The role of the microbiome for infectious diseases

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The exhaustive description of human microbiota and their relationship with health and disease: a major challenges in the twenty-first century

“We found that in 2011, there were more than 4 times as many citations referencing human gut microbiota than in 2005 (Figure 1A), when Eckburg et al. (2005) published the seminal large-scale gut metagenomics study. In addition, in 2011, there were approximately as many published items investigating human gut microbiota than during the 10 years between 1993 and 2002 (Figure 1B).”

## Glossary of terms (1)

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
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</tr>
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<td>Microbiome</td>
<td>The totality of the microbes with their genes that are harbored by the microbiota and the milieu in which they interact.</td>
</tr>
<tr>
<td>Metagenome</td>
<td>The genetic information of the whole microbiota, usually obtained by whole genome sequencing. This is the functional genetic potential provided by the genome of many individual organisms.</td>
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<tr>
<td>Metatranscriptome</td>
<td>Sum of genetic information in microbial mRNA, usually obtained by mRNA sequencing. Provides insights on what is functionally active in a microbial community.</td>
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<tr>
<td>Virome</td>
<td>Collection of all the viruses in an environment.</td>
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<tr>
<td>16S ribosomal DNA</td>
<td>A specific DNA gene that is unique to prokaryotic cells.</td>
</tr>
<tr>
<td>Taxonomy</td>
<td>The science of identifying species and arranging them into a classification.</td>
</tr>
<tr>
<td>Operational taxonomic unit (OTU)</td>
<td>Specific sequences based on sequence similarity (typically threshold is 97%) to reference genes. This is taken as a proxy for species-level divergence.</td>
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<td>Taxon</td>
<td>A group of phylogenetically related microbes that belong to the same taxonomic group, such as order, family, or genus.</td>
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<tr>
<td>Amplicon</td>
<td>An amplified fragment of DNA from a region of a marker gene (such as 16S rDNA) that is generated by PCR.</td>
</tr>
<tr>
<td>Sequencing</td>
<td>Technique allowing thousands or millions of DNA sequences to be obtained from a given sample.</td>
</tr>
<tr>
<td>Richness</td>
<td>Number of different taxa within a single population.</td>
</tr>
<tr>
<td>α Diversity</td>
<td>How many types of sequences in a sample.</td>
</tr>
<tr>
<td>β Diversity</td>
<td>How many different types of sequences are shared among samples.</td>
</tr>
<tr>
<td>Resilience</td>
<td>The capacity of the microbiome to absorb disturbance and reorganize itself while undergoing change, so as to retain essentially the same function, structure, and identity.</td>
</tr>
<tr>
<td>Dysbiosis</td>
<td>A condition in which the normal structure of the microbiome is disturbed, often through external pressures such as disease states or medication.</td>
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Humans: Meta-organisms

10-fold greater numbers of microbial than human cells, metabolically and immunologically integrated, with a biomass >1 Kg
A non-exhaustive overview of human gut microorganisms among bacterial, *Archaea*, viral, and *Eukaryota* domains

From: Lagier *et al*, *Front Cell Infect Microbiol*, 2012
Anatomic Regions of the Gut

• Upper GI tract: \(10^2 - 10^4\) cells/ml
  – Lactobacilli, streptococci, \(H.\ pylori\)

• Ileum: \(10^6 - 10^{12}\) cells/ml, upper bacteria plus
  – Facultative anaerobes: \(Enterobacteriaceae\)
  – Obligate anaerobes: \(Bacteroides, Veillonella, Fusobacterium\) and \(Clostridium\) species

• Colon: distal human colon is the most biodense natural ecosystem known \((10^{10} - 10^{12}\) cells/ml\)
  – Complex and diverse
  – Comprise most of our bacterial biomass
The influence of external factors determining the composition of the human gut microbiota

EFFECTS OF GUT MICROBIOTA ON HOST HEALTH

- Barrier effect
- Immunocompetence/Tolerance
- Protection against infections
- Synthesis
- Metabolic/Trophic function
- Drug metabolism
- Behavior conditioning
Intestinal bacteria confer indirect (immune-mediated) colonization resistance against enteric pathogens

a. *Bacteroides thetaiotaomicron* enhances expression of the peptidoglycan-binding C-type lectin regenerating islet-derived protein IIIγ (REGIIIγ), which is an antimicrobial peptide that primarily targets and kills Gram-positive bacteria

b. Segmented filamentous bacteria (SFB) closely associate with the intestinal epithelium and enhance IgA production by B cells, serum amyloid A (SAA)-dependent T helper 17 (TH17) cell differentiation, pro-inflammatory cytokine production and epithelial production of antimicrobial peptides. These processes confer protection against *Citrobacter rodentium*

d. *B. thetaiotaomicron* consumes carbohydrates used by *C. rodentium*, which contributes to the competitive exclusion of the pathogen from the intestinal lumen

e. *Bacteroides thuringiensis* secretes a bacteriocin that directly targets spore-forming Bacilli and Clostridia, including *Clostridium difficile*, through an unknown mechanism of action

f. Gram-negative bacteria, such as *Vibrio cholerae*, deliver toxic effector proteins directly to *Escherichia coli* through type VI secretion systems

g. A variety of *Bifidobacterium* spp. produce organic acids and peptides that impair growth and adhesion of pathogenic *E. coli* to enterocytes

