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Annals of Nutrition & Metabolism

Abstracts of the 10th Workshop on Probiotics and Prebiotics

Spanish Society of Probiotics and Prebiotics (SEPyP)
Las Palmas de Gran Canaria, Spain, February 6–8, 2019

Guest Editors

Guillermo Álvarez Calatayud, President of the Spanish Society of Probiotics and Prebiotics (SEPyP), Madrid

Ascensión Marcos, Research Professor at the Spanish National Research Council, Madrid

Luis Peña Quintana, Organizing Committee President of the 10th Workshop on Probiotics and Prebiotics, Las Palmas de Gran Canaria

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(Continued on next page)

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Annals of Nutrition & Metabolism

Preface

10th Workshop on Probiotics and Prebiotics

X Workshop of the Spanish Society of Probiotics and Prebiotics - SEPyP 2019

The modulation of gut microbiota to improve health has been empirically carried out since ancient times and fermented foods have been used against gastrointestinal infections since the year 76 BC. However, it was not until 1910 that Elie Metchnikoff observed that those inhabitants in the Balkans reached very advanced ages and related this fact with the habitual consumption of a fermented milk. He postulated that the bacteria involved in the fermentation of milk could be responsible for their longevity. Since then, the implication of the alterations of the microbiota in the origin and development of various diseases has promoted great research effort. This fact has been reflected in its clinical application with the use of probiotics and prebiotics. In recent years, techniques have been developed to modulate gut microbiota, with novel applications in medicine, such as faecal microbiota transplantation.

Today, healthcare professionals are increasingly aware of the benefits of a proper diet, which promotes the development of functional foods and, within them, those containing beneficial bacteria. However, despite the effort already made, we are still at the beginning of our knowledge about the microbiota and its influence on health and the emergence of diseases. The research in this field opens before us a very wide range of possibilities of development of new indications and therapeutic procedures, even personalized, that promise significant advances in our perception of what health means.

The Spanish Society of Probiotics and Prebiotics (*Sociedad Española de Probióticos y Prebióticos,* SEPyP) is a scientific non-profit organization founded in 2010, devoted to the development and promotion of scientific knowledge and research, the clinical application and the distribution of the microbiota within the body, together with the effects of probiotics and prebiotics on health. The professionals that form the organization (about a thousand) belong to different disciplines: physicians, pharmacists, veterinarians, microbiologists, basic researchers, immunologists, nutritionists, nurses, midwifes, etc. In addition, SEPyP is also supported by the most important companies of this area of knowledge.

Since it was founded, SEPyP annually organizes a workshop with the purpose of promoting the scientific knowledge of probiotics and prebiotics among the health professionals, in addition of being a unique forum for the exchange of the last research advances on the field of the microbiota and microbioma. This year the workshop is held from 6th to 8th February in Las Palmas de Gran Canaria (Canary Islands, Spain).

The scientific programme of the workshop (SEPyP-2019) is included in this special supplement of *Annals of Nutrition and Metabolism*. It includes 2 round tables, 3 extraordinary conferences, 7 workshops, 4 symposia, 20 oral presentations and the abstracts of the posters submitted. During the presentations, compelling topics for the professionals will be discussed, such as our different microbiota, fecal transplantation and sea microbiota, plus other topics of recent update like probiotics. During the Workshop the foundation of the Iberoamerican Society of Microbiota, Probiotics and Prebiotics is planned with the participation of experts on the subject on both sides of the Atlantic Ocean.

In conclusion, we want to express our gratitude to the participating companies for their invaluable help and specially, to the organizing and scientific committees. Also to the speakers, debate moderators of the round tables and workshops that have made this meeting possible.

Guillermo Álvarez Calatayud
Ascensión Marcos
Luis Peña Quintana
Editors

10th Workshop on Probiotics and Prebiotics

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10th Workshop on Probiotics and Prebiotics

WEDNESDAY, FEBRUARY 6TH

15:00 - 20:00 h

Distribution of Documentation

16:00 h

SATELLITE SYMPOSIUM

Sponsored by Heel

Microbiota and Probiotics in Dermatological Pathology

Speakers:

Microbiota in Dermatological Pathology: What we Know and Where we are Going

Diego Fernández Nieto

Gastroenterology Service. Ramón y Cajal University Hospital. Madrid.

New Evidence in Dermatological Pathology

Vicente Navarro López

Head of the Infectious Diseases Unit. Vinalopó de Elche University Hospital. Director of the Chair of Infectious Diseases and Human Microbiota. San Antonio Catholic University of Murcia.

17:00 h

SATELLITE SYMPOSIUM

Sponsored by Faes Farma

Chair:

Teresa Requena Rolanía

Research Institute in Food Sciences. CIAL (CSIC-UAM). Madrid.

Speakers:

The Effect Of Probiotics Across Different Physiologies, Pathologies and Life Stages – Evidence and Possible Mechanisms

Nigel Plummer

Cultech Limited.

18:00 h

SATELLITE SYMPOSIUM

Sponsored by Nutribén

Update on Microbiota: Paraprobiotics in Infant Feeding

Chair:

Luis Peña-Quintana

Chief, Pediatric Gastroenterology, Hepatology and Nutrition Unit. Maternal and Child University Hospital of Canarias. Las Palmas de Gran Canaria, Spain

Speakers:

Update on Microbiota: Probiotics vs. Non-Probiotics (Paraprobiotics and Posbiotics)

Abelardo Margolles Barros

Department of Microbiology and Biochemistry of Dairy Products. Dairy Research Institute of Asturias. Spanish National Research Council (IPLA-CSIC). Villaviciosa, Spain

New Trends in Infant Feeding: Paraprobiotics and the Prevention of Childhood Obesity

Rosaura Leis Trabazo

Coordinator, Pediatric Gastroenterology, Hepatology and Nutrition Unit. Santiago University Clinical Hospital. Santiago de Compostela, Spain

18:00 h

SATELLITE SYMPOSIUM

Sponsored by Stada

How Can We Use Probiotics in the Treatment of Genitourinary Disorders?

Speakers:

Knowing the Microbiota and Vaginal Probiotics

J. Evaristo Suárez Fernández

Professor, Microbiology Area. School of Medicine. Oviedo University. Asturias, Spain

Knowing the Scientific Evidence that Supports their Use

Ana Rosa Jurado

Coordinator, Work Group for Women Care of SEMERGEN

THURSDAY, FEBRUARY 7TH

08:30 - 09:00 h

Welcome and Opening

09:00 - 11:30 h

ROUND TABLE. OUR MICROBIOTA: CLINICAL APPLICATIONS

Chairs:

Daniel Ceballos Santos
Medical Director. Digestive System Service. Dr. Negrín University Hospital. Las Palmas de Gran Canaria, Spain

Ana Teresa Abreu y Abreu
Gastroenterology Service. Ángeles Pedregal Hospital. México DF, México

Speakers:

Intestinal Microbiota
Francisco Guarner Aguilar
Digestive System Research Unit. Vall d'Hebron University Hospital. Barcelona, Spain

Vaginal Microbiota
J. Evaristo Suárez Fernández
Professor, Microbiology Area. School of Medicine. Oviedo University. Asturias, Spain.

The Microbiome of Human Milk
Juan Miguel Rodríguez Gómez
Department of Nutrition and Food Science. Complutense University of Madrid. Spain

Oral Microbiota
María de los Desamparados Ferrer García
Postdoctoral Researcher of the Genomic and Health Area. Higher Center for Public Health Research (CSISP).

Microbiota of the Skin
Minia Campos Domínguez
Department of Dermatology. Gregorio Marañón University General Hospital. Madrid, Spain

Respiratory Microbiota
Rosa Rodríguez Fernández
Section of General Pediatrics. Gregorio Marañón Children's Hospital. Madrid, Spain

11:30 - 12:00 h

Coffee Break

12:00 - 14:00 h

CLINICAL USES SESSION: MICROBIOTA GUT-BRAIN AXIS LECTURE

Chairs:

Luis Serra Majem
Professor of Preventive Medicine. Director of the University Institute of Biomedical and Health Research. Las Palmas de Gran Canaria University. Spain

Armando Madrazo de la Garza
Postgraduate Professor. National Autonomous University of Mexico. Past president of LASPGHAN. President GENIS Foundation. México

Speaker:

Mental Diseases: Depression
Almudena Sánchez-Villegas
University Professor. Las Palmas de Gran Canaria University. Spain

Defense 6 Oral Communications

14:00 - 15:30 h

Work Lunch

15:30 - 16:30 h

Posters Presentation

16:30 - 18:30 h

WORKSHOPS

PEDIATRICS. Clinical Evidence

Chairs:

Enriqueta Román Riechmann
Chief, Pediatric Service. Pediatric Gastroenterology, Hepatology and Nutrition Unit. Puerta de Hierro University Hospital. Madrid, Spain

Félix Sánchez-Valverde Visus
Chief, Pediatric Gastroenterology, Hepatology and Nutrition Section. Hospital Complex of Navarra. Pamplona, Spain

Speakers:

Juan José Díaz Martín
Pediatric Gastroenterology, Hepatology and Nutrition Section. Central University Hospital of Asturias. Oviedo, Spain

Daniel González Santana
Pediatric Gastroenterology, Hepatology and Nutrition Unit. Maternal and Child University Hospital. Las Palmas de Gran Canaria, Spain

Beatriz Espín Jaime
Pediatric Gastroenterology, Hepatology and Nutrition Section. Virgen del Rocío Children's University Hospital. Sevilla, Spain

CLINICAL USES: ADULTS. Clinical Evidence

Chairs:

Francisco Guarner Aguilar
Digestive System Research Unit. Vall d'Hebron University Hospital. Barcelona, Spain

Ana Castellot Martín
Chief, Digestive System Service. Insular Maternal and Child University Hospital Complex. Las Palmas de Gran Canaria, Spain

Speakers:

Carlos Sánchez Vilar
Digestive System Service. Insular Maternal and Child University Hospital Complex. Las Palmas de Gran Canaria, Spain

Luis Peña Ferrera
Digestive System Service. Insular Maternal and Child University Hospital Complex. Las Palmas de Gran Canaria, Spain

VETERINARIAN. Marine Aquaculture and Biotechnology for the Testing and Development of Probiotics and Prebiotics

Chairs:

Cristina Ruano Rodríguez
University Institute of Biomedical and Health Research. Cultural Classroom Science and Gastronomy. Las Palmas de Gran Canaria University. Spain

Speakers:

Daniel Montero Vítores
Aquaculture Research Group (GIA). ECOAQUA University Institute. Las Palmas de Gran Canaria University, Spain

Juan Luis Gómez Pinchetti
Spanish Algae Bank (BEA). Institute of Oceanography and Global Change (IOCAG). Las Palmas de Gran Canaria University. Spain

Lorena Román Fuentes
Aquaculture Working Group (GIA). ECOAQUA University Institute. Las Palmas de Gran Canaria University. Spain

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18:30 - 19:30 h

General Meeting

FRIDAY, FEBRUARY 8TH

08:30 - 10:30 h

IMMUNONUTRITION SESSION

Chairs:

Ascensión Marcos Sánchez
Institute of Science and Technology of Food and Nutrition (ICTAN). CSIC. Madrid, Spain

Juan Rivera Medina
President of LASPGHAN. Gastroenterology Service. Child Hospital. Lima. Professor of the School of Medicine. Greater University of San Marcos and Cayetano Heredia Peruvian University. Lima, Perú

Speaker:

Prebiotics Modulation of Gut Microbiota and Immune Responses
Annick Mercenier
Science & Strategy Coordinator. Nutrition & Health Research. Nestlé Research Center

Defense 6 Oral Communications

10:30 - 11:00 h

Coffee Break

11:00 - 13:00 h

MICROBIOLOGY AND VETERINARIAN SESSION

Chairs:

Gaspar Pérez Martínez
Institute of Agrochemistry and Food Technology, IATA-CSIC. Spain

Mónica Peñate Bolaños
Digestive System Service. Insular Maternal and Child University Hospital Complex. Las Palmas de Gran Canaria, Spain

Speaker:

Fecal Transplant: State of the Art
Rosa del Campo Moreno
Microbiology and Parasitology Department. Ramón y Cajal University Hospital. Madrid, Spain

Defense 6 Oral Communications

13:00 - 14:30 h

CLOSING ROUND TABLE

Chairs:

Rafael Robaina Romero
Professor of Biology, Biology Department. Rector, Las Palmas de Gran Canaria University. Spain

Mónica de la Fuente del Rey
Professor of Physiology. Department of Genetics, Physiology and Microbiology. Complutense University of Madrid. Spain

Speakers:

Marine Microbiota
Carlos Pedrós-Alió
National Center for Biotechnology, CSIC. Cantoblanco, Madrid, Spain

Prebiotics in Functional Diets for Fish
Daniel Montero Vítores
University Professor. Aquaculture Working Group (GIA). ECOAQUA University Institute. Las Palmas de Gran Canaria University. Spain

The Potential Role of Algae as a Source of Functional Ingredients

Juan Luis Gómez Pinchetti

University Professor. Spanish Algae Bank. Institute of Oceanography and Global Change (IOCAG). Las Palmas de Gran Canaria University. Spain

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14:30 - 15:30 h

Work Lunch

15:30 - 16:30 h

Posters Presentation

16:30 - 18:30 h

WORKSHOPS

ALLERGY. Clinical Uses of Probiotics in Allergic Diseases

Sponsored by Allergy Therapeutics Iberica

Speaker:

Pedro Ojeda

Spanish Society of Allergology and Immunology

NUTRITION

Chairs:

Mónica Ruiz Pons

Head, Pediatric Nutrition Unit. Nuestra Señora de la Candelaria University Hospital. Santa Cruz de Tenerife, Spain

Mª Lourdes de Torres Aured

Head, Functional Unit of Dietetics and Nutrition. Miguel Servet University Hospital. Zaragoza, Spain

Speakers:

Acidified Milk and Food Intolerances. From Diagnosis to Reality

Rosaura Leis Trabazo

Professor of Pediatrics. Santiago de Compostela Univestity.
Coordinator, Pediatric Gastroenterology, Hepatology and Nutrition Unit. Santiago University Clinical Hospital.
Vice President of the Atlantic Diet Foundation-USC. Santiago de Compostela, Spain

Probiotics and Prebiotics in Infant Formulas

José Maldonado Lozano

Professor of Pediatrics. Granada University. Pediatric Gastroenterology, Hepatology and Nutrition Unit. Virgen de las Nieves University Hospital. Granada, Spain

Hydration and Probiotics

Adriana Ortiz Andrellucchi

International Chair of Advanced Studies in Hydration. University Institute of Biomedical and Health Research. Las Palmas de Gran Canaria University. Spain

FECAL TRANSPLANT

Chairs:

Rosa del Campo Moreno

Microbiology and Parasitology Department. Ramón y Cajal University Hospital. Madrid, Spain

Francisco Guarner Aguilar

Digestive System Research Unit. Vall d'Hebron University Hospital. Barcelona, Spain

Speakers:

Applications of Fecal Microbiota Transplant in the Clinic

Rosa del Campo Moreno

Microbiology and Parasitology Department. Ramón y Cajal University Hospital. Madrid, Spain

Standardization of the Collection, Transport and Preparation of Samples for Fecal Microbiota Transplantation

Borja Sánchez

Department of Microbiology and Biochemistry of Dairy Products. Institute of Dairy Products of Asturias. CSIC. Villaviciosa, Spain

Faecal Transplant Performance Protocol. Our Experience

Claudia Arajol González

Digestive Service. Bellvitge University Hospital. L'Hospitalet de Llobregat, Barcelona.

18:30 - 20:00 h

Presentation of the Ibero-American Society of Microbiota, Probiotics and Prebiotics

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21:00 h

Awards Ceremony

Certification

Requested the Recognition of Sanitary Interest of scientific acts (Reconocimiento de Interés Sanitario de actos de carácter científico) to the Ministry of Health, Consumption and Social Welfare.

Requested the Accreditation of Continuing Education of the Health Professions (Acreditación de Formación Continuada de las Profesiones Sanitarias) to the Government of the Canary Islands

Annals of Nutrition & Metabolism

Clinical Uses

INFLUENCE OF BREAST MILK MICROBIOTA ON COLONIZATION, GROWTH AND HEALTH OF INFANTS. EFFECTS OF PROBIOTIC INTERVENTION

J. Fonollá[1], B. Pastor-Villaescusa[2], J.A. Hurtado[3], M. Gil-Campos[4], J. Uberos[5], J.L. Leante[6], L. Affumicato[7], A. Iglesias-Deus[8], J.M. Garrido[9], A.D. Valero[1], C. Rodríguez[1], M.P. Díaz-Ropero[1], J.A. Maldonado-Lobón[1], M. Olivares[1]

[1]Research Department, Biosearch Life, Granada, Spain. [2]Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, Munich, Germany. [3]Department of Neonatology, Hospital Materno-Infantil, Granada, Spain. [4]Department of Metabolism and Pediatrics Research, Hospital Reina Sofía, Córdoba, Spain. [5]Department of Pediatrics, School of Medicine, Granada, Spain. [6]Department of Neonatology, Hospital General Universitario Santa Lucía, Cartagena, Spain. [7]Department of Neonatology, Hospital Regional Universitario, Málaga, Spain. [8]Department of Neonatology, Hospital Clínico Universitario, Santiago de Compostela, Spain. [9]Department of Pediatrics, Hospital Universitario, Salamanca, Spain.

Background/Aims: Infant's first years crucial in healthy gut bacteria. The objective of the present study is to evaluate the influence of breast milk microbiota on gut microbiota, growth and health of the infant.

Methods: A randomized double blinded controlled study including 625 women and their children during breastfeeding period was conducted. Women-infant pairs were distributed into two groups: in Lc40 group women received 1 capsule/day containing *L. fermentum Lc40* 3x10^{9}cfu; In Control group women received 1 placebo capsule/day containing maltodextrin. The intervention period was 16 weeks. Microbiota in breast milk and infant's feces was analysed and growth and health of infants was followed up during the intervention.

Results: Two hundred and ninety-one pairs of women-infant completed 16 weeks of intervention. A significant correlation was observed between the load of *Lactobacillus*, *Staphylococcus* and *Streptococcus* with the load of *Lactobacillus*, *Staphylococcus*, *Bacteroides* and *E. coli* in infant's feces ($p < 0.05$). The probiotic intervention modulated this effect. Regarding to growth of infants, superior weight z-scores were observed of infants which mothers had higher values of *Lactobacillus* in milk. *E.coli* load in infant's feces was also related to higher weight and height z-scores ($p < 0.05$). Load of *Staphylococcus* in breast milk and infant's feces was correlated with higher incidence of respiratory infection. The intervention with *L. fermentum* Lc40 reduce the effect of *Staphylococcus* on infant's health ($p < 0.05$). Finally, infant colic was significantly more likely to occur in infants which mother had higher level of anaerobes in milk ($p = 0.044$).

Conclusion: Breast milk microbiota influences infant colonization and is related with parameters of growth and health of the infants. Probiotic modulation of women microbiota might be a useful strategy to promote healthier patters of colonization in infants.

LACTOBACILLUS SALIVARIUS PS7, A PROBIOTIC STRAIN WITH POTENTIAL FOR PREVENTION OF OTITIS

N. Cárdenas[1], V. Martín[2], R. Arroyo[3], M. López[4], C. Badiola[5], J.M. Rodríguez[3]

[1]Probisearch SLU. [2]Centro Nacional de Microbiología, Instituto de Salud Carlos III. Majadahonda, Madrid. [3]Departamento de Nutrición y Ciencia de los Alimentos. Universidad Complutense de Madrid. [4]Centro de Salud Bermeo. Bermeo, Bizkaia. [5]Departamento Médico e I&D de Casen Recordati. Pozuelo de Alarcón, Madrid.

Background/Aims: Recurrent acute otitis media (rAOM) in children is a frequent condition with no satisfactory therapy. Recurrent use of antibiotics leads to an increasing antimicrobial resistance of otopathogens; therefore the use of probiotics in infection prevention become a new perspective. There is no evidence on the role of probiotics on rAOM in children. In a previous work, *Lactobacillus salivarius* PS7 was characterized as a potential probiotic strain and showed to exert a high antimicrobial activity against various bacterial species related to rAOM.

Methods: We have conducted a pilot study in 64 otherwise healthy children suffering from rAOM. All children received treatment with one daily dose of 10^9 CFU of *Lactobacillus salivarius* PS7 with milk or a milk product during 6 months. An external control group of children treated with standard therapy was used for comparison. Outcomes were the incidence and duration of AOM episodes and the pathogen carriage in the external ear canal.

Results: Sixty-one children (mean age 39 months) completed the study. At least one episode of AOM was diagnosed in 36% of treated children, compared to 65% in children treated with standard therapy. The incidence of AOM episodes during the treatment period, compared to the 6-month period before treatment was started, was reduced by 84%. The median duration of AOM episodes in treated children was 4 days, compared to 6 days in children treated with standard therapy. The microbial density in the external ear canal decreased from ≥ 3 $\log_{10}$ CFU at baseline to ≤ 2 $\log_{10}$ CFU at the end of the intervention period.

Conclusion: A strain of *L. salivarius* PS7 has been effective to reduce the incidence and duration of AOM episodes. These promising results should be confirmed in a controlled clinical trial.

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DEMOGRAPHIC AND CLINICAL CHARACTERISTICS INFLUENCING THE EFFECTS OF A CHOLESTEROL-LOWERING PROBIOTIC

J. Espadaler, S. Audivert, E. Navarro-Tapia, D. Buj

Innovation Department. AB-BIOTICS SA. Sant Cugat del Valles, Spain.

Background/Aims: Some probiotics have demonstrated cholesterol-lowering activity in randomized trials. However, the effect of demographic and clinical factors on such probiotics and potential interactions of concomitant medications has not been assessed.

Methods: Observational, 12-week prospective study, among patients initiating a probiotic containing strains *L. plantarum* CECT7527, CECT7528 and CECT7529 ($1.2x10^9$ CFUs u.i.d.). Patients initiating statin treatment, but not those already on statin treatment, were excluded. Multivariate regression was performed to assess the effect of demographic and clinical characteristics (baseline LDL and triglycerides, statin therapy intensity and other concomitant medications) on serum LDL and triglyceride change after 12 weeks, and on reported tolerability issues.

Results: 343 patients, median age 55 (19-85) years, 63% female, baseline LDL and triglycerides of 156.5 ± 43.8 mg/dL and 339.9 ± 227.8 mg/dL, respectively, were available for analysis. Use of statins, fenofibrates, antihypertensive, antidiabetic and antiplatelet medications was 46%, 5%, 57%, 32% and 20%, respectively. LDL reduction averaged 40.8 ± 42.5 mg/dL in the whole cohort, and 34.5 ± 37.5 mg/dL in the non-statin subpopulation (both $p< 0.001$). Baseline LDL ($p< 0.001$) and statin therapy intensity ($p= 0.028$), but not other demographic or clinical characteristics, significantly influenced LDL reduction in the whole cohort (bivariate $R^2= 0.37$). Reduction of serum triglycerides was also statistically significant in the whole cohort and in the non-statin subpopulation (both $p< 0.001$), and the effect was only dependent on baseline triglycerides ($p< 0.001$). 17% of patients reported tolerability issues (none of them severe), which correlated to antiplatelet use only (OR= 4.18, 95%CI 2.27-7.72; $p< 0.001$).

Conclusions: Probiotic treatment significantly reduced serum LDL cholesterol and triglycerides, regardless of age, gender, antidiabetic, antihypertensive or antiplatelet medication. Higher baseline LDL and triglycerides led to higher efficacy, and combination to statins further increased LDL reduction. Concomitant antiplatelet use increased the incidence of tolerability issues, mostly oily feces and flatulence.

EFFECT OF INTRAPARTUM ANTIMICROBIAL PROPHYLAXIS OVER BIFIDOBACTERIAL POPULATIONS IN HEALTHY FULL-TERM INFANTS: A QUANTITATIVE ASSESSMENT

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Background/Aims: The gut microbiota establishment begins at early life and disturbances on this process may pose implications for the health of the infant and on long-term. The main microbial transfer from mother to baby occurs during delivery, with the exposure to the first colonizers of the gastrointestinal tract, such as bifidobacteria. *Bifidobacterium* is one of the most abundant genus in the infant intestinal microbiota and it is known that this microbial group carries out key functions for maintaining a healthy status. Different external factors may affect the correct gut microbiota establishment. Among these, antibiotic exposure is common, with intrapartum antibiotics being present in over 30% of deliveries; even though its effect over the gut microbiota establishment is not still well-understood. In this context, the aim of this study was to study the effect of the intrapartum antimicrobial prophylaxis (IAP) on the bifidobacteria population establishment.

Methods: Faecal samples from 39 healthy full-term babies were collected at 2, 10, 30, 90 days of life. DNA was extracted and the levels of the most abundant bifidobacterial species (*Bifidobacterium longum*, *Bifidobacterium breve, Bifidobacterium bifidum, Bifidobacterium adolescentis, Bifidobacterium catenulatum, Bifidobacterium dentium, Bifidobacterium angulatum, Bifidobacterium animalis*) were determined by q-PCR.

Results: Despite the large inter-individual differences, our results showed a reduction in the levels of the most of bifidobacteria species in babies whose mothers received IAP, with respect to those whose mothers did not. The differences reached statistical significance ($p< 0.05$) for the species *B. longum* and *B. bifidum* during early days.

Conclusions: This study underlines the need to study the effect of the different perinatal factors affecting neonatal microbiota, not only at high taxonomical level but also at species level. This knowledge would help to develop rational strategies for favouring a healthy early microbiota development when this process is challenged.

All authors declare that no competing interest exits.

EFFECTS OF DAILY CONSUMPTION OF THE PROBIOTIC *BIFIDOBACTERIUM ANIMALIS* SUBSP. *LACTIS* CECT 8145 ON ANTHROPOMETRIC ADIPOSITY BIOMARKERS IN ABDOMINALLY OBESE SUBJECTS: A RANDOMIZED CONTROLLED TRIAL

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Background: The effects of probiotic *Bifidobacterium animalis* subsp. *lactis* CECT 8145 (Ba8145), and those of its heat-killed form (h-k Ba8145) on human anthropometric adiposity biomarkers are unknown. So that, to assess the effect of Ba8145 and h-k Ba8145 ingestion on anthropometric adiposity biomarkers was proposed to be studied.

Methods: A randomized, parallel, double-blind, placebo-controlled trial with abdominally obese individuals was performed. Participants (n= 135) consumed 1 capsule/day containing 10^{10} colony forming unit (CFU) of Ba8145, 10^{10} CFU of h-k Ba8145, or placebo (maltodextrin) for 3 months.

Results: Ba8145 ingestion decreased waist circumference, waist circumference/height ratio, and Conicity index ($P< 0.05$) versus its baseline. Changes versus the placebo group reach significance ($P< 0.05$) after the h-k Ba8145. Ba8145 decreased body mass index compared to baseline and to placebo group ($P< 0.05$). The decreases in visceral fat area after Ba8145 treatments reached significance ($P< 0.05$) only after h-k Ba8145. When analyses by gender were performed significance remained for women. Diastolic blood pressure and HOMA index decreased ($P< 0.05$) after h-k Ba8145. Gut microbiome analyses showed an increase in *Akkermansia* spp. after Ba8145, particularly in the live form, which was inversely related to weight ($P= 0.003$).

Conclusion: In abdominally obese individuals, the consumption of Ba8145, both as viable and, mainly, as heat-killed cells, improves anthropometric adiposity biomarkers, particularly in women. An increase in the gut *Akkermansia* genus appears as a possible mechanism. Our results support Ba8145 probiotic as a complementary strategy in obesity management.

SYMBIOTICS AS A NEW PERSPECTIVE IN THE TREATMENT OF LACTOSE INTOLERANCE

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Objective: Lactose intolerance, characterized by low levels of intestinal lactase, affects approximately 75% of the world population and is defined as a clinical syndrome characterized by pain and abdominal distention, flatulence, and diarrhea that occurs after lactose consumption. The use of specific probiotic strains, in particular those capable of expressing β-galactosidase enzymatic activity, has been proposed as an alternative treatment for subjects with lactose intolerance. The aim of this observational study was to investigate the efficacy of a symbiotic combination of lactic acid bacteria and inulin (SynGut®) in patients with lactose intolerance.

Materials and Methods: 38 adult subjects with physician-diagnosed lactose intolerance receiving a symbiotic combination of *Bifidobacterium lactis* W51, *Lactobacillus acidophilus* W22, *Lactobacillus plantarum* W21, *Lactococcus lactis* W19 and inulin (SynGut®) were assessed for symptom reduction using the SQLM (symptom questionnaire for lactose malabsorption) and changes in lactose consumption were evaluated. Secondary evaluations involved the changes in the proportion of patients with a positive hydrogen breath test (HBT).

Results: Treatment with SynGut® significantly decreased symptoms of lactose intolerance after 3 and 6 months of administration compared to baseline measurement. 71% of lactose intolerance patients reported an improvement of their symptoms after 3 month and 66% patients after 6 month of administration. Moreover, the proportion of patients with a negative HBT increased compared to baseline measurements: 0% at baseline, 65% after 3 months, and 81% after 6 months of administration.

Conclusion: This study shows that the symbiotic supplementation *Bifidobacterium lactis* W51, *Lactobacillus acidophilus* W22, *Lactobacillus plantarum* W21, *Lactococcus lactis* W19 and inulin (SynGut®) improves the symptoms of patients with lactose intolerance and opens new perspectives in the use of specific symbiotics in the treatment of lactose intolerance.

Immunonutrition

COMBINING TWO PROBIOTIC STRAINS WITH OLIGOFRUCTOSE AND INULINE ABOLISHES THEIR BENEFITS AGAINST *SALMONELLA* IN PIGLETS

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Background/Aims: The efficacy of two probiotic strains (*Bifidobacterium longum* subsp. *infantis* CECT 7210 (Laboratorios Ordesa S.L.) and *Lactobacillus rhamnosus* HN001) combined or not with a prebiotic mixture of oligofructose and inulin against *Salmonella typhimurium* was evaluated.

Methods: A total of 96 piglets (28 days) were distributed into 32 pens assigned to 5 treatments: one non-challenge (CTR+) and four challenged: same diet (CTR-), or supplemented with probiotic (> $3x10^{10}$cfu/kg each, PRO), prebiotic (5%, PRE) or their combination (SYN). After one week, animals were inoculated with *Salmonella*. Feed intake, weight and clinical signs were recorded. On days 4 and 8 post-inoculation (PI), one animal per pen was euthanized and samples collected for *Salmonella* counts, fermentation products, ileal histomorphology and serum TNF-α and PigMAP analysis.

Results: After the challenge, feed intake was decreased but more markedly in the SYN group that showed a lower final weight (Intake: 438^{a}, 315^{b}, 293^{b}, 237^{b} and 315^{b} g/d, P< 0.001; Final BW: 10.4^{a}, 9.3^{ab}, 8.9^{ab}, 8.2^{b} and 9.4^{ab} kg, P= 0.009; for CTR+, PRO, PRE, SYN and CTR-, respectively). PRE and SYN groups showed more liquid ileal consistency on day 8 PI (P= 0.009). A higher percentage of animals receiving PRO became negative to *Salmonella* in faeces at the end of the study (65% PRO vs 0 % CTR- (P= 0.03)). Lower amounts of ileal SCFA were observed in SYN (P= 0.025) and valerate was increased in PRE and SYN. At day 8PI a significant decrease in the ileal villous/crypts ratio was found in challenged groups except for PRO (1.06^{a} CTR+, 0.96^{ab} PRO, 0.84^{b} PRE, 0.84^{b} SYN, $0,85^{b}$ CTR-).

Conclusion: In conclusion, the combined probiotic strains were able to enhance the response of the animals against *Salmonella* with improved ileal histological architecture and a faster clear out of the pathogen from the gut. However, these beneficial effects disappeared when the probiotic was combined with the prebiotic mixture.

RELATIONSHIP BETWEEN FIBER CONSUMPTION, SHORT CHAIN FATTY ACIDS AND *CLOSTRIDIA* CLASS MEMBERS IN HEALTHY ADULTS

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Background: The promotion of beneficial commensal bacteria in the gut, along with an increased short chain fatty acid production (SCFA), have been postulated as potential mechanisms involved in the beneficial effects of a high fiber intake on health.

Objective: To assess the influence of fiber consumption on short chain fatty acid production and the abundance of bacteria belonging to Clostridia class in healthy adults.

Methodology: 261 adults aged between 25-50 y, not suffering chronic disease or following medical treatment were included (51% males). Short chain fatty acid production (acetic, propionic, butyric, isobutyric, valeric and isovaleric) was analysed by gas chromatography, and gut microbiota composition through 16S rRNA gene amplicon sequencing (V3+V4 gene regions. MiSeq 2x270 Illumina) and taxonomic analysis. Fiber consumption (FC) habits were analyzed by a food frequency questionnaire, and the following groups were considered: Low (< 15 g/day), Medium (15-25 g/day) and High (> 25 g/day). General linear models were used to assess fiber consumption effect on the studied variables, with FC groups, gender and BMI-fat groups as fixed factors, and age and energy as covariables. Bonferroni test was used for pairwise comparisons.

Results: The levels of acetic and butyric acid were higher in the high and medium FC groups compared to the low FC group, only reaching statistical significance for the latter (Medium vs. Low: P= 0.021 and P= 0.034, respectively). On the other hand, the high FC group showed higher levels of *Clostridiaceae* compared to the low FC group (P= 0.026), as well as higher *Faecalibacterium prausnitzii* levels (P= 0.018). In addition, both acetic and butyric acids were positively correlated to *F.prausnitzii* levels (P< 0.001).

Conclusion: Fiber consumption might exert beneficial effects on intestinal health, as observed in the increased levels of butyric acid and *F. prausnitzii*, both associated to gut anti-inflammatory effects.

DETECTION OF RIBOFLAVIN PRDUCTION BY *LACTOBACILLUS PLANTARUM* STRAINS DURING GROWTH AND ITS GASTROINTESTINAL SURVIVAL

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Introduction: Certain strains of lactic acid bacteria (LAB) can produce riboflavin, a water-soluble vitamin that belongs to group B. It is produced by plants and many microorganisms but higher animals lack the ability to biosynthesize it.

Methods: We have evaluated riboflavin production by five *Lactobacillus plantarum* strains, isolated from chicha, a fermented alcoholic beverage normally produced from maize, from Northwestern Argentina, and their isogenic riboflavin-overproducing mutants. Here we describe a method of fluorescence spectroscopy to quantify the production of riboflavin in real time in bacterial

culture supernatants. Thus, the riboflavin overproducing strain *L. plantarum* M5MA1-B2 was selected for further analysis. This strain was fluorescently labeled (mCherry) with the plasmid pRCR12 and its survival to digestive tract stresses in the presence of microbiota in the dynamic multistage BFBL gut model, and in a murine model using conventional adult BALB/c mice was analyzed.

Results: Among the B2 overproducing strains, *L. plantarum* M5MA1-B2 secreted the highest levels of riboflavin. This bacterium was fluorescently labelled by transfer of pRCR12 plasmid. The labelling with mCherry did not affect growth or riboflavin production. This strain, with or without plasmid, is able to adhere to Caco-2 cells and this interaction capacity can be quantified by fluorometry, in the case of the labeled strain. The survival of the isogenic strain M5MA1-B2[pRCR12] has also been demonstrated during in vitro simulation of gastrointestinal tract conditions and by analyzing the BALB/c intestinal content. The results indicated a satisfactory resistance of the strain to gastric and intestinal stress conditions but a low colonization capability. The developed method allows us to quantify the vitamin production, in real time, during bacterial growth, being a rapid and easy test for the selection of B2 vitamin-producing strains.

