THE NASAL MICROBIOTA: DIVERSITY, DYNAMICITY, AND VACCINE-MEDIATED EFFECTS

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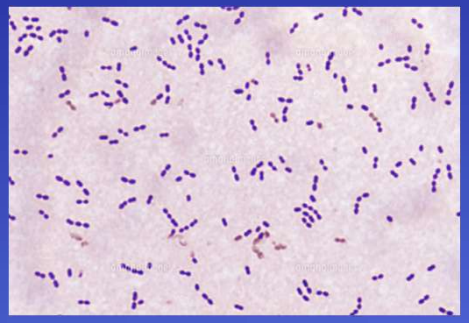
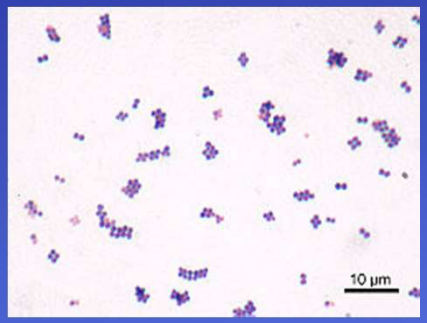
Why do we care about the nasopharyngeal microbiota?

- 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

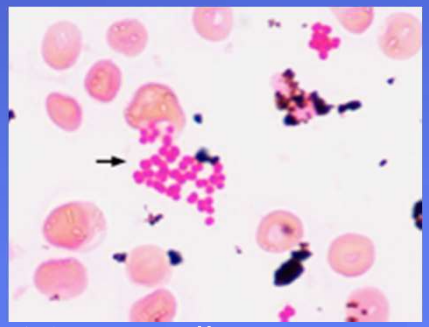
"The Big Four" in the nasopharynx



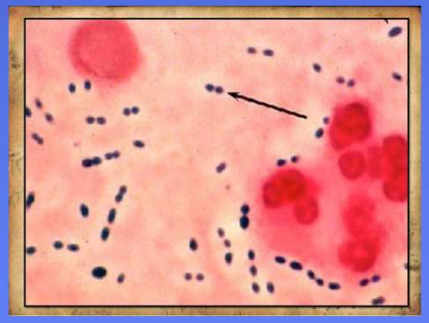
Streptococcus pneumoniae



Staphylococcus aureus



Moraxella catarrhalis



Haemophilus influenzae

Sources: http://imgtagram.com/rewtos2d-streptococcus-pneumoniae-bacteria/

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

- *Staph epi*
- *Propionobacteria*
- *Staph aureus*
- *Streptococcus pne*
- *Strept pyogenes*
- *Neisseria spp (inclu*
- *Haemophilus influe*
- *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

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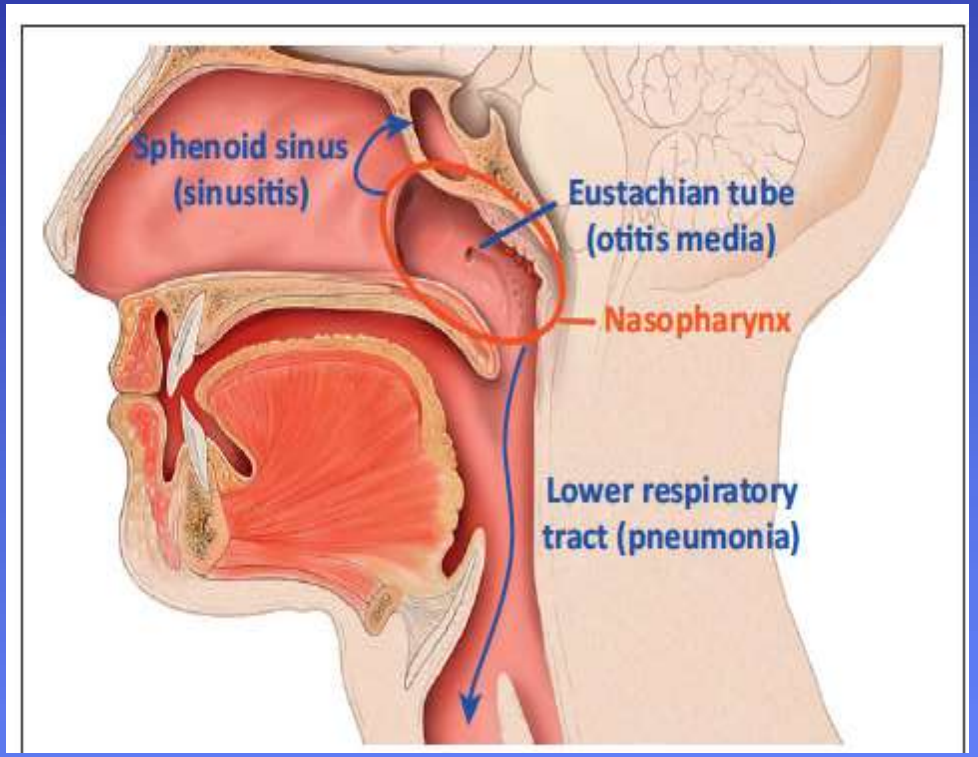
  - Antibiotics
- How does microbe tr
  invader