## Microbiota stimulates IMMUNITY through PRRs

### Luminal PAMPs
- ✓ LPS (Gram -)
- ✓ UPEC/Profilin
- ✓ Flagellin
- ✓ Peptidoglycan/lipopeptide
- ✓ Bacterial lipopeptide
- ✓ ds RNA
- ✓ Fibronectin (many bacteria)
- ✓ Lipoteichoic acid (Gram +)
- ✓ Lipoooligosaccharide

### Specific PRRs
- TLR-4
- TLR-11
- TLR-5
- TLR-1 e TLR-2
- TLR-2 e TLR-6
- TLR-3
- α5β1 integrin
- TLR-2
- PAF

### Endosomal PAMPs
- ✓ ss RNA
- ✓ CpG DNA

### Specific PRRs
- TLR-6 e TLR-7
- TLR-9

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From: Balfour and Sartor, Gastroenterology 2008, modified
Hospital deliveries, caesarean sections, special-care baby unit admissions, smaller family size, widespread use of antibiotics, good hygiene, nature of the maternal diet..

Lack of exposure of babies to bifidobacterial species and/or elimination of bifidobacterial species from the bowel (antibiotic therapy) could lead to an unbalanced maturation of the immune system (lack of Th2 response removal: immune deviation)

“Immunological Freudianism”

A total of 41 species and 20 genera were significantly associated with at least one potential interaction and associations were identified for all of the tested cytokines.

- Three stimulus-specific associations were observed, with *C. albicans* hyphae stimulation in connection with *Bacteroides xylanisolvens*, *Parabacteroides distasonis*, and *Coprococcus comes*.

- *Odoribacter splanchnicus* and the genus *Bilophila* were negatively correlated with TNFα production for LPS and *C. albicans* stimulations.
Role of Human Microbiome in Health

- Accumulating evidence reveals that the gut microbiota plays a major role in promoting health, as a result of which it is often referred to as the “forgotten organ” (O’Hara and Shanahan, 2006).

- The relationship between the host and microbiota is **symbiotic and mutualistic**, each deriving benefits from the other. In particular, mutualism is defined as “an interaction between species that is beneficial to both of them” and symbiosis as “the living together of two organisms in close association” (Ghosh, 2013).

- While the host provides the microbiota with a protected and nutrient-rich environment, the microbiota enhance, example digestion, immunity, and neuronal development.
Origins of the concept of colonization resistance

- Protection of the host intestines from exogenous pathogens by commensal bacteria — a phenomenon termed colonization resistance — was described more than five decades ago and was thought to result from microorganism-mediated direct inhibition.
- Antibiotic-associated susceptibility to secondary intestinal infections has been recognized for nearly as long as the therapeutic benefits of antibiotics.
- In the 1960s, it was shown that the minimum infective oral dose of *Salmonella enterica* subsp. *enteritidis* decreased 10,000-fold in mice following streptomycin therapy.
IN VIVO AND IN VITRO ANTAGONISM OF INTESTINAL BACTERIA AGAINST SHIGELLA FLEXNERI

I. CORRELATION BETWEEN VARIOUS TESTS*

DAVID J. HENTGES AND ROLF FRETER

From the Department of Microbiology, Stritch School of Medicine, Loyola University, Chicago, Illinois; and the Department of Microbiology, Jefferson Medical College, Philadelphia, Pennsylvania

Antagonism against pathogenic bacteria exerted by microorganisms of the normal human body flora has been the subject of numerous studies ever since the time of Metchnikoff’s original ideas on the benefits attributable to the presence of lactobacilli in the intestine. This degree of interest is only natural since, for many different reasons, the ability to control the bacterial flora of patients would be of great value to the physician. However, in spite of the amount of attention devoted to the subject, the “lactobacillus controversy” is still with us today. Likewise, the possible mechanisms involved in bacterial antagonisms in vivo are still in doubt. In the special case of interrelationships between intestinal bacteria, the subject of the present paper, many possible inhibitory mechanisms have been suggested which might enable one bacterial species to prevent the growth of another (reviewed by Waksman, 1945): competition for nutrients, toxic metabolites, and drug substances (colicin), change in pH, competition for oxygen, and lowering of the oxidation-reduction potential (Paine, 1958).

Received for publication June 27, 1961.
* This work was supported by contract N0000148-C-0230 from the Office of Naval Research and Jefferson Medical College and by grant no. 364 from the American Medical Association. A brief account of the present work was given in 1958 and in 1959 before the Society of American Bacteriologists (Hentges and Freter, 1958; Freter, 1959).

More than 50 years ago (1962!!), it was found that loss of obligate anaerobic bacterial populations from the lower gastrointestinal tract strongly correlated with susceptibility to infection, which suggests that these commensal organisms were providing colonization resistance.
Microbiota destruction and antibiotic-resistant infections

- Recommendations to combat the progression of antibiotic resistance included barrier and hygiene approaches to reduce transmission, limits on antibiotic use, and development of new and more effective antibiotics.
- A concern with these approaches, however, is that antibiotic resistance has grown despite their implementation.
- Recently, the White House provided a national action plan for combating antibiotic-resistant bacteria, which included specific milestones that introduced the potential role of the microbiome and the microbiota in combating antibiotic resistance (www.whitehouse.gov/sites/default/files/docs/national_action_plan_for_combating_antibiotic-resistant_bacteria.pdf).
The intestinal commensal microbiota provides colonization resistance against a wide range of pathogens by indirect and direct mechanisms.

