MICROBIOTA

9 BIOCODEX NEWSLETTER | MARCH 2020

BIOCODEX
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SUMMARY

EDITO

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> “ANTIBIOTICS NOT ONLY AFFECT PATHOGENS BUT ALSO THE COMMENSAL FLORA.”

Dear readers, Penicillin, the first antibiotic, was discovered by the Scottish bacteriologist Alexander Fleming in 1928. This discovery earned him, along with two other researchers, the Nobel Prize in Physiology and Medicine in 1945 and sparked a true revolution in the treatment of infectious diseases. The benefits brought by the dozens of molecules available represent an enormous progress which we are still taking advantage of today.

However, the World Health Organization (WHO) regularly warns us about the misuse of antibiotics. Both in human health and in the livestock industry (6,500 tons per year, not counting China and the United States), overuse has led to the emergence of resistant bacteria (environmental and pathogenic). “Never has the threat of antimicrobial resistance been more immediate and the need for solutions more urgent”, cautioned Dr. Tedros Adhanom Ghebreyesus, WHO Director-General, in early 2020.

In the wake of the WHO, the Centers for Disease Control and Prevention (Atlanta, Georgia, USA) published in 2019 a list of 18 resistant bacterial species – mostly pathogens – considered as concerning, serious and even urgent threats. Every year, these bacteria cause about 35,000 deaths in the United States and 33,000 in Europe (2015 data); some scientists estimate that these figures could rise to 10 million by the year 2050, and even surpass the number of deaths from cancer.

And the gut microbiota is not spared: antibiotics affect not only pathogens but also the commensal flora, altering its balance, sometimes durably: its return to “normal” can take 1 to 3 months and in some individuals may be incomplete. In this edition, Professor Francisco Guarner (Barcelona, Spain) explains the collateral damage of dysbiosis incurred by taking antibiotics and reviews the short, medium and long-term consequences to the individual and on the scale of populations.

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OVERVIEW

IMPACT OF ANTIBIOTICS ON THE GUT MICROBIOTA, DOES IT MATTER?

Massive misuse of antibiotics may lead not only to antimicrobial resistant infections but also to the spread of non-communicable, chronic diseases.

The use of antibiotics and vaccines have done more to extend life expectancy than any other medical innovation. Antibiotics are among the most recognized medical milestones according to experts appointed by the *British Medical Journal* [1]. After the introduction of measures for the prevention and treatment of infectious diseases, changes were gigantic. Infections typically caused 30% of all deaths, mostly in children aged under 5 years, but at the end of the 20th century less than 4% of deaths were due to infection [1].

However, two major concerns have recently emerged. First, treatments for a growing number of infections become less effective due to resistance. Antimicrobial resistance is now a major threat to human health, and its link with overuse of antibiotics is well documented [2]. Second, antibiotics meant to kill pathogenic microbes have had unintended consequences for the human microbial ecosystem, including changes that may be difficult to reverse [3]. The human body is home to a complex array of microbes known as the microbiome or microbiota, which play an important role in health. Microbiota alteration and the accompanying loss of functional attributes might result in the microbial communities of people living in industrialized societies being suboptimal for health [3]. These issues require awareness by the medical community and straightforward guidelines from health policy makers.

By Prof. Francisco Guarner
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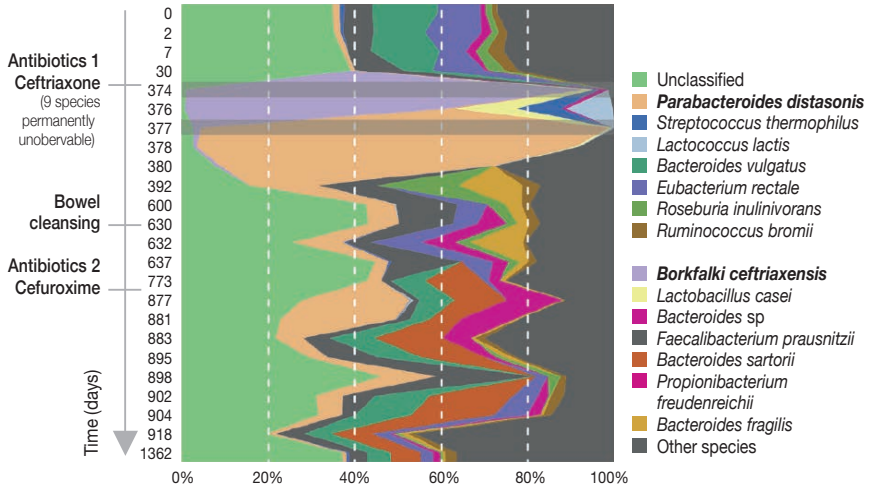
IMPACT OF MASSIVE USE OF ANTIBIOTICS

According to the WHO 2018 report [2], the overall amount of antibiotics consumed by humans is well above 6,500 tonnes per year (data from 65 countries; China and US not included). A median of 18 out of 1,000 inhabitants consume every day a defined dose of antibiotics, which means that 139 million doses are consumed every single day of the year. The median is lower in African countries (12 out of 1.000) than in Europe (17,8) or America (18,2), while infections cause up to 36,6% of total deaths in Africa but only 2,7% or 4,5% in Europe or America. Low-income countries still have high mortality rates from infectious diseases but low rates of antibiotic use. Limited access, use of wrong drugs or wrong treatment schedules, can contribute to resistant infections arising in low-income countries, like tuberculosis. In developed countries,

▶ FIGURE 1

Ceftriaxone induced mono-dominance of a single strain rising at 92% abundance in faecal samples on days 374 and 376, followed by mono-dominance of Parabacteroides distasonis on days 377, 378 and 380 [From 6].



as many as half of all antibiotic prescriptions can be considered inappropriate [2]. Unnecessary antibiotic consumption accelerates the development of resistances, and multi-drug resistant strains of *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*, etc., are on the rise [2].

ANTIBIOTIC-INDUCED DYSBIOSIS

Although most courses of antibiotics result in no immediate, obvious side-effects, there is concern about collateral damage altering the composition of the gut microbiota and its functions [3]. Antibiotic-associated diarrhoea is the most commonly recognized complication of antibiotics, and develops in 15 to 25% of patients. Most episodes of antibiotics-induced diarrhoea are mild and self-limited. However, an increasing number of cases develop more severe forms, including *Clostridioides difficile*–associated diarrhoea. Antibiotic-induced disturbances promote *C. difficile* spore germination within the intestine, overgrowth of vegetative forms and toxin production, leading to epithelial damage and colitis. Clinical presentation ranges from self-limiting diarrhoea to toxic megacolon, fulminant colitis and death [4].

