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Profiling pathogens in the preterm gut microbiota

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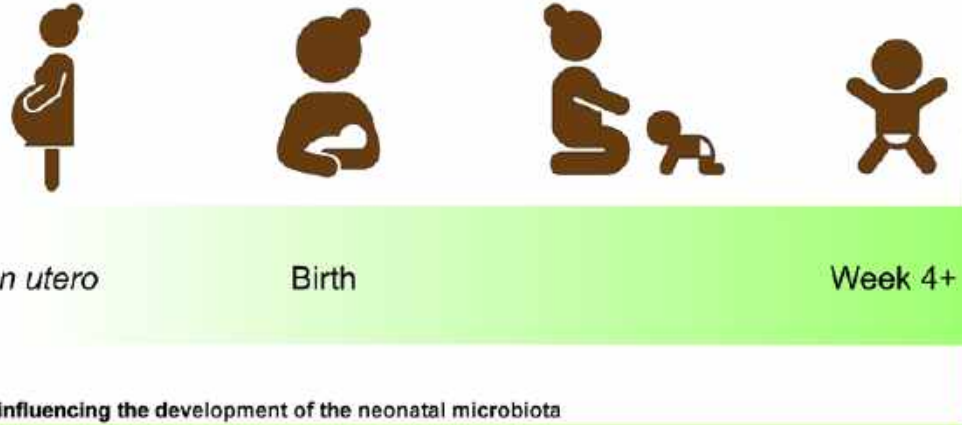
Future of the Microbiome

23-25th March 2021

The Neonatal Gut Microbiota



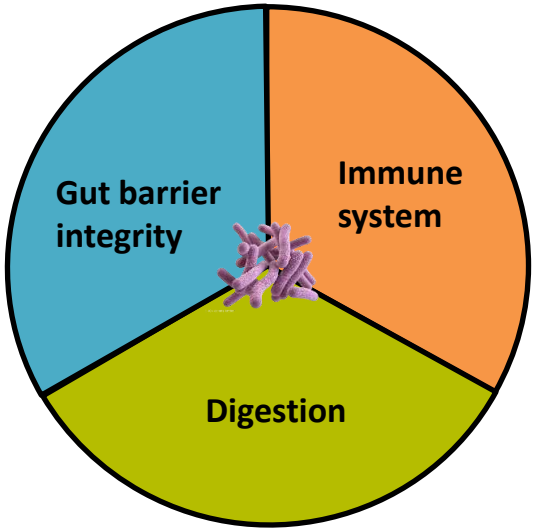
Factors influencing the development of the neonatal microbiota

Sterile womb? Uncertain presence and relevance of microbes before birth	Maternal prophylactic antibiotics through umbilical cord	C-section vs. vaginal birth: disrupted transfer of (faecal) maternal bacteria	Premature vs. term birth: extensive antibiotic treatment and hospital environment	Formula vs. breastfeeding: lack of human milk oligosaccharides (HMOs) & breast milk microbes	Antibiotic treatment: loss of beneficial antibiotic-sensitive species	Transfer of bacteria from siblings and other family members

Neonatal gut microbial signatures

'Healthy' vs. 'disturbed'	Bacterial species & strains resemble those found in the mother's gut vs. bacterial species & strains resemble skin and hospital bacteria	*Bifidobacterium* and *Bacteroides* dominate – low bacterial diversity vs. Higher bacterial diversity – *Staphylcoccus*, *Escherichia coli*, *Enterococcus* & *Klebsiella*	HMO-metabolising *Bifidobacterium* vs. simple sugar-metabolising bacterial strains such as *Escherichia coli*, & *Klebsiella*	*Bifidobacterium*-produced metabolites such as acetate – acidic environment vs. reduced short-chain fatty acids and more neutral environment

Function



Dalby MJ and Hall LJ. **Recent advances in understanding the neonatal microbiome**. F1000Research 2020, 9:422

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
  - Innate and adaptive

- C-section major delivery mode (~60%)
- Infants reside in 'sterile' Neonatal Intensive Care Units (NICUs)
  - Average stay is ~ 6 weeks
- Antibiotic prescribing common
  - exposure rates 75-95%

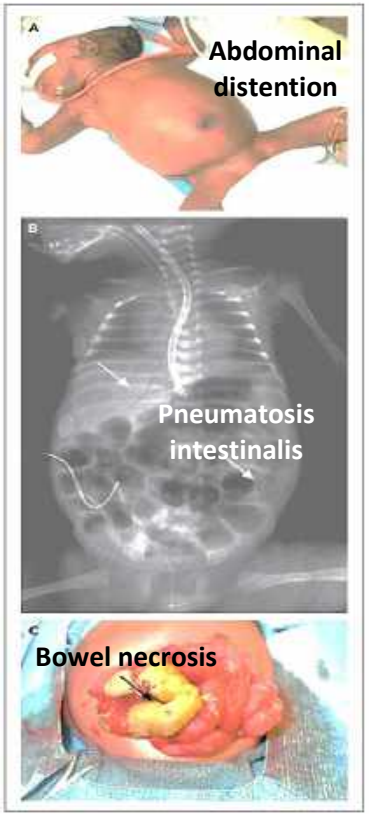
- **NICUs keeps premature infants alive**

- But what about the gut microbiota?

