MICROBIOTA

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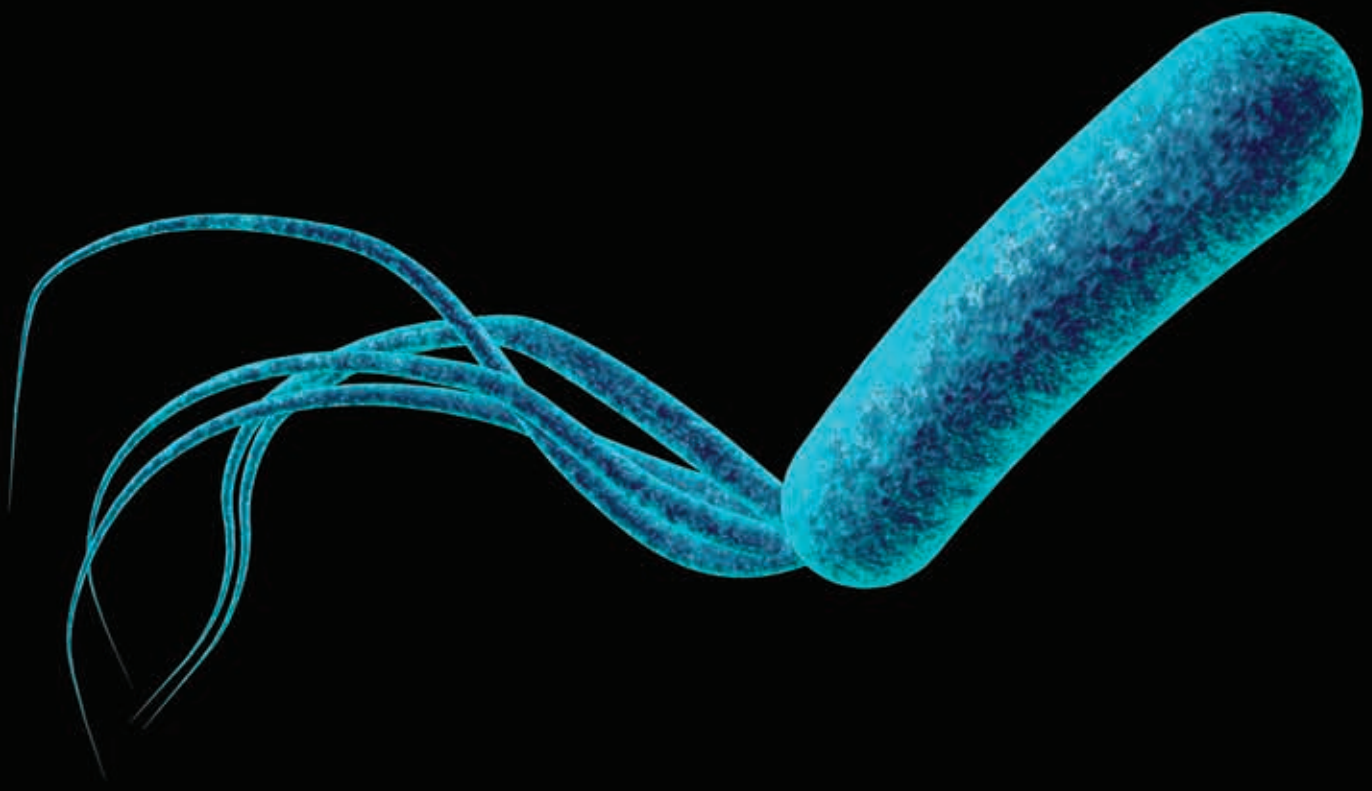

BIOCODEX Microbiota Institute

SUMMARY

EDITO

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Dear readers, celiac disease, first discovered by the English paediatrician Samuel Jones Gee more than 100 years ago, has since evolved in terms of status. Initially considered to be an exclusively paediatric disease characterized by malabsorption and exacerbated by certain dietary practices, it was later found to be a chronic autoimmune disease occurring at any age and characterized by systemic manifestations. The pro-inflammatory triggering antigen – gluten – as well as the pathophysiology of the disease, are now well understood. Nevertheless, two observations raise questions today and need to be addressed. The first concerns the recent, rapid rise in the worldwide prevalence of celiac disease over the past 50 years; the second relates to the fact that only 2-5% of genetically susceptible individuals actually develop the disease, whether from an early age or after decades of gluten consumption.

In addition, the risk of developing the disease is probably increased by other genetic factors that still have to be identified. It is also increased by environmental factors, such as being born in summer or having gastrointestinal infections, known to raise intestinal permeability and passage of immunogenic gluten peptides across the mucosa. Conversely, the age at introduction of gluten, the amount ingested, antibiotic exposure and the type of childbirth are not thought to have an impact on celiac disease development.

Why are some genetically predisposed individuals asymptomatic? What role does the gut microbiota play in the loss of gluten tolerance and the disease onset? In this issue, Professor Elena Verdú, a renowned expert in the field from McMaster University in Hamilton, Canada, suggests some ways to answer these questions and explains that an altered microbiota composition is detected in children at risk or suffering from celiac disease, although no specific microbial signature has been established to date. These are avenues that need to be explored to complete our understanding of celiac disease, and potentially delay its onset – or even prevent it.

“ WHAT IS THE ROLE OF THE GUT MICROBIOTA IN THE LOSS OF GLUTEN TOLERANCE AND THE DEVELOPMENT OF CELIAC DISEASE? ”

The link between diet and gut microbiota is also examined from another point of view in this issue: Professor Emmanuel Mas from the Hôpital des Enfants in Toulouse, France, comments on the impact, from birth, of diet and infant formula on the infantile gut microbiota and the short term consequences on overweight in young children. Lastly, Professor Harry Sokol from Hôpital Saint-Antoine, Paris, France shares the results of a study published in *Nature Microbiology* on the resilience of the gut microbiota of healthy young adults following antibiotic exposure.

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OVERVIEW

MICROBIOTA AND CELIAC DISEASE

Environmental factors are suggested to contribute to celiac disease pathogenesis, an autoimmune disease triggered by the ingestion of gluten. Clinical studies show alterations in the composition of the microbiota in celiac disease patients. Although some consistent findings across studies have been established, no celiac disease microbial signature has been defined. Using reductionist and gnotobiotic* animal models, recent research suggested that bacterial strains from celiac disease patients may have more pathogenic or inflammatory potential. Targeting the microbiota with the use of specific probiotics, shown to modify pathogenic mechanisms critical for celiac disease, could be an attractive therapeutic approach to complement the gluten-free diet.

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* Refers to laboratory animals obtained under conditions that allow perfect control of their microbial flora.

Celiac disease is a common inflammatory and autoimmune reaction that occurs in genetically predisposed individuals after consuming gluten (**Figure 1**). The characteristic lesion is the destruction of the finger-like projections of small intestinal lining (enteropathy). Clinical manifestations of the disease are varied and include both intestinal or extra-intestinal symptoms. Celiac disease is unique in that it is the only autoimmune disease where the triggering antigen (gluten) is known. The mechanisms that explain HLA genetic risk and the steps triggered by the dietary trigger that ultimately leads to the development of pro-inflammatory gluten specific T-cells and autoantibodies, are well understood (**Figure 2**). One unsolved question relates to the rapid recent increase in prevalence as well as the fact that celiac disease only develops in a fraction of genetically susceptible individuals, suggesting there must be additional genetic or environmental factors involved in activating the inflammatory cascade. In particular, there has been a growing interest in the role of the microbial factors in celiac disease development [1]. In this review we focus on bacterial alterations and how they could play a role in disease mechanisms as well as how they constitute potential therapeutic targets for celiac disease.

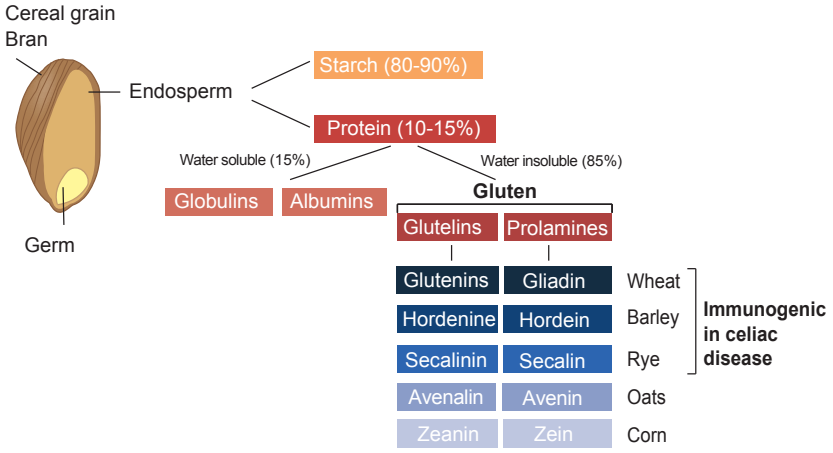
CORRELATION OF DYSBIOSIS AND CELIAC DISEASE: LESSONS FROM CLINICAL STUDIES

One of the first studies to suggest a microbial contribution to celiac disease described the presence of rod-shaped bacteria in duodenal biopsies of children born during a celiac disease "epidemic" in Sweden. These bacteria were not observed in children without celiac disease, or in children born following the epidemic and it was thought that their presence may have contributed to the increase in the disease incidence observed in Sweden [2]. However, the mechanisms underlying this association remain unknown.

A number of studies have since been published that analyzed the fecal and small intestinal microbiota composition in celiac disease patients compared to healthy

▶ FIGURE 1

Gluten is the term used to describe the mixture of storage proteins found, along with starch, within the endosperm of cereal grains. Gluten is made up of glutelins and prolamins, which are found in wheat, barley, rye, oats, and corn. However, due to their amino acid structure, only those glutelins and prolamins found in wheat, barley and rye are immunogenic for celiac disease patients.