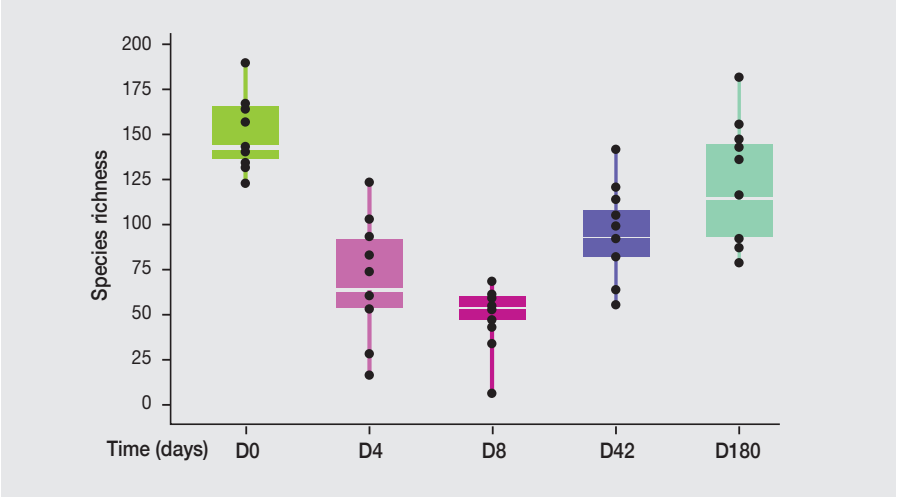
From birth onwards, the human gut microbiota rapidly increases in diversity during the first 3 years of age, before stabilizing to an adult-like state. Thereafter, the core composition is stable but bacterial abundances may fluctuate in response to external factors (diet, drugs, travel, etc.). Studies have shown that the effects of antibiotics result in very large shifts in relative abundances. In patients on β-lactams or quinolones, the core microbiota fell from 29 to 12 taxa, the total number of observed taxa decreased by 25%, and there was a shift from *Faecalibacterium* to *Bacteroides* as the dominant genus [5]. The use of antibiotics induced a decrease in microbial diversity (loss of richness in the ecosystem) and overgrowth of resistant species, which resulted in overall increase of microbial load, i.e. number of bacteria per gram of faeces [5]. Extreme cases of antibiotic-induced overgrowth have been reported showing mono-dominance of a single strain that bloomed to 92% relative abundance in faecal samples after intravenous ceftriaxone treatment [6]. (**Figure 1**).

In healthy volunteers, a 4-day antibiotic intervention led to blooms of enterobacteria and other pathobionts (*Enterococcus faecalis, Fusobacterium nucleatum*), and to the depletion of *Bifidobacterium* species and butyrate producers [7]. The gut microbiota only recovered to near-baseline composition within 1.5 months, although 9 common species, which were present in all subjects before the treatment, remained undetectable after 180 days. (**Figures 2 and 3**).

Dysbiosis is a compositional and functional alteration in the microbiota that perturbs the microbial ecosystem to an extent that exceeds its resistance and resilience capabilities [8]. The functional impact of antibiotics on short chain fatty acid producers, butyrate in particular, may have long-term consequences because of the rupture of the symbiotic balance between microbiota and host. Failure to produce butyrate increases the flow of oxygen towards the mucosa and perturbs the micro-ecosystem in a way that favours the survival of oxygen-resistant bacteria (enterobacteria) and precludes recovery of butyrate producers like *Faecalibacterium*, which are strict anaerobes [9]. Such changes critically affect the resilience capacity of the ecosystem and perpetuate the imbalance towards chronicity.

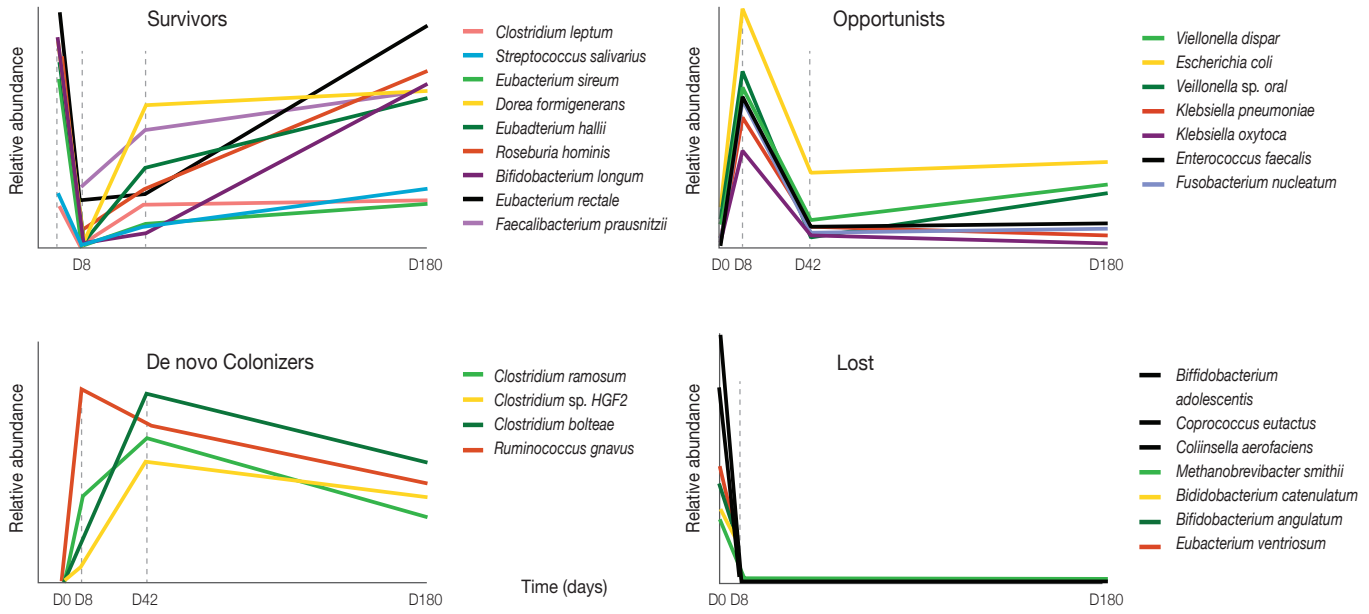
▼ FIGURE 2

Loss of bacterial richness and diversity in the gut microbiome after antibiotic treatment from day 0 to day 4 [From 7].



▼ FIGURE 3

Four-day antibiotic treatment induced large shifts in bacterial abundances. Four groups are marked according to their abundance pattern throughout the 180-day study period: survivors, opportunists, de novo colonizers and lost [From 7].



Unintended side-effects of antibiotics on the gut microbiota and the accompanying loss of functional attributes might result in rupture of the symbiotic balance between microbiota and host

THE RESISTOME

The resistome is the collection of all bacterial genes that directly or indirectly contribute to antibiotic resistance. Resistance genes do not seem to have been selected in response to recent exposure to antibiotics. Antibiotics date back hundreds of millions of years, so is resistance, and the number of genes in the resistome is a reflection of the continuous co-evolution of antibiotic-producing and target organisms. Composition of the resistome and prevalence of resistance genes in human-associated bacteria adapt to selective forces derived from human action.

Species that harbour β-lactam resistance genes are positively selected during and after antibiotic consumption [7]. Likewise, harbouring amino-glycoside resistance genes also increases odds of *de novo* colonization. Antibiotic resistance gene carriage modulates the recovery process after antibiotic consumption [7].

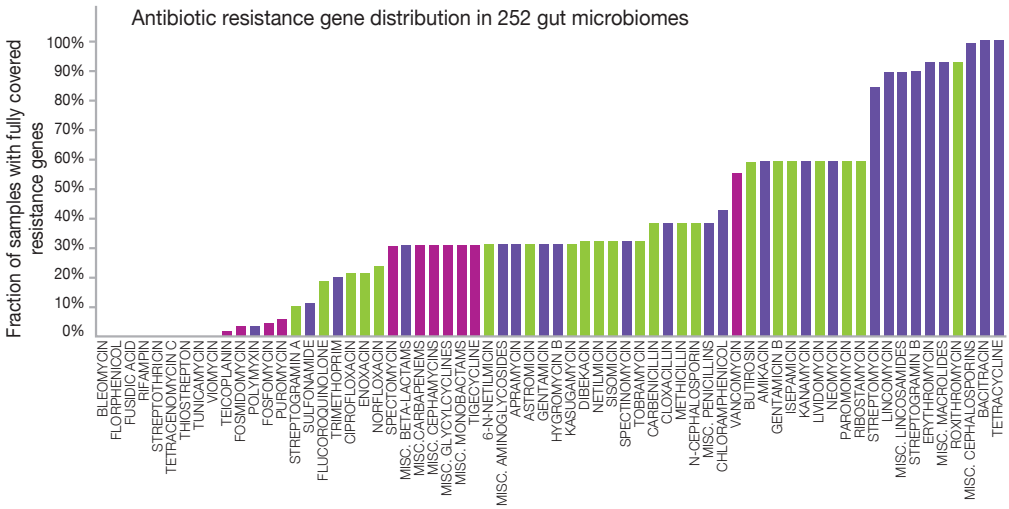
The human gut microbiome harbours a diverse repertoire of antibiotic resistance genes, which can be investigated by molecular sequencing technologies [10]. A study on 252 human faecal samples from different countries found that the most prevalent resistance genes in the microbiome are those corresponding to antibiotics also used in animals and to antibiotics available since a long time (**Figure 4**) [11]. Country-level data on antibiotic use in both humans and animals matched the observed country-specific differences in prevalence of resistance genes. Altogether, the data suggest a positive correlation between exposure to antibiotics and prevalence of antibiotic resistance genes.