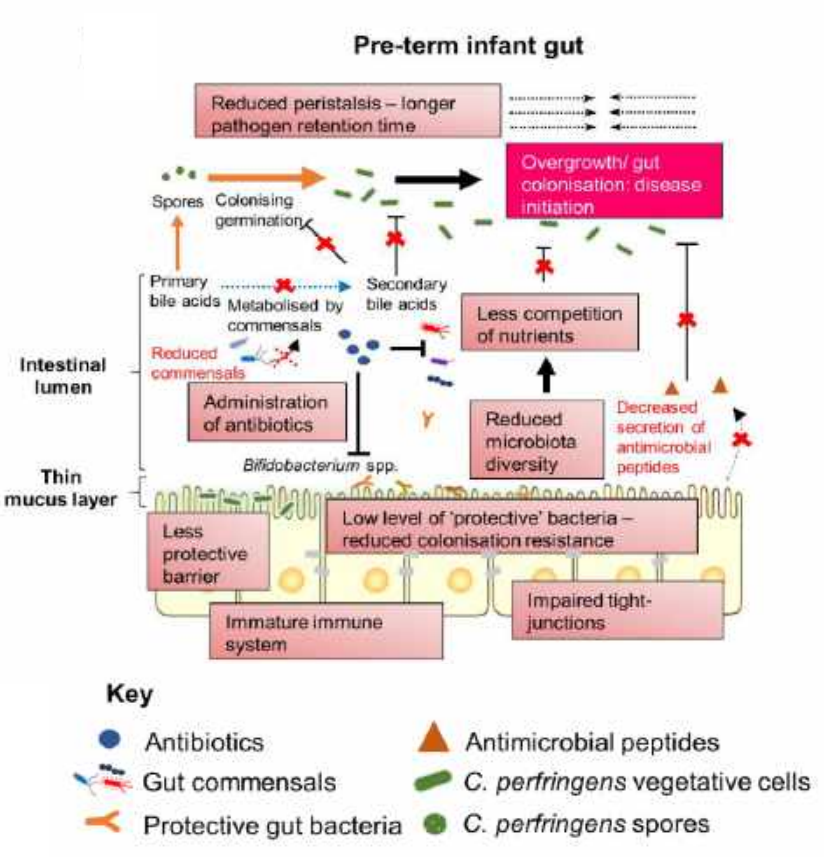
➢ Disrupted normal colonisation of infant gut
  - ➢ **Reduced levels of *Bifidobacterium***
  - ➢ **Increased levels of pathobionts (& hospital-acquired bacteria)**

Necrotising enterocolitis (NEC)

- Aberrant microbiota 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 overgrowth of pathobiont members of the preterm gut mictrobiota



Adapted from Neu and Walker, NEJM, 2011



Kiu and Hall. An update on the human and animal enteric pathogen *Clostridium perfringens.* Emerg Microbes Infect. 2018; 7: 141.

Rapidly profiling preterm microbiota for pathogen 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

