MICROBIOTA AND CHILD HEALTH

BIOCODEX Microbiota Institute

What are the links between child health and microbiota?

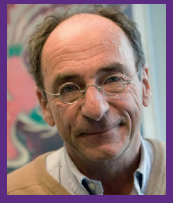
The answers are becoming clearer as scientists elucidate the multiplicity of factors at work in dysbiosis, whether the latter is the cause or the consequence of a childhood pathology. Without aiming to be exhaustive, this paper sheds light on current knowledge and prospects in four major areas: exposure to antibiotics, behavioral disorders, respiratory diseases and gastrointestinal disorders. Its purpose is to highlight the central role of the microbiota in the physical and mental development of children throughout their growth.

TA

1

IMPACT OF ANTIBIOTICS

BLE OF CONTENTS



1

IMPACT OF ANTIBIOTICS

Antibiotic therapy is a cornerstone of the modern therapeutic arsenal but it displays a number of side effects, in particular a detrimental action on the human microbiota and the creation of a reservoir of antibiotic resistance genes (resistome). While the resistome is still under-investigated, the impact of dysbioses caused by antibiotics is increasingly understood through scientific research, particularly in children. Let's explain further.

Antibiotics, child microbiota and long-term health effects

Scientific literature has confirmed that perinatal exposure to antibiotics disrupts the establishment of the intestinal microbiota and has potential consequences on the child's health throughout his/her growth.

GUT MICROBIOTA HOMEOSTASIS AND HEALTH

The intestinal microbiota is a complex and diverse ecosystem composed of microorganisms coexisting with their host in a collaborative relationship. This microbiota plays an important part in the proper functioning of the digestive system, but also in metabolic and immune homeostasis. Its particularities made it a key component in human health and a significant research field aiming at exploring the consequences of antibiotics-induced dysbioses. A literature review provided a better understanding of the effects of antibiotics on the intra- and postpartum development of the intestinal microbiota in children[1]. During that

THE GUT RESISTOME: AN AVENUE WORTH EXPLORING

The resistome designates all microbiota genes potentially coding for antibiotic resistance.

The growth of this gene reservoir has hardly been studied, but early acquired resistance could be related to the exposure to maternal and environmental microbes during and after birth.

The role of the resistome in the composition of the intestinal microbiota and its impact on individual or collective human health have yet to be defined.

period, exposure to antibiotics can take many different forms: mother treatment during pregnancy, cesarean delivery, postpartum treatment (especially in premature children), and even breastfeeding since antibiotics can change breast milk microbiota and/or be transmitted to the child.

HOST-MICROBIOTA INTERACTIONS: A SMALL WINDOW OF OPPORTUNITY

Several publications emphasize the importance of early intestinal colonization and the existence of a perinatal window of opportunity during which microbial exposure defines the "basic programming" of the future microbiota, and consequently the long-term health of the child. The beginning and duration of this window of opportunity has not yet been documented and represents an active field of research. It is apparently short, however, and encourages a limited use of antibiotics to restrict adverse effects. Research shows that not all antibiotic drugs have the same effect on the microbiota and that individual sensitivity plays an important role in the impact on health. That being said, most children in developed countries are exposed to them during their first year of life. This observation calls for research to dig deeper into early dysbiosis caused by antibiotic therapies in order to better manage the associated metabolic and autoimmune disorders.

ANTIBIOTICS ARE PERINATAL DETERMINANTS

Newborns are colonized at a very early stage in life by aerobic bacteria and facultative anaerobic bacteria, and later, by strictly anaerobic bacteria from the maternal microbiota and the environment.

Antibiotics, as well as mode of delivery, gestational age or feeding method, have an impact on this colonization.

An antibiotic treatment of over 3 days is a risk factor for colonization by antibiotic-resistant enteric bacteria, especially if a broad-spectrum drug is used.

[Source] : Marteau M, Doré J. *Gut microbiota, a full-fledged organ.* March 2017. John Libbey Eurotext

Focus on the risks of excess weight and obesity

Antibiotic-induced intestinal dysbiosis is thought to be the cause of some cases of excess weight in young children. Perinatal exposure or repeated treatments during the first 24 months of life are the main risk factors.

LITERATURE-BASED STUDY OF THE RISK OF EXCESS WEIGHT AT AN EARLY AGE

Excess weight or obesity at an early age is one of the metabolic risks associated with impairment of the intestinal microbiota that raises questions. Thirteen observational studies and meta-analyses, meeting previously defined inclusion criteria and identified from a corpus of 4,870 international publications, allowed this issue[2] to be clarified by monitoring bodyweight in 527,504 children exposed to anti-

[1] Nogack A, Salazar N, Arboleya S, et al. *Early microbiota, antibiotics and health*. Cell Mol Life Sci. 2018 Jan;75(1):83-91
[2] Rasmussen S, Shrestha S, Bjerregaard L, et al. *Antibiotic exposure in early life and childhood overweight and obesity: A systematic review and meta-analysis*. Diabetes Obes Metab. 2018 Jan 23

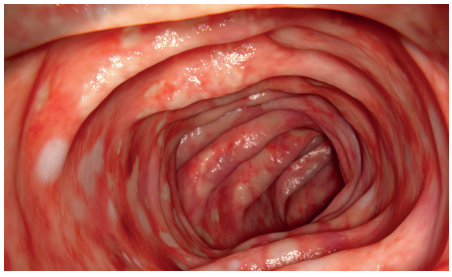
biotics in their first 24 months of life. This work was fine-tuned by studying in detail the six-month postnatal period, as well as the doses and type of antibiotics administered.

EARLY TREATMENT OR REPEATED CYCLES: INCREASED RISK OF EXCESS WEIGHT

Analysis of the data collected revealed a slight increase in the risk of excess weight or obesity during a postnatal treatment (in the six months after birth; OR [odds ratio]: 1.20) or during repeated administration of antibiotics (more than one treatment; OR: 1.24) before two years of age. Conversely, a single treatment or one occurring after the first six months of life does not appear to impact negatively weight progression. A question remains: is this a direct causal link? Does the effect of the antibiotic cause a weight problem in children? Or it is the opposite? Is childhood obesity likely to be associated with an increased risk of infection resulting in additional antibiotic treatment courses? Those who favor the first hypothesis consider that a detrimental intestinal colonization could play a specific role, in the light of its already documented involvement in the development of metabolic disorders. In any case, the research shows that in the perinatal period antibiotics should be administered with caution.