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



Shak et al, Trends in Microbiology (2013)

TABLE 1 Most frequent nasal swab OTUs[a]

OTU	Frequency (%)[b]
Unclassified *Moraxellaceae*	19.00
Streptococcus	17.86
Corynebacterium	7.04
Moraxella	6.46
Haemophilus	4.66
Unclassified *Pasteurellaceae*	4.09
Staphylococcus	3.84
Acinetobacter	3.44
Dolosigranulum	3.21
Propionibacterium	3.13
Unclassified *Proteobacteria*	2.59
Lactococcus	2.58
Neisseria	1.45
Actinomyces	1.24
Rothia	1.13
Veillonella	1.05

[a] Frequency of ≥1%.

[b] Percentage of total sequences per nasal microbial community, i.e., per child.

Children (6 mos. to 6 years old in Philadelphia

- 69% African-American
- 88% completed PCV7 vaccine
- All had URI symptoms

Laufer et al, mBio (2011)

Nasal microbiota composition

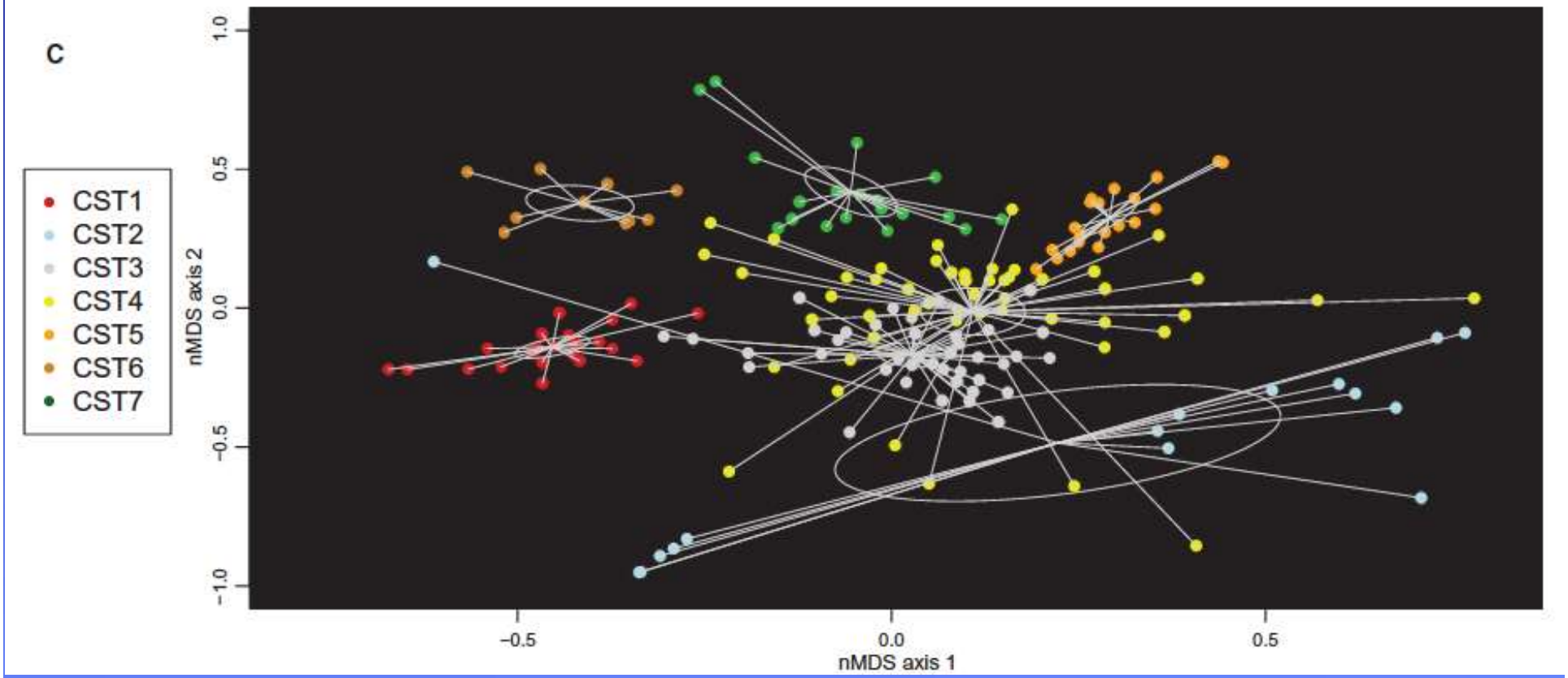
Danish Twin Registry study: (2015)

- Adults in Denmark
- Median nasal bacterial density ~$4x10^6$ 16S rRNA copies per nasal swab (range $6.7x10^5$ to $2.1x10^9$ copies)
  - Women had less than half the nasal density of men (2.97 vs. 7.94 x 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)



Liu et al, Science Advances, 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 nasophayngeal carriage rates of *S. aureus*, *H. influenzae*, and *M. catarrhalis* in children.