Conclusion: *L. plantarum* M5MA1-B2 is a suitable probiotic bacteria for the development of functional foods.

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PREDICTING OBESITY FROM GUT MICROBIAL COMPOSITION AND LIPID PROFILE: DOES GENDER MATTER?

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Aims: Different fatty acids could drive different changes in the composition and functionality of the intestinal microbiota, contributing to host's lipid metabolism and obesity development. Our aim was to address the connections between gut microbiota and different serum free fatty acids (FFA) in the context of obesity, by analyzing the possible interactions between all factors involved.

Methods: 66 subjects (age 52.7 ± 11.2y) classified according to Body Mass Index (BMI). Total and individual FFA were analyzed by colorimetric enzymatic assay and methyl-tert-butylether-based extraction protocol, respectively. Gut microbiota was determined by qPCR and diet through a food frequency questionnaire. Statistical analyses were performed, and predictive factors for obesity were obtained via classification by decision trees using machine learning methods.

Results: Subjects with higher serum levels of eicosapentaenoic acid (EPA) and higher fecal levels of *Bacteroides* belonged to normal weight group, with independence of gender. In males, levels of *Faecalibacterium* ≤ 6.456 or > 6.456 (log nº cells / gram of feces) were associated to normal and pre-obesity status, respectively. Furthermore, women with serum palmitic acid > 23.843 μg/mL and *Bifidobacterium* ≤ 6.729 (log nº cells / gram of feces), classified within the overweight group. In the same way, if EPA serum level was > 0.141 μg/mL, or ≤ 0.141 μg/mL running together with *Bifidobacterium* > 8.823 (log nº cells/gram of feces), women were normal weight.

Conclusion: These data point to serum EPA as a significant obesity indicator, independently of the rest of the variables. Furthermore, when the concentration of serum EPA is ≤ 0.235 μg/mL, the interaction between FFA and the gut microbiota seems to be gender-dependent, being associated to *Faecalibacterium* and *Bifidobacterium* for males and females, respectively. These results open the possibility of modifying the obese-linked FFA profile and the altered gut microbiota through dietary interventions attending to gender differences.

SEXUAL DIMORPHISM OF RAT GUT MICROBIOTA: KEY FACTOR IN NUTRITIONAL INTERVENTIONS

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Background and Aim: It is well known that there are gender-specific differences of immune system and microbiota composition in human and mice[1]. These differences may lead to gender-differential responses in nutritional interventions that act through modulation of microbiota composition (p.e. prebiotics and probiotics). The present study was aimed at analyzing sexual dimorphic differences in gut microbiota and intestinal immunity in adult rats.

Methods: For this purpose, cecal content from 12-week old female and male Wistar rats were collected to characterize microbiota composition by sequencing techniques. Moreover, IgA concentration was determined by ELISA in feces and gut wash obtained at the same time point. Fecal samples were also used to determine the IgA-coating bacteria.

Results: The qualitative metagenomic analysis evidenced that female rats have 1 phylum, 4 families and 13 genera that are not present in male rats. No male-specific colonization was observed. Regarding the quantitative analysis male rats showed significantly higher proportion of Firmicutes phylum which was associated with a higher *Lactobacillacea* and *Lactobacillus* at family and genus level, respectively ($p< 0.05$). Female rats had higher proportion of Verrucomicrobia phylum compared to the male rats which was due to a higher presence of *Akkermansiaceae* and *Akkermansia* ($p< 0.05$). Moreover, female rats have more diversity of microbiota than male rats. No sex-associated differences either on intestinal IgA nor on IgA-coated bacteria were observed.

Conclusion: It can be concluded that there is sexual dimorphism in composition and diversity of microbiota in adult rats fact that has to be considered in the design of preclinical studies.

Reference: 1. Elderman M, de Vos P, Faas M. Role of microbiota in sexually dimorphic immunity. Front Immunol. 2018; 9: 1018.

GALACTOOLIGOSACCHARIDE SUPPLEMENTATION AMELIORATES THE ACUTE GASTROENTERITIS PROCESS IN A PRE-CLINICAL MODEL OF DOUBLE ROTAVIRUS INFECTION

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Background and Aim: Group A rotaviruses (RV) are the leading cause of gastroenteritis among young children worldwide producing limited diarrhoea to severe dehydration and even death. After RV infection, immunity is not complete and less severe re-infections usually occur. These infections could be modulated by nutritional interventions with bioactive compounds, such as prebiotics. The aim of this study was to evaluate the influence of a particular galactooligosaccharide (GOS) on the RV symptomatology and immune response during two consecutive infections.

Methods: Lewis neonatal rats were inoculated with SA11 (first RV infection) on day 6 of life and with EDIM (second RV infection) on day 17 of life. GOS group was also orally administered with a daily dose of GOS between day 3 and 9 of life. Clinical and immunological variables were assayed during both infective processes until day 28 of life.

Results: During the first infection, the dietary intervention with GOS significantly reduced the incidence, duration and overall severity of the diarrhoea ($p< 0.05$). In addition, it reduced another severity indicator, the faecal weight output, during the diarrhoea period ($p< 0.05$). Second RV infection was not able to induce diarrhoea in any of the groups studied. Immune response during first infection with SA11 was not affected by GOS and had no impact on second infective process, but the dietary intervention significantly increased IFN-γ and TNF-α intestinal levels two weeks after the second infection ($p< 0.05$).

Conclusion: GOS supplementation is able to reduce the incidence and severity of the RV-associated gastroenteritis episode and to modulate the immune response against RV infections.

Microbiology and Veterinarian

IMPACT OF CURATIVE PELVIC RADIOTHERAPY ON THE GUT ENVIRONMENT OF PROSTATE CANCER PATIENTS

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Background: Gastrointestinal symptoms are frequent after pelvic radiotherapy and can greatly affect the quality of life of cancer survivors. The effect of radiation on the intestinal microbiome, and the implications of a radiotherapy-induced dysbiosis and the derived intestinal inflammation have received very little attention.

Aim: To perform a follow-up study in patients with prostate cancer for investigating alterations in gut microbiota and metabolites induced by pelvic radiotherapy and associations with inflammation and dietary changes.

Methods: Fourteen patients with prostate cancer undergoing pelvic radiotherapy were recruited and followed during the anti-cancer treatment until two months after finishing. Four stool samples were collected from each patient and changes in the bacterial communities were investigated by sequencing the V3-V4 region of the 16S rRNA gene with Illumina Technology (Miseq PE250), meanwhile short chain fatty acids (SCFAs) were analysed by gas chromatography. Additionally, calprotectin levels were determined using an ELISA kit and, evaluation of dietary intake was recorded by means of semi quantitative food frequency questionnaires.

Results: The composition of the gut communities changes along the radiation treatment, being the Bacteroidetes, the group more affected (p= 0.008, Wilcoxon test). Total SCFAs in feces was reduced with pelvic radiation. In particular, statistical differences were observed for butyrate and acetate excretion with respect to basal time. On the contrary, fecal calprotectin increased significantly during radiotherapy (p= 0.016). In addition, statistical differences in the energy intake were observed before and after two months of radiotherapy.

Conclusions: An impact of pelvic radiotherapy on gut microbiota composition and metabolites was observed in prostate cancer patients. Intestinal inflammation occurs at the same time that the microbiome shifts. The effect of radiation was partially, but not completely, restored after two months of finishing the anti-cancer therapy, with changes in the food ingestion patterns still noticeable at this time point.

A PRELIMINARY STUDY OF BACTERIAL GENETIC DETERMINANTS IN HUMAN GALLBLADDER. A FOCUS ON BILE RESISTANCE

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Background/Aims: The human gastrointestinal microbiota and its relationship with different physiological states has been characterized in detail in the last years. However, the microbiota of bile and gallbladder has been scarcely studied. Also, the functions of the autochthonous biliary microorganisms and the characteristics that allow the survival in this environment have not been investigated. In this context, we have constructed a metagenomic library from human bile of liver donors, in order to analyse the activities responsible for bacterial survival in presence of bile.

Methods: For the generation of the library we used total DNA isolated from human bile. Fragments with a size of approximately 20-30kb were cloned in the vector pCC1FOS™ (Epicentre®) and transformed into competent *Escherichia coli*. The pool of *E. coli* clones was tested in different concentrations of bile salts in order to detect highly resistant clones.

Results: Growth experiments allowed us to select five clones with a bile resistance phenotype. The selected clones were able to grow in a bile concentration at least 10 times higher than the *E. coli* strain containing the empty vector. The five fosmids were sequenced using Illumina technology and the bioinformatic analysis of the sequences showed the presence of a variety of potential genes that could play a role in bile resistance, coding for putative proteins involved in oxidative stress response, as well as DNA repair proteins, transmembrane pumps and lipopolysaccharide biosynthesis enzymes. Interestingly, the majority of DNA sequences displayed a low homology with genomic sequences of known microorganisms, suggesting that the native biliary microorganisms harbouring the DNA inserts could be new microbial taxons.

Conclusions: Five DNA fragments from biliary microorganisms, able to confer bile resistance in *E. coli*, were identified in our study. Further work is needed in order to unravel the specific DNA sequences responsible for the observed phenotype.

GUT MICROBIOTA AS A NEW PREDICTIVE FACTOR IN ACTIVE RELAPSING-REMITTING MULTIPLE SCLEROSIS

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Background: Microbiota influence on different illnesses has been studied for over 100 years. However, during the last 5-10 years significant efforts have been focused on demonstrate the relevance of microbiome in neurological diseases. Our recent findings show the effect of gut microbiota composition specifically in multiple sclerosis. In this study we have analyzed the gut microbiota in patients with relapsing-remitting multiple sclerosis and its relationship with the disease evolution.

Methods: The gut microbiota in a cohort of 16 patients with active relapsing-remitting multiple sclerosis and a control group of healthy population was compared by 16s rRNA massive sequencing. After a 24-month follow-up period study, a correlation analysis between microbiota data and the new relapses and new central nervous system injuries was performed in the group pf patients with the neurological disease. Besides, we analyzed the correlation of these new relapses or injuries and BMI.

Results: The gut microbiota of patients with multiple sclerosis differs from the healthy population. We have detected significant differences in the levels of some bacteria, including, but not only, the family *Ruminococcaceae*; and the genus *Gemmiger*, *Prevotella*, *Streptococcus*, *Ruminiclostridium*, *Ruminococcus*, *Succinivibrio* and *Sutterella*. Spearman's statistical analysis shows a positive correlation between some of these bacteria with the severity of the disease. On the other hand, we have not found a significant correlation between the BMI and the progression of the disease.

Conclusions: There is a clear difference in the microbiota of patients with active multiple sclerosis from the healthy population. Some of these microorganisms are associated with a worse prognosis, suggesting the role of microbiota composition as a predictive factor of the disease evolution.

EFFECT OF MICROBIOTA MEMBRANE VESICLES ON HUMAN DENDRITIC CELLS ACTIVATION AND DERIVED T-CELL EFFECTOR RESPONSES

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Background/Aims: Dynamic and complex interactions between the microbiota, the intestinal epithelium and the host immune system are well-known. The influence of gut microbiota on health and disease has been proven. There is scientific evidence that certain gut microbes, especially probiotics have a beneficial role in intestinal homeostasis. However, the microbiota factors that modulate host immune and defense responses have not been always well characterized. All bacteria release extracellular membrane vesicles (MVs) as a mechanism of intercellular communication. The objective of this study was to evaluate the immunomodulatory effect of MVs isolated from *Escherichia coli* strains of intestinal origin on dendritic cells (DCs) and the induced T-cell (LT) effector response.

Methods: MVs from five *E. coli* strains were isolated and tested: the probiotic *E. coli* Nissle strain 1917 (EcN), the derived mutant lacking the outer polysaccharide capsule (EcN: K5) and the commensals ECOR12, ECOR63 and ECOR53. Monocytes isolated from buffy coats from healthy donors were cultured and differentiated into DCs for 7 days, and then incubated with MVs for 24 h. Stimulated DCs were then co-cultured with isolated LT CD4+. DCs and lymphocytes were analyzed by flow cytometry and the supernatants collected for cytokine quantification by ELISA and MULTIPLEX inmunoassay.

Results: MVs from all the strains induced maturation of DCs ($p < 0.05$), although with different IL-10, IL-6 and TNF-α secretion profile ($p < 0.05$). After 4-day coculture (mDCs/LTs) MVs induced polarization of LTs towards a TH17 response ($p < 0.05$) with the exception of ECOR53 vesicles ($p < 0.05$). MVs from ECOR12 induced greater polarization to T regulatory response (Treg) than EcN MVs ($p < 0.05$), whereas the others did not cause any significant increase in Treg cells.

Conclusion: Microbiota MVs are sensed by immature DCs and specifically modulate LT cell responses, thus acting as key players in the modulation of the host immune system.

FEEDING TWO *BACILLUS* STRAINS IN COMMERCIAL SOWS: EFFECTS ON REPRODUCTIVE PERFORMANCE AND GUT MICROBIAL ECOSYSTEM

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The aim of the present study was to assess in breeding sows the effect of long term administration (three parities) of two different *Bacillus* strains on their reproductive performance and the gut microbial ecosystem. For this purpose, 90 Landrace x Yorkshire dams were selected and randomly allotted into 3 treatments: a control group (CON) fed a standard diet or supplemented with $5x10^8$ cfu/kg *B. subtilis* 25841 (PR1) or $5x10^8$ cfu/kg *B. amyloliquefaciens* 25840 (PR2). Reproductive parameters were recorded along the three reproductive cycles. Faecal samples (n= 13, n= 11 and n= 14 for CON, PR1 and PR2, respectively) were taken on days 8 and 21 of the third lactation for microbiota analysis by Illumina MiSeq 16S RNA. Both supplemented groups showed higher number of born piglets per litter (18.3, 19.5 and 20.6 for CON, PR1 and PR2, P= 0.01) and sows receiving PR2 also presented a higher number of born alive (15.7, 15.6 and 17.4, P= 0.01) and weaned piglets (13.9, 13.6 and 14.4; P= 0.002). Regarding colonic microbiota, no significant changes were registered in alpha, nor beta-diversity and scarce effects of the diets were found in the community structure (ANOSIM test, P= 0.08). However significant changes were observed at phylum level (Bacteroidetes; 22.4, 18.7, 18.6 %, P= 0.03) with decreases in the Prevotellaceae (9.4, 7.3, 7.3 %; P= 0.03) and increases in the Ruminococcaceae family (16.1, 13.1, 13.7 %; P= 0.04). Several genera were also modified by the probiotic strains including, *Prevotella, Ruminococcus, Megasphaera, Oscillospira, Dorea, Blautia* or *Roseburia*. In conclusion, the addition of *B. subtilis* 25841 and 25840 to the sow diet enhances their

reproductive performance in terms of an increased prolificacy. The strain 25840 also exhibit improvements in the viability of born piglets with increases in the number of weaned piglets per litter. Their administration along three parities demonstrated to have a clear impact on gut microbial ecosystem.

THE EFFECT OF DIETARY MODIFICATIONS ON GUT BACTERIAL MICROBIOME IN GROWING SWINES

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Background: Spanish swine industry is the forth potency in the world and the second potency in Europe (17.4% of European production; source: Ministry of Agriculture, Fisheries and Food). The improvement of the health status and growth performance of swine will revert in important economic benefits in the sector. An in-depth knowledge of the Spanish swine microbiome, followed by the assessment of the effects of dietary changes (i.e. probiotics and prebiotics) in the microbiota are recognized essential strategies to increase health and performance in pigs. In this study a characterization of Spanish swine gut microbiome and the prebiotic effect of diet have been analyzed.

Methods: A total of 32 Duroc x (LDxLW) growing-finishing pigs were included in the study. Animals were divided in two groups and fed with standard diet and with a potential prebiotic diet containing 24% citrus pulp, respectively. Faecal samples were obtained before the administration of the experimental feeds (time 0) and six weeks after. Data concerning weight and health status were also recorded. DNA was extracted with a modified commercial protocol and libraries obtained. Bacterial microbiome was obtained by MySeq platform (Illumina). Raw sequences were filtered based on quality, merged and annotated.

Results: In all cases, rarefaction curves reached a plateau, showing a complete saturation in the annotation of the sequences. Time 0 microbiome was analyzed to ensure that no significant differences existed before diet modification. The evolution of microbiome with standard and modified diet showed significant differences in genera, highlighting *Clostridium* and *Turicibacter*, which showed different evolution depending on the diet.

Conclusion: The results obtained let us firstly to characterize Spanish swine microbiome. Moreover, citrus pulp-diet showed gut microbiome differences after six weeks of consumption, preliminary pointing to a prebiotic effect of this compound, and support further studies on prebiotic and probiotic diet addition in swine.

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Clinical Uses

BENEFICIAL EFFECTS OF PREBIOTIC FORMULATION IN FRAILTY SYNDROME IN A RANDOMIZED AND PLACEBO-CONTROLLED TRIAL: ROLE OF CYTOKINES

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Background/Aims: Chronic administration of prebiotic formulation containing inulin and fructo-oligosaccharides reduces frailty syndrome in older people. The molecular mechanisms of these effects are unknown and we sought to evaluate the role of pro-inflammatory and anti-inflammatory cytokines in these effects.

Methods: We assessed frailty syndrome by the frail index (FI) score, and pro-inflammatory (TNF-alpha, IL-6 and IL-1beta) and anti-inflammatory (Il-4, IL-10) cytokines in individuals of the treatment (prebiotic formulation administered during 13 weeks) and control group in a randomized and placebo-controlled trial.

Results: At the 13-week follow-up, the placebo group had higher FI levels (preFI 0.23 ± 0.11, 35 postFI 0.24 ± 0.12, p= 0.012) and the intervention group had lower FI levels (preFI 0.22 ± 0.09, 36 postFI 0.20 ± 0.08, p< 0.001). Those individuals that showed an improvement in FI after administration of the prebiotic formulation showed a significant (p< 0.05) higher levels of anti-inflammatory cytokines in blood compared to individuals in the placebo group. In contrast the level of pro-inflammatory cytokines as well as leukocytes counts in blood were unchanged after prebiotic treatment.

Conclusion: Prebiotic administration reduces frailty levels through increasing the concentration of some peripheral anti-inflammatory cytokine levels. This clinical trail opens the possibility to modulate the chronic low-grade inflammation in frailty syndrome in older individuals through prebiotic administration.

LACTOBACILLUS GASSERI LA806 SUPPRESSES VISCERAL HYPERSENSITIVITY AND BARRIER DISRUPTION INDUCED BY CHRONIC STRESS IN RATS

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Introduction: Irritable bowel syndrome (IBS) is a common functional disorder. Underlying mechanisms include gut microbiota unbalance and altered intestinal permeability with subsequent bacterial translocation. Specific probiotic strain ability to adhere well to intestinal cells may play a pivotal role in restoring the microbiota and gut barrier, which might be of significant value in IBS treatment.

Objective: To assess in a model of IBS the efficacy of *Lactobacillus gasseri* LA 806 previously characterized *in vitro* for its capacity to adhere to gut epithelial cells and to reinforce the epithelial barrier.

Methods: Male Wistar rats were treated orally with 1 mL of NaCl 0.9% alone or containing *L. gasseri* LA806 (10^9 UFC/mL) during 15 days and were submitted to a 4-day water avoidance stress (WAS) session on day 12 of treatment. A group of rats not submitted to WAS and orally treated with the vehicle was used as control (basal condition). Visceral sensitivity was measured (myoelectrical activity in response to colorectal distensions [CRD]) and intestinal permeability assessed using ^{51}Cr-EDTA as a marker.

Results: *L. gasseri* LA806 reduced significantly WASinduced hypersensitivity to CRD at the two highest volume of distension (18 ± 3 vs. 31 ± 5 for WAS + vehicle at 0.8 mL and 20 ± 2 vs 29 ± 2 for WAS + vehicle at 1.2 mL). When administered orally at 10^9 UFC/day during 15 days, *L. gasseri* LA806 reduced significantly WAS-induced hyperpermeability: 1.95 ± 0.14 for WAS + vehicle/LA806 vs. 2.93 ± 0.21 for WAS + vehicle (p= 0.0018). No significant difference was observed between the group no WAS + vehicle and the group WAS + vehicle/LA806.

Conclusion: *L. gasseri* LA806 is able to decrease abdominal pain (visceral sensitivity) in part by restoring intestinal epithelial barrier function (intestinal permeability decrease) in a rat model of IBS.

SYMBIOTIC, CONTAINING *BACILLUS COAGULANS* LMG-S-24828 REDUCES GASTROINTESTINAL ADVERSE EFFECTS IN PATIENTS USING MIGLUSTAT OR TK-INHIBITORS

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Introduction: Some tyrosine kinase inhibitors (TK-i) used in the treatment of chronic leukemias and substrate inhibitors (Miglustat), an iminosugar used in lysosomal diseases, can cause gastrointestinal disorders such as diarrhea, distension and abdominal pain. These adverse effects decrease the quality of life related to health and lead to early withdrawal of treatment. Some probiotics have shown an improvement in the symptoms mentioned in patients with func-

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tional digestive disorders [1]. Hypothesis: a symbiotic, containing *Bacillus coagulans* LMG-S-24828, minimizes the gastrointestinal adverse effects associated with the use of TK-i or Miglustat.

Objectives: To evaluate in patients treated with TK-i or Miglustat the effect of the controlled administration of the symbiotic for a month, regarding to rhythm and type of stools.

Method: Randomized cross-over trial in which 15 patients with TK-i or Miglustat were blinded to placebo or symbiotic in a daily dose, with a "washing" phase of two months between the administration of each of them. The patients were asked to complete the Bristol Stool Chart (BSC) every day during the study period. This score allows to identify the stool form using seven different images with accompanying written descriptors. The frequency of withdrawal of treatment with TK-i and Miglustat in each group will also be evaluated. The analysis of the results will be carried out using the Student's t-test, considering the statistical significance of the differences with p-value < 0.05. The protocol was approved by the Autonomous Committee of Ethics.

Results: The study period has not yet concluded before deadline. The results and conclusions will can be provided in the meeting.

References: 1) Simrén M, Barbara G, Flint HJ, et al. Intestinal microbiota in functional bowel disorders: a Rome foundation report. Gut. 2013; 62: 159-76.

EFFECT OF THE ADMINISTRATION OF A PROBIOTIC WITH *LACTOBACILLUS* AND *BIFIDOBACTERIA* ON ANTIBIOTIC-ASSOCIATED DIARRHEA

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Background/Aims: Antibiotics treatment is related to diarrhea (AAD). There is a lack of studies demonstrating the beneficial effect of using a specific probiotic combination: Pearls IC[a] (*Lactobacillus acidophilus* NCFM, *Lactobacillus rhamnosus* Lr-32, *Bifidobacterium breve* M-16V, *Bifidobacterum longum* BB536, *Bifidobacterium lactis* BI-04 and *Bifidobacterium bifidum* Bb-02). The aim of the study is to analyze the effect, safety and acceptability of this combination of probiotics on diarrhea associated with treatment with amoxicillin-clavulanic acid (CA).

Methods: Pilot, unicentric, randomized, double-blind, parallel group, placebo-controlled study (probiotic vs. placebo for 30 days). *Target population:* Patients older than 18 years, both sexes treated with CA (850 mg/125 mg every 8 h/orally) for 7 days. *Sample size:* n = 40. Considering a prevalence of the antibiotic effect in the stools of 75% and a reduction by the antibiotic effect of 35% (80% powered and 95% confidence). *Subjects:* Adult patients who attended the Emergency Department (Dexeus Hospital, Barcelona) between January and April of 2018 with prior informed consent with a follow up in primary care at 30 days. *Variables:* The differences between day 0 and day 30 of the number of daily stools and duration of diarrhea were evaluated; Stool consistency according to Bristol Stool Form, Quality of intestinal life (GIQLI). Subjective evaluation and evaluation of adverse effects of the product through a specifically designed questionnaire. *Statistical methods:* U-Mann-Whitney test. Significance level of 5%. Software R v3.4.2.

Results: Thirty-six subjects were included (18 per group). Pearls IC[a] delayed between 4 and 5 days the appearance of the diarrheic episode vs placebo ($p < 0.001$) with a tendency to decrease the number of daily bowel movements and a better subjective assessment.

Conclusions: Pearls IC[a] demonstrated its beneficial effect on DAA by delaying the onset of diarrhea and showed a tendency to decrease the number of daily stools vs placebo.

PATIENT CHARACTERISTICS INFLUENCING INFANT COLIC AMELIORATION UNDER A PROBIOTIC TREATMENT

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Background/Aims: Probiotic interventions are gaining clinical evidence for the treatment of infant colic and other functional gastrointestinal diseases (FGIDs). However, patient characteristics facilitating or preventing response to probiotic intervention in colicky babies have not been studied.

Methods: Prospective, observational trial in babies diagnosed for colic and/or functional constipation, and initiating treatment with an oil suspension containing *B. longum* CECT7894 and *P. pentosaceus* CECT8330 ($\geq 10^9$ cfus daily). Exclusion criteria included preterm delivery, antibiotic or probiotic use within 2 weeks of enrollment, and concomitant acute or chronic medical conditions. Severity of colic, constipation and other FGIDs was rated by pediatricians on a 5-point Likert scale each, at baseline and after 2 weeks. Parental anxiety was measured with the GAD-7 scale. Effect of patient characteristics was assessed by multivariate linear regression.

Results: 36 babies (64% female, 53% cesarean delivery, age range 1-40 weeks) were available for analysis. 85% had moderate-to-severe colic symptoms, 63% had concurrent functional constipation and 45% had other FGIDs. Prevalence of breastfeeding, formula-feeding and mixt feeding were 38%, 38% and 24%. Moreover, 33% of babies had previously failed other treatments for colic symptoms, and 50% of them had at least one parent with anxiety. Colic severity was reduced by 1.4 ± 0.9 points ($P< 0.001$) and constipation severity was also reduced. Colic improvement was significantly higher in babies with higher baseline scores, and lower in babies having one or more parents with anxiety or also displaying constipation (multivariate $R^2= 0.55$, individual factors p-values ranging 0.049 to < 0.001). Type of feeding, mode of delivery, gender, body weight and previous failure of other colic interventions did not influence the change in colic severity in babies taking this particular probiotic formula.

Conclusions: Baseline severity, concomitant FGIDs and parental anxiety can influence treatment response in babies receiving a probiotic intervention for infant colic.

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ITS-SEQUENCING REVEALS ALTERATIONS ON THE ABUNDANCE OF SPECIFIC GUT *BIFIDOBACTERIUM* POPULATIONS IN PREMATURE BABIES

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Background/Aims: The correct gut microbiota colonization at the beginning of life is a key event for the foundation of early and future health. This process is compromised in premature babies due to different aspects: immaturity, long hospital stay, medications, difficulties for oral feeding, etc. Several studies have identified the alterations on this process, being *Bifidobacterium* one of the most affected microbial groups. *Bifidobacterium* is one of the first colonizers and dominant in the intestinal microbiota of breast-fed healthy babies. Moreover, some strains are widely used as probiotics, with premature babies being among the population groups that could benefit more from the development of probiotics for promoting a correct microbiota establishment. Here we aimed at characterizing the colonization and development of bifidobacterial microbiota in premature babies exposed to different perinatal factors.

Methods: Faecal samples were collected at 2, 10, 30 and 90 days of life from 40 premature- and 40 full term-babies (for comparison). DNA was extracted, used for PCR-amplification of the ITS region, submitted to next-generation-sequencing and the sequences were annotated against an improved bifidobacterial ITS database (Milani et al. 2014).

Results: We found noticeable differences in the abundance of bifidobacterial species between premature and full term babies along the first three months of life. Among the different perinatal factors studied, delivery mode and feeding, further affected the composition of bifidobacteria in premature babies.

Conclusions: ITS region allowed to monitor the gut bifidobacteria colonization during the first months of life. This work confirm that different perinatal factors affect the microbiota development in preterm babies and extends this observation to the specific influence on the bifidobacterial microbiota. This would allow improving the development of bifidobacteria as probiotic for promoting a correct gut microbiota colonization in these infants.

SURVEY ABOUT KNOWLEDGE AND USE OF PROBIOTICS AND PREBIOTICS BY PEDIATRICIANS

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Introduction: The contribution of probiotics, prebiotics and symbiotics to the organism allows to reinforce a healthy balance between microorganisms that form intestinal microbiota. Therefore, given their beneficial effects on nutrition and health, health professionals tend to use them more frequently. The aim of the study was to establish the general knowledge of physicians and nurses specialized in pediatrics about prebiotics, probiotics and symbiotics, as well as to determine their use in routine clinical practice.

Method: The Spanish Society of Probiotics and Prebiotics (SEPyP) developed an online survey in November 2017 that consisted of 14 questions about the relationship between breast milk modulatory nutrients, infant milk formulas and intestinal microbiota. The survey was sent by email to 6134 pediatricians and pediatric nurses registered in the database of the course sponsored by Nutricia: "Nutrition in Pediatrics", obtaining a total of 537 responses, received in two rounds. All data were analyzed with the statistical package SPSS.

Results: 75% of the surveyed professionals were pediatricians, of which 52% belonged to primary care. 86% knew correctly the definition of probiotics and a 74% knew the one of prebiotics. However, 5% confused the two terms. Up to 31% of professionals were unaware that both compounds are present in breast milk. An 87% of the participants used probiotics in their clinical practice. The most commonly used forms of administration were pharmacological preparations and infant formulas (30% and 21% respectively). 52% used prebiotics frequently, mainly pharmacological preparations. Only 40% of the participants used symbiotics in their clinical practice, though just 79% of the participants could identify them correctly.

Conclusion: Despite the fact that most of professionals dedicated to Pediatrics know, distinguish and use probiotics and prebiotics, there is still a great lack of knowledge about the fundamental concepts of the relationship between the microbiota and health, as is the case with breastmilk. As a positive data, 99% of these professionals consider it necessary to continue receiving training on intestinal microbiota, probiotics and prebiotics.

Conflict of interest: This work has been funded by the company Nutricia-Danone.

THE INVOLVEMENT OF THE PEDIATRICIAN IN EMERGENCIES, ESSENTIAL IN THE ADHERENCE TO THE RECOMMENDATION OF PROBIOTICS

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Background: Probiotics are not funded products, whose acquisition depends on the recommendations of the pediatrician, being very important their training and involvement for information to families. Despite the scientific evidence, its recommendation is not done systematically, and the consequences are worse health outcomes, increased use of human resources and health care cost. The objective of the study is to verify the adherence to the recom-

mendation of probiotics, in a pediatric emergency service, participating in a project to improve the quality of care by systematically recommending them.

Methods: Quality improvement project.

- All children 0-14 years old, attended in Pediatric Emergency, for infectious pathology that equals oral antibiotherapy and GEA. Recommendation in writing of probiotics.
- Professionals: 7 pediatricians of the Emergency Department.
- Start of the project: Noviembre 2017, continues in active.
- Verification of adherence to the recommendation through a telephone survey of 192 randomly selected families.

Results:

- Probiotic acquisition 173/192 (90,1%). 19 cases have not acquired the most frequent cause not consider it necessary or unavailable in pharmacy. The price was the cause at 26%.
- In 90.1% the recommended probiotic was acquired. In 11 cases, another was acquired on the recommendation of the pharmacist. The price was only in 1 cases the reason of the change.
- 18% did not complete the treatment. The most frequent cause (71%) was not considered necessary and 29% difficulties for its administration to the child.

Conclusions:

- High adherence to the recommendation (90%).
- Good filling (82%).
- False idea that the price hinders the acquisition of the product.
- The involvement of pediatricians in the recommendation of probiotics is essential to improve the quality of care in the emergency department.

USE OF GUTALIVE® AND ITS IMPACT ON THE STANDARDIZATION OF DOWNSTREAM MICROBIOTA-BASED STUDIES

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Aim: The aim of this work was to evidence that the use of different stool collection strategies has a deep impact on the viability and diversity of the fecal microbiota that is recovered in the laboratory, notably if oxygen toxicity is not considered. This is particularly critical if the purpose of the sample is to envisage personalized biotherapeutic purposes, such as autologous fecal microbiota transplant or designing personalized biotherapeutics.

Methods: In order to analyze differences in the viability of fecal microbial populations during the whole delivery procedure, the same fecal specimens were sampled in conventional stool containers and GutAlive® devices, which minimize exposure of fecal microbiota to oxygen. Samples from five healthy donors were used and 150 differential colonies between the two devices were recovered. Differences included those associated to the two collection devices and individuals. All colonies were identified by 16S rRNA gene sequencing. Complete genomes of two extremely oxygen sensitive (EOS) bacteria recovered were sequenced with the Illumina MiSeq Sequencing System.

Results: Microbial diversity obtained was notably higher using GutAlive®. This device was able to maintain the viability of EOS species such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii* and a possible new member of the *Clostridiales* order. These obligate anaerobes were not recovered using the conventional stool container. Remarkably, GutAlive® allowed culturing and identifying an anaerobic isolate which may represent a new lineage within *Clostridiales*. This shows the importance of a personalized approach in microbiota-based therapies, as this novel isolate was recovered only from feces of one of the donors.

Conclusion: Using GutAlive® for stool sampling and transport allowed higher recovery of viable EOS bacteria by limiting oxygen exposure during the whole process. By standardizing the sampling and transport of the fecal specimens to the lab, GutAlive® can be applied for the normalization of microbiota-based studies, analysis and developments.

Competing interests: NM is a full-time employee of Microviable Therapeutics; CH-C, SD, AM and BS are co-founders and SAB members of Microviable Therapeutics, and also co-inventors on at least one patent regarding microbiome stool collection kit and applications.

SCREENING OF *LACTOBACILLI* STRAINS OF HUMAN ORIGIN CANDIDATES FOR THE PREVENTION OF URINARY TRACT INFECTIONS

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Background/Aims: Intestinal and vaginal pathogen reservoirs are thought to play a key role in recurrent urinary tract infections (rUTIs) in women. Preventive probiotic interventions are gaining interest to avoid excessive antibiotic use, and disruption of the normal microbiota. We sought to identify and characterize candidate probiotic strains capable of reducing pathogen reservoirs for the prophylaxis of rUTIs.

Methods: Screening for survival in in vitro simulated gastric (pH 3), intestinal and vaginal conditions, antagonistic capacity against urinary pathogens, presence of bacteriocins genes and resistance to specific antimicrobial agents was performed on *Lactobacillus* isolates from human origin and compared with *L. rhamnosus* ATCC 55826 reference strain (vaginal isolate).

Results: *L. plantarum* strains CECT8675 and CECT8677 displayed the best characteristics. They showed similar growth to *L. rhamnosus* ATCC55826 strain under simulated vaginal and intestinal conditions, but increased survival to simulated gastric conditions ($p< 0.05$ and $p< 0.10$, respectively). A significant decrease in growth was observed for rUTI pathogens *E. coli* UPEC, *P. mirabilis* S. saprophyticus, *K. pneumoniae* and *E. faecalis* when co-cultured with the supernatants of candidate probiotic

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strains, both unadjusted and adjusted to the same pH as control media (pH 6). The pathogen inhibition profile differed for these two candidate probiotic strains. Our results showed inhibition not only due to lactic acid, but also to the presence of different plantaricin genes: *plnEF* in *L. plantarum* CECT8675 and *plnW* in *L. plantarum* CECT8677. Moreover, CFU/mL counting of *E. coli*, *S. saprohyticus*, *P. mirabilis* and *K. pneumoniae* after co-incubation showed not only a bacteriostatic effect, but also bactericidal activity after 24 hours of co-incubation (higher than *L. rhamnosus* ATCC55826). Finally, both *L. plantarum* strains showed a safe antibiotic resistance profile according to ISO 10932:2010 IDF 2010.

