Quadram Institute
Science Health
Food Innovation

Preterm microbiota: modulation and diagnostics

@hall_lab

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HallLab

Swedish Neonatal Quality Register (SNQ) Meeting. 12th-13th March 2020

Preterm birth and the gut microbiota

- Born under 37 weeks gestation
  - Low birth weight (< 1500g)
  - 1:9 live births globally are defined as preterm
- Gut physiologically underdeveloped
- Immature immune system

- %)
-

- **NICUs keeps premature infants alive**

- But what about the gut microbiota?
  - Disrupted normal colonisation of infant gut
    - **Reduced levels of *Bifidobacterium***
    - **Hospital-aquired bacteria are not your friend…**

Necrotising enterocolitis (NEC)

- Aberrant colonisation appears pivotal to NEC development
  - Most common gastrointestinal emergency in NICU (5-15%)
    - huge burden in terms of mortality (40%)
    - serious long-term health problems
  - NEC linked to *Clostridium perfringens* and/or *Klebsiella pneumoniae* overgrowth

Probiotics* have shown significant value for prevention of preterm NEC

*including *Lactobacillus* and *Bifidobacterium*

Only 10/58 UK NICUs using 'probiotics'

BAMBI study

x240

± probiotics

B. bifidum
L. acidophilus

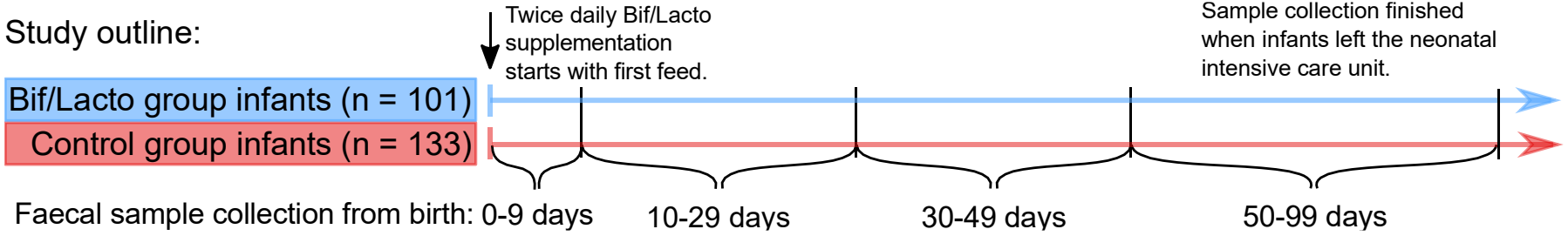
Cristina Alcon
PhD student

Dr Matthew Dalby

- *longitudinal sampling (birth to 3 years)*
- *microbiota and opportunistic pathogen strain profiling*
- *immune & metabolite analysis*
- *correlate to clinical outcomes*

BAMBI study – data outline for this talk

Study outline:

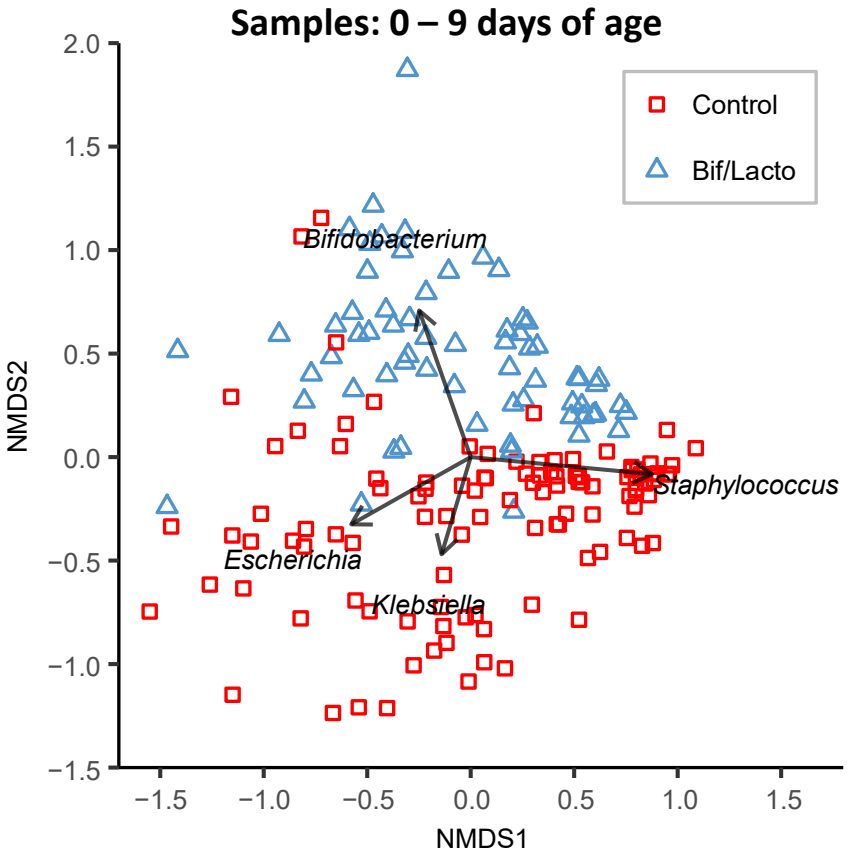


"Bif/Lacto infants"
Infants given Infloran twice daily

"Control infants"
Standard care with no supplemented bacteria

Alcon-Giner/Dalby et al. 2019. bioRxiv [preprint]: doi.org/10.1101/698092

Does supplementation impact the preterm gut microbiota?



Compare the overall microbiota composition of all samples

Non-metric multidimensional scaling (NMDS)

- Collapse information from multiple dimensions into just a few
  - ➢ visualised and interpreted

Based on Bray-Curtis dissimilarity index

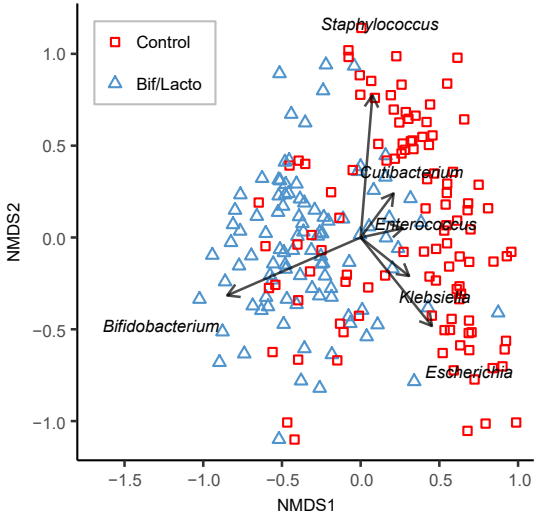
- A statistic used to quantify differences in populations
- Similarity based on abundance