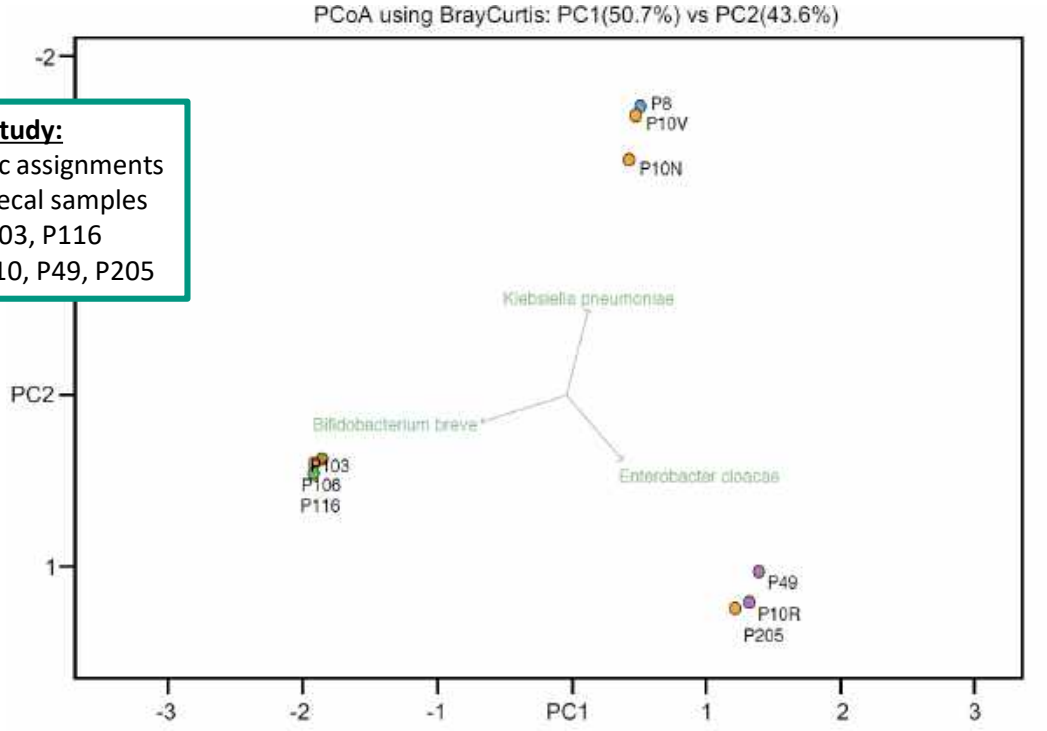
- **Oxford Nanopore MinION sequencing platform offers portable and near real time DNA analysis**
  - attractive for in-field or clinical deployment, e.g. rapid diagnostics

- **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

Samples used in the study:
PCoA based taxonomic assignments of MinION preterm faecal samples
- Healthy – P106, P103, P116
- NEC/sepsis – P8, P10, P49, P205