Conclusions: Candidate strains *L. plantarum* CECT8675 and CECT8677 deserve further evaluation in clinical studies for rUTI prophylaxis.

SELECTION AND VALIDATION OF *LACTOBACILLUS RHAMNOSUS* CECT 8800 AS AN OPTIMAL PROBIOTIC STRAIN FOR VAGINAL APPLICATIONS

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Background/Aims: Bacterial strains (normally lactobacilli) vaginally administered have been extensively tested on the treatment of different vaginal conditions, although the selection and characterization of these bacterial strains are commonly based on poor criteria. The aim of these work was to perform a comprehensive analysis of the optimal strain to be selected for this purpose.

Methods/Results: Forty-four strains were obtained out of fourteen samples of healthy women's vaginal swabs. Molecular identification of each strain was performed by RAPD and 16S rRNA sequencing. Six of these strains were lactobacilli from species *L. casei*, *L. jensenii* and *L. rhamnosus*. In addition, collection strains *L. crispatus* CECT 4840 and *L. iners* DSM 13335 were included in this study. First, pH reduction was measured in pure and co-cultures of these strains and strains *L. rhamnosus* CECT 8800 and *L. casei* BPL013 were selected for further studies. Both strains are producers of short chain fatty acids (SCFAs), being *L. rhamnosus* CECT 8800 the most efficient. Further studies with *L. rhamnosus* CECT 8800 regarding protection from pathogens infection of endometrial primary cultures (G. *vaginalis, A. vaginae, P. acnes* and *S. agalactiae*), quantification of secreted pro-inflammatory cytokines, antibiotic resistance, and resistance to pharma products for IVF treatment and toxicology were performed. *L. rhamnosus* CECT 8800 resulted as an efficient protector from main vaginal pathogens, reducing the pro-inflammatory cytokines levels produced by a non-lactobacilli dominated microbiota, no carrying genetic information for antibiotic resistance and being resistant to progesterone treatment. *In vitro* cytotoxity test and in vivo retarded hypersensitivity and vaginal mucosa irritation tests were performed with the selected strain showing an optimum safety profile to be used vaginally.

Conclusion: *L. rhamnosus* CECT 8800, is an optimal candidate for vaginal microbiota restorer treatments. The strain has shown to be the most efficient from the group studied in terms of SCFAs production, high pH reduction phenotype, anti-inflammatory and anti-infective properties and excellent toxicological and safety profile.

CREATION OF AN ONLINE PLATFORM FOR THE SPREAD OF KNOWLEDGE ABOUT THE USE OF PROBIOTICS AND PREBIOTICS IN CLINICAL PRACTICE (ELPROBIOTICO.COM)

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Introduction: El Probiótico (www.elprobiotico.com) is a website launched in January 2014. The purpose was to spread information for health professionals about the evidence and clinical practice of probiotics and prebiotics.

Material and methods: Under the coordination of the Scientific Committee (led by the doctors Francisco Guarner and Guillermo Álvarez Calatayud), are published in the portal weekly contents of free access in relation to the topicality of microbiota, probiotics and prebiotics. Since the launch, the offer of programs accredited by the Commission for continuing training of SNS and CCFCPS has been extended periodically, with three major accredited courses currently available: "Probiotictherapy in Gastroenterology ", "Scientific evidence and clinical practice guidelines for the use of probiotics, prebiotics and symbiotics" and "Probiotics and prebiotics in health and disease" as well as other monografic review topics.

Results: Five years after the launch of the Web, 123 short articles, 8 review topics and 19 clinical cases, as well as multimedia content and other formats have been published. 25,151 users have been registered on the platform and about 6,200 different people visit each month, with an average time per visit of 4 minutes and 17 seconds. The most consulted contents are those of the formative area, with more than 15,000 evaluation tests carried out among all the users since the launch.

Conclusions: The increase observed in registration and participation statistics the training activities of the portal confirms the efficacy of the Web probiotic as a tool to spread knowledge about the microbiota and the clinical use of probiotics and prebiotics among health professionals and suggests an increase in the interest of this group for these issues in recent years.

EVALUATION OF PROBIOTIC POTENTIAL IN STRAINS ISOLATED FROM HUMAN MILK AND INFANT FECES

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Background/Aims: Breast milk is a component of high food value due to the presence of essential nutrients and elements such as: immune cells, immunoglobulins, antimicrobial peptides and microorganisms both diners and beneficial, among these, the Lactic acid Bacteria (BAL) of the genera *Streptococcus, Enterococcus, Lactococcus, Lactobacillus, Weissella, Bifidobacterium* and *leuconostoc*, recognized for having probiotic potential, that is to say, that administered in adequate quantities are capable of influence the health of the individual. In Colombia, mainly in Antioquia there are few studies that characterized the natural microbiota of biological fluids such as breast milk and the feces of exclusively breastfed infants, so from the realization of a study in this population, obtained 34 isolates.

Methods: They underwent in vitro tests to evaluate their probiotic potential and were identified using biochemical and molecular techniques.

Results: It was verified that the BAL isolates of both milk and feces have good probiotic potential, resisting hostile conditions of acid pH and bile salts at 0.3%, in addition to good susceptibility to antibiotics and as antagonists against microorganisms pathogens. Of the 10 molecularly identified strains, the best bacteria with probiotic potential of 100% were: *Enterococcus faecium* isolated from breast milk, *Enterococcus faecalis* isolated from the feces of infants from which also isolated a *Lactobacillus rhamnosus* with a potential of 93.75%.

Conclusion: The consumption of breast milk as a first food is very important not only from the nutritional point of view but also microbiological due to the presence of BAL with probiotic capacity but it is recommended to use molecular tests for a total trace of probiotics In both biological sources.

CHANGES CONCERNING TO THE LEVEL OF KNOWLEDGE AND PRACTICES ABOUT PROBIOTICS AMONG ARGENTINE DOCTORS IN THE LAST 5 YEARS

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Introduction: Practices about probiotics have changed drastically in the last decade due to increasing knowledge on scientific evidence. In Argentina there are no published data about this topic.

Aim: To evaluate knowledge, attitudes and practices of physicians about probiotics in two periods of time.

Methods: A closed-ended structured questionnaire was implemented in two periods of time (2012-2017) to physicians in the city of Buenos Aires. Target and sample size: 120 and 95 doctors were interviewed (Pediatricians and Gastroenterologists).

Results: More than 50% of doctors are familiar with probiotic use, showing statistically differences between 2012 and 2017 survey (44% vs 69,4%; p 0.0003 Z proportion Test). Probiotic definition according to FAO/WHO criteria was correct in both groups (71,8% vs 75,6%), showing better recognition as a yeast in the 2017 group (p 0.002). Probiotics characteristics were better recognized as to adhering to the mucosa and alive microorganisms in the second group (p 0.01) and *Saccharomyces boulardii*/*Lactobacillus casei* have been proven to work in acute infectious diarrhea and antibiotic associated diarrhea (39,7% and 21,2%; p 0.004). Although doctors recommend probiotics to their families (42,10%) and themselves (29,4%), this tendency was increased in the last 5 years in both groups (67,3% and 70,5% respectively; p 0.0001). There is a high level of confidence in both groups (83% and 82,2%) considering probiotics safety (80% and 69,3%) and being used in children (66% and 68,3%) with no statistical differences between groups. Reasons for not recommending or prescribing probiotics are: no official guidelines (20,3% and 23%) and not having enough experience (26,8% and 28%). Overall 87% and 82% doctors in both groups read at least 1 article/year about probiotics. Information resources are: pharmaceutical industry (23%), congresses (20,5%) and journals (20,1%) in the first group and congresses (31,5%), internet (20.2%) and journals (18,4%) in the second one, showing statistically differences between the groups (p 0.002; Z proportion Test)

Conclusions: Most doctors feel well informed about probiotics with high level of confidence on their safety. More than 70% recommend probiotics for their relatives or themselves. Lack of information is a key obstacle for not prescribing probiotics even though more than 80% read at least 1 article/year related to probiotics. Continuous medical education is key to promote the use of them and to increase awareness among the scientific community.

Immunonutrition & Veterinarian

COMBINING TWO PROBIOTIC STRAINS WITH OLIGOGALACTOSE ABOLISHES THEIR BENEFITS AGAINST F4+ETEC IN PIGLETS

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Background/Aims: This study evaluates the efficacy of combining two probiotic strains (*Bifidobacterium longum* subsp. *infantis* CECT7210 (Laboratorios Ordesa S.L.) and *Lactobacillus rhamnosus* HN001) with oligogalactose to improve their efficacy against enterotoxigenic *Escherichia coli* (ETEC) F4+.

Methods: A total of 96 piglets of 21 days were distributed into 32 pens assigned to 5 treatments: one non-challenge treatment (CTR+) and four challenged: same diet (CTR-), or supplemented with probiotic strains (> $3x10^{10}$ cfu/kg each) (PRO), prebiotic (5%) (PRE) or their combination (SYN). After one week, piglets were orally inoculated with F4+ ETEC ($1.2x10^{10}$ cfu). Feed intake, weight gain, fecal consistency and rectal temperature were recorded. On days 4 and 8 post-inoculation (PI), one animal per pen was euthanized and samples of blood, feces and tissues collected. Enterobacteria and coliform counts, fermentation products, ileal histomorphology and serum TNF-α and PigMAP were analyzed.

Results: Group PRO performed better after the challenge than SYN, reducing the number of enterobacteria and coliforms in ileal mucosa scrapes. Fermentation was scarcely modified by the treatments. The challenge promoted an important decrease of villous height at day 4 PI similar in all diets. At day 8 PI, PRO fed animals showed improved recovery of villi height (337^{a} CTR+, 292^{ab} PRO, 272^{b} PRE, 269^{b} SYN, 267^{b} CTR-; P< 0.001). Acute phase protein PigMAP was markedly increased by SYN treatment at day 8 PI (0.51^{b} CTR+, 0.57^{b} PRO, 0.56^{b} PRE, 2.43^{a} SYN, 0.62^{b} CTR- mg/ml, P= 0.003). Pro-inflammatory cytokine TNF-α was also increased by SYN at day 4 PI (86^{ab} CTR+, 98^{ab} PRO, 75^{b} PRE, 118^{a} SYN, 77^{b} CTR- mg/ml, P= 0.013).

Conclusion: To conclude, results show that animals receiving the probiotic combination performed better against the F4+ challenge showing a higher recovery of ileal mucosa. However, these benefits disappeared when probiotics were combined with oligogalactose leading to detrimental effects on growth rate and pro-inflammatory markers.

THE USE OF PROBIOTICS IN SWINE: AN ALTERNATIVE FOR PREVENTING DISEASES

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The use of probiotics is an alternative to the therapeutic treatment of pathological problems in pigs. The source of many of these disorders lies in an alteration of the intestinal microbiota that may be associated with management changes, such as weaning, since the passage from a liquid to a solid diet besides the suppression of the mother's immune contribution constitute the beginning of any of these alterations.

The mechanisms of action of the probiotic strains are based on competitive exclusion phenomena between these and the invading pathogens, the stimulation of the immune response, as well as the optimization of the use of the nutrients received through the diet.

This study aims to make a first approach to the knowledge of the use of probiotics in the porcine species. For that purpose, a series of experimental tests were designed to be carried out in livestock farms that house the different stages of production (lactation and transition), in order to determine the benefits they bring, such as improvement of production rates or decrease in classic medical treatment.

The said components were administrated by means of the formulation of a commercial fermented feed that incorporates these ingredients and that was applied in the aforementioned phases. For sampling, different moments were established according to the objectives set.

The results obtained in the trials conclude that the use of product tested can have positive effects on piglet in peri-weaning by reducing the possible inflammatory effects of the lipopolysaccharides of the Gram-negative bacteria and also increased the natural immune response against the Gram-positive bacteria. Finally, the integrity of the intestinal mucosa was favoured, as seems to be inferred from the increase in the height of the intestinal villi.

FERMENTED DAIRY FOODS: IMPACT ON INTESTINAL MICROBIOTA AND HEALTH-LINKED BIOMARKERS

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Aims: The intake of fermented foods is gaining increasing interest due to their health-promoting benefits. Among them, fermented dairy foods have evidenced association with obesity prevention, and reduction on the risk of metabolic disorders and immune-related pathologies. Fermented foods could lead to these health benefits by providing to the consumer with both, easily metabolizable nutrients and beneficial microorganisms. Our aim was to evaluate the possible relationship between the consumption of fermented dairy products and the intestinal microbiota, serum lipid profile and the pro-oxidant/inflammatory status.

Methods: 151 healthy adults (age 57.9 ± 17.4 y) were evaluated. Dietary fermented food intake was assessed by an annual

food frequency questionnaire (FFQ), including 26 fermented dairy products. Levels of the major phylogenetic types of the intestinal microbiota were determined by qPCR. Serum glucose and lipid profile, as well as serum malondialdehyde (MDA), C-reactive protein (CRP) and leptin levels were determined by standardized protocols.

Results: Among fermented dairy foods, whole natural yogurt (71.0 ± 99.5 g/day), flavoured skimmed yogurt (5.9 ± 32.6 g/day) and flavoured fermented milk (2.8 ± 23.0 g/day) were the most consumed. While whole natural yogurt showed a positive association with fecal levels of *Akkermansia* and *Bacteroides* group, skimmed yogurt intake was inversely related to every analysed microbial group. Yogurt consumers showed higher levels of *Akkermansia* and *Lactobacillus* group than non-consumers. Furthermore, CRP serum levels were significantly lower in yogurt consumers.

Conclusion: Yogurt consumption was associated with higher fecal levels of certain microorganisms such as *Bacteroides* and *Akkermansia,* for which health benefits have been reported. Also, yogurt consumers showed lower CRP concentrations pointing to the need of exploring, through human intervention studies, the possible anti-inflammatory effect of these foodstuffs.

MODULATION BY *LACTOBACILLUS RHAMNOSUS* GG OF MICROBIOTA AND INNATE IMMUNE FUNCTIONS IN CHRONOLOGICAL OLD AND ADULT PREMATURE AGEING MICE

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Background and Aims: Ageing is characterized by an impairment of the homeostatic (nervous and immune) systems and by changes in the intestinal microbiota. The objective of the study was to analyse differences in the gut microbiota and in several innate immune functions in old and prematurely ageing mice (PAM) when compared with adults, and the modulatory effects of supplementing with *Lactobacillus rhamnosus* GG.

Methods: Prematurely aging mice (PAM), old and adult mice (females ICR-CD1) were divided into two groups (N = 8-10), which received LGG (10^9 CFU/mice/day; lyophilized in skim milk and added to drink water) or skim milk without the probiotic LGG (controls). After 4 weeks of LGG supplementation, mice were analysed for several innate immune parameters (chemotaxis, phagocytosis and natural killer activity). Faecal samples were collected for microbiota analysis at 0, 2 and 4 weeks of LGG supplementation.

Results: Ageing in ICR-CD1 female mice was related with increase of *Lactobacillus* and *Blautia coccoides*/*Eubacterium rectale* and decrease of *Akkermansia*. LGG supplementation was related with changes in *Bifidobacterium*, *Faecalibacterium* and *Ruminococcus* counts. PAM and old mice showed lower values of the immune functions analysed than adult mice. After 4 weeks of LGG ingestion all these functions improved, achieving values similar to those in adults.

Conclusion: *L. rhamnosus* GG can be considered a probiotic supplement useful for upgrading the immune system in ageing and, consequently, for reaching a healthy longevity.

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IMPROVEMENT IN PRODUCTIVE AND HEALTH INDICATORS IN IBERIAN PIGS SUPPLEMENTED WITH INGUBAL®

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Background: During the last years, the introduction of probiotics into the field of animal production is receiving great interest, given the constant changes in the livestock sector. Thus, the need to enhance the productivity to satisfy the increasing demand in the animal products together with the emergence of infectious diseases negatively affecting the production and the restrictions on the use of antimicrobials has led to the search for new approaches. Ingubal® is a fermented supplement containing yeast and different strains of lactic acid bacteria with proven probiotic properties and granted QPS status.

Aim: The objective was to assess the effect of Ingubal® as an alternative to the use of antimicrobials in animal feed.

Methods: Two homogeneous groups of Iberian pigs were formed according to age, sex and health status. The study group was fed with a standard feed supplemented with Ingubal® from birth to weaning and the control group received only the standard feed. Whole blood and serum were collected for complete blood count and biochemical parameters determination. Faecal swabs were collected for counting total mesophilic and lactic acid bacteria. Iberian pigs were weighed at different time points to estimate the effect on final weight and average daily gain (ADG).

Results: Our most important findings show that ADG was significantly higher in supplemented Iberian pigs. In this group, higher haematocrit, haemoglobin count, mean corpuscular volume and mean corpuscular haemoglobin were found but there were no differences in the erythrocyte count regarding the control group. A lower white blood cell count was also found in the study group.

Conclusions: These results show that the use of fermented feed with probiotic action (Ingubal®) has led to an improvement in the productive and health indicators, thereby favouring an improvement on farm profitability.

LACTOBACILLUS RHAMNOSUS MP01 AND *LACTOBACILLUS PLANTARUM* MP02 PREVENT GASTROINTESTINAL INFECTIONS IN CANINE PUPPIES AFTER WEANING

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The probiotic potential of *Lactobacillus rhamnosus* MP01 and *Lactobacillus plantarum* MP02, two strains isolated from canine milk, was evaluated through different assays, including survival to conditions similar to those found in the canine gastrointestinal tract, production of antimicrobial compounds, adherence to intestinal mucin, degradation of mucin, and pattern of antibiotic sensitivity. Globally, both strains showed a high *in vitro* probiotic potential. Subsequently, acute and repeated doses toxicity was tested in a rat model, which confirmed the safety of the strains. Finally, a clinical trial was performed to evaluate the potential of the strains to prevent episodes of infectious gastroenteritis when administered for 2 months to 1-month-old puppies. A group (n= 12, 6 males and 6 females) of German shepherd puppies received *L. rhamnosus* MP01 while a second group (n= 12, 6 males and 6 females) received *L. plantarum* MP02. The same trial was performed with Yorkshire puppies and one placebo group for each breed (German shepherd, n= 12; Yorkshire, n= 12) was also included. The results showed that administration of the strains was associated to a significant reduction of gastroenteritis episodes in both breeds. This preventive effect was associated to statistically-significant increase in the populations of lactobacilli and Faecalibacterium spp., and in the fecal concentration of butyrate, acetate and propionate. Probiotic treatment had no statistically-significant effect neither on the body weight of the puppies nor on the fecal IgA concentration, when compared with the same parameters in the control group.

Microbiology

MICROBIOLOGIC TECHNOLOGY WORKS FOR CLEANING AND DISINFECTING

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Aims: Microbiologic technology works for cleaning and disinfecting.

Methods: Trials were performed in triplicate and for each of the microorganisms separately. The chemical cleaning product was sodium hypochlorite, which is used routinely in daily cleaning. After performing each application methodologies for products section C, we proceeded to the collection of samples from the surfaces of the tiles in order to proceed to the quantitative evaluation of the presence of the pathogenic strains inoculated in each case and the remaining microorganisms. The culture media and conditions used, were the traditional environmental tests in microbiology and medium surfaces. The method for evaluating the presence of microorganisms were by contact plates or plates Roda.

Results: Microbes cleaners are good for cleaning and disinfecting. When cleaning with microbes' cleaners you have obviously more microbes left (10) in the surface than cleaning with a chemical (4) in time 0. But in time 24H once you introduce pathogens, the growth is more important in the chemical (30) cleaned surface than the one with microbes (12).

Conclusion: We learn that even using chemicals surface is never 100% free of microbes. After 24 h microbiology content on the surface are in all cases higher than before. Pathogens develop quicker when the surface has been previously cleaned by a chemical than a microbe cleaning product. It means that pathogens find less resistance and then develop quicker. The persistence of bacteria of the genera Lactobacillus or Bacillus Kocuria (included in the cleaner product formulation) does not determines a negative effect. When doing a recount, we need to use a system for detecting bacteria that facilitates screening of these groups of microorganisms considered capable of controlling pathogens, as has been shown in studies in vivo even with both men and animals.

Study done by Dr. Maria Angel Calvo (Barcelona University, Microbes Department).

COMPARISON OF DIFFERENT MODES OF REGULATION IN EXPRESSION OF DEXTRANSUCRASE FROM *LEUCONOSTOC LACTIS AV1N* AND *LACTOBACILLUS SAKEI* MN1 STRAINS

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Introduction: Lactic acid bacteria (LAB) dextransucrases, encoded by *dsr* genes, synthesise dextrans. The demand for "natural food" has led to increased use of dextrans for the development of functional foods, as they improve food texture and have immunological and antiviral properties.

Methods: In this work, the dextran-producing *Leuconostoc lactis* AV1n, isolated from avocado, was investigated. The polymer was purified from culture supernatants, its structure determined by methylation, FTIR and GC analyses, and quantified by the phenol-sulphuric method. The putative P_{dsrLL} promoter and *dsrLL* (pRCR20) or P_{dsrLL} alone (pRCR21), were cloned into the pRCR plasmid upstream of *mrfp*, the mCherry coding gene. Plasmids were stablished in *Lactococcus lactis*, transferred to other LAB by electroporation, and mCherry fluorescence was then measured spectrophotometrically.

Results: Upon transfer of pRCR20 to the dextran non-producing *Leuconostoc mesenteroides* CM70, the bacterium became red due to the mCherry expression and produced dextran, confirming that the *dsrLL* encodes the AV1n dextransucrase. The production of dextran in AV1n was temperature-dependent and in the presence of sucrose, reached ten-fold higher levels at 20°C than at 37°C. Influence of temperature and carbon source in gene expression was monitored by measurement of the mCherry levels in AV1n[pRCR21]. Furthermore, expression from the P_{dsrLS} promoter of the dextran-producing *Lactobacillus sakei* MN1, was investigated. Thus, AV1n carrying the pRCR15 plasmid, with the P_{dsrLS}-*mrfp* fusion, was also analysed. The results confirmed an induction of expression from P_{dsrLL}-*mrfp* at low temperature in the presence of sucrose, glucose, maltose or fructose. Sucrose also induced expression from P_{dsrLL}. However, the fusion P_{dsrLS}-*mrfp* was activated by increasing the temperature (20-37°C) in the presence of sucrose, though no influence of temperature was detected when exposed to other sugars.

Conclusion: Two types of regulation of expression of LAB dextransucrases have been identified, suggesting two different mechanisms for environmental adaptation.

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CHARACTERIZATION OF PROBIOTIC PROPERTIES OF *LACTOBACILLUS SALIVARIUS* PS7

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Background/Aims: *Lactobacillus salivarius* is a well-characterized bacteriocin producer frequently isolated from human gastrointestinal tracts (GIT), milk, female genital tract and other sources. Several strains have gained attention as promising probiotics due to their ability to modulate gut microbiota, produce antimicrobial substances, stimulate protective immune response, inhibit faecal enzymatic activity and produce short chain fatty acids allowing an advisable acidification of the gut, among others.

Hence, the aim of this study was to isolate, identify and characterize a *Lactobacillus salivarius* strain with potential probiotic activity.

Methods: *In vitro* studies including survival after transit through an *in vitro* gastrointestinal model, assays of adhesion to epithelial cell lines, production of beneficial molecules and antimicrobial activity were performed. Furthermore, a safety characterization, including metabolic activities, antibiotic resistance and animal toxicity assays in rat models were conducted.

Results: The strain identification was confirmed by sequencing the 16S rRNA and by MALDITOF. *L. salivarius* PS7 was deposited in the Colección Española de Cultivos Tipo (CECT) and named *L. salivarius* CECT 9422. The viability of the strain after exposition to conditions simulating those found in the gastrointestinal tract was high. The strain was susceptible to antibiotics and did not produce histamine, tyramine, putrescine or cadaverine. The *in vivo* acute and repeated dose ingestion study demonstrated a lack of mortality and morbidity after the inoculation of rats with the PS7 strain. This strain has shown to exert a high antimicrobial activity against *Alloiococcus otitis, Staphylococcus aureus, Staphylococcus epidermidis*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*, regular causal agents of acute otitis media (AOM). PCR analysis revealed that this strain contains structural genes of bacteriocin Abp-118 in its genome.

Conclusion: Our results suggest that *L. salivarius* PS7 should be considered as probiotic strain and a potential alternative for the prevention of AOM.

ANALYSIS OF SOCIAL AND MICROBIOLOGICAL FACTORS IN THE MEDELLÍN MILK BANK POPULATION TO STRENGTHEN PUBLIC LACTATION POLICY

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Introduction: In the early stages of life, in Colombia, malnutrition and morbidity-mortality is an evident problem, therefore, it is appropriate to conduct impact research, thus generating a synergy academy-society. For the integral development of the individual in the physiological and psycho-social, lactation is critical, for the nutrients that strengthen the digestive and immune system, and provides a beneficial microbiote to the intestine of the infant, including Lactic Acid Bacteria.

Background: The last report of the bank Hospital General de Medellín (HGM), demonstrated the effectiveness of lactation by reducing the incidence of Necrotic Enteritis from 3.33% in 2015 to 1.8% in 2017, evidencing the importance of orienting public policies that allow to increase the indicators of breastfeeding.

Objective: To relate breastfeeding with sociocultural and biological mother-child factors that allow identifying critical variables that contribute to the discussion on public policies, being the experience of the Brazilian network of Human Milk Banks a reference.

Methods: The population and microbiological parameters of 67 donors and their infants were used, both external and those of the HGM bank, carried out by researchers from the National University of Colombia-Medellín. The databases were analyzed with the variables obtained through surveys, with the R software.

Results: The critical variables were socioeconomic factors, exclusive breastfeeding for 3 months and vaginal births in 63%, evidence the decrease of cases in these variables to the detriment of breastfeeding.

Conclusion: It was proven that social and microbiological factors do influence lactation and consequently in the formulation of public policies, highlighting milk banks as a facilitating tool for the fulfillment of the goals established in the different national plans for health promotion and prevention.

A FECAL-CULTURE MODEL, MONITORING GAS PRODUCTION, FOR ASSESSING PREBIOTICS' FERMENTABILITY IN NORMAL-WEIGHT AND OBESE SUBJECTS

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Background/Aims: The gut microbiota is altered in different conditions, which leads to the interest in prebiotics to restore it and to identify the best suited compounds. The use of fecal culture models provides an interesting strategy, allowing to identify prebiotics with suitable fermentation profiles, and appropriate microbiota-modulation properties, for each population. Here we aimed at comparing the fermentability and microbiota-modulation ability of different prebiotics, including the novel prebiotic 1-Kestose, in normal-weight and obese adults.

Methods: Fresh fecal samples from 9 normal-weight (BMI< 25 kg/m^2) and 10 obese (BMI> 40) volunteers were collected, transported to the laboratory (under anaerobic conditions), homogenized, diluted (10% v/v) into a carbohydrate-free basal medium and stabilized at 37°C in an anaerobiosis cabinet. The prebiotics tested (Actilight, Synergy, P95, Inulin, GOS and 1-kestose) were added (0.3%, w/v) to the stabilized fecal cultures and incubated at 37°C for 24 hours. Gas production along incubation was monitored in real-time by using the ANKOM RF System. Samples were taken at 0 and 24 hours of incubation for pH measurements, determination of short-chain-fatty-acids by gas chromatography and gut microbiota analyses by qPCR.

Results: In both volunteers' groups kestose resulted the more fermentable prebiotic as indicated by the largest accumulation

of gas and the lowest pH after incubation. On the contrary, inulin was the less fermentable compound. Interestingly, intestinal microbiota from obese individuals tended to show a lower ability to produce gas than that from normal-weight volunteers. The different prebiotics were able to induce changes in microbiota composition in both volunteer groups, although showing differences among them.

Conclusions: The fecal culture model used, with real-time monitoring of gas production, constitutes a fast and easy method for assessing the fermentability of prebiotics in different population groups. 1-kestose showed good characteristics suggesting its applicability as a readily fermentable prebiotic substrate for intestinal microbiota modulation.

IMPROVING ROBUSTNESS OF PROBIOTIC *LACTOBACILLUS PENTOSUS* STRAINS BY ADAPTATION TO EDIBLE OILS

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Background/Aims: Probiotic *Lactobacillus pentosus* were isolated throughout fermentation process of naturally fermented Aloreña green table olives. These strains exhibited high tolerance to low pH and high concentrations of bile salts as well as antimicrobial activity against several pathogens. To enhance their probiotic activity against several stresses, pre-adaptation of *L. pentosus* strains was done using edible oils.

Methods: In this study, we determined the effect of edible oils on survivability and stress tolerance of adapted *L. pentosus* strains such as acid and bile tolerance. Further, we analysed stress gene expression in adapted *L. pentosus* strains versus non-adapted *L. pentosus* strains by qRT-PCR.

Results: Seven *L. pentosus* strains were adapted to different edible oils (i.e., sunflower, olive and argan). We determine that survival and growth of probiotic *L. pentosus* strains in the presence of vegetable-based edible oils was dependent on the strain tested and the oil used. However, pre-adaptating the strains to the corresponding oils significantly increased their cell viabilities. As such, we examine whether pre-adaptating probiotic *L. pentosus* strains with oils will improve probiotic properties of *L. pentosus* strains such as tolerance to acids or bile. Improvements in stress resistance were observed in some pre-adapted strains with oils such as acidic and bile conditions; further, pre-adaptations with some oils induced stress gene expression for moonlighting proteins involved in several stress responses and other functions.

Conclusions: These results showed that pre-adaptation with vegetable edible oils may represent a novel approach to enhance robustness of probiotic bacteria improving their stability in probiotic products.

LACTIC ACID IS PARTLY RESPONSIBLE OF THE ANTIPROLIFERATIVE EFFECT OF *L. ACIDOPHILUS* OVER CACO-2 AND HT29 CELLS

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Background: Several independent studies have addressed the beneficial role of lactic acid bacteria over different cancer types, both *in vitro* and *in vivo*. However, and to our knowledge, no molecular study has been performed to understand how this inhibitory effect may take place.

Methods: Different fractions from several lactic acid bacteria and bifidobacteria were obtained in order to test their effect over two well-known epithelial cell lines, Caco-2 and Ht29, using the RTCA device. Effect of this extract over cell cycle and the process of necrosis/apoptosis was checked by flow cytometry. Molecular mechanism of action was elucidated by RNASeq.

Results: The most active fraction was obtained from *Lactobacillus acidophilus*, and RNASeq analysis revealed an activation of several detoxification/xenobiotic pathways in epithelial cells. Nuclear magnetic resonance revealed a quite complex extract with the presence of alanine and lactic acid. Alanine had no effect over proliferation but buffering of the extract suggested that some of the observed inhibitory effect was due to lactic acid. The antiproliferative effect was visible from 300 mg/L lactic acid, and 2,7 g/L were enough to induce complete culture death in Ht29 an Caco-2.

Conclusion: Although it remains to known the role of other compounds in the anti-proliferative effect of the *L. acidophilus* extract, we have shown for the first time that CaCo-2 and Ht29 cell lines are sensible to lactic acid concentrations lower than those observed for instance in yoghourt (9 g/L), suggesting that carcinogenic cells may be more sensitive to lactic acid than normal cells. Production of lactic acid in the colon is therefore a molecular mechanism of action by which lactic acid bacteria exert their beneficial role in the colon.

ALPHA DIVERSITY STUDY IN PATIENTS WITH *CLOSTRIDIUM DIFFICILE* INFECTION AND ASYMPTOMATIC CARRIERS

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Background: *Clostridium difficile (CD)* is a gram-positive, anaerobic, spore-forming bacillus, considered the leading cause of nosocomial diarrhea and pseudomembranous colitis in hospitalized patients. The pathogenicity is associated with the use of antibiotics and a decreased of the immune response, due to age and comorbidities. Intestinal colonization of *CD* can lead to a situation such

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as the absence of symptoms to mild-moderate diarrhea or pseudomembranous colitis cases. The aim of this study is to contrast the alpha diversity of intestinal microbiota in patients diagnosed with symptomatic *CD* infection (CDI) versus asymptomatic colonized by *CD*. The hypothesis is that the colonization of certain groups of key bacteria can be related to the overgrowth of *CD* or the transition from asymptomatic carrier (AC) to patients with CDI.

Methods: Alpha diversity was compared at the genus level using the Shannon and Simpson indexes and the Chao1 and Abundance-based Coverage Estimator (ACE) richness estimators of two groups of patients composed of 15 patients with CDI and 15 asymptomatic carriers of *CD*. The composition of the intestinal microbiota was obtained by amplification of bacterial gene DNA 16S, its massive sequencing and taxonomic allocation of the obtained sequences.

Results: Alpha diversity indexes showed both groups with a medium-low diversity without significant differences (Shannon: CDI = 2.1 vs AC = 1.9; Simpson: CDI = 0.8 vs AC = 0.7). There were no statistically significant differences in terms of species richness (Chao1: CDI = 47.2 vs AC = 51.6; ACE: CDI = 47.2 vs AC = 51.6). Additionally, these results have been compared with a group of healthy people.

Conclusions: Due to similarities in terms of a decreased alpha diversity between CDI and AC groups, it is possible that colonization would pose a risk in the progression from a carrier to an infected state of *CD*.

SUSCEPTIBILITY OF *SERRATIA MARCESCENS* ISOLATES CAUSING NEONATAL SEPSIS TO THE PREDATORY BACTERIA *BDELLOVIBRIO BACTERIOVORUS*

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Background/Aims: *Serratia* sp. are emerging as a leading cause of sepsis in neonatal intensive care units (NICU), usually presenting resistance to multiple antibiotics. The predatory bacteria *Bdellovibrio bacteriovorus* can feed from a broad range of human pathogens, as it has been proven in mammalian cell lines and animal models, being non-toxic and non-pathogenic for host cells. We aimed to assess the susceptibility of *S. marcescens* isolates causing neonatal sepsis to *B. bacteriovorus* predation in order to explore ecological antimicrobial alternatives to antibiotics.

Methods: Eight *S. marcescens* clinical isolates obtained from the NICU of a tertiary hospital were used as preys. Whole genome sequencing was applied to assess the phylogenetic relationship and genotypic determinants of antimicrobial resistance of the isolates. Also, antimicrobial susceptibility testing was perform by the MicroScan Walkaway system. *B. bacteriovorus* HD100 strain was used as predator. Co-culture of prey and predator were performed using a 96-well Bioscreen plate. Thirty μL of *Bdellovibrio* suspension cells (10^8 or 10^9 PFU/mL) were added to prey cultures in HEPES broth and incubated at 30°C for 48h with shaking. The capability to prey was evaluated by quantifying the amount of reduction in optical density at 600 nm of the cultures. *Pseudomonas putida* KT2440 was used as a positive predation control.

Results: *S. marcescens* isolates were found to be genetically closely related, but not identical. Antimicrobial resistant determinants were found in their genomes, some within mobile genetic elements. Phenotypically, the strains exhibited different patterns of resistance. All *S. marcescens* strains showed similar significant reductions in their population density. However, residual cells of prey were detected in all experiments.

Conclusion: *B. bacteriovorus* efficiently predates *in vitro S. marcescens* isolates recovered from preterm neonatal patients affected with late-onset sepsis, regardless their antibiotic resistance profile. Predatory bacteria might be explored as a novel adjuvant to antibiotherapy.