➢ **Calculate the genus 'driving' the separation between groups**

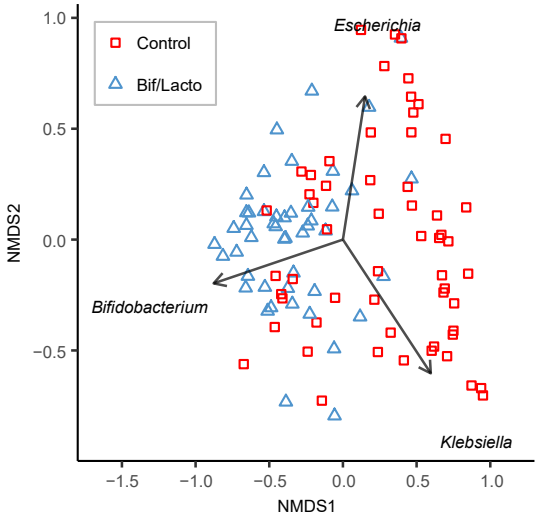
Bif/Lacto infants: ***Bifidobacterium***

Control infants: ***Staphylococcus***
Escherichia
Klebsiella

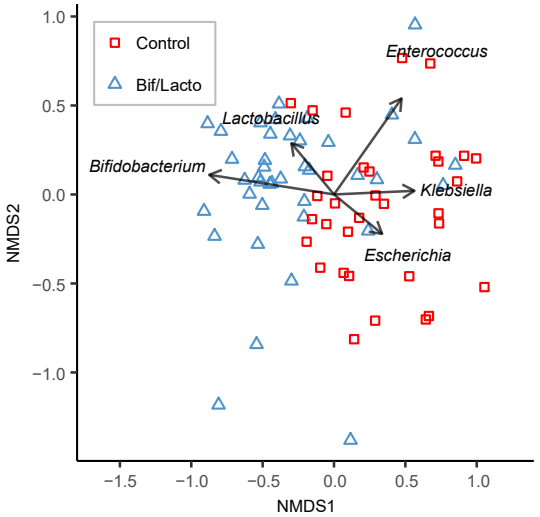
Samples: 10 – 29 days of age



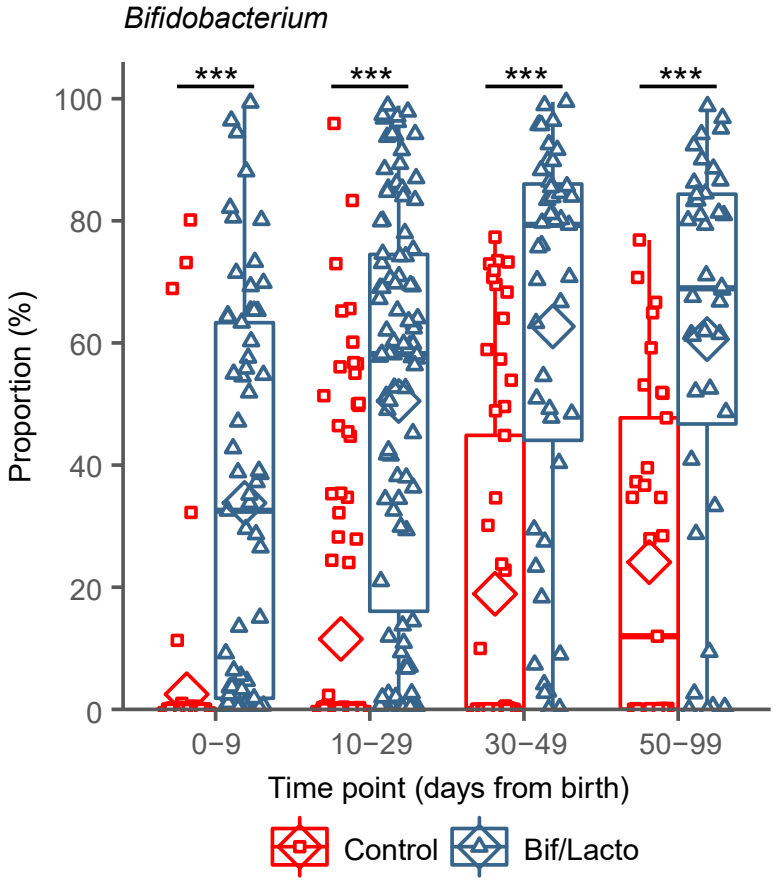
Samples: 30 – 49 days of age



Samples: 50 – 99 days of age



Bif/Lacto infants cluster separately from non-supplemented infants throughout study period

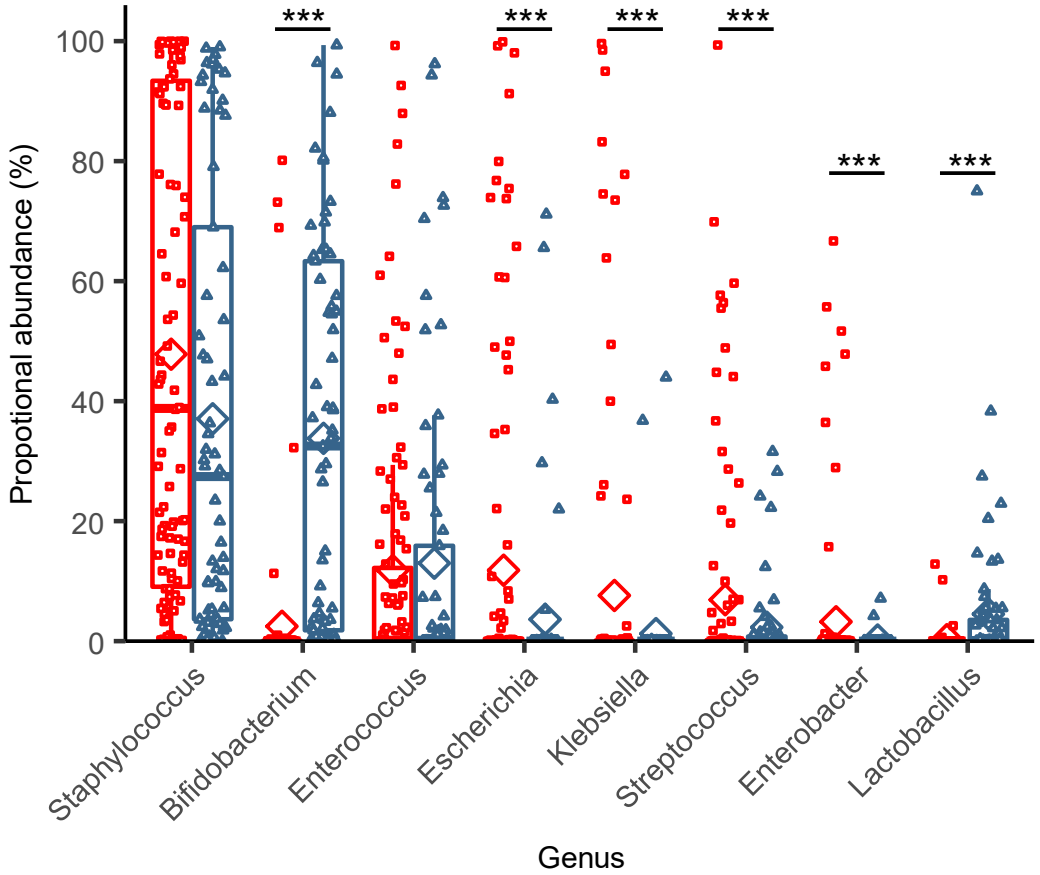


- ***Bifidobacterium* relative abundance higher in Bif/Lacto at all time points**