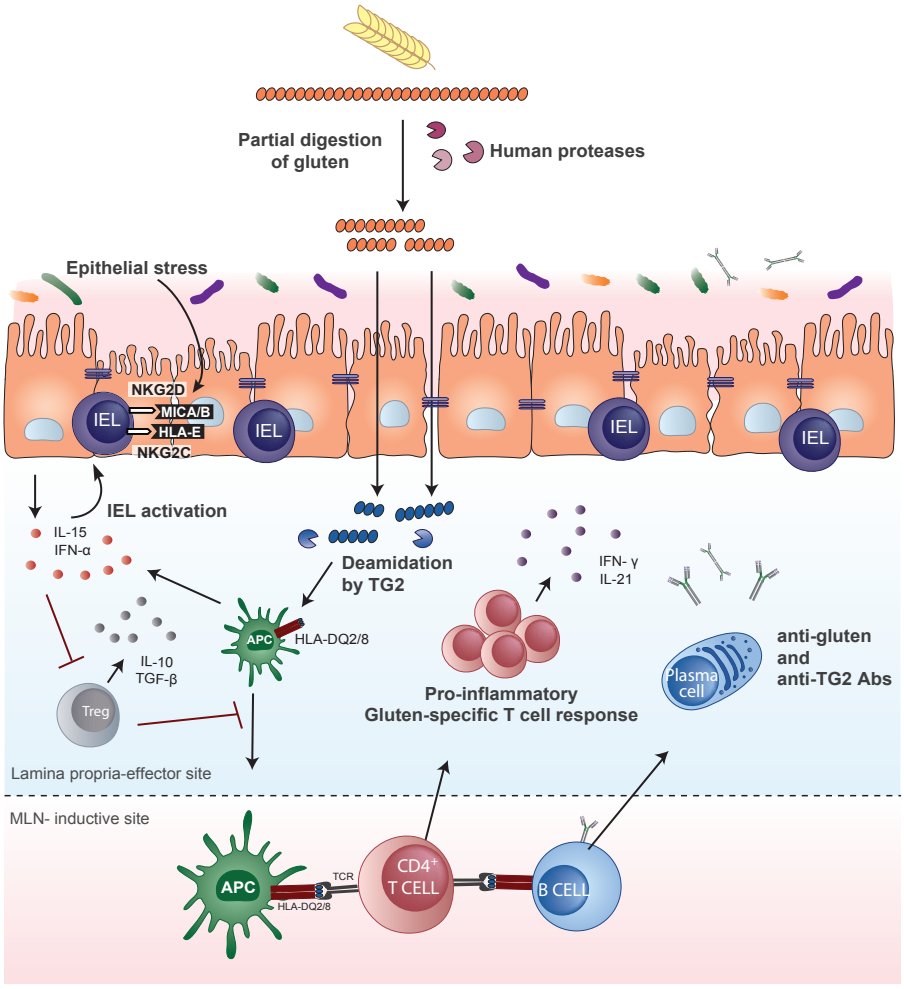


controls. Some relatively consistent findings across studies include increases in proportions of *Bacteroides* and members of Proteobacteria, and decreases in *Lactobacillus* and *Bifidobacteria* in celiac patients compared to controls [1]. Increased abundance of Proteobacteria was also found in patients who suffered from persistent symptoms, despite adhering to a gluten-free diet [3]. More recently, children at a high genetic risk for developing celiac disease were shown to have a different microbiota composition compared to children who were at a low genetic risk [4-6]. Finally, at-risk children who went on to develop celiac disease were suggested to have higher basal microbial diversity that did not further diversify over time, suggestive of a "premature maturation" of the gut microbiota [7]. While the results suggest that an altered early trajectory of the microbiota could predispose to celiac disease, larger trials with increased sample sizes are needed to confirm the findings. Despite the findings of an altered composition of the microbiota in celiac disease or in at-risk children, no celiac "microbial signature" has been established. Differences in the locations of study population, status of control subjects, fecal *vs.* small intestinal samples, and methodology may contribute to inconsistencies between studies. Inconsistent findings have also been reported regarding associations between events that can alter the development of the microbiota and celiac disease development. While early studies suggested antibiotic use and delivery by C-section could increase celiac disease risk, more recent larger clinical studies did not confirm these associations [8].

The long-term follow-up of at-risk infants may provide insight into the factors that may contribute to disease onset. Despite no evidence for causation, these clinical associations have stimulated the study of basic causative mechanisms in reductionist systems and animal models.

▼ FIGURE 2

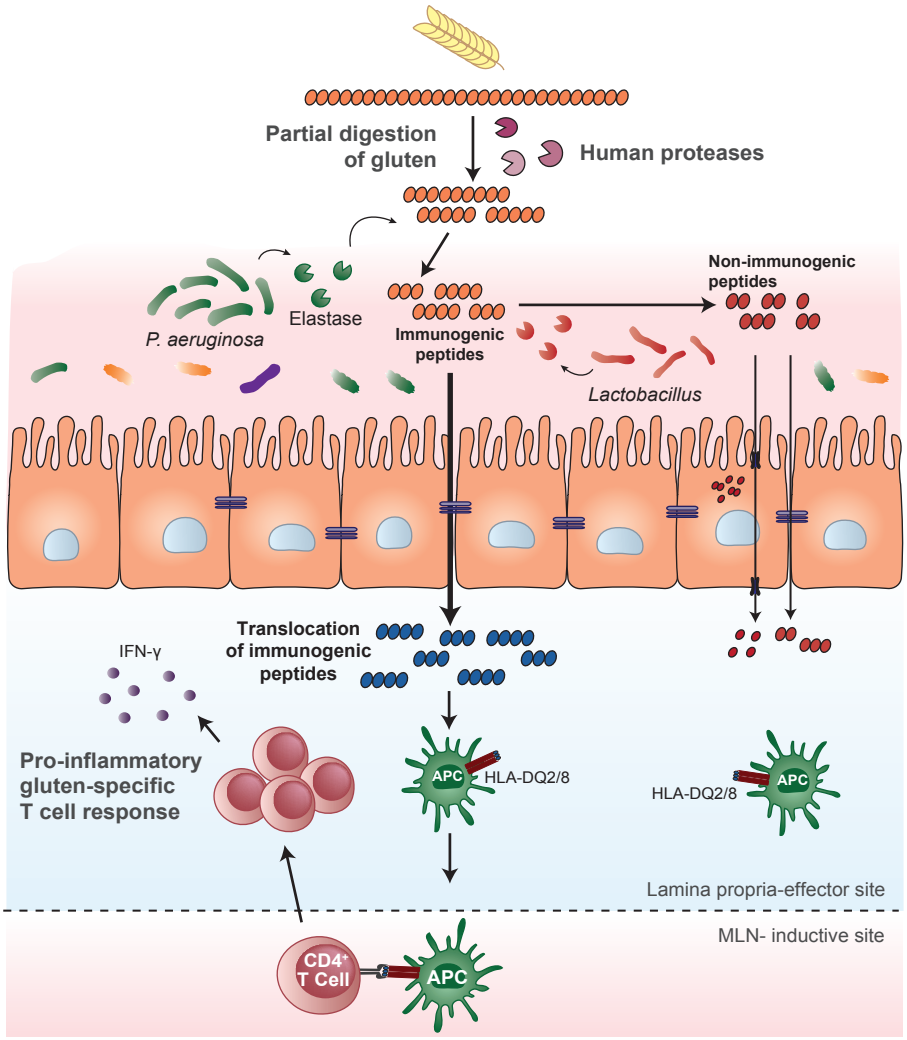
The pathophysiology of celiac disease includes an innate immune response that involves the activation of cytotoxic intraepithelial lymphocytes (IELs) and a gluten-specific inflammatory T cell response. Adapted from [8].



IEL: intraepithelial lymphocyte; APC: antigen presenting cell; MLN: mesenteric lymph node; TG: transglutaminase

▼ FIGURE 3

Microbes may modulate celiac disease pathogenesis through gluten digestion. Gluten peptides partially digested by human enzymes can be further digested by bacterial-derived enzymes, producing peptides with different immunogenic properties. Adapted from [12].



APC: antigen presenting cell; MLN: mesenteric lymph node

MECHANISMS BY WHICH MICROBIOTA ALTERATIONS CAN INFLUENCE CELIAC DISEASE: LESSONS FROM BASIC RESEARCH

How microbes could contribute to the pathogenesis or development of celiac disease can be better understood by studying the function of the microbial community in celiac disease patients *vs.* healthy subjects. Isolation of bacteria from the human small intestine allows for translation into reductionist models. For instance, strains of *Enterobacteriaceae* isolated from celiac patients were more virulent than those isolated from healthy controls [9]. Moreover, *E. coli* strains isolated from celiac children had more *in vitro* pro-inflammatory capacity compared to strains of *Bifidobacterium* that were isolated from control children [10]. Microbiota "humanized" mouse models of germ-free mice add a layer of complexity and allow *in vivo* comparison of phenotypes induced by a microbiota of interest. Moreover, these mice can express features of the human immune system (such as MHC-class II expression) that are critical for celiac disease development. Transgenic mice that express the human celiac risk gene, *HLA-DQ8*, were protected from gluten-induced pathology when they were minimally colonized with a microbiota that was free from pathogens or opportunistic bacteria. However, if an adherent strain of *E. coli*, isolated from the celiac gut, was added to the protective bacteria, mice developed gluten-induced pathology. Similarly, treatment of mice harbouring a diverse murine microbiota with the antibiotic vancomycin led to an increase in Proteobacteria, including *E. coli*, and a worsening of gluten-induced pathology [11].