Zoom on the risks of IBD

There is an increased risk of Inflammatory Bowel Disease (IBD) in young children who have been exposed intrapartum to antibiotics. The physiological disruptions caused by dysbiosis, in particular within the intestinal mucosa and the immune system, facilitate the development of this type of rare inflammatory diseases.



Endoscopic image of the colon affected by ulcerative colitis (UC)

EXPOSURE TO ANTIBIOTICS AND EARLY IBD

The excess weight issue underlines the complexity in elucidating the impact of dysbiosis in some medical fields. Causal relations have been more detailed in the gastroenterology field, where a link has been established between the composition of the intestinal microbiota and IBD. This connection leads researchers to increasingly take dysbioses into account when attempting to understand disorders with poorly identified etiology, especially ulcerative colitis (UC) and Crohn's Disease in young children (under six years of age), whose global incidence is increasing regularly. As the cause of this progression cannot be found in known genetic and environmental factors, intestinal weakness associated with disruptions in the microbiota has been suggested.

TWOFOLD HIGHER RISK OF IBD AFTER INTRAPARTUM ANTIBIOTIC TREATMENT

Swedish researchers[3] explored this hypothesis by studying a cohort of 827,239 children born between 2006 and 2013. This was an extensive analysis based on cross-checking the Swedish National Registers of births, patients and drug prescriptions. In total, 17% of subjects were exposed intrapartum to antibiotics (including 5% repeatedly) and 65% after birth, most of them more than once (7 out of 10). Fifty-one children were affected by Crohn's disease or UC. Compared to the control population, children exposed to antibiotics during pregnancy had an elevated risk (aHR[4] = 1.93) of developing early IBD.

[3] Örtqvist A, Lundholm C, Halfvarson J et al. *Fetal and early life antibiotics exposure and very early onset inflammatory bowel disease: a population-based study*. Gut. 2018 Jan 10.

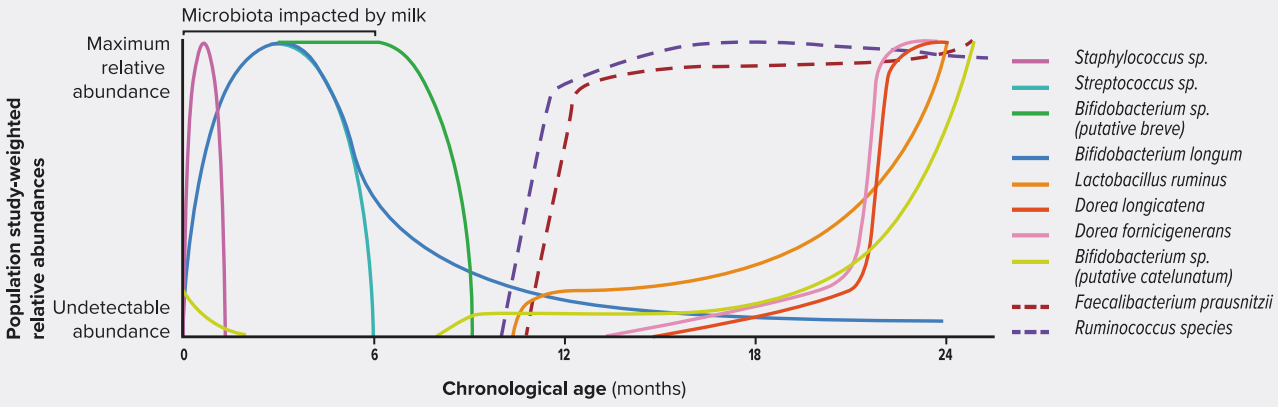
FETAL IMPACT

Intrauterine exposure to antibiotics is believed to trigger a disruption of early bacterial colonization in children, characterized by a low concentration of commensal bacteria, in particular *Faecalibacterium prausnitzii* and Ruminococcaceae, and an increase in pathogenic bacteria. This dysbiosis could cause significant physiological changes due to the interaction between microbiota and host through production of SCFA[5] (particularly butyrate), induction of the immune system of the intestinal mucosa, stimulation of the local nervous system and maintenance of the intestinal barrier function. These are all malfunctions which are liable to trigger inflammatory disorders.

IMPROVING ANTIBIOTIC PRESCRIPTION TO PRESERVE THE MICROBIOTA

The data mentioned above and those derived from a growing number of studies and publications show that a diversified microbiota displaying a high commensal/pathogenic bacteria ratio contributes to an adequate development in children by limiting the risk of occurrence of some diseases, especially metabolic or inflammatory. This observation should not lead to a dismissal of antibiotic therapy, whose efficacy and benefits prove invaluable in many cases, as emphasized by all healthcare providers. However, optimizing prescriptions, spectrum of the drugs employed, duration of treatment and modes of administration are good ways of limiting the impact of antibiotics and the transfer of resistance to the intestinal microbiota in order to preserve child health in the short and long term.

CHANGES IN THE INTESTINAL MICROBIOTA DURING THE FIRST TWO YEARS OF LIFE[6]



[4] Ajusted Hazard Ratio
[5] Short-chain fatty acids. They are products of carbohydrate fermentation (organic anions and saturated fatty acids) carried out by anaerobic bacteria in the colon.
[6] *Op.* cit. p.5

2

BEHAVIORAL DISORDERS

The relationship between behavioral disorders and microbiota is the subject of a growing number of studies. Some microorganisms are thought to produce substances that can cross the blood-brain barrier, and are therefore likely to be implicated in central nervous system impairments. A better understanding of these interactions would help clarify the etiology of some psychiatric disorders that are still poorly understood.

The role of oral and intestinal microbiota and mycobiome in autism

Autistic children have distinct bacterial and fungal populations in the intestines, as well as oral dysbiosis. These two complementary research areas could help structure diagnostic approach and therapeutic care.

DISRUPTED GASTROINTESTINAL SYSTEM

Autism is a neurodevelopmental disorder that generally appears in early childhood, and is characterized by behavioral disorders: difficulties in establishing social relationships, communication problems and OCD (Obsessive Compulsive Disorders). The mechanisms of the disease remain unclear, but the recurrent presence of gastrointestinal problems in autistic children could suggest a possible link with the intestinal microbiota. Studying this hypothesis could help clarify etiology, which is currently based mainly on genetic and environmental factors.

BACTERIAL ALTERATIONS...