Some antibiotic resistance genes are readily exchanged between bacteria through horizontal gene transfer. Studies have shown that under antibiotic-induced stress, rising opportunist bacteria spread resistance genes among the microbial community. A longitudinal study of the gut microbiome in Finnish children observed that the use of antibiotics promoted the expansion of antibiotic resistance genes in the gut, due to overgrowth of bacteria harbouring resistance genes and increased mobili-

Under antibiotic-induced stress, opportunist bacteria spread resistance genes among the gut microbial community. The human gut microbiota is an accumulator of resistance genes potentially providing them to pathogens

▶ FIGURE 4

The human gut resistome: the most prevalent resistance genes are those for antibiotics that are also used in animals and for antibiotics that have been available for longer [From 11].



Probiotics may prevent the overgrowth of resistant species during antibiotic treatment and minimise the spread of antibiotic resistance genes

zation of resistance genes by plasmids [12]. Antibiotic resistance genes carried on microbial chromosomes showed a peak in abundance after antibiotic treatment followed by a sharp decline, whereas abundance of resistance genes carried on mobile elements persisted long after antibiotic therapy ended. This might be explained by the fact that episomal genes can be broadly distributed across multiple species by horizontal gene transfer.

The human gut microbiota may be the most accessible reservoir of resistance genes to pathogens. Early life antibiotic treatment is associated with reduced microbial diversity but also with an increased risk of antibiotic resistance development.

ANTIBIOTICS AND RISK OF DISEASE

Perturbations of the gut microbial ecosystem during early life combined with genetic susceptibility may have a long-lasting impact on the immune system leading to disease or predisposition to disease later in life. Indeed, it has been shown that inflammatory bowel diseases, metabolic disorders (type 2 diabetes, obesity), and atopic diseases are associated with an altered composition of the gut microbiota.

A leading hypothesis regarding the pathogenesis of inflammatory disorders is that alterations of the gut microbiota caused by repeated exposure to antibiotics trigger inflammation. Infants receiving antibiotics before one year of age were found to have a 5.5 times higher risk of developing IBD than unexposed children [13]. Likewise, antibiotic exposure in the first 2 years of life, when host adipocyte populations are developing, are associated with a diagnosis of childhood obesity [14]. Reduced gut microbial richness has been associated with increased adiposity, insulin and leptin resistance, and a more pronounced inflammatory phenotype.

CONCLUSION

Despite the resilience of the intestinal microbiota, the spread of antibiotic resistance genes is now a major threat to human health, and overuse of antibiotics seems to be the leading cause. In addition, there is growing evidence linking fragility of the human microbiota in industrialized countries with the coincident spread of non-communicable, chronic diseases [3]. Antibiotic exposure is again an obvious cause for such microbiota derangement. Shifting current trends towards more sustainable medical practices is a major challenge for public health in the 21st century.

Unquestionably, restricted and rational use of antibiotics is the best and most efficient way of preventing detrimental imbalances of the human gut microbiome. Interestingly, use of a probiotic with proven efficacy for prevention of antibiotic-induced diarrhoea has been shown to constrain the overgrowth of resistant species during antibiotic treatment [15]. Potentially, this strategy could also minimise the spread of antibiotic resistance genes.

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COMMENTED ARTICLE
ADULTS' SECTION

By Prof. Harry Sokol
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WHOLE-VIROME ANALYSIS SHEDS LIGHT ON VIRAL DARK MATTER IN INFLAMMATORY BOWEL DISEASE

Commentary on the original publication by Clooney et al. (Cell Host & Microbe 2019) [1]

The human gut virome is thought to significantly impact the microbiome and human health. However, most analyses have been performed on a limited fraction of known viruses. Using whole-virome analysis on a published inflammatory bowel disease (IBD) cohort and an in-house ulcerative colitis dataset, the authors shed light on the composition of the human gut virome in IBD beyond this identifiable minority. They observed IBD-specific changes to the virome and increased numbers of temperate phage sequences in individuals with Crohn's disease. Unlike prior database-dependent methods, no changes in viral richness were observed. Among IBD subjects, the changes in virome composition reflected alterations in bacterial composition. Furthermore, incorporating both bacteriome and virome composition offered greater classification power between health and disease. This approach to analyzing whole virome across cohorts highlights significant IBD signals, which may be crucial for developing future biomarkers and therapeutics.

WHAT DO WE ALREADY KNOW ABOUT THIS SUBJECT?

The virome is probably one of the main forces that shape the human gut microbiome, but it may also be its least understood component. The virome is composed mostly of bacteriophages (phages), which play a key role in many microbial ecosystems by stimulating diversity, helping to replenish nutrients and facilitating horizontal gene transfer. Understanding the role of bacteriophages in the structures of microbial communities will be essential if we are to understand and control the alterations in the human gut microbiome that are associated with various diseases. Many gut bacteria (and potential phage hosts) are difficult to grow in the laboratory, which means that virome analysis is highly dependent on metagenomic sequencing and bioinformatic approaches.

However, phages lack universal marker genes (like the 16S rRNA gene found in bacteria), and there is a lack of taxonomic information with sparse databases, which means that methods are needed that are independent of databases. The first metagenomic studies revealed the diversity of the human gut virome, but could only classify a very small fraction (2%) of the DNA sequenced [2]. Improvements in high throughput sequencing technologies now make it possible to analyze the virome with an unprecedented level of detail. It has been confirmed that the virome is incredibly diverse, that the majority do not align to any reference virus sequences in databases (referred to as viral dark matter), and that the composition is unique to individuals.

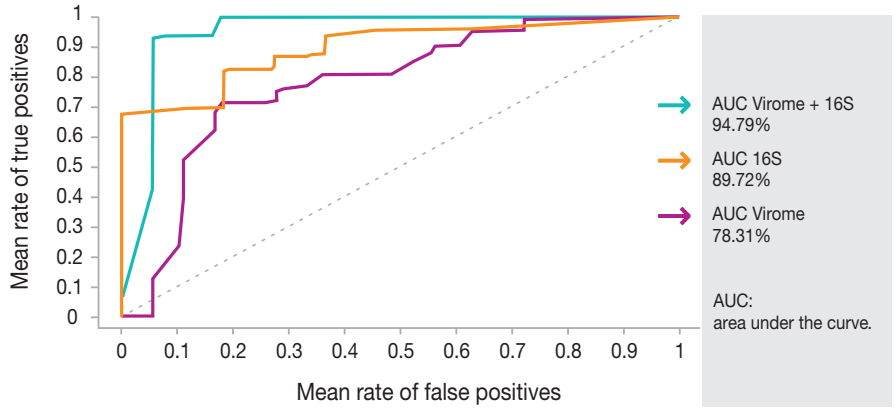
Although the etiology of IBD remains unclear, these diseases are multifactorial

KEY POINTS

- A large majority of the gut virome cannot be studied because it is absent from the databases
- The database-independent method described here allows analysis of the whole gut virome
- In IBD, the gut virome is altered, less stable, and dominated by temperate phages
- The gut virome could be used as biomarker or therapeutics in the future

▼ FIGURE 1

Classification between healthy subjects and IBD patients using gut virome and bacterial microbiota by 16S sequencing (cohort from [2]).
ROC curve for statistical models using the virome alone, the bacterial microbiota alone (16S) or both together.



and associated with alterations in the gut microbiome. Emerging data now indicate that the gut virome is altered in IBD [3] with greater overall diversity and an increased relative abundance of the order Caudovirales. However, almost all the results have been based on changes in the composition of the identifiable fraction of the virome, which can represent as little as 15% of the dataset [3]. This limits our overall understanding of the virome and hinders the identification of potential disease biomarkers. An analytical method independent of databases is essential if we want to fully characterize the alterations in the gut virome.