Each point is an infant sample

Box plots = median and interquartile range

Diamonds = mean

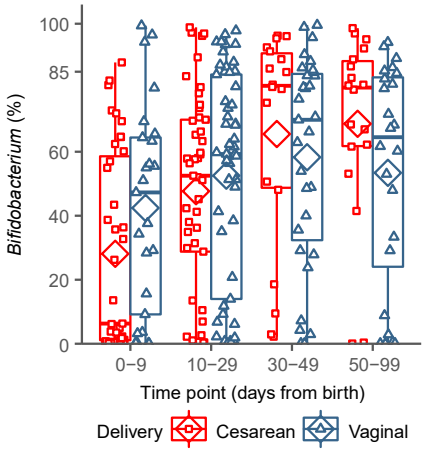


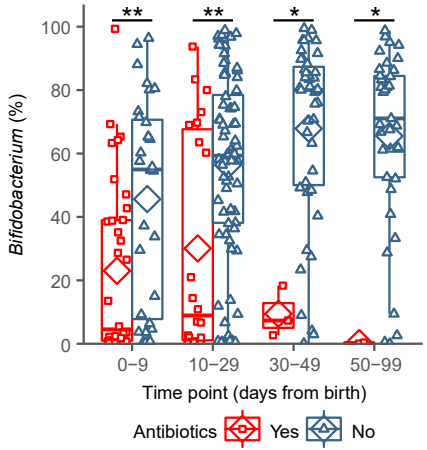
Infants: 0 – 9 days of age

Bif/Lacto vs. Control

Lower:
Escherichia
Klebsiella
Streptococcus
Enterobacter

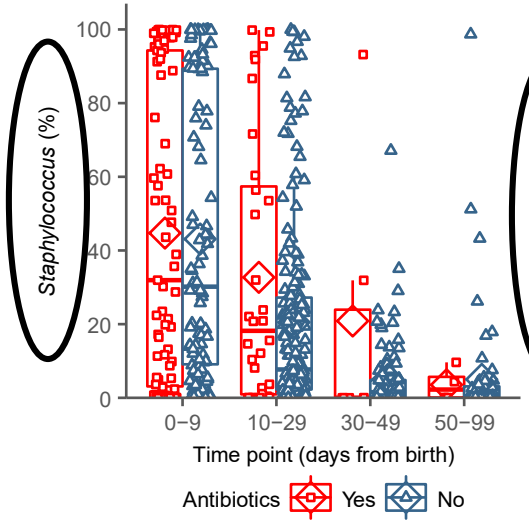
Higher:
Bifidobacterium
Lactobacillus

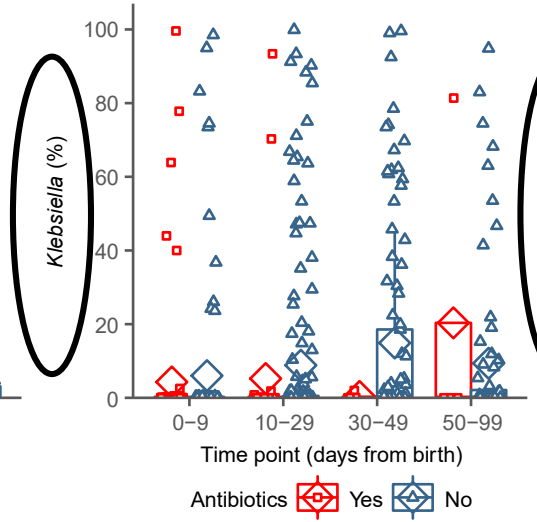


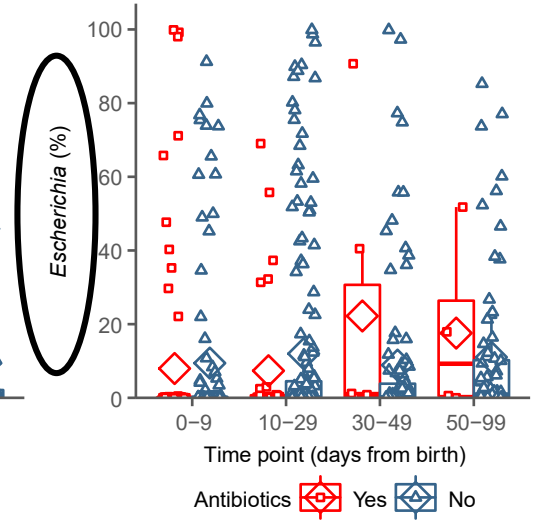


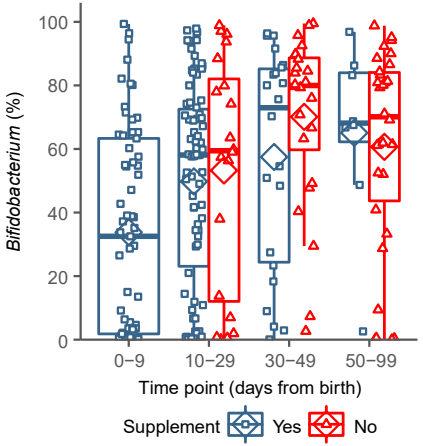
Factors influencing *Bifidobacterium* in Bif/Lacto infants:

Delivery – **no**
Antibiotics – **yes**
Birthweight – **yes**
Gestational age – **yes**



