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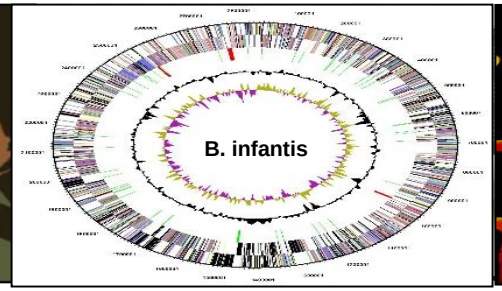

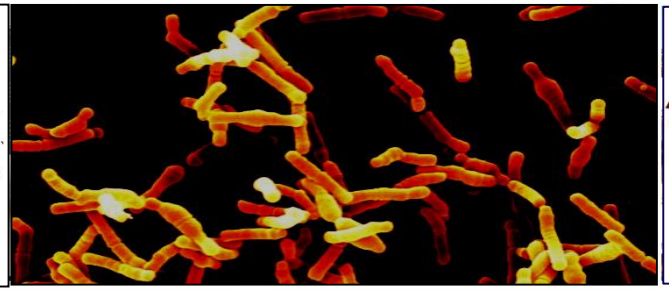

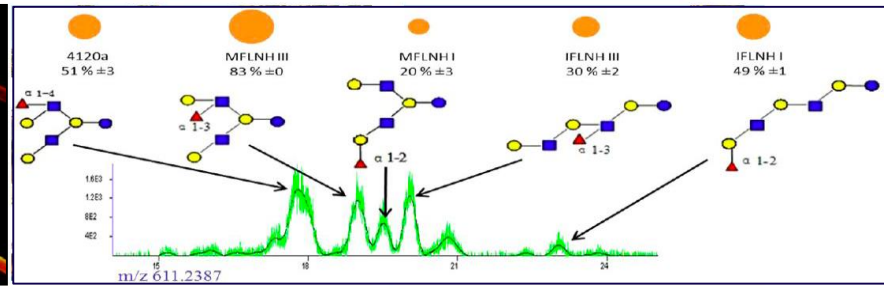

Bifidobacterium, Breastfeeding, and Infant Health

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Outline

- **Part 1: *Bifidobacterium* and antimicrobial resistance genes in infants**
- **Part 2: *Bifidobacterium longum* subsp. *infantis* and infant vaccine response**
- **Part 3: *Bifidobacterium longum* subsp. *infantis* around the world**

Background: Infant Microbiome 100 Years Ago

A century ago, *Bifidobacterium* were practically a monoculture in smears of breast-fed infant poop, but infant poop is much more variable in western countries today…

Image from Logan, WR. "The Intestinal Flora of Infants and Young Children", Journal of Pathology and Bacteriology V18. Sept. 3, 1913

Bifidobacterium and Infant Health – Why care?

- **Antimicrobial Resistance (AMR) is a major public health problem, as of 2013 in USA:**
  - **Minimum 2,049,442 illnesses per year**
  - **Minimum 23,000 deaths per year**

CDC 2013 Antibiotic Resistance Threats in the United States

Even infants harbor AMR genes!!!

Public domain image, https://en.wikipedia.org/wiki/Infant#/media/File:Human-Male-White-Newborn-Baby-Crying.jpg

Bifidobacterium and AMR

AMR is not a common trait in *Bifidobacterium*

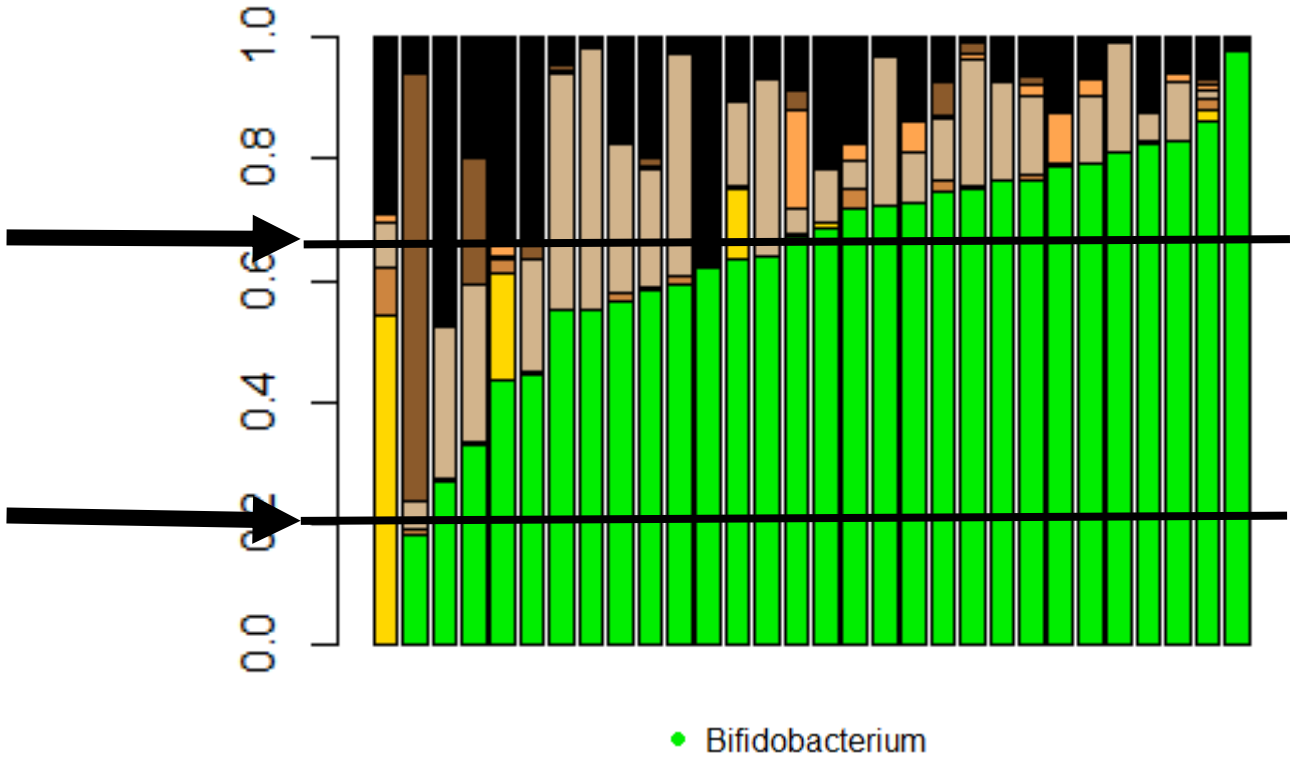
Hypothesis: Infants with microbiomes dominated by *Bifidobacterium* will have lower levels and diversity of AMR genes

