
<tr>
<td><em>Bacteroides</em></td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>1.36</td>
<td>1.85</td>
</tr>
<tr>
<td><em>Streptophyta</em></td>
<td>1.21</td>
<td>1.79</td>
</tr>
</tbody>
</table>

*Tarabichi et al, Microbiome (2015)*
Top 5 most changed genera

Interferon-stimulated genes

Type I IFN

Type II IFN

LAIV

Control

Activity

LAIV

Control

Activity
LAIV is associated with increased abundance of Staphylococcus
IFN may enhance *S. aureus* colonization

Administered MRSA intranasally to WT mice and knockout strains for type I interferon receptor (IFNAR KO) and type II interferon receptor (IFNGR KO)

- Examined persistence of MRSA
- IFNAR animals had significantly lower intranasal load of MRSA
Conclusions

- The nasal-pharyngeal microbiome is of significance to public health and to vaccine developers
  - Composition may impact the development of lower respiratory tract and other invasive infections (otitis media, meningitis, sinusitis, etc.)
  - Involved in maintenance and transmission of pathogens throughout a community
- The composition on the whole is remarkably robust to environmental changes
- However, external perturbations – such as viruses or vaccines – can promote the emergence of specific bacterial taxa
  - Which may be mediated by host responses
Conclusions (cont.)

- We need a better mechanistic understanding of inter-microbial interactions
  - How elimination or reduction of individual microbial populations alters presence, abundance, diversity, and behavior of others
  - Long-term view of vaccinations – alter carriage patterns in populations over time
  - Short-term benefits versus long-term implications
- How host factors alter the acquisition and/or elimination of individual taxa
  - Immune responses
  - Individual ecological factors
Acknowledgements

UCLA
• Scott Hu, M.D., M.S.C.R.
• Yasir Tarabichi, M.D., M.S.C.R.
• Wing Lung
• Connie Yuen, M.S.
• DOM Statistical Core
  • David Elashoff, Ph.D.
  • Xiaoyan Wang, Ph.D

J. Craig Venter Institute
• Barbara Methe, Ph.D.
• Kelvin Li, Ph.D.

NIH Genomic Sequencing Centers for Infectious Diseases
• Maria Giovanni, Ph.D.

New York University
• Elodie Ghedin, Ph.D.

Funding: Cal Tech-UCLA Joint Center for Translational Medicine TAG Award, UCLA CTSI seed grant, NIH R01 HL10894901; NIAID; UCLA STAR Program
THANK YOU!