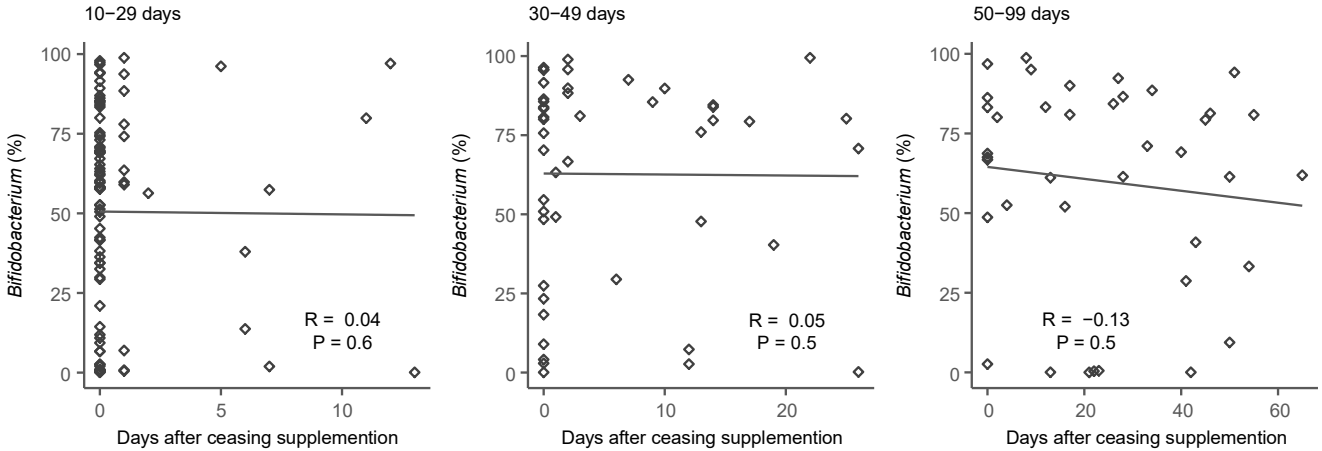


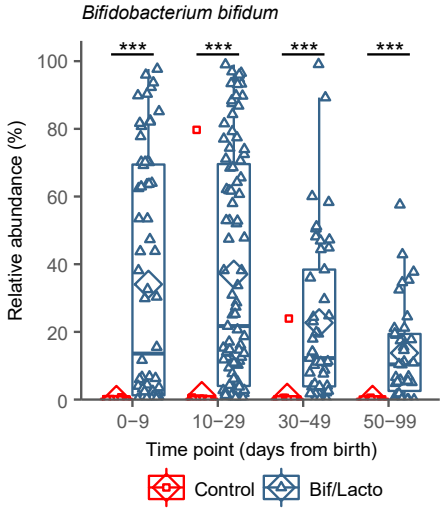


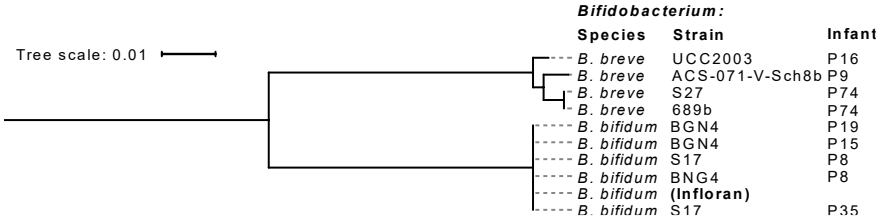


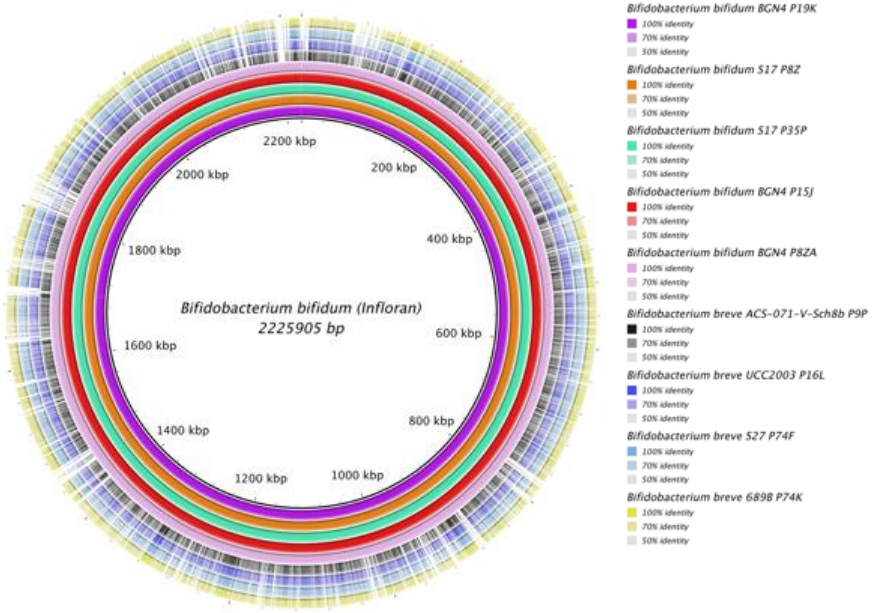
Bifidobacterium persistence after supplementation ceases?

- Infloran supplementation stopped when infants reached the equivalent of 34 weeks gestational age