Dr Cristina Alcon

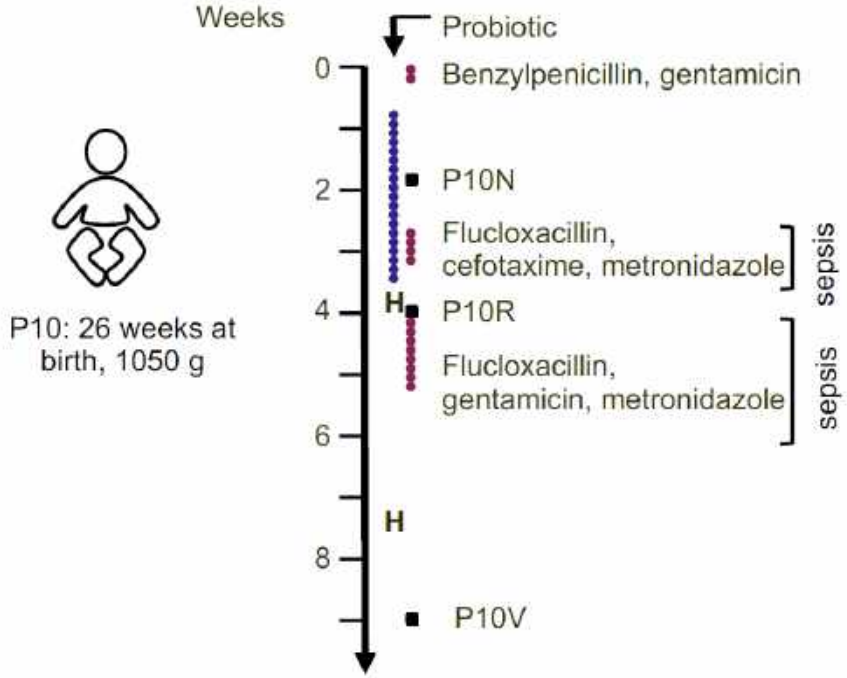
Dr Richard Leggett

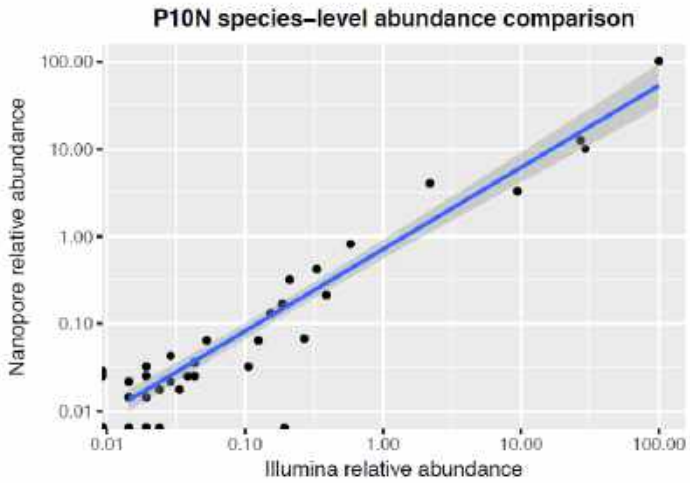
Dr Darren Heavens

Prof Matt Clark

Leggett & Alcon-Giner at al. 2020. Nature Microbiol. 5 430-442

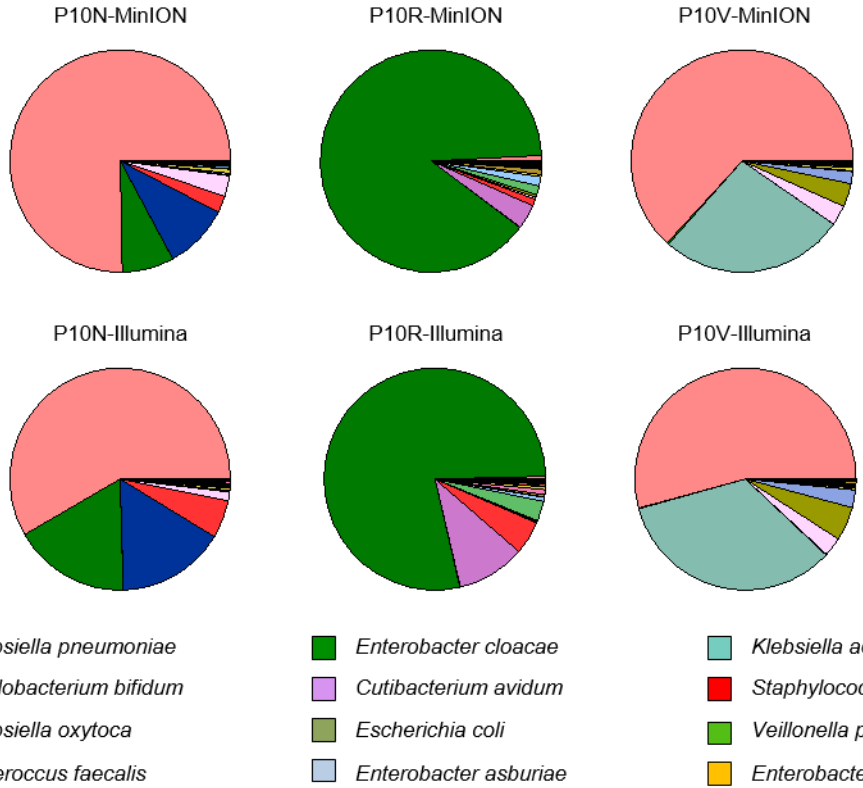
Longitudinal profiling of preterm gut microbiota

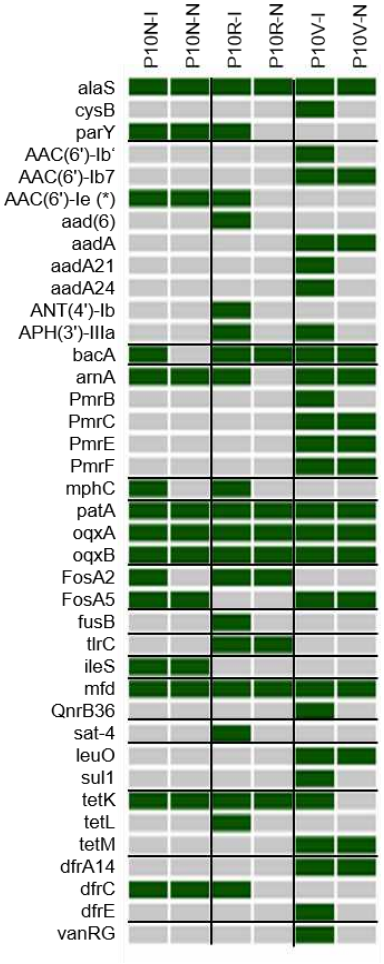




Correlation plot of normalised species abundance in taxonomic assignments of Illumina vs. Nanopore → high correlation with expected proportions