Study [ref]	Study description	Ages examined	*S. aureus*	*H. influenzae*	*M. catarrhalis*
Madhi et al. 2007 [45]	Randomised controlled trial of PCV9 in South Africa	mean age 5.64 years (5.3 years after third dose of vaccine)	No differences in carriage between PCV9 and placebo groups	No differences in carriage between PCV9 and placebo groups	ND
Prymula et al. 2009 [69]	Randomised controlled trial of PCV11 in Czech Republic and Slovakia	6, 12–15, 13–16, 15–18, 19–22, and 24–27 months	ND	Lower carriage in the PCV11 group (10%) compared to control (18%) at 15–18 months; no longer significant when molecular assays differentiating NTHi and *H. haemolyticus* applied	ND
Lee et al. 2009 [33]	Prospective observational study in two time periods following PCV7 introduction (2–3 and 5–6 years post-PCV7) in the United States	Mean age 2.7 years	Carriage rate remained stable at 14% in both time periods examined	ND	ND
van Gils et al. 2011 [65,66]	Randomised controlled trial of PCV7 in the Netherlands	6 weeks and 6, 12, 18, and 24 months	Higher carriage in the 2+1 dose group (10%) compared to unvaccinated controls (5%) at 12 months	No differences between vaccinated children and unvaccinated controls	Lower carriage in the 2+1 dose group (61%) compared to unvaccinated controls (68%) at 12 months
Prymula et al. 2011 [70]	Randomised controlled trial of PCV10 in Czech Republic	12–15, 13–16, 15–18, 19–22, and 24–27 months	ND	Lower carriage of NTHi (differentiated from *H. haemolyticus*) in the PCV10 group (10%) compared to unvaccinated controls (16%) at 24–27 months	ND
Dunne et al. 2012 [59]	Randomised controlled trial of PCV7 with or without 23 valent polysaccharide booster (23vPPS) in Fiji	17 months	ND	No differences in carriage between PCV7 (with or without 23vPPS) and unvaccinated controls	No differences in carriage between PCV7 (with or without 23vPPS) and unvaccinated controls
Ho et al. 2012 [64]	Cross-sectional study in Hong Kong	Mean age 3.9 years	No difference in carriage between PCV7 vaccinated and unvaccinated children	ND	ND
Dukers-Muijrers et al. 2012 [32]	Cross-sectional study in the Netherlands	6 weeks to 4 years	No difference in carriage between PCV7 vaccinated and unvaccinated children	ND	ND
Spijkerman et al. 2012 [63]	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	11–12 months and 24 months	Higher carriage in both post-PCV7 time periods (9% and 14%) compared to pre-PCV7 (5%) at 11–12 months	Higher carriage in both post-PCV7 time periods at 11–12 months (65% and 65% post-PCV7 compared to 46% pre-PCV7) and at 24 months (73% and 76% post-PCV7 compared to 52% pre-PCV7)	Higher carriage 4–5 years post-PCV7 (80%) compared to pre-PCV7 (59%) at 24 months

Note: Only statistically significant differences are reported. ND = not determined.

Dunne et al, Vaccine (2013)

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Lee et al. 2009 [33]					
van Gils et a 2011 [65,6					
Prymula et a 2011 [70]					
Dunne et al. 2012 [59]					
Ho et al. 2012 [64]					
Dukers-Muij 2012 [32]					
Spijkerman 2012 [63]					

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Conclusions from a number of epidemiological studies:

- Widespread use of PCV has *coincided* with increased incidence of MRSA infections
- Carriage *S. aureus* has been shown to increase or not change following introduction of PCV
  - H_2O_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

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

Taxa	All Subjects	PHiD-CV Group (N = 25)[a]		Control Group (N = 29)[a]		Day 180-Day0
	Day 0	Day 0	Day 180	Day 0	Day 180	Comparison (p-value)[b]
Proteobacteria	**56.9% (33.7–70.6)**	**58.6% (31.4–70.2)**	**61.7% (46.2–78.3)**	**53.8% (36.1–70.6)**	**57.1% (43.6–69.8)**	0.74
Haemophilus influenzae	*1.6% (0–9.8)*	*1.6% (0–7.9)*	*1.0% (0–4.9)*	*2.0% (0–13.8)*	*2.5% (0–12.6)*	0.85
Moraxella catarrhalis	*12.3% (3.7–24.5)*	*15.7% (3.4–28)*	*12% (1–24.6)*	*9.2% (3.7–18.8)*	*4.2% (1.4–13.1)*	0.65
Moraxella nonliquefaciens	*2.1% (0.6–10)*	*2.5% (1.2–9.5)*	*4.0% (0.8–14)*	*1.4% (0.3–10.2)*	*2.4% (0.1–8.9)*	0.47
Firmicutes	**25.9% (15–46.8)**	**20.1% (11.8–44.8)**	**18.2% (8.6–46.6)**	**26.6% (19.9–46.9)**	**31.6% (15.6–41.2)**	0.66
Streptococcus pneumoniae	*4.4% (0.2–25.4)*	*4.0% (0.3–32.3)*	*10.3% (0.4–37.7)*	*4.9% (0–21.1)*	*10% (0.9–35.3)*	0.67
Actinobacteria	**7.8% (1.8–21.6)**	**8.5% (1.5–15.8)**	**5.1% (0.9–9.2)**	**6.9% (2.3–22.1)**	**2.1% (0.5–15.2)**	0.18
Corynebacterium spp.	*5.6% (1.7–19.8)*	*8.5% (0.9–15.4)*	*3.8% (0.8–7.7)*	*5.2% (2–21.1)*	*2.1% (0.4–15.1)*	0.45
Bacteroidetes	**0.4% (0.1–3.8)**	**0.7% (0.2–4.1)**	**1.0% (0–4.2)**	**0.3% (0.1–2.4)**	**0.3% (0–3.3)**	0.92
Other Phyla	**0% (0–0.2)**	**0% (0–0.3)**	**0.1% (0–0.2)**	**0.1% (0–0.2)**	**0% (0–0.2)**	0.15