Some studies, such as those of an Italian team[7], have tried to validate the hypothesis of a dysbiosis. Fecal samples from 40 children with severe autistic disorders and 40 "neurotypical" controls led to the characterization of the bacterial populations by amplification of 16S rRNA genes. The analyses confirm the pertinence of the original hypothesis: a significant increase in the Firmicutes/Bacteroidetes ratio, traditionally associated with an increased risk

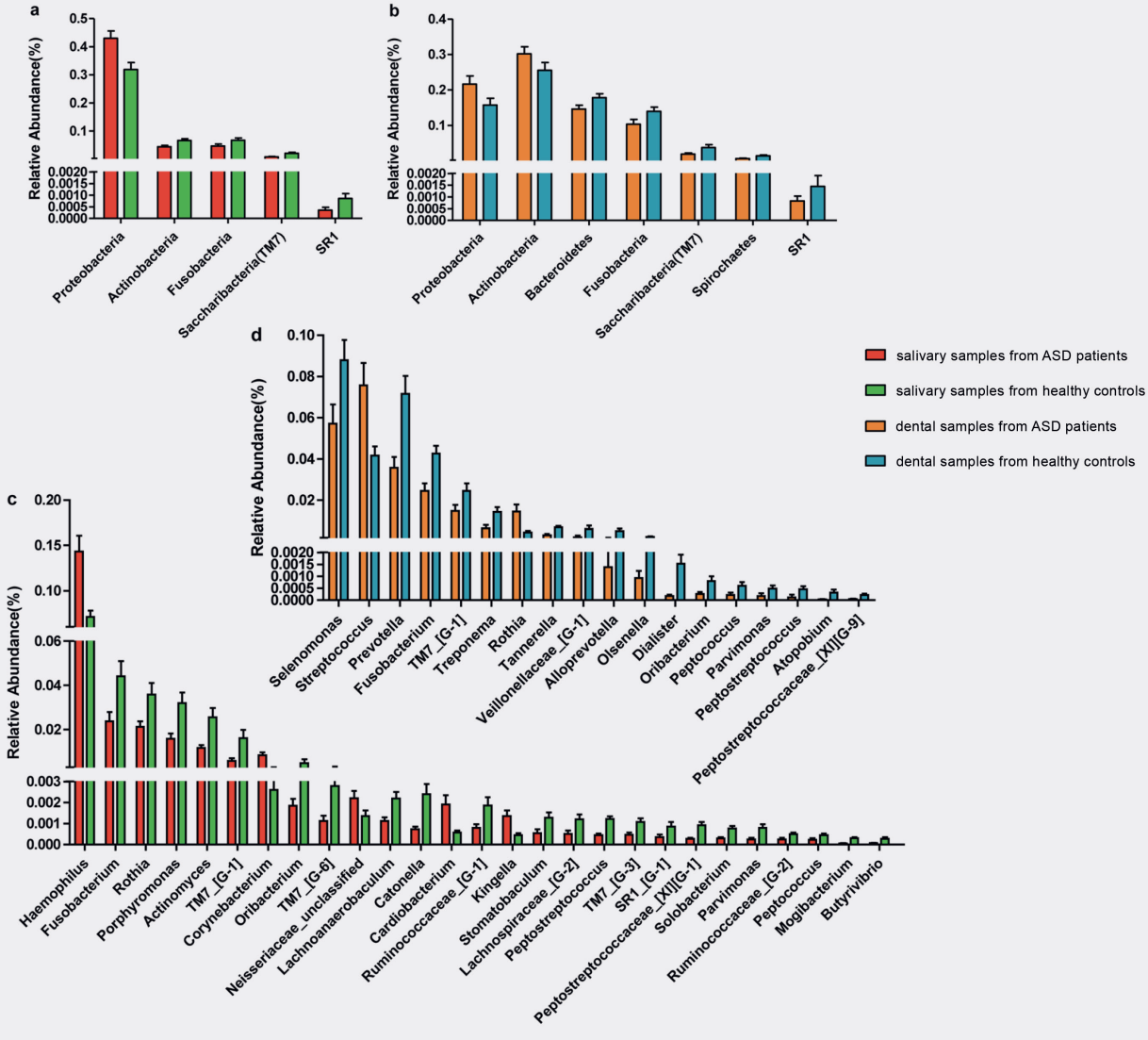
[7] Strati F, Cavalieri D, Albanese D, et al. *New evidences on the altered gut microbiota in autism spectrum disorders*. Microbiome. 2017 Feb 22;5(1):24. doi: 10.1186/s40168-017-0242-1

of developing inflammatory disorders, was observed in autistic children. At the genus level, a depletion of *Alistipes, Bilophila, Dialister, Parabacteroides* and *Veillonella*, and an increase in *Collinsella, Corynebacterium, Dorea* and *Lactobacillus* were observed. In autistic subjects suffering from constipation (a symptom of gastrointestinal problems that is common in this pathology), an abundance of *Escherichia, Shigella* and *Clostridium* was also observed.

... AND FUNGAL ALTERATIONS

Analysis of the fungal community also demonstrated disparities between the autistic and control subjects, with the former having a twofold higher proportion of *Candida*. This observation must be tempered, as this type of fungus is naturally found in humans, to the point that the discrepancy is not very significant. Fungal dysbiosis seems nevertheless confirmed. It could influence bacterial development and vice versa, as the two communities develop within the same microbiota.

COMPOSITION (*PHYLA* AND GENERA) OF THE ORAL MICROBIOTA (SALIVA AND DENTAL PLAQUE): AUTISTIC VS. HEALTHY CHILDREN[8]



[8] Qiao Y, Wu M, Feng Y, et al. *Alterations of oral microbiota distinguish children with autism spectrum disorders from healthy controls*. Sci Rep. 2018 Jan 25;8(1):1597. doi: 10.1038/s41598-018-19982-y

IS THE ORAL MICROBIOTA ALSO INVOLVED?

The intestinal microbiota is not the only one being implicated in the development of autism. Researchers are also examining the microbial populations of ENT areas, which contain a large diversity of taxa (more than 700 in the oral cavity alone) and act as a reservoir of infections for other parts of the body, including the central nervous system. Since previous works have shown oral dysbioses in patients with Parkinson's, Alzheimer's, MS, or migraine, researchers decided to characterize the oral microbiota of autistic children to characterize any microbial specificities[9].