"Commensal bacteria activate innate immune defenses in the mucosa, produce or modify host-derived metabolites, deplete nutrients, or produce substances that are directly toxic to competing bacteria."

Pamer EG et al., Science, 2016
Three major pathogens will be analysed for its relationships with microbiota

- *Clostridium difficile*
- *Enterococcus faecalis*
- *Staphylococcus aureus*

Finally, the possible role of Faecal Microbiota Transplantation as therapeutic option will be stressed
• Nested case–control study including 25 CDI cases and 25 matched controls. Fecal specimens collected prior to disease onset were evaluated by 16S rRNA gene amplification and pyrosequencing to determine the composition of the intestinal microbiota during the at-risk period.

• The diversity of the intestinal microbiota was significantly reduced prior to an episode of CDI. Sequences corresponding to the phylum Bacteroidetes and to the families Bacteroidaceae and Clostridiales Incertae Sedis XI were depleted in CDI patients compared to controls, whereas sequences corresponding to the family Enterococcaceae were enriched.

• In multivariable analyses, cephalosporin and fluoroquinolone use, as well as a decrease in the abundance of Clostridiales Incertae Sedis XI were significantly and independently associated with CDI development.

• This study shows that a reduction in the abundance of a specific bacterial family – Clostridiales Incertae Sedis XI - is associated with risk of nosocomial CDI and may represent a target for novel strategies to prevent this life-threatening infection.
• Here we correlate loss of specific bacterial taxa with development of infection, by treating mice with different antibiotics that result in distinct microbiota changes and lead to varied susceptibility to *Clostridium difficile*.

• Mathematical modelling augmented by analyses of the microbiota of hospitalized patients identifies resistance-associated bacteria common to mice and humans.

• Using these platforms, we determine that *Clostridium scindens*, a bile acid 7α-dehydroxylating intestinal bacterium, is associated with resistance to *C. difficile* infection and, upon administration, enhances resistance to infection in a secondary bile acid dependent fashion.

• These findings have implications for the rational design of targeted antimicrobials as well as microbiome-based diagnostics and therapeutics for individuals at risk of *C. difficile* infection.
Conserved microbial taxa associated with *C. difficile* inhibition in mouse (A) and in humans (B)
Phylogenetic tree constructed from representative sequences of intestinal bacteria associated with resistance to *C. difficile* infection (blue), including cultured representatives subsequently used in adoptive transfer experiments (bold)
LETTER

Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*

Charlie G. Buffle1,2, Yanni Douc1,3,4, Richard R. Stein2, Peter T. McKenna1,2, Lilian Ling2, Asia Gobourn2, Daniel No2, Hui Liu2, Melissa Kinnebrew1,2, Agnes Viale2, Eric Littmann2, Marcel R. van den Brink2,3, Robert C. Troy2, Ying Liu2,3, Chris Sanders2, Justin R. Cross2, Nora C. Trussard2,3, Joao B. Xavier2,3 & Eric G. Pamer1,2,8

8 January 2015 | Vol 517 | Nature
Antibiotic-induced alterations in gut microbial metabolism decrease colonization resistance against *C. difficile*

Antibiotic treatment alters the gut microbiota structure, specifically decreasing bacteria that are able to deconjugate and dehydroxylate primary bile acids into secondary bile acids. The loss of secondary bile acid metabolism and competition from the gut microbiota allow for *C. difficile* outgrowth, toxin production, and disease.
• Antibiotic-mediated microbiota destruction and the consequent loss of colonization resistance can result in intestinal domination with vancomycin-resistant *Enterococcus* (VRE), leading to bloodstream infection in hospitalized patients.

• Clearance of VRE remains a challenging goal that, if achieved, would reduce systemic VRE infections and patient-to-patient transmission.

• A precisely defined consortium of commensal bacteria containing the *Clostridium* cluster XIVa species *Blautia producta* and *Clostridium bolteae* restores colonization resistance against VRE and clears VRE from the intestines of mice.

• These findings suggest that therapeutic or prophylactic administration of defined bacterial consortia to individuals with compromised microbiota composition may reduce inter-patient transmission and intra-patient dissemination of highly antibiotic-resistant pathogens.
Adoptive transfer of bacterial consortia containing a mixture of *C. bolteae*, *B. producta*, *Blautia_unclassified*, *E. dolichum*, *A. muciniphila*, *P. distasonis*, and *B. sartorii* (7-mix) prevents VRE colonization.
Relative abundance levels of *B. producta* and *C. bolteae* in feces from mice administered with the 7-mix, CBBP, or bacterial mixtures lacking *C. bolteae*, *B. producta*, or *P. distasonis* (Ps) and *B. sartorii* (Bs). \( \Delta \) indicates the absence of corresponding strain(s).