Methods - Included Subjects

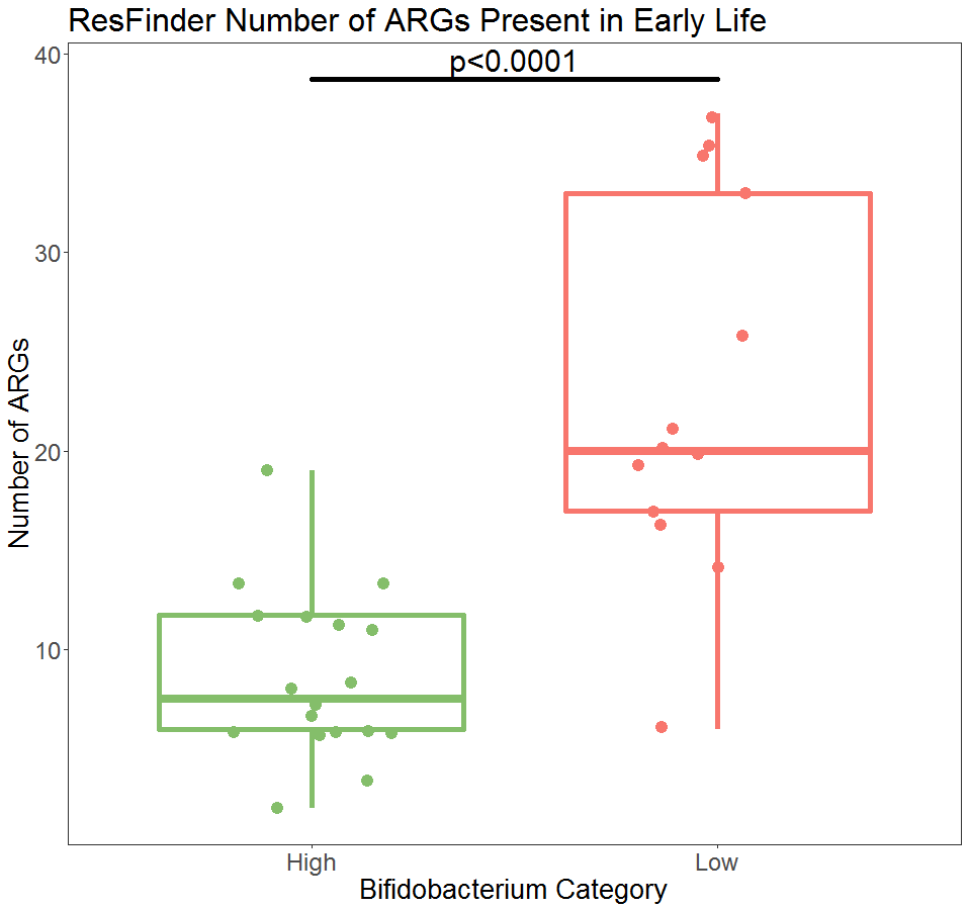
High *Bifidobacterium* infant samples had >65% *Bifidobacterium* relative abundance

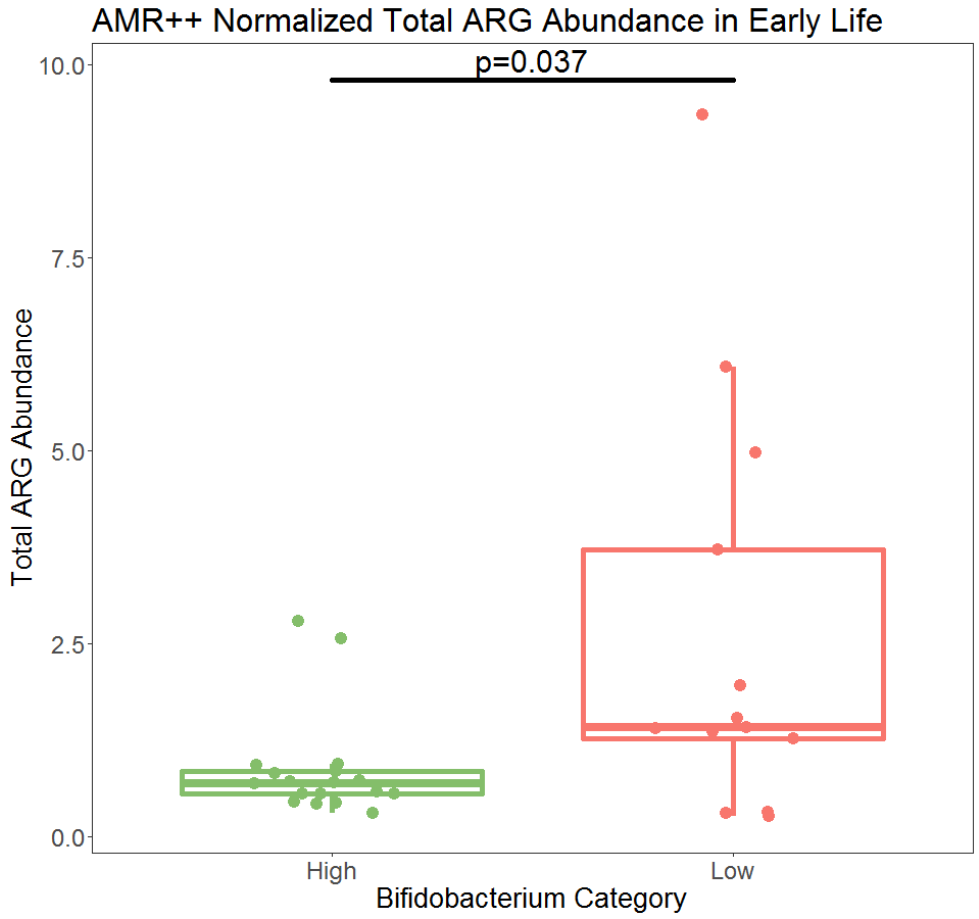
Low *Bifidobacterium* infant samples had <20% *Bifidobacterium* relative abundance