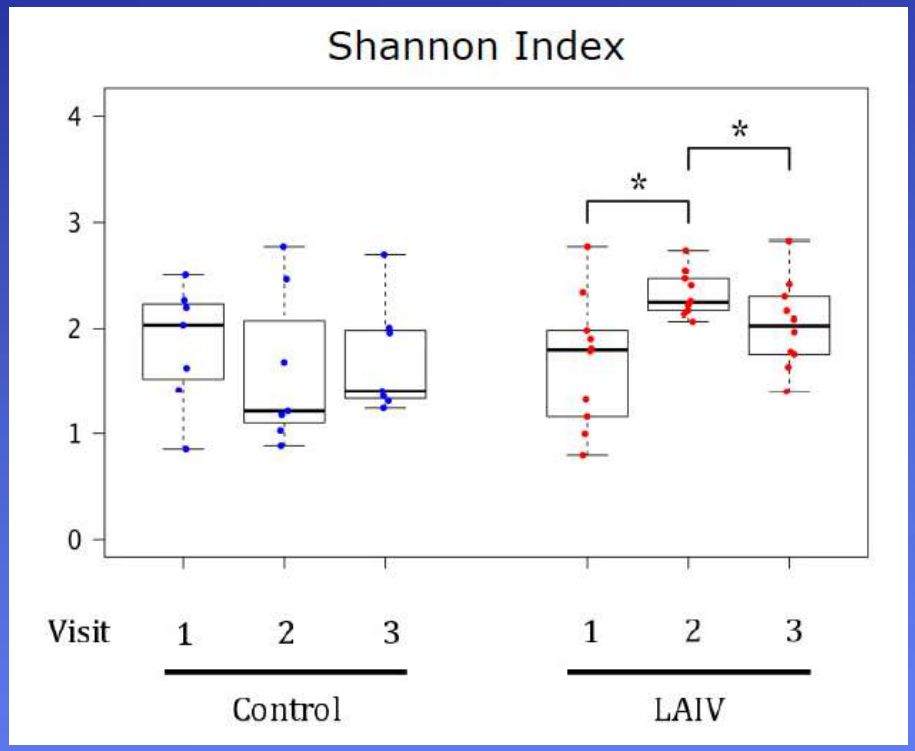
Feazel et al, Plos One, (2015)

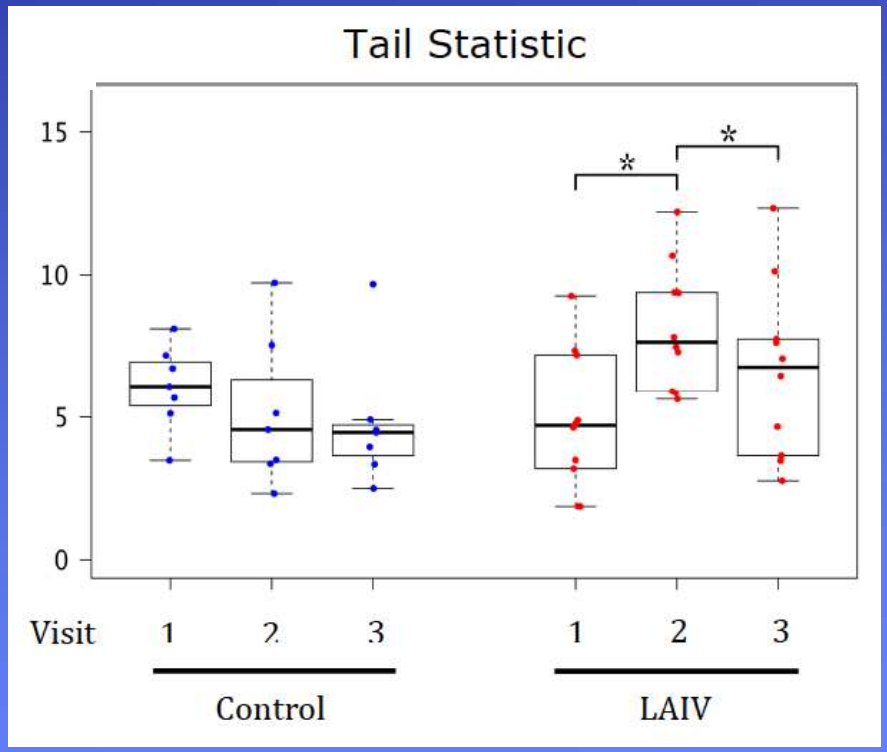
Changes in microbiome following viral vaccine