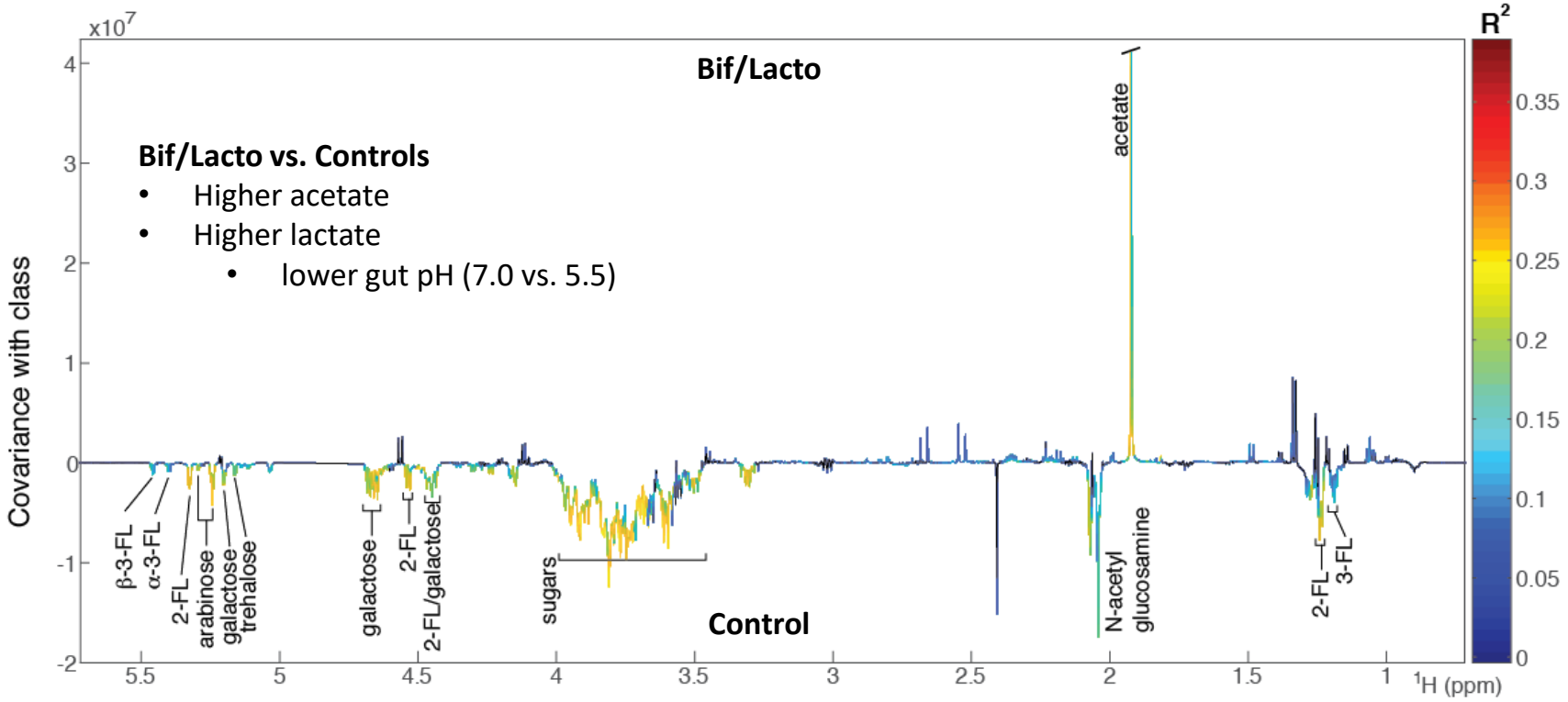






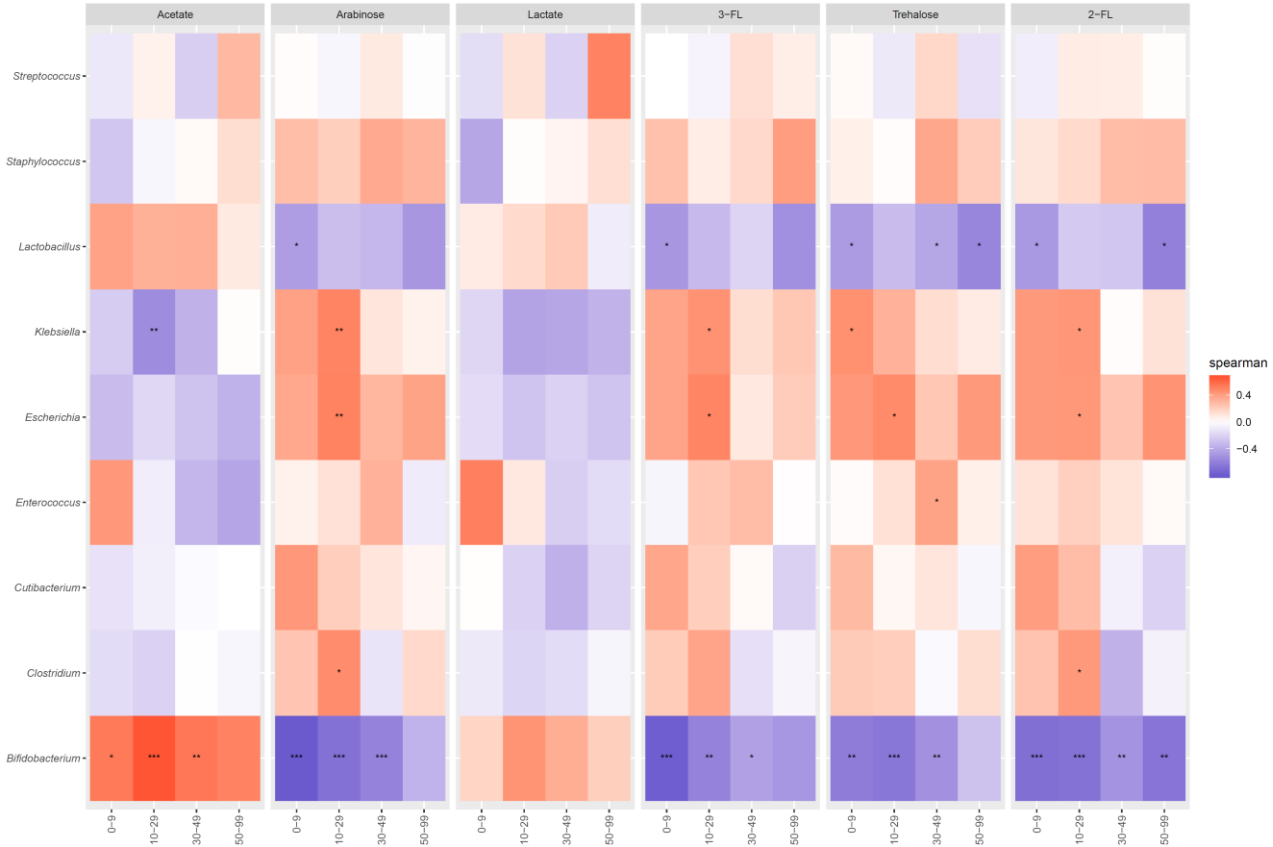
- **Genome analysis confirmed *B. bifidum* isolated from Bif/Lacto infant samples is identical to *B. bifidum* in Infloran**

Bif supplementation alters faecal metabolite profiles



Dr Fahmina Fardus-Reid & Dr Jon Swann (Imperial College London)

OPLS-DA model [combined]: $Q^2Y = 0.587$; $p = 0.01$ (100 permutations)



Spearman correlation heat map

- displaying main faecal metabolites (rows) versus the most abundant bacterial groups (columns)
- Red denotes positive correlation and blue denotes for negative correlation

- *Bifidobacterium* associated with
  - high levels of acetate
  - low amounts of 2-FL, 3-FL, arabinose, and trehalose

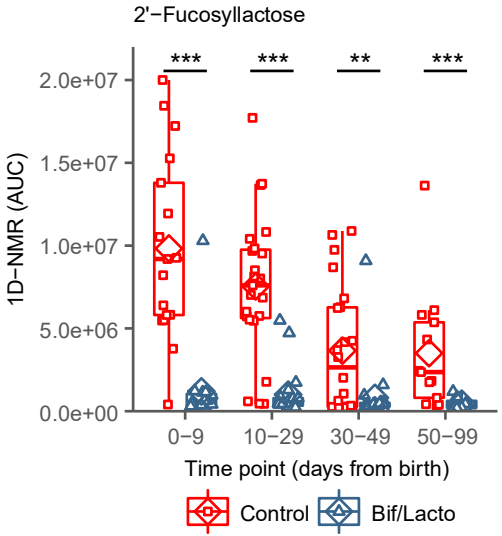
➢ Acetate and lactate metabolic by-products of *Bifidobacterium*

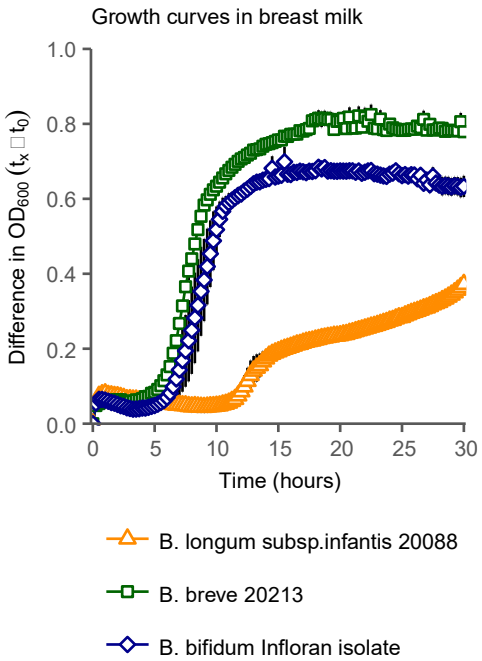
➢ 2-FL and, 3-FL common components of HMOs