30 Randomly Selected Bangladeshi Infant Stool Microbiomes at Week 6



AMR genes, Early Life





Family of Origin and AMR

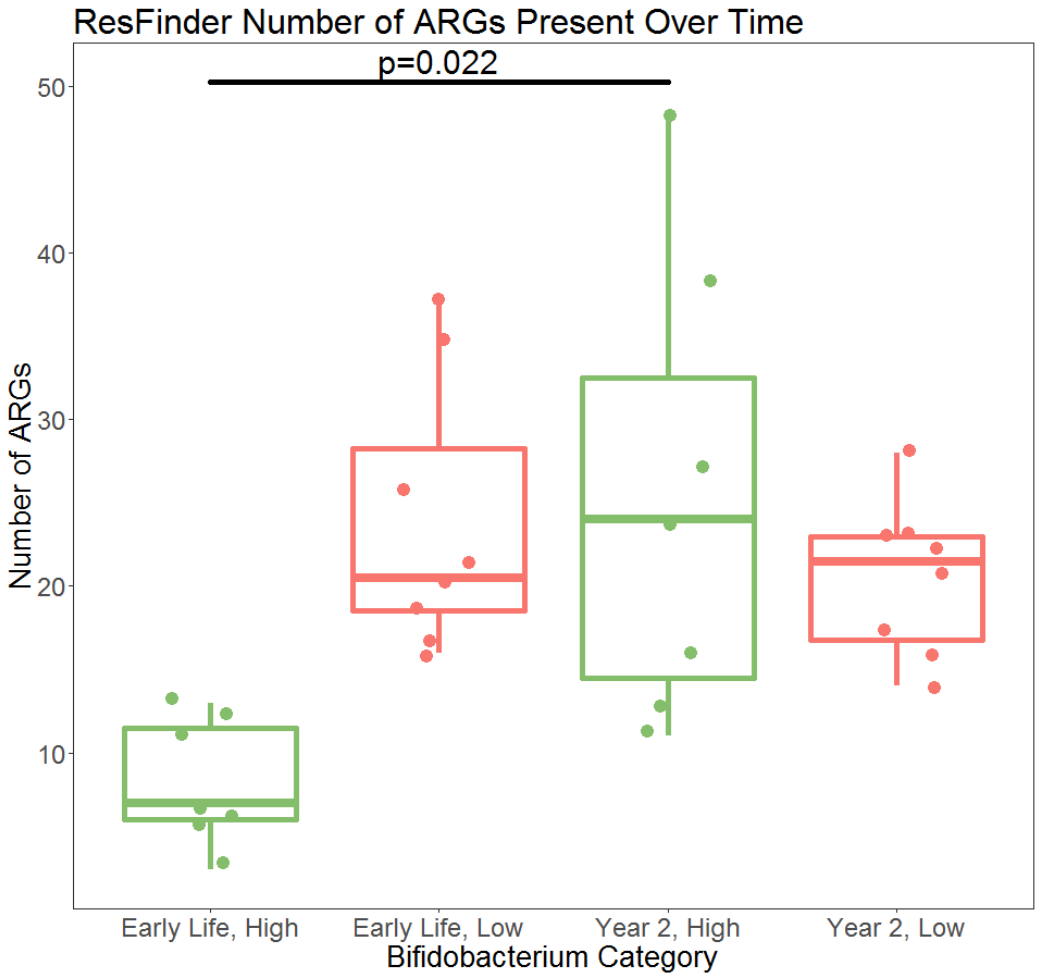
Enterobacteriaceae

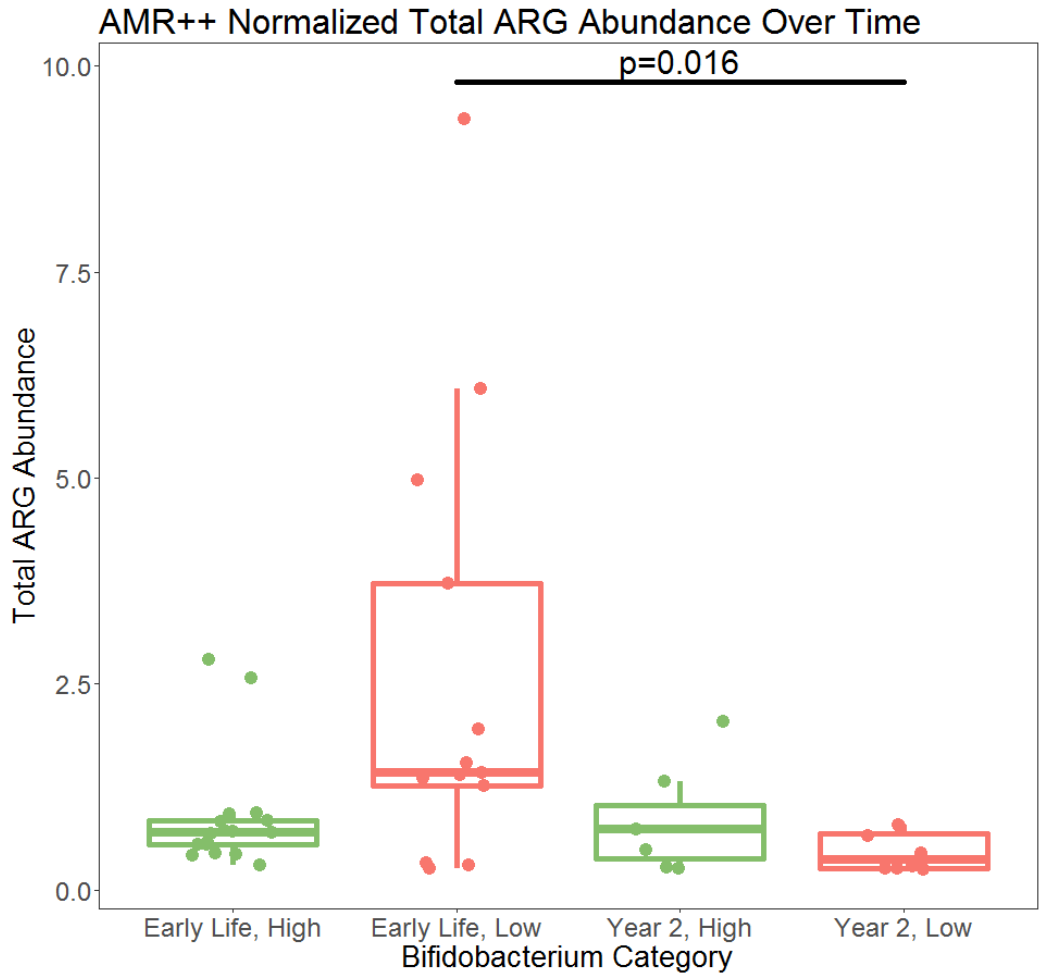
- >30 Different AMR genes
- Resistance to:
  - Aminoglycosides
  - Beta-lactams
  - Fluoroquinolones
  - Fosfomycin
  - Macrolides
  - Phenicol
  - Sulfonamides
  - Tetracyclines
  - Trimethoprim

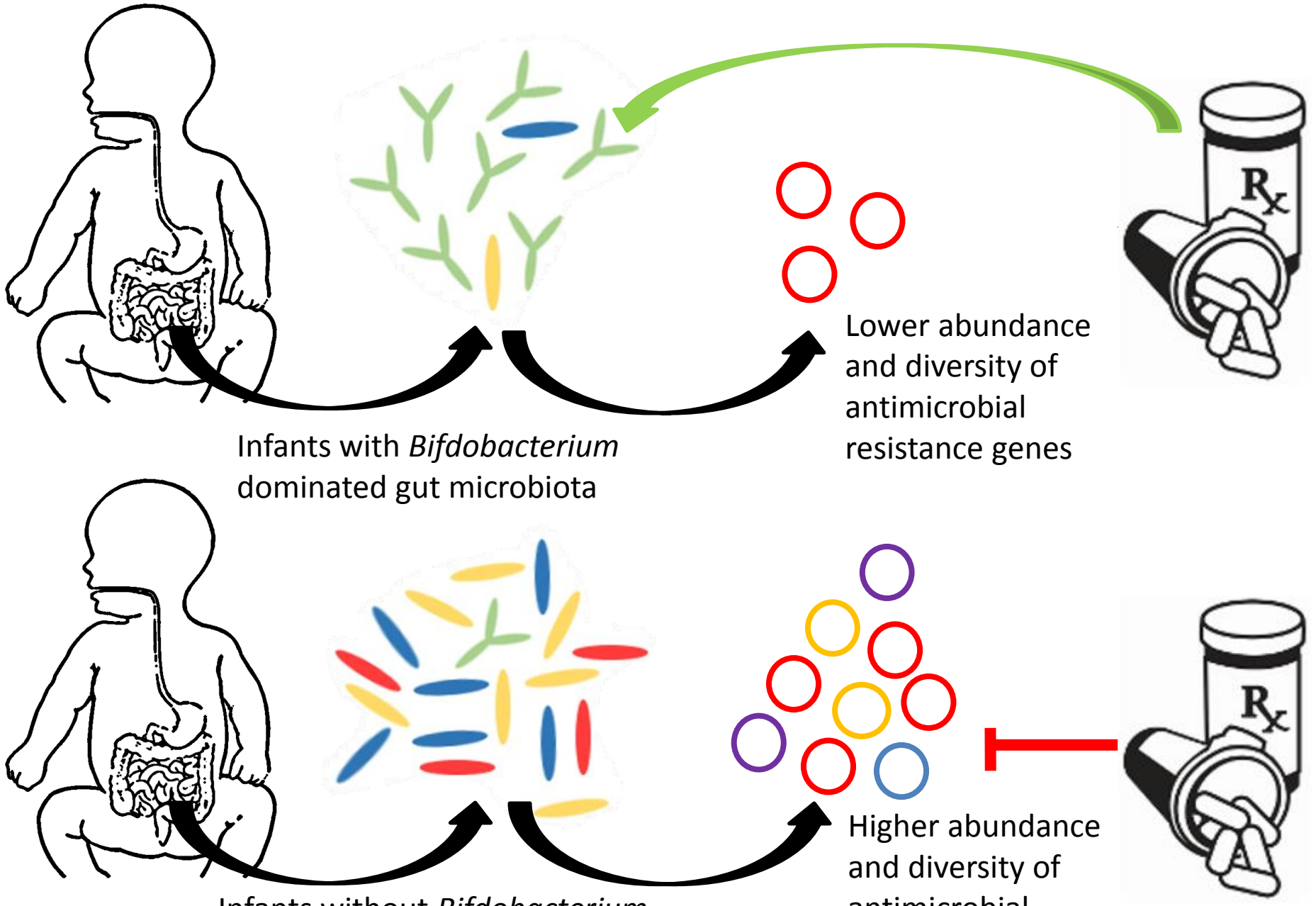
Bifidobacteriaceae

- Only 3 AMR
  - erm(X): Macrolides
  - tet(W) and tet(O): Tetracyclines

AMR Gene Abundance Over Time






Rx
Lower abundance and diversity of antimicrobial resistance genes
Infants with *Bifdobacterium* dominated gut microbiota
Rx
Higher abundance and diversity of antimicrobial resistance genes
Infants without *Bifdobacterium* dominated gut microbiota