MICROBIOTA OF PRE AND POST-PASTEURIZED HUMAN MILK AND FECES OF INFANTS FROM A MILK BANK

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Background/Aims: Human milk is considered as an ideal and complete nourishment for newborns and children in the early stages of life. It contains a potential probiotic microbiota derived from the digestive system of the mother that is transmitted by the entero-mammary pathway, which helps with the metabolism of nutrients, maturation of the immune system, competition or antagonism against pathogens, and is also present in the infant feces. However, in some cases infants can't receive it for many reasons, which is why the Human Milk Banks are in charge of the promotion, protection, collection and processing of the aliment, that is donated by mothers both internal and external to the hospital, to be administrated to those infants who need it. The microbiota of the pre and post pasteurized human milk from donors of a milk bank and the feces of their infants, was studied.

Methods: 40 samples of breast milk were collected at each processing step in the bank and also 40 samples of infant feces. For the isolation of the microorganisms, serial dilutions were made and then were inoculated in selective culture media according to each of the microorganisms studied.

Results: The isolates with probiotic potential were identified by biochemical tests or molecular analysis. *Lactobacillus brevis* from infant feces and *Enterococcus faecium* from post-pasteurized milk were identified. A reduction in milk microbiota including the potential probiotics was observed after pasteurization.

Conclusion: It was found that breast milk contains an autochthonous microbiota that includes commensal and potential probiotic microorganisms. In addition, it is necessary to implement faster and safer analysis for the identification of Acid Lactic Bacteria (LAB) in human milk to avoid unnecessary discarding of milk in banks.

CHARACTERIZATION OF MICROBIOTA OF HUMAN MILK AND INFANT FECES

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Background/Aim: Human breast milk is a very complex food, enriched by a wide diversity of nutrients and other elements that favor the immune and gastrointestinal system development, among them, a native microbiota coming from the mother's gastrointestinal system through the enteromammary pathway and wich will be part of the gastrointestinal tract microbiota of the infant, among these beneficial microorganisms lactic-acid bacteria (LAB), which have potential probiotic species. Having in mind the importance of a good nutrition at the first years of human life and the strong scientific evidence that supports the probiotics as a natural alternative to enhance the nutrient metabolism, the development of the immune system and the antagonism versus pathogens both in nurslings and children under five years old, the probiotics are presented as a better alternative to reduce the antibiotic consumption and thus counteract the increasing resistance to them. The aim is characterize the microbiota of human breast milk and the feces of infants.

Methodology: 27 breast milk and 27 infant feces samples were studied by means of cultivation in selective agars for the microbiota of interest. For the isolates identification, a convencional microbiologic characterization, molecular and biochemical tests and probiotic potential tests were made. The ability to behave as probiotics was evaluated.

Results: The presence with probiotic potential of *Lactobacillus rhamnosus* in colostrum, *Lactobacillus plantarum* in mature breast milk and *Enterococcus faecalis* in infant feces were identified.

Conclusion: The studied strains in this investigation were viable facing the probiotic potential tests, for their ability to resist in vitro gastrointestinal conditions, besides having a good antimicrobial activity versus various pathogens and susceptibility to widely used antibiotics. This opens the door to brand new biotechnological works with probiotic strains obtained from human sources for the benefit of the consumer.

ANTIMICROBIAL ACTIVITY AGAINST HUMAN PATHOGENIC STRAIN OF *STAPHYLOCOCCUS AUREUS* CECT4013 BY *LACTOBACILLUS GASSERI* STRAINS

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Background/Aims: The objective of this work was to isolate, identify and characterize *Lactobacillus* strains from human samples (human milk and feces of breast feeding babies and their mothers) as potential probiotics with antimicrobial activity against *Staphylococcus aureus* CECT4013.

Methods: Three strains of *Lactobacillus gasseri* (ORD0529, ORD0713 and ORD0714), isolated from feces of breast feeding babies, were selected to assess this activity, and compared with *Lactobacillus salivarius* CECT5713 (positive control), a strain with antibacterial activity against *S. aureus*. Broth inhibitory assays were carried out in order to asses this antibacterial activity using neutralized cell-free culture supernatants (CFCS) and so demonstrate that the antibacterial activity was not due to the production of organic acids. The antimicrobial activity was measured by optical density (OD) and plate counting expressed as CFU/ml.

Results: More than three hundred probiotic strains from human samples were isolated, identified and characterized through several molecular biology techniques, including sequencing of gene 16S RNA, Random Amplification of Polymorphic DNA (RAPD) and also biochemical tests (API 50CH, API ZYM, catalase test, oxidase test, animopeptidase test and Gram stain). Neutralized supernatants of all three selected probiotics showed a significant reduction of growth of *S. aureus* CECT4013 expressed as CFU/ml along the time, being significant at 3 and 6 hours with the neutralized supernatant of *L. gasseri* (ORD0529) compared with all strains tested, included the positive control.

Conclusions: The three strains of *Lactobacillus* isolated from feces of breastfeeding babies, and particularly *L. gasseri* (ORD0529) possess antibacterial activities that results in a reduction of growth of *S. aureus* CECT4013.

GAMMA-AMINOBUTYRIC ACID PRODUCTION BY *BIFIDOBACTERIUM* STRAINS FROM HUMAN ORIGIN

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Background/Aims: The gut-brain axis (GBA) consists of bidirectional communication between the central and the enteric nervous system, linking emotional and cognitive centers of the brain with peripheral intestinal functions, gut microbiota can influence these interactions. The objective of this research work is detecting probiotic bacteria able to synthesize biochemical compounds capable to interact with the brain through this gut- brain axis. For this purpose, we evaluate the ability to produce gamma-aminobutyric acid (GABA) of a probiotic strain collection, belonging to *Bifidobacterium* and *Lactobacillus* genera, from human milk and feces of breast feeding babies and their mothers.

Methods: A simple test for screening of bacteria with the key enzyme Glutamate decarboxylase (GAD) was made and the positive strains for this enzyme test were grown in medium containing different concentrations of monosodium glutamate (MGS) (0, 20, 30 and 50mg/ml). This experiment was carried out two times in triplicate. GABA production was measured by mass spectrometry.

Results: More than three hundred probiotic strains from human origin were isolated, identified and characterized through several molecular biology techniques and also biochemical tests. 16 strains of *Bifidobacterium* genus were positive for the GAD enzyme, but only 6 strains were able to export GABA, including species of *Bifidobacterium angulatum* and *Bifidobacterium adolescentis*.

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Conclusions: GABA production was strain-dependent. *B. angulatum* was the best producing strain of GABA among the range of strains tested. These results provide novel opportunities to consider these strains as functional ingredients.

SCREENING OF GABA PRODUCTION IN MEMBERS OF THE GENUS *BIFIDOBACTERIUM*

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Gamma-amino butyric acid (GABA) is the main inhibitor neurotransmitter in the central nervous system, regulates multiple physiological processes in the human body and its dysfunctions has been linked to anxiety and depression disorders. In recent years, dietary supplementation with GABA has been associated with antihypertensive, analgesic and antidepressant properties and thus there is an increasing interest in identifying probiotic strains with GABA production capabilities, which could act as delivery vectors of this neurotransmitter in the human gut. Several lactic acid bacteria, including lactobacilli and streptococcal strains, have been reported capable to produce GABA, though this activity has been scarcely explored in members of the genus *Bifidobacterium*.

In this work the GABA production capability was studied among members of the genus *Bifidobacterium*. First, an *in silico* analysis was performed on available *Bifidobacterium* genomes, to determine how widespread GABA production genes are within this group of bacteria. Based on the results, a screening of GABA production capability was performed in a collection of 58 *Bifidobacterium* strain, mainly comprised of members of the species *B. adolescentis*. For this purpose, the strains were grown overnight in the presence of the GABA precursor, monosodium glutamate and then, GABA and residual monosodium glutamate concentrations were determined through high-performance liquid chromatography in cell-free supernatants. The presence of the *gadB* and *gadC* genes, encoding a glutamate decarboxylase and a glutamate/GABA antiporter system, respectively, in the GABA producing strains was determined through PCR.

The *gadB* and *gadC* genes were commonly detected in strains belonging to the species *B. adolescentis.* Besides, 25 % of the analyzed strains were capable to transform almost all monosodium glutamate provided, to GABA. In conclusion, GABA production is a relatively common trait in *B. adolescentis* strains, which could represent potential candidates for GABA delivery *in vivo*.

LACTOBACILLUS SALIVARIUS LPM01 (DSM 22150) REDUCES INFLAMMATION IN CELLULAR MODELS OF INTESTINAL EPITHELIUM

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Background/Aims: Gut dysbiosis is associated with pathologies that occur with inflammation. Administration of probiotics is a therapeutic strategy to modulate microbiota composition. *Lactobacillus salivarius* LPM01 is a probiotic isolated from human breast milk. Previous studies performed in vitro or in vivo models have shown that this strain inhibits growth of pathogenic bacteria. Administration of this probiotic also improves health status in immunocompromised people. At present no information is available about the anti-inflammatory effects on human intestinal epithelial cells, an important issue to reinforce its role as a probiotic.

Methods: The intestinal epithelial cell line HT29 was seeded ($1x10^5$ cells/mL) in 12-well plates and grown for 7 days. Two models were set up to mimic an inflammatory Background: (i) addition of TNF-alpha (10 ng/ml, 2-h incubation), and (ii) incubation with enteropathogenic *Escherichia coli* (EPEC, MOI 100) for 3h. The probiotic was grown, washed and added to HT-29 culture at a concentration of $1x10^7$ UFC/mL. The secreted pro-inflammatory cytokine IL-8 was quantified by ELISA. Probiotic-free supernatant and isolated membrane vesicles (MV) have also been tested. MVs were visualized by Cryo-TEM.

Results: *L. salivarius* LPM01 reduce IL-8 secretion induced by TNF-alpha when HT-29 cells were first incubated with this cytokine followed by incubation with the probiotic, indicating that this strain may have a curative effect. The analysis performed with HT-29 cells simultaneously incubated with *L. salivarius* and EPEC, showed that this probiotic also significantly reduces the EPEC-induced IL-8 secretion, confirming the protective effect on pathogen-associated inflammation. Addition of MVs or bacteria-free supernatant instead of *L. salivarius* did not reduce IL-8 levels. This effect could not be attributed to a secreted factor.

Conclusion: *L. salivarius* LPM01 displays anti-inflammatory effects. This probiotic reduces IL-8 secretion by 45% when HT-29 cells were treated with EPEC and by 50% when inflammation was induced by TNF-alpha.

TAXONOMICAL AND FUNCTIONAL CHARACTERIZATION OF THE INTESTINAL MICROBIOTA ASSOCIATED TO OBESITY

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Background and Aims: The intestinal microbiota has been pointed out as a key factor in obesity, since it contributes to meta-

bolic and inmunological homeostasis in the host. The dysbiosis of the gut microbiota linked to obesity has been related to a higher Firmicutes to Bacteroidetes ratio, a lower gene richness and an alteration of the metabolic functions of the gut microbiota [1, 2, 3]. The main objective of this work was to outline the significance of the compositional and/or functional changes in the obese-associated intestinal microbiota.

Methods: The taxonomic composition of the fecal microbiota of obese (Ob) and normal weight (Np) individuals was determined by high-throughput sequencing analysis using the Illumina Seq platform. Amplification of the 16S rRNA gene was carried out using as primers sequences directed towards the V3-V4 regions of the gene. Functional characterization was determined by measuring short chain fatty acids (SCFAs) by High Performance Liquid Chromatography (HPLC) and the ammonium concentration spectrophotometrically.

Results: Data analysis did not show significant differences between Ob and Np individuals in the dominant phyla Bacteroidetes (13%), Firmicutes (76%), Actinobacteria (10%) and Proteobacteria (1.8%). Nevertheless, the proportion of Verrumicrobia was significantly higher in normal weight individuals (Np). At genus level, *Collinsella*, *Alistipes*, *Clostridium*, *Clostridium* XIVa, *Romboutsia* and *Oscillibacter*, among others, showed significant differences between both groups of individuals. Bacterial diversity according to the Chao1 index was significantly higher in Np individuals. Regarding the functional characterization, the fecal samples of Ob individuals showed the highest concentrations of acetic and butyric acids.

Conclusion: In spite of the great interindividual variability, the microbiota of obese individuals is characterized by a higher or lesser proportion of certain microbial groups and a higher formation of SCFAs in relation to the microbiota of normal weight individuals.

Acknowledgments: Finantial support by AGL2016-75951-R.

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INTRODUCTION OF MILK CHANGE LACTIC BACTERIA AND PROTEIN METABOLITES IN INFANTS OUTGROWING COW'S MILK ALLERGY

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Background: Cow's milk protein allergy (CMPA) is very common in infancy. Currently, the only therapeutic option is a dairy elimination diet. Standardized oral milk challenges are performed each 6 months to determine possible tolerance adquistion.

Aim: To analyze the intestinal changes in feces of infants with non-IgE mediated CMPA after successful milk challenges and introduction of dairy product in their diet.

Methods: Twelve allergic children (between 1 and 2 years old) that were initially consuming extensively hydrolyzed formulas provided stool samples before oral milk challenges, and a week and a month after. Changes in the intestinal microbiota populations were determined by high-throughput sequencing of the 16S rRNA gene, meanwhile diverse microbial metabolites (short chain fatty acids and indoles) were quantified by chromatographic methods.

Results: The introduction of milk in infants with outgrowing non-IgE CMPA increased significantly the levels of fecal lactic acid bacteria, in particular the genus *Lactococcus*. Microbial metabolites derived from the catabolism of proteins, such as escatol (produced from tryptophan) enhanced, meanwhile branched chain fatty acids diminished.

Conclusions: The introduction of dairy products is accompanied by modifications in the infant gut environment through changes in the microbiota and protein metabolic end-products according to this kind of dietary change.

DOES MATERNAL PSYCHOSOCIAL DISTRESS INFLUENCE HUMAN MILK MICROBIOTA?

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Background: Human milk microbiota participates in the correct development of newborn infants as it is the source of a diverse population of commensal bacteria. The microbiota present in human milk depends on different maternal factors such as gestational age, antibiotic therapy, diet, geographical origin and health status. Maternal distress postpartum affects a relatively high number of women. The objective of this work was to assess the evolution of the composition of human milk microbiota and to determine whether maternal psychosocial distress affects this bacterial community.

Methods: Participants (n= 51) of the BINGO study collected milk samples at weeks 2, 6, and 12 and completed questionnaires about maternal stress, anxiety and depression at week 6 postpartum. Bacterial composition and diversity in milk samples was determined by 16S rRNA sequencing (V3-V4 region) using Illumina MiSeq technology.

Results: There was a wide variation in the bacterial profile of individual milk samples. At the phylum level, *Firmicutes* and *Proteobacteria* were found in every sample. *Firmicutes* had also the

highest relative abundance (72% at week 2 and 45% at week 12). Globally, the bacterial diversity in milk samples, measured as the Shanon diversity index, increased over time, but differences were found between women with low and high psychosocial distress. In samples from women with high psychosocial distress, there was no change in bacterial diversity with time, in contrast to the increase registered in women with low psychosocial distress. These changes were not related to differences in the relative abundance of the main bacterial genera.

Conclusions: There was a progressive and significant increase in bacterial diversity during the first three months postpartum. The relative abundance of *Firmicutes,* in particular *Staphylococcus* but not *Streptococcus,* decreased during this period. Low maternal psychosocial distress was associated to higher bacterial diversity (Shanon diversity index) in milk samples taken at week 12.

PROBIOTIC BIOFILMS CAN MODULATE THE ADHESION OF PATHOGENS

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Background: Biofilms are microbial communities that grow attached to surfaces. Their formation on medical devices is a common source of hospital-acquired infections. Especially concerning are those found inside the nasogastric enteral tubes that are used for feeding preterm children whose suckling ability is not yet developed. The type of biofilms described in such devices are quite complex, including members of the *Enterobacteriaceae* family, *Pseudomonas* spp., staphylococci, lactic acid bacteria and *Bifidobacterium* spp. Very often these communities become dominated by *Enterobacteriaceae*, leading to neonatal infections. We hypothesize that the outcome could be different if the surfaces were previously colonized with probiotic strains.

Methods: Biofilms of probiotic microorganisms isolated from human milk and feces of healthy lactating children were developed in multi-well PVC microplates. Attached population and biomass (cell + matrix) were measured over time. Biofilms 3D structure was visualized by CLSM. To study the role of the presence of probiotic strains in pathogen adhesion, PVC surfaces were first conditioned with the former biofilms and then were used as adhesion substratum for *Serratia marcescens*, a ubiquitous microorganism frequently isolated from NICU environments and an increasing cause of preterm sepsis.

Results: All the tested strains were able to form dense biofilms although at a different pace. Several strains of *Bifidobacterium* spp. and *Lactococcus lactis* were the faster biofilm formers. When their biofilms were conditioning the PVC surfaces, *S. marcescens* adhesion was strongly reduced.

Conclusions: Probiotic biofilms could modulate the adhesion of pathogens to nasogastric enteral tubes, being good candidates as first colonizers in these medical devices.

TARGET-SPECIFIC PROBIOTICS: A NEW ALTERNATIVE FOR THE CONTROL OF HYPERURICEMIA AND THE PREVENTION OF GOUT?

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Background: Uric acid is a final product of purine catabolism that plays a key role as antioxidant in human plasma. Absorption at intestinal level of purines from ingested food may also contribute to uric acid levels in plasma. Uric acid concentrations above normal levels in plasma are referred to as hyperuricemia, and are associated with the development of gout. At present, pharmacological treatment for hyperuricemia is the standard option but it produces undesirable side-effects. The aim of this work was to assay the ability of a collection of lactic acid bacteria and bifidobacteria to transport and metabolize purines and uric acid.

Methods: The intake and intracellular transformation of inosine and guanosine and the production of xanthine, hypoxanthine and guanine after incubation at 37°C was evaluated in a collection of 17 strains (*Lactobacillus*, *Bifidobacterium*) isolated from human milk of healthy women. The concentration of purine metabolites was carried out using HPLC. Other probiotic properties (pH and bile resistance, antibiotic sensibility, antimicrobial activity and biofilm formation kinetics were also determined *in vitro*.

Results: A preliminary screening revealed that most *Lactobacillus* isolates had high ability to uptake inosine and guanosine (> 95% of the concentration available in the media), in contrast to *Bifidobacterium* isolates. Selected strains from this initial screening (*Lactobacillus plantarum* MP05 and four strains of *Lactobacillus salivarius* (MP07, MP49, MP71 and MP312) showed a complete transformation of the inosine and guanosine or uric acid added. *L. salivarius* MP07, MP49 stood out by having the highest acid and bile resistance, respectively, and the fastest biofilm formation kinetics.

Conclusions: Nucleoside intake and transformation by *L. plantarum* MP05 and *L. salivarius* MP07, MP49, MP71 and MP312, as well as resistance to acid pH and bile salts, antimicrobial activity and biofilm formation ability, make them good candidates for the control of hyperuricemia.

A PEA OLIGOSACCHARIDE PREPARATION EXERTS *IN VITRO* BIFIDOGENIC PROPERTIES ON INFANT FECAL MICROBIOTA

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Background: There is a growing interest in identifying dietary plant-derived carbohydrates capable of modulating the composition and metabolic activities of the infant gut microbiota. Non-digestible carbohydrates, including α-galacto-oligosaccharides (α-GOS) from plant sources like pea, are resistant to hydrolysis and absorption in the small intestine, and might contribute to microbiota modulation.

Methods: Gas chromatography with flame ionization detection (GC-FID) was used for the separation and quantitation of the main pea α-GOS from a commercial preparation named AlphaGOS®. By using fecal homogenates of six infant donors (from 6 months to 2 years old), *in vitro* incubation studies of pea α-GOS in comparison with commercially available β-galacto-oligosaccharides derived from lactose (GOS-La) were carried out. α-GOS metabolism during the fermentation period (0-24 h) and their modulatory effect on infant fecal microbiota was evaluated.

Results: Pea α-GOS composition was mainly comprised by manninotriose (Gal-(α1→6)-Gal-(α1→6)-Glc, 49% on dry matter) and verbascotetraose (Gal-(α1→6)-Gal-(α1→6)-Gal-(α1→6)-Glc, 43% on dry matter), followed very distantly by melibiose (Gal-(α1→6)-Glc, 4% on dry matter). Quantitative analysis demonstrated that pea α-GOS were extensively and rapidly fermented by infant fecal microbiota. A decrease of pH in fermentation media accompanied by a statistically significant growth of bifidobacteria similar than GOS-La was observed; pea α-GOS showed a selective significant increase of *Bifidobacterium longum* and *Bifidobacterium catenulatum/pseudocatenulatum*.

Conclusion: These data support a potential use of plant-derived α-GOS as prebiotic compounds in infant formula.

Annals of Nutrition & Metabolism

Our microbiota

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Human beings have a large amount of bacteria (approximately the same number as their own cells) in their organism.[1] These colonize, above all, the skin and those body cavities that communicate with the exterior, fundamentally, the vagina and the digestive tract, the largest proportion of them being found in the colon. With this combination of microorganisms, which we call autochthonous microbiota [2], we maintain a symbiotic relationship that provides us with a series of advantages going from protection against invasion by pathogenic microbes and the development of immune defensive system to the collaboration in the digestion of components of the diets and the provision of vitamins and other essential nutrients [3].

Thanks to the work of Theodor Escherich, for more than a century we have known that the functions exercised by the microbiota are essential for our life [4]. Nonetheless, the study of microbiota and microbiome remained quite stagnant for most of the twentieth century because sufficient technologies were not developed to allow for the adequate analysis of the complex microbial communities inhabiting our organism and the enormous variety of interactions that occur as a result. The emergence of genomic techniques and with them of proteomics and metabolomics has helped to mitigate that deficiency and has promoted an enormous worldwide effort to learn about the world so close to us but at the same time so overlooked [5, 6].

Every individual possesses a distinct microbial community that depends on their genotype, early exposure to environmental microorganisms as well as on diet and lifestyle changes and therapy against infections and other illnesses. This implies that colonization from birth will differ depending on factors such as type of childbirth, breastfeeding model, rural or urban environment, being born in a developed or developing country, use of antibiotics, especially those used to fight infections during childbirth and early childhood, etc. [7]. An inadequate development of our digestive microbiota during the first years of life due to the increase in the number of caesarean sections [8], the premature abandonment of breastfeeding [9], or abuse of antibiotics [10], an inadequate diet [11] and the process of aging [12] in the adult life, can lead to a state of dysbiosis with an alteration both qualitative (species different from the usual) and quantitative (lower concentration of their beneficial microorganisms) microbiota. This will lead to a decrease in their beneficial effects and frequently the appearance of diseases such as diarrhea. Equally, the vaginal microbiota alteration can also cause infectious diseases and predispose to Papilloma virus infection, among others [13].

All the mucous membranes and epithelium exposed to the outside world have a microbial community. This means that the skin surface and its annexes and the surface covering the digestive, respiratory and genitourinary tract are the home of a microbiota that can vary notably in complexity and density, This will depend on each tract, device or system, of each part of the same device, each host and its situation at a given time (age, state of health, etc.). The different tracts (and parts of tracts) are not watertight

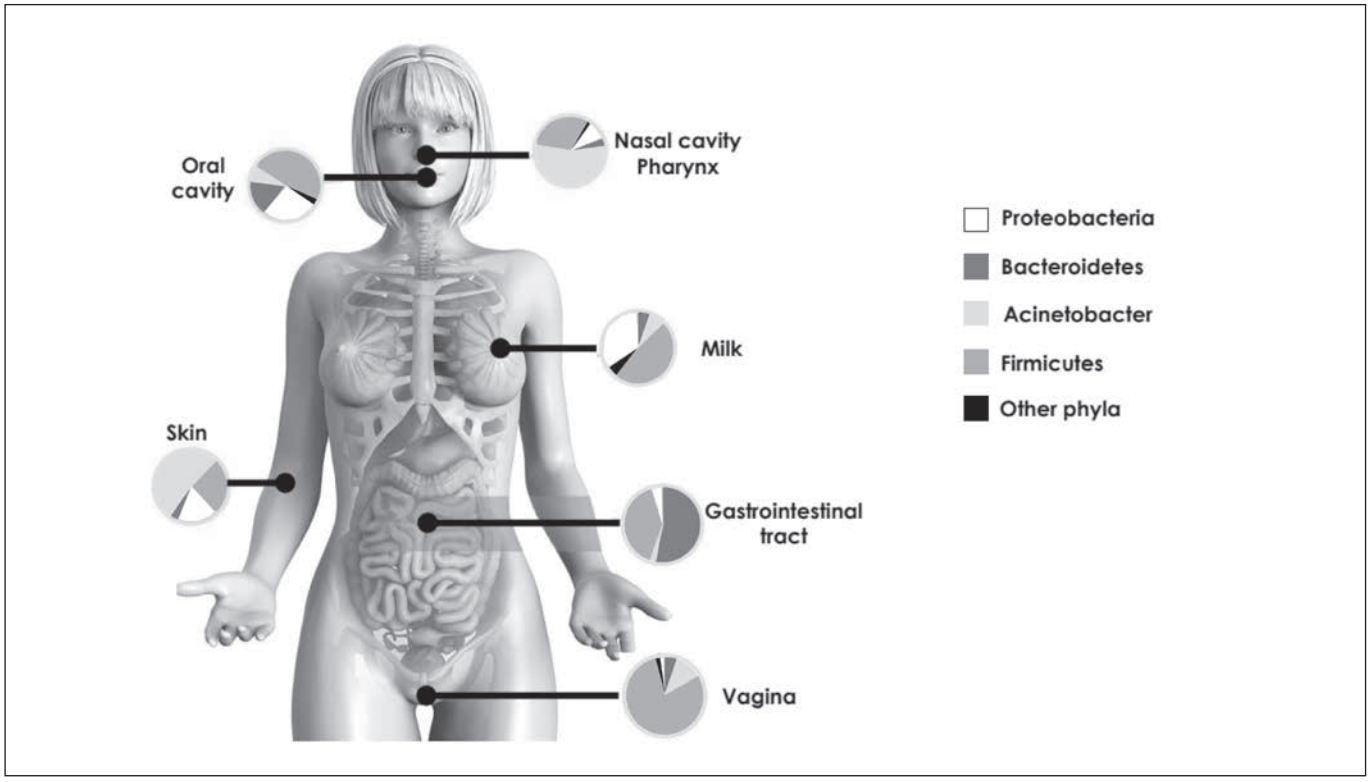

Figure 1. The human body contains a site-specific microbiota and microbiome in different organs. Within a given organ, the composition may change depending on the part of the organ and on different host, microbial and environmental factors. As an example, the composition of the skin microbiota changes depending on the part of the body that is sampled. *Source of the phyla graphs: Aagaard et al. (2016).*

compartments, but they can share microorganisms, in such a way that it is very difficult to specify the origin of a specific microorganism (Fig. 1) [14]. However, the conditions of each site are usually very specific so that, in spite of interindividual variability, each one of them contains, in physiological conditions, a characteristic set of microbes and of functions associated to that microbiota [15].

As an example, we extract figure 2 from the Human microbe Project Consortium (HMP) where we can observe the principal coordinates plot that shows the variation among samples. This figure demonstrates that primary clustering is by body area, separating the oral, gastrointestinal, skin and urogenital habitats; the nares bridges oral and skin habitats [5]. In this review, we deal with the microbiota studied most (digestive tract, vagina, skin, respiratory and mammary), this resulting in the fact that there are already scientifically contrasted clinical applications for many illnesses in many cases. However, there are also microbiota that occupy locations considered sterile until recent years, so that in the future, their impact on health can be equally relevant and, in fact, more and more attention is being given to them [16].

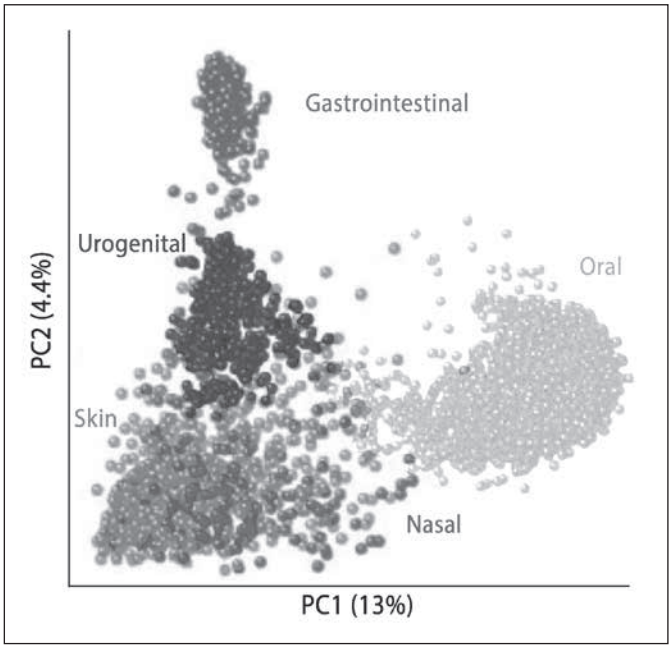

Figure 2. Diversity of the human microbiome is concordant among measures, unique to each individual, and strongly determined by microbial habitat. *Source of the graph: Huttenhower et al. (2012).*

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Intestinal Microbiota, Antibiotics and Risk of Disease

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Abstract

Microbes have lived in and on animal hosts since multicellular life evolved about 1 billion years ago. Hosts provide habitat and nutrition to the microbial communities, and derive many benefits from their guests. Microbial colonizers of the gut contribute with metabolic (recovery of energy and nutrients), protective (barrier effect against invaders) and trophic (immune regulation, neuro-endocrine development) functions. Loss of species diversity and gene richness in the gut microbiome is commonly reported in individuals affected by chronic non-communicable diseases of increasing incidence in modern society, including metabolic, inflammatory and neoplastic disorders. Developing and maintaining gut microbiota diversity is a novel clinical target for health promotion and disease prevention.

Gut Microbes

Chronic microbial colonization that inflicts no evident harm on the host only attracted minor clinical attention during the past century [1]. However, animals are in permanent association with microbial communities maternally inherited at birth or acquired from the environment. Such communities remained unexplored before the age of molecular techniques because of the difficulties to cultivate and isolate a large majority of the microbial species. Development of novel gene sequencing technologies as well as availability of powerful bioinformatic analysis tools have allowed a dramatic proliferation of research studies over the past few years.

All epithelial surfaces of mammals are colonized by microorganisms, but the gastrointestinal tract harbors the largest microbial burden. The human gastrointestinal tract houses over 10^{14} microbial cells with over 1,000 microbial species, most of them belonging to the domain Bacteria [2]. Hence, the gastrointestinal mucosa is the body's principal site for interaction with the microbial world. The human gastrointestinal mucosa exhibits a large surface, comparable to a tennis court, and contains adapted structures and functions for bi-directional communication with microorganisms, including a number of preformed receptors, microbial recognition mechanisms, host-microbe cross-talk pathways, and microbe-specific adaptive responses [3].

The stomach and duodenum harbor very low numbers of microorganisms adhering to the mucosal surface or in transit, typically less than 10^3 bacteria cells per gram of contents. Acid, bile, and pancreatic secretions kill most ingested microbes, and the phasic propulsive motor activity impedes stable colonization of the small bowel lumen. There is a progressive increase in numbers of bacteria along the jejunum and ileum, from approximately 10^4 in the jejunum to 10^7 cells per gram of contents at the ileal end. The large intestine is heavily populated by anaerobes with numbers in the region of 10^{12} cells per gram of luminal

contents. The colonic transit time is slow (30 to 70 hours) and microorganisms have the opportunity to proliferate by fermenting available substrates derived from either the diet or endogenous secretions. By far, the colon harbours the largest population of microbial symbionts, which contribute to 60% of solid colonic contents [4].

Several hundred grams of bacteria living within the intestinal tract certainly affect host homoeostasis. Some resident bacteria in the human gut are associated with toxin formation and pathogenicity when they become dominant, e.g. *Clostridium difficile*. Some other resident species are potential pathogens when the integrity of the mucosal barrier is functionally breached. However, the normal interaction between gut bacteria and their host is a symbiotic mutualistic relationship, defined as mutually beneficial for both partners. The host provides a nutrient-rich habitat, and intestinal bacteria confer important benefits on the host's health [5]. Several beneficial features of gut bacteria are widely recognized, including production of short chain fatty acids, vitamin synthesis, secretion of bacteriocins and inhibition of pathogens through a multiplicity of mechanisms [4, 6]. The primary functions of the gut microbiota are ascribed into three categories, i.e. metabolic, protective and trophic functions.

Metabolic functions assist in the fermentation of non-digestible dietary substrates and endogenous mucus. Gene diversity among the microbial community provides a variety of enzymes and biochemical pathways that are distinct from the host's own constitutive resources. Fermentation of carbohydrates is a major source of energy in the colon for bacterial growth and produces short chain fatty acids that can be absorbed by the host. This results in salvage of dietary energy, and favours the absorption of ions (Ca, Mg, Fe) in the cecum. Metabolic functions also include the production of vitamins (K, B12, biotin, folic acid, pantothenate) and synthesis of amino acids from ammonia or urea [7].

Protective functions include the barrier effect that prevents invasion by pathogens. The resident bacteria represent a crucial line of resistance to colonization by exogenous microbes or opportunistic bacteria that are present in the gut, but their growth is restricted. The equilibrium between species of resident bacteria provides stability in the microbial population, but use of antibiotics can disrupt the balance (for instance, overgrowth of toxigenic *Clostridium difficile*). The barrier effect is based on the ability of certain bacteria to secrete antimicrobial substances, the bacteriocins, which inhibit the growth of pathogens, and also in the competition for ecological niches.

Trophic functions of the gut microbiota include the control of epithelial cell proliferation and differentiation.

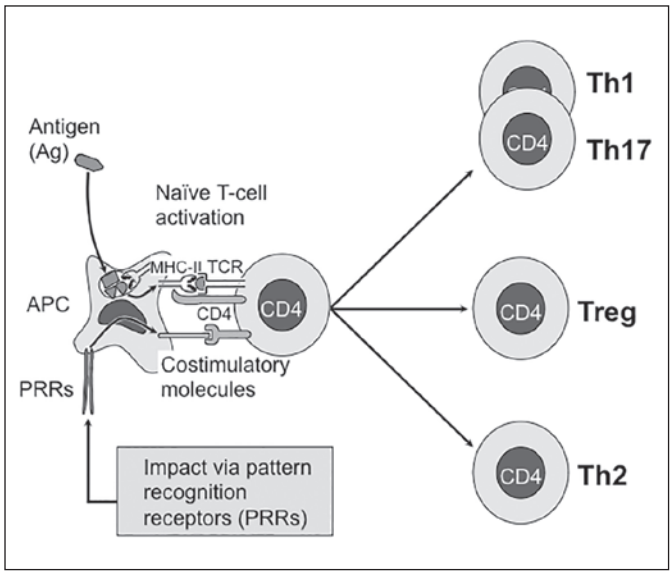

Figure 1. The specialized lymphoid follicles of the gut mucosa are the major sites for induction and regulation of immune responses. Gut microbes stimulate clonal expansion of lymphocytes, which may differentiate into Th1, Th2, Th17 or Treg cells, with different effector or regulatory capabilities. Innate recognition of microbe associated molecular patterns by antigen-presenting cells (APC) plays a decisive role for the induction of either effector or regulatory pathways.