THE ORAL CAVITY, A SPECIAL ENVIRONMENT

The peculiarity of the oral cavity is that both soft tissue (mucous membranes) and hard tissue (teeth) coexist in it. Sampling of both saliva and dental plaque provided a more detailed identification of the bacterial populations of 111 samples taken from 32 autistic children and 27 controls. Like with the intestinal microbiota, large differences were observed between the two groups of participants. The oral microbial community of autistic subjects is characterized by an overall bacterial depletion and a rise in pathogens such as *Haemophilus* in the saliva and *Streptococcus* in the dental plaque, as well as a decrease, in both areas, in several commensal bacteria: *Prevotella, Selenomonas, Actinomyces, Porphyromonas* and *Fusobacterium*. The dental plaque also displays a significant decrease of all Prevotellaceae, a family capable of interacting with the immune system, and a high concentration of *Rothia*, a bacterium frequently associated with dental diseases in the literature.

THE MICROBIOTA: A NEW DIAGNOSTIC AND THERAPEUTIC TOOL IN PSYCHIATRY?

Thanks to the oral bacterial populations identified in autistic disorder, a diagnostic model has been developed based on the main oral biomarkers. This tool, which displayed a 96.3% efficacy rate with saliva, could prove particularly useful and relevant in modern psychiatry. This biological approach could complement the usual criteria derived mostly from DSM-5 (Diagnostic and Statistical Manual of Mental Disorders), which is based on a consensus on clinical symptoms that are difficult to measure. More extensive investigations into the microbiota of autistic children could make possible new diagnostic approaches, and the development of innovative therapeutic strategies.

Gut-brain axis and ADHD in adolescents

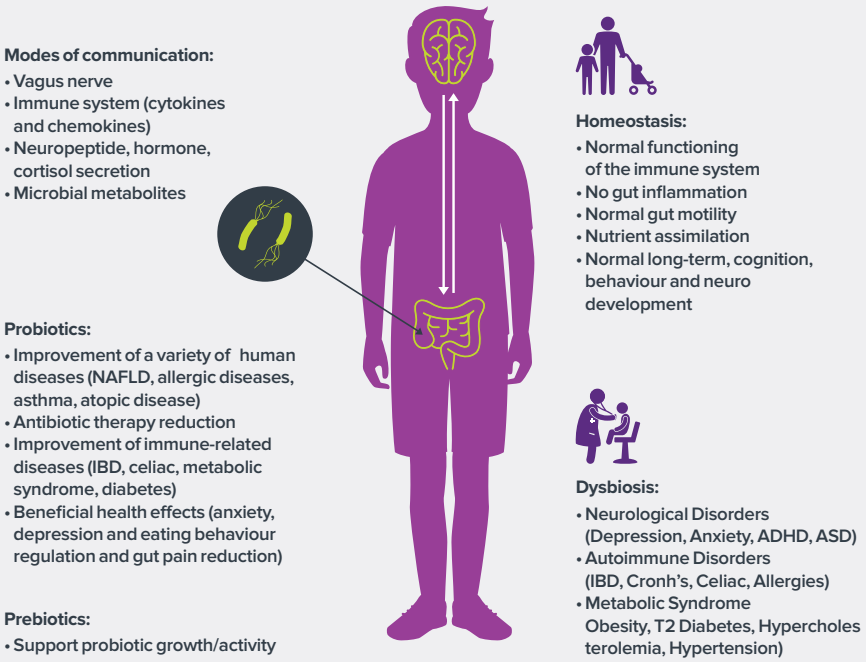
Some harmful systemic changes linked to intestinal dysbioses could present themselves in the form of attention deficit hyperactivity disorder. Dopamine precursors synthesized in the intestine could reach the central nervous system (CNS) and heighten the risk of developing this type of pathology.

IMPAIRED NEUROTRANSMISSION

Attention deficit hyperactivity disorder (ADHD) is another class of neuropathies possibly involving the human microbiota. It is associated with abnormal dopamine neurotransmission and reward processing deficits *via* the underlying neural circuits, in particular in the ventral striatum. The microbiota could contribute to these changes through the gut-brain axis[10]. The hypothesis was explored through characterization of the intestinal microbiota of adolescents and young adults

[9] Article mentioned p.9
[10] The gut-brain axis includes the means used by the intestine to communicate with the brain (especially the NCS), and conversely

SCHEMATIC REPRESENTATION OF THE BIDIRECTIONAL COMMUNICATION BETWEEN GUT AND BRAIN [11]



GUT-BRAIN AXIS

The intestinal microbiota is involved in the communication between gut and brain through many compounds and various pathways.

Its role in the maturation of the neuroendocrine axis that controls stress and regulates emotions has been clearly established.

Several studies suggest that dysbioses could contribute to the pathophysiology of CNS diseases such as anxiety and depression disorders, autism spectrum disorders and some neurodegenerative diseases (Parkinson's, Alzheimer's, MS).

[Source] : *Op.* cit. p. 5

with attention deficit hyperactivity disorder, in addition to an analysis of cerebral responses to emotional stimulation[12].

HIGHER LEVELS OF DOPAMINE PRECURSOR

Analysis of the intestinal microbiota by 16S rRNA sequencing has provided an identification of the bacterial populations in adolescents with ADHD. Functional imaging was used to compare the intestinal microbial populations and cerebral responses to stimulation of reward processes in some participants, independently of the initial diagnosis. Characterization of the microbiota revealed changes, in particular an increase in *Bifidobacterium* in ADHD subjects. These bacteria, which are predominant in their intestines, possess a gene encoding cyclohexadienyl dehydratase, an enzyme involved in the synthesis of phenylalanine, a dopamine precursor capable of crossing the blood-brain barrier. Consequence: an increase in brain dopamine levels leading to an increased risk of neurological disruption.

MOTIVATIONAL DEFICIT

This increased production of monoamines driven by the intestinal microbiota is correlated with a decrease in neural mechanisms of reward anticipation in the ventral striatum, another ADHD marker. This link was confirmed by imaging: subjects presenting with intestinal dysbiosis displayed "intolerance to delayed reward" (inability to tolerate deferral of a reward), considered by many experts to be the fundamental mechanism of the pathology. The intestinal microbiota thus seems to be a new factor in the etiology of attention deficit hyperactivity disorder, as its composition has a direct impact on the synthesis of cyclohexadienyl dehydratase. Whilst the possibility of dysbiosis linked to ADHD is not excluded, further investigations are required to specify the level of interactions, as well as the functional effects of microbiota on psychiatric disorders in general (OCD, phobias, anxiety disorders...).

