The impact of antibiotics on the gut microbiota

Dr Paul Cotter

paul.cotter@teagasc.ie
Teagasc Food Research Centre, Moorepark
& Alimentary Pharmabiotic Centre (APC), University College Cork
Cork, Ireland
Intro - Culturing of gut microbes reveals only the tip of the iceberg

Only 30-40% of gut microbes are culturable

Need for the development of culture-independent (DNA-based) approaches

Field revolutionized through the advent of Next Generation DNA Sequencing (NGS) & bioinformatics
Intro - Next Generation DNA Sequencing (NGS)

16S gene Sequencing

Sample (usually faecal)

DNA extraction

Shotgun Sequencing

16S specific PCR

Randomly shear DNA

Composition

Functional Potential

- Sutterella
- Lachnospira
- Bifidobacterium
- Faecelibacterium
- Clostridium
- Bacteroides
- Enterobacter
- Streptococceae
- Ruminococcus
- Lactobacillus

RNA processing
Chromatin structure
Energy production
Cell Division
Amino-acid metabolism
Nucleotide metabolism
Carbohydrate metabolism
Coenzyme metabolism
Lipid metabolism
Translation
Transcription
Replication
Cell wall/membrane biogenesis
Cell motility
PTMs, protein folding and turnover
Inorganic ion metabolism
Secondary metabolite biosynthesis
General function prediction only
Function unknown
Signal-transduction mechanisms
Intracellular trafficking
Defense mechanisms
Cytoskeleton
Impact of Antibiotics on the Gut Microbiota

Thanks to these technologies, the contribution of the gut microbiota to health has become even more apparent (as highlighted earlier by Prof Azpiroz)

This has also highlighted the detrimental effects of the over-use of antibiotics

2 representative studies from our laboratory

1. Impact of antibiotics on the infant microbiota
2. Impact of anti-C. difficile treatment on the gut microbiota
Part 1:
Short term impact of broad spectrum antibiotics on the infant gut microbiota

Gut microbiota evolves rapidly during the first 2 yrs of life

Factors affecting gut microbiota at birth:
- Preterm vs. full term
- Mode of delivery
- Maternal weight/maternal diet
- Hospital environment
- Contact with mother/healthcare staff
- Antibiotic use (during pregnancy or in early days of life)
- Feeding choice

Factors affecting the development and acquisition of the infant gut microbiota (up to 24 months):
- Breastfeeding vs. Formula feeding
- Use of probiotic/prebiotic supplemented feeds
- Antibiotic exposure
- Timing of weaning and foods chosen
- Home structure e.g. number of siblings

Increase in diversity

Age 0 12 months 24 months
Increase in stability

There is emerging evidence that disruption of the microbiota during this time of life can have detrimental consequences later in life including atopic disease – Allergy, Asthma, Eczema, Obesity (?) & others

Fouhy et al. Gut Microbes 2012
Infant microbiota study

Controls

Antibiotic treated

- No antibiotic
- Ampicillin + gentamicin
- 48 hrs
- Assess microbiota
- 4 wks
- Assess microbiota
- 8 wks
- Assess microbiota

= ?
Antibiotics are responsible for changes in the infant microbiota (phylum).

**Significant Changes**

- * Week 4
- * Week 8
- ♦ Recovery

**Week 4**

- Treated: Proteobacteria (3%), Firmicutes (43%), Actinobacteria (54%)
- Controls: Proteobacteria (5%), Firmicutes (24%), Actinobacteria (37%)

**Week 8**

- Treated: Proteobacteria (3%), Firmicutes (43%), Actinobacteria (54%)
- Controls: Proteobacteria (5%), Firmicutes (24%), Actinobacteria (37%)
Antibiotics are responsible for changes in the infant microbiota (genus)

- **Week 4**
  - **Treated**
    - 5% ♦
    - 13% ♦
    - 75% ♦ *
  - **Controls**
    - 10% ♦ *
    - 38% ♦ *
    - 25% ♦ *
    - 4% ♦ *

**Legend**:
- Parabacteroides
- Lactobacillus
- Gemella
- Sutterella
- Bilidobacterium
- Barnesiella
- Bacteroides
- Phascolarctobacterium
- Oscillibacter
- Anaerococcus
- Peptostreptococcus
- Corynobacteriaceae
- Micrococcaceae
- Anaerotruncus
- Rikenella
- Members of the Enterobacteriaceae family
- Alistipes
- Peptostreptococcaceae incertae Sedis
- Streptococcus
- Clostridium
- Enterococcus
- Lachnospiraceae incertae sedis
- Veillonella
- Finegoldia
- Actinobacter
- Lactococcus
- Fusobacterium
- Peptoniphilus
- Mucuspirrillium
- Akkermansia
- Prevotella
- Weisella
- Porphyromonas
- Faecalibacterium
- Ruminococcus
But the (desirable) bifidobacteria are not recovering completely