Epithelial cell turnover is reduced in colonic crypts of germ-free animals as compared with colonized controls. Cell differentiation is highly influenced by the interaction with resident microorganisms as shown by the expression of a variety of genes in germ-free animals mono-associated with specific bacterial strains [8], and in humans fed with probiotic lactobacilli [9]. Gut bacteria have important trophic effects on mucosal immunocompetent cells and are critical for the development of a healthy immune system. Multiple and diverse interactions between microbes, epithelium and gut lymphoid tissues are constantly reshaping local and systemic mechanisms of immunity. Commensal microbes play a major role in the induction of regulatory T cells in gut lymphoid follicles [10] (Fig. 1).

Antibiotics and Risk of Disease

Every day, 10 to 30 out of 1,000 inhabitants of developed countries consume a defined daily dose of antibiotics as ambulatory patients [11]. In other words, today an average of 10 million West Europeans and 6 million North Americans living in the community will take a dose of antibiotics. Although most courses of antibiot-

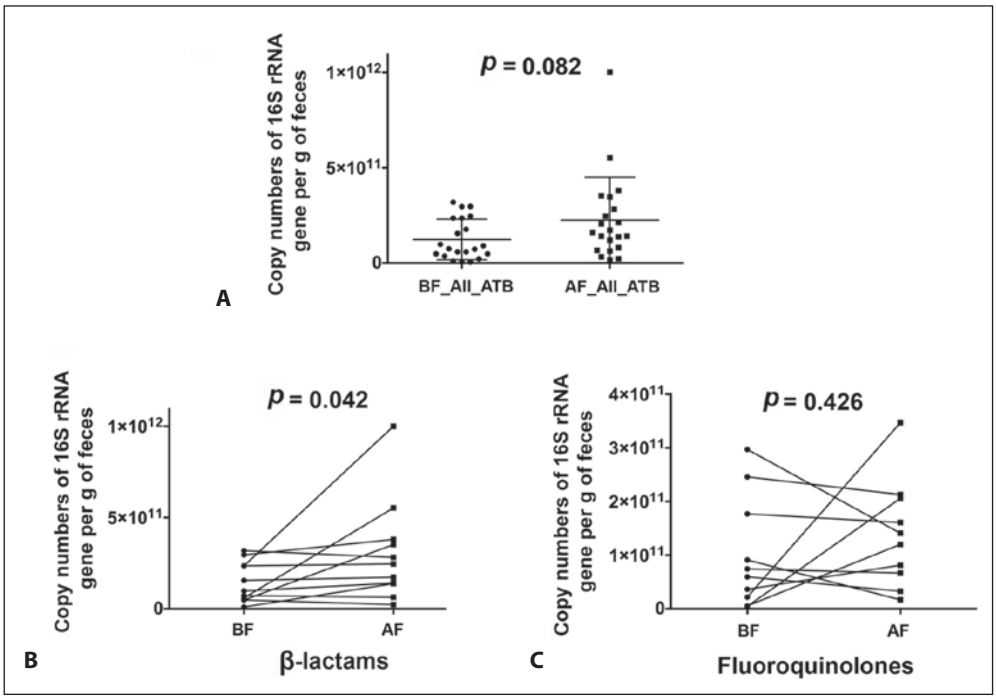

Figure 2. Changes in bacterial load before and after antibiotic treatment in fecal samples from 21 human subjects. A) Bacterial load as assessed by quantitative real-time PCR (qPCR) of the 16S rRNA gene with universal primers. B) Effect of beta-lactam antibiotics. C) Effect of fluoroquinolones. BF_ATB = before treatment; AF_ATB = after treatment (Data extracted from reference 14)

ics result in no immediate, obvious side-effects, there is a concern that antibiotic collateral damage altering the composition of the microbiota will interfere with the functions of this microbial community. Antibiotic-associated diarrhea is the most commonly recognized complication of antibiotics, and develops in 15 to 25% of patients on antibiotics. Most episodes of diarrhea induced by antibiotics are mild and self-limited in a few days. However, an increasing number of cases develop more severe forms, including *Clostridium difficile*–associated diarrhoea. The antibiotic-induced disturbance of the intestinal microbiota promotes *C. difficile* spore germination within the intestine, vegetative growth, and toxin production, leading to epithelial damage and colitis. Clinical presentation ranges from self-limiting diarrhoea to toxic megacolon, fulminant colitis and death, but even milder cases may suffer recurrent episodes of diarrhoea overtime. Antibiotics directed against *C. difficile* can decrease the load of the pathogen and toxin production inducing clinical remission. However, if the microbiota is unable to restore resistance to colonization by *C. difficile*, the patients will develop recurrent episodes of infection [12]. Hence, *C. difficile* infection is the paradigm of how antibiotics can disturb the protective function of the human gut microbiota against pathogens.

There is particular concern with the use of antibiotics during childhood. Antibiotics are among the most prescribed medications during early life. Data from the Centers for Disease Control and Prevention indicate that the average child in the U.S. receives about 3 antibiotic treatments in the first 2 years of life and approximately 11 by the age of 10 [13]. Repeated exposure to antibiotics for the treatment of ear, sinus, and throat infections is common before the age of 3. This is also a period during which the gut microbiota is shaped. Indeed, from birth to 3 years of age, the composition of the gut community undergoes continuous changes, with a gradual increase in phylogenetic diversity. The introduction of solid meals is associated with an increase in the abundance of Bacteroidetes and a switch from genes facilitating lactate utilization to those linked to carbohydrate utilization, vitamin biosynthesis, and xenobiotic degradation. Superimposed on these patterns of gradual change, the effects of antibiotics result in large shifts in the relative abundance of taxonomic groups [14]. Use of antibiotics induces a decrease in microbial diversity (loss of richness in the ecosystem) and overgrowth of resistant species, which may even result in an overall increase of microbial load (Fig. 2).

Perturbations of the gut microbial ecosystem during its period of development 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 (IBD), metabolic disorders (type 2 diabetes, obesity),

and atopic diseases are associated with an alteration of the gut community composition.

The incidence and prevalence of childhood IBD is increasing worldwide. A leading hypothesis regarding the pathogenesis is that alterations of the gut microbial community caused by repeated exposure to antibiotics trigger inflammation. Several retrospective and nationwide cohort studies have examined the potential correlation between the use of antibiotics and IBD. Those infants receiving antibiotics before one year of age were found to be more likely to be diagnosed with IBD than non-users [15]. This association appeared to be strongest in the first 3 months after use and among children with more than 7 courses of antibiotic treatment [16]. No definitive link between the type of antibiotic used and IBD was made in any of the studies.

Although it has been demonstrated that human genetics and diet play an important role in determining body weight, it is now widely accepted that the increase in the prevalence of obesity over the past 30 years is also attributable to the alteration of the gut microbial community composition. The demonstration that the obesity phenotype can be transferred to germ-free recipient mice via microbiome transplantation provided evidence that the gut microbial community contributes to obesity perhaps by increasing caloric recovery from consumed foods. Indeed, obesity has been associated with an alteration of the composition and function of the gut microbial community [17]. Interestingly, reduced diversity and lower gene counts in the microbial gut community has been associated with increased adiposity, insulin and leptin resistance, and a more pronounced inflammatory phenotype [18]. These traits are also found after repeated antibiotic treatments. For instance, antibiotic exposure in early life, when host adipocyte populations are developing, has been associated with the development of adiposity in humans [19]. Since the 1950s, low dose antibiotics have been widely used as growth promoters in animal husbandry. Experiments using mice have shown that low dose antibiotics increase fat mass and the percentage of body fat [20]. Coincidentally, the period of accelerated increase in prevalence of obesity in the US overlaps with both increased dietary caloric intake and antibiotic exposure through food.

Antibiotics are powerful medicines to fight against pathogens and cure infectious diseases. However, despite the well-documented resilience of the gut microbiota, treatment with these drugs may be associated with persistent changes in microbial composition and with potential long-term consequences for host immunity and metabolic activities. Many of these unintended consequences come about from the use of antibiotics in early life, during microbial community acquisition, a period which in turn is involved in the education of the host's immune system.

Investigation of the Gut Microbiota in Clinical Practice

Breath tests, culture or microscopic examination of fecal samples, or PCR quantification of targeted species, do not provide an overall insight on the whole spectrum of microbial communities that inhabit the human gut. They are suitable for well-known clinical uses as diagnostic tools for malabsorption of sugars, assessment of oro-cecal transit time, small intestine bacterial over growth, infectious entero-colitis, parasitic disease, etc., but do not reflect properly the structure and functions of the human gut microbial ecosystem. These tools do not allow evaluation of the dysbiotic changes that are associated with chronic non-communicable diseases.

Progress in developing useful diagnostic applications based in microbiome testing is still behind expectations because of a number of unresolved problems. The procedure most commonly used to investigate dysbiosis in the human gut is based in DNA extraction from fecal samples, 16S rRNA gene amplification, and sequencing of the amplicons using next generation sequencing equipments. However, there is no full standardization of this procedure and the results achieved vary widely from laboratory to laboratory. Different technological approaches, as well as confounding factors such as ethnicity, diet, drugs, colonic transit time may account for the lack of consistency. The lack of a defined healthy range for the composition of the human gut ecosystem makes this issue challenging to address, as there are large variations between individuals considered to be in a healthy status. Thus, current approaches for identifying microbial markers for diagnostic purposes need to relay in comparisons with parallel control groups of healthy individuals. Diagnostic markers for defining dysbiosis are still to be properly validated, i.e. assessment of sensitivity and specificity of the proposed test for a defined condition.

In conclusion, today there are no standardized tools for routine evaluation of the human gut microbiota in the clinical setting. Although a healthy gut microbiota is usually linked to phylogenetic and functional diversity, in depth research is needed to ascertain proper biomarkers for identifying a healthy versus a dysbiotic gut microbiome. Research protocols based on molecular technologies, metabolomics or both, will be needed for a better knowledge of the contributions of microbial symbionts to host health and identification of deviations leading to disease.

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The Vaginal Microbiota

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The Leading Role of Steroids in Vaginal Microbiota Colonization

The composition of the vaginal microbiota is reliant on the environmental conditions of the cavity, which are in turn conditioned by the secretion of steroid hormones. During **infancy** hormone production has not started and consequently the mucosal layer is thin and quite dry, thus allowing not true colonization but rather contamination by microorganisms of the enteric tract (streptococci and other strict or facultative anaerobic cocci and bacilli) and the skin (staphylococci). **Menarche** is characterized by the onset of hormone production, which facilitates the thickening of the mucosa and the generation of a nutrient rich fluid that becomes acidified by proton secretion from the ectocervix cells. Concomitantly, the glycogen present in the exudate is degraded to glucose by enzymes secreted by the parietal epithelium. Overall, this promotes lactobacilli colonization, which enhances the initial drop in pH and provokes elimination of the previous microbial contamination. During the **fertile stage** the vagina is colonized by one or a few species of lactobacilli, the most common in descending order being: *L. crispatus*, *L. iners*, *L. jensenii*, *L. gasseri* and *L. vaginalis*. These generate lactic acid, which maintains the pH of the cavity in between 4.5 and 4.0 and protects the mucosal surface from invasion by undesirable microorganisms. During **pregnancy** the concentration of lactobacilli increases further and the pH lowers to 3.5-3.0, thus maximizing the protection of the cavity and protecting the foetus from infection. Finally, **menopause** marks the cessation of steroid hormone production, which leads to thinning of the mucosal walls, increased dryness and, as a consequence, loss of most of the microbiota.

The influence of hormone variation on the vaginal microbiota is also observed during the **menstrual cycle**. The point of the lowest level of secretion marks the beginning of menstruation and the greatest reduction in the concentration of the resident lactobacilli. As a consequence, the concentration of *Gardnerella* and other potentially pathogenic bacteria increases. At the end of menstruation, the onset of the production of oestrogen during the follicular phase allows the recovery of the lactobacilli and the re-acidification of the exudate, thereby controlling pathogen proliferation. Finally, during the luteal phase the predominance of lactobacilli starts to decline, although this does not usually compromise the protection of the mucosa.

The Mutualistic Relation between Humans and Vaginal Lactobacilli

Lactobacilli protect the vagina against infection throughout the fertile stage, this **microbial antagonism**

being so beneficial that the cavity itself creates the conditions that promote colonization, as mentioned above.

The mechanisms by which this protection is effected can be grouped into three categories:

– *Colonization interference*: Lactobacilli form a biofilm on the mucosa that hinders attachment by other microorganisms.
– *Production of antimicrobial compounds*: The antimicrobial effect of lactic acid has already been commented. In addition, most vaginal lactobacilli produce hydrogen peroxide, an antiseptic that is toxic for anaerobic and microaerophilic microorganisms. Finally, there are strains that produce bacteriocins, which are antimicrobial peptides that attack the plasma membrane and inhibit the cell-wall synthesis of other bacteria.
– *Co-aggregation*: Most vaginal lactobacilli form clumps and capture other microorganisms within these aggregates, thus impeding them from coming into contact with the mucosa, as well as enhancing the harming effect of the antimicrobials they produce.

The Retreat of Lactobacilli: Causes and Consequences

The depletion of lactobacilli has two main causes:

- *Physiological*: Menstrual discharge has a neutral pH, which is toxic for lactobacilli and is one of the reasons why their numbers fall during the period. Sometimes they are not able to recover afterwards, particularly since *Gardnerella* and other microorganisms, levels of which pick during menstruation, produce biogenic amines that provoke the consistent raising of vaginal pH.
– *Non-physiological*: These include the use of barrier contraceptives that liberate copper cations, which are toxic to not only sperm cells but also to lactobacilli; vaginal douching, which may break the biofilm that protects the mucosa; the use of systemic antibiotics and cancer chemotherapeutics that permeate into the vaginal exudate; abdominal radiotherapy, etc.

The reduction in the colonization by lactobacilli almost invariably has pathological consequences, the most common being:

– *Vaginosis*: Produced by a variety of vaginal bacteria such as *G. vaginalis*, *Mobiluncus* spp. and *Atopobium* spp. Characterized by a thin exudate with a pH above 4.5, a fishy odour and/or the presence of vaginal cells covered in bacteria which are not lactobacilli.
– *Fungal vaginitis*: Usually produced by *Candida albicans*, a yeast indigenous to the vagina that changes its morphology during infection to become filaments and generates inflammation of the mucosa accompanied by itching and other symptoms.
- *Aerobic vaginitis*: Produced by several enteric bacteria such as *Escherichia coli* or Bacteroidetes. Characterized by a yellowish, strong-smelling (but not fishy) exudate, with a pH close to neutral, within which parabasal and polymorphonuclear cells can be observed.
- *Infections of the lower urinary tract*: Mainly produced by *E. coli*. In its migration from the anus to the urethral orifice, the healthy vagina acts as a barrier due to the low pH created by the lactobacilli, but in their absence it becomes a staging post.

Treatment of Vaginal infections

Several approaches may be taken towards the repopulating of vaginal lactobacilli and restoration of vaginal eubiosis:

- Acids: They lower the pH and are toxic for most vaginal pathogens. In addition, they create the acidic conditions that promote lactobacilli recolonization and attenuate the strong odour that accompanies vaginal pathologies. The most widely used is lactic acid. In the case of fungal vaginitis, boric acid is used instead. In the past, vinegar (source of acetic acid) and yogurt were used.
– Antimicrobials: Metronidazol and clindamycin are most frequently used for the treatment of vaginosis, while several azoles can be used for candidiasis. In cases of aerobic vaginitis and infections of the lower urinary tract, it is advisable to conduct an antibiogram, given their diverse aetiology.
– Probiotics: Vaginal probiotics include one or several *Lactobacillus* strains, ideally H_2O_2 producers that attach to the vaginal epithelium. They transiently colonize the mucosal surface, where they produce lactic acid, thus creating the appropriate conditions for recolonization by the indigenous lactobacilli. In addition, they probably multiply on the mucosa, which prolongs their therapeutic effect.

The use of Vaginal Probiotics

Probiotic lactobacilli may be administered orally or vaginally. The advantages of the first are the ease of administration and the fact that treatment does not have to be suspended during menstruation. However, the probiotic bacteria has to survive passing through the digestive sys-

tem. The vaginal route, on the other hand, insures that all the administered lactobacilli will reach the cavity. Consequently, oral preparations have to contain at least 10 times more lactobacilli and be applied for longer periods than those instilled vaginally.

A substantial proportion of vaginal infections are produced by microorganism indigenous to the cavity and, consequently, adapted to it. This may be one of the reasons behind their frequent relapses. To fight them, it may be advisable to apply the treatment over several cycles.

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Annals of Nutrition & Metabolism

The Microbiome of Human Milk

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Human milk · Microbiota · Microbiome · Lactation

Abstract
Studies carried out in the last 15 years have demonstrated that human milk represents a continuous supply of commensal, mutualistic and/or potentially probiotic bacteria to shed the infant gut and drive the assembly of a healthy infant gut microbiome. Indeed, once in the infant gut, these bacteria may play several key roles, contributing –among others– to the protection against infections and the maturation of the immune system functions. Bacteria found in human milk may have different origins and some studies suggest that specific bacteria present in the maternal digestive tract could reach the mammary gland during late pregnancy and lactation. The microbiota composition in human milk has implications not only on the infant but also on the mammary health. In fact, mammary dysbiosis may lead to mastitis, a frequently underrated and underdiagnosed condition that represents the first medical cause for undesired weaning. In the future, a better knowledge on the human milk microbiota and their influencing factors could be exploited to rationally design novel microbiota-based strategies to fine tune it, and (or) to develop novel probiotics derived from human milk, with the final aim to improve maternal and infant health.

Microbial Diversity in Human Milk

The short- and long-term health-promoting effects of breastfeeding have been known for decades and apply both to developing and developed countries. Historically, these effects were partly attributed to the presence of the so-called "*bifidogenic factors*", leading to the predominance of microorganisms of the genus *Bifidobacterium* in the gut of breast-fed babies. However, the role of human milk as a complex ecological niche and as a relevant source of bacteria to seed the infant gut had remained overlooked until recently.

Culture-Dependent Studies

The first microbiological studies on human milk, carried out during the second half of the past century, were mainly focused in the detection of potentially harmful microbes and their role as a source of infant infections. However, over the last decade, the presence of viable commensal, mutualistic, or potentially probiotic bacteria in human milk from healthy individuals has been described [1]. This has lead to an increasing interest in the human milk microbiota and, particularly, the potential of breastfeeding as a mean of transferring beneficial bacteria from the mother to the breastfed infant, and their role in the maternal and/or infant health. It also stimulated the search for novel milk

bacterial strains with potential to be used as probiotics for the mother-infant dyad.

The cultivable bacteria usually isolated from human milk include Gram-positives belonging to the genera *Staphylococcus*, *Streptococcus*, *Corynebacterium* and *Propionibacterium*. At a lower extend lactic acid bacteria (*Lactobacillus, Lactococcus*, *Leuconostoc*, *Weissella*, *Enterococcus*, among others) and bifidobacteria are also commonly isolated from human milk [2-6]. *Lactobacillus* (*L. salivarius*, *L. gasseri*, *L. fermentum, L. reuteri,* among others) and *Bifidobacterium* (*B. longum* and *B. breve*) species isolated from human milk have received the highest scientific attention because of the recognised potential of some strains belonging to these groups as probiotics. Noticeably, isolates from these two bacterial genera seem to be more abundant in human milk samples from locations with a low use of antibiotics [7]. Even though globally, more than 200 different bacterial species, belonging to approximately 50 different genera have been isolated from human milk up to the present [Fernández et al., 2013].

The microbial load in human milk may range from 10^1 to 10^6 CFU/mL, depending on the health status of the mother (e.g., mastitis) and, also, on the milk collection method. As an example, the use of milk pumps may result in high concentrations of contaminating Gram-negative bacteria (*Enterobacteria*, *Pseudomonas*, *Stenotrophomonas,* among others) and yeasts appearing from rinsing water and/or poor hygienic manipulation practices [8].

Some limitations of culture-based analyses are due to their inability to assess viable but non-cultivable organisms although, in contrast, they enable the isolation, preservation and characterization of bacterial strains [5, 9]. The availability of bacterial strains isolated from human milk, together with the novel genetic tools, is allowing the sequencing and annotation of its genomes, facilitating further functional studies and future applications.

From pioneer human milk studies to the most recent culture-based analyses aimed at isolating human-milk strains for potential probiotic applications, the use of culture-methods has unveiled human milk as a complex ecological niche and potential source of probiotics. In addition, we should not forget that human milk might contain yeasts, moulds and viruses. The transmissions of three specific viruses (CMV, HIV, and HTLV-I) to the infants through breastfeeding are of particular concern, and are taken into consideration during management of human milk banks. In addition, human milk may contain bacteriophages, which might play a role in modulating the human milk microbiota [10]. Moreover, the human milk ecosystem also contains a complex population of human cells, which may interact with the microorganisms, both in human milk and in the infant gut, although host-microbe interactions within the human milk have been scarcely explored.

Culture-Independent Studies

Cultivable microorganisms are recognized to represent a limited fraction of the microbial communities encountered at a specific ecological niche. In this context, the application of culture-independent molecular techniques, including quantitative PCR, denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and Next Generation Sequencing (NGS) approaches, from metataxonomics (16S rRNA amplicon analysis) to metagenomics (total DNA sequencing), has provided insightful additional observations on the microbial populations encountered in human milk [11]. Notably, such techniques have their own limitations as they detect nucleic acids, which may belong to either live or dead organisms, and they tend to over- or underestimate some microbial groups due to the fact that different microbial groups may exhibit variations in DNA extraction and PCR efficiencies, but also due to current limitations inherent to the bioinformatics analysis [11, 12].

Globally, culture-independent studies have confirmed the presence of DNA from bacterial groups previously identified with culture-dependent techniques, such as *Staphylococcus, Streptococcus, Corynebacterium, Propionibacterium. Lactococcus, Leuconostoc, Weissella, Bifidobacterium* and/or *Lactobacillus* spp. [10, 13-17]. In addition, some studies have also reported the presence of DNA from strictly anaerobic gut-associated microbes (*Bacteroides*, *Blautia, Clostridium*, *Collinsella, Coprococcus*, *Eubacterium, Faecalibacterium, Roseburia*, *Ruminococcus, Veillonella*, among others), which are either non-culturable or very difficult to culture in the laboratory and, therefore, may not be detected using traditional culture-based methods [10, 12, 15, 16].

Microbiome studies focused on human milk have also revealed the presence of DNA belonging to a third group of bacterial genera typically associated with soil and water samples, including *Acinetobacter*, *Bradyrhizobium*, *Methylobacterium*, *Microbacterium*, *Novosphingobium*, *Pseudomonas*, *Ralstonia*, *Sphingopyxis*, *Sphingobium*, *Sphingomonas*, *Stenotrophomonas* and *Xanthomonas* [14, 15]. However, it has been recently shown that high-resolution molecular techniques used to study low abundance microbiomes (such as that of human milk from healthy women) have a high susceptibility to false positives because of potential contamination with DNA sequences from the water- and soil-associated bacterial genera cited above.

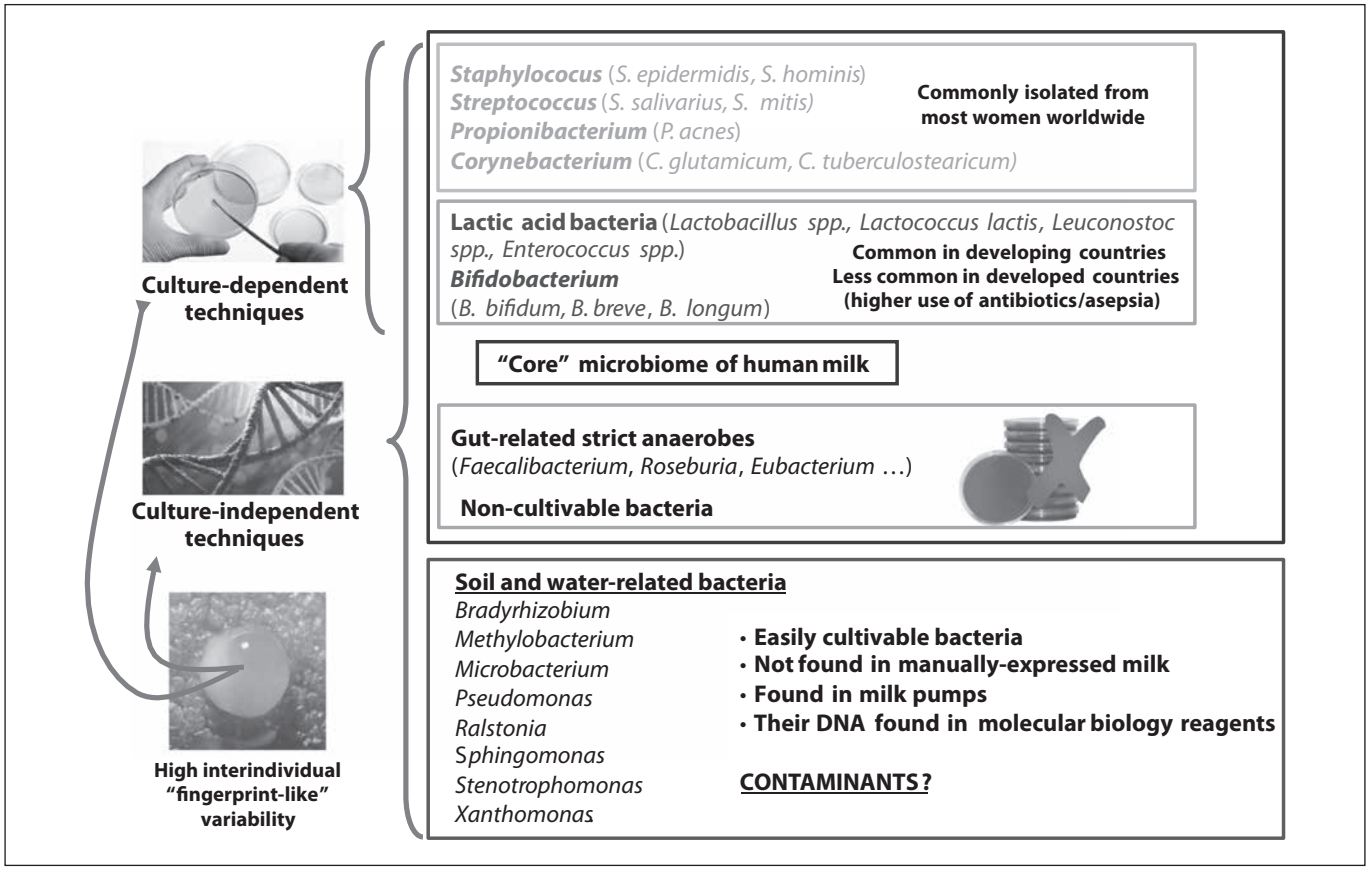

Figure 1. Main bacterial genera and species that can be isolated from or which DNA can be detected in human milk.

Presence of contaminating DNA in the working environment, PCR reagents, DNA extraction kits and molecular biology grade water are a particularly relevant challenge when working with samples containing low microbial load since, upon amplification, the low amount of starting material may be widely overcome by the contaminating DNA and lead to inaccurate results and conclusions. In fact, most DNA sequence-based studies describing microbial communities in low-biomass environments neither report sequencing of negative controls, nor describe their contaminant removal procedures.

Considering the DNA contamination issue and the fact that soil and water-associated Gram-negative bacteria have been seldom isolated from human milk, it is highly probable that the core bacteriome of human milk is actually constituted by *Staphylococcus*, *Streptococcus*, *Lactobacillus*, *Corynebacterium*, *Propionibacterium* and gut-associated obligate anaerobes (*Bifidobacterium*, *Bacteroides*, *Roseburia, Eubacterium*, *Faecalibacterium*, *Ruminococus*) (Fig. 1).

The fact that sequences from lactobacilli, bifidobacteria and strict anaerobes can be detected in some studies and are scarce or absent in others may also be attributable to differences in the high-throughput sequencing techniques, but also to genetic, environmental, medical or dietary differences among subjects.

Factors Influencing Microbiota/Microbiome Composition in the Human Milk

The quantitative and/or qualitative composition of many components of human milk (peptides, proteins, lipids, immunological compounds, oligosaccharides, etc.) may be influenced by several factors, including genetic background, geographical location, maternal nutrition, part of the feeding (foremilk, hindmilk), gestational age, circadian rhythm, lactation stage, and others. However, little is known on the interaction and impact of these and other factors on microbial communities composition in the human milk [12, 18] (Fig. 2).

Some studies have investigated the impact of a variety of factors in the human milk microbiota/microbiome com-

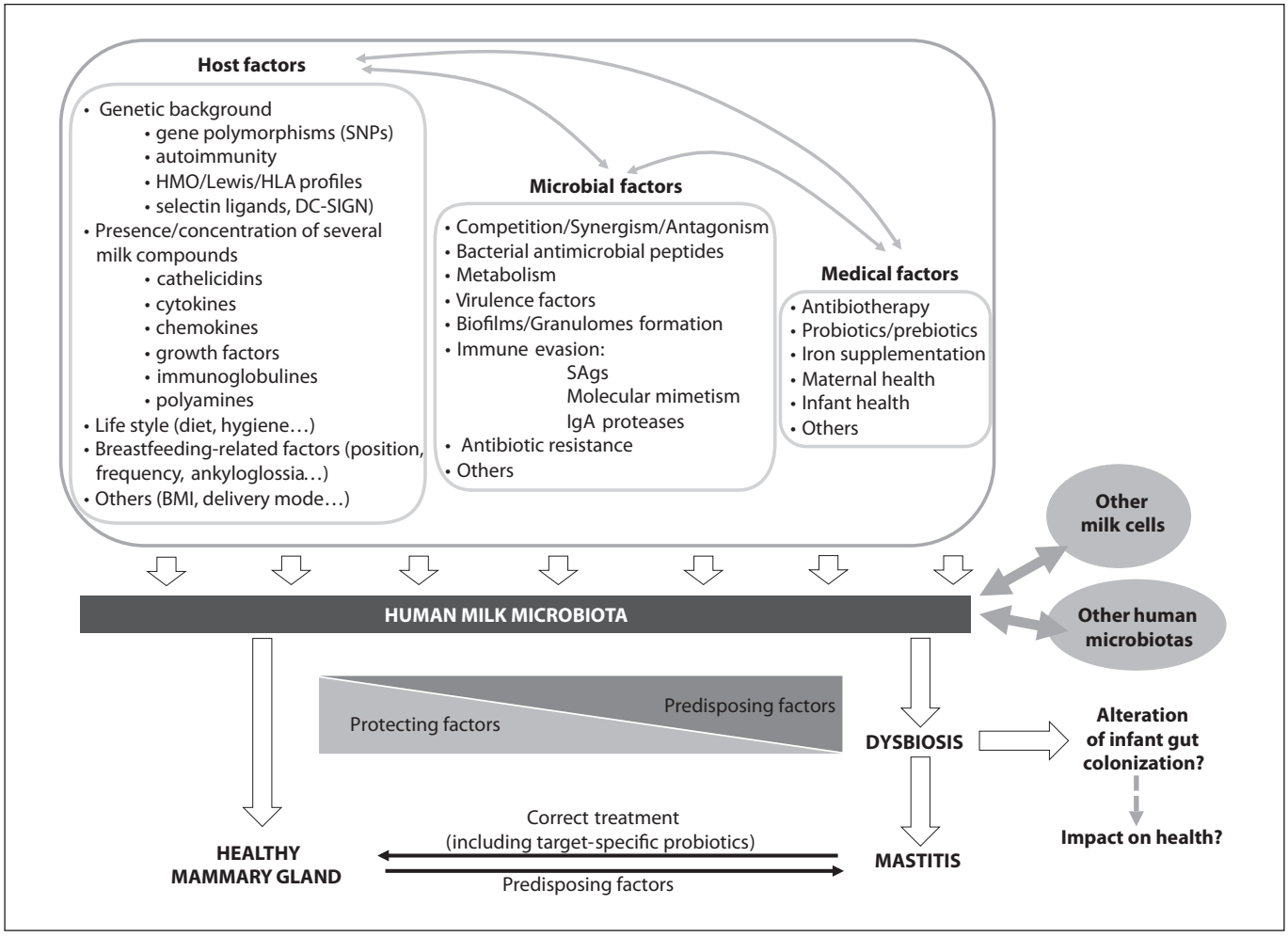

Figure 2. Factors that may play a role in the composition of the human milk microbiota and in protecting or predisposing to mastitis.

position. Such factors include HMO profile, gestational age, postpartum period geographical location, mode of delivery, maternal diet, maternal health status (healthy, mastitis, metabolic syndrome, obesity, allergy, celiac disease, HIV-positive women), medical treatments (antibiotics, chemotherapy) and use of pumps and other devices for sampling [7, 8, 14, 15, 17]. Some of the studies found significant differences between the compared groups while others did not. Conflicting and controversial results have also been obtained when different research groups have compared the effect of the same factor on the human milk microbiome. So, while it is becoming evident that human milk microbiome may be influenced by several factors and, also, that microbiota found in human milk may exert a strong influence on other milk components and, globally, on maternal/infant health, the exact triggers or drivers of differences in the composition of the human milk microbiota/microbiome need to be elucidated. Conflicting results between studies can be explained, at least partially, by host factors, environmental factors, perinatal factors, differences in milk collection and storage procedures, growth media and conditions, DNA extraction and amplification protocols, DNA sequencing methods, and bioinformatics analysis, among other factors [12]. International and collaborative research, sharing common protocols from recruitment criteria to bioinformatics, is required in order to enable the comparison of results between groups and to evaluate the actual impact of the factors cited above [11, 12].

Origin of Bacteria in Human Milk

Traditionally, it was believed that any prokaryote found in human milk was just the result of contamination from

the infant's oral cavity or the mother's skin. However, the detection of live bacterial cells and/or DNA from anaerobic species that are usually related to gut environments and that cannot survive in aerobic locations has fuelled a scientific debate on the origin of milk-associated bacteria. These findings suggest that at least some of the bacteria present in the maternal digestive tract could reach the mammary gland through an endogenous route, involving complex interactions between bacteria, epithelial cells and immune cells [19]. Although the pathway and mechanisms that some bacteria could exploit to transit from the oral and/or intestinal epithelium to reach the mammary gland and other locations has not been elucidated yet, some works have offered a plausible scientific basis [20, 21].

An increased bacterial translocation from the gut to mesenteric lymph nodes and mammary glands in pregnant and lactating mice has been described previously [20]. Bacteria could be observed histologically in the subepithelial dome and interfollicular regions of Peyer's patches, in the *lamina propria* of the small bowel, and associated with cells in the glandular tissue of the mammary gland. In the same study, acridine orange staining of human milk and blood cytopreparations identified bacterial cells in association with maternal mononuclear cells. In addition, other studies have reported that oral administration of *L. reuteri*, *L. gasseri*, *L. fermentum* and *L. salivarius* strains isolated from human milk to lactating women led to their presence in human milk [22, 23]. In fact, the microbiome of the different human body locations can be regarded as a dynamic network of interrelated communities and thus, although the infant's mouth or the maternal skin may provide some bacteria to the milk, this is not incompatible with the fact that human milk represents a source of bacteria to the infant.

Mother-to-Infant Transfer of Bacteria through Human Milk

After birth, the bacterial colonization process represents the first massive contact with microbes; and different studies have established a link between early gut microbial colonization patterns and the risk of developing certain metabolic, inflammatory and chronic diseases later in life, underlining the important role of the microbiota-host interactions in the neonatal period. By providing a supply of live microorganisms, together with different bioactive substances including carbohydrates to be selectively fermented by beneficial commensal microbes in the infant gut, human milk seems to play a pivotal role in the proper establishment and further development of the infant microbiota and, consequently, on important host functions including nutrient absorption, formation of host barriers against pathogens, or maturation of the immune and nervous systems [24].