Recent translational work in mice expanded on the observation that bacteria are capable of degrading gluten (**Figure 3**). The study used gnotobiotic mice that were colonized with either opportunistic pathogens, such as *Pseudomonas aeruginosa*, or with commensals, such as *Lactobacillus*. The authors showed that different bacteria can degrade gluten *in vivo*, but the protein fragments they produce are distinct. The study further demonstrated that enzymes from *P. aeruginosa*, which was isolated from a celiac disease patient, could degrade gluten. This digestion process produced gluten fragments that stimulated an inflammatory immune response in cells isolated from celiac disease patients and were better able to cross the small intestinal barrier, where interaction with immune cells would occur. Several peptides generated by *P. aeruginosa* digestion that were subsequently digested with *lactobacilli*, isolated from a healthy subject and a core member of the healthy microbiome, no longer induced inflammatory immune responses *in vitro*. This study provided a key mechanism where both opportunistic pathogens and commensals may modify the repertoire and immune properties of gluten peptides in the gut, thereby impacting disease susceptibility [12].

GLUTEN METABOLISM BY BACTERIA

- Gluten is highly resistant to breakdown by host digestive enzymes in the small intestine due to its amino acid structure.
- This leaves large gluten fragments that are capable of inducing immune responses once they cross the epithelial barrier in genetically susceptible individuals.
- The gastrointestinal tract harbours bacteria that are able to breakdown gluten, and these bacteria may differ between celiac disease patients and healthy subjects [17].

CAN MICROORGANISMS BE USED TO TREAT OR PREVENT CELIAC DISEASE?

A diagnosis of celiac disease means strict, life-long avoidance of gluten-containing foods because exposure to small amounts of gluten can trigger a variety symptoms and enteropathy in affected people. Gluten is used ubiquitously in processed foods, which makes strict adherence difficult, and has prompted the search for alternative or adjuvant therapies. Given the key role for microorganisms in regulating immunity and the association between celiac disease and altered composition and function of the microbiota, the therapeutic potential of several probiotics has been tested. A strain of *Bifidobacterium longum*, previously shown to have anti-inflammatory effects *in vitro* [10-13], was tested in children on a gluten-free diet in a double-blind placebo-controlled trial. Administration of the probiotic led to some immune changes, as well as lower levels of potentially harmful bacteria (*B. fragilis*). However, no changes in symptoms were observed between children that received the probiotic compared to those that received placebo [14]. Because the probiotic was administered together with the gluten-free diet, it is difficult to discern between changes induced by the dietary restriction or the probiotic. Two other studies tested the effects of a strain of *Bifidobacterium infantis*. The first randomized double-blind placebo-controlled trial demonstrated that patients receiving the probiotic showed significant improvement of symptoms following the 3-week trial, but no differences were found in intestinal permeability [15]. A follow-up trial tested whether the same probiotic could modulate innate immune responses, which could be responsible for the symptomatic improvement previously observed. Administration of a strain of *B. Infantis* led to a decrease in the number of small intestinal Paneth cells, that paralleled a decrease in antimicrobial peptides. These effects of the probiotic were independent of the gluten-free diet [16]. Due to the nature of these few studies including only small groups, there is no evidence to date to recommend any probiotic in celiac disease. Moreover, probiotics consumed by patients with celiac disease need to be rigorously certified gluten-free, and this is not the case for every over the counter preparation. Prior to patient consumption, we need a better understanding of mechanisms of action, and those chosen for further testing should be selected due to their involvement in celiac disease pathways. For example, bacteria that aid in the detoxification of gluten could be selected and used to complement the gluten-free diet. However, to date, no single bacterium tested has shown optimal gluten digestion *in vitro*. Studies have focused on bacterial strains that produce enzymes capable of degrading gluten, but fungal species such as *Aspergillus niger*, also produce gluten-degrading enzymes, and rational combinations of fungal and bacterial organisms may offer an attractive avenue of therapeutic research in celiac disease.

CONCLUSION

The role of the intestinal microbiota in celiac disease has become evident. Expanding on clinical associations, reductionist systems and gnotobiotic animal models have provided evidence that specific microbes can modulate key steps in celiac disease pathogenesis. The continued use of these systems to study specific microbe-host and microbe-gluten interactions as well as larger clinical studies where at-risk individuals are followed over time are critical for understanding how microbes could trigger disease. This can allow for microbe-targeted preventative strategies or adjuvant therapies to the gluten-free diet.

References

1. Verdu EF, Galipeau HJ, Jabri B. Novel players in coeliac disease pathogenesis: role of the gut microbiota. *Nat Rev Gastroenterol Hepatol* 2015 ; 12 : 497-506.
2. Ou G, Hedberg M, Hörstedt P, *et al.* Proximal small intestinal microbiota and identification of rod-shaped bacteria associated with childhood celiac disease. *Am J Gastroenterol* 2009 ; 104 : 3058-67.
3. Wacklin P, Laurikka P, Lindfors K, *et al.* Altered duodenal microbiota composition in celiac disease patients suffering from persistent symptoms on a long-term gluten-free diet. *Am J Gastroenterol* 2014 ; 109 : 1933-41.
4. Olivares M, Neef A, Castillejo G, *et al.* The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing coeliac disease. *Gut* 2015 ; 64 : 406-17.
5. Sellitto M, Bai G, Serena G, *et al.* Proof of concept of microbiome-metabolome analysis and delayed gluten exposure on celiac disease autoimmunity in genetically at-risk infants. *PLoS One* 2012 ; 7 : e33387.
6. Olivares M, Benítez-Páez A, de Palma G, *et al.* Increased prevalence of pathogenic bacteria in the gut microbiota of infants at risk of developing celiac disease: The PROFICEL study. *Gut Microbes* 2018 ; 9 : 551-8.
7. Olivares M, Walker AW, Capilla A, *et al.* Gut microbiota trajectory in early life may predict development of celiac disease. *Microbiome* 2018 ; 6 : 36.
8. Tye-Din JA, Galipeau HJ, Agardh D. Celiac disease: a review of current concepts in pathogenesis, prevention, and novel therapies. *Frontiers in Pediatrics* 2018 ; 6 : 350.
9. Sánchez E, Nadal I, Donat E, *et al.* Reduced diversity and increased virulence-gene carriage in intestinal enterobacteria of coeliac children. *BMC Gastroenterol* 2008 ; 8 : 50.
10. De Palma G, Kamanova J, Cinova J, *et al.* Modulation of phenotypic and functional maturation of dendritic cells by intestinal bacteria and gliadin: relevance for celiac disease. *J Leukoc Biol* 2012 ; 92 : 1043-54.
11. Galipeau HJ, McCarville JL, Huebener S, *et al.* Intestinal microbiota modulates gluten-induced immunopathology in humanized mice. *Am J Pathol* 2015 ; 185 : 2969-82.
12. Caminero A, Galipeau HJ, McCarville JL, *et al.* Duodenal bacteria from patients with celiac disease and healthy subjects distinctly affect gluten breakdown and immunogenicity. *Gastroenterology* 2016 ; 151 : 670-83.
13. Laparra JM, Olivares M, Gallina O, Sanz Y. Bifidobacterium longum CECT 7347 modulates immune responses in a gliadin-induced enteropathy animal model. *PLoS One* 2012 ; 7 : e30744.
14. Olivares M, Castillejo G, Varea V, Sanz Y. Double-blind, randomised, placebo-controlled intervention trial to evaluate the effects of Bifidobacterium longum CECT 7347 in children with newly diagnosed coeliac disease. *Br J Nutr* 2014 ; 112 : 30-40.
15. Smecuol E, Hwang HJ, Sugai E, *et al.* Exploratory, randomized, double-blind, placebo-controlled study on the effects of Bifidobacterium infantis natren life start strain super strain in active celiac disease. *J Clin Gastroenterol* 2013 ; 47 : 139-47.
16. Pinto-Sánchez MI, Smecuol EC, Temprano MP, *et al.* Bifidobacterium infantis NLS super strain reduces the expression of α-defensin-5, a marker of innate immunity, in the mucosa of active celiac disease patients. *J Clin Gastroenterol* 2017 ; 51 : 814-7.
17. Caminero A, Nistal E, Herrán AR, *et al.* Differences in gluten metabolism among healthy volunteers, coeliac disease patients and first-degree relatives. *Br J Nutr* 2015 ; 114 : 1157-67.

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COMMENTED ARTICLE
ADULTS' SECTION

RESILIENCE OF HEALTHY ADULT GUT MICROBIOTA FOLLOWING ANTIBIOTIC EXPOSURE

Comments of the original article of Palleja et al. (Nature Microbiology 2018) [1]

By Prof. Harry Sokol
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To minimize the impact of antibiotics, gut microorganisms harbour and exchange antibiotics resistance genes, collectively called their resistome. Using shotgun sequencing-based metagenomics, we analysed the partial eradication and subsequent regrowth of the gut microbiota in 12 healthy men over a 6-month period following a 4-day intervention with a cocktail of 3 last-resort antibiotics: meropenem, gentamicin and vancomycin. Initial changes included blooms of enterobacteria and other pathobionts, such as *Enterococcus faecalis* and *Fusobacterium nucleatum*, and the depletion of *Bifidobacterium* species and butyrate producers. The gut microbiota of the subjects recovered to near-baseline composition within 1.5 months, although 9 common species, which were present in all subjects before the treatment, remained undetectable in most of the subjects after 180 days. Species that harbour β-lactam resistance genes were positively selected for during and after the intervention. Harbouring glycopeptide or aminoglycoside resistance genes increased the odds of *de novo* colonization, however, the former also decreased the odds of survival. Compositional changes under antibiotic intervention *in vivo* matched results from *in vitro* susceptibility tests. Despite a mild yet long-lasting imprint following antibiotics exposure, the gut microbiota of healthy young adults are resilient to a short-term broad-spectrum antibiotics intervention and their antibiotics resistance gene carriage modulates their recovery processes.