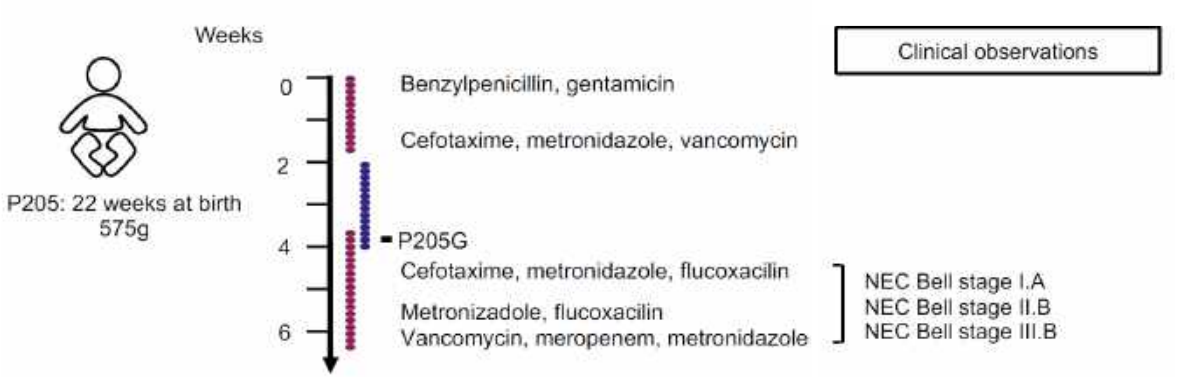
MinION vs. Illumina taxonomic and AMR assignments are comparable