[11] From Cerdó T, Ruíz A, Suárez A, Campoy C. *Probiotic, Prebiotic, and Brain Development*. Nutrients. 2017 Nov 14;9(11). pii: E1247. doi: 10.3390/nu9111247

[12] Aarts E, Ederveen T, Naaijen J, et al. *Gut microbiome in ADHD and its relation to neural reward anticipation*. PLoS One. 2017 Sep 1;12(9):e0183509. doi: 10.1371/journal.pone.0183509. eCollection 2017

3

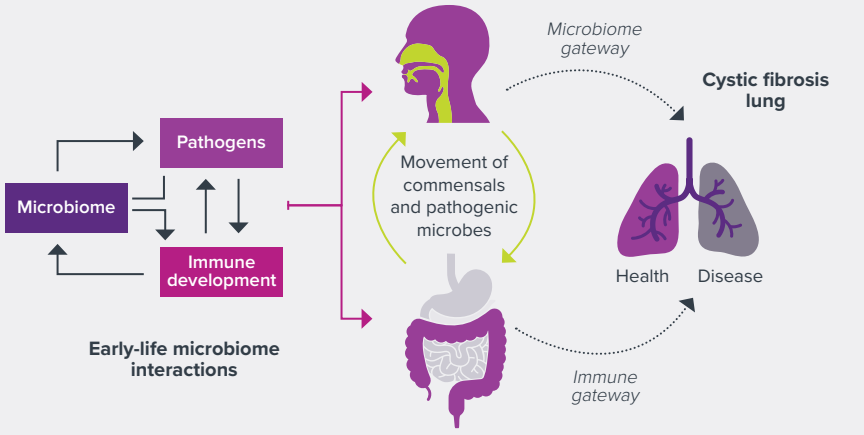
RESPIRATORY DISEASES

In children, the development of cystic fibrosis has already been correlated with changes in respiratory tract microbial populations. This relation could provide answers to improve the understanding of the pathogenic mechanisms of other respiratory pathologies. Better characterization of the different microbiota could also help develop more targeted, less invasive assessment, diagnosis and treatment methods.

Respiratory microbiota and pulmonary infections associated with cystic fibrosis

Detailed characterization of the respiratory tract microbiota demonstrates the specificity of the bacterial populations of children with cystic fibrosis. This could be a path towards identification of risk factors for pulmonary infections and better patient care.

SCHEMATIC OF MICROBIOME AND IMMUNE INTERACTIONS WITH PATHOGENS IN CHILDREN WITH CYSTIC FIBROSIS[13]



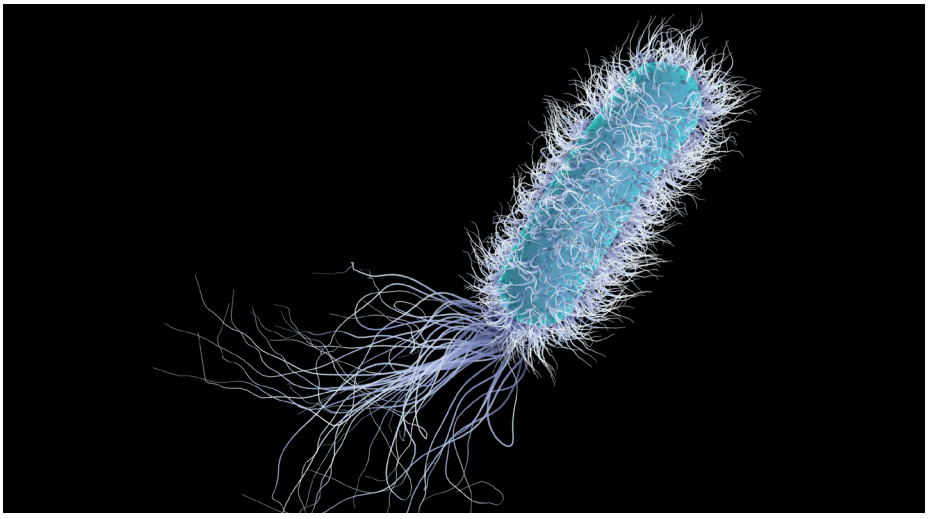
A MORE ADVANCED TECHNOLOGY

Recent technical progress in molecular screening, sequencing and metagenomic analyses are contributing to the detailed characterization of human respiratory tract microbiota. This growing precision is accompanied by a better understanding of the complex relationships between the different populations of microorganisms and the respiratory system of their host, whether the latter is healthy or not. In this respect, cystic fibrosis is a good illustration. Pulmonary infections, which play a determining role in the pathogenesis of the disease, display changes over time. Relatively benign infections with *Staphylococcus aureus* and *Haemophilus influenzae* in early childhood, can progress to persistent infections with Gram-negative bacteria such as *Pseudomonas aeruginosa*, which become increasingly severe with age. This progression is accompanied by harmful changes in the respiratory tract microbiota and raises an underlying question: are these dysbioses the consequences of infectious episodes, or do they contribute to their development?

[13] From Segal LN, Blaser MJ. *Harnessing the Early-Life Microbiota to Protect Children with Cystic Fibrosis*. J Pediatr. 2015 Jul; 167(1): 16–18.e1. Published online 2015 Apr 29. doi: 10.1016/j.jpeds.2015.03.055. With the kind permission of the editor

DIVERSITY ISSUE

In the absence of answer regarding causal relations, a literature review has identified the differences between a healthy respiratory microbiota and a microbiota disrupted by cystic fibrosis[14]. The first is diversified and characterized by a predominance of one or two bacterial genera among the following: *Staphylococcus, Dolosigranulum, Corynebacterium, Haemophilus, Streptococcus* and *Moraxella*. The last three expose patients to a higher risk of developing acute respiratory infections. In contrast, children with cystic fibrosis have a less dense and less diversified bacterial environment, marked by the predominance of *Corynebacterium* and *Streptococcus*. This dysbiosis seems to become more pronounced with time and disease severity, and the terminal phase can be associated with the presence of only one or two pathogens.



Pseudomonas aeruginosa

HOW TO IMPROVE PATIENT CARE

Once a better understanding of the characteristics and progression of the respiratory tract microbiota in individuals with cystic fibrosis was obtained, the question of the interactions between dysbioses and structural and functional development of pulmonary infections arose. A detailed elucidation of the mechanisms at work could help identify the potential pathogenic or protective factors in order to strengthen prophylactic and therapeutic approaches.