WHAT ARE THE MAIN INSIGHTS FROM THIS STUDY?

The authors of this study re-analyzed a set of published keystone data [3] from a Crohn's disease and ulcerative colitis cohort and from healthy controls. The problem of high interindividual variation was overcome by using protein homology and a specific algorithm (Markov cluster) to group the viral sequences into presumed taxonomic ranks. This made it possible to describe changes in composition across the whole virome beyond the identifiable minority. The authors suggest that unlike the core virome of healthy subjects composed of virulent phages, the virome of IBD subjects is altered, less stable, and dominated by temperate phages. They show that changes in the virome reflect alterations in the bacteriome and that the use of both the bacteriome and virome composition was more effective at differentiating between IBD and healthy subjects (**Figure 1**). The results were validated on a longitudinal ulcerative colitis cohort. This database-independent approach could be used to shed light on viral dark matter from many published studies.

WHAT ARE THE CONSEQUENCES IN PRACTICE

These findings confirm that the human gut virome is altered in IBD and that this may be associated with members of the bacterial microbiota; this could be used as a diagnostic or even prognostic biomarker. In addition, the observed alterations suggest that the virome could be partly responsible for the alterations in the bacterial microbiota seen in IBD. To this end, the gut virome could therefore be a future target or a future therapeutic tool in IBD.

CONCLUSION

Using a database-independent method, the whole gut virome can be studied whereas usually only a minority is analyzed. While the core virome in healthy subjects consists of virulent phages, the virome in IBD is altered, less stable, and dominated by temperate phages. Using both the virome and the bacterial microbiota composition makes it possible to differentiate IBD patients from healthy subjects more effectively than by using only one or the other. These results open new perspectives for the use of the virome as biomarker or therapeutic target in IBD.

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COMMENTED ARTICLE
CHILDREN'S SECTION

LONG-TERM BENEFIT OF MICROBIOTA TRANSFER THERAPY ON AUTISM SYMPTOMS AND GUT MICROBIOTA

Commentary on the original publication by Kang et al. (Sci Rep 2019) [1]

By Prof. Emmanuel Mas
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Many studies have reported abnormal gut microbiota in individuals with Autism Spectrum Disorders (ASD), suggesting a link between gut microbiome and autism-like behaviors. Modifying the gut microbiome is a potential route to improve gastrointestinal (GI) and behavioral symptoms in children with ASD, and fecal microbiota transplant could transform the dysbiotic gut microbiome toward a healthy one by delivering a large number of commensal microbes from a healthy donor. The authors had previously performed an open-label trial of Microbiota Transfer Therapy (MTT) that combined antibiotics, a bowel cleanse, a stomach-acid suppressant, and fecal microbiota transplant, and observed significant improvements in GI symptoms, autism-related symptoms, and gut microbiota. Here, the authors report on a follow-up with the same 18 participants two years after treatment was completed. Notably, most improvements in GI symptoms were maintained, and autism-related symptoms improved even more after the end of treatment. Important changes in gut microbiota at the end of treatment remained at follow-up, including significant increases in bacterial diversity and relative abundances of *Bifidobacteria* and *Prevotella*. Their observations demonstrate the long-term safety and efficacy of MTT as a potential therapy to treat children with ASD who have GI problems, and warrant a double-blind, placebo-controlled trial in the future.

WHAT DO WE ALREADY KNOW ABOUT THIS SUBJECT?

It is known that children with autism spectrum disorders suffer from a variety of gastrointestinal problems including constipation, diarrhea, and bloating. These children also have a dysbiotic gut microbiome characterized by an increased ratio of Firmicutes/Bacteroidetes due to a low relative abundance of Bacteroidetes. This dysbiosis alters the gut-brain axis, promoting both the gastrointestinal problems and the characteristic autism-like behaviors.

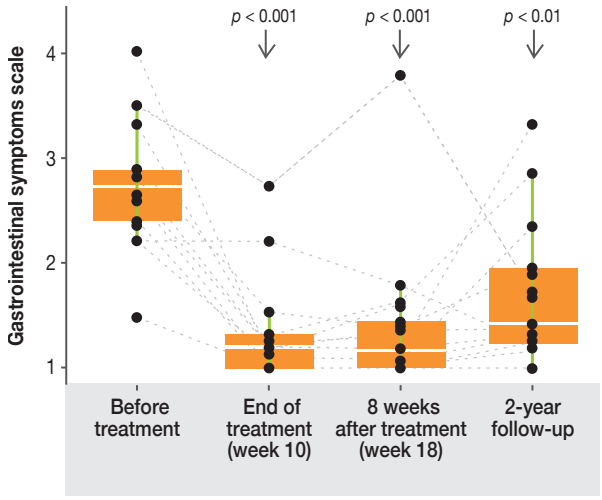
Microbiota transfer therapy consists of an initial gastrointestinal preparation with a 14 day course of vancomycin and a bowel cleanse on day 15, followed by fecal microbiota transplantation using a high initial dose of standardized human gut microbiota (by the oral or rectal route) and then a low maintenance dose for 7-8 weeks, concurrently with a proton pump inhibitor from day 12. Kang *et al.* previously reported that this treatment led to an 80% reduction in gastrointestinal symptoms and a smaller reduction in behavioral symptoms in children with ASD, in addition to a modification of the gut microbiota, at the 8-week follow-up [2].

WHAT ARE THE MAIN INSIGHTS FROM THIS STUDY?

This article presents a follow-up evaluation of the 18 autistic children two years after the initial microbiota transfer therapy. Improvements in gastrointestinal symptoms, as assessed by the *Gastrointestinal Symptom Rating Scale* questionnaire, were maintained with a 58% reduction (**Figure 1**). Improvements were observed in all gastrointestinal symptoms (abdominal pain, indigestion, diarrhea, and constipation). Transit remained improved, with a 26% reduction in the percentage of days of abnormal stools.

▶ FIGURE 1

Change in gastrointestinal symptoms assessed by the Gastrointestinal Symptom Rating Scale questionnaire.



The families reported that autism-related signs steadily improved. ASD severity was 47% lower than baseline when assessed by the *Childhood Autism Rating Scale* (**Figure 2**). On the *Aberrant Behavior Checklist* the scores continued to improve and were 35% lower at two years compared to 24 % at the 8-week follow-up.

The gut microbiota was analyzed by 16S RNA analysis for 16 of the 18 children. Bacterial diversity was higher at two years than after eight weeks of follow-up (**Figure 3**). A higher relative abundance of *Bifidobacterium* and *Prevotella* persisted at the 2-year follow-up, while *Desulfobivrio* abundance did not persist significantly.

WHAT ARE THE CONSEQUENCES IN PRACTICE?

These findings indicate that microbiota transfer therapy has a durable, long-term effect on the gut microbiota. In addition, it significantly and durably improves gastrointestinal and behavioral symptoms of autism spectrum disorders.
It is now essential to carry out randomized, controlled, double-blind studies in children with ASD who do or do not have gastrointestinal problems. Indeed, dysbiosis may be present and impact the gut-brain axis even in the absence of gastrointestinal symptoms. The results of this study should be confirmed before this approach is used in clinical practice.