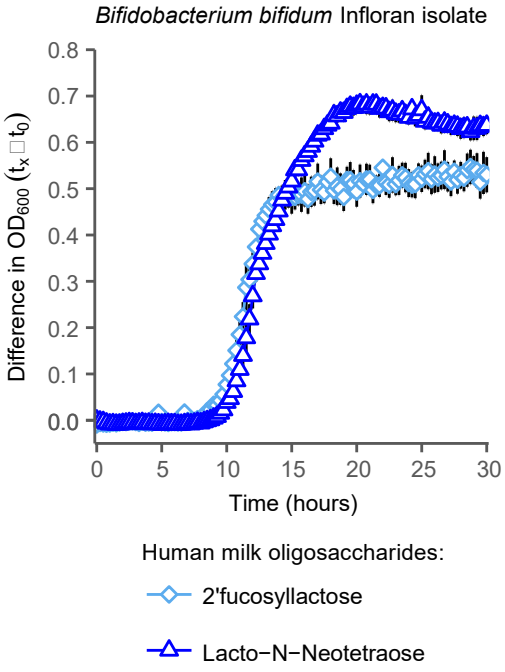
➢ **Does high relative abundance of *Bifidobacterium* in Bif/Lacto infants correlate with HMO metabolism?**

Key point: The link to diet!

Almost all infants were given at least some mothers breast milk or donor milk







- ***B. bifidum* 'probiotic' strain can utilise whole breast milk and individual HMOs for growth**

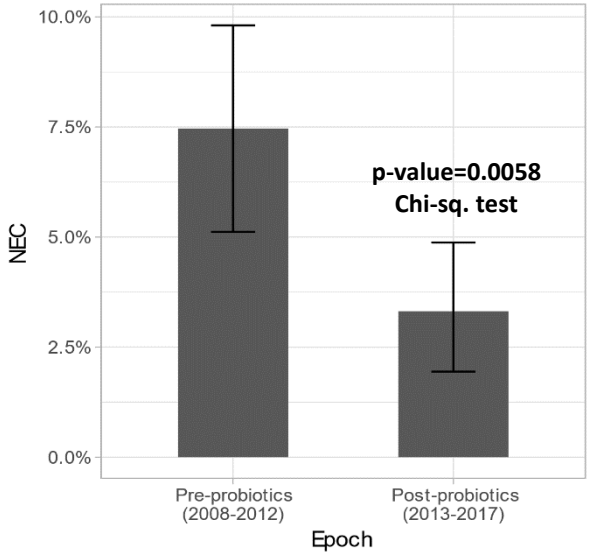
Does this Bif/Lacto supplementation impact preterm health outcomes?

YES!

Prof Paul Clarke

Dr Claire Robertson

- Irrespective of NEC classification system used, there were significant drops in NEC rates
  - pre-Bif/Lacto: 35/469 (7.5%)
  - post-Bif/Lacto: 17/513 (3.3%)



Robertson C et al. Incidence of necrotising enterocolitis before and after introducing routine prophylactic *Lactobacillus* and *Bifidobacterium* probiotics. Archives of Disease in Childhood - Fetal and Neonatal Edition. October 2019. doi: 10.1136/archdischild-2019-317346

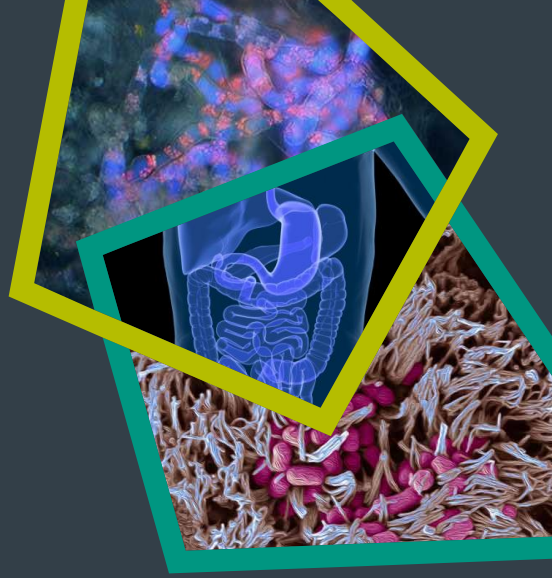
Conclusions (1)

- Preterm microbiota is dominated by *Staphylococcus*, *Streptococcus*, *Enterococcus, Enterobacteriaceae* and *Clostridium*
- Supplementation of preterm infants with *B. bifidum* and *L. acidophilus* positively alters microbiota profiles
    - reduction in potentially pathogenic bacteria correlates with increase in *Bif*
        - *Bif* providing colonisation resistance – why reduction in NEC incidence?
- *Bifidobacterium* abundance is significantly influenced by birth weight, antibiotics and diet
- Birth mode (i.e. vaginal or C-section) does not appear to influence microbiota or *Bif* composition
- >50% reduction in NEC rates since introducing Bif/Lacto supplementation
    - effect-size mirroring RCT meta-analyses and corroborating ongoing use of bacterial therapies or 'probiotics' to prevent NEC in high-risk neonates

Highlights important role that *Bifidobacterium* supplementation may play in modifying early life microbiota development in order to positively impact health

Quadram Institute Science Health Food Innovation

Can we utilise new technologies to rapidly profile preterm microbiomes?



Rapidly profiling preterm microbiota for pathogen diagnostics

- **Oxford Nanopore MinION sequencing platform offers portable and near real time DNA analysis**
  - attractive for in-field or clinical deployment, e.g. rapid diagnostics

- **Preterm associated NEC and sepsis are difficult to diagnose at early stages, and are often associated with sudden serious deterioration**
  - most common pathogens linked include *C. perfringens*, group B streptococcus, *E. coli*, *Enterobacter* spp., and *Klebsiella pneumoniae*
  - huge rise in antimicrobial resistance (AMR) also highlights need for new technologies able to identify at-risk individuals, diagnose infectious agents, and suggest optimised treatments

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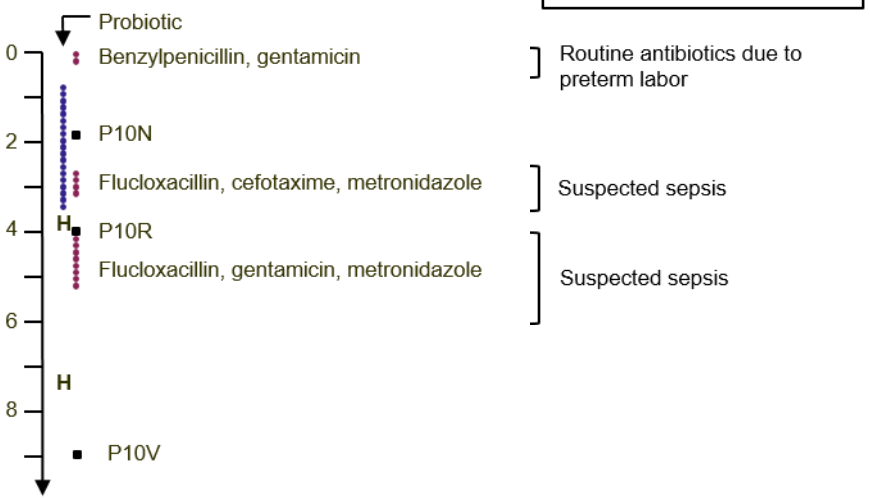
- **Good diagnostic method must be able to confidently identify;**
  - microbes to species level for accurate diagnosis
  - species abundance within the microbiota (as these bacteria can be present within the wider community, but not cause disease when at low levels)
  - AMR gene repertoires