PERSON-YEARS, NUMBER OF DEATHS AND MORTALITY RATE PER AGE GROUP. FRENCH REGISTRY OF CYSTIC FIBROSIS FOR THE PERIODS 1992- 1996 TO 2007-2011.[15]

Age (years)	1992-1996			1997-2001			2002-2006			2007-2011		
	PY	Number of deaths	Mortality rate (‰)	PY	Number of deaths	Mortality rate (‰)	PY	Number of deaths	Mortality rate (‰)	PY	Number of deaths	Mortality rate (‰)
0-4	2,309.0	6	2.6	2,712.7	6	2.2	4,138.7	6	1.4	4,870.4	6	1.2
5-9	2,727.7	21	7.7	3,478.8	11	3.2	3,735.5	13	3.5	4,401.1	3	0.7
10-14	2,164.9	38	17.6	3,537.6	52	14.7	4,189.6	32	7.6	4,107.1	16	3.9
15-19	1,630.3	32	19.6	2,787.5	58	20.8	3,968.8	65	16.4	4,295.9	50	11.6
20-24	978.0	39	39.9	1,988.0	60	30.2	3,007.9	74	24.6	3,817.1	62	16.2
25-29	480.9	21	43.7	1,205.2	57	47.3	2,060.3	45	21.8	2,841.1	64	22.5
30+	323.6	11	34.0	1,187.8	51	42.9	2,762.1	70	25.3	4,758.3	107	22.5
Total		**168**			**295**			**305**			**308**	

PY : person-years

[14] Frayman K, Armstrong D, Grimwood K, et al. *The airway microbiota in early cystic fibrosis lung disease*. Pediatr Pulmonol. 2017 Nov;52(11):1384-1404. doi: 10.1002/ppul.23782. Epub 2017 Aug 16

[15] Bouet S et al. *Cystic fibrosis mortality: analysis of the French registry data*, 1992-2012. BEH. 2015;38-39:710-7

Nasal microbiota: a reliable marker of bronchiolitis severity

The severity of bronchiolitis in newborns may be assessed by characterizing their nasal microbiota. This could be an alternative, simpler and less invasive method than a nasopharyngeal aspirate, which is conventionally used.

ASPIRATE *VS.* SWAB

As is the case for bronchiolitis, characterization of the airway microbiota takes on a particular importance in some pulmonary pathologies. The severity of the disease is directly correlated with the local microbial populations. Consequently, accurate identification of the microorganisms present proves essential and requires high-quality sampling. Standard protocol (nasopharyngeal aspirate) offers good diagnostic and prognostic efficacy. But the procedure is invasive and proves tricky to perform in children, in particular in infants. Simpler to perform and less traumatic, nasal swab is a promising alternative. Its efficacy was studied[16] by comparing the nasopharyngeal and nasal microbiota of infants with bronchiolitis (composition / capacity to predict the severity of the inflammatory episode). Both sampling techniques were assessed in 815 hospitalized children; bacterial populations were characterized by amplification of 16S rRNA genes.

DIFFERENT POPULATIONS, BUT SIMILAR PREDICTABILITY

The analyses show a disparity between the microbial populations of the two areas. The nasal microbiota is composed mainly of the genera *Staphylococcus* (40.8%), *Corynebacterium* (10.4%), *Moraxella* (9.3%), *Haemophilus* (7.4%), *Dolosigranulum* (5.2%), *Streptococcus* (5%) and *Enterobacter* (4.7%). However, the nasopharyngeal microbiota is dominated by the genera *Moraxella* (30.7%), *Streptococcus* (30.5%) and *Haemophilus* (19.7%). These differences however should be put in perspective: predominance of *Moraxella* or *Haemophilus* in the nasal area is also found in the nasopharynx, making these two sites a good tool for predicting the severity of bronchiolitis. Children with a *Haemophilus*-dominant profile display an increased risk of requiring intensive care or prolonged hospitalization (≥ 5 days). In comparison, subjects with a *Moraxella*-dominant profile seem to be the least at risk. This predictive capacity is not however applicable to other bacterial genera. The nasal swab presents many practical advantages, and can therefore be considered a valid alternative to nasopharyngeal aspirate in assessing the severity of bronchiolitis in infants.

[16] Luna P, Hasegawa K, Ajami N, et al. *The association between anterior nares and nasopharyngeal microbiota in infants hospitalized for bronchiolitis*. Microbiome. 2018 Jan 3;6(1):2. doi: 10.1186/s40168-017-0385-0

4

GASTROINTESTINAL DISORDERS

A close relationship has been established between childhood gastrointestinal disorders and intestinal microbiota. The composition of the intestinal microbiota impacts the risk of developing certain disorders, and conversely, a dysbiosis caused by a gastrointestinal disorder can play a role in promoting, worsening or increasing the recurrence of the underlying disorder. Research is focused on gaining a better knowledge of etiopathogenesis in order to develop new therapeutic and prophylactic solutions.

Infectious diarrhea and intestinal microbiota

The fight against infectious diarrhea is a priority of healthcare systems in developing countries, since this pathology is the second cause of child mortality in these regions. Describing the intestinal microbiota and its dysbioses are part of this fight and could help offer personalized treatments.

RISKS ASSOCIATED TO DIARRHEA

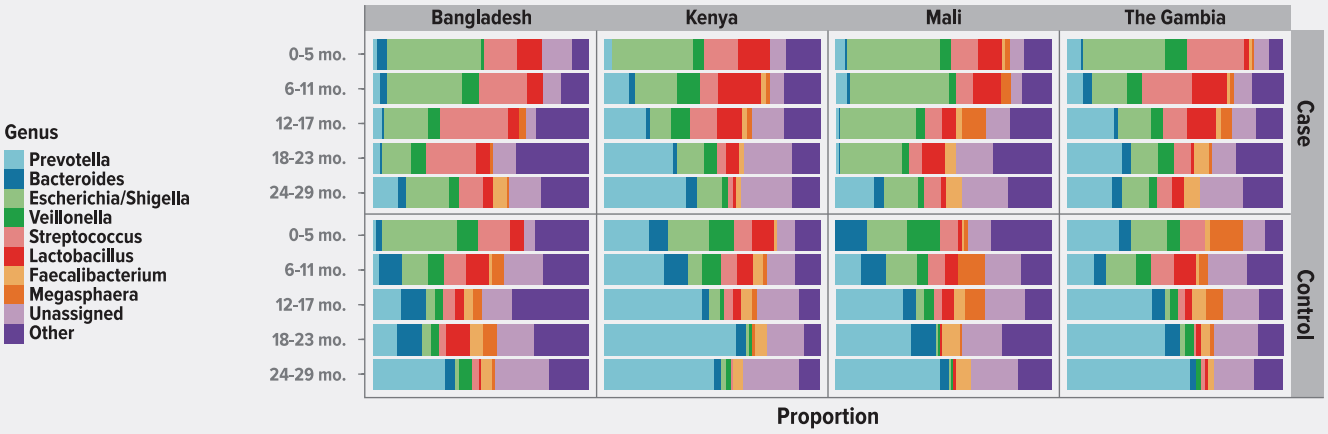
Recurrent or prolonged diarrhea episodes increase the risk of malnutrition and stunted growth. They are also associated to numerous comorbidities: cognitive disorders, cardiovascular diseases, glucose intolerance, etc. Despite a regular decrease, near 525,000 children under 5 years of age die every year in the world. Understanding the etiology of diarrhea is a health priority, but the wide variety of types and potential causes requires further investigation, especially in terms of correlation with the intestinal microbiota. A recent study focused on Vietnamese population was added to the existing body of scientific literature on infectious diarrhea. It characterized the bacterial communities of 145 children with diarrhea and 54 controls[17].

