Gut Microbiome Modulation: Alternative to Antibiotics?

OIE Headquarters, 2nd International Symposium ATA

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Our background: Diarrhea in pediatrics

20 years of scientific collaboration with NRC

*E. coli* diarrhea is largely antibiotic-resistant:

we studied alternative treatment options
Introducing beneficial bacteria by:

- Fecal transplantation, probiotics

Modifying nutrient input:

- Diet, fibres, prebiotics, milk oligosaccharides

Eliminating undesired bacteria:

- Phages, bacteriocins, antibodies

Targeting →pathogen or →microbiota or →host with nutritional interventions

Pathogen-Microbiota-Host Interaction determines Infection Outcome

Microb Biotech 9:553
Passive immunization with bovine milk antibodies

Dairy cows as bioreactors: modified concept of P. Ehrlich & E.v. Bering (1890)

° We vaccinated cows with rotaviruses and E. coli

° Dam immunization stimulates broadly cross-reacting antibodies

° Cows transfer antibodies to the calf via colostrum

° We collected tons of colostral milk

° We isolated 10 kg immunoglobulins per 1 t of milk

° Clinical trials showed treatment effects against RV diarrhea

J.Clin. Micro. 25, 982;
J. Gen. Virol. 69, 1647;
J. Infect. Dis. 156, 158;
Pediatr. Infect. Dis. J. 17,1149;
Scand. J. Gastro. 35, 711;
BMC Microbiol 7, 86
Preclinical work: dose effect in mice

BMC Microb 7: 86
Preclinical work: intestinal histology (mouse)

BMC Microb 7: 86
Clinical trials in Germany, USA and Bangladesh showed treatment effects against RV diarrhea...

Attenuated fluid loss in g/kg/day:

<table>
<thead>
<tr>
<th></th>
<th>IIBC (n = 40)</th>
<th>Placebo (n = 40)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 1</td>
<td>87 ± 10*</td>
<td>105 ± 10</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>78 ± 14</td>
<td>115 ± 14</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>62 ± 10</td>
<td>110 ± 14</td>
<td>0.005</td>
</tr>
<tr>
<td>day 4</td>
<td>54 ± 11</td>
<td>84 ± 12</td>
<td>0.06</td>
</tr>
</tbody>
</table>

...but it did not work against *E. coli* diarrhea

JID 156:158

PIDJ 17: 1149

SJG 35: 711
Health-promoting lactobacilli: Modified concept of E. Metchnikoff (1905)

Isolation of *Lactobacillus paracasei* from infant feces → safety studies → RCT

Yogurt: lactic streptococci and lactobacilli

J. Bacteriol. 189, 1311; 189, 8109; 190, 3161
Pediatrics 116, e221
Patient enrolment scheme of probiotic trial at icddr,b

NIH selected dietary trial: RCT, WHO diarrhea criteria, pathogen-specific

no clinical effect

Ped 116: e221
Clinical outcome in non-rotavirus patients

Low amounts of probiotic were detected in stool, but fecal microbiota was not investigated.

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>L. paracasei (n=27)</th>
<th>Placebo (n=36)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cumulative stool output g/kg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-24 h</td>
<td>40 ± 26</td>
<td>68 ± 43</td>
<td>0.01</td>
</tr>
<tr>
<td>0-48 h</td>
<td>79 ± 53</td>
<td>130 ± 91</td>
<td>0.01</td>
</tr>
<tr>
<td>0-72 h</td>
<td>118 ± 82</td>
<td>189 ± 127</td>
<td>0.01</td>
</tr>
<tr>
<td>0-96 h</td>
<td>151 ± 118</td>
<td>248 ± 155</td>
<td>0.01</td>
</tr>
<tr>
<td>0-120 h</td>
<td>188 ± 167</td>
<td>318 ± 193</td>
<td>0.01</td>
</tr>
<tr>
<td>0-144 h</td>
<td>225 ± 218</td>
<td>381 ± 240</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Cumulative stool frequency (number)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-24 h</td>
<td>6.2 ± 3.8</td>
<td>8.9 ± 5.6</td>
<td>0.04</td>
</tr>
<tr>
<td>0-48 h</td>
<td>11.7 ± 6.4</td>
<td>16.8 ± 11.3</td>
<td>0.04</td>
</tr>
<tr>
<td>0-72 h</td>
<td>17.1 ± 8.5</td>
<td>24.2 ± 15.4</td>
<td>0.04</td>
</tr>
<tr>
<td>0-96 h</td>
<td>21.5 ± 10.7</td>
<td>31.0 ± 19.5</td>
<td>0.03</td>
</tr>
<tr>
<td>0-120 h</td>
<td>25.5 ± 13.6</td>
<td>37.3 ± 23.1</td>
<td>0.02</td>
</tr>
<tr>
<td>0-144 h</td>
<td>27.9 ± 17.0</td>
<td>42.5 ± 26.0</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Feeding and delivery mode influence gut microbiota composition

Is it possible to modify the gut microbiota composition in C-section delivered infants with a probiotic?