- 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





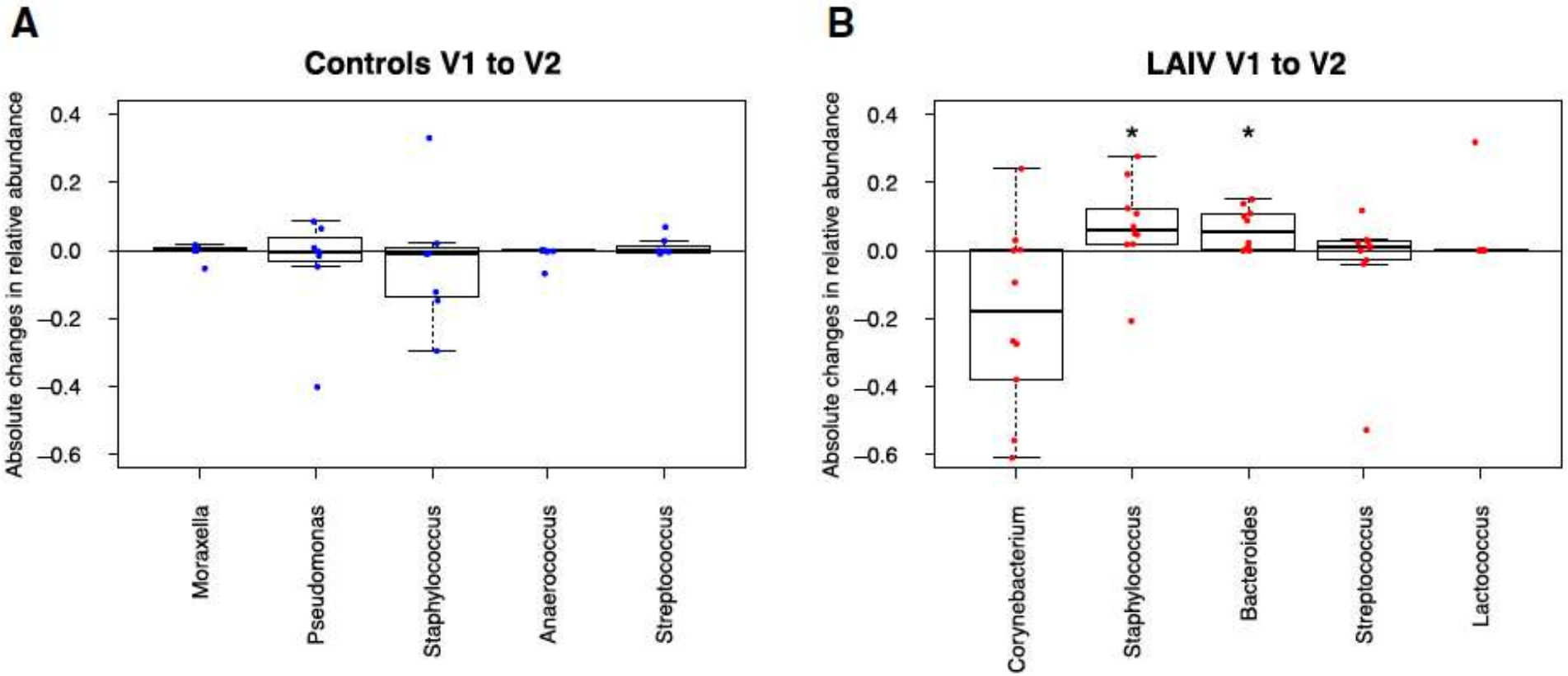
Tarabichi et al, Microbiome (2015)