KEY POINTS

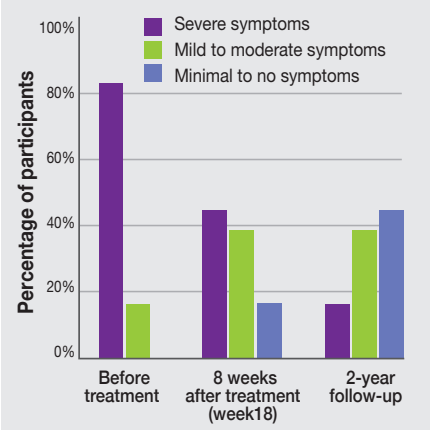
- Microbiota transfer therapy has a persistent effect on gastrointestinal symptoms two years after the initial treatment.
- It also has an effect on autism behaviors two years after the initial treatment
- Further research is needed to determine whether microbiota transfer could improve autism behaviors even in the absence of gastrointestinal problems.

CONCLUSION

This study confirms the benefits of microbiota transfer therapy in children with autism spectrum disorders. The effects of the initial treatment were maintained at the 2-year follow-up on both the gut microbiota and on gastrointestinal symptoms, with even a continued improvement in autism-like behaviors.

▼ FIGURE 2

Severity of autism spectrum symptoms.



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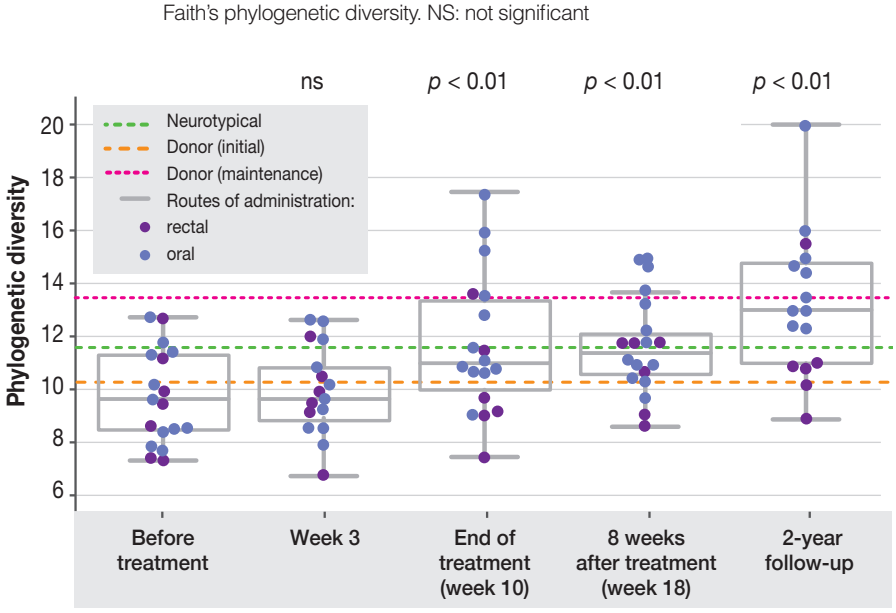
▼ FIGURE 3

Evaluation of gut microbiota.



CONGRESS REVIEW

UNITED EUROPEAN GASTROENTEROLOGY ueg week

By Dr. Paul Cardenas
Institute of Microbiology, Universidad San Francisco de Quito, Ecuador

INTESTINAL MICROBIOTA: INFLUENCING FACTORS AND ROLE IN CERTAIN PATHOLOGIES

OCTOBER 2019

BARCELONA, SPAIN

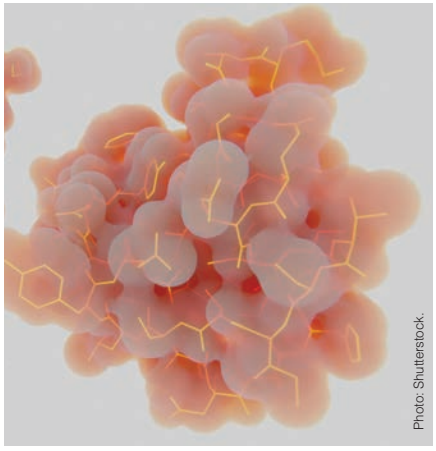


Photo: Shutterstock.

The UEG week on its 2019 edition brought interesting findings on how the gut microbiota health is related to the prevention, development and cure of major diseases.

DEVELOPMENT AND VARIATIONS OF THE HEALTHY GUT MICROBIOTA

The microbiota is a complex microbial community established in individually variant ecosystems (as the human gut). For that reason, its shaping depends of a wide range of influences and insults as presented by Georgina Hold (University of South Wales, Australia). Trillions of microbes have co-evolved with humans, and are in a continuous adaptation towards the human physiology. Since birth, such variations depend of factors like delivery mode, diet, geography, early exposures (pollution and antibiotics), ageing and host genetics. However, environmental factors seem to play a more important role in microbiota modelling than host genetics [1]. Early life microbiota is an important determinant to understand chronic diseases development, particularly in urban societies, for example asthma, allergies, eczema, inflammatory bowel disease (IBD), coeliac disease, obesity.

There is not 'one' normal microbiota pattern in healthy individuals, since in the microbiota metabolic and functional patterns are not species driven. Likewise, in the gut microbiota shaping, inter-country variants are more important than inter-individual variants [2]. Self-reported results from the HELIUS cohort by Stijn Meijnikman (Academic Medical Centre, Netherlands) showed that bacterial diversity is related to ethnic background (probably driven by diet and ancestries). It is considered that a high-Bacteroides/low-Prevotella ratio is related with a westernized diet; however, microbiota functionality analysis usually shows contradictory results. As a consequence 'dysbiosis' is an imprecise term if "healthy", "unhealthy" or just "different" microbiota are not defined on each case.

MICROBIOTA AND INTESTINAL DISEASE

The interaction between the microbiota and the host is a 2-way communication, Lipopolysaccharide (LPS) is an important mediator produced by Gram-negatives that triggers intestinal inflammation, besides adipose cell-proliferation and insulin resistance as explained by Remy Burcelin (Paul-Sabatier University, France). Bacterial translocation to the adipose tissue is also an important feature in metabolic syndrome. Furthermore, high concentrations of bacterial DNA on adipocytes can be considered as molecular biomarkers of type 2 diabetes.

Irritable bowel syndrome (IBS) is a complex disease where the microbiota and the host interplay in its physiopathology as presented by Magnus Simrén (Sahlgrenska University Hospital, Sweden). There are IBS patients where there is not a clear microbiota signature when comparing with healthy controls. However, some specific patterns have been associated with symptoms severity [3]. By modulating the microbiota patterns on IBS patients (by probiotics or non-absorbable antibiotics) symptoms can be improved.

EFFECT OF DRUGS INTAKE ON THE GUT MICROBIOTA

Drugs intake interacts directly with the gut microbiota as explained by Rinse K. Weersma (University Medical Center Groningen, Netherlands). There are three scenarios: the drug affects the gut microbiota changing its composition/function, the microbiota metabolizes the drug making it to activate/inactivate, or the microbiota has indirect effects on the drug response [4].

In the first case, the use of proton pump inhibitors has shown the increase of potentially harmful bacteria (*Enterococcus, Streptococcus, Staphylococcus* and *Escherichia*). Other drugs have reported to have a significant impact in the gut microbiota like metformin, laxatives, antidepressants and antibiotics. In the second scenario the most commonly studied drugs are sulfasalazine (which is activated by the microbiota), and digoxin (which is inactivated by specific bacterial strains).

