What does it all mean?
Making sense of microbiome data

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Microbiome – hottest area in science?

48,164
1 April 2018

~100
Promise of the microbiome

• Change our understanding of what it means to be human
• Provide new diagnostics, therapies and dietary solutions across multiple health targets (cancer, metabolic, inflammatory, infection, stress)
• Support and inform personalised medicine
• Inform human development and ageing (Extremes of Life)
Human milk oligosaccharides (HMOs), a family of complex sugars which the baby cannot digest.
Your microbiota has been selected stochastically from all of the microbes you have encountered during your life, from or perhaps even before your birth. It has also been modified by a number of variables, including your genome, your birth mode, your diet, your health status, your environment and many other factors.

At this moment in time it is in a particular configuration as a result of your multiple encounters with nature and nurture. It is unique to you, and hopefully it is relatively stable and resilient.
Gut microbiome of 170 elderly subjects

Claesson, .. Hill... et al, 2011, PNAS 108; 4586-4591
<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Bacteria</th>
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<tr>
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<td>Bacteroides</td>
</tr>
<tr>
<td>Species</td>
<td>coli</td>
<td>perfringens</td>
<td>intestinalis</td>
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16S rRNA OTU

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<table>
<thead>
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<td>Megalopygidae</td>
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<tr>
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<td>sapiens</td>
<td>opercularis</td>
</tr>
<tr>
<td>strains</td>
<td>Veiled lizard</td>
<td></td>
<td>Flannel moth caterpillar</td>
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</tbody>
</table>
Carriage of microbial taxa varies but metabolic pathways (as measured by gene families) remain stable within a healthy population.

Different actors, same functions?
We are only just beginning to document gut microbial diversity across populations. In our study, more than 33% of the total Hadza GM genera remain unidentified. Such taxonomic uncertainty holds exciting prospects for discovering yet unknown microbial genetic arrangements.
Microbiome – not just the bacteriome

- Residents (permanent)
- Visitors (temporary)
- Tourists (probiotics)
- Invaders (pathogens)
Microbiome-associated diseases

- Acne
- Antibiotic-associated diarrhoea
- Arthritis
- Asthma/allergies
- Atherosclerosis
- Autism
- Autoimmune diseases
- Cancer
- Dental caries
- Depression and anxiety
- Diabetes
- Eczema
- Gastric ulcers
- Infections
- Inflammatory bowel diseases
- Malnutrition
- Metabolic syndrome
- Necrotizing enterocolitis
- Obesity
- Parkinson’s disease
- Psoriasis
- Vaginosis
Does the microbiome shape or shadow human health?

Causality or association?

• GWAS
• Epidemiology
• Pharmacology
Microbiome and inflammatory disease

Over 200 individuals with IBS or IBD vs controls
Intra-individual distances

Obvious changes, but noisy signals, no single microbe driving disease
Multiple potential direct and indirect bi-directional pathways by which the gut microbiome can modulate the gut–brain axis. They include endocrine (cortisol), immune (cytokines) and neural (vagus and enteric nervous system) pathways.
A bacterial driver–passenger model for colorectal cancer: beyond the usual suspects

Harold Tjalsma, Annemarie Boley, Julian R. Marchesi and Bas E. Dutilh

Abstract | Cancer has long been considered a genetic disease. However, accumulating evidence supports the involvement of infectious agents in the development of cancer, especially in those organs that are continuously exposed to microorganisms, such as the large intestine. Recent next-generation sequencing studies of the intestinal microbiota now offer an unprecedented view of the aetiology of sporadic colorectal cancer and have revealed that the microbiota associated with colorectal cancer contains bacterial species that differ in their temporal associations with developing tumours. Here, we propose a bacterial driver–passenger model for microbial involvement in the development of colorectal cancer and suggest that this model be incorporated into the genetic paradigm of cancer progression.