Table 2 Mean relative abundance of detected phyla and genera by group and visit

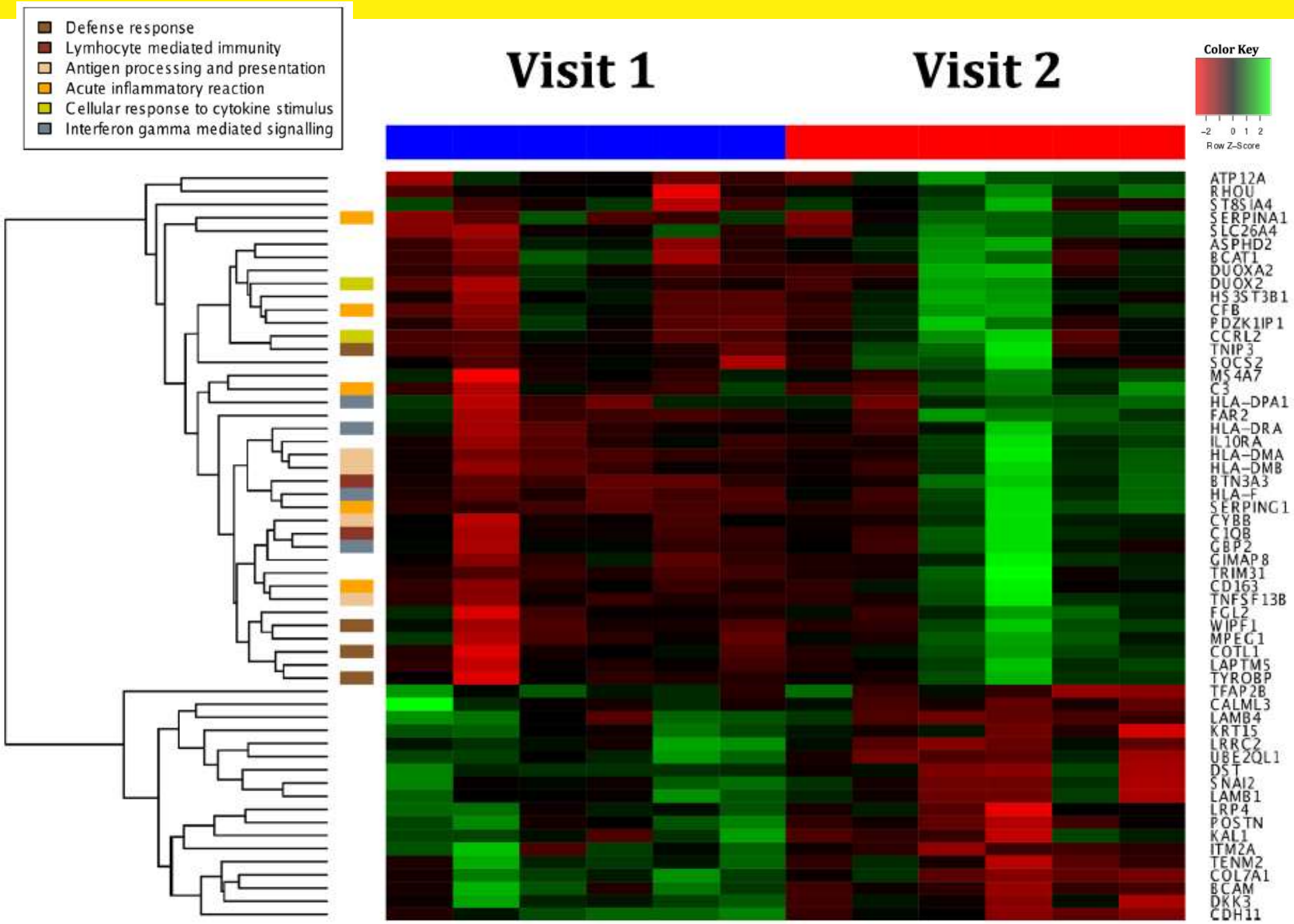
	Controls			LAIV		
Phylum	Visit 1 (%)	Visit 2 (%)	Visit 3 (%)	Visit 1 (%)	Visit 2 (%)	Visit 3 (%)
Genera (in *italics*)						
Actinobacteria	37.46	38.94	35.42	45.97	23.52	38.96
Corynebacterium	24.89	25.75	25.16	34.44	15.40	30.86
Propionibacterium	10.29	11.09	8.00	6.66	6.21	5.35
Actinomycetales	1.41	1.62	1.60	3.09	1.42	2.16
Firmicutes	32.18	25.56	41.56	40.71	51.34	44.99
Staphylococcus	16.14	12.79	25.28	19.04	26.37	24.53
Streptococcus	1.11	2.14	0.49	8.37	4.68	4.26
Bacilli Class	2.67	2.10	4.10	3.60	4.80	4.32
Bacillales	1.87	1.37	2.74	1.85	2.56	2.55
Proteobacteria	23.91	30.29	13.34	5.28	6.92	5.01
Moraxella	11.66	22.16	10.51	0.68	0.02	0.12
Pseudomonas	7.59	3.12	0.87	0.03	1.26	0.07
Enterobacteriaceae	0.92	1.20	0.18	3.29	1.69	0.47
Bacteroidetes	1.40	0.85	0.04	2.60	7.87	4.95
Bacteroides	0.00	0.00	0.01	0.00	6.26	4.13
Cyanobacteria	1.36	1.85	1.41	1.04	3.66	3.65
Streptophyta	1.21	1.79	1.40	1.03	3.58	0.88

Tarabichi et al, Microbiome (2015)