The indirect effect of the gut microbiota on the drug response has been reported in antitumoral immunotherapies as presented by Harry Sokol (Saint-Antoine Hospital, France). The use of anti PD-1 immunotherapy on melanoma, non-small cell lung cancer, renal carcinoma and others is directly affected by the use of antibiotics. Additionally, the positive effect of ipilimumab on melanoma is directly related with the presence of *Faecalibacterium prausnitzii* [5]. Other studies have reported similar results but with different bacteria like *Akkermansia muciniphila*, however the mechanisms seem that these bacteria have an important anti-inflammatory effect via CTL4 pathway.

Photo: Shutterstock.

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CONGRESS REVIEW

By Prof. Gourdas Choudhuri
Fortis Hospital, Haryana, India

THE GROWING IMPORTANCE OF GUT MICROBIOTA ON DIGESTIVE HEALTH

DECEMBER 2019

KOLKATA, INDIA

The Asian Pacific Digestive Health Conference held in Kolkata, India, between December 12-15, focused on the particular subject of gut microbiota in digestive health. Attended by over 5,000 participants from across the globe, the conference witnessed packed halls in both the major symposia that dealt with this topic.

GUT MICROBIOTA IN HEALTH AND DISEASE

The first symposium started with an insightful overview by Dr Holtmann from Australia. He emphasized that the large number of commensal bacteria normally residing in the human gut far exceeds the number of cells in the human body and drew attention to the key role microbes play in maintaining human health.

Gut microbes belong to three taxonomic classes: Bacteria, Archaea and Eukaryota; although most are difficult to cultivate, they perform the crucial functions of food digestion (especially fibre), production and absorption of vitamins, absorption of nutrients, protection of mucosa from pathogen colonization, regulation of the host immune system and intestinal peristalsis.

Dr Holtmann said that although stool microbiota collected from stools (luminal microbes) has until now been most researched, scientists are now recognizing the presence of a "mucosa-associated microbiote" that is more difficult to extract, characterize and culture, and yet seems to play a much stronger role in regulating our gut health and immune system.

There is growing evidence linking SIBO (small intestinal bacterial overgrowth) as well as dysbiosis associated with several diseases. Luminal antimicrobial therapy has been shown, for example, to improve liver functions in patients with chronic liver

disease and primary sclerosing cholangitis, and the clinical response often noted in patients with irritable bowel syndrome (IBS)/disease (IBD).

He highlighted the strong links that have emerged between gut microbiota and a variety of gastro-intestinal (GI) and non-GI conditions, and showed growing evidence how interventions targeting gut microbiota could cure or control currently incurable diseases.

HOW TO STUDY THE GUT MICROBIOTA?

As the understanding of Gut microbiota is increasing, so are tools to study it. Dr Ayesha Shah from the University of Queensland discussed how traditional tools such as jejunal aspiration and breath tests are becoming outdated due to their bothersome methods or lack of specificity, and paving the way for newer culture-independent molecular methods such as bacterial density load (qPCR) and microbial community profiling using sequencing.

Prof. Peter Gibson from Melbourne while discussing the role of Gut microbiota modulation by way of treatment, spelt out what could be an ideal strategy. At the start one needs to define microbial or functional dysbiosis in the individual by analyzing the microbiota of the stool or biopsied mucosa or by functional assays of metabolites. This could help determine the desired change in Gut microbiota, such as altering the specific communities or the total abundance. Subsequently a method from the armantum catalogue could be employed to achieve the desired change, such as use of antibiotics, probiotics, diet or fecal microbiota transfer.

An example that he shared to drive home this approach included a method to increase diversity of gut bacteria by certain diets. Each food item, especially vegetables and fruits, seems to encourage growth of a select variety due to the type of prebiotics each contains; increasing the variety of vegetables and fruits in each meal could be a simple method of increasing diversity of flora in our guts.

Probiotics may help boost the relative abundance of specific bacteria for certain conditions. The ones that have been tested and proven to be of value include *Bifidobacteria, Faecalobacterium prausnitzii,* and certain species of *Lactobacillus*. On the other hand, antibiotics such as rifaximine can be used to reduce abundance of certain undesirable bacteria which breakdown sulphates or protein and be related to disease.

IBS, the commonest GI condition thought to be linked to food, and hence in turn, suspected to be contributed by the Gut microbiota, has been the subject of several RCTs using various probiotics such as different strains of *Lactobacillus, Bifidobacteria, Saccharomyces* and combination preparations. Despite the heterogeneity of the condition of IBS large and unlikeliness of large benefits, some probiotics have shown efficacy in RCTs: the front runner is a specific strain of *Bifidobacterium infantis* strain which fed for 4 to 8 weeks showed > 20% overall benefit in symptoms of pain, bloating and satisfaction of stool evacuation. Benefit was also noted with the use of a specific strain of *B. animalis*, and *L. plantarum.*

ANTIOBIOTICS AND MICROBIOTA PERTURBATION

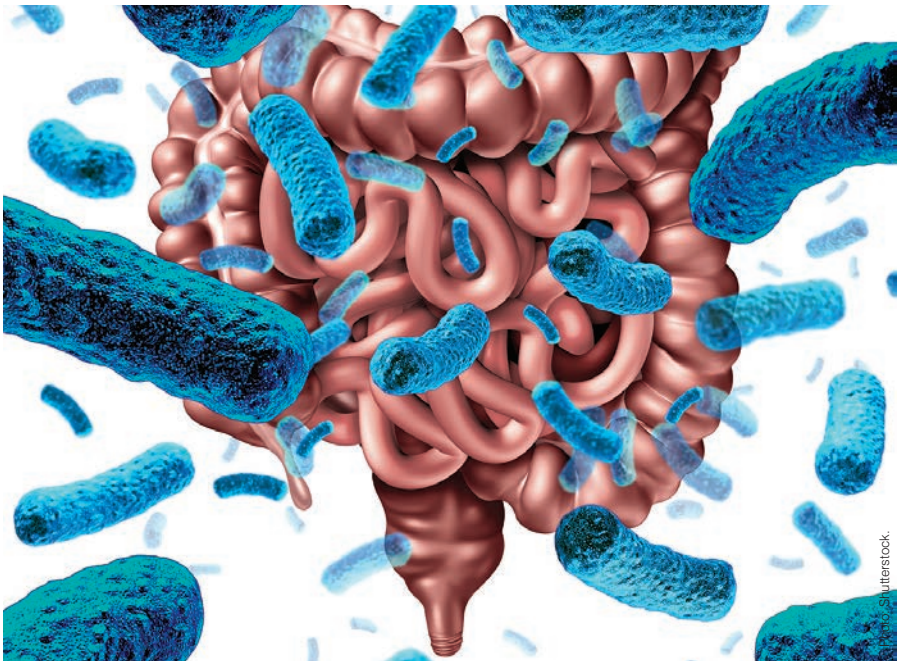
The Biocodex Pharma symposium on "Antibiotics and microbiota perturbation" chaired by Dr Henry Cohen (Uruguay) and Dr Kentaro Sugano (Japan), was a well-attended and interesting session. Dr K.L. Goh (Malaysia) outlined the magnitude, diversity and role of Gut microbiota and highlighted two features by way of comparison: the microbial genome has around 3,300,000 genes compared with 22,000 genes of the human one, and the inter-individual difference was 80% in the former compared to 0.01% between human cells!

Disruption of this hugely biodiverse Gut microbiota by use of antibiotics has been shown to has been associated with several health issues. Apart from the commonly known consequence of encouraging and stimulating *Clostridium difficile* infection, it often leads to a state of dysbiosis, which in turn predisposes to developing a "leaky gut" and to immunoactivation.