**Figure credit:**
O. Sakwinska, NRC

**Legend:**
- Bifidobacterium longum group
- Bifidobacterium pseudocatenulatum group
- Bacteroides
- Streptococcus thermophilus group
- Streptococcus agalactiae group
- Klebsiella
- Escherichia
- Granulicatella
- Clostridium butyricum
- Lachnospiraceae
- Remaining

**Abbreviations:**
- VD: vaginal delivery
- CS: C-section

**Statistical Significance:**
- p=0.001
- p=0.029
- p=0.04
Clinical trial in Greek infants with *Lactobacillus reuteri* probiotic

The probiotic does not dominate the gut microbiota, but shifts the microbiota in supplemented C-section infants to that of vaginal-delivered infants.
Phage therapy: Renaissance of another old concept

Bacteriophages as anti-microbial agents: concept of F. d’Herelle (1920)

- **Collection of phages**
  Ecological survey in Bangladesh

- **In vivo tests in mice**
  Lytic activity of oral phage in the gut

- **In silico safety evaluation**
  Genome sequencing and risk assessment

- **Phase I trials in humans**
  Safety and pharmacokinetics in volunteers

  *Phase II efficacy trial in humans*
  Acute *E. coli* diarrhea in children at icddr,b

Antimicrobial Agents Chemother. 48, 2558; 49, 2874
J. Bacteriol. 186, 8287; 186, 8276; 189, 8206
Virology 388, 21; 393, 16; EBioMedicine 4,124
Ann Rev Virol 2, 599
Phage therapy: An appealing concept that works well in the laboratory

Time scale: all is done in 20 min

T4 phage on *E. coli*
T4 Phage goes into the fermentation unit

AEM 80: 1469

Virol 434:222
Healthy Humans: Safety test of T4 phage in Switzerland and Bangladesh

Kinetics of oral phage appearance in stool

Followed by three safety trials at icddr,b (healthy adults, older, then younger children): no adverse events

Stool microbiota composition is not affected by oral phage

EMI, in press
A double blinded placebo controlled randomized phage therapy trial at icddr,b

T4 phage: 3.6 x 10^8/ml

Microgen phage: 1.4 x 10^9/ml

Placebo

Planned: Efficacy evaluation after 375 patients

Safety evaluation after 75 patients

Outcome: Oral phage is safe in diarrhea patients

The trial was sponsored by:

Nestlé Health Science
Lack of phage amplification and clinical efficacy

A. Stool Weight

B. Stool Frequency

C. Total ORS

D. Phage-positive stools

E. Phage counts

EBioMed 4: 124
Stool microbiota: more streptococci than *E. coli* in ETEC

**EBioMed 4: 124**

- **Streptococcus**
- **Escherichia**
- **Others**
- **Bifidobacterium**

**Streptococcus** R=0.449 P=0

**Escherichia**

**It-ETEC**

**Abundance (%)**

**relative abundance**

**cfu/g**
The failure of the phage therapy trial:
*Is not an argument against phage therapy,
*but against using ETEC diarrhea as target

Indeed, when investigating other forms of childhood diarrhea, we identified patients with endogenous T4 phages replicating to high titers on outgrowing intestinal *E. coli*

* killing the winning hypothesis
* importance of target bacteria above phage replication threshold
Prebiotics: milk oligosaccharides

Conceptual framework

The oligosaccharides:

Figure credit: N. Sprenger, NRC
Stool microbiota in breastfed, formula-fed and BMO-supplemented FF infants

**BF**  |  **FF**  |  **BMO**

<2w  |  6w  |  12w

Env. Microbiol. 18: 2185

- Proteo
- Firmicutes
- Bacteroides
- Bifido
PCA of stool microbiota compositional shifts

EMI 18: 2185

Bifidobacterium

E. coli

Streptococcus

Ongoing:
RCT at icddr,b
HMO treatment of acute childhood diarrhea
*There is hope for intervention into the pathogen-microbiota-host interaction network,
*These treatment options need still to be developed,
* …and adapted to veterinary use,
*There are currently more descriptive than interventional microbiota studies
*These interventions need a case by case development, don’t expect universal solutions
Growth promotion and gut microbiota: insights from antibiotic use

Antibiotics in feed → Growth promotion → Why?

Proposed mechanisms:
- Decrease of pathogen load
- Reduction of microbial antimetabolites
- Reduced feed use in the gut microbial loop
- Enhanced uptake of nutrients due to changed gut histology

Germ-free animals: + Gut microbiota

Weight development:
- GF > Conv. by ~ 15%
- Microbes cause growth retardation, also seen with antibiotic treatment of conventional chicken
- Conv. = GF (weight)
- Increase in adiposity

Bacterial degradation of plant polysaccharides → absorbable monosaccharides → induction of lipogenesis

…issue was also intensively discussed in childhood malnutrition & obesity → interdisciplinary cross-talk
Acknowledgements

At icddr,b:
S. A. Sarker (PI), S. Sultana, F. Qadri, A. Cravioto

At NRC:

Other Nestlé units: D. Moine, P. Decombes (NIHS); L. Philippe (CDU); N. Conus, T. v.d. Weid (NN); M. Kuslyss (NHS)

…and the children of Bangladesh participating in the clinical trials