Bacteria present in human milk are among the first colonizers of the infant gut and, accordingly, several studies have reported a vertical mother-to-infant transfer of microorganisms through human milk (at the species and strain level), using both culture-dependent [2, 3, 4, 25], and culture-independent techniques [6]. In fact, the initial microbiota of healthy breastfed babies resemble closely that found in the mother's milk and, remarkably, the networks established between the intestinal microorganisms and the host in breast-fed babies are different from those found in formula-fed infants.

Until recently, it was thought that the development of a more diverse gut microbiota in breastfed infants started at the weaning period. However, a recent work showed that stopping breastfeeding -rather than introducing solids- drives maturation of the infant gut microbiota [26]. These researchers found more adult-like taxa in the microbiomes of babies who stopped breastfeeding earlier, while the microbiota of babies breastfed for longer periods were dominated by bacteria present in breast milk.

From Physiology to Pathology: Lactational Mastitis

The process of lactation has been remarkably successful since the earliest mammals, allowing thousands of species to occupy a vast range of ecological niches. However, mastitis remains as a common feeding complication among most, if not all, mammalian species. Mastitis represents the first medical cause of undesired premature weaning, with an incidence among lactating women as high as 35%.

The lactating mammary gland ecosystem is hospitable to many microorganisms, including bacterial groups that have the potential to cause mastitis [1]; however, upon disturbance of this balanced state, infection can occur and, in fact, recent studies suggest that mastitis is a process characterized by a mammary bacterial dysbiosis [18] (Fig. 2).

In this context, microbiological analysis of milk is the only method that allows an etiological diagnosis of mastitis. It may seem simple but is not an easy issue, partly due to the absence of uniform or standard protocols for the collection of this biological fluid, the doubts that often arise for the interpretation of the results and, in humans, the lack of tradition in milk microbiological analysis. The collection of a representative sample for microbial analysis is of outmost importance in order to get a correct diagnosis since there are many sampling-related factors that may

affect the result [23]. The etiopathogenesis of the different types of lactational mastitis (acute mastitis, subacute mastitis, granulomatous mastitis…) and the factors that predispose to or protect from suffering them have been reviewed by Fernández et al. [18].

Since resistance to antibiotics and ability to drive the formation of biofilms are common properties among mastitis-causing bacteria, many cases are refractory to antibiotic therapy. As a consequence, alternative strategies are required to improve mastitis healing rates while reducing the use of antibiotics. In this context, the development of new strategies for mastitis management based on human milk probiotics, as an alternative or complement to antibiotic therapy, is particularly appealing [18, 23].

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Annals of Nutrition & Metabolism

The Skin Microbiome

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Abstract

The skin is the human body's largest organ and provides the first line of defence against environmental attack and pathogen invasion. It is colonized by a diverse milieu of microorganisms at different body sites, which play important roles in sensing the environment, protecting against colonization and infection of pathogens, and guiding the host immune system in response to foreign invasions. Colonization is driven by the ecology of the skin surface, which is highly variable depending on topographical location, endogenous host factors and exogenous environmental factors. The development of molecular methods has led to an emerging view of the resident skin microorganisms as highly diverse and variable. The skin microbiome is largely variable between individuals and body sites, with several core commensal members commonly shared among individuals at the healthy state. The cutaneous innate and adaptive immune responses can modulate the skin microbiota, but the microbiota also functions in educating the immune system. These microbial commensals are essential to skin health and can potentially lead to disease when their abundances and activities change due to alterations in the environment or in the host. An enhanced understanding of the skin microbiome is necessary to gain insight into microbial involvement in human skin disorders and to enable novel promicrobial and antimicrobial therapeutic approaches for their treatment.

Skin Microbial Community

The skin is not only the largest organ in the human body but also a host for hundreds of microorganisms, including bacteria, viruses and eukaryotes like fungi and arthropods[1]. Inmediately following birth, our skin is colonized with microbiota. The composition of the skin microbiome is diverse and variable between different locations of the skin. The exact composition of skin microbes varies from individual to individual, but remains somewhat stable over time in the same person[2]. The dominant types of bacteria that reside on the skin appear to be relatively stable, with the rare, less abundant types of bacteria accounting for the variability.

Classifications of cutaneous microbiota focused on skin microbes only as pathogens or opportunistic pathogens. However, the community of microorganisms on human skin is more complex than once thought. The development of methods based on sequencing technologies, independent of the need for cultivation of microbes has changed our understanding of skin microbiota.

To date, the majority of culture- and sequencing-based microbiome studies have focused on characterizing the skin bacterial and fungal communities. Based on sequencing analysis of phylogenetic marker genes, such as the bacterial 16S ribosomal RNA (rRNA) and fungal internal transcribed spacer (ITS), the bacterial residents identified

mainly belong to four phyla: Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes[3], while the majority of fungal species identified are from a single genus, *Malassezia*[4]. The majority of the identified bacterial genera are *Corynebacterium*, *Propionibacterium* and *Staphylococcus*[3].

Other eukaryotes that colonize the human skin belong to the phylum Arthropoda. *Demodex* mites favor lipids of the sebum. *Demodex folliculorum* is found in hair follicles in clusters with other mites of the same species. The smaller mite *Demodex brevis* resides alone in sebaceous glands or in meibomial glands which are located at the rim of the eyelids[5].

Less is known about the human virome. Detecting methods have largely focused on metagenomic sequencing of total DNA, making it unlikely to detect RNA viruses. Double stranded DNA (dsDNA) eukaryotic viruses, including herpesviruses, papillomaviruses, polyomaviruses, circoviruses, adenoviruses, anelloviruses and paroviruses, have been identified in the healthy skin microbiota[6]. In addition, prokaryotic viruses of the major skin bacteria, in particular *P. acnes* and *S. epidermidis* phages, were found at multiple skin sites. Phages are prokaryotic viruses that infect bacterial hosts and are a dominant part of the skin virome. They are commonly found at multiple skin sites, naturally co-occurring with their preferred bacterial hosts. Sequencing analysis suggested that *Propionibacterium* and *Staphylococcus* phages are the most abundant skin phages, while other phages, such as *Streptococcus* and *Corynebacterium* phages, are also present but at lower relative abundances[7]. In summary, many studies suggest the existence of a complex and dynamic virome on the human skin.

Factors Influencing the Composition of the Skin Microbiome

Despite variations in the skin microbiome due to multiple contributing factors, skin microbial communities of healthy individuals appear relatively stable over at least several months[8]. The stable nature of the human skin microbiome and persistence of core skin microorganisms suggest important functions of the commensal microbiota in skin health. The composition of the human skin microbiome is influenced by multiple factors. Similar to the microbial communities at other body sites, individual variation is the major factor differentiating the skin microbiome among the populations. Age, sex and hygiene practice also contribute to the individual variation of the skin microbial composition. The uniqueness of each individual´s microbial composition seems to be stable over time.

The spatial site is another factor affecting the skin microbiome composition. The skin provides many niches in which large populations of microbes are subjected to variable ecological pressures including moisture, sweat level, temperature, pH and the composition of antimicrobial peptides and lipids. Each site represents an ecological niche that favours the growth of its own unique collection of microorganisms. The microbial communities at dry, moist and lipid-rich sites are largely different. In addition, skin structures such as hair follicles, sebaceous, eccrine and apocrine glands constitute discrete niches that harbor unique microbiota[9].

In dry and exposed sites, mixed populations of bacterial species of β-Proteobacteria and Flavobacteriales are part of the resident microbiota. In moist sites such as the axilla, *Corynebacterium* species predominate, although *Staphylococcus* species are also present. The lipid-rich areas of the skin, such as the sebaceous sites on the face and upper trunk exhibit the lowest microbial diversity and are predominated by *Propionibacterium* species and *Staphylococcus* species. Lipophilic fungi such as *Malassezia* species, as well as the *Demodex* mite, *Demodex folliculorum* can also be found [3].

In addition to individual differences, topographical variation, and environmental influences, the host health status and the skin condition can also affect the composition of the microbiota. Shifts in skin bacterial and fungal communities have been linked to a number of skin diseases and conditions including psoriasis, atopic dermatitis, acne, dandruff, and damaged or wounded skin. The parasitic mite *Demodex folliculorum*, as well as its own associated microbiota, has been implicated in rosacea.

The Role of the Skin Microbiota in Shaping Skin Functions

Keratinocytes, the cells that coat the outer skin layers, constantly monitor the skin surface to recognize foreign or pathogen-associated molecular patterns (PAMPs), and in their presence, initiate an innate immune response via TLRs and Nod-like receptors, resulting in the production and secretion of cytokines, chemokines and AMPs[10]. The skin microbiota plays an important role in shaping host immunity and aiding in the stimulation of host immune responses to defend against the colonization of pathogenic microorganisms.

The composition, dynamics and function of the skin microbiota have a significant impact on skin health and function. The resident microorganisms sequester nutrients from skin secretions and form a dynamic ecological system

with the host skin through complex interactions within the microbial communities and with the host. The mechanisms responsible for homeostasis between the microbiome and the host remain largely unknown. Furthermore, complex ecological interactions with the environment and competition between microbial species are important for the maintenance of a healthy microbiome.

From sequencing information we know that microbial communities are more complex than previously expected from culture based studies. Microbes profit from their host as they are provided nutrients and a stable ecological niche. The interactions of microbes within the host can be divided in three categories of relationships: commensalism, mutualism and detrimental. An example of a beneficial relationship between bacteria and the skin is the stimulation of Toll-like receptors (TLRs). Stimulation of TLRs induces distinct patterns of gene expression in the host that leads to the activation of a variety of immune responses (pro-inflammatory and anti-inflammatory).

Disruption of the normal composition of microbes, called dysbiosis, can affect host-microbe crosstalk and may result in disease. Besides, with changes to the condition of the skin, including injury or the introduction of medical devices such as implants or catheters, some of the resident microorganisms can behave as opportunistic pathogens.

Key Players of the Commensal Skin Microbiota

To date, the dominant and most extensively studied members of the healthy skin microbiota include *Staphylococcus*, *Propionibacterium*, *Streptococcus*, *Corynebacterium* and *Malassezia*. Changes in the abundances of these organisms are often linked to diseased states. Studies have also implicated phages as potential modulators of the skin bacterial community.

Staphylococcus Epidermidis

The Gram-positive bacterium *S. epidermidis* is a dominant skin resident found at multiple body sites. Sequencing of *S. epidermidis* has revealed a high level of strain diversity, with nearly 600 sequence types currently identified. Unlike its coagulase-positive relative *Staphylococcus aureus*, coagulase-negative *S. epidermidis* is widely accepted as a beneficial skin microorganism of low pathogenicity[11]. *S. epidermidis* has several beneficial actions in skin health. *S. epidermidis* produces and secretes a number of antimicrobial peptides (AMPs) which can directly prevent the colonization of skin pathogens including Group A *Streptococcus* (GAS) and *S. aureus*[12]. It also functions as a bacterial primer on the skin, regulating and promoting host inflammatory responses via Toll-like receptor (TLR) signalling. In case of co-colonization with pathogenic *S. aureus*, commensal *S. epidermidis* not only upregulates AMP expression but also abolishes the inhibition of NF-κB signaling asserted by *S. aureus*, leading to amplified host immunity in response to pathogen invasion. *S. epidermidis* can enhance host immune responses in defence against other bacterial pathogens in addition to *S. aureus*, such as GAS, as well as against viral infections, such as vaccinia virus and human papillomavirus (HPV), while maintaining its own colonization on the skin. Despite being typically considered a commensal organism, *S. epidermidis* can act as an opportunistic pathogen, with biofilm formation as a pathogenic mechanism.

Propionibacterium Acnes

Gram-positive lipophilic *P.acnes* is a dominant species at sebaceous sites, such as the face, neck and upper trunk. Propionibacteria are believed to play a beneficial role in maintaining skin health via their ability to metabolize triglycerides in sebum to short chain fatty acids (SCFAs). SCFAs exhibit antimicrobial properties and contribute to emolliate the skin and to the acidic cutaneous pH, thus preventing the colonization of pathogenic skin species including *S. aureus*[13]. In addition to the production of SCFAs, some *Propionibacterium* species are capable of producing bacteriocins. *P. acnes* bacteriocins have been shown to inhibit the growth of some *P. acnes* strains as well as other bacteria.

Malassezia Species

The predominant fungus detected by using phylogenetic markers belongs to the species *Malassezia* including the most frequent isolates *M. globosa*, *M. restricta* and *M. sympodialis*[4]. *Malassezia* are lipophilic yeasts that colonize sebaceous areas of the skin and degrade sebum. Similar to the bacterial distribution on the skin, the distribution of *Malassezia* is dependent on the characteristics of the respective habitat. *M. globosa* predominates on the back, occiput and inguinal crease, whereas *M. restricta* is found on the scalp, in the external auditory canal, retroauricular crease and glabella. Genome analysis of *M. restricta* and *M. globosa* has revealed an abundance of lipases and phospholipases that are believed to aid in fatty acid metabolism. One of the by-products from fatty acid metabolism by *Malassezia* species is azelaic acid, which exhibits antimicrobial properties against skin bacteria and fungi. Similar to other skin commensal microorganisms, *Malassezia* species have also been linked to a number of skin diseases. *M. sympo-*

dialis has been implicated in atopic dermatitis, whereby it contributes to skin inflammation via the release of allergens. *M. restricta* has been controversially associated with dandruff, an inflammatory scalp disorder. Despite associations with skin inflammatory conditions, the prevalence of *Malassezia* species on healthy skin suggests that these species are commensals and may become harmful when unfavorable conditions are presented. Further understanding of the functions of these fungal species will provide important insight in skin health and disease.

The Skin Microbiome in Cutaneous Disease

Acne

Altered bacterial colonization is considered to be one of the main elements contributing to the development of acne[14]. Historically, *P. acnes* has been implicated in the pathogenesis of the common skin disease, acne, mostly due to a high frequency of isolation of the species from acne lesions. Yet this association remains a topic of much debate due, in part, to the dominance of the species on healthy, non-acneic skin. *P. acnes* colonizes sebaceous follicles that contain microcomedones providing the bacterium an anaerobic and lipid-rich environment. A study found that acne patients showed no difference in the relative abundance of *P. acnes* versus healthy controls. However, only certain *P. acnes* strains were highly associated with acne. Sequencing and comparative genome analysis of large collections of *P. acnes* strains isolated from acne patients and healthy individuals have since revealed significant phylogenetic diversity within this species. Certain lineages of strains have been associated with disease while others are associated with health[15].

Another mechanism for acne pathogenesis, involving a host–bacteria interaction via metabolites has been recently described. In the presence of externally available vitamin B12, *P. acnes* was shown to repress its own vitamin B12 biosynthesis and shunt the metabolic flow towards the production of porphyrins, a group of bacterial metabolites inducing inflammation in host tissues and leading to acne development[16]. This suggests that the skin microbiota constantly senses the host metabolite level, reacts to its changes, and in turn plays a role in skin health or disease.

Atopic Dermatitis

A hallmark of atopic dermatitis (AD) that has been known for decades is that patients have increased colonization with bacteria and are particularly susceptible to infections with *S. aureus* and viruses such as herpes and vaccinia. It has been hypothesized that the alteration in surface microbial composition is due to dysfunction of the skin barrier. There is also a decreased expression of antimicrobial peptides (AMPs) in the skin. Flares are associated with a lower skin bacterial diversity and effective treatment increases bacterial diversity (*Corynebacterium*, *Streptococcus* and *Propionibacterium*)[17].

Rosacea

In rosacea, microbes other than bacteria may take advantage of impaired homeostasis between host and skin microbiota. The *Demodex* mite found on healthy skin is significantly increased on the skin of rosacea patients. The mite may participate in the exacerbation of disease either by disrupting the skin barrier or by triggering TLR2 activation through the chitin in the insect´s cuticle. Furthermore, it has been reported that bacteria that live in the digestive tract of *Demodex* are released into the surrounding skin tissues, thereby triggering further tissue degradation and inflammation[18].

Seborrhoeic Dermatitis and Dandruff

The predominant fungus of the skin microbiota, *Malassezia*, is postulated to take part in seborrheic dermatitis. Dandruff is mainly associated with *M. restricta* and *M. globosa*. The fungus is known to secrete a lipase that splits triglycerides into irritant fatty acids that may induce hyperproliferation and scaling.

Role of Microbiome in Skin Therapy

With the many health benefits conferred by commensal microorganisms, research has turned towards exploiting the properties of commensal skin microorganisms, such as those with potential probiotic properties, to manipulate the skin microbiota and enhance skin health. Examples include the topical application of the commensal skin bacterium *Janthinobacterium lividum* to treat athlete's foot,

Commensal skin microorganisms can be exploited to correct dysbiosis in the skin microbiota in diseases. *S. epidermidis* has been suggested as a probiotic in treating acne[19]. While *S. epidermidis* and *P. acnes* naturally co-exist on the skin, it was found that commensal *S. epidermidis* can inhibit the overgrowth of *P. acnes*, which has been linked to acne. On the other hand, the health-association of certain *P. acnes* strains implies that supplementation with health-associated strains may help to treat acne and to

maintain skin health. While typical acne treatments include antibiotic administration, the extensive use of antibiotics has led to the emergence of antibiotic-resistant strains and thus increased rate of treatment failure. Exploiting probiotic and prebiotic therapeutics will ultimately reduce the prevalence of antibiotic resistance in the population and potentially result in better treatment outcomes.

Additionally, non-pathogenic microorganisms that are not usually part of the normal skin microbiota have been investigated for their potential applications in enhancing immune responses. *Vitreoscilla filiformis*, a Gram-negative bacterium recognized by keratinocytes, can stimulate antioxidant and antimicrobial defence mechanisms via TLR-2 signalling[20]. Application of topical *V. filiformis* to lesional skin significantly improved the skin condition in atopic dermatitis patients by inducing high levels of the anti-inflammatory cytokine IL-10.

In summary, an enhanced understanding of the skin microbiome is necessary to gain insight into microbial involvement in human skin disorders and to enable novel promicrobial and antimicrobial therapeutic approaches for their treatment.

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Respiratory Microbiota in Children

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Abstract

Studies have shown that the human microbiota plays an important role in maintaining the host cell homeostasis as well as modulating the host immune response [1].

Until recently, the lower respiratory tract was thought to be a sterile environment, but it is know now that just after birth and during the neonatal period the airway is colonized by bacteria that constitute the respiratory microbiota. In recent years studies have shed light into the composition of the respiratory microbiota during the first years of life, highlighting the numerous factors that may influence the diversity and abundance of the microbiota composition [2, 3]. Factors such as breastfeeding, mode of delivery, use of antibiotics and age, could affect the respiratory microbiota composition, which is a dynamic process and changes throughout the first two years of life [4, 5].

Current evidence suggests that the respiratory microbiota, by modulating the host immune response, may affect disease severity in infants with bronchiolitis [6]. In addition, studies have shown that it could also play an important role in the development and pathogenesis of childhood asthma [7-9].

Introduction

A trillion of bacteria inhabit the human body maintaining the homeostasis but also modulating the host immune response. In fact, it is known that alterations in the microbiota composition have implications for human health. Indeed, some authors suggest that the microbiota should be considered part of the human genome due to its physiologic implications [1, 10].

In recent decades, there have been important advances in the knowledge of the human microbiome, largely due to the development of molecular techniques for the identification and characterization of this ecosystem. It is estimated that only 30% of the bacteria living in the human body can be cultivated with conventional methods. The most widespread method for studying the human microbiome is the sequence of the 16S ribosomal (r)RNA for the reasons outlined below. Bacteria are prokaryotic cells that contain in the small subunit of the ribosome the 16S RNA gene. The 16S rRNA is particulary suitable for microbial detection because is highly conserved across different bacterial species, but also confers bacterial specificity allowing the identification of the bacteria at the genus or species level. By simultaneously amplifying and characterizing different bacteria, 16S rRNA sequencing not only allows to analyze the composition of the microbiome but also its function [1].

For years, the gastrointestinal microbiota has been the main focus and widely studied in relation to the development or the pathogenesis of certain diseases. However, the role of the airway microbiota in human health and disease, has gained great interest in recent years. Few studies have been able to characterize the "sterile" healthy microbiome of the lower respiratory tract, since obtaining lung samples

not contaminated with microbes of the upper airway is a relatively difficult task. Charlson et al [11] observed that the composition of the lung microbiome was similar to the bacterial composition in the upper respiratory tract, but with a lower biomass, suggesting that the pulmonary microbiota probably develops following microaspiration or inhalation from the upper respiratory tract.

Health and Respiratory Microbiota in Children

In 2010, researchers showed that lungs are not sterile [12]. With an area of 75 m^2 and in direct contact with the environment, lungs are one of the anatomical sites most exposed to bacteria [13]. The composition of the airway microbiota during respiratory health appears to be influenced by the migration and clearance of microbes rather than the growing conditions influencing the rates of bacterial growth. In healthy individuals, aspiration from the oral cavity seems to be the principal mechanism favoring the colonization of the lower airway by bacteria. Nevertheless, there are other factors that contribute to the composition of the respiratory microbiota in healthy infants including age, mode of delivery, use of antibiotics, and especially breastfeeding.

The upper respiratory tract is colonized during the first years of life with an abundant number of bacterial communities including commensal and potentially pathogenic bacteria, such as *Streptococcus pneumoniae, Haemophilus influenzae,* and *Staphylococcus aureus* [3]. In general, these bacteria remain in the nasopharynx without causing any symptoms. However, when the balance between the host and the pathogen is altered, the bacteria can spread to the airway or even into the bloodstream causing clinical disease [3]. Biesbroek et al characterized in an unselected cohort of healthy children, the upper respiratory tract microbiota composition sequentially, at 1.5, 6, 12, and 24 months of age. They identified, as early as 1.5 months of age, eight predominant respiratory microbiota profiles that varied during the first two years of life in these healthy children. The most stable profiles were characterized by the presence and/or abundance of *Moraxella* and *Corynebacterium/Dolosigranulum* also associated with breastfeeding as well as with fewer respiratory infections in the first months of life. Less stable profiles were characterized by the abundance of *Haemophilus* or *Streptococcus*. The results of this study emphasize the importance of breastfeeding, as it has a significant impact on the composition of the nasopharyngeal respiratory microbiota in the first weeks of life [3].

Teo et al went a step further, and in a selected birth cohort of children at high risk for asthma, studied the nasopharyngeal microbiota composition sequentially in periods of health and acute respiratory disease caused by RSV or rhinovirus, during the first year of life. Of the main six genera integrating the respiratory microbiota that authors identified (*Haemophilus, Streptococcus, Moraxella, Staphylococcus, Alloiococcus and Corynebacterium*), they found that in the first weeks of life most healthy infants were colonized with *Staphylococcus* or *Corynebacterium*, to be later replaced by *Moraxella* or *Alloiococcus*. *Haemophilus, Streptococcus* were more frequent in older children [7].

Also using 16S rRNA sequencing, Mortensen et al characterized the dynamics of the airway microbiota at 1 week, 1 month, and 3 months of life in a large cohort of 700 infants. Using hypopharyngeal aspirates, they found five predominant pneumotypes belonging to these genera: *Staphylococcus, Streptococcus, Moraxella*, and *Corynebacterium* [14].

Table 1 summarizes the major studies describing the composition of the respiratory microbiota during "respiratory health" in children, which is mostly composed by *Moraxella, Streptococcus, Haemophlius* and *Corynebacterium*. In the majority of these studies it was observed that: a) the composition of the respiratory microbiota varied sequentially with age during the first year of life, and b) that it was affected by different factors such as the mode of delivery [15], breastfeeding, age [16, 17], and season of the year.

Respiratory Microbiota and Disease in Children

Importance of Respiratory Microbiota in Bronchiolitis Severity

Respiratory syncytial virus (RSV) bronchiolitis is the first cause of hospitalization in infants less than one year of age. It is estimated that by two years of age, almost all children have had a primary infection by RSV. There are patient populations at risk for severe RSV disease, such as infants born prematurely, and those with bronchopulmonary dysplasia and congenital heart disease. However, the majority of children hospitalized with RSV are previously healthy, and host factors alone do not completely explain the variability in disease severity. It has been postulated that viral factors, such as viral loads or viral genotypes [18], also contribute to RSV bronchiolitis severity. Most likely the severity of the disease is influenced by a combination of both viral factors and a dysregulated or impaired host innate immune response [19].

Whether the respiratory microbiota could contribute to the severity of RSV bronchiolitis is a topic of increas-

Table 1. Airway microbiota in healthy children.

Study year	Age	Cohort	Type of sample	Technique	Predominant Genera/Phila	Conclusions
Bogaert D 2011	18 m	96 healthy infants (unselected)	Nasopharynx	16 S rRNA V5-V6	*Moraxella, Haemophilus, Streptococcus, Flavobacteria, Dolosigranulum, Corynebacterium,* and *Neisseria*	*Proteobacteria*/*Fusobacteria* profiles predominated in winter, whereas *Bacteroidetes*/*Firmicutes* profiles predominated in spring.
Biesbroek G 2014	1.5 m and 6 m	202 infants	Nasopharynx	16S r RNA	*Moraxella, Haemophilus, Staphylococcus, Streptococcus,* and *Corynebacterium/ Dolosigranulum*	*Dolosigranulum* and *Corynebacterium* might contribute to the protective effect of breastfeeding against respiratory infections.
Teo SM 2015	< 12 m	234 healthy Selected birth cohort (at risk for asthma)	Nasopharynx	16S rRNA	*Moraxella* (31.2%), *Streptococcus* (15.5%), *Corynebacterium* (13.5%) *Staphylococcus* (10.3%), *Haemophilus* (9.7%) and *Alloiococcus* (8.8%;	Most infants initially colonized with *Staphylococcus* or *Corynebacterium* before stable colonization with *Alloiococcus* or *Moraxella*.
Dominguez-Bello MG 2016	< 1 month	10 healthy	Nasopharynx	16S rRNA	Vaginal: *Lactobaccillus*, *Prevotella*, *Sneathia* C-section: *Staphylococcus*, *Corynebacterium*, *Propionibacterium*	Mode of delivery determines the composition of the nasopharynx microbiota
Mortensen MS 2016	< 3 m	695 healthy infants	Hypopharynx	16S r RNA	Most frequent phyla were *Firmicutes* (61%), *Proteobacteria* (30%), *Actinobacteria* (6%), and *Bacteroidetes* (2%)	Bacterial colonization of the airway is frequent in healthy individuals. Early colonization is important for the development of the microbiota later in life
Salter SJ 2017	< 24 m Birth cohort	21 healthy infants	Nasopharynx	16 sRNA	Five taxa: *Moraxella*, *Streptococcus*, *Haemophilus*, *Corynebacterium* and *Flavobacteriaceae*.	*Staphylococcus* and *Corynebacterium* present in the first few months of life while abundance of *Moraxella* and *Flavobacteriaceae* increase in proportionally with age.
Wang W 2018	< 12 y	115 healthy	Nasopharynx, anterior nares and oropharynx	16S rRNA and PCR amplification	*Moraxella*, *Staphylococcus*, *Corynebacterium*, *Streptococcus*, and *Dolosigranulum*	*Corynebacterium, Streptococcus, Staphylococcus, Moraxella,* and *Dolosigranulum* predominate in the ANs and NP microbiota while the dominant bacterial components the OP differs

OP: oropharynx; NP: nasopharynx; AN: anterior nares.

ing interest. Suarez Arrabal et al [20] investigated the relationship between nasopharyngeal (NP) colonization with potentially pathogenic bacteria *(S. pneumoniae, S. aureus, H. influenzae and M. catarrhalis)* and RSV disease severity in previously healthy children. They found that nasopharyngeal colonization with these bacteria (except for *S. aureus*) was significantly more common in infants hospitalized with RSV bronchiolitis than in age-matched healthy controls. Colonization with Gram-negative bacteria was particularly more frequent. Moreover, they found that RSV+ infants colonized with Gram negative vs. Gram-positive bacteria had greater leukocytosis, higher plasma concentrations of IL 6 and IL 8, and a more prolonged requirement for supplemental oxygen. Subsequently, de Steenhuijsen et al [6] evaluated a cohort of 132 infants with RSV infection to determine if the different NP microbiota profiles were associated with specific host responses by analyzing whole genome blood transcriptional profiles, and correlated those profiles with clnical disease severity. They identified five predominant clusters: *Haemophilus influenzae*, *Streptococcus*, *Corynebacterium, Moraxella,* and *Staphylococcus aureus.* RSV disease severity, as defined by the need for hospitalization was associated with predominance of *H. influenzae and Streptococcus*. Moreover, infants with RSV bronchiolitis, and with predominance of *H. influenzae* or *Streptococcus,* had blood transcriptional profiles that, in addition to expression of the interferon genes shared by all RSV bronchiolitis, were distinctly characterized by overexpression of innate immunity genes related to Toll-like receptor (TLR)-2, 4, 5 and 8 and

neutrophil and macrophage activation pathways. All these data, indicate that the respiratory microbiota during RSV bronchiolitis could modulate the host immune response and modify disease severity.

Rosas Salazar et al [21] analyzed the nasopharyngeal microbiota composition in infants with bronchiolitis caused by rhinovirus or RSV. Authors found that the microbiota composition was largely dominated by *Moraxella, Streptococcus, Corynebacterium, Haemophilus,* and *Dolosigranulum* irrespective of the etiology of the bronchiolitis, however, infants with RSV vs those with rhinovirus infection had greater abundance of *Haemophilus*. Other authors [21-26] also found that predominance of *Haemophilus* or *Streptococcus* was associated with enhanced disease severity of viral bronchiolitis (RSV or other viruses), confirming these findings.

Table 2 summarizes different studies that have documented the composition of the respiratory microbiota in children with acute bronchiolitis caused by RSV or another viruses [27].

Importance of Respiratory Microbiota in the Pathogenesis of Asthma

Asthma is a chronic inflammatory and immunologic disorder of the respiratory tract that affects one in ten children in western countries. Geographical disparity combined with a generational increase in prevalence, emphasizes that changing environmental exposures could play an important role in the genesis of this disease. Different hypotheses have explained the association between airway microbiota and the development of asthma:

– The "microflora hypothesis" suggests that early exposures in life interrupt the "normal" composition of the microbiota, and consequently, promote immune dysregulation and hypersensitivity disorders. Research studies in animal models support the role of the microbiota in the development of asthma and atopic diseases and identifies a critical window in the first 100 days of life when intestinal microbial dysbiosis is more influential in the promotion of hypersensitivity disorders [8, 9].
  Studies showed that germ-free mice had an exaggerated Th2 immune response, airway eosinophilia and hyperreactivity and mucosal hypersecretion compared with mice that were kept in a normal non-germ-free environment.
  Teo et al [7] found that early and asymptomatic colonization of the airway by *Streptococcus* at 2 months of age was significantly associated with the development of wheezing at 5 years of age. These findings are consistent with the results of "The Copenhagen Prospective Study on Asthma in Childhood –CPSAC–" [28] that was conducted in children of asthmatic mothers. In this selected cohort authors found that children colonized in the first month of life with *S. pneumoniae, M. catarrhalis and/or H. influenzae* had almost a 5-fold higher prevalence of asthma at 5 years of age than those non-colonized (33% vs 10% for colonized vs. non-colonized, respectively).
  Bisgaard et al conducted a study in a birth cohort of 321 newborns born from asthmatic mothers were enrolled, and followed during the first 5 years of life for the development of wheezing. In this study, hypopharyngeal samples were collected at one month of age and cultured for *S. pneumoniae, H. influenzae, M. catarrhalis, and S. aureus* [8]. Authors found that early colonization with *Haemophilus, Streptococcus or Moraxella,* but not *Staphylococcus* was associated with persistent wheezing (hazard ratio, 95% CI: 2.99 [1.66-5.39]). In this study, eosinophilia as well as total IgE were significantly increased in children colonized with *S. pneumoniae, M. catarrhalis, H. influenzae* at 4 years of age, but this relationship was not found with total IgE. Prevalence of asthma, as well as the reversibility of airway resistance after treatment with beta 2 agonists at 5 years of age was significantly greater in children colonized with these organisms during the neonatal period.
  In agreement with these findings, others have also found that abundance of *Haemophilus, Streptococcus or Moraxella* were related to the presence of asthma in children [7-9].
– The "disappearing microbiota" hypothesis suggests that as the prevalence of ancient commensal microorganisms decreases, the risk of becoming more susceptible to potentially pathogenic microorganisms increases. The hypothesis of the "disappearing microbiota" has been studied in relation to the increase and decrease of the prevalence of several diseases in developed countries [29].

Respiratory Microbiota in Pneumonia

Pneumonia is the leading cause of mortality in children under five years of age worldwide. Traditionally only a single bacterial pathogen was thought to be the causative agent of pneumonia (i.e. *S. pneumoniae*) in children. However this concept is evolving and, it is currently proposed that pneumonia occurs due to alterations in homeostasis of the respiratory tract, where more than one pathogen could be present [30, 31]. A study conducted in 383 children hospitalized with pneumonia, compared the respiratory microbiota identified from induced sputum samples vs those from samples obtained from the nasopharynx and oropharynx according to age. Authors found that in chil-

Table 2. Airway microbiota and viral bronchiolitis.