In the long term, research in this field may lead to the discovery of innovative therapeutic interventions that decrease the incidence of CRC, such as the selective removal of potential CRC-driving bacteria and/or stimulation of health-promoting bacteria before CRC has been initiated.
Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients

V. Gopalakrishnan, 1, 2, 3 C. N. Spence, 1, 2, 3 L. Neel, 1, 2 A. Keubler, 1, 2 M. C. Andrews, 1 T. V. Karpinets, 1, 2 P. A. Prieto, 1 D. Vicente, 1 K. Hoffman, 1 S. C. Wei, 1 A. P. Cogdill, 1, 2 L. Zhao, 1 C. W. Hudgens, 1, 2 D. S. Hutchison, 1, 2 M. Tanza, 1 P. Patacchia de Macedo, 1 T. C. Cotechinis, 1 T. Kumar, 1 W. S. Chen, 1 S. M. Reddy, 1 H. Szczepanski, 1 S. T. Sionano, 1 J. Galleway-Pena, 1 H. Jiang, 1 P. L. Chen, 1 E. J. Shappi, 1 R. Periampal, 1 M. A. Loyner, 1 E. P. Chmaly, 1 S. Sheshurani, 1 L. M. Venet, 1 F. C. Okugawa, 1 V. B. Jensen, 1 A. G. Swennes, 1 F. McAllister, 1 E. Marcelo Riquelme, Sanchez, 1 X. Zhan, 1 E. Le Chatelier, 1 I. Zlotog, 1 N. Pour, 1 J. L. Austin, 1, 2 L. S. Haydn, 1 E. M. Burton, 1 J. M. Gardner, 1 E. Smarras, 1 J. Hu, 1 A. J. Lazir, 1 T. Tsuikawa, 1 A. Dian, 1 T. Tavish, 1 T. L. Gilbert, 1 W. T. Green, 1 S. P. Patil, 1 S. E. Woodman, 1 R. N. Marla, 1 M. A. Davle, 1 J. F. Gershon, 1 P. H. Huy, 1 J. E. Lee, 1 J. Zhang, 1 L. E. C. Coop, 1 Z. A. Furtal, 1 C. R. Daniel, 1 N. J. Alm, 1 J. F. Petrosino, 1 T. Tetzlaff, 1 P. Sharma, 1 J. P. Allison, 1 R. R. Jeng, 1 J. A. Wargi 1, 2, 3

Preclinical mouse models suggest that the gut microbiome modulates tumor response to checkpoint blockade immunotherapy, however, this has not been well characterized in human cancer patients. Here we examined the oral and gut microbiome of melanoma patients undergoing anti-programmed cell death 1 protein (PD-1) immunotherapy. (n = 112). Significant differences were observed in the diversity and composition of the patient gut microbiome of responders versus non-responders. Analysis of patient fecal microbiome samples (n = 43, 30 responders, 13 non-responders) showed significantly higher alpha diversity (P < 0.01) and relative abundance of bacteria of the Luminobacteraceae family (P < 0.01) in responding patients. Meta-generics studies revealed functional differences in gut bacteria in responders, including enrichment of anabolic pathways. Immune profiling suggested enhanced systemic and antitumor immunity in responding patients with a favorable gut microbiome as well as in germline mice receiving fecal transplants from responding patients. Together, these data have important implications for the treatment of melanoma patients with immune checkpoint inhibitors.
The human superorganism
Human cells + microbial cells
Genome + Microbiome

FIXED       MUTABLE

The microbiome represents both a target and a source of therapeutic or prophylactic interventions to restore or protect health.

- Acne
- Antibiotic-associated diarrhoea
- Arthritis
- Asthma/allergies
- Atherosclerosis
- Autism
- Autoimmune diseases
- Cancer
- Dental caries
- Depression and anxiety
- Diabetes
- Eczema
- Gastric ulcers
- Infections
- Inflammatory bowel diseases
- Malnutrition
- Metabolic syndrome
- Necrotizing enterocolitis
- Obesity
- Parkinson’s disease
- Psoriasis
- Vaginosis
Therapeutic options

- Antibiotics
- Prebiotics
- Probiotics
- Faecal microbiota transplant
- Pharmaceuticals
- Diet
- Lifestyle

Microbiome

Health

Host

Diet

Lifestyle

Pharmaceuticals
‘Mine’ the Microbiome for anti-infectives

If the microbiome is a significant player in health and disease, then it should be possible to mine interventions from this niche

- faecal microbiota transplants
- microbial consortia
- probiotics
- bacteriophage
- pharmabiotics
  - (bioactives, bacteriocins, etc.)
FMT Faecal microbiota transplants

Fecal microbiota transplantation

The gut microbiota is the collection of microorganisms that live in the stomach and intestines. When the healthy gut microbiota is altered, *C. difficile* may cause an infection that can be difficult to cure.