ETIOLOGIC AGENTS WERE IDENTIFIED

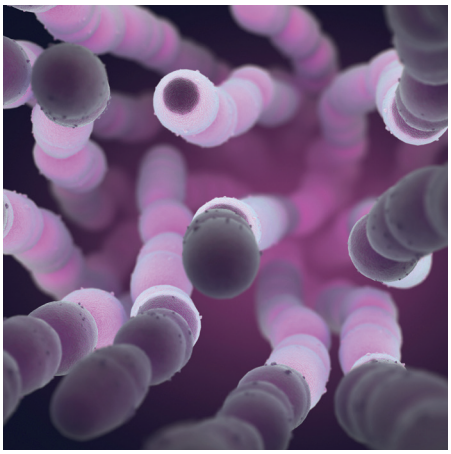
Bacterial analysis of fecal samples revealed four major types of bacterial predominance in children with diarrhea: *Bifidobacterium, Bacteroides, Streptococcus* and *Escherichia*. The first two categories, usually asymptomatic, are

[17] Florez P, Jie S, Pham Thanh D, et al. *Assessing gut microbiota perturbations during the early phase of infectious diarrhea in Vietnamese children*. Gut Microbes. 2017 Aug 2:1-17. doi: 10.1080/19490976.2017.1361093

COMPARISON OF THE COMPOSITION OF THE FECAL MICROBIOTA FROM NEWBORNS AND YOUNG CHILDREN WITH DIARRHEA AND HEALTHY CONTROLS.
STUDY CARRIED OUT IN FOUR DEVELOPING COUNTRIES [18]



close to the composition of the microbiota of control children. Predominance of *Streptococcus* and *Escherichia* in the microbiota of children who had the most severe symptoms, confirmed previous studies that associated these microorganisms with a higher risk of gastrointestinal disorders. Bacterial depletion was also observed in all categories. Nineteen taxa are concerned (mainly belonging to the Clostridiales and Erysipelotrichales orders) and especially *Blautia hansenji* which is known for its ability to produce SCFA[19], essential to homeostasis.



Streptococcus

INDIVIDUAL VULNERABILITIES

Age, nutritional status, intake of breast milk and etiology seem to contribute to the composition of bacterial communities during the early phase of diarrhea episodes. *Streptococcus* are more largely associated to young children (under 2) and bacterial infections, while *Escherichia* are found in older children and/or children with poor nutritional status. This extensive characterization is part of the analysis of the complex influences of infectious diarrheas on the intestinal microbiota and guides research towards new therapeutic approaches.

KEY FIGURES FOR DIARRHEA

Definition: passage of three or more loose or watery stools per day
- acute watery diarrhea lasting several hours or days
- watery and bloody diarrhea, or dysentery
- persistent diarrhea (14 or more consecutive days)

There are about 1.7 billion cases of diarrhea annually in children in the world.

2nd cause of death and major cause of malnutrition in children under

525,000 deaths per year of children aged 5 or less in the world

[Source] : WHO

[18] Pop M, Walker AW, Paulson J, et al. *Diarrhea in young children from low-income countries leads to large-scale alterations in intestinal microbiota composition*. Genome Biol. 2014; 15(6): R76. Published online 2014 Jun 27. doi: 10.1186/gb-2014-15-6-r76

[19] Short-chain fatty acids. They are products of carbohydrate fermentation (organic anions and saturated fatty acids) carried out by anaerobic bacteria in the colon

Is intestinal dysbiosis the cause of persistent diarrhea?

Persistent diarrhea is a major cause of child mortality but its origin remains controversial. The composition of the intestinal microbiota is a hypothesis which could lead to numerous answers regarding this disease with an unclear pathogenesis.

IS PERSISTENT DIARRHEA AN INFECTIOUS DISEASE...

Persistent diarrhea is a particular form of the disease, defined as an episode lasting more than 14 days (beyond 30 days, it is called "chronic diarrhea"). The mortality rate attributed to persistent diarrhea has been determined (54% of all deaths caused by diarrhea) but its pathogenesis is still poorly understood: is it the progression of an acute episode or a disease in its own right? Through a literature review[20] it was revealed that the majority of researchers consider persistent diarrhea to be an infectious disease associated to an intestinal colonization by pathogenic bacteria, be it followed by an acute or another episode. This colonization could be promoted by malnutrition and other factors (especially exposure to antibiotics) susceptible to cause intestinal dysbiosis.

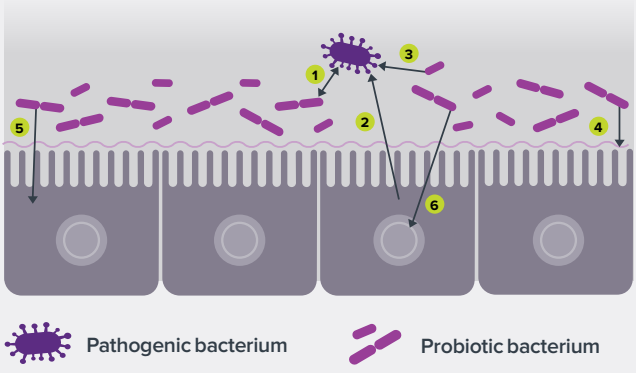
... OR IS IT DUE TO MICROBIAL DYSBIOSIS?