- Taken together, these observations suggest that reestablishment of colonization resistance in the ampicillin-treated mouse model requires two cooperative interactions.
- *B. sartorii* and *P. distasonis* inactivate ampicillin, allowing engraftment of ampicillin-sensitive *C. bolteae*, which in turn supports engraftment of *B. producta*. 
**Blautia producta** directly inhibits VRE growth

VRE growth in cecal content supplemented with cultures of the indicated CBBP strains prior to seeding with VRE
Intestinal Domination and the Risk of Bacteremia in Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplantation

Characterization of the intestinal microbiota during allogeneic hematopoietic stem cell transplantation
Intestinal Domination and the Risk of Bacteremia in Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplantation

Ying Tour,1,* Jose S. Xavier,1 Lauren Lipuma,2 Carlos Udels,3 Jenna Goldberg,4 Asia Gobourne,4 Youn-Jee Lee,5 Krista A. Dohin,5 Nicholas D. Sarvet,1 Agnes Viale,1 Miguel-Angel Perales,6 Robert R. Jenq,7 Marcel M. van den Brink,4,8 and Eric G. Palmer1,9
CID 2012:55 (1 October)

Table 3. Association of Intestinal Domination With Bacteremia

<table>
<thead>
<tr>
<th>Dominating Taxon</th>
<th>VRE Bacteremia</th>
<th></th>
<th></th>
<th>Gram-negative Bacteremia</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P</td>
<td>HR (95% CI)</td>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Enterococcus</strong></td>
<td>9.35 (2.43–45.44)</td>
<td>.001</td>
<td>1.35 (2.5–5.08)</td>
<td>.690</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Streptococcus</strong></td>
<td>0.21 (.00–1.75)</td>
<td>.184</td>
<td>0.82 (.09–3.65)</td>
<td>.823</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Proteobacteria</strong></td>
<td>0.75 (.01–6.14)</td>
<td>.837</td>
<td>5.46 (1.03–19.91)</td>
<td>.047</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio; VRE, Vancomycin-resistant Enterococcus.

* Bacteremia for each organism was defined as at least one positive blood culture within the study period.

b Intestinal domination was analyzed as a time-varying predictor.
Nasal carriage of *Staphylococcus aureus* predisposes to invasive infection, but the mechanisms that permit or interfere with pathogen colonization are largely unknown.

Whereas soil microbes are known to compete by production of antibiotics, such processes have rarely been reported for human microbiota.

Nasal *Staphylococcus lugdunensis* strains produce lugdunin, a novel thiazolidine-containing cyclic peptide antibiotic that prohibits colonization by *S. aureus*.

Lugdunin is bactericidal against major pathogens, effective in animal models, and not prone to causing development of resistance in *S. aureus*.

Notably, human nasal colonization by *S. lugdunensis* was associated with a significantly reduced *S. aureus* carriage rate, suggesting that lugdunin or lugdunin-producing commensal bacteria could be valuable for preventing staphylococcal infections.

So, human microbiota should be considered as a source for new antibiotics.
**Human commensals producing a novel antibiotic impair pathogen colonization**

**Table 1 | Lugdunin spectrum of activity**

<table>
<thead>
<tr>
<th>Species and strain</th>
<th>Resistance</th>
<th>Lugdunin MIC (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> USA300 (LAC) + 50% human serum</td>
<td>MRSA</td>
<td>1.5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> USA300 (NRS384)</td>
<td>MRSA</td>
<td>1.5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> Mu50</td>
<td>GISA</td>
<td>3</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> SA113</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> RN4220</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em> BK463</td>
<td>VRE</td>
<td>3</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> VRE366</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em> ATCC19118</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> ATCC49619</td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> 168 (trpC2)</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> PAO1</td>
<td></td>
<td>&gt;50</td>
</tr>
<tr>
<td><em>Escherichia coli</em> DH5α</td>
<td></td>
<td>&gt;50</td>
</tr>
</tbody>
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MRSA, methicillin-resistant *S. aureus*; GISA, glycopeptide intermediate-resistant *S. aureus*; VRE, vancomycin-resistant *Enterococcus*. 
Lugdunin has efficacy in a mouse skin infection model
Lugdunin-producing wild-type *S. lugdunensis* IVK28 restricts the growth of *S. aureus in vitro* (A) and *in vivo* (B) in cotton rats.
Human commensals producing a novel antibiotic impair pathogen colonization

Table 2 | S. aureus and S. lugdunensis distribution in hospitalized patients

<table>
<thead>
<tr>
<th></th>
<th>S. lugdunensis-positive</th>
<th>S. lugdunensis-negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus-positive</td>
<td>1</td>
<td>59</td>
<td>60</td>
</tr>
<tr>
<td>S. aureus-negative</td>
<td>16</td>
<td>111</td>
<td>127</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>170</td>
<td>187</td>
</tr>
<tr>
<td>Risk</td>
<td>0.059*</td>
<td>0.347*</td>
<td>0.321</td>
</tr>
</tbody>
</table>

Significant differences between S. lugdunensis-positive and S. lugdunensis-negative patients were analysed by the Chi-squared test.

$\frac{0.059}{0.347}$ versus $0.347$: risk ratio, 0.169; 95% confidence interval 0.025–1.147.