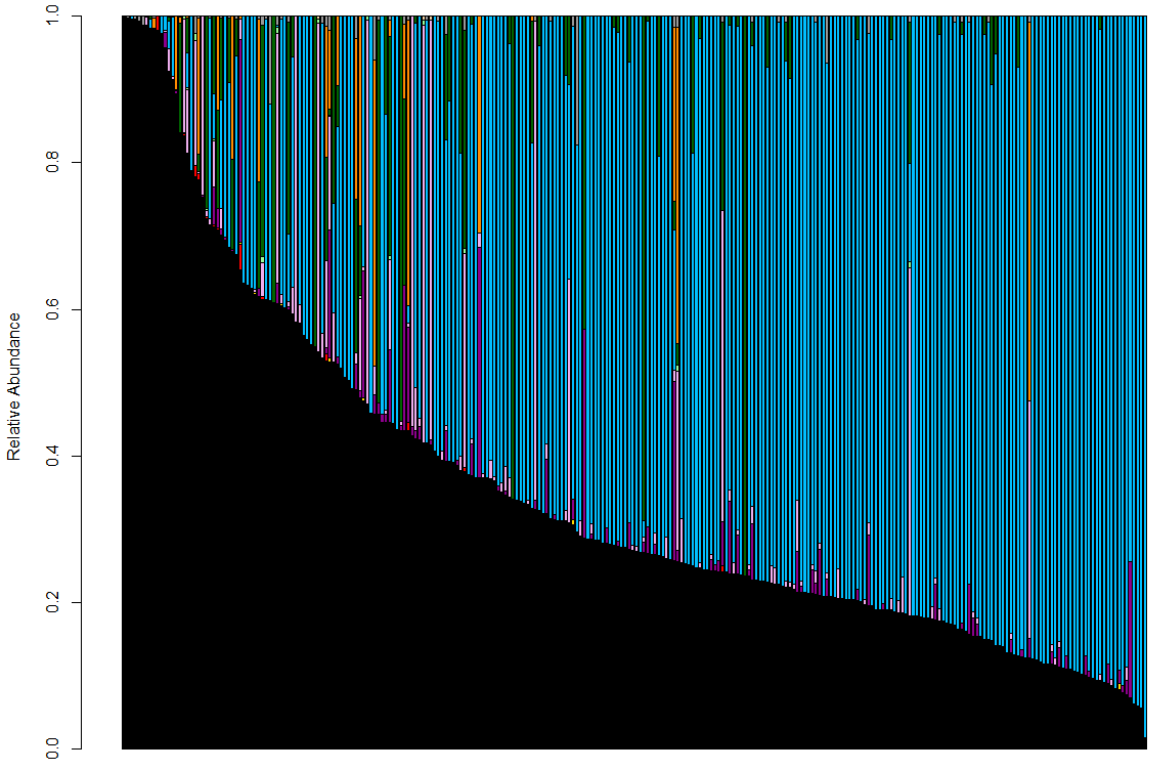
Conclusions Part 1

- **High levels of *Bifidobacterium* reduce abundance and diversity of ARGs in infants in early life**
- **Infants with low levels of *Bifidobacterium* experience a drop in ARG abundance at weaning**
- **The diversity of ARGs increases at weaning in infants with high levels of *Bifidobacterium***
- **Need further studies to better understand the timing of how *Bifidobacterium* help to shape the infant resistome**

Bifidobacterium and Infant Health

- **Vaccination saves 6 million lives per year (PMID: 12531324)**
- **To be effective, vaccinated individuals must mount a robust immune response to the vaccine**

Bifidobacterium Species Colonizing Bangladeshi Infants



- Other Genera
- B. adolescentis
- B. animalis
- B. bifidum
- B. breve
- B. dentium
- B. longum ssp infantis
- B. longum ssp longum
- B. longum unknown ssp
- B. pseudocatenulatum
- Unknown Bifidobacterium

Dhaka, Bangladesh, Infants at age 1.5 months.

Hypothesis

Higher levels of *Bifidobacterium* correlate with improved vaccine response

Nazmul Huda

Vaccine Response Correlates with *Bifidobacterium*

Bifidobacteria€¥	BCG				TT					HbV					Polio												SEB		
	6w	15w		2y	15w		2y			15w		2y			15w	2y											6w	15w	2y
																plasma								stool					
	CD4 T-cell SI	CD4 T-cell SI	Skin test	CD4 T-cell SI	CD4 T-cell SI	ALS IgG	CD4 T-cell SI	Plasma IgG	IgG AI	CD4 T-cell SI	ALS IgG	CD4 T-cell SI	Plasma IgG	Plasma IgG AI	ALS IgG	P1 IgA	P2 IgA	P3 IgA	P1 IgG	P2 IgG	P3 IgG	IgA AI	IgG AI	P1 IgA	P2 IgA	P3 IgA	CD4 T-cell SI	CD4 T-cell SI	CD4 T-cell SI
Bifidobacterium		■		■	■		■	■		■													■	■	■	■		■	
B. longum		■			■	■				■								■										■	
B. longum subsp *infantis*		■			■					■																		■	
B. longum subsp *longum*														■	■														
B. breve											■					■													
B. bifidum																			■	■	■								

Color key: ■ Positive association ■ Negative association

Conclusions Part 2

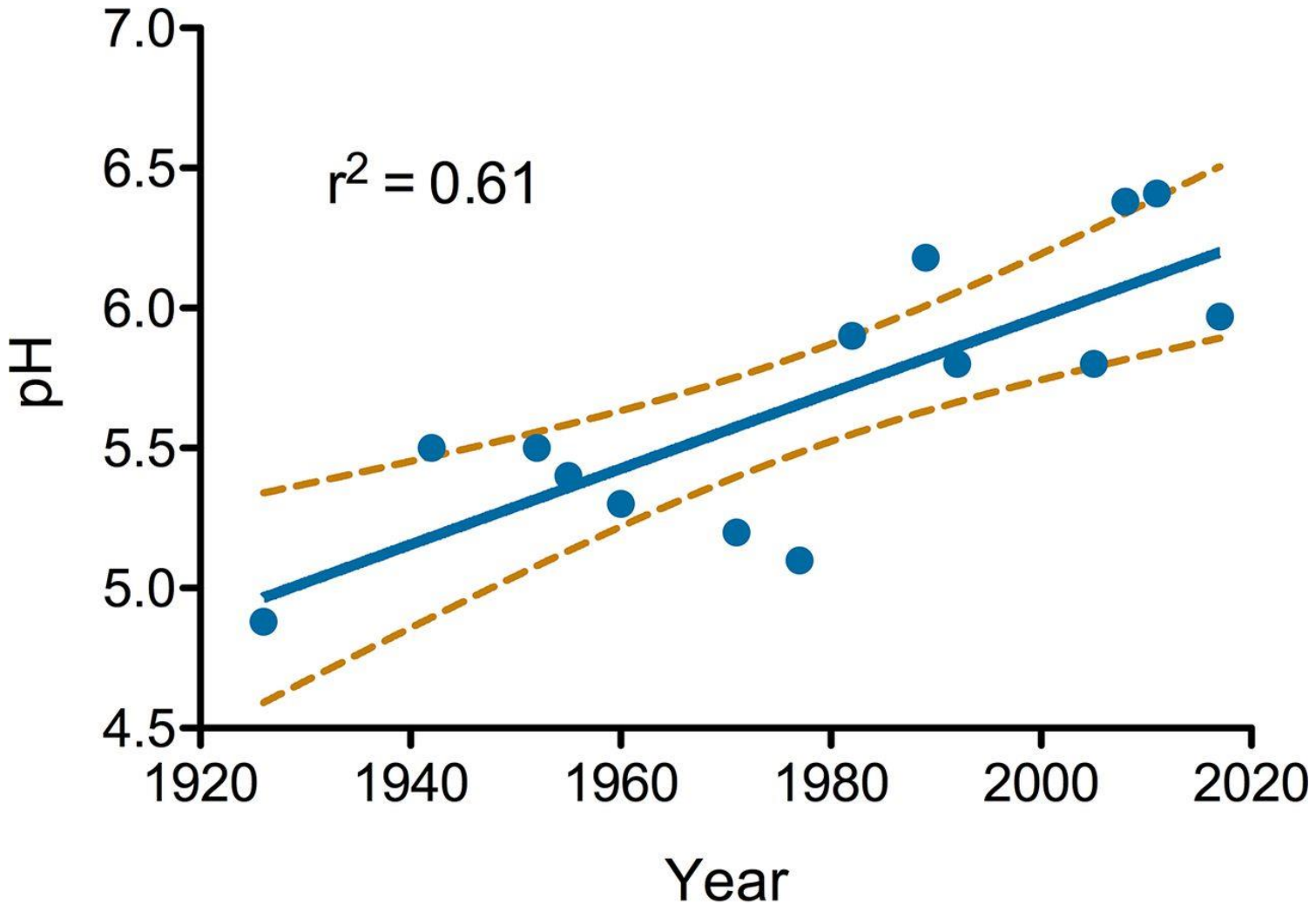
- **High levels of *Bifidobacterium* at ages week 6, week 11, and week 15 correlate with improved vaccine response in early life**
- **This improvement in vaccine response is durable, with infants who had high levels of *Bifidobacterium* in early life exhibiting improved vaccine response at age 2 years**
- **Species of *Bifidobacterium* matters!**
  - **Sustained improvement with early colonization by *B. longum* subspecies *infantis*, but some *Bifidobacterium* species were negatively correlated with vaccine response**