Among other etiologic hypotheses, the theory of a bacterial outbreak is gaining momentum. It was born from the observation that an abundant presence of commensal bacteria, especially *Escherichia coli*, disrupts lactose absorption. Connecting intestinal dysbiosis, levels of *E. coli* and persistent diarrhea could provide several clues to elucidate it pathogenesis. On this topic, certain probiotics have shown their efficacy to reduce the duration of acute diarrhea and persistent diarrhea[21] in children[22].

IDENTIFIED RISK FACTORS

An analysis of scientific literature provided a draft model of the disease pathogenesis. The first risk factor category includes very young age (under 1, risk x 3), malnutrition (risk x 2) and low birth weight (risk x 1.8). The second category includes an infection in the previous month (risk x 2) and a history of persistent diarrhea (risk x 3 to 6). The third category is related to eating habits (diet, breast milk substitutes...) which can multiply the risk by 4. Other factors have also been identified, including administration of antibiotics. Potential etiologies and confirmed risk factors underline the importance of a close collaboration between pediatricians, gastroenterologists, nutritionists and biologists. Interdisciplinarity could help obtain a deeper understanding of the relationships between infection, dietary habits and pathogenic and commensal microorganisms.

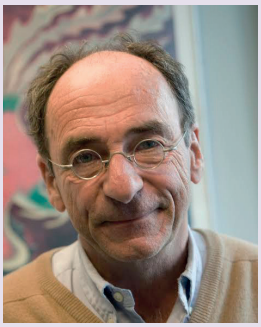
SCHEMATIC REPRESENTATION OF INTERACTION MECHANISMS BETWEEN PROBIOTICS AND THE INTESTINAL EPITHELIUM [23]



1 Competitive exclusion of adherence of pathogenic bacteria
2 Stimulation of the protective humoral immune response
3 Antimicrobial activity, for example by modifying the pH or by producing bacteriocins
4 Enhanced mucus barrier function through the prevention of mucus degradation
5 Synthesis of polyamines which have a trophic action by stimulating proliferation and differentiation of epithelial cells
6 Inhibition of the host's inflammatory response through the modulation of signaling pathways (for instance NF-kappa B or MAP kinase pathways)

[20] Sarker S, Ahmed T, Brüssow H. *Persistent diarrhea: a persistent infection with enteropathogens or a gut commensal dysbiosis*? Environ Microbiol. 2017 Oct;19(10):3789-3801. doi: 10.1111/1462-2920.13873. Epub 2017 Sep 14
[21] Dinleyici EC, Kara A, Ozen M, Vandenplas Y. *Saccharomyces boulardii* CNCM I-745 in different clinical conditions. Expert Opin Biol Ther. 2014;14:1593-609
[22] Basu S, Chatterjee M, Ganguly S, Chandra PK. *Effect of Lactobacillus rhamnosus GG in persistent diarrhea in Indian children: a randomized controlled trial*. J Clin Gastroenterol. 2007 Sep;41(8):756-60
[23] From Girardin M, Frossard JL. *Place des probiotiques dans le traitement des maladies inflammatoires intestinales.* Rev Med Suisse 2012; volume 8. 1674-1678

PROFESSOR OLIVIER GOULET



Professor Olivier Goulet is the Head of Gastroenterology, Hepatology and Nutrition at the Necker Children's Hospital in Paris, France. His scientific approach focuses, among others, on the establishment of the intestinal microbiota from birth. Thanks to this research field it is possible to identify the parameters likely to modify or disrupt this early colonization and study its impact on the onset of certain pathologies.

GASTROINTESTINAL DISORDERS IN CHILDREN: THE NEED TO ACT

What are the main childhood gastrointestinal disorders involving the microbiota?

I count five of them. The first two are infectious (or post-infectious) diseases related to an infestation by pathogens, and inflammatory bowel diseases, which are due to a conflict between the immune system and commensal bacteria. But intestinal microbiota is also involved in food allergies, functional disorders (irritable bowel syndrome, constipation), as well as in obesity. Whether intestinal dysbioses are the cause or the consequence of these diseases has yet to be determined. Any change in the microbiota is likely to lead to functional changes, or even organic alterations, and conversely. Simply put, we estimate that infections can generate a dysbiosis and that chronic inflammatory bowel diseases can cause, as well as be caused by, a dysbiosis. The mechanisms involved in allergies are quite unclear but they could develop even before the maturation of the immune system, while later and/or prolonged changes could be involved in gastrointestinal functional disorders or obesity..

Is France especially exposed to this type of disease?

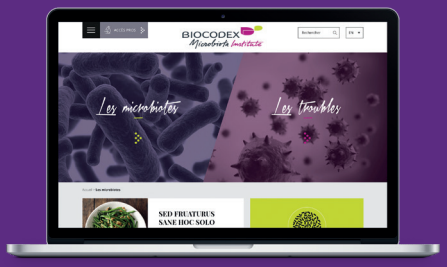
These observations are unquestionable, but they are not specific to France. The incidence of diseases mentioned earlier has increased in the past 20 years and I believe it could be called an "epidemic". What changed? The number of C-sections, antibiotic administration and

« We should adopt a true "culture of prevention" that includes the protection of intestinal microbiota. »

antacids. Our dietary habits and the composition of the food we eat, especially in terms of preservatives, also play a role. All these elements are involved in the disruption of our intestinal microbiota. We should not draw simplistic conclusions, but we should bear in mind that a wide range of arguments and studies correlate these changes to a significant increase in certain childhood diseases. The challenge is even more critical in pediatrics since events that happen at birth or early childhood tend to have a lasting impact on the microbiota composition and long-term consequences on health.

How can we improve this situation and improve patient treatment?

Health professional believe that the emergency is to obtain an easier and less expensive access to intestinal microbiota metagenomics analysis methods. This could provide a qualitative comparison of the microbiota composition of the same individual at different times. Identifying changes between “baseline microbiota” and that observed during the course of a disease could help us identify pathophysiological mechanisms and develop personalized or targeted therapeutic responses. In this context, the probiotics approach seems to be effective in certain intestinal disorders and deserves to be further investigated. Some countries are starting to show interest in this topic, but France must not be left behind, especially within the European Union. Moreover, we should adopt a true “culture of prevention”, with the support from public authorities, that includes the protection of intestinal microbiota. This would contribute to limit exposure to risk factors that are likely to generate dysbioses (C-sections, antibiotics, antacids, inadequate diet, etc.) and would decrease the number of several childhood diseases. ●



Find us also on our website

bmi-pro.com

BIOCODEX
Microbiota Institute

biocodexmicrobiotainstitute.com/pro

Code SAP 98985