MinION can be used to profile preterm metagenomics samples

*Initially benchmarked efficacy of MinION technology by profiling a bacterial mock community of staggered abundance

Timeline of preterm P10 indicating faecal sample collection points (P10N, P10R and P10V)

P10: 26 weeks at birth, 1050 g



nature microbiology

Article | Open Access | Published: 16 December 2019

Rapid MinION profiling of preterm microbiota and antimicrobial-resistant pathogens

Richard M. Leggett, Cristina Alcon-Giner, Darren Heavens, Shabhonam Caim, Thomas C. Brook, Magdalena Kujawska, Samuel Martin, Ned Peel, Holly Acford-Palmer, Lesley Hoyles, Paul Clarke, Lindsay J. Hall & Matthew D. Clark

Nature Microbiology **5**, 430–442(2020) | Cite this article

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Dr Richard Leggett
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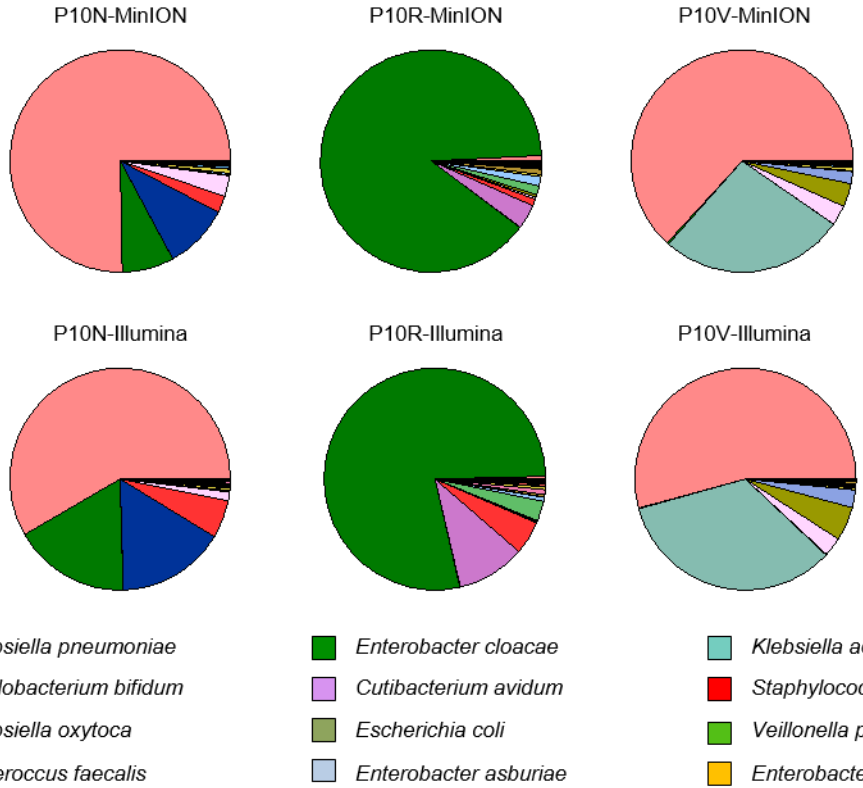
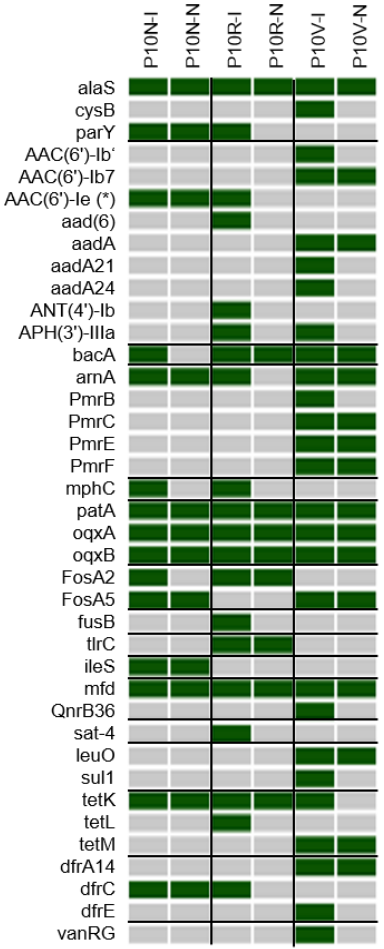
Darren Heavens
EI Team Leader

Dr Matt Clark
NHS Research Leader

MinION vs. Illumina taxonomic and AMR assignments are comparable



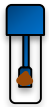


R7.3 flow cells

MinION + NanoOK RT allows ‘real time’ diagnostics

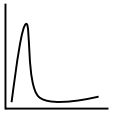
Presentation of NEC symptoms
Prescription of GENERAL antibiotics

0:00



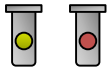
Sample collection and DNA extraction

1:30



DNA quality control

2:15



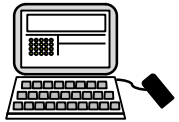
1D Library preparation)

4:00

4:08 Detection of *K. pneumoniae* specific SHV variants (1,500 reads)

Prescription of BESPOKE antibiotics

5:00 Pathogen identified and pathogen-specific AMR hits detected (*Klebsiella pneumoniae,* 20,000 reads + AMR genes)



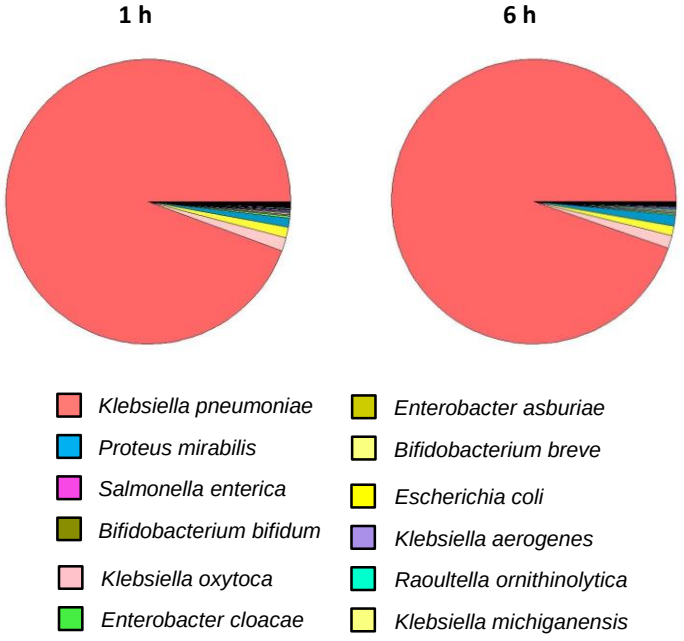
NanoOK RT software
CARD antibiotic database