Study	Age	Study subjects	Site	Technique/ Other assays	Predominant Genera/Phila	Conclusions
Suarez Arrabal MC 2015	< 12 m	136 RSV 23 healthy controls Unselected	Nasopharynx	Bacterial culture Plasma concentration of IL8 and IL6	Gram-positive bacteria: *Staphylococcus aureus*, *Streptococcus pneumoniae*, β-hemolytic *Streptococcus*. Gram-negative bacteria: *Moraxella catarrhalis* and *Haemophilus influenzae*	Infants with RSV bronchiolitis colonized with potentially pathogenic bacteria had increased numbers of mucosal and systemic inflammatory cells. Colonization with GNB was associated with higher concentrations of IL6 and IL 8 and longer o2 need.
Teo SM 2015	< 12 m	234 infants (Birth cohort/ selected) RSV and rhinovirus bronchiolitis	Nasopharynx	16S rRNA	*Moraxella* (31.2%), *Streptococcus* (15.5%), *Corynebacterium* (13.5%) *Staphylococcus* (10.3%), *Haemophilus* (9.7%) and *Alloiococcus* (8.8%)	Most infants initially colonized with *Staphylococcus* or *Corynebacterium* before stable colonization with *Alloiococcus* or *Moraxella*. Incursions of *Streptococcus*, *Moraxella* or *Haemophilus* when infants had RSV or rhinovirus infection.
Hasegawa K 2016	< 12 m	1005 viral bronchiolitis/ unselected	Nasopharynx	16S rRNA	*Haemophilus*, *Moraxella*, and *Streptococcus*	Infants with a *Haemophilus*-dominant profile had greater disease severity than those with *Moraxella*-dominant profile.
Rosas Salazar C 2016	< 6 m	135 infants 52 rhinovirus and 83 RSV	Nasopharynx	16 s rRNA-V4	*Moraxella* (32.9%), *Streptococcus* (18.4%), *Corynebacterium* (10.7%) *Haemophilus* (10.2%), and *Dolosigranulum* (4.6%)	Significant differences in the nasopharyngeal profile between infants with RV and RSV. Greater abundance of Staphylococcus and Haemophilus in RSV infants
de Steenhuijsen W 2016	< 2 y	106 RSV 26 healthy Unselected	Nasopharynx	16S r RNA and Blood host gene expression profiles	*Haemophilus influenzae, Streptococcus, Corynebacterium, Moraxella*, or *Staphylococcus aureus*	Hospitalization associated with *H. influenzae* and *Streptococcus* predominant profile. Blood transcriptome profiles of children with RSV and *H. influenzae* or *Streptococcus* predominant profiles had greater overexpression of TLR, neutrophil and macrophage activation and signaling.
Hasegawa K 2017	< 12 m	40 infants most with RSV or rhinovirus 110 healthy	Nasopharynx	16s rRNA	Four profiles: *Moraxella* (37%), *Corynebacterium*/*Dolosigranulum* (27%) *Staphylococcus* (15%), mixed profile (20%)	Infants with *Moraxella* and *Corynebacterium*/ *Dolosigranulum*-profile had lower likelihood of bronchiolitis than those with *Staphylococcus* predominant profile.
Stewart CJ 2017	< 12 m	144 infants most with RSV or rhinovirus	Nasopharynx	16 s rRNA and Whole genome shotgun sequencing	*Haemophilus*, *Moraxella*, and *Streptococcus*	Metabolomics is able to predict bronchiolitis severity. Enrichment of sphingolipid metabolites positively correlated with *S. pneumoniae*
Hu Q 2017	< 12 m	27 infants RSV 22 healthy	Oropharynx and faecal	16 S rRNA V3 V4	Two bacterial pathogens: *Streptococcus pneumoniae* and *Haemophilus*	Imbalanced FM/OP microbiota in children with viral bronchiolitis, The OP microbiota in healthy and RSV+ infants dominated by *Streptococcus*
Luna PN 2018	Infants	815 infants with viral bronchiolitis	Nasopharynx	16 S rRNA	Four profiles: *Haemophilus*, *Moraxella*, *Streptococcus*, and mixed profiles	Association between *Haemophilus*-dominant microbiota and increased severity of bronchiolitis. *Moraxella*-dominant profile wa identified as protective against PICU admission.
Ederveen THA 2018	< 6 m	54 RSV 21 healthy	Nasopharynx	16s rRNA qPCR	Haemophilus (30.5%) *Streptococcus* (29.4%), *Moraxella* (10.7%), *Corynebacterium* (6.89%), and *Staphylococcus* (2.9%)	The nasopharyngeal microbiota in young infants with RSV infection is marked by an overrepresentation of *Haemophilus*

FM: faecal microbiota; OP: oropharynx; PICU: Pediatric Intensive Care Unit; RSV: respiratory sincitial virus; RV: rhinovirus; TLR: toll-like receptor; IL: interleukin.

dren <5 years of age, abundance of *Actinomyces, Veillonella, Rothia*, and *Lactobacillales* was associated with shorter length of stay, while the abundance of *Haemophilus* and *Pasteurellaceae* was associated with higher risk of PICU admission. In children > 5 years of life, identification of *Porphyromonadaceae, Bacteriodales, Lactobacillales,* and *Prevotella* was associated with longer length of stay [30]. Overall, these data suggest that certain pathogens, at different ages during childhood can exert the opposite effects, emphasizing the complexity of pathogen-host interactions in children with respiratory infections.

Respiratory Virome in Children

The majority of viruses that inhabit in humans are bacteriophages, that are viruses that infect bacteria. However, the identification of the human virome is challenging because of the lack of a common target in viruses, such as the 16S rRNA gene in bacteria. More modern techniques are being implemented and allow the analysis of all DNA sequences in a sample. Specifically, high-throughput next-generation sequencing (NGS) has become a powerful tool for pathogen detection, since it allows the examination of the entire virome, and even of viruses that are not identified by regular culture [32].

Wang et al in 2015 [33] characterized the respiratory virome using metagenomic analyses in children with severe acute respiratory infection vs. healthy children. They found that the most represented species were Paramyxoviridae, Coronaviridae, Parvoviridae, Orthomyxoviridae, Picornaviridae, Anelloviridae and Adenoviridae (> 50% genome). Viral populations in healthy children without airway infection were less diverse, and composed of viruses mainly from the Anelloviridae family with only a small proportion of frequent respiratory viruses. In another study, Lysholm et al [34] analyzed the virome in patients (70% children < 7 years old, and 30% with a median age of 44 years) with severe lower respiratory tract infection and found three predominant families: Paramyxoviridae, Picornaviridae and Orthomyxoviridae, and the predominant genera included RSV, influenza A virus and human rhinoviruses .

As these new tools for sequencing are implemented in clinical practice, it will open new perspectives to our understanding of the respiratory virome in connection with the microbiome and its implications in respiratory disease.

Conclusions

The composition of the respiratory microbiota in healthy infants is influenced by a number of factors including age, mode of delivery, use of antibiotics, and especially breastfeeding. The respiratory microbiota by modulating the host immune response may contribute to the severity of bronchiolitis in infants, and appears to play an important role in the pathogenesis of asthma. Currently, the composition and contribution of the respiratory virome to the development of airway disease in children is being studied, but there is increasing evidence of the importance of the airway virome in respiratory diseases.

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Prebiotic Modulation of Gut Microbiota and Immune Responses: Development of an Innovative Natural Immunomodulatory Health Ingredient from Carrots

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Health ingredient · Polysaccharide · Immune function · Infection resistance · Microbiota

Functional ingredients that support immune function and help protecting against infections in humans have long be searched for. Activity-guided fractionation led to the identification of an active constituent of a Traditional Chinese Medicinal extract described as delivering these benefits and we further established that this active compound can also be isolated from carrot pomace, a side stream of carrot juice production. This discovery solves critical sourcing, cost, as well as regulatory issues and makes application of this plant derived bioactive in dietary supplements and functional foods economically feasible.

Preclinical studies have shown that the ingredient modulates local and systemic immune function and protects from a *Salmonella* infectious challenge in mice. A human proof of concept study in 60 healthy volunteers showed a dose-dependent enhancement of innate immune function as well as modulation of the microbiota. Currently, two placebo controlled clinical trials are ongoing that test the efficacy of two doses of the compound (NL-01) given as dietary supplement. One trial addresses the protective effect of NL-01 in 156 healthy volunteers experimentally challenged with a common cold virus. The other trial tests protection against acute respiratory infections during the winter season 2018/2019 in 600 older adults. Microbiota profiling is being performed on the fecal samples collected in the second study.

Since the initial observation that a relatively low dose of plant derived polysaccharides induced changes in microbiota of mice and healthy humans, NutriLeads pursued studies to better understand the role NL-01 can play as a fibre or as a prebiotic, beyond its direct influence on the host immune system. The first step in this approach was to launch experiments of *in vitro* faecal fermentation. These data will next be expanded in a human study, currently being designed. The major challenge at this stage is to better understand how microbiota-mediated and direct effects on the host act together (or not) to provide a benefit to the host. NutriLeads also aims at establishing a solid pipeline between preclinical and clinical research of its lead ingredients.

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Fecal Transplant: The State of the Art

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The therapeutic efficacy of transfer of fecal material for the treatment of diarrheas caused by *Clostridium difficile* has been documented since the year 2013 when a Dutch group demonstrated that cure levels of 90% were obtained with this technique while the cure level was 30% with the standard antibiotic treatment (van Nood et al., 2013). After the publication of this work, the European Society of Gastroenterology established some guides for the performance of this technique, which was implanted in many hospitals due to it simplicity and above all due to it important clinical efficacy. Our hospital began to use it in March of 2015, and we have conducted a total of 30 interventions, all by colonoscopy and with a cure rate of 90%. During these years, many articles have been published on this technique, most of them stressing the absence of side effects, the safety of the procedure and the high cure rates. However, some undesired effects have also been described and that should be taken into consideration as well as the absence of long-term clinical series, so that we cannot rule out the appearance of side effects in the long term. An update of the European guides has been published recently, without important changes, but which deserves a comment on the current situation of the fecal transplant.

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Fecal Microbiota Transplantation: Historical Perspective and Future Trends

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Abstract
The human gut microbiota is composed of the different microorganisms of our intestinal ecosystem. It has an important metabolic and nutritional role, and it is involved in the correct development of the gut and the maturation of our immune system. Currently, the application of fecal microbiota transplantation (FMT) has allowed us to establish causality in microbiome studies, and we know that microbiota can cure diseases or induce the appearance of disease symptoms, or even determine the success of some health treatments. But knowledge of the curative effects of feces has been documented many centuries ago. In the present article we want to make a brief review of the history of FMT, focusing on its effect on the human microbiome and its capacity to act on human physiology. We also want to place special emphasis on aspects that must be taken into account to translate microbiome research to medicine, related to safety, regulation and standardization of methods, as well as the importance of being able to predict the success of FMT.

Introduction and Early Evidence

In the last five years, since the publication of the work by van Nood and coworkers in *The New England Journal of Medicine*, the fecal microbiota transplantation (FMT) has attracted the attention of clinicians and fundamental researchers working on microbiota [1]. In clinical practice there is a growing interest in FMT, which is due to their great potential to cure pseudomembranous colitis (the infection caused by *Clostridium difficile*; CDI), as well as the promising results that are being obtained in the treatment of many other diseases, such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), or metabolic syndrome. But knowledge of the healing power of stool is not something new. It is believed that the first evidence that supports the application relating to the transplantation of feces appeared in China. In the fourth century, the so-called "yellow soup" was used for the treatment of intestinal problems, and different applications related to the utilization of feces in the treatment of diseases have been used in traditional Chinese medicine for centuries. Some of these therapies were used in Europe centuries later, both in veterinary and human medicine [2, 3]. Obviously, the studies carried out in those times with fecal suspensions lacked adequate experimental designs to draw clear conclusions and solid scientific evidence, but

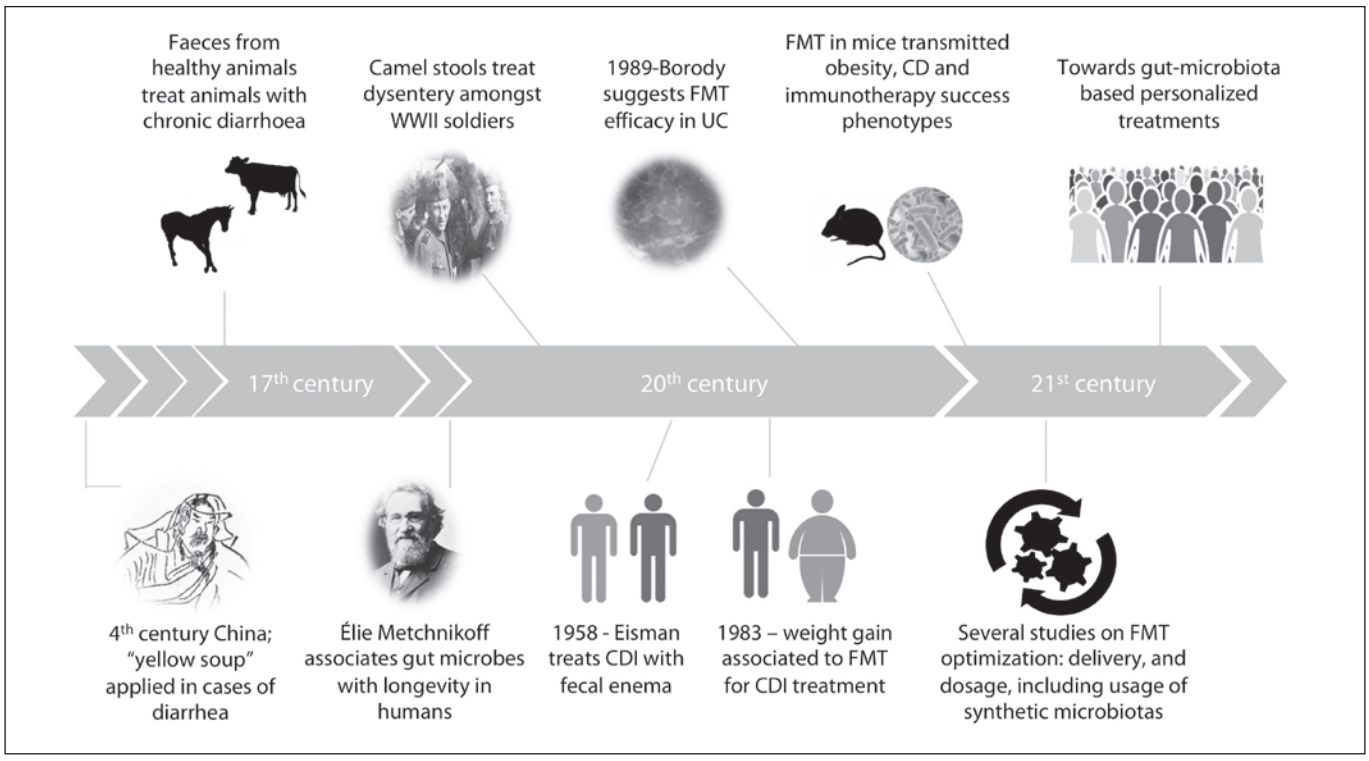

Figure 1. Timeline representing key landmark facts and discoveries in the field of faecal microbiota transplantation and human health.

they laid the foundations that connected the gut microbial ecosystem with the pathophysiology of intestinal diseases.

FMT in the 20th Century

At the end of the 19th century there was already enough knowledge to state that microorganisms were the causal agents of infectious diseases. However, some scientists pointed out that beneficial microbes are much more abundant than harmful ones, and that without them life as we know it would not be possible. At the beginning of the 20th century, and almost simultaneously, lactobacilli and bifidobacteria were discovered. The Austrian paediatrician Ernst Moro characterized for the first time a strain of *Lactobacillus acidophilus,* and the French paediatrician Henry Tissier isolated a *Bifidobacterium* strain from infant feces [4]. Soon after, in 1908, Elie Metchnikoff, considered by many as the father of probiotics, published his book entitled "The prolongation of life, optimistic studies", in which he compiled observations showing that the inhabitants of the Balkans were living longer, and he associated this longevity with the consumption of "Bulgarian bacillus" fermented milk (currently *Bulgarian bacillus* is *Lactobacillus delbrueckii* subsp. *bulgaricus*, one of the starters of yogurt). Thus, at the beginning of the 20th century key knowledge was being generated for the development of modern intestinal microbiology, and the first concrete evidence of the beneficial effects of certain microorganisms was being shown. At that time, the transfer of gastrointestinal content from healthy to sick animals was being used in veterinary science. Meanwhile, in the African campaign developed by the Germans in the Second World War, the intake of fresh camel feces was an effective remedy for the treatment of bacterial dysentery, an effect that was associated with the presence of *Bacillus subtilis* in the camel stool [5]. By the middle of the century, some scientific papers were published using FMT as therapy for the treatment of pseudomembranous enterocolitis. In 1958, a group from the University of Colorado published an article showing the results of the treatment of four patients with enemas from healthy donors, observing a rapid recovery in all of them [6]. Many other similar studies followed the work of Eisman and co-workers, also with very promising results. In the latter part of the 20th century, these procedures also began to be used in non-infectious diseases, such as Ulcerative Colitis [7]. Although in most cases it was observed that patients responded positively to the treatment and underwent a clinical recovery, the first cases of secondary effects due to FMT also began to be recorded. For example, since 1983, cases of weight gain have been observed in patients

Table 1. Relevant clinical applications of FMT. Results retrieved from human intervention trials performed in last years.

Pathology		Patients and studies	Main observations
Gastrointestinal conditions	Pseudomembranous colitis	CDI (patients not responding to antibiotic treatment)	Overall (90%) successful resolution
	Inflammatory bowel disease	Crohn´s disease	More of 50% of clinical remission
		Ulcerative colitis	Low rate of clinical remission
	Irritable bowel syndrome	Few patients and studies	Doses and delivery mode should be taken into consideration
	Chronic constipation	Randomized, controlled trial	Increase in clinical improvement rate
Extra-intestinal conditions	Metabolic syndrome	Obese	Positive results, but transient effects
	Neuropsychiatric disorders	Parkinson (case report)	Symptomatic improvement
		Multiple sclerosis (few case reports)	Normalization of symptoms
		Autism (two cases in children)	Symptoms remission
		Chronic fatigue syndrome (cohort)	More than 50% responded with complete resolution
	Autoimmune disease	Idiopathic thrombocytopenic purpura (case report)	Improvement and normalization

who have received an FMT for the treatment of CDI [8]. Thus, by the end of the last century, it was becoming clear that a careful evaluation of the pros and cons of FMT must be considered before CDI treatment, and before broadening the application to other diseases.

FMT in the 21st Century

The advancement and refinement of high-throughput sequencing technologies in the last twenty years has allowed the study of the human microbiome in a myriad of diseases. Today, we know that microbial dysbiosis (a dysbiosis is an imbalance in the microbial population and/or their functions) have been detected in more than 100 diseases and/or disorders [9]. However, the vast majority of studies that have been carried out so far are observational studies, which establish associations between a physiopathological condition and the composition of the intestinal microbiota. In these types of studies causality, that is, the determination of whether the dysbiosis is the cause of the disease, or is the consequence of it, cannot be established. In this sense, FMT was very useful to be able to establish causality. Nowadays, using animal models, we know that the obesity phenotype can be transmitted through FMT, that an intestinal microbiota of mice suffering from Crohn's disease is able to induce the appearance of symptoms of the disease in healthy mice, and that different compositions of the intestinal microbiota determine the success of immunotherapy treatments in cancer, among other examples [10-12]. But in addition to the preclinical experimentation, which has allowed us to deepen our understanding of the mechanisms by which the microbiota is related to diseases, or may favor their treatment, numerous human intervention trials have been conducted and shed light on the FMT's potential in the treatment of diseases. The following sections include some of the most relevant results related to the application of FMT in CDI and other pathologies.

FMT in the Treatment of CDI

As indicated in previous sections, FMT has been used for decades for the treatment of CDI with very good results. However, the work that has attracted greater attention in the scientific community is that published by a multidisciplinary group from the Netherlands in 2013, that is the first randomized clinical trial for CDI treatment using FMT [1]. Van Nood and co-workers enrolled 43 patients in their study. From the patients that completed the evaluation (41), 12 patients received a standard vancomycin regime, 13 patients took a standard vancomycin regime followed by a bowel lavage, and the third group (16) received vancomycin, a bowel lavage and an infusion of donor feces through a nasoduodenal tube. More than 90% of the patients of the third group responded positively to the FMT treatment: 13 patients (81%) were cured (the primary outcome was cure without relapse within 10 weeks after the initiation of therapy) after the first infusion and two patients were cured after a second

infusion. In the other two groups, the percentage of curation was lower than 35%. This study shows a clear benefit for the patients treated with FMT using a nasoduodenal probe, but other ways of treatment (e.g. colonoscopy or oral administration) have also shown similar efficacy. In this regard, a primary cure rate higher than 90% was reported in CDI patients using colonoscopic FMT [13]. On the other hand, the efficacy of oral ingestion and colonoscopy as a way of delivery was also compared, showing that treatment with oral capsules containing concentrated fecal material was similar to colonoscopy for preventing recurrent CDI over 12 weeks [14]. Small pilot studies using synthetic microbiotas (a defined group of bacteria cultivated separately in the laboratory and mixed in adequate amounts to resemble a healthy human microbiota) were also successful in the treatment of CDI [15]. A systemic review and meta-analysis of the efficacy of different FMT protocols concluded that the way of delivery, the dosage and the number of doses may have an effect on the effectiveness of treating recurrent CDI. In general, the efficacy of colonoscopy is higher than duodenal delivery, and several infusions increase the efficacy rate compared with a single infusion [16].

Application of FMT in Other Diseases

The success obtained with FMT in the treatment of CDI has led to exploring its applications in other intestinal and extra-intestinal diseases. Among these, in the last few years, FMT has been successfully used in the treatment of IBS, IBD and metabolic syndrome, among others. The effectiveness of FMT in IBD is variable and it depends on the disease (Crohn, ulcerative colitis and pouchitis). Clinical remission seems to be higher in Crohn's disease than in ulcerative colitis. It has also been shown that remission in ulcerative colitis patients improves when several infusions are delivered through colonoscopy [17]. The success of microbiota restoration therapies in IBS, one of the most commonly diagnosed gastrointestinal disorders, has also been reported, in which resolution or improvement of symptoms have been shown after FMT, although few studies have been performed and some concerns have been raised relating to the application of FMT in IBS [18]. Obesity and metabolic syndrome is one of the best examples of extra-intestinal diseases in which FMT has shown positive effects. Kootte et al. reported an improvement in insulin sensitivity in obese patients with metabolic syndrome when receiving a fecal transplant from lean donors, although the effect seems to be transient and the response of the recipient depends on the type of microbiota present in the donor [19]. In summary, the application of FMT in diseases other than CDI shows promising perspectives, although at the moment there is a limited number of studies, and well-designed randomised clinical trials are required to draw solid evidence-based recommendations.

Conclusions and Future View

The procedures for the restoration of the intestinal microbiota are currently an effective alternative for the treatment of CDI, and have shown very promising results in the treatment of other diseases [20]. However, a common position regarding FMT is lacking and the universality of its application in clinical practice is being hampered by several factors. One of the main hurdles is the lack of homogeneous regulation in different countries. In the USA, it is not necessary to file an Investigational New Drug application for FMT being used in CDI. Although FMT retains the drug status, the current Food and Drug Administration policy of tolerance allows patients suffering from CDI to be treated by FMT. In Europe, the European Commission does not have a common regulation, and it states that each Member State is free to regulate FMT. Thus, while in some countries, such as France, Austria and Belgium, competent authorities have issued national positions, most EU countries have not expressed their opinion [21]. In this respect, and in the absence of a common regulation in Europe, consensus documents have been drawn up in which groups of experts have laid down some recommendations for the implementation of FMT. In this regard, although the use of fresh or frozen fecal samples does not seem to influence the efficacy in CDI, the use of fresh or properly stored samples to maintain the microbiota viability could be important in other applications such as IBD [16, 22, 23].

In recent years we have also begun to understand which factors determine the success of FMT and microbiota engraftment in the recipient. By conducting a follow-up of the patient's microbiota after the transplant, it was found that the biodiversity of the gut microbial ecosystem increases, and these changes induced in the microbiome after donation are maintained for several weeks at least [24]. However, in order to optimize efficacy, it is very important to be able to predict if establishment in the intestine of the new microbiota will be successful. Today, we know that analysis of the donor microbiota before the donation could help us select the right microbial profile with more chances of colonizing the patient's gut [19]. Of course, other aspects related to FMT safety must be taken into account, and, although zero risk does not exist, the absence of biological hazards that could be responsible for disease transmission must be guaranteed as much as possible. Also, the lack of standard operating procedures to analyse the microbi-

ome (from the stool sample collection to the physician's report, including the transport and storage of the samples, the methods of lysis of the microorganisms and DNA extraction, the sequencing techniques used, the curation of metagenomics databases and the bioinformatics pipelines for sequence analysis) is, nowadays, one of the main obstacles to translate microbiome research to medicine.

In summary, FMT offers us a new tool to modify human physiology and it is one of the new trends in medicine and personalized nutrition. In this regard, the use of minimal (synthetic) microbiotas composed of well characterized strains in terms of safety and functionality, that can be specifically designed and adapted to different pathologies, represents an interesting alternative and minimizes the biological risks associated to FMT. Also, at present we have the necessary knowledge to store our intestinal microbial ecosystem, which gives us the opportunity to use it in the future for customized applications, such as microbiota autotransplants, or as a source of personalized probiotics. Thus, regulatory authorities should take a step forward in order to make the implementation of FMT for the treatment of specific diseases possible.

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Author Contributions

All authors contributed to write sections of the manuscript and to manuscript revision. All authors read and approved the submitted manuscript.

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Faecal Microbiota Transplantation Update: From a Microbiological Perspective

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Gut Microbiota · Remodelling Therapies · *Clostridioides difficile* Infection

Abstract
The classic infectious diseases concept, a microorganism that causes an infection, has renovated in recent years with the assumption that the gut microbiota ecosystem is capable of influencing our health and our disease during our whole life. The scientific and medical interest of human intestinal microbiota has increased considerably, particularly with the support of the recent molecular tools that allowed us to decipher its real composition. The effectiveness of Faecal Microbiota Transplant (FMT) to cure the diarrhoea caused by *Clostridioides difficile* infection (CDI) has been known for a long time, although in recent years it has been extensively applied demonstrating their potential for the microbiota remodelling. At this time, the unique scientific indication of FMT is CDI, although many questions remain unanswered, including the lack of knowledge about the basis of its mechanism of action. Recent advances have suggested the possible use of alternative therapies, trying to minimize the inoculation of the entire faecal ecosystem. The criteria for selecting the best microbiota donor are not well established, although in the case of CDI success is assured with any donor and any of the different procedures that can be used. Some aspects that should be improved have been identified, such as the routine monitoring of the composition of the microbiota by massive sequencing throughout the FMT process, as well as the urgent need to regulate and control the FMT practices.

Introduction

In 2013, van Nood *et al.* published promising results from their clinical trial about efficacy of Faecal Microbiota Transplantation (FMT) for the treatment of *Clostridioides difficile* Infection (CDI) *versus* the convectional antibiotic therapy with vancomycin [1]. Since then, numerous authors have focused their research on this fascinating field in which different medical specialities interact, such as Gastroenterology, Infectious Diseases and Clinical Microbiology. However, some important issues about the FMT, mainly from a microbiological point of view, remain still unanswered. In this review, we summarize current knowledge about this technique and future insights that should be considered.

The gut microbiota is a functional organ, but its particular characteristics prevent Human Transplant Organizations from considering it as a transplantation entity. Nowadays, recurrent CDI is the main indication for FMT, and although numerous hospitals have implemented this intervention, there is a lack of consensus on the methodology and in its regulation [2]. The European FMT working group published in 2017 consensus guidelines based on the previ-

ously published scientific evidences [3]. In addition there are available other important reviews on this field [4-6].

Scientists and clinicians have recently dimensioned the huge metabolic, endocrine and neurological potential of the gut microbiota, as well as the unquestionable influence of this community of microorganisms on human health and disease [7]. From a microbiological perspective, FMT represents not only an ecological opportunity to restore/rearrange a non-functional dysbiotic ecosystem [8] (Lemon 2012), but also a healthcare challenge that must be controlled to prevent short and long-term undesirable effects. To this, it is critical to maximize the selection of donors, as we hypothesize that numerous diseases could be related to the composition or functionality of the gut microbiota. However, presumptive healthy donors could later develop some microbiota-related condition impossible to detect at the donation time [9]. On the other hand, since the do-it-yourself tutorials are available on social media, FMT seems to be a common practice in the community, underestimating the possible secondary effects. Health authorities and clinicians should raise awareness of the potential risks associated to unregulated practices.

Donor Selection and Sample Processing

Donor's selection for CDI is usually performed among relatives or households, in order to implant a similar graft to what the patient had before *C. difficile* infection. Microbiota is a highly individualized ecosystem, and subjects have their particular composition, so the donor selection is a critical decision [10]. Nevertheless, in FMT for CDI it has been reported similar success rates using faecal microbiota from anonymous donors [11] or even commercial preparations [12]. Faecal donor selection is an exhaustive process perfectly detailed in the European guidelines by Cammarotta *et al*. [3].

A definitive way to prepare the samples has not yet been established [13], although the simplicity of the process ensures their success. While the initial published protocols used a mechanical faeces mixer, the latest trends are more efficient preventing the death of anaerobes mediated by oxygen penetration. In fact, the oxygen exposure should be kept to a minimum, even during the sample collection and transportation.

The amount of faeces is also a relevant factor. The minimum has been set to 50 gr, although up to 100-150 gr could be used depending of their hydration grade [14]. Fresh faeces (between 6 and 24 h after deposition) are the most suitable sample, but similar results were published for frozen faeces [15], even in extensive frozen periods [16] and including refractory CDI [17]. The major advantage of frozen stools is their availability in critically ill patients or emergencies, facilitating the FMT procedure. On the contrary, the lyophilised product had a slightly lowered efficacy [18].

The best faecal diluent is water that is readily absorbed in the colon, although other diluents, including saline solution, yogurt, or milk, have been used in the past. Stools should be solubilized in a minimal volume of 300-500 ml to moisten the entire colon. To obtain the final infusion, it is enough to hydrate the stool during 10-15 min and then perform a mild manual homogenization that prevents the oxygen penetration. The final objective of this process is to achieve an aqueous solution containing the highest bacterial concentration and free of solid residues that could potentially obstruct the colonoscopy. To eliminate those residues, a soft spin or simply a filtration with a strainer can be performed. It is crucial to coordinate the sample processing with the colonoscopy group to adjust the time to the minimum, ensuring again the lowest level of exposure to oxygen. The faecal solution must be transport at room temperature, never refrigerated, to avoid temperature contrasts during the colonic implantation.

The most accepted delivery route is the instillation by colonoscopy, though a catheter or the working channel, because it has been observed higher efficiency in comparison to other routes [19,20]. Enemas and nasoduodenal administration were used in the past and, although are not currently recommended, could be considered in problematic patients in whom colonoscopy is contraindicated due to intestinal perforation risk.

Despite that preparation of faecal material is simple, fast and highly effective, their standardization in each centre is frequently challenging. The use of capsules has demonstrated high rate of success, even in refractory patients, and could be used as a simplified alternative to colonoscopy in FMT [21-25]. Their unique limitations are the duodenal liberation of the faecal content and a more complex manufacturing of the capsules. The encapsulation process involves the use of two gastric-resistant capsules, of which the smaller one is filled with faecal preparation, and in turn is introduced inside the larger one. The high number of pills needed to achieve a sufficient bacterial load, has been partially reduced by bacterial concentration through centrifugation [26].

Microbiota Engraftment

The new molecular tools have revealed the extremely complex ecosystem that our gut harbours [27]. However,

most of works have been only assessed this complexity by determining the bacterial taxonomic composition by massive sequencing of the 16S rDNA gene. This approach alone may overlook underlying functional and ecological roles with true impact on health and disease. In any case, the gut microbiota of CDI patients is completely altered in terms of taxonomic composition. In fact, CDI occurs as consequence of a deeply microbiota perturbation, usually after antibiotic administration, but the extended diarrhoeic process aggravates the aberrant dysbiosis. Pathological low proportions of *Bacteroidetes* were reported, whereas *Proteobacteria* are significantly incremented [28-30]. More significantly, the metabolic processes in which resides the gut microbiota functionality are disrupted during the CDI [31]. Looking at lower taxonomic level, *Veillonella, Clostridium, Lactobacillus, Streptococcus, Ruminococcus,* and unclassified bacteria similar to *Erysipelothrix* have been described as the major genera of these patients [23,32,33]. FMT completely restores the microbiota composition and their metabolic functionality [34]. As is expected, the microbiota taxonomic profile in the receptor used to be almost identical to those from the donor, at least in the first days after the FMT process. It has been described that, when a single donor was used to FMT in several receptors, a homogenous pattern is maintained during at least three months [35], although not the entire donor's microbiota is completely implanted [29].

The gut virome is also revealing interesting data in the FMT field, where the *Caudovirales* bacteriophage has shown to be transmissible [36,37]. The current challenge on the gut microbiota is the fungiome [38] and their characterization during FMT.

Other Remarkable Aspects

During the FMT process, the individualization of each donor and each receptor must be considered. In fact, it is impossible to obtain universal patterns in all CDI patients. Even though the microbiota of a single donor is inoculated in several subjects, different patterns of implantation will be observed in each of them. In the same way, it is recognized that all *C. difficile* isolates are not equal and microbiota particularities has been observed when the hipervirulent ribotypes 027 and 078 provokes the diarrhoea [39, and unpublished data from our group]. The complete eradication of *C. difficile* after FMT was recently observed by Allegretti *et al.* [40], but in our experience the colonization by this pathogenic microorganism is not interrupted after FMT, although their density is dramatically reduced.

Remarkable metabolic alterations that occur during CDI are resolved after FMT, such as the altered short chain fatty acids (SCFAs) production [41], acidosis [42], urine infection [43] and the composition of billiard acids [44]. The bile composition substantially affects the *C. difficile* germination, being stimulated by taurocholic acid and inhibited by lithocholic and ursodeoxycholic acids, although can exist different effects related to *C. difficile* lineages. Noticeably, the normalization of the bile content could also contribute to the management of CDI as unique treatment or in combination with FMT.

Clinical benefits on probiotics use to remodelling the gut microbiota have been described for numerous clinical conditions, but not for CDI. Scientific evidences were only described regarding the *Saccharomyces boulardii* and *Lactobacillus* spp. intake, that prevent CDI and reduce their recurrence risk [45,46]. Mills *et al.,* have extensively reviewed this item recently [47]. Others probiotic strains that might be useful for CDI are Lactobacilli, Bifidobacteria and *Escherichia coli* Nissle, but specific probiotic recommendation must be perform taking into account the individual microbiota profile of each patient. Other possibilities for gut microbiota remodelling are the use of bacteriophages [48], predator bacteria or *quorum sensing* modifications, although these alternative strategies are still under development.

An important factor underestimated in the FMT practice is the Immune System response to the implanted microbiota. Only mild complications have been reported for FMT in CDI and almost never related to an immunological complication, but undesirable long-term effect should not be rule out. The immunological alterations after FMT have been scarcely reported, only detecting changes on IL23 expression [49].

FMT practices also provoke beneficiary secondary effects, being some of the most interesting the decontamination of antibiotic multiresistant microorganisms [50-53], the decrease of the entire resistome [54], and the colonization prevention [55]. The influence in other remote ecosystems has been also suggested, demonstrating the powerful of this procedure. The main research focus on FMT might be to gain knowledge and to control the actual FMT impact on the microbial communities of our body [56,57].

Final Remarks

The clinical efficacy of FMT in the CDI treatment is overwhelming. Nevertheless, from a microbiological point of view there are some questions that should be clarified [58]. The instilled faecal solution dramatically reduces the

C. difficile populations, but it has been described that a filtrate solution free of microorganisms is equally efficient [59]. The single bacteriotherapy might also be explored, being the major candidate *Bacteroides ovatus* producing a bile salt hydrolase able to inhibit the *C. difficile* growth [60]. The combinations of different approaches could be a priority in the future, trying to avoid the transfer of microorganisms to prevent undesirable long-term side effects [61,62].

Gut microbiota of donors and receptors should be screened before and after FMT [63,64] to dimension the actual effect by monitoring all microbial communities. As this technology is not available in all centres, routinely FMT might be performed in specialized centres with trained personnel and stool bank [65]. The donor selection should be guided in base of their microbiota, also including immunological compatibility tests. In a near future, the use of mathematical models could be useful to modelling the gut microbiota [66] with a more ecological strategy. Finally, it is essential to establish an FMT regulation, also considering their ethical aspects [67].

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Clinical Aspects of Faecal Microbiota Transplantation

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Clostridioides difficile · Ribotype 027 · Faecal microbiota transplantation · Side effects

Abstract
Despite *Clostridioides difficile* infection (CDI) is probably a poorly diagnosed pathology, in recent years its overall incidence has increased dramatically. The attributable causes are the high antibiotic consumption, the aging of the population, changes in food habits, but mainly the recent irruption of hyper-virulent genetic lineages. Molecular typing of *C. difficile* isolates is necessary to describe their epidemiology and to trace the transmission routes inside hospitals and healthcare-associated centres. The current treatment are based on oral antibiotic administration, and new molecules aiming the recurrence reduction are developing. Faecal microbiota transplantation (FMT) has demonstrated to be a safe and effective treatment for CDI recurrences, although their standardization is a current priority. FMT represents a promising strategy to bioremediate gut microbiota dysbiosis linked to numerous pathologies, but its healthy benefit is still not well established.