High throughput sequencing – specific sequencing of the bifidobacteria populations (*rpoB* is encodes the β subunit of the bacterial RNA polymerase)

*B. longum* is more dominant in the treated samples

*B. breve* present to a higher extent in controls
Summary

Antibiotic administration early in life resulted in:
Massive increase in Proteobacteria
Reduced Actinobacteria
Bacteroidetes very poor recovery over 8 weeks
After 8 weeks infant gut microbiota not fully recovered
Bifidobacteria recover but the type of *Bifidobacterium* differs

What are the long term implications?
Undesirable interactions between the host and the microbiota
Atopic disease – Allergy, Asthma, Eczema, Obesity (?) & others (increased risk of infection?)
Part 2:
Impact of antimicrobials employed to control *Clostridium difficile* infection

Rea et al. PNAS 2011 108 Suppl 1:4639
Targeting *Clostridium difficile* in the gut microbiota

Why *Clostridium difficile* as target?

- Major GI infectious agent
- Sensitive to metronidazole and vancomycin.
- Increasingly associated with GI disorders
- Causes 15-25% of all antibiotic associated diarrhoea
- Toxin producer which can be fatal in the elderly
- Incidence is on the increase
Assessing the potential of Antimicrobial peptides

Simulated human distal colon model performed for 5 hours prior to addition of lacticin 3147.

The Magic bullet: a narrow spectrum antimicrobial?

Overlaid with Clostridium difficile

Bacillus thuringiensis

Thuricin CD; a two component bacteriocin

30,000 sporeformers

Overlaid with Clostridium difficile

Rea et al., PNAS 2010 107:9352
Thuricin CD is a novel two component bacteriocin, with three sulphur to $\alpha$-carbon bridges in each peptide (NMR structure solved by Sit and Vederas, U. Alberta).

Target specificity of Thuricin CD:

- Clostridium difficile
- Lactobacillus paracasei
- Bifidobacterium lactis BB12
- L. johnsonii LA1
- L. rhamnosus GG
- L. casei (Yakult)
- C. difficile
- B. lactis Bb12
- L. casei (Actimel)
In vitro distal colon model to further test specificity*

20% human faecal slurry

\[ 10^6 \text{ Clostridium difficile} \]

control

Thuricin CD (90uM)

control

Vancomycin (90uM)

Metronidazole (90uM)

24h

24h

24h

24h

Total DNA purified, amplified V4 region of 16S rRNA, 454 sequencing, MEGAN

Rea et al. PNAS 2011 108 Suppl 1:4639

*Gibson lab
Thuricin CD exhibits potent \textit{ex vivo} activity

- Thuricin CD (90 uM)
- Vancomycin (90 uM)
- Metronidazole (90 uM)
Thuricin CD specificity confirmed

Phylum
- Bacteriodetes
- Firmicutes
- Proteobacteria
- Actinobacteria
- Spirochaetes
- Lentisphaerae
- Ternicutes

Family
- Enterobacteriaceae
- Pasteurellaceae
- Desulfovibrionaceae
- Alcaligenaceae
- Bacteroidaceae
- Porphyromonadaceae
- Streptococcaceae
- Leuconostocaceae
- Carnobacteriaceae
- Lachnospiraceae

Rea et al. PNAS 2011 108 Suppl 1:4639
Summary

Confirmation that broad spectrum antibiotics:

Bring about a massive increase in Proteobacteria

Negatively impact on overall gut microbial diversity

Identification of a new narrow spectrum anti-\textit{C. difficile} antimicrobial

What are Overall Conclusions?

Broad spectrum antibiotics need to be used with care and should ideally be substituted with narrow spectrum antimicrobials (or used in conjunction with approaches to protect/encourage the recovery of the gut microbiota)
Teagasc
Dr Mary Rea
Dr Fiona Fouhy
Prof Paul Ross
Dr Catherine Stanton
Dr Orla O’Sullivan

University College Cork
Prof Colin Hill
Prof Fergus Shanahan
Prof Ger Fitzgerald

Funding

The Irish Agriculture and Food Development Authority