Background: Infant Microbiome 100 Years Ago

A century ago, *Bifidobacterium* were practically a monoculture in smears of breast-fed infant poop

Image from Logan, WR. "The Intestinal Flora of Infants and Young Children", Journal of Pathology and Bacteriology V18. Sept. 3, 1913

Fecal pH reported in studies along with the average, standard deviation, and numbers of samples measured (where reported) plotted by year of study publication.



Bethany M. Henrick et al. mSphere 2018;
doi:10.1128/mSphere.00041-18

Background: Infant Feeding 100 Years Ago

- **Safe infant formula and modern sanitation are relatively recent inventions.**
- **100 years ago infants faced a stark choice: Human breastmilk or risk death**

Public health poster promoting breastfeeding, from Wolf, JD, "Low Breastfeeding Rates and Public Health in the United States" AJPH 93 (12) December 2003, 2000-2010. PMID: 14652321

Background: Composition of Breastmilk

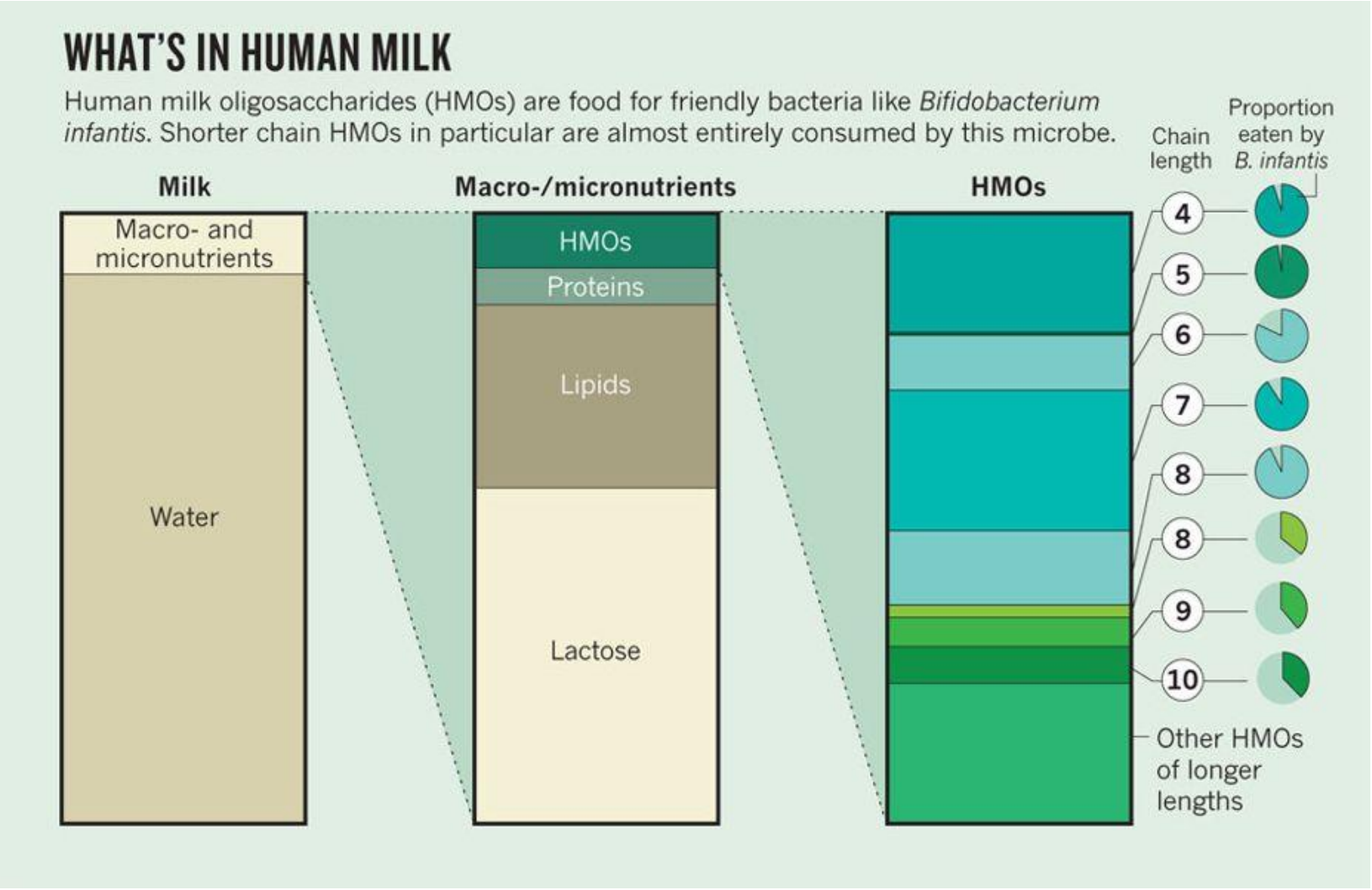
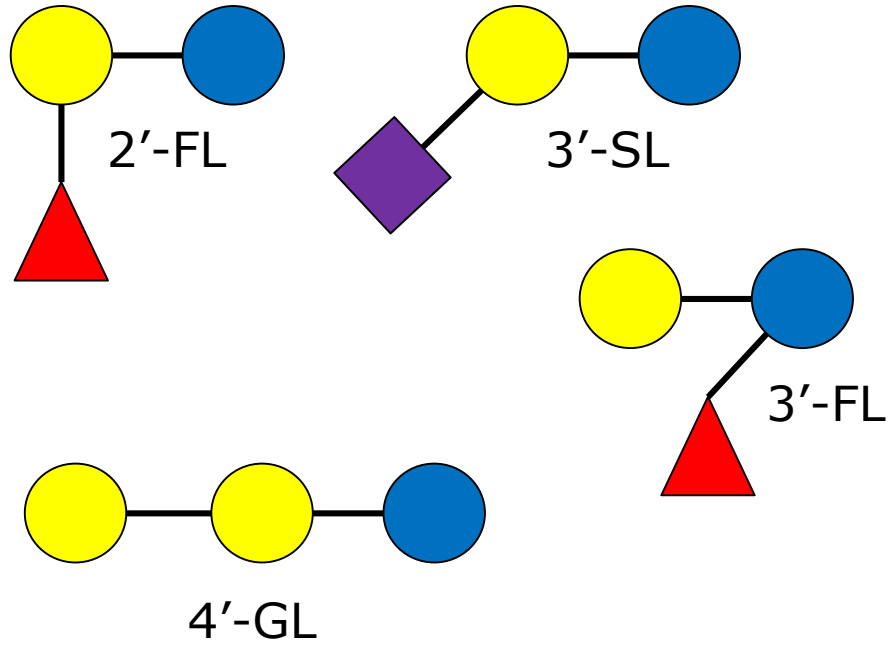
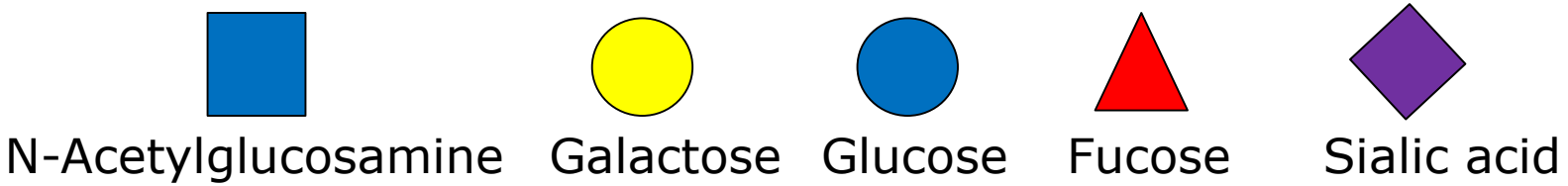