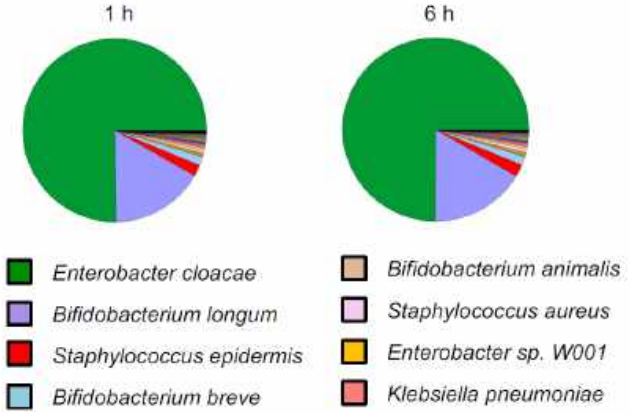


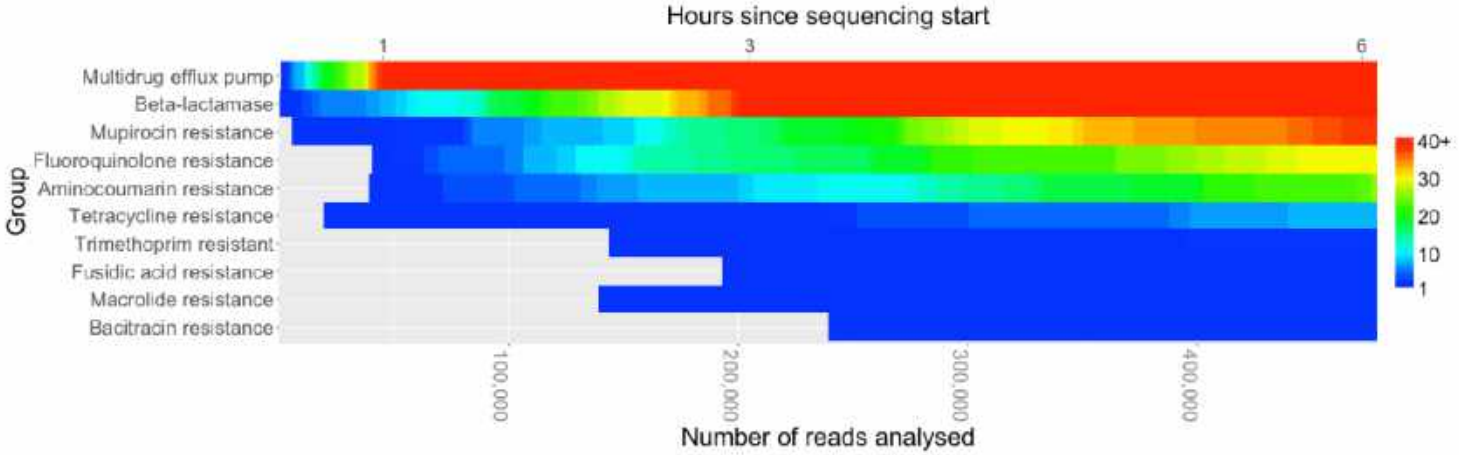


R7.3 flow cells

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



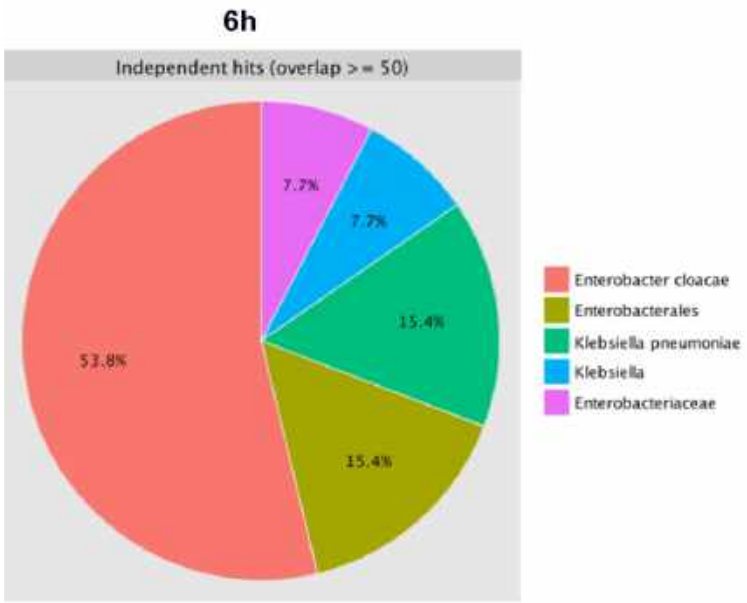




Heat map displaying number of CARD database (i.e. AMR) hits among the most common AMR genes found in P205

Correlating AMR hits with bacteria taxa

'Walk-out' analysis in P205

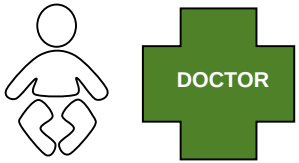


- **For clinically actionable data – also need to know 'who' is carrying AMR determinants**
  - added 'Walkout' analysis NanoOK RT tool
  - examines each read that has a good quality hit to an AMR gene to see if it also has an 'independent' hit to the nt (or bacterial alias) database
  - defined independence as being a match that stretched at least 50 bases away from the AMR gene in either direction

Real time microbiome profiling – how fast can we go?

Presentation of NEC symptoms
Prescription of GENERAL antibiotics

Preterm P8



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)