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WHAT IS ALREADY KNOWN ABOUT THIS TOPIC?

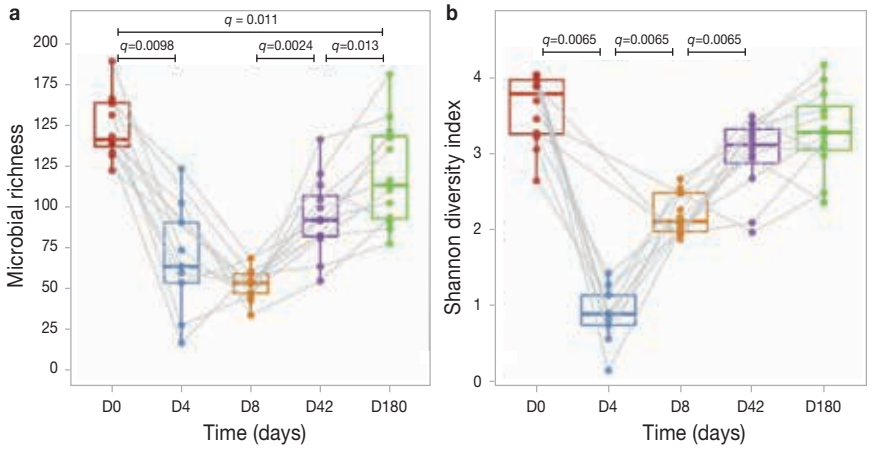
The human gut microbiota forms a complex and balanced ecosystem. Perturbations of this ecosystem can play a role in the development of infections, obesity, diabetes as well as neurological and inflammatory disorders. It is estimated that antibiotics have added 2 to 10 years to our life expectancy, but early exposure to these drugs has also been associated with noxious metabolic, inflammatory and neurological effects, both in animal models and in humans. When microbial communities are exposed to antibiotics, not only do they react by shifting their composition, but also by evolving, optimizing and disseminating antibiotic resistant genes (ABR genes) which collectively form the resistome [2]. The human gut microbiota is a reservoir of ABR genes which are exchanged between the resident strains, thereby propagating resistance [3]. The development and spread of antibiotic-resistant bacteria constitute a serious threat to public health. Only a few studies have investigated the effects of specific antibiotics on intestinal ecosystems and their associated resistomes. In previous work it was shown that antibiotic administration induces a decrease in microbiota diversity and an increase in the carriage of ABR genes [4, 5]. However, the effects of a combination of antibiotics on the microbiota and the role of ABR genes in microbial persistence have not yet been studied. In this study, 12 healthy men aged 18 to 40 years received a cocktail of three last-resort antibiotics (vancomycin, gentamicin and meropenem). The authors analysed the impact of this treatment on the gut microbiota by shotgun sequencing

KEY POINTS

- The intestinal microbiota of healthy young adults is resilient to 4 days of broad-spectrum antibiotic treatment with an approximate 6-month recovery of most bacterial communities.
- The recovery of individual species is modulated by ABR gene carriage.
- The impact of prolonged or repetitive antibiotic treatment requires further study, especially in paediatric populations.

▼ FIGURE 1

Gut microbiota diversity recovers after treatment with broad spectrum antibiotics. Microbial richness (**a**) and Shannon diversity index (**b**). Boxplots represent the diversity measures for the 12 volunteers (median, first and third quartiles). P values adjusted by the FDR (*False Discovery Rate*) are indicated between consecutive samples (two-sided Wilcoxon test).



of faecal samples taken before and at four time points over a 6-month period following antibiotic administration.

WHAT ARE THE MAIN RESULTS OF THIS STUDY?

At D4, immediately after the intervention, microbiota diversity and richness were notably reduced compared to baseline. However, despite the use of very broad spectrum antibiotics, many species were still detectable at D4 (**Figure 1a**). By D8, microbiota diversity (measured by the Shannon index) had considerably increased, suggesting that surviving microorganisms had begun to regrow (**Figure 1b**). At 6 months, microbiota diversity was almost completely restored to baseline levels but this was not the case for richness, suggesting that some strains had been permanently (or at least extendedly) eradicated.

Some of the early observable changes included blooms of normally subdominant commensals like *Escherichia coli, Veillonella spp., Klebsiella spp., Enterococcus faecalis* and *Fusobacterium nucleatum* and a sharp depletion of butyrate-producers like *Faecalibacterium prausnitzii, Roseburia hominis, Anaerostipes hadrus, Coprococcus spp.* and *Eubacterium spp.* These shifts in microbiota composition were no longer significant at D42.

The authors then investigated the role of ABR genes in microbiota recovery. In particular, they found that β-lactamase-harbouring metagenomic species had significantly higher odds of survival (OR = 1.64 [1.24-2.17]) at D8. In addition, metagenomic species not detected at baseline had better odds of subsequent *de novo* colonization if they harboured ABR genes against one of the three antibiotic classes used.

WHAT ARE THE PRACTICAL CONSEQUENCES?

These findings indicate that the gut microbiota of healthy young adults is resilient to a 4-day intervention with broad spectrum antibiotics with recovery of the majority of bacterial communities after about 6 months. The recovery of individual species is modulated by carriage of ABR genes. Further studies are needed to assess the impact of repetitive perturbations and/or over longer periods and to determine whether these findings also hold true in children whose immune system and microbiota are immature. It is possible that repetitive use of antibiotics over long periods selects for bacteria carrying ABR genes at the expense of other commensals, with prolonged or permanent effects on the microbiota. In such cases, corrective intervention with exogenous supply of microorganisms could be considered. The effects of antibiotics on the intestinal microbiota are therefore important and their use must be rationalized.

CONCLUSION

Broad spectrum antibiotics negatively impact the gut microbiota in an immediate, significant and durable manner for some species. In healthy young adults, the gut microbiota is resilient but near-complete recovery takes about six months. Modulated by ABR gene carriage, the capacity of species to regenerate is more favourable to diversity than to richness.

References

1. Palleja A, *et al.* Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nature Microbiol* 2018 ; 3 : 1255-65.

2. Wright GD. The antibiotic resistome: the nexus of chemical and genetic diversity. *Nat Rev Microbiol* 2007 ; 5 : 175-86.

3. van Schaik W. The human gut resistome. *Phil Trans R Soc* 2015 ; B 370 : 20140087.

4. Rashid MU, *et al.* Determining the long-term effect of antibiotic administration on the human normal intestinal microbiota using culture and pyrosequencing methods. *Clin Infect Dis* 2015 ; 60 : S77–S84.

5. Zaura E, *et al.* Same exposure but two radically different responses to antibiotics: resilience of the salivary microbiome versus long-term microbial shifts in feces. *mBio* 2015 ; 6 : e01693-15.

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FEEDING PRACTICES FROM BIRTH TO 12 MONTHS: IMPACT ON THE GUT MICROBIOTA AND THE RISK OF BEING OVERWEIGHT

Comments of the original article by Forbes et al. (JAMA Pediatr 2018) [1]

By Prof. Emmanuel Mas
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The aim was to characterize the association between breastfeeding, microbiota, and risk of overweight during infancy.
The study included 1,087 infants; fecal microbiota at M3 to M4 and/or M12 were characterized by 16S ribosomal RNA sequencing. At M3, infants who were exclusively formula fed had an increased risk of overweight. At M12, microbiota profiles differed significantly according to feeding practices at M6; among partially breastfed infants, formula supplementation was associated with a profile similar to that of nonbreastfed infants, contrary to the introduction of complementary foods without formula. Breastfeeding may be protective against overweight by modulating the gut microbiota. Subtle microbiota differences emerge after brief exposure to formula in the hospital. Formula feeding appears to stimulate changes in microbiota that are associated with overweight, whereas other complementary foods do not.

WHAT IS ALREADY KNOWN ABOUT THIS TOPIC?

From birth, certain factors influence the development of obesity. Breastfeeding has a protective effect, partly because breast milk is low in protein. The gut microbiota (GM) must also be taken into consideration because it is involved in food absorption and energy metabolism. The GM is formed during the first 2 to 3 years of life, and the method of feeding (breast milk *vs.* infant formula milk) is one of the main factors that modulates GM composition. The GM of obese adults is less diverse and has a higher ratio of *Firmicutes/Bacteroidetes*.

WHAT ARE THE MAIN RESULTS OF THIS STUDY?

This study is based on data from 1,087 infants included in the CHILD birth cohort (*Canadian Healthy Infant Longitudinal Development*). Infant faecal samples were collected for microbial analysis at 3 to 4 months (n=996), 12 months (n=821), and at both time points (n=730). Mothers completed questionnaires on the method of feeding at 3 and 6 months, which made it possible to define different groups according to breastfeeding practices (**Table 1**). Among these infants, 74.2% were delivered vaginally, and 39.8% of mothers were overweight or obese. Rates of exclusive breastfeeding were 53.8% at 3 months and 17.6% at 6 months.

At 3 months, exclusive breastfeeding protected against the risk of being overweight at 12 months (defined by measured weight: expected weight ratio > 85th percentile) compared to exclusively formula-fed infants: 19.2% *vs.* 33.3%, respectively, with no significant effect of adjustment (**Table 1**). At 6 months, formula milk in addition to breastfeeding increased the risk of being overweight at 12 months, but solid foods did not have the same effect. Prolonged breastfeeding was found to confer a protective benefit.