Figure from Petherick, Anna "Development: Mother's Milk: A Rich Opportunity." Nature 468, December 2010, S5-S7. PMID: 21179083

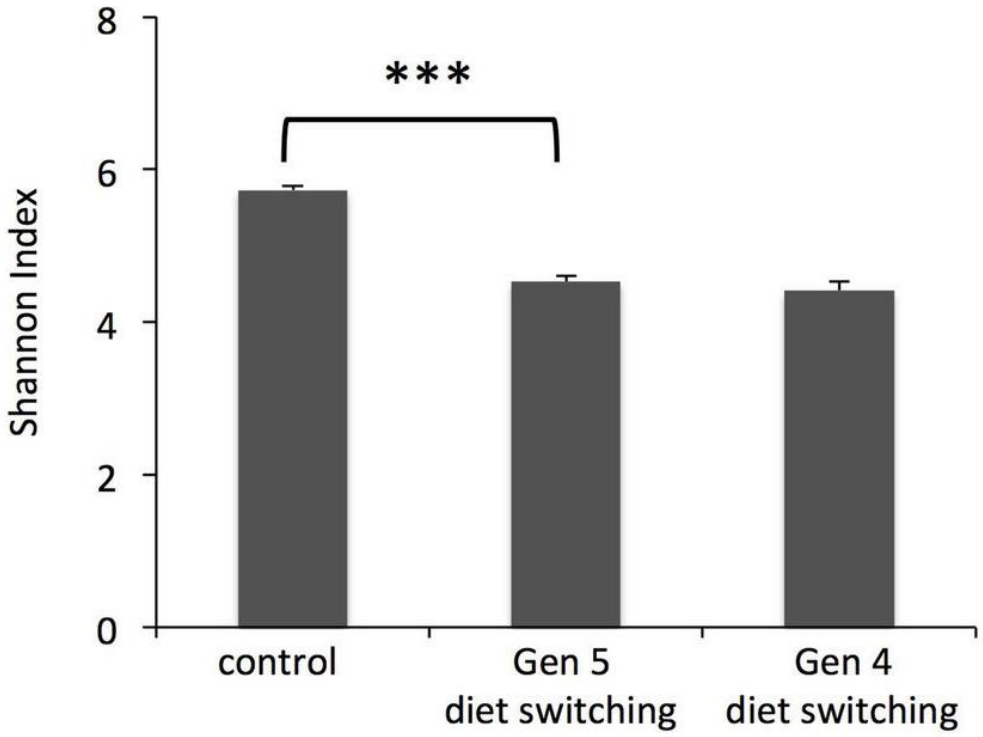
Background: Breastfeeding and *Bifidobacterium*

- **Human Milk Oligosaccharides (HMOs)**
  - **3rd most abundant component of human milk**
  - **Not digested by the infant**
  - **Are consumed by some species of *Bifidobacterium*, especially *B. longum ssp. infantis***





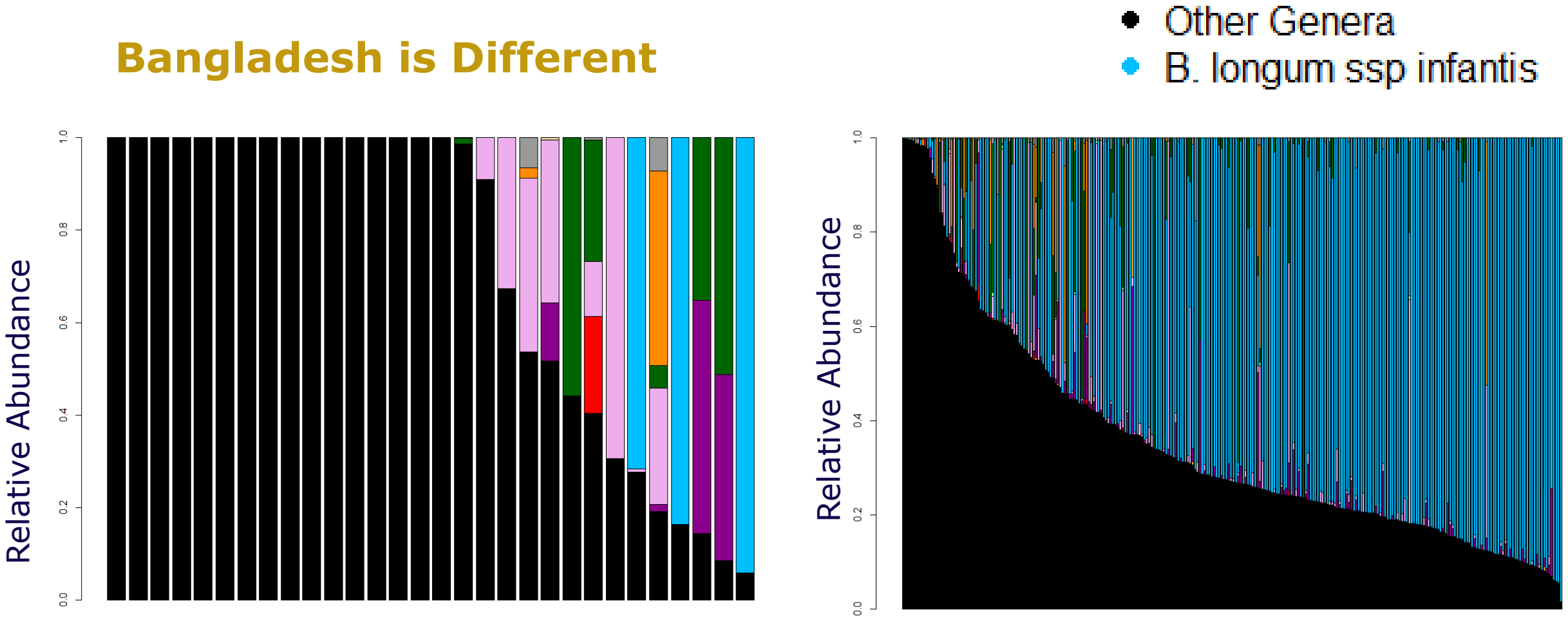
Background: Diet and Commensal Extinction



- **Microbiota Accessible Carbohydrates (MACs)**
  - **Not human digested**
  - **Consumed by microbes**
  - **Low MAC diet causes microbe extinctions**
- **Infant formula does not have HMOs: it's low MAC!**

Sonnenburg et al., "Diet-induced extinctions in the gut microbiota compound over generations" Nature 529, January 2016, 212-215, PMID: 26762459