*P = 0.015.
Faecal Microbiota Transplantation (FMT): state of the art

• The gastrointestinal microbiota, its active role in health and disease and the therapeutic potential of FMT are areas of great global interest undergoing rapid developments and advances

• The efficacy of FMT in multiple relapsing CDI and its superiority over antibiotic therapy is now unequivocal

• FMT is an exciting potential therapy for IBD with encouraging reports especially in ulcerative colitis, including a recent clinical trial performed in Canada

• FMT may have therapeutic applications for a wide range of not only gastrointestinal but also systemic conditions (e.g. diabetes, obesity, autoimmune phenomena) found to have their pathogenesis related to gastrointestinal dysbiosis

• There are several products under development, and in the future, FMT will likely shift from the use of crude, fresh whole stool to highly processed, filtered, frozen and potentially cultured formulations

From: Borody et al., Curr. Opin. Gastroenterol., 2014
Duodenal Infusion of Donor Feces for Recurrent Clostridium difficile


From:
the Departments of Internal Medicine (E.N., A.V., M.N., P.S.), Microbiology (C.E.V.), Gastroenterology (J.E.W.M.B., J.J.K.), and Cardiology (J.G.P.T.) and the Clinical Research Unit (M.G.W.D.), Academic Medical Center, University of Amsterdam, Amsterdam; the Laboratory of Microbiology, Wageningen University, Wageningen (S.F., E.G.Z., W.M.V.); the Department of Experimental and Medical Microbiology, Leiden University Medical Center, Leiden (E.J.K.); and the Department of Gastroenterology, Hagaziekenhuis, The Hague (J.J.K.) - all in the Netherlands -
and the Department of Bacteriology and Immunology, Medical Faculty, University of Helsinki, Helsinki (W.M.V.).
Rates of Cure without Relapse for Recurrent *Clostridium difficile* Infection

Five weeks after the initiation of therapy, there was a recurrence of infection in 1 of 16 patients (6%) in the infusion group, 8 of 13 (62%) in the vancomycin-alone group, and 7 of 13 (54%) in the group receiving vancomycin with bowel lavage.

Of 16 patients in the infusion group, 13 (81%) were cured after the first infusion of donor feces. The 3 remaining patients received a second infusion with feces from a different donor; of these patients, 2 were subsequently cured. Overall, donor feces cured 15 of 16 patients (94%).

Resolution of infection occurred in 4 of 13 patients (31%) in the vancomycin-alone group and in 3 of 13 patients (23%) in the group receiving vancomycin with bowel lavage.

The overall cure rate ratio of donor-feces infusion was 3.05 as compared with vancomycin alone (99.9% confidence interval [CI], 1.08 to 290.05) and 4.05 as compared with vancomycin with bowel lavage.
Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent *Clostridium difficile* infection

G. Cammarota*, L. Masucci†, G. Ianiro*, S. Bibbò*, G. Dinoi*, G. Costamagna†, M. Sanguinetti† & A. Gasbarrini*

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**Table:**

<table>
<thead>
<tr>
<th>FMT</th>
<th>V</th>
<th>V, BC*</th>
<th>V, BC</th>
<th>Faecal inf.</th>
<th>Faecal infusion every 3 days in patients with PMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanc</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
</tr>
</tbody>
</table>

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**Figure 1** | Timeline of scheduled treatments after patient randomisation.

- **V** = vancomycin 125 mg by mouth four times per day
- **BC** = bowel cleaning
- **VPR** = vancomycin pulse regimen (125–500 mg/day every 2–3 days)
- *According to patient clinical condition*
Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent *Clostridium difficile* infection

G. Cammarota*, L. Masucci†, G. Ianiro*, S. Bibbò*, G. Dinoi*, G. Costamagna†, M. Sanguinetti† & A. Gasbarrini*

**Figure 2** | Percentage of patients cured.
Fecal Microbiota Transplantation and Successful Resolution of Multidrug-Resistant-Organism Colonization

Nancy F. Crum-Cianflone,¹,²,³ Eva Sullivan,⁴,⁵ Gonzalo Ballon-Landa⁴

Infectious Disease Division, Scripps Mercy Hospital, San Diego, California, USA; Infectious Disease Division, Naval Medical Center San Diego, San Diego, California, USA; Pharmacy Department, Scripps Mercy Hospital, San Diego, California, USA

June 2015 Volume 53 Number 6

1/11/2013 10/30/2013

Death

15 weeks post FMT

6/6/2012 9/21/2012

15 weeks post FMT

Admitted to hospital

1/11/2013

- CRE K. pneumoniae
- MDR A. baumannii²
- MDR P. stuartii²
- MDR A. baumannii²
- MDR A. baumannii²
- MDR A. baumannii²
- MDR A. baumannii²
- MDR P. stuartii²
- MDR P. stuartii²
- MDR P. rettgeri²
- MDR P. rettgeri²
- MRSA³
- MRSA³
- MRSA³
- MRSA³
- CRE K. pneumoniae