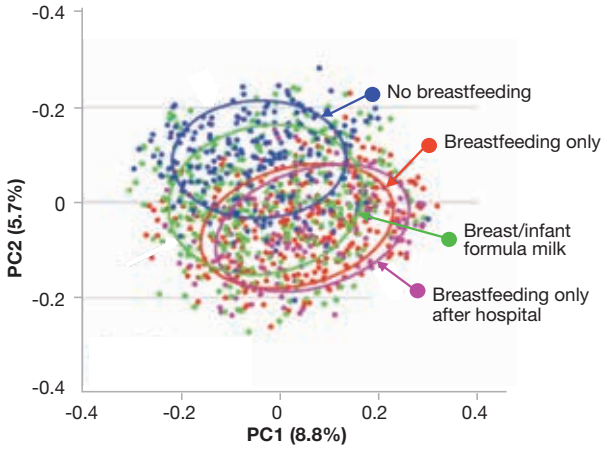
As expected, GM richness and diversity at age 3 to 4 months differed according to the method of feeding, and the composition was significantly different between exclusively breastfed and non-breastfed infants (**Figure 1**). An increase in breastfeeding was accompanied by an increase in relative abundance of *Bifidobacteriaceae* and *Enterobacteriaceae* and a decrease in *Lachnospiraceae, Veillonellaceae,* and *Ruminococcaceae*.

KEY POINTS

- Breastfeeding protects against becoming overweight at 1 year.
- This effect is mediated by the composition of the gut microbiota.
- Studies are needed to determine whether this effect persists over a longer term.

▶ FIGURE 1

Analysis of the principal components of gut microbiota at age 3 to 4 months.



P = 0.01

Pseudo *F* = 10.9

By 12 months, the GM had become more homogeneous but there were still differences relative to the method of feeding at 6 months, *i.e.* increased richness for infants at least partially fed with formula milk, and a relative abundance of *Actinobacteria* and *Proteobacteria* that was higher in exclusively breastfed infants and lower in non-breastfed infants.

Greater GM richness at 3 to 4 months correlated with an increase in excess weight or a risk of becoming overweight at 12 months, particularly with regards to *Lachnospiraceae*, with a median relative abundance of 5.9% (overweight), 4.7% (risk of becoming overweight), and 1.9% (normal weight) (*p*=0.01) (**Figure 2**).

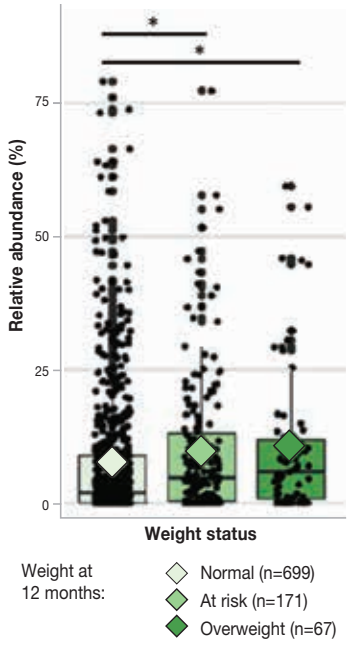
WHAT ARE THE PRACTICAL CONSEQUENCES?

This study firstly demonstrates the benefit of breastfeeding on gaining excess weight at 1 year, and secondly that this benefit is related to modulation of the GM.

Additionally, it is important to promote exclusive breastfeeding from birth, by limiting supplementation with infant formula milk in the maternity unit. This benefit is enhanced by prolonged breastfeeding. Whereas the use of formula milk has a negative impact in infants, this is not the case for solid foods.

▼ FIGURE 2

Relative abundance of Lachnospiraceae at age 3 to 4 months according to weight at 12 months.



▼ TABLE 1

Raw and adjusted association between infant feeding modes and weight status at 12 months

Breastfeeding	Prevalence of excess weight, n (%)	Raw OR (95%CI) (n=1,020)	Adjusted OR (95%CI) with missing data (n=1,087)[a]
Breastfeeding at 3 months			
None (formula only)	53/193.3 (33.3)	2.11 (1.39-3.19)	2.02 (1.18-3.45)
Mixed (breastfeeding and formula)	84/304(27.6)	1.61 (1.13-2.30)	1.63 (1.09-2.44)
Only after leaving the maternity unit	35/171 (20.5)	1.09 (0.68-1.69)	1.13 (0.68-1.89)
Exclusively (breastfeeding only)	74/386 (19.2)	1 (reference)	1 (reference)
Breastfeeding at 6 months		**(n=1,001)**	
None (formula ± food)	77/249 (30.9)	2.11 (1.33-3.42)	1.59 (0.92-2.74)
Mixed (breastfeeding and formula ± food)	81/296 (27.4)	1.77 (1.13-2.85)	1.43 (0.87-2.37)
Mixed without formula (breastfeeding and food)	55/279 (19.7)	1.16 (0.71-1.90)	0.96 (0.57-1.64)
Exclusively (breastfeeding only)	31/177 (17.5)	1 (reference)	1 (reference)
Duration of breastfeeding		**(n=978)**	
< 6 months[b]	68/219 (31.1)	2.02 (1.39-2.93)	1.64 (1.06-2.52)
6 to < 12 months	85/309 (27.5)	1.70 (1.21-2.41)	1.47 (0.99-2.18)
≥ 12 months	82/450 (18.2)	1 (reference)	1 (reference)

OR: adjusted odds ratio

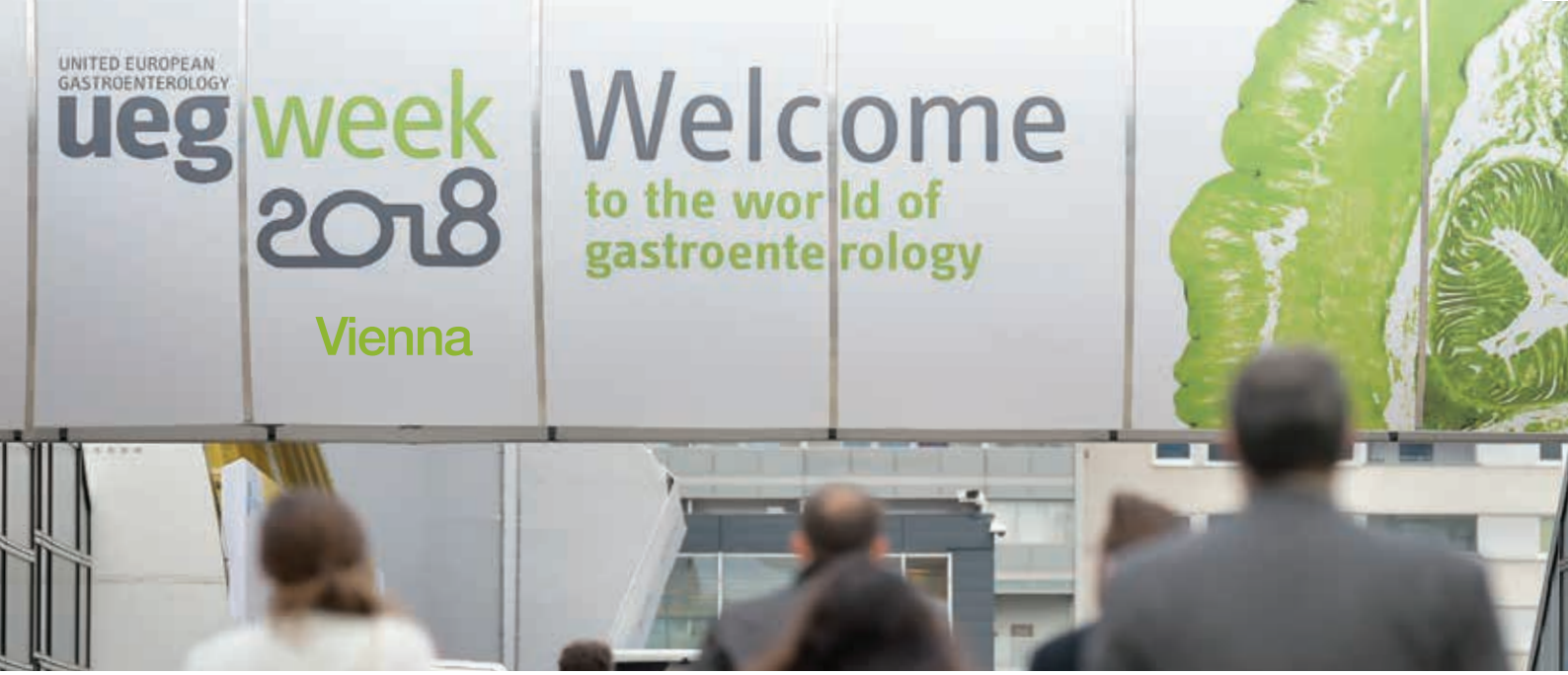
a: adjusted for maternal BMI, smoking, level of education, race/ethnicity, Caesarean delivery, presence of a dog in the home, infant's gender, oral antibiotherapy before 12 months, and site of inclusion (Manitoba, Vancouver, Edmonton). - **b**: exclusion of infants who were never breastfed. Breastfeeding refers to human milk directly at the breast or bottled.

CONCLUSION

Breastfeeding, especially when prolonged, has a protective effect against becoming overweight at 1 year. Infant formula milk, even if used to supplement breastfeeding, increases GM richness and diversity at age 3 to 4 months, particularly *Lachnospiraceae*, and increases the risk of becoming overweight at 1 year.

Reference

1. Forbes JD, Azad MB, Vehling L, Tun HM, Konya TB, Guttman DS, Field CJ, Lefebfre D, Sears MR, Becker AB, Mandhane PJ, Turvey SE, Moraes TJ, Subbarao P, Scott JA, Kozyrskyj A, for the Canadian Healthy Infant Longitudinal Development (CHILD) Study Investigators. *JAMA Pediatr* 2018 ; 172 : e181161.



CONGRESS REVIEW

UNITED EUROPEAN GASTROENTEROLOGY ueg week

By Prof. Danny De Looze
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University Hospital Gent, Belgium

FECAL TRANSPLANTATION - READY FOR PRIME TIME?