Another major concern is acquisition/transmission of antibiotic resistance by horizontal gene transfer. The perturbation of the innate gut flora and settlement of "abnormal" ones could predispose to a variety of disorders like obesity and diabetes as well.

Saccharomyces boulardii (Sb) has held sway as the prime remedy for treatment of AAD. Discovered in 1920 by the French microbiologist Henri Boulard, this species has continued to prove useful in protecting the gut from perturbations caused by antibiotic use, and restore the disturbed state to normalcy.

The APDW meeting witnessed a strong research thrust and elucidation of role of gut microbiota in human health and disease. Presentations by international experts showed how gut microbiota has moved from a newly recognized enigma and observations to the emergence of a subspecialty with thorough in-depth research and planned interventions, opening up new therapeutic possibilities.



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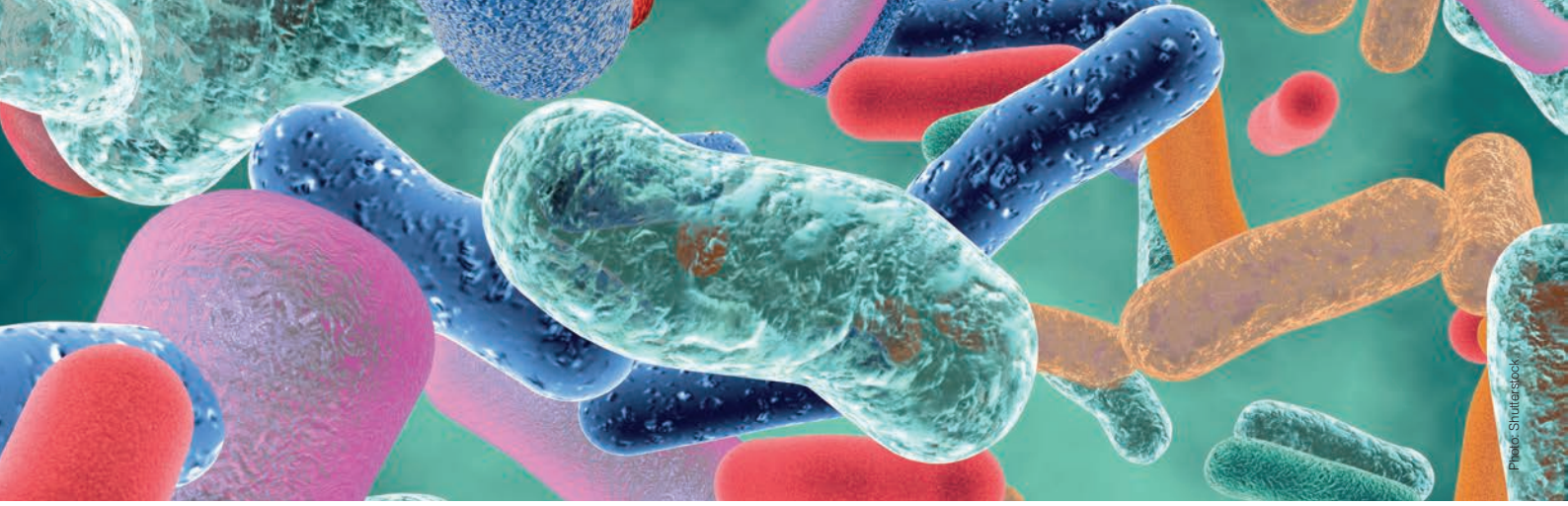

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LITERATURE SELECTION

By Prof. Markku Voutilainen
Turku University Faculty of Medicine; Turku University Hospital, Department of Gastroenterology, Turku, Finland

VAGINAL MICROBIOTA

IMPLICATION OF VAGINAL MICROBIOTA IN BACTERIAL VAGINOSIS AND CANDIDIASIS

Rosca AS, Castro J, Sousa LGV, *et al.* Gardnerella and vaginal health: the truth is out there. *FEMS Microbiol Rev* 2019

Vaginal microbiota is classified in five major subtypes (community state types). Four of them are composed of *Lactobacillus* species. The vaginal innate immune system, epithelial cells, Toll-like receptors, and natural antimicrobial peptides are other components of the defensive system against pathogens.
Lactobacillus genus has a central role in vaginal defense mechanisms *via* production of lactic acid and bacteriocins, and preventing adhesion of pathogenic bacteria. Bacterial vaginosis (BV) is characterized by an overgrowth of pathogens and a polymicrobial biofilm that adheres vaginal epithelium. *Gardnerella* spp. is the predominant specie at the biofilm and has the highest virulence. BV is treated with metronidazole, clindamycin, or tinidazole. Many *Gardnerella* spp. isolates and other pathogens are resistant to metronidazole. Adjuvant therapy e.g. with *Lactobacillus* probiotics may increase the therapeutic effect of metronidazole.

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Studies to understand polymicrobial interactions among vaginal pathogens could lead to ecologically based treatments.

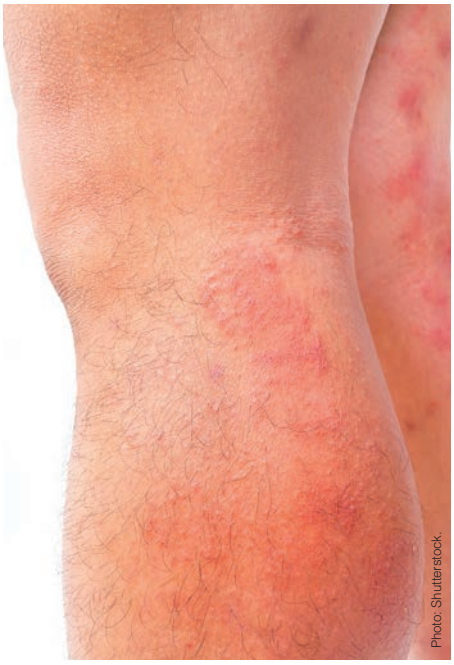
Tortelli BA, Lewis WG, Allsworth JE, *et al.* Associations between the vaginal microbiome and Candida colonization in women of reproductive age. *Am J Obstet Gynecol* 2019

Candida albicans (CA) is detected in vaginal microbiome in about 30% of women. Of 255 non-pregnant reproductive-aged women, 42 women (16%) were colonized by CA. The commonest vaginal microbiomes were classified as *Lactobacillus crispatus*-dominant (20%), *L. iners*-dominant (39%), and diverse (38%). Compared with white women and *L. crispatus*-dominant communities, CA was more common in black women and *L. iners*-dominant communities. *In vitro*, *L. crispatus* produced more lactic acid and inhibited more significantly pH-dependent growth of CA.
The main result was that *Lactobacillus* species have different interactions with CA, and *L. crispatus* may prevent CA colonization more effectively than *L. iners* through higher lactic acid production.

WHICH ROLE IN ATOPIC DERMATITIS AND ACNE?