Stool from a healthy donor is transferred to a patient with recurrent *C. difficile* infection.

A healthy microbiota is restored in the patient’s colon.

FMT success rates in CDAD

<table>
<thead>
<tr>
<th>Reference</th>
<th>Percentage resolution</th>
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<tr>
<td>Cammarota (2015)</td>
<td>100</td>
</tr>
<tr>
<td>van Nood (2013)</td>
<td>95</td>
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<tr>
<td>Youngster (2014)</td>
<td>90</td>
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<tr>
<td>Kelly (2014)</td>
<td>85</td>
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<tr>
<td>Rubin (2013)</td>
<td>80</td>
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<tr>
<td>Matilla (2012)</td>
<td>75</td>
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<tr>
<td>Kassam (2012)</td>
<td>70</td>
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<tr>
<td>Kelly (2012)</td>
<td>65</td>
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<tr>
<td>Jorup-Ronstrom (2012)</td>
<td>60</td>
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<td>Hamilton (2012)</td>
<td>55</td>
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<td>Brandt (2012)</td>
<td>50</td>
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<td>Wilcox (2011)</td>
<td>45</td>
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<tr>
<td>Garborg (2010)</td>
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<td>Yoon &amp; Brandt (2010)</td>
<td>35</td>
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<td>Rehle (2010)</td>
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<td>Arkkila (2010)</td>
<td>20</td>
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<tr>
<td>Macconnachie (2009)</td>
<td>15</td>
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<tr>
<td>Keller (2009)</td>
<td>10</td>
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<td>Borody (2003)</td>
<td>5</td>
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<td>Aas (2003)</td>
<td>0</td>
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<td>Faust (2002)</td>
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<td>Petersen (1994)</td>
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‘Mine’ the Microbiome for anti-infectives

If the microbiota is a significant player in health and disease, then it should be possible to mine interventions from this niche

- faecal microbiota transplants
- **microbial consortia**
- probiotics
- bacteriophage
- **pharmabiotics**
  - (bioactives, bacteriocins, etc.)
Microbial consortia

- Seres Therapeutics’ lead Phase 3 development candidate, SER-109, is an investigational oral microbiome therapeutic for the prevention of recurrent *Clostridium difficile* infection in adults with multiply recurrent CDI.

- The FDA has granted SER-109 both Breakthrough Therapy and Orphan Drug designations.

- SER-109 is an ecology of bacterial spores enriched and purified from healthy, screened human donors.

www.serestherapeutics.com/pipeline/ser-109
Microbial consortia and porcine salmonellosis

- Isolated 10,000 strains from uninfected pigs in a *Salmonella* infected herd
- Chose 5 strains with *in vitro* anti-*Salmonella* activity (4 × *Lactobacillus*, 1 *Pediococcus*)
- Conducted a blinded trial (N=10) where pigs were fed the microbial consortia before being deliberately infected with *Salmonella* on 3 consecutive days

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![Graph showing % Diarrhoea (day 4), Clinical scores (blinded), Salmonella/g faeces (day 15), and Weight gain/kg (day 15)]

- % Diarrhoea (day 4)
  - Control: 75%
  - Consortium: 25%
  - **p < 0.001**

- Clinical scores (blinded)
  - Control: 40
  - Consortium: 10
  - **p < 0.001**

- Salmonella/g faeces (day 15)
  - Control: 10^7
  - Consortium: 10^6
  - **p < 0.001**

- Weight gain/kg (day 15)
  - Control: 25
  - Consortium: 15
  - **p < 0.001**
‘Mine’ the Microbiome for anti-infectives

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Evaluating the evidence by types of acute diarrhoea suggests that probiotics significantly reduced antibiotic-associated diarrhoea by 52% (95% CI 35–65%), reduced the risk of travellers’ diarrhoea by 8% (–6 to 21%), and that of acute diarrhoea of diverse causes by 34% (8–53%).

Probiotics reduced the associated risk of acute diarrhoea among children by 57% (35–71%), and by 26% (7–49%) among adults.