1/11/2013 10/30/2013

Death

15 weeks post FMT

6/6/2012 9/21/2012

15 weeks post FMT

Admitted to hospital

1/11/2013

- CRE K. pneumoniae
- MDR A. baumannii²
- MDR P. stuartii²
- MDR A. baumannii²
- MDR A. baumannii²
- MDR A. baumannii²
- MDR A. baumannii²
- MDR P. stuartii²
- MDR P. stuartii²
- MDR P. rettgeri²
- MDR P. rettgeri²
- MRSA³
- MRSA³
- MRSA³
- MRSA³
- CRE K. pneumoniae
Fecal bacteriotherapy eliminates established *K. pneumoniae* and VRE intestinal domination

From: Caballero et al., PLoS Pathogens, 2015
# European consensus conference on faecal microbiota transplantation in clinical practice


**Box 1 Key issues to select potential donors at the preliminary interview**

<table>
<thead>
<tr>
<th>INFECTIOUS DISEASES</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>History of, or known exposure to, HIV, HBV or HCV, syphilis, human immunodeficiency virus I and II, malaria, trypanosomiasis, tuberculosis</td>
<td></td>
</tr>
<tr>
<td>Known systemic infection not controlled at the time of donation</td>
<td></td>
</tr>
<tr>
<td>Use of illegal drugs</td>
<td></td>
</tr>
<tr>
<td>Risky sexual behaviour (anonymous sexual contacts, sexual contacts with prostitutes, drug addicts, individuals with HIV, viral hepatitis, syphilis; work as prostitute; history of sexually transmissible disease)</td>
<td></td>
</tr>
<tr>
<td>Previous reception of tissue/organ transplant</td>
<td></td>
</tr>
<tr>
<td>Previous (&lt;12 months) reception of blood products</td>
<td></td>
</tr>
<tr>
<td>Recent (&lt;6 months) needle stick accident</td>
<td></td>
</tr>
<tr>
<td>Recent (&lt;6 months) body tattoo, piercing, earing, acupuncture</td>
<td></td>
</tr>
<tr>
<td>Recent medical treatment in poorly hygienic conditions</td>
<td></td>
</tr>
<tr>
<td>Risk of transmission of diseases caused by prions</td>
<td></td>
</tr>
<tr>
<td>Recent parasitism or infection from rotavirus, Giardia lamblia and other microbes with Gi involvement</td>
<td></td>
</tr>
<tr>
<td>Recent (&lt;6 months) travel in tropical countries; countries at high risk of communicable diseases or traveller’s diarrhoea</td>
<td></td>
</tr>
<tr>
<td>Recent (&lt;6 months) history of vaccination with a live attenuated virus, if there is a possible risk of transmission</td>
<td></td>
</tr>
<tr>
<td>Healthcare workers (to exclude the risk of transmission of multidrug-resistant organisms)</td>
<td></td>
</tr>
<tr>
<td>Individual working with animals (to exclude the risk of transmission of zoonotic infections)</td>
<td></td>
</tr>
</tbody>
</table>

| GI METABOLIC AND NEUROLOGICAL DISORDERS |  |
| History of IBS, IBD, functional chronic constipation, coeliac disease, other chronic GI disorders |  |
| History of chronic, systemic autoimmune disorders with GI involvement |  |
| History of, or high risk for, GI cancer or polyposis |  |
| Recent appearance of diarrhoea, hematocoezha |  |
| History of neurological/neurodegenerative disorders |  |
| History of psychiatric conditions |  |
| Overweight and obesity (body mass index >25) |  |

| DRUGS THAT CAN IMPAIR GUT MICROBIOTA COMPOSITION |  |
| Recent (<3 months) exposure to antibiotics, immunosuppressants, chemotherapy |  |
| Chronic therapy with proton pump inhibitors |  |
European consensus conference on faecal microbiota transplantation in clinical practice

Giovanni Cammarota,1 Gianluca Ianiro,1 Herbert Tilg,2 Mirjana Rajilić-Stojanović,3 Patricia Kump,4 Reetta Satokari,9 Harry Sokol,6 Perttu Arkkila,7 Cristina Pintus,8 Alisa Hart,9 Jonathan Segal,9 Marina Alos,10 Luca Masucci,11 Antonio Molinari,12 Franco Scaldati,1 Giovanni Gasbarrini,1 Antonio Lopez-Sanromán,13 Alexander Link,14 Pieter de Groot,15 Willem M de Vos,5,16 Christoph Högenerauer,4 Peter Malferttheiner,14 Eero Mattila,17 Tomica Milosavljević,18 Max Nieuwdorp,17,18 Maurizio Sanguinetti,11 Magnus Simren,20 Antonio Gasbarrini,1 The European FMT Working Group

Box 3  Blood and stool testing to check donors for any potentially transmittable disease

GENERAL BLOOD TESTING
- Cytomegalovirus
- Epstein-Barr virus
- Hepatitis A
- HBV
- HCV
- Hepatitis E virus
- Syphilis
- HIV-1 and HIV-2
- Entamoeba histolytica
- Complete blood cell count with differential
- C-reactive protein and erythrocyte sedimentation rate
- Albumin
- Creatinine and electrolytes
- Aminotransferases, bilirubin, gamma-glutamyltransferase, alkaline phosphatase

BLOOD TESTING IN SPECIFIC SITUATIONS
- Human Immunodeficiency virus type 1 and 2 antibodies
- Strongyloides stercoralis