48:00 End of the run

***Added real-time functionality to NanoOK software, creating NanoOK RT tool**

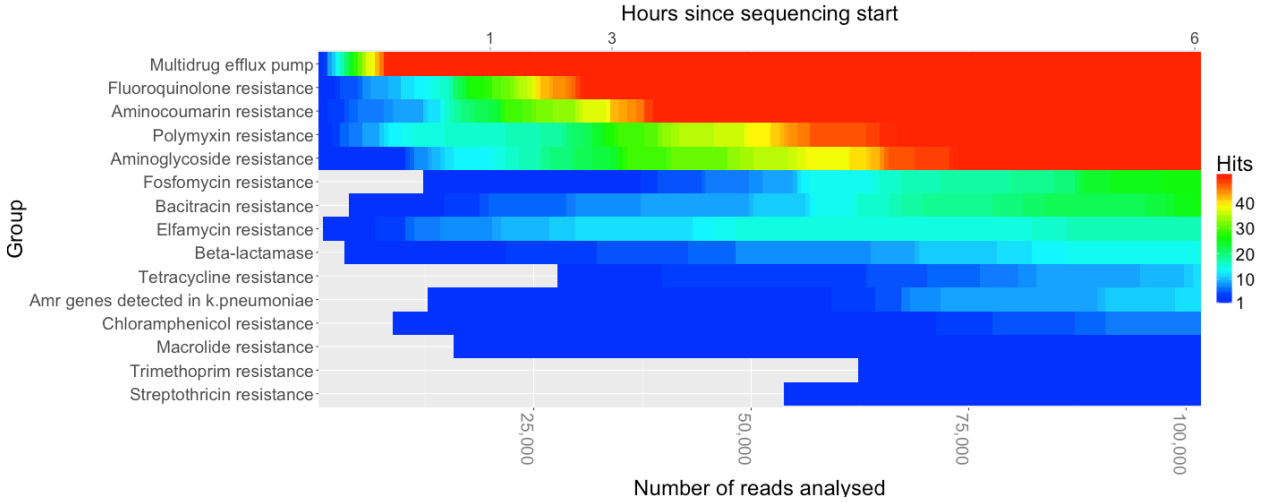
https://nanook.readthedocs.io/en/latest/reporter

Real time taxonomic profiles

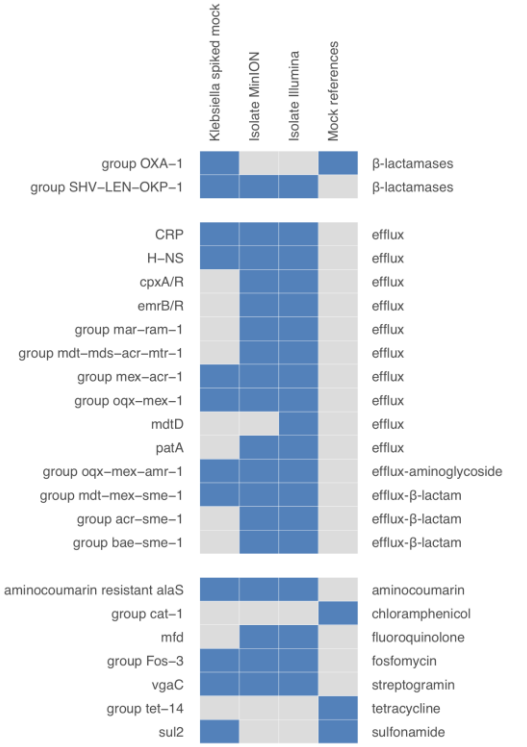


MinION + NanoOK RT allow ‘real time’ AMR profiling

- NanoOK Reporter ‘walkout’ analysis indicated ~90% of AMR genes within P8 mapped to *K. pneumoniae*
- Multidrug exporters (*acrB* and *oqxA*, conferring resistance to tetracycline, chloramphenicol, and fluoroquinolones), *vanSC* (resistance to vancomycin), and *tet 41* (resistance to tetracycline)



Heat map displaying number of CARD database hits detected among most common groups of antibiotic resistance genes found in preterm P8. Top and lower panel indicate the hours since sequencing started and the number of reads analysed.



Known mock community comprising 8 bacteria and P8 isolate of *K. pneumoniae* sequenced using a MinION and analysed with NanoOK RT tool.

MinION generated AMR profiles can be phenotypically validated

- Isolated *K. pneumoniae* strains from patient P8
  - performed Illumina and MinION WGS and assembly on *K. pneumoniae*
- AMR genes including, *FosA* (fosfomycin resistance), *acrA, oqxA*, and *oqxB* (efflux pumps), and *SHV-185* (extended-spectrum β-lactamases, ESBLs), correlated between WGS data and walk-out analysis

- Tested antibiotic resistance phenotypes to link to AMR genotypes with commonly used antibiotics in NICUs
- *K. pneumoniae* had higher minimum inhibitory concentration (MIC) breakpoint value for those antibiotics that were prescribed to P8
  - benzylpenicillin, amoxicillin, metronidazole, gentamicin and vancomycin
- Data correlates with AMR data generated by NanoOK reporter and 'walkout analysis'

Antibiotic	MIC mg/L	Eucast (mg/L)
Gentamicin	3.12	2
Benzylpenicillim	780	ND
Amoxicillin	3900	>512
Metronidazole	1250	ND
Vancomycin	1562	ND
Meropenem	6.25	0.125
Cefotaxime	0.19	0.25

Conclusions (3)

- Used 20-species human microbiota mock community to demonstrate how Nanopore metagenomic sequence data can be reliably and rapidly classified
- In single patient time course, we captured the diversity of the immature gut microbiota
  - observed how complexity changes over time in response to interventions
    - probiotic, antibiotics and episodes of suspected sepsis
- Performed 'real-time' runs from sample to analysis using faecal samples of critically ill infants and of healthy infants receiving probiotic supplementation
- Real-time analysis was facilitated by new NanoOK RT software package
  - reliably identified potentially pathogenic taxa (e.g. *K. pneumoniae*)
  - and corresponding AMR gene profiles within as little as one hour of sequencing
  - validated using mock communities, pathogen isolation, whole genome sequencing and antibiotic susceptibility testing

Our results demonstrate that this pipeline can process clinical samples to a rich dataset able to inform tailored patient antimicrobial treatment in <5 hours

Acknowledgments

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Derek Pickard
Gordon Dougan

Quadram Institute
George Savva

Glycome

Earlham Institute
Richard Leggett
Darren Heavens

NHM
Matt Clark

Nottingham Trent
Lesley Hoyles

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

BBSRC bioscience for the future

wellcome

Marie Skłodowska-Curie Actions

crohn's and colitis UK NACC Inform Support Research