The effect on acute diarrhoea is dependent on the age of the host and genera of strain used.
Probiotics could prevent infection by:

- Direct antagonism
- Immunomodulation
- Exclusion
Probiotics produce a number of potential effector molecules

- Some may be delivered in the initial dose (e.g. bacterial structures)
- Some may require active metabolism in the GI tract (e.g. SCFA)
Probiotic mechanism of action

Bacteriocin production as a mechanism for the antiinfective activity of Lactobacillus salivarius UCC118


*Alimentary Pharmabiotic Centre and Department of Microbiology and School of Pharmacy, University College Cork, Cork, Ireland

Edited by Todd R. Klaenhammer, North Carolina State University, Raleigh, NC, and approved March 1, 2007 (received for review January 17, 2007)

Rare instance in which a single molecule has been identified as the probiotic mechanism
Bovine mastitis is the most persistent disease in dairy cattle. Treated with broad spectrum antibiotics, milk must be withheld. Billions of euros every year.

Infused infected quarters with *Lactococcus lactis* DPC3147 (produces lacticin 3147). A single application led to complete recovery within 3 days (11/11 animals). No withholding of milk.
Compared live culture against leading antibiotic treatment (N=25)

Clinical cure rates

L. Lactis 64% (16/25)
Antibiotic 72% (18/25)

Klostermann et al., 2008 J Dairy Res 75:365-373
Crispie et al., 2008 J Dairy Res 75:374-384
A randomized synbiotic trial to prevent sepsis among infants in rural India

4,556 infants

We observed a significant (40%) reduction in the primary combined outcome of death and neonatal sepsis, from 9% in the placebo arm to 5.4% in the treatment arm.

Significant reductions were also observed for culture-positive and culture-negative sepsis and lower respiratory tract infections.

Lactobacillus plantarum and FOS

<p>| Table 2: Effect of synbiotic treatment on sepsis and other morbidities in the first 60 days of life |
|-----------------------------------------------|------------------|------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Outcome category</th>
<th>Control (n=2,718)</th>
<th>Synbiotic (n=2,280)</th>
<th>RR (95% CI)</th>
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</thead>
<tbody>
<tr>
<td>Death and sepsis (primary outcome)</td>
<td>46/206 (2.2%)</td>
<td>25/123 (2.0%)</td>
<td>0.56 (0.36-0.84)</td>
</tr>
<tr>
<td>Death</td>
<td>15 (0.7%)</td>
<td>6 (0.3%)</td>
<td>0.42 (0.26-0.68)</td>
</tr>
<tr>
<td>Sepsis (A + B + C)</td>
<td>47/202 (2.3%)</td>
<td>27/117 (2.3%)</td>
<td>0.75 (0.50-1.15)</td>
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<tr>
<td>A. Sepsis/pSBI—culture-positive sepsisemia</td>
<td>17/66 (2.6%)</td>
<td>6/0 (0)</td>
<td>0.22 (0.00-2.94)</td>
</tr>
<tr>
<td>Gram-negative sepsis</td>
<td>16/67 (2.4%)</td>
<td>10/0 (0)</td>
<td>0.69 (0.28-1.75)</td>
</tr>
<tr>
<td>Gram-positive sepsis</td>
<td>11/63 (1.7%)</td>
<td>2/0 (0)</td>
<td>0.20 (0.03-1.39)</td>
</tr>
<tr>
<td>B. Sepsis/pSBI—culture-negative sepsis (culture-negative clinical sepsis; worse, hospitalization and IV antibiotics)</td>
<td>23/101 (22.8%)</td>
<td>19/99 (19.1%)</td>
<td>0.93 (0.55-1.58)</td>
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<tr>
<td>C. Sepsis/pSBI—LRTI (LRTIs requiring antibiotic therapy)</td>
<td>48/167 (28.6%)</td>
<td>25/113 (22.0%)</td>
<td>0.85 (0.57-1.28)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>59 (2.6%)</td>
<td>12 (0.5%)</td>
<td>0.20 (0.11-0.32)</td>
</tr>
<tr>
<td>Local infections (including &gt;30 postnatal, oral thrush, conjunctivitis)</td>
<td>32 (1.5%)</td>
<td>16 (0.7%)</td>
<td>0.41 (0.27-0.63)</td>
</tr>
<tr>
<td>Abscess, cutis media</td>
<td>11 (0.5%)</td>
<td>5 (0.2%)</td>
<td>0.46 (0.26-0.84)</td>
</tr>
<tr>
<td>Ophthalmia</td>
<td>13 (0.6%)</td>
<td>3 (0.1%)</td>
<td>0.23 (0.03-0.81)</td>
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</table>
Next-generation probiotics: the spectrum from probiotics to live biotherapeutics