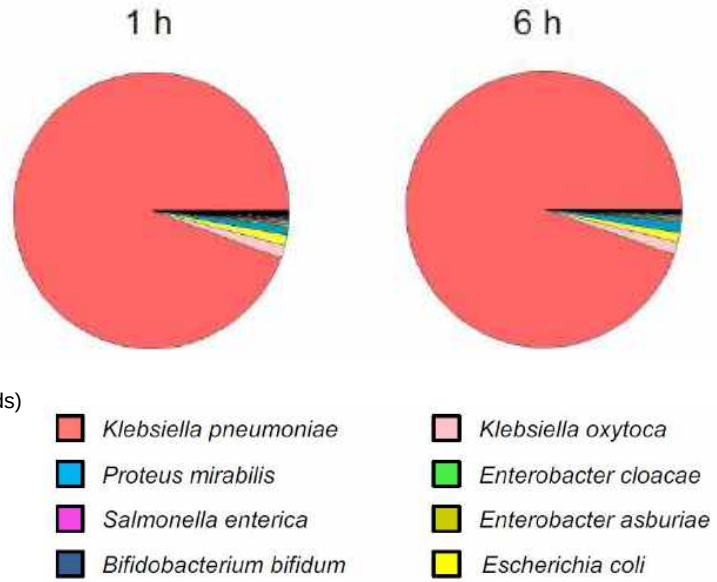
Prescription of BESPOKE antibiotics

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

Real time taxonomic profiles; using NanoOK RT

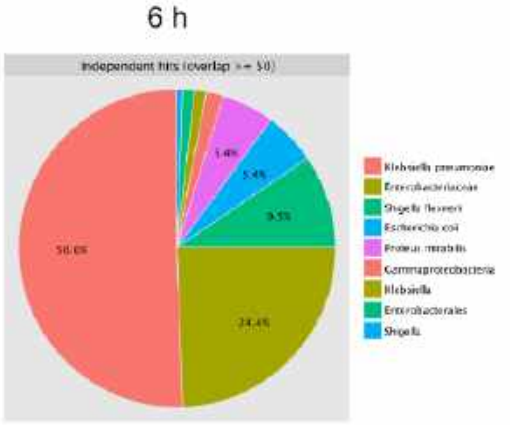


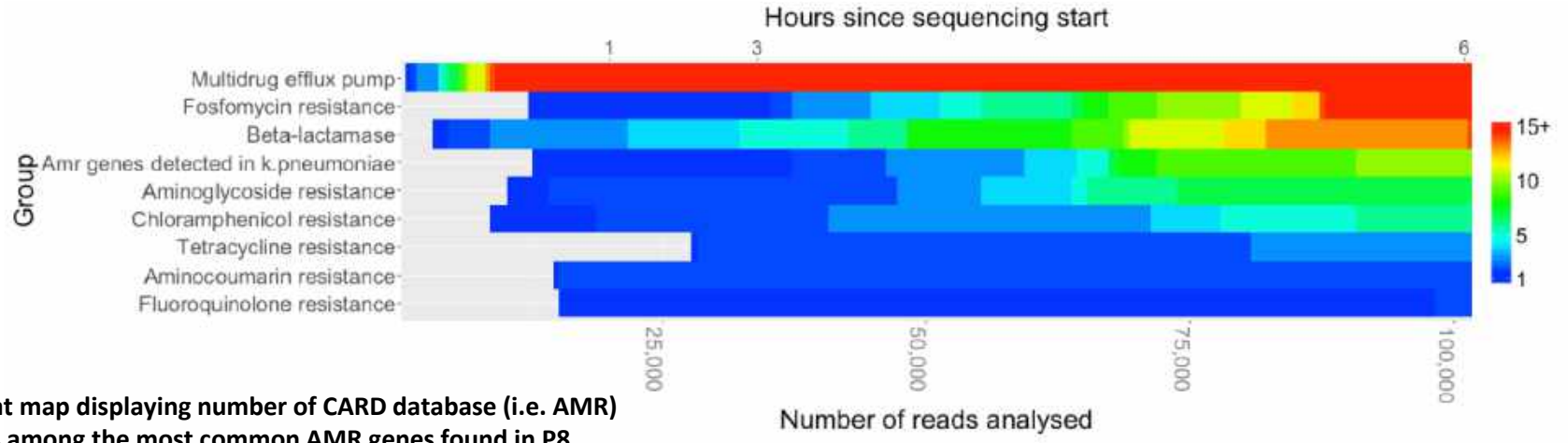
‘Real time’ AMR profiling and benchmarking

- NanoOK Reporter ‘walkout’ analysis indicated ~75% of AMR genes within P8 mapped to *Klebsiella*

→ *Klebsiella* is of particular concern due to increasing emergence of multidrug-resistant isolates that cause severe infection

Walkout analysis showing taxa encoding AMR genes; independent bacterial hits (defined as > 50bp away from AMR sequence



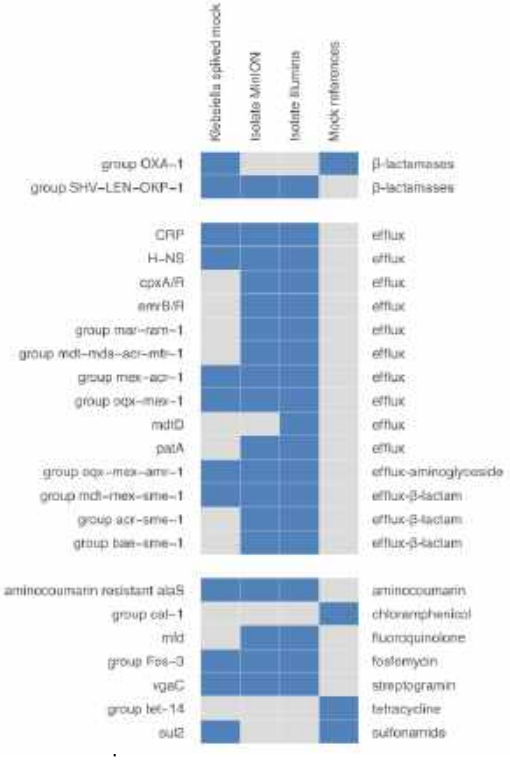


Heat map displaying number of CARD database (i.e. AMR) hits among the most common AMR genes found in P8

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'