GENERAL STOOL TESTING
- Detection of Clostridium difficile
- Detection of enteric pathogens, including Salmonella, Shigella
- Campylobacter, Escherichia coli 0157 H7, Yersinia, vancomycin-resistant enterococci, methicillin-resistant Staphylococcus aureus, Gram-negative multidrug-resistant bacteria
- Norovirus
- Antigens and/or acid fast staining for Giardia lamblia and Cryptosporidium parvum
- Protozoa (including Blastocystis hominis) and helminths
- Faecal occult blood testing

STOOL TESTING IN SPECIFIC SITUATIONS
- Detection of Vibrio cholera and Listeria monocytogenes
- Antigens and/or acid fast staining for Isospora and Microsporidia
- Calprotectin
- Helicobacter pylori faecal antigen
- Rotavirus
Possible problems for donors recruitment?

- So, potential FMT donors need to be tested for some basic infectious agents, both blood borne (syphilis, human immunodeficiency virus, hepatitis B and C viruses) or enteric (C. difficile, Salmonella, Shigella, Yersinia, Campylobacter, Escherichia coli O157:H7, enteric viruses, ova, and parasites)

- However, it also is preferable that donors do not have any gastrointestinal disorders, metabolic syndrome, allergic or autoimmune diseases, or neurologic or psychiatric problems

- When faced with such rigorous qualifications, most recipients are not able to identify and recruit a large number of donors. In some centers (Bakken, Clin. Gastroenterol. Hepatol., 2011) that select donors based on these described criteria, it was found that more than 90% of potential donors do not pass the screening evaluations
In this paper, it was reported the clinical experience with 43 consecutive patients who were treated with FMT for recurrent CDI.

The donor identification and screening was simplified by moving from patient-identified individual donors to standard volunteer donors.

Material preparation shifted from the endoscopy suite to a standardized process in the laboratory, and ultimately to banking frozen processed fecal material that is ready to use when needed.

Standardization of material preparation significantly simplified the practical aspects of FMT without loss of apparent efficacy in clearing recurrent CDI.

Approximately 30% of the patients had underlying inflammatory bowel disease, and FMT was equally effective in this group.
Routes of administration

• Upper GI tract
  – Endoscopy;
  – Nasogastric/nasointestinal tubes;
  – Ingestion of pills

• Proximal colon
  – Colonscopy

• Distal colon
  – Enema
  – Rectal tube
  – Sigmoidoscopy
Routes of administration: pros and cons

• Nasogastric or nasointestinal routes may be uncomfortable and less appealing to the patient, may require radiology assistance to confirm tube placement, and carry some risk of vomiting and aspiration.

• Retention enema is inexpensive and has little procedural risk, but it may be difficult for some patients to retain the donor material and may require multiple treatments.

• Endoscopic routes of administration are well tolerated and have the advantage of allowing examination of the colonic mucosa.

• Endoscopic delivery carries some procedural risk and increases health care utilization and costs, although a cost-effectiveness study showed that FMT dominated (i.e., was less costly and more effective) compared with vancomycin for initial CDI (Varier et al., CMI, 2014).
Open-label, single-group, preliminary feasibility study conducted from August 2013 through June 2014 at Massachusetts General Hospital, Boston. Twenty patients with at least 3 episodes of mild to moderate *Clostridium difficile* infection and failure of a 6- to 8-week taper with vancomycin or at least 2 episodes of severe *C. difficile* infection requiring hospitalization were enrolled.

Healthy volunteers were screened as potential donors and FMT capsules were generated and stored at $-80^\circ$ C.

Patients received 15 capsules on 2 consecutive days and were followed up for symptom resolution and adverse events for up to 6 months.
• No serious adverse events attributed to FMT were observed. Resolution of diarrhea was achieved in 14 patients after a single capsule-based FMT. All 6 non-responders were re-treated; 4 had resolution of diarrhea, resulting in an overall 90% rate of clinical resolution of diarrhea (18/20). Self-ranked health scores improved significantly on a scale of 1 to 10 from a median of 5 (IQR, 5-7) for overall health and 4.5 (IQR, 3-7) for gastrointestinal-specific health on the day prior to FMT to 8 (IQR, 7-9) after FMT administration for both overall and gastrointestinal health (P = .001).

• This preliminary study among patients with relapsing *C difficile* infection provides data on adverse events and rates of resolution of diarrhea following administration of FMT using frozen encapsulated inoculum from unrelated donors. Larger studies are needed to confirm these results and to evaluate long-term safety and effectiveness.
### Adverse Events in Published Series of More Than 5 Patients Receiving FMT

