Stunted Neonatal Gut Microbiota and Pathogen Colonisation Associated with Caesarean Births

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Gut Microbiota Acquisition in Very Early Life

- **Local species pool**
  Vaginal, faecal, skin, oral, breastmilk

- **Maternal microbiota**
  Vaginal, faecal, skin, oral, breastmilk

- **Local environment**
  Hospital, midwives, medical device, home, pets, parents, sibling microbiota

- **Role of early life events?**
  Mode of delivery, breast/formula feeding, antibiotics, weaning
Global Trends of Elective Caesarean Sections

- Major abdominal surgery
- Maternal intrapartum antibiotics
- Elective/planned C-section frequently performed without medical indication
- Epidemiological association with autoimmune diseases (e.g. asthma)
- Hypothesis: Perturbed gut microbiotas of C-section babies predispose them to health issues later in life

WHO guideline on C-section rates: 10-15%, No evidence of mortality risk reduction above this threshold
Metagenomic Sampling of the Neonatal Period

UK birth cohort (n > 2k) 2016 - 2018

601 term babies and 176 mothers

Shotgun metagenomics of 1,683 stool samples

BHR LEI UCLH

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High Resolution Gut Microbiome Profiling

Phylogeny of the Human Gut Microbiota

Comprehensive Database of Reference Genomes
- Lawley Lab Human Microbiota Culture Collection (~200 novel species)
- Quality filtered public genomes (e.g. HMP collection)

$k$-mer-based Read Classification (Kraken/Bracken)
- Scalable for large-scale shotgun metagenomics
  ~1% computing resource versus deep sequencing & de novo assembly
- Improved sensitivity and taxonomic resolution
  ~30% high recall than HMP
  Majority of the human gut microbiota metagenomic sequences
classified at species (~80%) and strain level (~50%)

Forster and Kumar et al. Nat Biotechnol, in press
Permutational Multivariate Analysis of Variance

Age-dependent microbiome maturation

Species composition (beta diversity)

Effect size of clinical factors stratified by age

Day 4, $R^2 = 7.9\%$

- Delivery mode
- Postnatal antibiotics
- Maternal antibiotics in delivery
- Postnatal stay in hospital
- Feeding mode

Shao et al., unpublished data
Effect of Delivery Mode on the Neonatal Gut Microbiota

Mode of delivery:
- Largest explanatory power on microbiome variation
- Effect size: 7.9% – 2.5% over the neonatal period
- Statistically significant at 6 months of age ($R^2 = 1.1\%$)
- Vaginal microbiota-associated *Lactobacillus* spp. at very low abundance (<2%) in both groups
- Stable developmental trajectory dominated by commensal *Bifidobacterium*, *E. coli* and *Bacteroides*
- Environmental-associated bacteria ~80% on day 4
- Delayed *Bifidobacterium* spp. colonisation
  98.6% C-section newborns without *Bacteroides* spp. 48.9% by 6 months of age

![Day 4, n = 310](image)

![Day 7, n = 532](image)

![Day 21, n = 325](image)

Vaginal, $n = 584, 315$ babies

C-section, $n = 582, 286$ babies

Shao et al., unpublished data
Maternal *Bacteroides* Transmission Disrupted in C-section

*Strain transmission threshold:* 5% sharing of unique rare SNPs within mother-baby sample pairs (*MIDAS*)

**a**

- *Bacteroides vulgatus*
- *Bifidobacterium longum*
- *Escherichia coli*
- *Bacteroides uniformis*
- *Bifidobacterium bifidum*
- *Bifidobacterium adolescentis*
- *Bacteroides caccae*
- *Bacteroides fragilis*
- *Bacteroides ovatus*
- *Parabacteroides distasonis*
- *Faecalibacterium prausnitzii*
- *Bacteroides faecis*
- *Bacteroides plebeius*
- *Bacteroides stercoris*
- *Prevotella copri*
- *Blautia wexlerae*
- *Eubacterium rectale*
- *Bacteroides cellulosolyticus*

**b**

*Delivery mode*
- Red: Vaginal
- Blue: C-section

**c**

*C-section Lack of late colonisation*

*Shao et al., unpublished data
*MIDAS: Nayfach & Pollard, Genome Res (2016)*
Opportunistic Pathogen Colonisation in C-section Babies

Differential species in C-section babies

Associated with healthcare environment
Rare, low carriage in mothers

WGS of 836 pathogen isolates
Faecal biobank access
Culturing and isolation from raw stools

Combined result of the first 21 days

Shao et al., unpublished data
Strain Diversity, Virulence and AMR of *E. faecalis*

**Whole-genome Phylogeny of *E. faecalis***

- **Isolate Source**
  - UK - Infant Gut Microbiota
  - UK - Hospital bloodstream infection
  - Global Collection

- **E. faecalis MLST groups**
  - ST179
  - ST16
  - ST30
  - ST10
  - ST191
  - ST23
  - ST18
  - ST6

**UK babies (n = 356)**
- UK hospitals (n = 168)
- Global (n = 672)

- 70.5% of baby isolates carried multiple genetic determinants for hospital adaptation

- ~15% of total isolates in dominant lineages

- No carriage of vancomycin resistance

- Dominant lineages: enrichment of tetracycline and aminoglycoside resistance genes

Shao et al., unpublished data
Early Life Microbiota Acquisition and Development

Hygiene/Missing Microbiota Hypotheses
- Missing environmental and host genetic factors unmeasured
- Stochastic colonisation process: ecological drift and priority effect

Association → Causation
- Long-term host outcome via health data linkage
- Mechanistic studies in germ-free and gnotobiotic mice

Adapted from Blanton et al. Science (2016)
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Population Metagenomics

Mother-infant Dyads
Neonatal Period (0-3 wks)
Longitudinal Sampling to 6 mos

n = 1683

Colonisation dynamics in very early life

Targeted Culturing

Fecal Biobank Access
Selective Media and Condition

Microbiota perturbations

Pathogen phlogeny and gene carriage

Bacterial WGS

Pure Culture Collection
Expand Reference Genome DB

Colonisation succession
Pathogen resistance