Fyhrqvist N, Muihead G, Prat-Nielsen S, *et al.* Microbe-host interplay in atopic dermatitis and psoriasis. *Nat Commun 10* 2019

The authors compared atopic dermatitis (AD) and psoriasis (PSO) microbiota with that of healthy volunteers. The authors detected 26 and 24 microbes typical for AD and PSO, respectively. The most discriminative taxa for AD were genus *Staphylococcus*, and most discriminating microbes for PSO were *Corynebacterium simulans*, Neisseriaceae g. spp., *C. kroppenstedtii*, *Lactobacillus* spp. and *L. iners*.
AD is characterized by *S. aureus* abundance. In PSO, many different bacteria such as *Corynebacterium* may be involved. The depletion of *Lactobacillus* is typical for both diseases. In AD, loss of strictly anaerobic bacteria is typical with diminished production of lactic and short chain fatty acids leading to increased skin pH. Microbe-host interactions are important both in skin homeostasis and disease pathogenesis.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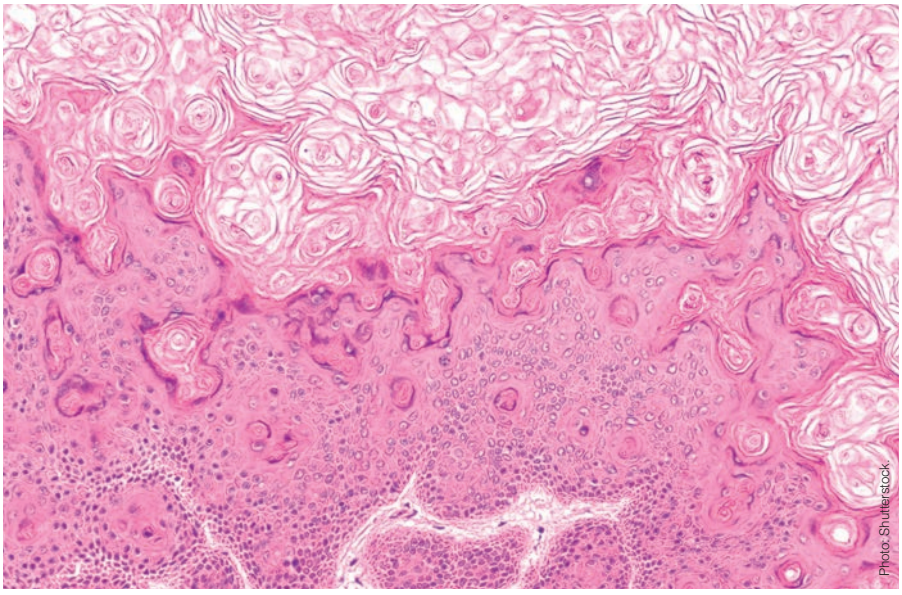
Claudel JP, Affret N, Leccia MT, *et al*. Staphylococcus epidermidis: a potential new player in the physiopathology of acne? *Dermatology* 2019

The interplay between skin and cutaneous microbiota is essential to differentiate between commensal and pathogenic bacteria. During puberty, over-colonization of skin pilosebaceous units (PU) by *Cutibacterium* acnes (CUA) may cause acne.
Some strains of *S. epidermidis* modulate host innate immune reactions, and some isolates have antimicrobial activity against CUA. Conversely, some CUA strains have antimicrobial activity against *S. epidermidis* which may also control CUA via succinic acid. The use of topical antibiotics may result in the development of antibiotic-resistant strains of CUA and *S. epidermidis*. Eliminating only CUA may lead to proliferation of *S. aureus* and *S. epidermidis* increasing the risk of infections.

Lactobacillus may be efficient in acne and other inflammatory skin diseases. The authors suggest that regular oral or topical supplementation of skin microbiota could be treatment option in acne.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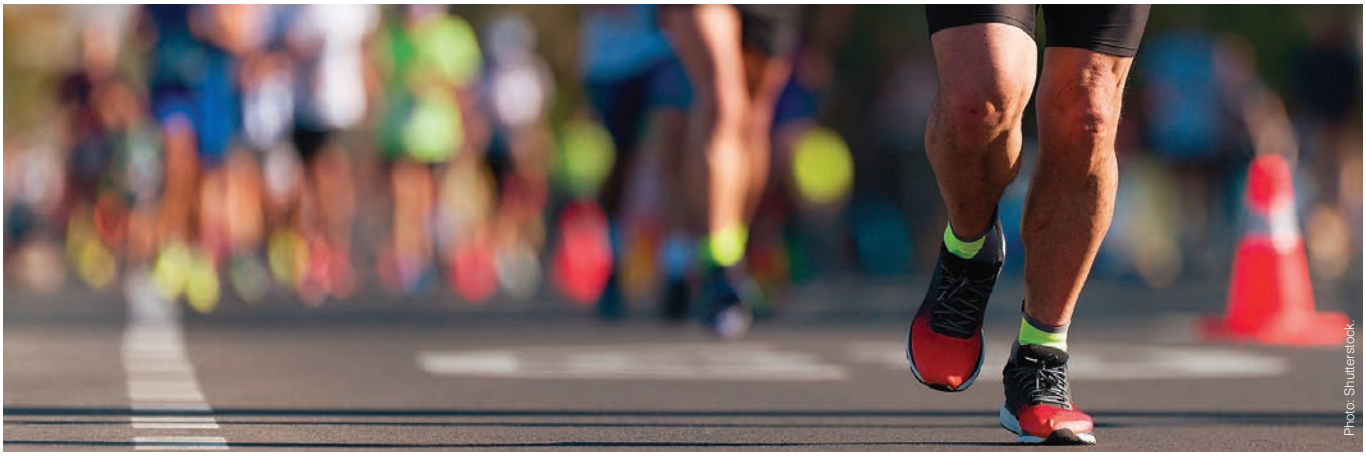
GUT MICROBIOTA

MODULATING GUT MICROBIOTA IN METABOLISM DISORDERS AND ALCOHOLIC HEPATITIS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Liu Y, Wang Y, Ni Y, *et al.* Gut microbiome fermentation determines the efficacy of exercise for diabetes prevention. *Cell Metabolism* 2019

The impact of exercise on gut microbiota was examined in prediabetic men. Exercise responders had a decrease in fasting insulin and insulin resistance (HOMA-IR), whereas in non-responders they remained unchanged or even deteriorated. Exercise caused increased abundance of Firmicutes, Bacteroides, and Proteobacteria. Alterations of the gut microbiota correlated with the reduction of HOMA-IR. DNA synthesis, amino acid (AA) metabolism, and short chain fatty acid (SCFA) synthesis enhanced in responders. In non-responders, AA fermentation was shifted to production of colonic gases and detrimental compounds, which associate with increased insulin resistance. Increased serum short chain fatty acids, but decreased branched chain amino (BCAA) and aromatic amino acids were detected only in responders. SCFAs have a beneficial role in energy and glucose metabolism, whereas increased BCAAs associate with insulin resistance.

In conclusion, exercise responders' gut microbiome had enhanced capacity to produce short chain fatty acids but increased breakdown of BCAAs, whereas the microbiome of non-responders produced metabolically detrimental compounds.

Duan Y, Llorente C, Lang S, *et al.* Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. *Nature* 2019

Alcoholic hepatitis (AH) patients have increased fecal *Enterococcus faecalis* (EF), 80% of AH patients are positive for EF. Germ-free mice on ethanol diet were colonized with cytolysin-positive (CL) EF feces of AH patients. Those infected with CL + feces developed a more severe ethanol-induced liver damage. Mice having overgrowth of intestinal enterococci and on ethanol diet were given bacteriophages lysing CL + EF. They developed less severe liver damage. Thus phage therapy may attenuate ethanol-related liver disease caused by CL + EF and improve prognosis in severe AH.


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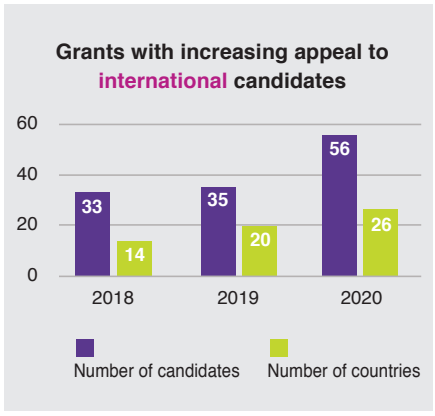
International grants 2019 on the topic "Gut Microbiota and Drug Metabolism": geographical distribution of application



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Graphic design:
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BIOCODEX Microbiota Institute

BMI 20-01 (03-20) - Code SAP: 98624 - Achevé d'imprimer par Corlet, Imprimeur, S.A. en mars 2020