Paul W. O’Toole, Julian R. Marchesi and Colin Hill

The leading probiotics currently available to consumers are generally drawn from a narrow range of organisms. Knowledge of the gut microbiota and its constituent actors is changing this paradigm, particularly given the phylogenetic range and relatively unknown characteristics of the organisms under investigation as novel therapeutics. For this reason, and because their development is likely to be more amenable to a pharmaceutical than a food delivery route, these organisms are often operationally referred to as next-generation probiotics, a concept that overlaps with the emerging concept of live biotherapeutic products. The latter is a class of organisms developed exclusively for pharmaceutical application. In this Perspective, we discuss what lessons have been learned from working with traditional probiotics, explore the kinds of organisms that are likely to be used as novel microbial therapeutics, discuss the regulatory framework required, and propose how scientists may meet this challenge.

Figure 2 | Schematic diagram summarizing some differences in the history and route to market of probiotics, next-generation probiotics and live biotherapeutic products.
If the microbiome is a significant player in health and disease, then it should be possible to mine interventions from this niche:

- faecal microbiota transplants
- microbial consortia
- probiotics
- bacteriophage
- pharmabiotics
  - (bioactives, bacteriocins, etc.)
Thuricin CD: A narrow spectrum bacteriocin for *C. difficile*

Isolated >50,000 sporeformers from faecal specimens

Overlaid with *Clostridium difficile*

*Thuricin CD*: a two component bacteriocin

*Thuricin CD*: a two component sactibiotic, highly active against *Clostridium difficile*
Thuricin CD is a novel two component sactibiotic, with three sulphur to $\alpha$-carbon bridges in each peptide.
Thuricin CD also works as well as frontline antibiotics, but without collateral damage.

Faecal fermentation (artificial colon model)
If the microbiome is a significant player in health and disease, then it should be possible to mine interventions from this niche:

- faecal microbiota transplants
- microbial consortia
- probiotics
- bacteriophage
- pharmabiotics
  - (bioactives, bacteriocins, etc.)
Phage Planet

- Discovered in 1917

- \(10^{31}\) phage on earth (1,000,000,000,000 phage for every grain of sand in the world)

- If all phage were laid end to end they would extend for 200 million light years

- Most abundant biological entities in/on human body

- Vast majority uncharacterised
Phage – life cycle

Self-assembling, Natures’ nanomachines

1 bacterium → 256 bacteria
1 phage → $10^{16}$ phage
4h → 10,000,000,000,000,000,000
Phage therapy

- Staphylococcus aureus - MRSA
- Mycobacterium avium
- Clostridium difficile
- E. coli O157:H7
- Salmonella enterica
- Pseudomonas aeruginosa
- Pediococcus damnosus
- Acinetobacter baumanii
- Pectobacterium, Cronobacter
- Campylobacter jejuni
- Listeria monocytogenes
Isolated 2 phage active against *Pseudomonas aeruginosa*

RCT (N=24) for treatment of chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa* (United Kingdom)

Tom Patterson USA
*Acinetobacter baumannii*

16y old male (France)
*Staphylococcus aureus*
Phage enzyme therapy

Purified fractions of CHAP$_K$

CHAP$_K$

w/ Aidan Coffey, Cork Institute of Technology
Three donors (various time points)

13 successful FMT recipients at T0 and followed for 12 months
Phage and FMT

Draper et al., unpublished
Efficacy of Sterile Fecal Filtrate Transfer for Treating Patients With *Clostridium difficile* Infection

**RESULTS:** In all 5 patients, FFT restored normal stool habits and eliminated symptoms of CDI for a minimum period of 6 months.