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

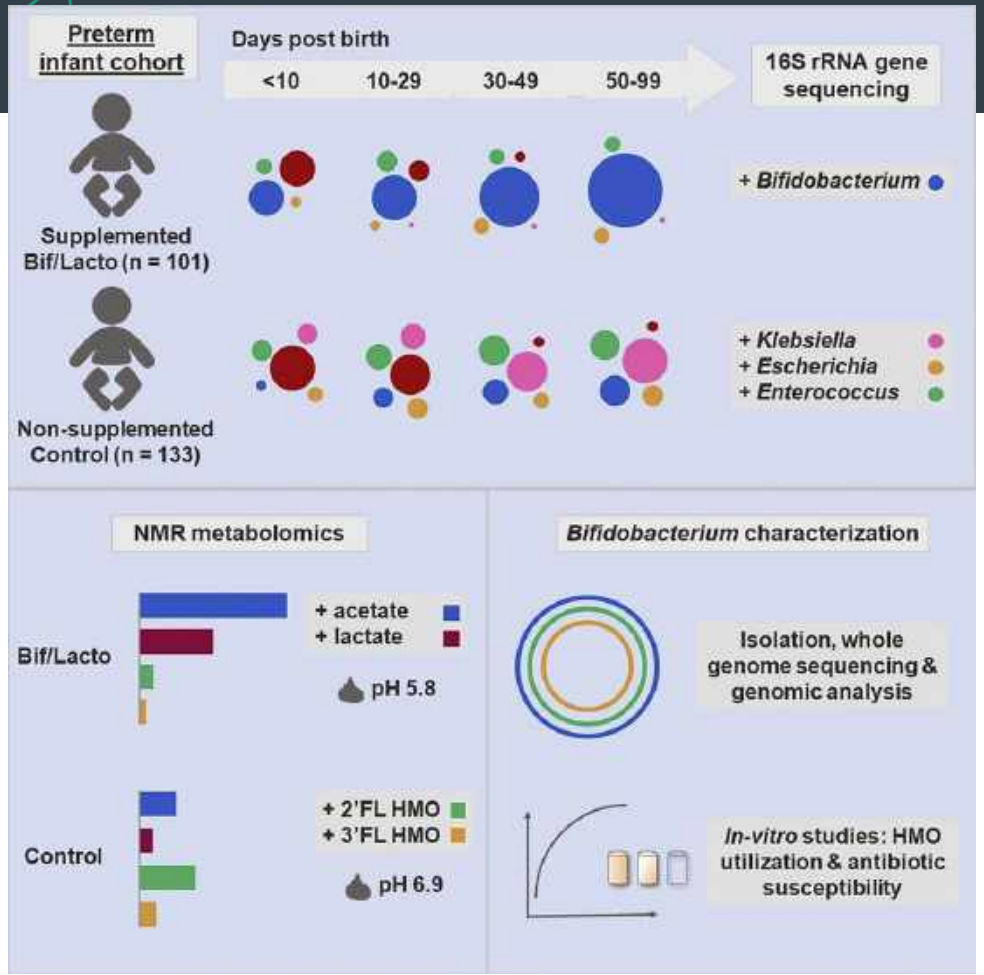
Summary

- **Nanopore metagenomic sequence data can be reliably and rapidly classified**
- **In patient time courses, we captured diversity of the preterm 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

Also used the Flongle and new RAD kits → increased speed and lower cost

Our results demonstrate that this pipeline can process clinical samples to a rich dataset able to inform tailored patient antimicrobial treatment in <4 hours

The BAMBI Study



→ *Bifidobacterium* dominates the gut microbiota in supplemented preterm infants

→ Supplemented preterm infants have lower abundance of potential pathogens

→ Metabolomic analysis show higher faecal acetate and lower pH in supplemented infants

→ *In vitro* and genomic analysis confirms HMO metabolism in *Bifidobacterium* supplement

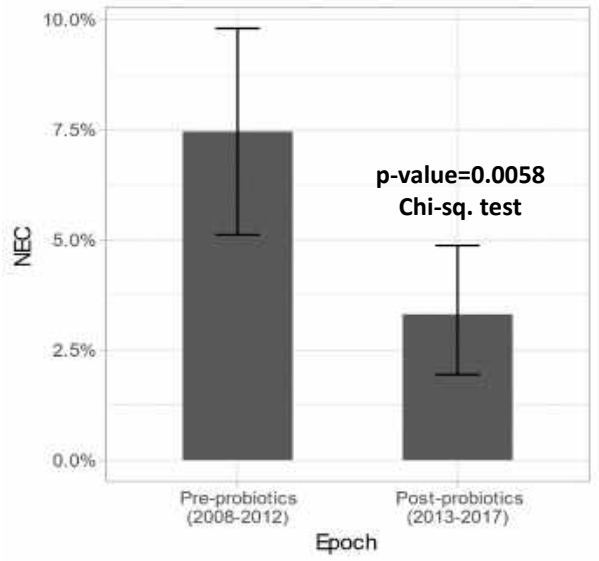
Alcon-Giner/Dalby et al. 2020. Cell Reports Medicine. doi.org/10.1016/j.xcrm.2020.100077

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. 2019. doi: 10.1136/archdischild-2019-317346

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