Top 5 most changed genera



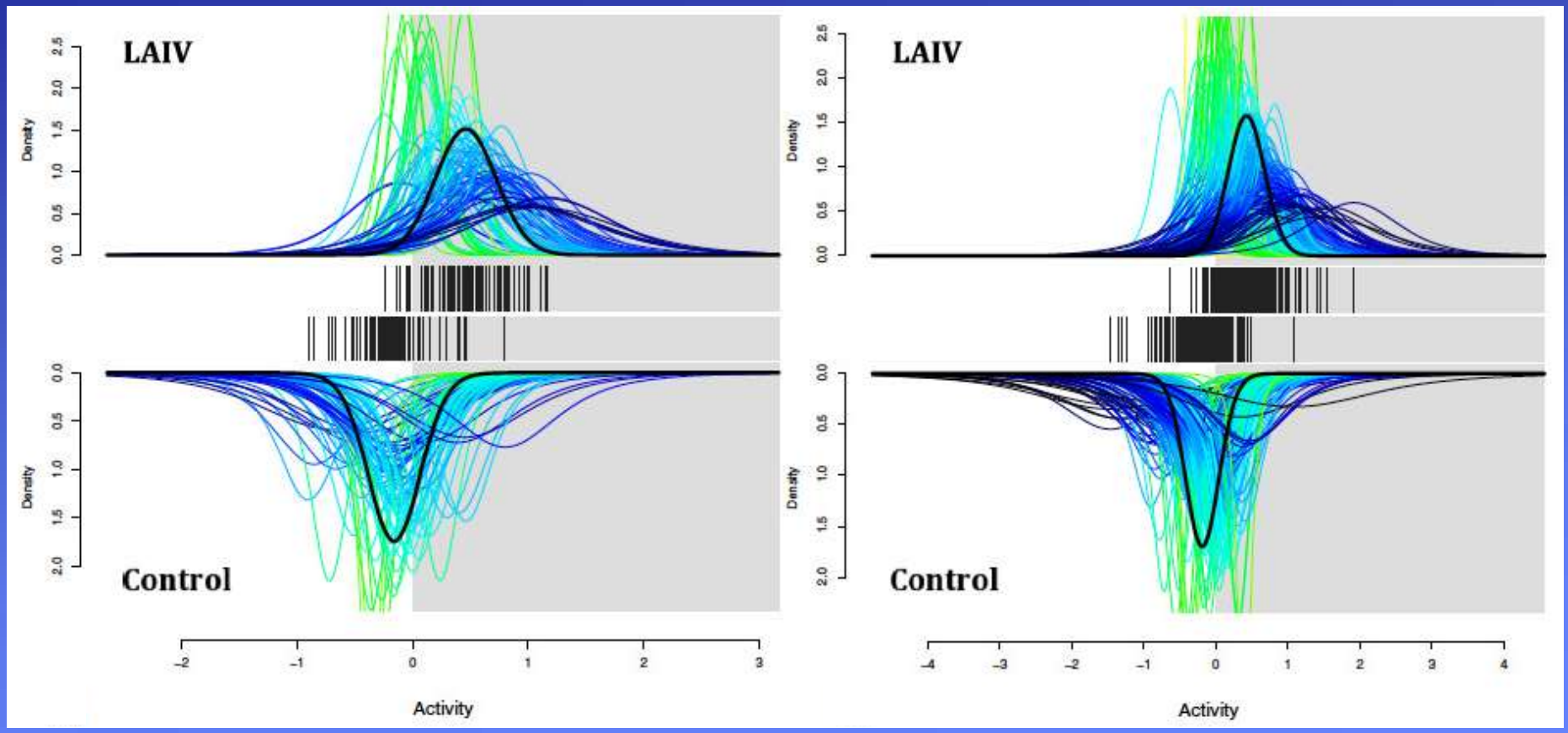
Tarabichi et al, Microbiome (2015)


Defense response
Lymhocyte mediated immunity
Antigen processing and presentation
Acute inflammatory reaction
Cellular response to cytokine stimulus
Interferon gamma mediated signalling
Visit 1
Visit 2
Color Key
−2 0 1 2
Row Z-Score
ATP12A
RHOU
ST8SIA4
SERPINA1
SLC26A4
ASPHD2
BCAT1
DUOXA2
DUOX2
HS3ST3B1
CFB
PDZK1IP1
CCRL2
TNIP3
SOCS2
MS4A7
C3
HLA-DPA1
FAR2
HLA-DRA
IL10RA
HLA-DMA
HLA-DMB
BTN3A3
HLA-F
SERPING1
CYBB
C1QB
GBP2
GIMAP8
TRIM31
CD163
TNFSF13B
FGL2
WIPF1
MPEG1
COTL1
LAPTM5
TYROBP
TFAP2B
CALML3
LAMB4
KRT15
LRRC2
UBE2QL1
DST
SNAI2
LAMB1
LRP4
POSTN
KAL1
ITM2A
TENM2
COL7A1
BCAM
DKK3
CDH11