OCTOBER 2018

VIENNA, AUSTRIA

Despite the fact that we still do not know all the secrets and mysteries about the gut microbiota, a lot of hope is put on treating GI diseases with intestinal microbiota. Fecal microbiota transplantation seems to be the Holy Grale. But is it? At 2018's UEGW in Vienna numerous lectures were dedicated to it. An overview.

THE IDEAL DONOR

Despite the fact that up until now nobody really knew how to precisely define the normal gut microbiota ("eubiosis"), we do know that high microbial diversity and gene richness are of key importance in the host-microbiota equilibrium. Therefore, the ideal fecal donor should be screened for bacterial richness. A surrogate marker for this property was proposed by Marie Joossen (Leuven, Belgium) who pointed out that the presence of *Blastocystis hominis* correlates with a higher microbial richness [1]. This finding – if confirmed by others – may change our current practice to avoid carriers of this commensal as fecal donors. Enriching the donor's microbiota by prebiotics or using multiple donors may also ensure (theoretically) a higher baseline diversity of the donated material. This was also observed by Karakan *et al.* (Ankara, Turkey) who performed an open trial in ulcerative colitis with an overall complete response rate of 32% which was particularly influenced by a high bacterial diversity in the donated fecal material.

THE NEW BROWN GOLD

Strict adherence to current guidelines for selection of donors for fecal material necessitates the rejection of most donors. Terveer *et al.* (Leiden, the Netherlands) report that only 3,5% of possible donors are suitable at the end of the line [2]. The main reasons for not being accepted as a donor are: age above 50, high BMI and carrier status of non-pathogenic germs *(Blastocystis hominis, Dientamoeba fragilis)* and MDROs (multidrug resistant organisms) [2].

CLOSTRIDIUM DIFFICILE

The main indication for fecal microbiota transplantation remains refractory infection with *Clostridium difficile (C. difficile).* Ianiro *et al.* (Rome, Italy) showed, in a retrospective series of 282 *C. difficile* patients comparing antibiotic treatment and fecal transplant, that this latter treatment resulted in significant shorter hospital stay, significantly lower mortality and specifically less sepsis-related mortality.

Antonio Gasbarrini (Rome, Italy) therefore suggests that the time has come to promote fecal microbiota transplantation as the first line therapy in *C. difficile* infection.

EXPANDING THE SCOPE OF FMT

Antonio Gasbarrini gave a nice overview of promising indications for fecal microbiota transplantation. It has been shown that fecal transplants restore the human microbiota better than probiotics after antibiotic-induced dysbiosis in humans. This was also the case following combined chemotherapy and antibiotic induced dysbiosis in the setting of hematological stem cell therapy and in patients with liver cirrhosis receiving antibiotics. Mice models even show evidence for restoration of immune function and intestinal integrity after chemotherapy induced intestinal damage. Therefore, Antonio Gasbarrini makes the case for pre-emptive stool conservation for subsequent autologous fecal microbiota transplantation, *e.g.* following antibiotic treatment or bone marrow transplantation. *In vivo* evidence from clinical trials must be awaited before this futuristic but not unrealistic strategy can be implemented. Anyhow, it seems quite logical that collecting one's own stool for autologous transplantation later in life, is the way to go.

ULCERATIVE COLITIS

In 3 out of 4 published randomized controlled trials fecal microbiota transplantation was superior to placebo in refractory ulcerative colitis (UC) patients [3]. Mean remission rate in these studies, however, was only 25-30 % and Rainer *et al.* (Graz, Austria) presented a study with similar complete remission rates, showing no added value of administering fresh stool in these patients. Nevertheless, up until now there was no standardized stool transplantation protocol in UC. Remission rates of 30% seem low but to put things in perspective: this is also the remission rate that is achieved with the expensive and widely used biologicals […]. The importance of colonic microbiota in UC was demonstrated by Herrera-de Guise *et al.* (Barcelona, Spain) who showed that patients in long-term stable remission (more than 5 years) present with an abundance of *Akkermansia muciniphila* and *Faecalibacterium prausnitzii,* similar to healthy controls. These authors even suggest that we should think of a paradigm shift in treating UC: our therapeutic endpoint should perhaps no longer be immune suppression, but we should aim to reach eubiotic microbiota characteristics.

IRRITABLE BOWEL SYNDROME

Irritable bowel syndrome (IBS) is certainly the condition for which the expectations of a cure by fecal microbiota transplantation are very high in both patients and health care practitioners. Still, conflicting results from randomized controlled trials [4, 5] do not currently support a widespread use of this treatment in IBS. Intestinal dysbiosis is present in IBS but a clear causality between these microbial changes and symptoms are lacking. Halkjaer *et al.* (Copenhagen, Denmark) performed a randomized controlled trial in 52 adult IBS patients; an increase in biodiversity (comparable to the donors) was observed in the patients being actively transplanted (5). Unfortunately, the placebo group had a significantly better clinical outcome at 3 and 6 months than the patients receiving fecal capsules [5]. In a small cohort of 16 IBS patients, Holster *et al.* (Orebro, Sweden) were also unable to demonstrate efficacy for fecal microbiota transplant *vs.* placebo. They also studied rectal sensitivity by means of a barostat and showed no difference between the active and control groups, concluding that changing the microbiota does not contribute to the visceral hypersensitivity in IBS.

BACTERIAL PILLS

Ianiro *et al.* performed an open label trial in *C. difficile* infection with a synthetic microbiota suspension (10 patients only) demonstrating efficacy. Khanna *et al.* (Rochester, USA) have also demonstrated efficacy in prevention of *C. difficile* infection's relapse with a non-frozen, lyophilized, orally administrated microbiota restoring drug (RBX7455) in an open-label phase 1 trial. These are promising results that need to be confirmed in large scale randomized trials.

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CONCLUSION

The UEGW 2018 added more insight into the desired eubiotic characteristics of fecal material and the promising new indications for fecal microbiota transplantation that may arise the upcoming years. Up until now *C. difficile* infection remains the only clear-cut indication for this treatment which is currently not ready for prime time in other conditions.

References

1. Tito RY, Chaffron S, Caenepeel C, *et al.* Population-level analysis of *Blastocystis* subtype prevalence and variation in the human gutmicrobiota. *Gut* 2018 [Publication en ligne avant l'impression].
2. Terveer EM. How to establish and run a stool bank. *Clin Microbiol Infect* 2017 ; 23 : 924-30.
3. Imdad A, Nicholson MR, Tanner-Smith EE, *et al.* Fecal transplantation for treatment of inflammatory bowel disease. *Cochrane Database Syst Rev* 2018 ; 11:CD012774.
4. Johnsen PH, Hilpüsch F, Cavanagh JP, *et al.* Faecal microbiota transplantation versus placebo for moderate-to-severe irritable bowel syndrome: a double-blind, randomised, placebo-controlled, parallel-group, single-centre trial. *Lancet Gastroenterol Hepatol* 2018 ; 3 : 17-24.
5. Halkjær SI, Christensen AH, Lo BZS, *et al.* Faecal microbiota transplantation alters gut microbiota in patients with irritable bowel syndrome: results from a randomised, double-blind placebo-controlled study. *Gut* 2018 ; 67 : 2107-15.

Association Tunisienne des Sciences de la Nutrition

5th International Congress on Nutrition

"Microbiota and non-communicable diseases"

November 9-11, 2018
Hammamet - Tunisia

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CONGRESS REVIEW

Association Tunisienne
des Sciences de la Nutrition

By Prof. Jaafar Heikel
African Centre for Health Research and Studies, Mohammed-VI University Polytechnique, Morocco

THE GUT MICROBIOTA: ISSUES AND CHALLENGES IN THE MANAGEMENT OF METABOLIC DISORDERS

NOVEMBER 2018

HAMMAMET, TUNISIA

The 5th International Congress on Nutrition, organized by the Tunisian Association of Nutrition Sciences, was held on November 9-11, 2018 in Hammamet, Tunisia. This edition sheds light on the link between the gut microbiota and metabolic disorders such as diabetes and obesity.

THE MICROBIOTA: A NEW PLAYER IN OUR UNDERSTANDING OF METABOLIC DISORDERS

Like most African countries, Maghreb countries are experiencing a demographic, epidemiological, and nutritional transition. In 2018, as in developed countries, there were more deaths from non-communicable diseases (75%) than from infectious diseases. Excess weight, obesity, diabetes, and hypertension have become a public health concern, with prevalence exceeding 50, 20, 10 and 30%, respectively. Classic approaches to management have proved to be inadequate due to a number of determinant factors.

Although the first published studies on gut microbiota date back to the 1960s, it has only been in the last fifteen years or so that new work has highlighted the role of the microbiota in the maintenance of chronic inflammatory states, insulin resistance, or obesity, by acting through different mechanisms [1]. Phenomena such as metabolic endotoxemia and bacterial translocation, resulting from the passage of lipopolysaccharides (LPS) into the systemic circulation, appear to be implicated.

Whether the disorder is diabetes, obesity, or metabolic syndrome, the quantity, quality, and diversity of microbiota (especially the phyla *Firmicutes, Bacteroidetes,* and *Actinobacteria*) underlie the cascade of responses leading to increased intestinal permeability ("leaky gut"), mobilization of pro-inflammatory cells, and induction of specific cell transport proteins. Gut microorganisms are even thought to play a positive role in the immune system through exposure to bacterial LPS, which may be tolerated in some cases. Metabolic disor-

ders, such as those caused by a high-fat diet, for example, could be avoidable by inhibiting LPS receptors (CD14/Toll-like receptor 4; TLR-4).