A key advantage of FFT is the avoidance of all risks inherent to the transfer of living microorganisms. Further advantages include the potential for standardization and for the development of a robust, inexpensive, and patient-friendly formulation (i.e., capsules filled with freeze-dried FFT preparations without the need to conserve living bacteria or spores). When contemplating the intersection of successful therapies related to FMT (classic FMT, spores, and FFT), it appears plausible that the active agent(s) of any FMT therapy are not living bacteria, but rather bacterial components, antimicrobial compounds of bacterial origin (e.g., bacteriocins), or bacteriophages contributing to the normal intestinal microenvironment. These could be common to all successful FMT therapies and even rather unspecific regarding the bacterial strain(s) used for therapies.
Microbiome solutions for MRSA

- Bacteriocins
- Bacteriophages
- Live biotherapeutics
- Phage lysins
- Traditional antibiotics

© by author
Combination therapies

- Not a binary choice, could use microbiome-based solutions with standard small molecule antimicrobials as combination therapies

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**MRSA**

- **Control**
- **Nisin 1µg/ml**
- **Methicillin 32µg/ml**
- **Nisin 1µg/ml**
  **Methicillin 32µg/ml**

**Ellis et al, unpublished**

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Fighting biofilms with lantibiotics and other groups of bacteriocins

Harsh Mathur1,2, Des Field1,2,3, Mary C. Rea1,2, Paul D. Cotter1,2, Colin Hill1,2,3 and R. Paul Ross2,4

Biofilms are sessile communities of bacteria typically embedded in an extracellular polymeric matrix. Bacterial cells embedded in biofilms are inherently recalcitrant to antimicrobials, compared to cells existing in a planktonic state, and are notoriously difficult to eradicate once formed. Avenues to tackle biofilms thus far have largely focussed on attempting to disrupt the initial stages of biofilm formation, including adhesion and maturation of the biofilm. Such an approach is advantageous as the concentrations required to inhibit formation of biofilms are generally much lower than removing a fully established biofilm. The crisis of antibiotic resistance in clinical settings worldwide has been further exacerbated by the ability of certain pathogenic bacteria to form biofilms. Perhaps the most notorious biofilm formers described from a clinical viewpoint have been methicillin-resistant Staphylococcus aureus (MRSA), Staphylococcus epidermidis, Pseudomonas aeruginosa, Gardnerella vaginalis and Streptococcus mutans, the latter of which is found in oral biofilms. Due to the dearth of novel antibiotics in recent decades, compounded by the increasing rate of emergence of resistance amongst pathogens with a propensity for biofilm formation, solutions are urgently required to mitigate these crises. Bacteriocins are a class of antimicrobial peptides, which are ribosomally synthesised and often are more potent than their antibiotic counterparts. Here, we review a selection of studies conducted with bacteriocins with the ultimate objective of inhibiting biofilms. Overall, a deeper understanding of the precise means by which a biofilm forms on a substrate as well as insights into the mechanisms by which bacteriocins inhibit biofilms is warranted.
Combinations and biofilms

Fig. 2. Anti-biofilm activity of nisin and polymyxins against *P. aeruginosa*: Inhibition of biofilm formation of *P. aeruginosa* PA-01. a) In the presence of nisin (1/3× MIC), colistin (1/2×, 1/5× MIC) and combinations thereof and b) in the presence of nisin (1/4× MIC) and polymyxin B (1/2×, 1/5× MIC) and combinations thereof, when assessed in microtiter plates and subjected to crystal violet (CV) staining for the detection of biofilm formation. (Adapted from Field et al. 2016b) under the terms of the Creative Commons Attribution License)
Conclusions

• Our microbiomes provide an opportunity to influence health and disease.

• Microbiome composition influences some/many treatment outcomes. May help stratification of patients and choice of most effective therapy.

• Microbiome-based solutions for infection could be deployed alone or in combination with small molecule antibiotics, could ‘rescue’ existing therapies.

• Microbiome may deliver ‘traditional’ blockbusters, but more likely to involve personalised or targeted solutions.

• Paradigm shift needed, current regulatory system is not appropriate for microbiome-based interventions aimed at impacting health; which will include personalised solutions, complex compositions and food based solutions.
What lies before us and what lies behind us are small matters compared to what lies within us (microbiome?). And when we bring what is within out into the world, miracles happen.

APC Microbiome Ireland colleagues
UCC Bacteriophage Laboratory
UCC Bacteriocin Laboratory
Paul Ross, UCC