Introduction

The incidence of both nosocomial and community-acquired diarrhoea caused by *Clostridioides difficile* (formerly *Clostridium difficile* [1]) is increasing worldwide, constituting a major health problem involving several medical specialities [2]. Global data demonstrates that about 20% of patients suffering from *C. difficile* infection (CDI) have life threatening recurrences [3,4]. This pathology is disabling for patients and families, and causes high consumption of health resources [5]. Faecal microbiota transplantation (FMT) has been recognized as the most efficient treatment for recurrent CDI [4,6]. In the present chapter, we aimed to summarize the current knowledge about CDI, their treatment with FMT, as well as other indications for FMT.

C. difficile Infection

Several factors contribute to CDI incidence, being the most relevant the broad-spectrum antibiotic consumption (mainly clindamycin, cephalosporins, penicillins and fluoroquinolones), the elderly multi-pathological patients, and the female sex [7,8]. In the last years, substantial changes have been documented in the epidemiology of this microorganism, since the irruption of hyper-virulent and hyper-epidemic lineages [9,10]. In Europe, the estimated incidence is 0.7-28.7 per 10.000 patient-bed days [11,12], whereas half a million of USA patients were affected in 2010 by this pathology, causing 15,000-20,000 deaths per year and $1 billion of overcharge, being the most common healthcare-associated infection in this country [5]. In addition to its high mortality, CDI is characterized by high morbidity and loss of quality life.

In 2018, scientific evidence about the relationship of the increased virulence of ribotypes 027 and 078 and the consumption of trehalose as a food additive has been reported [13]. The epidemic worldwide spread of the hyper-virulent ribotype 027 in hospitals have been documented since the early 2000s, at the same time this food additive was approved. Epidemiological studies demonstrated the worldwide dispersion of this ribotype, and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) coordinates along with the European Centre for Disease Prevention and Control (ECDC) the European Study Group on *C. difficile*. These platforms are useful to know the current lineages causing CDI, but also to be aware of the emergence of new genetic variants, as the zoonotic ribotype 078 with a 39 bp deletion in the *tcdC* gene [14]. Although 027 is the most frequent ribotype in Europe, its global distribution is country-depending, being the most equally distributed 001/072 and 014/020 ribotypes [11]. Latest data strongly suggest an increased incidence in both community acquired CDI and in non-risk population [15,16]. Recent epidemiological studies indicated that some European countries have successfully controlled the dissemination of the 027 clone, particularly driven by a restriction in fluoroquinolone prescription [17].

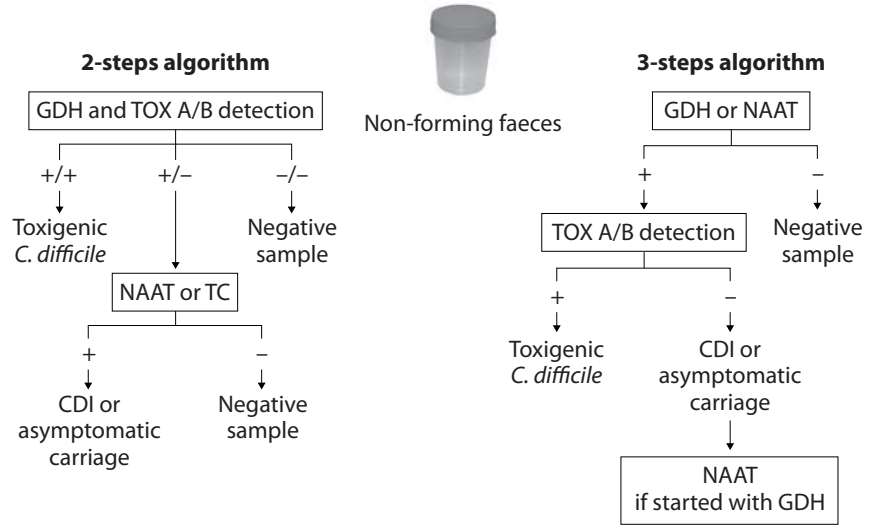
The main virulence factors of *C. difficile* are the high-molecular-weight clostridial toxins. Toxin A (TcdA) and toxin B (TcdB) bind and enter the colonic epithelium, causing proinflammatory chemokine and cytokine production, influx of neutrophils, and disruption of tight junctions, fluid secretion and epithelial cell death. Some strains, including so-called hyper-virulent strains (PCR ribotypes 027 and 078), additionally produce an additional binary toxin (*cdtA*/*cdtB*), whose the significance remains to be elucidated [18].

Faecal carriers of *C. difficile* may remain clinically asymptomatic without any recommendation for eradication. Its known that colonization rates in infant population can be high, although the clinical significance is questionable. In adult population, rates of carriers decline but depend on previous admissions and the length of stay in hospitalized patients [19]. In that sense, routinely screening of CDI on admission is not recommended [20] and *C. difficile* screening must be only realized on unformed stools, particularly from patients with a history of at least 3 days of hospitalization or a recent previous admission in a hospital or a healthcare institution. The habitual source of infection is the hospital environment, where the spores survive and spread with high efficiency despite the extreme measures of isolation and cleaning, such as a standard laundry processing of hospital bed sheets [21]. It has been observed that the transmission efficiency is ribotype-dependent, being 027 the most epidemic [22,23].

CDI is probably an underdiagnosed condition, mainly due to insensitive tests or lack of clinical suspicion. CDI diagnosis is based on both clinical manifestations and microbiological confirmation of toxigenic *C. difficile* isolates or free toxins in faeces [24]. CDI clinical manifestations can be stratified in mild (only diarrhoea), moderate (fever and abdominal cramps and discomfort), severe (hypoalbuminemia, leukocytosis or abdominal peritonism), and fulminant (hypotension with need of vasoactive agents, toxic megacolon, fever, leukocytosis/leukopenia, renal or respiratory failure) [6]. The microbiological gold standard techniques to CDI diagnosis are the cell cytotoxicity neutralization assay or toxigenic culture, but these methods are not available in most laboratories. The common diagnostic algorithm is represented in Figure 1, and it is based on the glutamate dehydrogenase (GDH) determination combined with enzyme immunoassays for toxins A/B. Diagnosis, when possible, might be completed with specific PCR for genes that codify the toxins (*tcd*A, *tcd*B, and binaty toxin) [14]. Capillary gel-based electrophoresis method is the reference method for *C. difficile* typing [25], and its routinely implementation is highly recommended for local and global epidemiology.

Molecular typing techniques help us to classify the recurrence as relapse (infection by the same strain) or reinfection (different strain), being the whole genome sequencing (WGS) the most suitable approach for this purpose [26]. In addition, the determination of the gut microbiota profile allowed the definition of patterns linked to recurrences: lower values of bacterial richness and species diversity combined with low proportions of *Bacteroides, Prevotella, Lachnospiraceae* and *Bifidobacteria* [3].

Antibiotic treatment reaches curating rates of 80% when using in a first CDI episode. The available options are metronidazole, vancomycin, and fidaxomicin, being the final decision influenced by the clinical status of the patients and the risk of recurrence [27]. Vancomycin has demonstrated higher efficiency than metronidazole in moderate-severe CDI [28], and fidaxomycin reduces the recurrence incidence comparing to vancomycin except when the infection is produced by the 027 ribotype [29]. Economic factors also make vancomycin the more suitable drug. The use of bezlotoxumab (monoclonal antibodies anti toxin B) added to the antibiotic treatment is effective in the recurrence prevention [30], reducing the global costs of the disease [31]. Several parenteral vaccines targeting both toxins TcdA and TcdB have been tested in clinical trials (2 in phase III), and it is likely to be available soon for human use. Novel coming therapies still under research are surotomycin (an



CDI: *Clostridioides difficile* infection; GDH: Glutamate dehydrogenase; NAAT: nucleic acid amplification test; TOX A/B: production of toxin A and B; TC: toxigenic culture.

Figure 1. Microbiological diagnostic algorithm for toxigenic *C. difficile* infection.

oral daptomycin-derivated lipopeptide), cadazolid (a novel hybrid of oxazolidinone and fluoroquinolone), and ridinilazole (a novel compound). Probiotics can represent an ecological alternative to limit the population density of C. difficile. *Lactobacillus rhamnosus* GG and *Saccharomyces boulardii* have demonstrated utility in CDI prevention, although few results have been obtained during the active infection [32].

FMT for CDI

Several clinical trials [33-35] and meta-analysis [36-40] have concluded that FMT is the most effective therapy for CDI. The cure rates reach 85-90%, especially when performed by colonoscopy, although other available administration routes have similar success rates. FMT is commonly performed during the second or the third CDI recurrence episode [41,42], although the possibility to realize FMT during the first episode, aiming to reduce the number of recurrences, has been suggested [43]. No criteria of exclusion have been set, although the general fragility of the CDI patients, particularly in elderly, might be account at least to limit the colonoscopy complications. The use of pills instead of colonoscopy might represent an innocuous alternative for those patients. FMT is an ecological strategy highly effective for the cure of CDI, resulting in cost savings for the health system and improving the life quality of patients [44]. The standardization of the FMT process is a current priority [45,46], and the major limitation to compare the results obtained in the different published articles.

FMT Safety and Secondary Effects

The secondary effects of FMT intervention can be divided into short and long term adverse effects. It is accepted that the immediately secondary effects are scarce and generally mild, as it is reported in different studies [33,47-50]. The most common side effects occurring immediately after FMT include diarrhoea, flatulence, abdominal pain, constipation, vomiting, pruritus, paraesthesia, elevation of C-reactive protein, and headache. Infectious complications are also reported, as the diarrhoea caused by norovirus transmission [51], or the reactivation of ulcerative colitis process [52]. On the other hand, beneficial effects directly derived from FMT practice are also described, such as the eradication of multiresistant bacteria [53,54] the reduction of the resistome [55], or even the cure of chronic urinary tract infections [56,57].

On the other hand, long-term side effects of FMT are not yet well dimensioned, since the current series are still very short and most of the populations are of advanced age. The worst scenario would be that FMT patients could develop diseases related to the donor's microbiota, and this fact is not considered in the current procedures.

Other FMT Indications

FMT is already a promising therapy for gastrointestinal disorders as inflammatory bowel disease (IBD), chronic constipation, and irritable bowel syndrome (IBS). Recent findings have also implicated the gut microbiota in extraintestinal disorders as obesity, multiple sclerosis, metabolic alterations, HIV infection, and autism, where FMT could also represent a therapeutic via.

Ulcerative colitis is the non-CDI condition in which the therapeutics possibilities of FMT have been more investigated [58-61] using repeated faecal infusions from a single or various donors. The rates of endoscopic remission after FMT *versus* placebo are promising (24 vs 5%, 30 vs 20%, 27 vs 8%, 32 vs 9%, respectively), but clearly insufficient, and in consequence FMT is not currently recommended as therapy for ulcerative colitis [62]. In the case of Crohn's disease, patients have responded well to intestinal decontamination with ciprofloxacin and rifaximin [63], which supports the relevant role of bacteria (*Firmicutes* reduction and *Proteobacteria* increase) [64] and also fungi (*Basidiomycota/Ascomycota* ratio and *Candida albicans* increase, and *Saccharomyces cerevisiae* decrease) [65] in this disease. Nevertheless, in IBD the altered immune response seems to be more decisive in the course of the disease, and consequently FMT have less potential. Some important FMT aspects should be better adjusted and personalized to reach a higher remission rate in IBD, including a better selection of donors, who should be immunologically-compatible with receptors, its early application in mild forms of the disease, the decontamination of autochthonous gut microbiota, and the control over confounding factors that might mask the results.

FMT has also obtained promising results as a therapeutic option for constipation [66,67], although the engraftment of the donor's microbiota has not been well established yet.

In regard to IBS, its ethiopathogeny is not elucidated, being influencing by SCFAs production combined with a proinflammatory bacterial profile (abundance of *Firmicutes* and reduction of *Bacteroidetes*) [68] and a permeable intestinal barrier [69]. A recent meta-analysis of the FMT application in IBS showed that it is safe and effective to attenuate its clinical manifestations [70], although the results of on-going studies can be crucial to decide the therapeutic FMT indication for IBS.

Cirrhosis pathophysiology conditioned a gut microbiota dysbiosis [71] that combined with hyperammonemia provokes a neuronal dysfunction denominated hepatic encephalopathy. Their current treatment is the administration of lactulose and rifamixin to decrease the bacterial metabolism of gut microbiota. Preliminary results indicate that FMT is a safe and valid alternative to treat this pathology, using both the enema and the faecal capsules intake [72].

Finally, FMT is a promising option for extraintestinal disorders in which the contribution of the gut microbiota has been demonstrated, as in metabolic syndrome [73,74], HIV infection [75], autism [76], or even in cancer, as an adjuvant to immunotherapy [77,78]. However, the available research in these fields is limited and a large number of scientific studies are needed to demonstrate the efficiency of FMT in these pathologies.

Final Remarks

CDI incidence is increasing worldwide due in part by the higher antibiotics consumption, the aging of the population and the food habits, but genetics *C. difficile* changes are also contributing. The characterization of the strains at molecular level it is necessary to realize epidemiological studies and to identify routes of transmission in the hospital and healthcare-associated centres. FMT has demonstrated to be an effective, cost-efficient, ecological, and safe therapeutic option to treat CDI, although it is not well optimized for other pathologies.

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Fecal Microbiota Transplantation in Children

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Abstract

There has been a growing interest in fecal microbiota transplantation (FMT) over recent years, in part to the expanding association of intestinal dysbiosis with a wide range of human diseases. Adult studies have shown that FMT is an effective treatment for recurrent *Clostridium difficile* infection and might have applications in other diseases. However, there is a paucity of data available in children, who may differ from adults. FMT has particular interest in pediatrics, given the concerns about rates of adverse events with existing therapeutic options, and the greater cumulative medication burden associated with childhood-onset disease. Published literature on the use of FMT in pediatrics is sparse. We review published studies looking at FMT in children in some specific gastrointestinal conditions. At present, evidence on FMT in paediatric disease is not strong enough to recommend its use as part of routine treatment.

Introduction

There has been a growing interest in fecal microbiota transplantation (FMT) over recent years, in part due to the increasing prevalence of *Clostridium difficile* infection (CDI) and expanding association of intestinal dysbiosis with a wide range of human diseases. Adult studies have shown that FMT is an effective treatment for recurrent CDI, what may have applications in other illnesses. However, there is a paucity of data available in children, who may differ from adults [1].

Gut microbiota contains at least 1014 bacteria, with approximately 1,000 to 1,200 different bacterial species that exist in symbiosis with their host. This complex community of microorganisms perform vital functions for their host including synthesis of vitamins, fermentation of dietary carbohydrates, metabolism of bile and host hormones, competitive exclusion of pathogens, and the development and maturation of the immune system [1]. Microbiome of children has differences with adult microbiome, especially in very young children. Initial colonization of the infant gut and dynamic microbiome development over the next few years set the stage for a relatively stable microbiome in the healthy adult.

Human microbiome research is a rapidly developing field. Knowledge of the association of dysbiosis, or perturbations of the intestinal microbiome, with a wide range of diseases is increasing. Reported cases with FMT because gastrointestinal (GI) conditions in children are shown in Table 1, these conditions include:

- Inflamatory bowel disease (IBD)
- Recurrent clostridium infection (CDI)
- Allergic colitis
- Autism spectrum disorders
- An other diseases

Table 1. Pediatric studies of fecal microbiota transplantation (TMF).

Author	Indication for FMT	Comorbilities	Num of children	Age (yr)	Delivery method	Cure rate %	Follow up	Adverse events
Kahn (2012) [12]	Recurrent CDI	None	1	2	Colonoscopy	100	2 months	None
Rubin (2013) [18]	Recurrent CDI	None	2	6-8	Upper tract (not specified)	50	2 months; relapsed 1 patient	None
Kunde (2013) [3]	Ulcerative colitis	None	10	7-21	Enemas			None
Kelly (2014) [19]	Recurrent CDI	Immunocompromised (5)	5	6-16	Not specified	70	12 weeks	No infectious complication
Russell (2014) [14]	Recurrent CDI	IBD (3) None (7)	10	1-13	Nasogastric (2) Colonoscopy (8)	90	1 month – 4 years	None
Pierog (2014) [20]	Recurrent CDI	Muscular dystrophy (1) Crohn´s disease (1) Hirschprung disease (1) Gastrostomy tube (1) None (1)	5	1-12	Colonoscopy	100	1 year	None
Wallia (2014) [21]	Recurrent CDI	Prematurity (2) Gastrostomy tube (2)	2	1-2	Colonoscopy	100	8-27 months	None
Suskind (2014) [4]	Ulcerative colitis	None	4	13-16	Nasogastric	No clinical or laboratory benefit		
Wang (2015) [23]	Recurrent CDI	None	1	1	Nasojejunal	100	4 months	None
Kronman (2015) [13]	Recurrent CDI	IBD (3) Cerebral palsy (1) NEC (1) Wilms tumor (1) None (4)	10	1-13	Nasogastric	90	0,4-23 months	2 day of mucoid stools (1)
Hourigan (2015) [22]	Recurrent CDI	IBD (5) Mithocondrial disease and cecostomy (1) None (2)	8	6-17	Colonoscopy	100	6 months	Mild abdominal pain (2)
Suskind (2015) [4]	Crohn disease	None	9	13-16	Nasogastric	77%	12 weeks	None
Kellermayer (2015) [5]	Ulcerative colitis	None	4	14-16	Colonoscopy and serial enemas	100	4 weeks	None
Lahtinen (2017) [24]	*Salmonella* resistant to antibiotics infection	None	1	17	Colonoscopy	100	4 months	None
Liu (2017) [15]	Allergic colitis	Non specified	19	5-11 months	Colonoscopy	100	15 months	Eczema (1)
Sierra Salinas (2017) [7]	VEOIBD	None	1	6	Ileostomy	100	5,5 months	None
Dow (2018) [25]	Recurrent CDI	Pompe disease and B cell immunodeficiency	1	1	Gastrojejunal	100	5 years	None

FMT for Inflamatory Bowel Disease in Children

Inflamatory bowel disease (IBD) is a chronic inflammatory condition of the gut characterized by alternating periods of remission and relapse. IBD comprises Crohn's disease (CD), ulcerative colitis (UC) and IBD-Unclassified (IBD-U). IBD´s incidence has increased steadily worldwide affecting both children and adults, approximately 25% of IBD patients are diagnosed before the age of 18 years [2].

IBD has been associated with underlying intestinal dysbiosis, with a decreased microbiome diversity in Crohn's disease and ulcerative colitis (UC). In adults, several systematic reviews of published studies showed that in general FMT in IBD is safe with a variable efficacy. However, there are limited data for the treatment of IBD in children with FMT [1].

First cases report of FMT in IBD was published in 2012 by Kunde et al [3]. Indication for FMT was UC and fecal microbiome was delivered by serial enemas. Clinical response was maintained at least 1 month in six of nine patients, defined by a decrease in Pediatric Ulcerative Colitis Activity Index (PUCAI) score of >15. FMT was found to be safe and well tolerated although transient mild–moderate side effects were experienced. In comparison, Suskind et al [4] didn´t find any clinical or laboratory improvement in 4 children with UC who received a single FMT via NG tube for UC. Same authors in a separate report showed that seven of nine patients receiving FMT via NG tube for the treatment of Crohn's disease had engraftment of donor stool microbiome and a clinical response with improvement of Pediatric Crohn's Disease Activity Index score. These patients tolerated the procedure without adverse events.

Kellermayer et al [5] reported transient withdrawal of immunotherapy in three children with UC giving high frequency serial FMT by colonoscopy and subsequent enemas. Moreover, serial FMT induced a transient engraftment of the donor microbiome in the recipient. Vandenplas [6] also reported a case 18-month-old girl baby presenting with an early-onset colitis with UC-like phenotype, who responded after 7 serial FMT infusions with donor stool from and age-matched niece and older brother. Although this patient did experience systemic adverse reactions that were transient, serial FMT was shown to be effective.

In 2016, a case report describes an 11-year-old girl with corticosteroid-dependent UC who responds after serial FMT infusions every 2–4 weeks over a 10-month period. Patient remained in clinical remission at 40 weeks post final FMT and showed complete endoscopic healing. A further 2016 case report described a 3-year-old girl baby with acute severe UC who was refractory to aminosalicylates and all immunosuppressive drugs. She received 6 successive FMT enemas and 4 FMT via nasoduodenal tube over 10 days, but ultimately required colectomy. Donor faecal microbiota was not identified in the patient, and the authors concluded that due to the severely damaged colonic epithelium, paucity of crypts and overall decrease of mucous in the outer layer, the donor microbiota could not be retained by the recipient. The authors suggest that patients with mild disease might be better candidates for FMT.

Recently, in Spain, Sierra et al reported first FMT in children with IBD-U refractory who responded after 2 FMT infusion via dissociated ileostomy (Maxwell´s technique). A 6 year old boy showed clinical response with increase in weigth and decrease protein-C reactive [7].

Finally, Haiming Fang et al [8] published an meta-analysis with all studies publicated at the moment: systematic retrospective review and complete cohort study and randomized controlled trials (RCT) to critically evaluate the efficacy and safety of FMT on IBD. They concluded that the pooled estimate of clinical remission rate of FMT for paediatric UC was 10% (95% CI: 0%-43%) and 45% for paediatric CD (95% CI: 24%-66%), while it was 26% (95% CI: 10%-48%) for adult UC and 22% (95% CI=3%-52%) for adult CD. Compared to adults, paediatric population has a dynamic developing gut microbiome and IBD typically have a more aggressive course in the paediatric age group suggesting that paediatric IBD phenotype may have a distinct pathophysiology from adult-onset IBD.

Compared to adults, here is a paucity of data available in children. Paediatric IBD and the paediatric microbiome have several unique features that suggest that microbial based therapies could be particularly effective.

Although FMT trials in IBD, especially in children, are still in their early stages, apparently procedure is safe and well tolerated although moderate but transient side effects have been reported [1]. Efficacy reported is variable, but FMT may offer some benefit in certain patients inducing transient improvement in underlying dysbiosis. There are many unanswered questions, such as the optimal frequency and periodicity of FMT infusion needed, and whether different protocols or routes of administration are requiered depending on IBD phenotype or based on disease location. Moreover, IBD is a complex disease with varying phenotypes and genotypes associated with certain shifts in microbial composition; this questions whether patients with differing IBD genotypes and phenotypes would differentially respond to FMT warrants further investigation.

Fecal Microbiota Transplantation for *Clostridium Difficile* Infection in Children

Clostridium difficile is the main cause of infectious nosocomial diarrhea in developed countries. *Clostridium difficile* infection (CDI) is defined by the presence of symptoms (usually diarrhea) and either a stool test positive for *C. difficile* toxins or detection of toxigenic *C. difficile*, or colonoscopic or histopathologic findings revealing pseudomembranous colitis [9]. CDI can be very severe and has a significant risk of mortality. In the last years the risk of acquiring infection in the community in increasing. Recurrent CDI, defined as new onset of symptoms with positive *C. difficile* testing less than 60 years after finishing primary treatment for CDI [10], is rising disproportionately to primary CDI and resistance of conventional treatment is a real problem.

Pathophysiology of Clostridium difficile infection

Clostridium difficile is an anaerobic, gram-positive, toxin-producer bacillus that lives in the large intestine. Exotoxins are responsible for causing colitis in susceptible patients. Heat and acid spores are vehicles of infection via fecal-oral route. CDI is rare under 2 years of age but are frequently asymptomatic pathogen carriers (73% by 6 months of age decreasing with time with rare asymptomatic colonization in adults) [11].

Diagnosis

Because of the high prevalence of asymptomatic carriage of toxigenic *C. difficile* in infants, testing for CDI should never be recommended for infants < 12 months of age with diarrhea, not even in children of 1-2 years of age unless other causes (infectious or noninfectious) have been excluded. In children > 2 years of age with prolonged diarrhea and risk factors (recent antibiotic use, immunocompromised individuals, cirrhosis, inflammatory bowel disease, etc.), *C. difficile* testing is reccomended [9].

Treatment of Clostridium difficile infection in children

Metronidazole or vancomycin are recommended to treat children with an initial episode or first recurrence of nonsevere CDI. An increasing rate of patients who do not achieve cure with conventional treatment could be candidates for other modalities of treatment such as fecal microbiota transplantation (FMT) [9]. Restore intestinal dysbiosis is the goal of FMT in CDI because favourable colonic microbiome prevents *C. difficile* from flourishing [11].

FMT data in adults have been rising in the last decade but trials in children are still lacking. First reported case in children is published by Kahn [12] in 2012 in a 2 years old boy with recurrent CDI who was successfully treated with FMT.

Successful rates are high, up to 90-100% in the 2 largest pediatric case series published by Kronman [13] and Russel [14] in 2015 and 2014 respectively. Preferred delivery route is colonoscopy (24/47) but with no differences in symptom resolution comparing with nasogastric/nasojejunal access (18/47), in 5 patients delivery route was not specified. FMT for recurrent CDI was not effective in symptom resolution in 3 of the cases reported: a 5 years old boy with underlying IBD, a 4 years immunosuppressed girl and a 6 years old boy. A 13 months old boy is the youngest reported child receiving a FMT for recurrent CDI [9].

Successful rates are also high in patients with recurrent CDI with underlying IBD, 12/47 patients reported in recurrent CDI series with symptom resolution in 11/12. FMT for IBD is described in a different section. No serious adverse events have been reported in this case series, only mild symptoms such as transient vomiting, mild abdominal pain or mucous stools independently on immune status.

Fecal Microbiota Transplantation for Allergic Colitis in Children

Allergic colitis (AC) is a common disorder in infants caused by immune reactions in the digestive system. Typical manifestations include rectal bleeding, mucous stools, diarrhea and abdominal pain. Food allergens (cow´s milk proteins principally), dysbiosis and immune system disbalances contribute to AC. Conventional therapy is based in avoiding exposition to allergenic proteins by using a hydrolysed formula with a high rate of success [15].

Unfortunately, response in some patients is not satisfactory and need other therapies. In 2017, Liu [15] published the first cases series in 19 infants with refractory AC that received a FMT. Recipients were 11 boys and 8 girls aged from 4-11 months. AC was diagnosed based on clinical symptoms: rectal bleeding, exclusion of other causes of rectal bleeding (infectious colitis, anal fissure…), clinical remission after milk exclusion and recurrence after re-challenge and chronic inflammation with eosinophilic infiltration in intestinal mucosa. Patients selection was done after incomplete remission after conventional therapy. FMT delivery route was rectal tube in left colon. In all patients, clinical remission was observed within 2 days after FMT. After more than 15 months of follow-up, no relapse was notified (two patients who were lost in follow up). After FMT all patients continued with hydrolysed formula.

In 10 patients fecal microbiota was analysed before FMT and during follow up with detection of an increase in microbiota diversity in 6/10 patients. This is the only study that indicates FMT for allergic colitis in infants and more studies are needed to demonstrate this new indication for FMT.

FMT Autism Spectrum Disorders

Autism spectrum disorders (ASDs) are complex neurobiological disorders that impair social interactions and communication and lead to restricted, repetitive, and stereotyped patterns of behaviour, interests, and activities. While ASD diagnoses are increasing, with 1-2% of children worldwide, causes of this disorder remain poorly understood and appear to involve a complex interaction of genetic and environmental factors, among those who are microbiome, that is an environmental factor that is partially inherited from the mother.

Many children with ASDs also experience significant gastrointestinal (GI) symptoms, such as constipation, diarrhea, and alternating intestinal pattern. These GI symptoms appear to be due, in part, to dysbiotic gut microbiota and perhaps their missing roles on modulating metabolites (e.g., 4-ethylphenylsulfate, indolepyruvate, and corticosterone) that affect GI function and neurobiological conditions. Many children with ASD undergo an increase in number of oral antibiotic treatment received during the first 3 years of life, which is thought to destabilize their gut microbiota and provides opportunities for colonization to competitive potential pathogens. A number of studies reported that children with ASD have altered gut bacteria profiles compared with neurotypical children, although in certain cohorts, no significant difference has been showed. Children with ASD have lower amount of fermentative bacteria (e.g., *Prevotella copri*), and lower overall bacterial diversity, it has also been hypothesized that lack of beneficial gut microbiota impairs neurological health.

Experiments done in an ASD mouse model demonstrated that increase with *Bacteroides fragilis* alone could alter gut microbiota and blood metabolite profiles, correct increased gut permeability (gaps in cell-to-cell junctions), and improve ASD-associated behaviours. In children with ASD, a small open-label study found that 8 weeks of treatment with oral vancomycin (a non-absorbable antibiotic which acts only in the gut) led to major improvements in both GI symptoms and ASD symptoms, although the benefits were lost within a few weeks after treatment was stopped.

Interest in rebalancing human gut microbiota to treat ASD is growing. However, only temporary symptom improvements have been reported from vancomycin treatment, and probiotics have had mixed clinical results with minimal microbiota analysis or long-term follow-up. ASD's GI and behavioral symptoms may derive, at least in part, from gut microbiota dysbiosis and FMT may effectively rebalance the gut microbiota and improve some GI and ASD symptoms.

The study done by Kang et al [16], although it had some limitations because it was a observational study not double-blind placebo controlled, they showed the FMT is safe and well-tolerated in children with ASD ages 7–16 years. FMT led to significant improvements in both GI- and ASD-related symptoms, and those results were sustained at least 8 weeks after treatment. At the same time with these clinical improvements, both microbiota and phage from the donors appear to have engrafted, at least partially. This shifted gut microbiota of children with ASD toward that of neurotypical children is consistent with the hypothesis that gut microbiota may be at least partially responsible for GI and ASD symptoms. These results are promising and provide a crucial step for understanding the connection between the microbiome and ASD. A randomized, double-blind, placebo-controlled study is the next step to investigate the value of FMT in treating children with ASD and GI problems.

Fecal Microbiota Transplantation for Other Diseases

Interest in FMT therapy in children in increasing and may play a significant role in future treatment of other diseases linked to gut microbiota dysbiosis such as type 2 diabetes mellitus, metabolic syndrome, obesity, colonization of the gastrointestinal tract by multi-resistant microorganism, depression, autism spectrum disorders… More trials in this field are needed in adults and in children to demonstrate new indications for FMT [17].

Conclusions

At present, evidence on FMT in paediatric disease is not strong enough to recommend its use as part of routine treatment. Initial reports appear encouraging that FMT appears to be effective for recurrent CDI and safe, at least in the short term, in IBD. Preliminary results are promising and more studies are needed to define the best indications, optimal timing, frequency, mode of delivery, and the most appropriate donor for each patient. Further researches, especially RCT studies, are needed to identify a safer, efficacious, and economical method of FMT and its potential mechanism.

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Marine Microbiota

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The ocean is the largest ecosystem in the world and is governed by microorganisms. There are 10 raised to the 29 cells of bacteria and archaea. In each marine sample there are about ten times more different taxa than in the human gut microbiota. The total number of species of marine microorganisms is unknown, but some estimates reach 10 to the 12 species in total. Marine microorganisms are responsible for half of the planet's primary production and more than 90% of respiration in the ocean. They also control various mechanisms of climate regulation, such as the biological carbon pump or the dimethyl sulfide cycle, and they even influence the geology of the planet. In addition, each cell has a genome with between 1,000 and 10,000 genes with a huge potential to provide useful genes. A prominent example is that of rhodopsins. These proteins, capable of using the energy of light to generate the proton gradient through the cell membrane, were discovered in archaea of hypersaline environments in the 70s of the last century. At the beginning of this century their extraordinary abundance was discovered in the surface ocean, where approximately half of the cells have this protein. Currently, rhodopsins from different microorganisms are one of the basic elements of optogenetics, one of the most promising technologies in biomedicine.

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Prebiotics in Functional Diets for Fish

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The successful replacement of fish meal (FM) and fish oil (FO) by land-based meals and oils in feeds for marine fish species is a determining factor to achieve a sustainable aquaculture sector development. However, their use has been associated with variable side-effects on fish growth performance and health associated to decrease levels of essential fatty acids, increased percentages of 18:C fatty acids and alterations in n-3/n-6 ratio in the different tissues. Fatty acids are involved directly in immune events, by its structural role in membranes and for being precursors of eicosanoids. Dietary imbalances lead to alterations in the immune system functionality, generally decreasing resistance to pathogens. The strategy of inclusion of prebiotics allowed the increase of the immune potential and disease resistance in European seabass (*Dicentrarchus labrax*) fed diets based in a high replacement level of marine ingredients by terrestrial ingredients. This species is one of the most important cultured species in the Mediterranean region. Dietary supplementation of mannanoligosaccharides (MOS) in European sea bass diets enhanced growth performance and a higher response of the Gut Associated Lymphoid Tissue (GALT). Anti-iNOS and anti-TNFα gut immunopositivity patterns were influenced by MOS supplementation, with an up-regulation of TNF-α, cyclooxygenase-2 (COX-2), CD4 and IL10 in distal intestine. Synbiotic supplementation with a probiotic resulted in a reduction of MOS-induced gut humoral proinflammatory response by increasing the expression of some cellular-immune system related genes. Fish mortality after *Vibrio anguillarum* infection was reduced in fish fed MOS compared to fish fed the non-suppelmented diet after 90 days of feeding.

Thus, overall pointing to the combination of MOS and a probiotic (acting as a synbiont) as a viable tool to potentiate European sea bass juvenile's growth and disease resistance when supplemented in low FM and FO diets.

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The Potential Role of Algae as a Source of Functional Ingredients

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Different studies have correlated the effects that diet, considering also marine organisms, shows on some diseases, therefore highlighting the enormous potential of traditional and alternative foods in the prevention and progression of chronic diseases beyond meeting basic nutritional needs. In the last years, nutritional research has also confirmed micro- and macroalgae (or seaweed) as rich sources of potentially valuable, health-promoting compounds in a scenario where consumers are increasingly aware of the relationship between diet, health and disease prevention. Combining both ideas, nutritional needs vs. health promotion and disease risk reduction, a high number of micro- and macroalgae species are shown as a very interesting source of functional ingredients (bioactive compounds or phytochemicals) that may benefit health beyond the role of basic nutrition.

Due to the increased interest in prebiotics as functional foods with recognized health benefits, algae in general are suggested as a rich source of polysaccharides and other metabolites with complex structures such as polyphenols. Polysaccharides from red, green and brown seaweed such as carrageenans, agars, ulvans, alginates or fucoidans, among others, can be considered as dietary fibres (being a majoritarian component of the biomass) as they are resistant to digestion by human enzymes, therefore stimulating growth of beneficial gut bacteria. Phlorotannins (polyphenols with complex structures) occurring in brown algae can be also transformed into beneficial bioactives metabolites when they are ingested. Fermentation processes of seaweed components has been also described as a source of beneficial metabolites such as short chain fatty acids (SCFA) protecting from different diseases, as it has been suggested by different *in vitro* and *in vivo* studies.

Microalgae and cyanobacteria are being also considered as a rich source of varied and complex, not very well known, polysaccharides including bglucans, exopolysaccharides (EPS) and proteins with potential prebiotic properties. Different strains of the genera *Arthrospira* (*Spirulina*), *Porphyridium*, *Isochrysis* or *Chlorella* have been described as promoters of dietary metabolic changes and morphological modifications when supplied to animal models.

The aim of this presentation is to systematically review the scientific evidences related to the characteristics and effects of dietary prebiotics from micro- and macroalgae biomass consumption on a range of health outcomes.

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