BACTERIA-HOST CELL COMMUNICATION: IMPACT ON METABOLISM

When subjected to a high-fat, low-fibre diet, the bacteria that compose the gut microbiota undergo changes on their surface (LPS) that trigger immune responses and local inflammatory reactions. These events increase intestinal permeability, allowing inflammatory components to enter the bloodstream [2]. Recent work has highlighted this role of dietary fat in dysbiosis and endotoxemia, initially in the oral microbiota. The process is thought to be triggered first by glycoprotein CD36 (increased sensitivity to the taste of fat), and then augmented at the level of the microbiota of the gustatory papillae (rich in *Streptococci*), thus creating a local inflammatory process identical to that seen in the intestinal wall. In addition, the texture and type of fat – saturated or polyunsaturated – and the involvement of bile salts have also been suggested as factors that could help account for metabolic disorders and the risk of obesity.

The role of certain bacterial phyla such as *Firmicutes, Bacteroidetes,* and *Actinobacteria* in metabolic endotoxemia, based on studies in both axenic mice and humans, is well established. For instance, in the mouse, a high-fat diet increases the concentration of circulating LPS, leading to metabolic changes linked to obesity, and analysis of gut microbiota reveals a significant decrease in *Bifidobacterium* spp. and *Bacteroides*-associated gut bacteria. In addition, a negative association has been observed between endotoxemia and the number of *Bifidobacteria*; the latter can reduce the level of LPS and improve intestinal barrier function [3-5], as well as intestinal barrier integrity, which is crucial to prevent passage of bacterial components from the intestinal lumen into the bloodstream and host tissues.

WHAT IS THE IMPACT ON MANAGEMENT OF METABOLIC DISORDERS?

The major focus is currently the identification of specific bacteria, with a view to offering clinicians the tools to prevent or manage patients at risk of, or already suffering from, a metabolic disorder [6-7]. Restoring equilibrium to the intestinal ecosystem or re-balancing the microbiota is a challenge in patients with intestinal dysbiosis, which is determined by epigenetics, the environment, diet, lifestyle, history of antibiotic treatments, and state of health.

Thus, *Firmicutes* and *Bacteroidetes*, which account for the majority of our gut microbiome, affect the risk of metabolic diseases, relative to their abundance. Moreover, recent studies on specific bacteria associated with energy and carbohydrate metabolism have been carried out in the laboratory. These studies seem to show, for example, that *Akkermansia muciniphila*, even pasteurized, improves intestinal barrier function and the thickness of the mucus layer, and may thus influence insulin resistance and obesity. The oxygen sensitivity of this species has so far limited its ability to be cultured and restricted its study in humans.

Other gut bacteria, such as *Faecalibacterium prausnitzii*, play a beneficial role and may offer therapeutic strategies based on the use of specific probiotics.

In addition, other elements should be considered, such as the interactions between the host, microbiota, and brain, leading to the concepts of the taste cortex, pleasure circuit, and microbial agents that mediate obesity, which are of particular importance.

The significant failure to manage obesity may be explained by approaches that lack optimal management of the dynamic states of the gut microbiota over time.

Consequently, today's challenge is to better understand "obesogenic and diabetogenic microbiotic factors" in order to shape the way low-fat and low-carbohydrate nutritional diets, appropriate physical activity, probiotic and prebiotic supplementation, as well as faecal transplantation may be used in the future.

The role of these factors needs to be clarified with regards to their complementarity and based on an integrated preventative, but also therapeutic, approach [8].

Sufficiently large cohort studies should help confirm the role of the oral and gut microbiota in the development of transient and chronic inflammatory states underlying metabolic disorders – and probably other pathological states, such as cancers or some psychiatric conditions.


Photo: Adobe Stock.

References

1. Feng Q, Chen WD, Wang YD. Gut microbiota: an integral moderator in health and disease. *Front Microbiol* 2018 ; 9 : 51.
2. Cani PD, Everard A. Talking microbes: When gut bacteria interact with diet and host organs. *Mol Nutr Food Res* 2016 ; 60 : 58-66.
3. Gomes JMG, Costa JA, Alfenas RCG. Metabolic endotoxemia and diabetes mellitus: A systematic review. *Metabolism* 2017 ; 68 : 133-144.
4. Cani PD, Neyrinck AM, Fava F, *et al.* Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* 2007;50:2374-83.
5. Cani PD, Amar J, Iglesias MA, *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007;56:1761-72.
6. Harsh IA, Konturek PC. The role of gut microbiota in obesity and type 2 and type 1 diabetes mellitus: new insights into "old" diseases. *Med Sci* 2018 ; 6 : 32.
7. Crommen S, Simon MC. Microbial regulation of glucose metabolism and Insulin resistance. *Genes* 2017 ; 9 : pii: E10.
8. Dao MC, Clement K. Gut microbiota and obesity: concepts relevant to clinical care. *Eur J Intern Med* 2018 ; 48 : 18-24.

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LITERATURE SELECTION

PROTON PUMP INHIBITORS MODIFY GUT MICROBIOME

By Prof. Markku Voutilainen
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Proton pump inhibitors (PPIs), which are nowadays ones of the most widely used medicines even if about half of the prescriptions lack an evidence-based indication, have a central role in the treatment of peptic ulcer and gastro-oesophageal reflux disease. PPIs inhibit acid secretion from gastric parietal cells. PPI-induced hypochlorhydria may increase the risk of infections.

Mishiro *et al.* investigated the impact of 20 mg daily esomeprazole for 1 month on saliva, periodontal pocket fluid and fecal microbiota in 10 healthy volunteers [1]. Colonic microbiota contained the greatest number of species. Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria were the most abundant species in faeces, whereas Firmicutes, Proteobacteria, Bacteroidetes and Fusobacteria were the most common in saliva and periodontal pocket fluid. PPI caused a significant reduction in the diversity of salivary microbiota. *Streptococci*, which are predominantly found in the upper gastrointestinal tract, were increased in faeces and also in saliva and periodontal pocket after PPI treatment [1].

Antibiotics and acid-suppressive medications cause dysbiosis. Stark *et al.* performed a retrospective study with 333,353 US children [2]. PPI prescriptions were associated with obesity, each additional antibiotic class increased the risk of obesity, and each additional 30-day prescription of acid-suppressive medication strengthened the association with obesity.

Mailhe *et al.* examined the gastrointestinal microbiota composition of 6 patients who underwent gastroscopy and colonoscopy [3]. Samples were obtained from stomach, duodenum, ileum, and colon. Culturomics was performed with mass spectometry MALDI-TOF and metagenomics targeting the V3-V4 region in the 16S rRNA. In all, 368 bacterial species were observed (37 new species): 110 from the stomach, 106 from the duodenum and 235 from the left colon. The upper gut contained less aero-intolerant species and less rich microbiota compared with the lower gut. Three patients used long-term PPI treatment; their gastric pH and bacterial diversity were higher compared with those not using PPI.

Investigators from Cleveland, have reviewed the impact of PPIs on gut microbiome [4]. The main consequence of PPI treatment is the increase in gastric pH. PPI treatment may lead to excess *Streptococcus* gastric colonisation, which may cause dyspeptic symptoms. Small bowel bacterial overgrowth risk is moderately increased during PPI treatment [4]. PPI and antibiotics increase *Clostridium difficile* infection (CDI) risk. PPI treatment may also increase spontaneous bacterial peritonitis risk in hepatic cirrhosis. A statistical association between PPI use and the incidence of *Salmonella* and *Campylobacter* infections has been reported.

Observational studies reporting associations between PPI use and side effects do not necessarily prove causal relationship. PPI users are often sicker than non-users, which could partially explain the increase of side effects. Anyhow, PPIs should be used only on evidence-based indications with lowest effective doses and should be stopped when the treatment response has been achieved.

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GUT MICROBIOTA INVOLVED IN THE PATHOGENESIS OF NON-ALCOHOLIC FATTY LIVER DISEASE

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in western countries and affects 25-30% of the general population. NAFLD is classified into simple fatty liver disease with no or minimal inflammation, and steatohepatitis (NASH), which is characterized by steatosis, inflammation and fibrosis. NASH may lead to cirrhosis, which is a risk factor for hepatocellular carcinoma (HCC). NAFLD is the hepatic manifestation of metabolic syndrome.

Puri and Sanyal reviewed the role of the intestinal microbiome in NAFLD [5]. Increased adipose tissue mass with activation of the innate immune system leads to insulin resistance. Altered gut microbiota and increased intestinal permeability cause immune activation. Microbiome may also affect extra-intestinal organs by translocation, gut-derived neurohumoral signalling, and altering the nutritional substances absorbed from the intestine.

Chen and co-workers examined gut microbiota in the bile acid metabolism [6]. Microbiota produces enzymes that in the intestines convert primary bile acids (synthesised and conjugated in the liver) into secondary bile acids. Dysbiosis may lead to decreased synthesis of secondary bile acids, which in turn diminishes the activation of nuclear receptors such as farnesoid X receptor (FXR), pregnane X receptor, Takeda G-protein-coupled bile acid protein 5 (TGR5) and vitamin D receptor. These receptors play important roles in energy regulation and their dysfunction may play a role in the pathogenesis of NAFLD. Dysbiosis leads to increased bile acid deconjugation and is associated with disturbed lipid and cholesterol metabolism, weight increase and disturbed signalling [6]. Gut microbiota metabolises bile acids, conversely, bile acids are needed to maintain normal gut microbiota.

The gut microbiota is changed in NAFLD, but there is no uniform pattern [6]. Bacteria converting primary bile acids (*C. leptum* for example) are decreased in the faeces of NAFLD patients. Decreased FXR increases the synthesis of primary bile acids, gluconeogenesis, triglycerides and very-low-density lipoprotein synthesis. Thus decreased FXR as well as TGR5 may be involved in NAFLD pathogenesis. Modulation of gut microbiota could be an option for the treatment of NAFLD. Probiotics could adjust the whole bile acid pool instead of individual nuclear receptors [6].