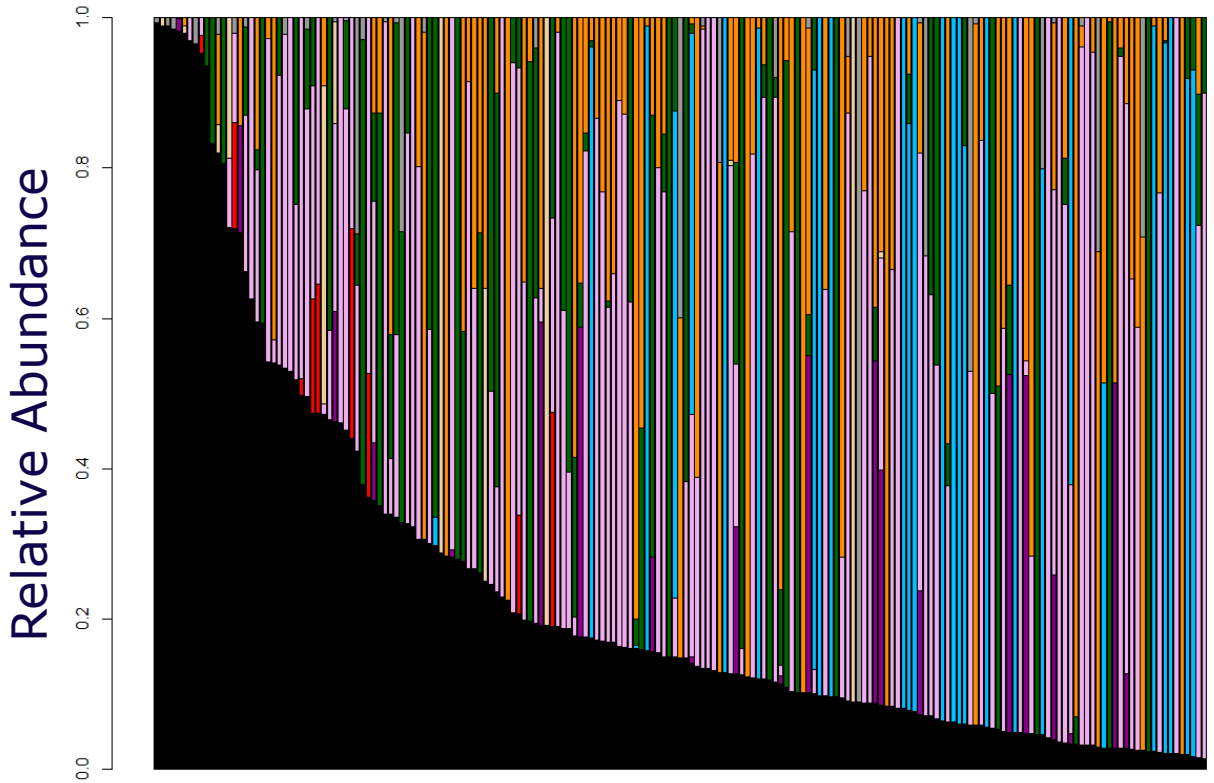
Bangladesh is Different



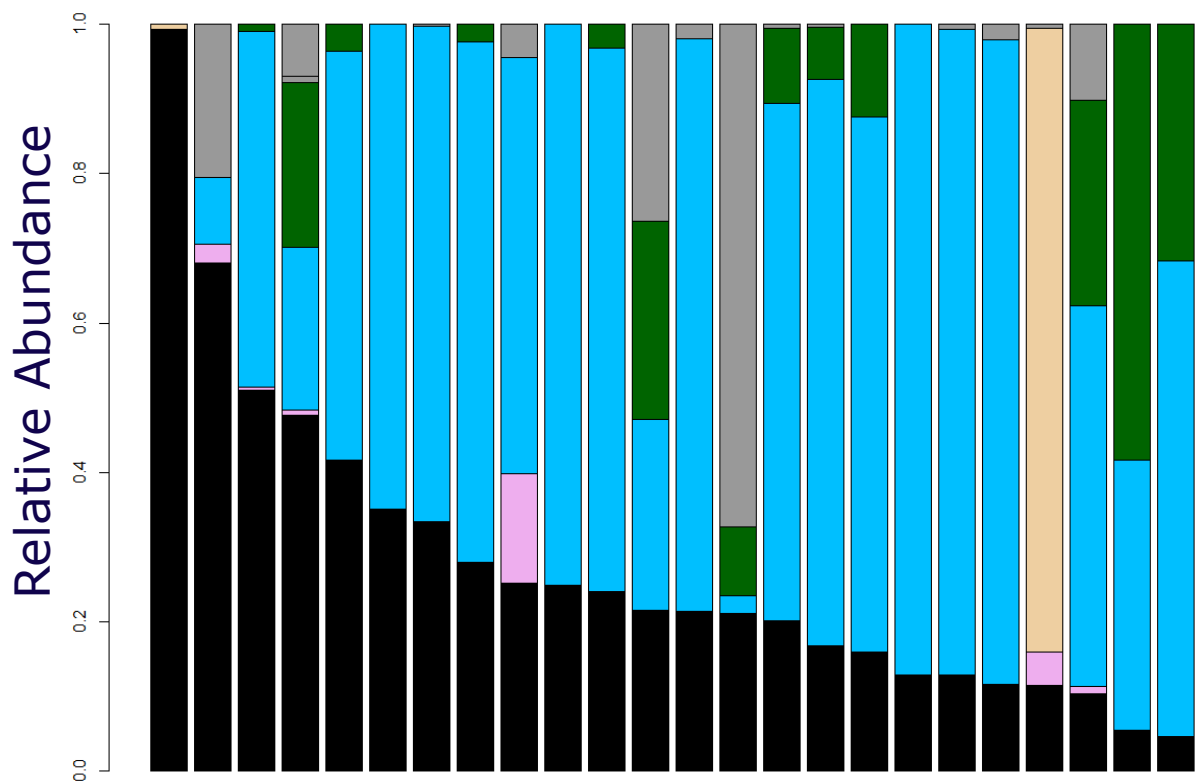
Davis, CA, Infants at age 2 months.

Dhaka, Bangladesh, Infants at age 1.5 months.

The Infant Microbiome Today

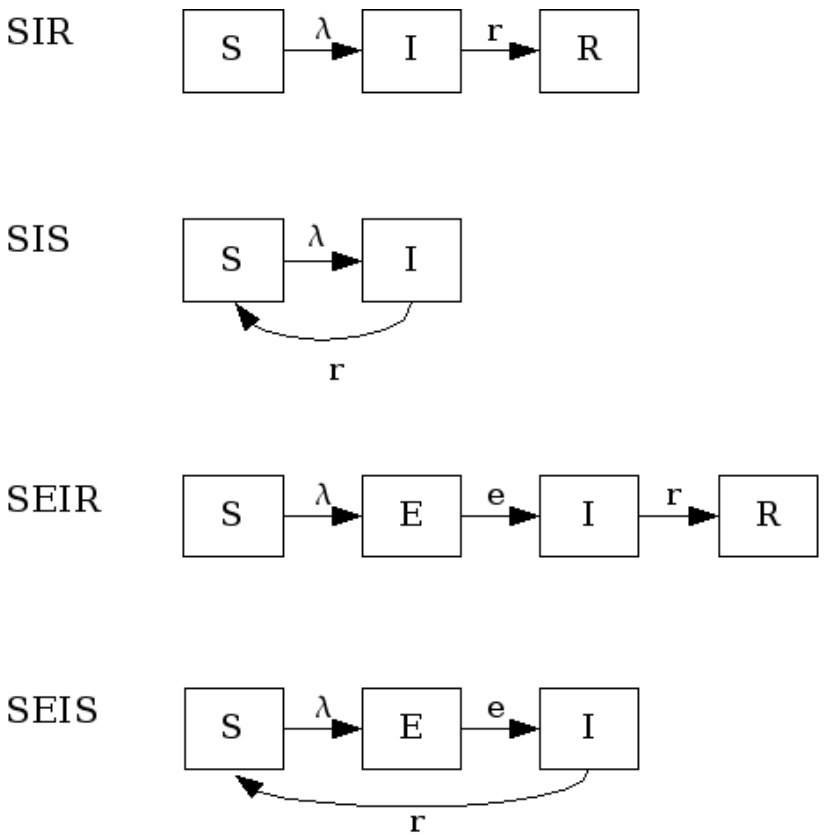


Switzerland, Infants at age 2 months.