<table>
<thead>
<tr>
<th>Authors</th>
<th>No.</th>
<th>Method of delivery</th>
<th>Follow-up</th>
<th>Adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Nood et al</td>
<td>16</td>
<td>Duodenal infusion</td>
<td>70 days</td>
<td>Diarrhea, 5; abdominal cramps, 5; belching, 3; nausea, 1; symptoms resolved in all within 3 hours</td>
</tr>
<tr>
<td>Youngster et al</td>
<td>20</td>
<td>Nasogastric tube or colonoscopy</td>
<td>6 mo</td>
<td>Mild abdominal discomfort/bloating, 4; transient fever (day 2), 1</td>
</tr>
<tr>
<td>Rubin et al</td>
<td>75</td>
<td>Nasogastric tube</td>
<td>60 days</td>
<td>No adverse events or deaths</td>
</tr>
<tr>
<td>MacConnachie et al</td>
<td>15</td>
<td>Nasogastric tube</td>
<td>4–24 wk</td>
<td>No adverse events “related to transplant”; upper Gl bleeding during the first month after FMT</td>
</tr>
<tr>
<td>Aas et al</td>
<td>18</td>
<td>Nasogastric tube</td>
<td>90 days</td>
<td>Peritonitis in patient on peritoneal dialysis on day 3 (died “shortly thereafter”); pneumonia in patient with chronic obstructive pulmonary disease (died on day 14)</td>
</tr>
<tr>
<td>Mattila et al</td>
<td>70</td>
<td>Colonoscopy</td>
<td>1 y</td>
<td>No serious adverse events; approximately one-third noted irregular bowel movements and excessive flatulence during the first couple of weeks after FMT</td>
</tr>
<tr>
<td>Hamilton et al</td>
<td>43</td>
<td>Colonoscopy</td>
<td>1 y</td>
<td>1 complication: “perforation” from colonoscopic biopsy that resolved without surgery</td>
</tr>
<tr>
<td>Patel et al</td>
<td>31</td>
<td>Colonoscopy</td>
<td>1 y</td>
<td>No adverse events</td>
</tr>
<tr>
<td>Yoon and Brandt</td>
<td>12</td>
<td>Colonoscopy</td>
<td>3 wk to 8 y</td>
<td>No complications of FMT</td>
</tr>
<tr>
<td>Pathak et al</td>
<td>12</td>
<td>Colonoscopy, nasoduodenal, enema</td>
<td>2–29 mo</td>
<td>No adverse events</td>
</tr>
<tr>
<td>Dutta et al</td>
<td>27</td>
<td>Enteroscopy plus colonoscopy</td>
<td>10–34 mo</td>
<td>Low-grade fever, 5; bloating, 3; resolved within 12–24 h</td>
</tr>
<tr>
<td>Lee et al</td>
<td>94</td>
<td>Enema</td>
<td>6–24 mo</td>
<td>No significant adverse events; 10% experienced transient constipation and excessive flatulence</td>
</tr>
<tr>
<td>Emanuelsson et al</td>
<td>23</td>
<td>Rectal catheter</td>
<td>23</td>
<td>“A few” patients experienced temporary constipation (apparently soon after FMT)</td>
</tr>
<tr>
<td>Silverman et al</td>
<td>7</td>
<td>Enema</td>
<td>4–14 mo</td>
<td>No adverse events but reported 1 patient with “postinfectious” IBS (mixed pattern)</td>
</tr>
<tr>
<td>Schwartz et al</td>
<td>13</td>
<td>Colonoscopy</td>
<td>Not stated</td>
<td>Norovirus, 2 (2 days and 12 days after FMT); investigators speculated person-to-person rather than FMT transmission</td>
</tr>
<tr>
<td>Kelly et al</td>
<td>80</td>
<td>Mixed</td>
<td>12 wk</td>
<td>Potentially related adverse events: Death: aspiration during colonoscopy with respiratory failure Hospitalizations: IBD flares, 4; postcolonoscopy abdominal pain, 1; fever, diarrhea, encephalopathy, pancytopenia in patient with lymphoma, 1 Nonserious adverse events: abdominal pain/bloating immediately after FMT, 3; “mucosal tear” at colonoscopy, 1; self-limited diarrhea, 3; fever, 1; IBD flare, 1</td>
</tr>
</tbody>
</table>

*Immunocompromised patients (eg, immunosuppressive therapy for IBD, organ transplant, cancer with antineoplastic therapy).*

From: Kelly et al., Gastroenterology 2015
• Sixty-four patients (39 women; mean age 74 years) were included. Of them, 44 (69%) were cured by a single faecal infusion, whereas 20 (31%) needed repeat infusions. Overall, FMT cured 62 of 64 (97%) patients.
• In the subgroup of patients with severe CDI, only eight of 26 (30%) were cured with a single infusion. At multivariate analysis, severe CDI (OR 24.66; 95% CI 4.44-242.08; \( P < 0.001 \)) and inadequate bowel preparation (OR 11.53; 95% CI 1.71-115.51; \( P = 0.019 \)) were found to be independent predictors of failure after single faecal infusion.
• Severe CDI and inadequate bowel preparation appear to be independent predictors of failure after single faecal infusion in patients treated with FMT by colonoscopy for recurrent CDI.
• The results may help to optimize protocols and outcomes of FMT in patients with recurrent CDI.
Conclusions

• Human microbiota study is one of the most intriguing and exciting research topics in the last years.
• In the near future the association of specific microbiota types with specific diseases will permit to make treatment able to restore the “good microbiota” modified by therapeutic approaches.
• The assessment of the microbiota composition that has to be used for FMT by -omics will permit to patient tailor this therapeutic procedure.
• The use of frozen samples derived from volunteer donors with well-characterized microbiota may allow the reduction of the costs and the standardization of FMT.
For any question please contact me!!

maurizio.sanguinetti@unicatt.it