Variable definitions, histologic assessments and methods, as well as different bioinformatics approaches have been used. Thus it is difficult to draw generalisable conclusions of the microbiota changes in the pathogenesis of NAFLD [5]. The mechanisms that link microbiota changes to NAFLD pathogenesis are increased energy extraction in the gut and increased free fatty acid hepatic uptake, altered gut barrier function and endotoxemia with inflammation, altered bile acid and choline metabolism.

Loman and co-authors analysed the impact of pre- and probiotic treatment on NAFLD [7]. They identified 25 studies that fulfilled the PICOS* criteria: 9 assessed prebiotic, 11 probiotic and 7 symbiotic treatments. These therapies significantly reduced body mass index (BMI), hepatic transaminases and ν-glutamyltransferase, cholesterol and triglycerides levels. The effect of pro- and prebiotics were similar on BMI, liver enzymes and high-density cholesterol. The major weaknesses of the studies were the lack of intestinal microbiota analysis, the heterogeneity of treatments, and their short duration. The present meta-analysis was, however, the first one to report simultaneous changes induced by microbiota treatment on weight, lipid metabolism and inflammation in NAFLD.

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* Criteria for inclusion and exclusion of studies: Patients – Intervention – Comparator – Outcomes - Study Design

References

1. Mishiro T, Oka T, Kuroki Y, *et al*. Oral microbiome alterations of healthy volunteers with proton pump inhibitor. *J Gastroenterol Hepatol* 2018 ; 33 : 1059-66.
2. Stark CM, Susi A, Emerick J, Nylund CM. Antibiotic and acid-suppression medications during early childhood are associated with obesity. *Gut* 2019 ; 68 : 62-9.
3. Mailhe M, Ribaconi D, Vitton V, *et al*. Repertoire of the gut microbiota from stomach to colon usin culturomics and next-generation sequencing. *BMC Microbiol* 2018 ; 18 : 157.
4. Singh A, Cresci GA, Kirby DF. Proton pump inhibitors: risks and rewards and emerging consequences to the gut microbiome. *Nutr Clin Pract* 2018 ; 33 : 614-24.
5. Puri P, Sanyal AJ. The intestinal microbiome in nonalcoholic fatty liver disease. *Clin Liver Dis* 2018 ; 22 : 121-32.
6. Chen J, Thomsen M, Vitetta L. Interaction of gut microbiota with dysregulation of bile acids in the pathogenesis of nonalcoholic fatty liver disease and potential therapeutic implications of probiotics. *J Cell Biochem* 2018.
7. Loman BR, Hernández-Saavedra D, An R, Rector RS. Prebiotic and probiotic treatment of nonalcoholic fatty liver disease: a systematic review. *Nutr Rev* 2018 ; 76 : 822-39.

MOROCCAN MICROBIOTA DAY

By Moulay Skali
Sales Manager Biocodex Morocco

In collaboration with the AMECHO (Moroccan Association of Sonographers), Biocodex Morocco organized the "First Moroccan Microbiota Day" on Sunday, November 11, 2018.

This meeting, which took place at the School of Medicine and Pharmacy in Casablanca, was attended by nearly 300 general practitioners from several cities throughout the country who came to hear presentations by a variety of speakers with a diverse set of expertise.

The speakers were selected from among internationally renowned experts in the field:

- Prof. Jaâfar Heikel (African Center for Health Research and Studies, Mohammed-VI University Polytechnique, Morocco)
- Dr. Alexis Mosca (Hôpital Robert-Debré, Pediatric Gastroenterology Unit. AP-HP, France)
- Prof. Philippe Marteau (Hôpital Saint-Antoine, Gastroenterology-Hepatology Unit. AP-HP, France)
- Prof. Jean-Marc Sabate (Hôpital Avicenne, Gastroenterology-Hepatology Unit. AP-HP, France)
- Dr. Philippe Nuss (Hôpital Saint-Antoine, Psychiatrics Unit. AP-HP, France)

The presentations addressed a number of topics focusing on the microbiota:

- epidemiological data from Morocco on non-communicable diseases;
- the impact of antibiotics on gut microbiota in children and its short- and long-term consequences;
- the relationship between intestinal dysbiosis and hepatogastric disorders in adults;
- the involvement of the microbiota and the role of probiotics in the management of irritable bowel syndrome
- the role of the gut-brain axis in neurological pathologies.

This event was intended to be lively and interactive. Following the successive presentations, the audience and the speakers exchanged views at length. The goal was to enable health professionals to access useful scientific knowledge and envision concrete responses to real clinical situations.

In view of its success, this unprecedented initiative could be renewed in 2019 in other Moroccan cities to meet other doctors and associations. A perspective in line with the mission to which Biocodex has been committed for a number of years, with particular thanks to the creation of the Biocodex Microbiota Institute and the Biocodex Microbiota Foundation.

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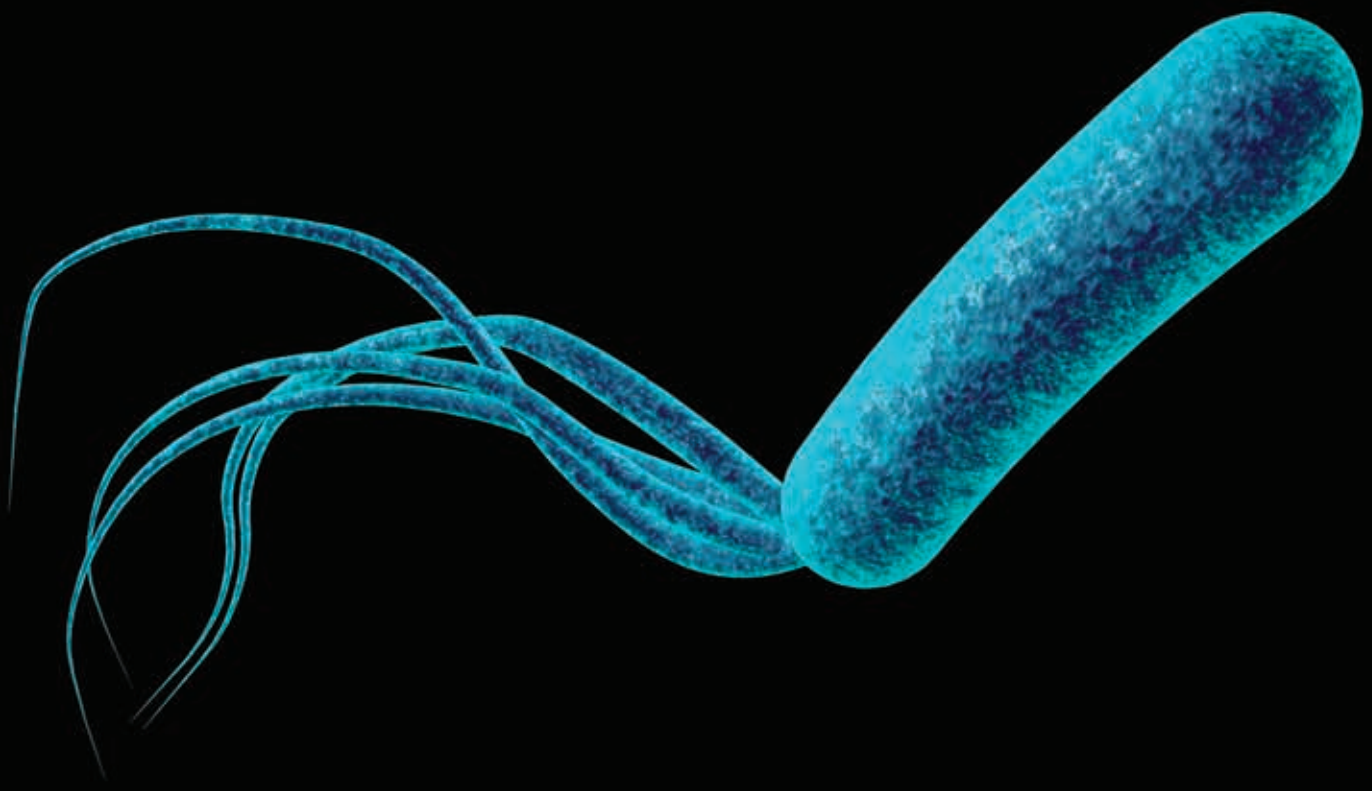

BIOCODEX Microbiota Institute

SUMMARY

CALL FOR PROPOSALS

BIOCODEX MICROBIOTA FOUNDATION

The 2019 international call for proposals was closed last November 30th. Under the theme "Gut microbiota and drug metabolism", it received 35 applications from 19 different nationalities. The winner will be announced in April and will be awarded a €200,000 grant. A new edition is already scheduled for 2020 for which the theme will be decided by the International Scientific Committee.

The 2018 national calls for proposals in Canada, the United States, Finland, France, Morocco, Mexico, Russia, Turkey and Ukraine have all been closed and the winners announced. These calls will be renewed in 2019.

• **Visit www.biocodexmicrobiotafoundation.com to find out more.**

"PRO" OF THE NET

BIOCODEX MICROBIOTA INSTITUTE

The Institute's website offers health professionals a new thematic folder: Gut microbiota and immune defenses. This particularly well documented folder describes the latest advances on the link between gut flora and immune defenses. It sheds light on current knowledge and perspectives around three main topics, namely involvement of the microbiota in the development of the immune system, its role in diseases with a strong immune and inflammatory component (type 1 diabetes, IBD, among others), and the benefits of modulating commensal gut bacteria in preventing infections.

The general public pages of the site present a thematic paper on gastroenteritis and infectious diarrhea aimed at all audiences. Is it possible to act on the microbiota to combat diarrhea? What are the culprits responsible for damaging our flora? This thematic paper aims to provide some insights.

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