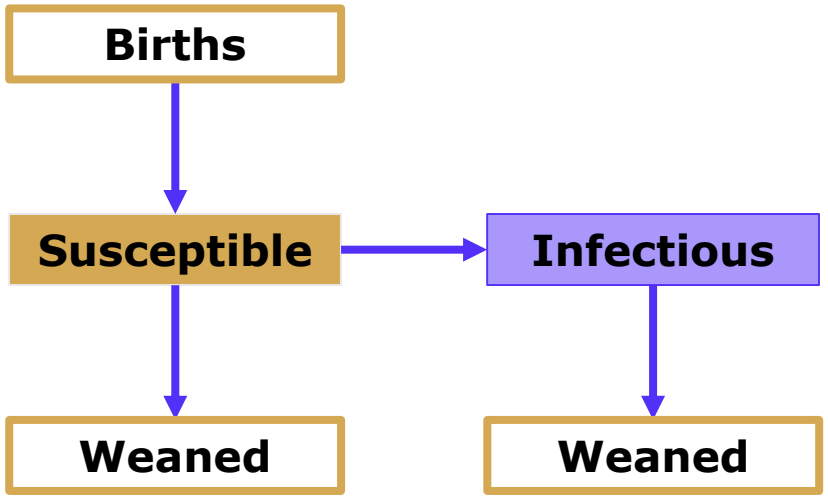
The Gambia, Infants at age 1 month.

Deterministic Epidemiological Models



- **Deterministic models divide the population into compartments, and then use differential equations to predict the flow of people between compartments**
- **As shown, these models are missing something important: demographics!**

Creating SI Models of *B. infantis*



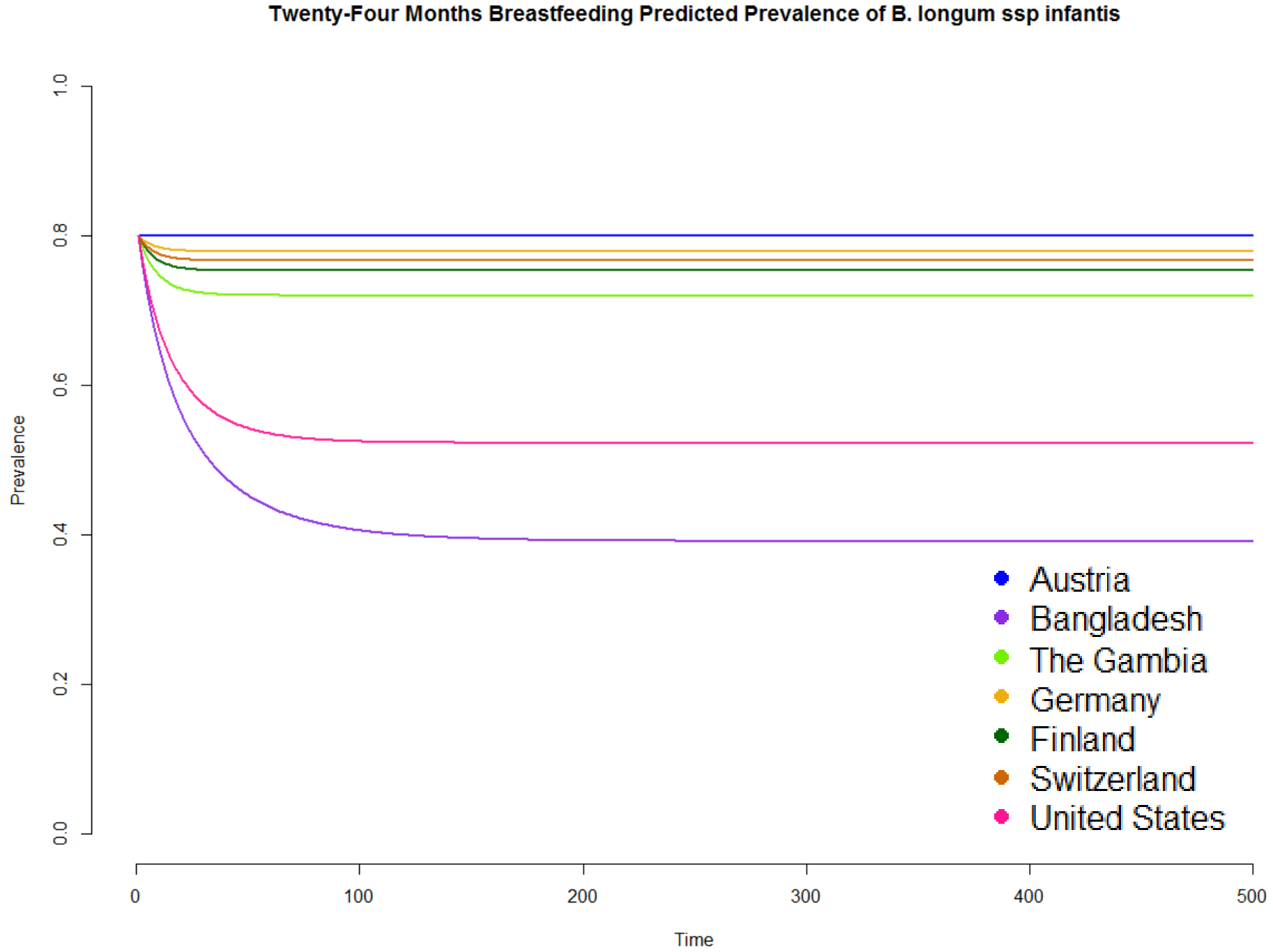
- **Used an SI model**
- **Assumptions:**
  - ***B. infantis* is transmitted in the community**
  - **All infants are born susceptible to *B. infantis*, but vertical transmission is relatively rare**
  - **Random mixing in population**
  - **Population is at a constant size (birth rate=weaning rate)**

Wait, RARE vertical transmission?

- **Many species of *Bifidobacterium* are vertically transmitted**
- **In Davis, no *B. infantis* at week 1 or month 1 in tested infants. First detected at age 2 months**
- ***B. infantis* drops below the limit of detection when infant is no longer breastfed**

B. infantis around the world

Country	Total Number of Included Infants	Number of Infants Colonized with at least 5% B. longum ssp infantis	Prevalence of B. longum ssp infantis in the population	R_0
Austria	122	4	3.3%	1.02
Bangladesh	274	219	80%	2.00
Finland	135	1	0.74%	1.00
Gambia	23	21	91%	2.67
Germany	149	3	2.0%	1.01
Switzerland	189	26	14%	1.08
United States	30	3	10%	1.05


Twenty-Four Months Breastfeeding Predicted Prevalence of B. longum ssp infantis
Prevalence
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
Time
Austria
Bangladesh
The Gambia
Germany
Finland
Switzerland
United States

Conclusions Part 3

- **Population prevalence of *Bifidobacterium longum ssp. infantis* is tied to breastfeeding duration**
  - **Longer duration breastfeeding, higher rates of colonization with *B. infantis***
- **Need approximately 2 years breastfeeding duration to maintain high levels of *B. infantis* in most populations**
- **There are unknown factors that change transmission dynamics across populations**
  - **Hygiene practices?**
  - **Use of antibiotics?**
  - **Rates of exposure to other infants?**
  - **More data is needed!!!**
- **Examined transmission only a single subspecies of *Bifidobacterium*, unknown to what extent other species may compensate for the loss of *B. infantis***

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