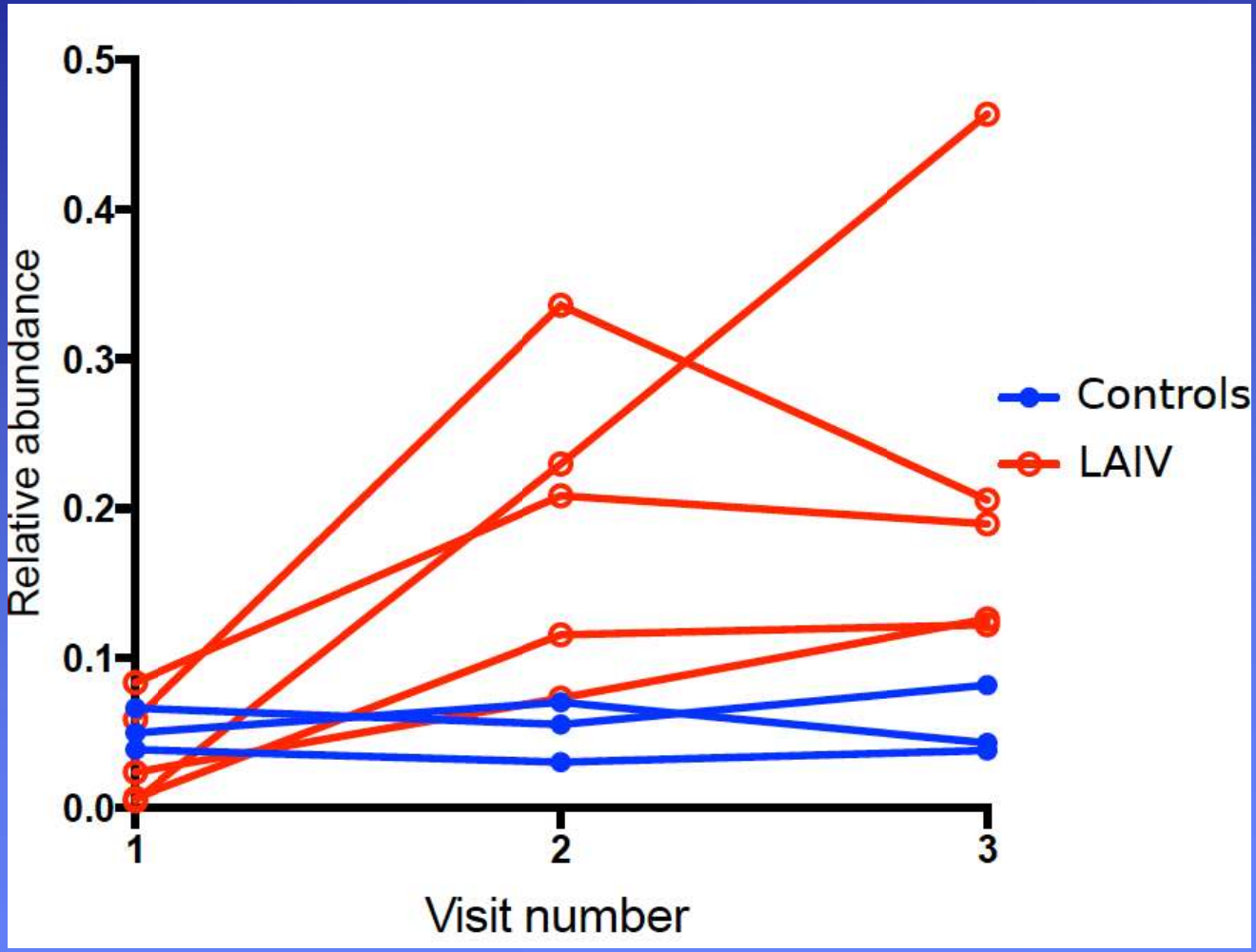
Interferon-stimulated genes

Type I IFN

Type II IFN



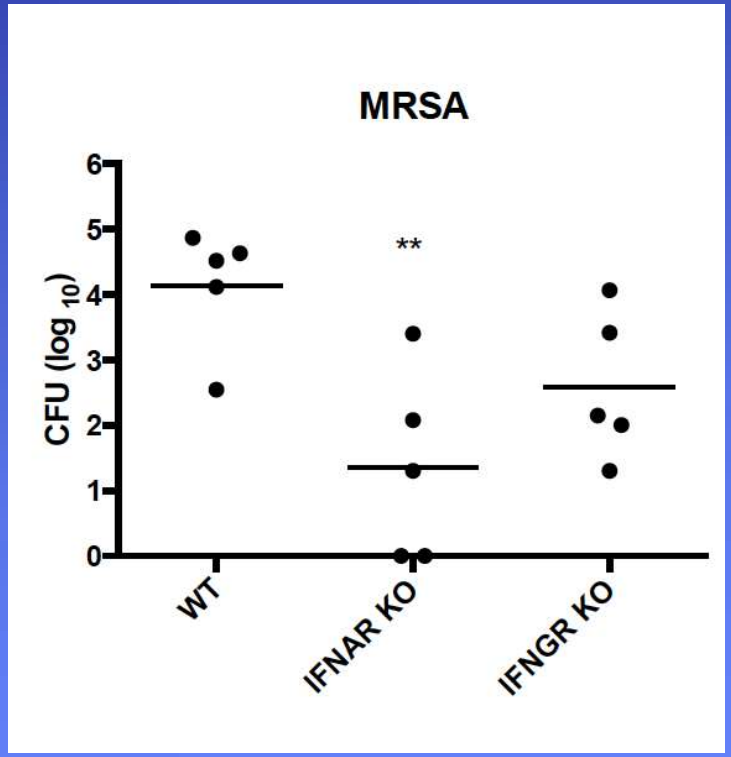
LAIV is associated with increased abundance of Staphylococcus



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

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