RAPPORTI ISTISAN 25|1

ISSN: 1123-3117 (cartaceo) • 2384-8936 (online)

Microbiome in humans, animals, food and the environment: implications for risk assessment in food safety

Edited by
A. Silenzi, L. Busani

AMBIENTE
E SALUTE

ISTITUTO SUPERIORE DI SANITÀ

Microbiome in humans, animals, food and the environment: implications for risk assessment in food safety

Edited by
Annalisa Silenzi, Luca Busani
Centro di riferimento Medicina di Genere

ISSN: 1123-3117 (cartaceo) • 2384-8936 (online)

Rapporti ISTISAN
25/1

Istituto Superiore di Sanità
Microbiome in humans, animals, food and the environment: implications for risk assessment in food safety.
Edited by Annalisa Silenzi, Luca Busani
2025, 41 p. Rapporti ISTISAN 25/1

The European Food Safety Authority (EFSA) highlights the necessity of incorporating microbiome data into food safety risk assessment to elucidate substance-microbiome interactions and their health implications. This integration should extend to the microbiomes of animals, food, and the environment within the One Health framework. Achieving this necessitates collaboration among specialized research groups and regulatory agencies, including EFSA and the Food and Drug Administration (FDA), to embed microbiome science into risk assessment and One Health policy. Italian experts, coordinated by the Istituto Superiore di Sanità (the National Institute of Health in Italy) and the Italian Focal Point of EFSA, outline key microbiome components across human, animal, food, and environmental domains, proposing methodologies for their systematic inclusion in risk assessment and regulatory processes.
Key words: Microbiome; Risk assessment; One Health; Food safety; Gut microbiota; Host-microbiome interactions

Istituto Superiore di Sanità
Microbioma nell'uomo, negli animali, negli alimenti e nell'ambiente: implicazioni per la valutazione del rischio nella sicurezza alimentare
A cura di Annalisa Silenzi, Luca Busani
2025, 41 p. Rapporti ISTISAN 25/1 (in inglese)

L'Autorità Europea per la Sicurezza Alimentare (*European Food Safety Authority*, EFSA) evidenzia l'importanza di integrare i dati sul microbioma nella valutazione del rischio per la sicurezza alimentare, al fine di chiarire le interazioni tra sostanze chimiche e microbioma e le loro implicazioni per la salute. Tale integrazione dovrebbe includere i microbiomi umani, animali, alimentari e ambientali, in linea con l'approccio One Health. Per realizzare questo obiettivo, è necessaria una stretta collaborazione tra gruppi di ricerca e agenzie regolatorie, come l'EFSA e la Food and Drug Administration (FDA), per incorporare le conoscenze sul microbioma nelle politiche di valutazione del rischio e One Health. Gli esperti italiani, coordinati dall'Istituto Superiore di Sanità e dal Punto Focale italiano dell'EFSA, propongono metodologie per l'inclusione sistematica del microbioma nei processi di valutazione del rischio e nelle normative, delineando le sue componenti chiave nei vari settori coinvolti.
Parole chiave: Microbioma; Valutazione del rischio; One Health; Sicurezza alimentare; Microbiota intestinale; Interazione ospite-microbioma

Questo lavoro è il risultato di un finanziamento ricevuto dal Focal Point italiano dell'EFSA.

Per informazioni su questo documento scrivere a: annalisa.silenzi@iss.it

Il rapporto è accessibile online dal sito di questo Istituto: www.iss.it

Citare questo documento come segue:
Silenzi A, Busani L (Ed.). *Microbiome in humans, animals, food and the environment: implications for risk assessment in food safety.* Roma: Istituto Superiore di Sanità; 2025. (Rapporti ISTISAN 25/1).

Legale rappresentante dell'Istituto Superiore di Sanità: *Rocco Bellantone*
Registro della Stampa - Tribunale di Roma n. 114 (cartaceo) e n. 115 (online) del 16 maggio 2014

Direttore responsabile della serie: *Antonio Mistretta*
Redazione*: Sandra Salinetti*
La responsabilità dei dati scientifici e tecnici è dei singoli autori, che dichiarano di non avere conflitti di interesse.

viale Regina Elena, 299 – 00161 Roma

TABLE OF CONTENTS

INTRODUCTION

Annalisa Silenzi, Luca Busani, Roberta Masella
Centro di riferimento Medicina di Genere, Istituto Superiore di Sanità, Rome, Italy

Many different microorganisms populate the human body, collectively called the human microbiota. Because of the vast diversity and enormous amount of microbial life that colonizes the human body, humans are now considered to be ecosystems consisting of distinct ecological niches or habitats, each harbouring a discrete collection of coevolved microorganisms extensively interacting with each other and the host. Coevolution has led to an interdependent relationship: the human microbiome contributes to a wide range of essential functions for the host, influencing physiological, immunological, and metabolic processes. In turn, the host's lifestyle and behaviours influence the composition and function of the microbiome (1). For example, age and diet play a primary role in gut microbiota variation, while local ecological conditions, particularly water and nutrient availability, determine the specific states of the skin microbiota community. All these factors and others – such as genetics, gender, socioeconomic status, geography, physical activity, pregnancy status, disease status, and environmental exposure –appear to play a role in shaping the composition and function of microbial communities (2). Recent surveys and meta-analyses that explored changes in the human gut microbiome in health and disease have revealed that such changes are highly personalized and follow distinct temporal trajectories.

The role of the human microbiome in maintaining human health is not yet fully understood. However, research is beginning to elucidate the associations between perturbations in the human microbiome and human diseases, and the factors that might be responsible for those perturbations, raising the question of the need to consider the microbiome when a specific Risk Assessment is carried out (3). Until now, estimates of the risk to human health associated with exposure to environmental, food chemical or biological factors have been performed without involving microbiome communities (4).

Risk Assessment, as intended by the *Codex Alimentarius* and included in the European food safety regulation, is a science-based process that consists of 4 steps:

1. *Hazard identification*
   identifying a particular agent's known or potential health effects.
2. *Hazard characterization*
   qualitative and/or quantitative assessment of adverse effects associated with biological, chemical, and physical agents that may be present in foods.
3. *Hazard exposure assessment*
   a qualitative and/or quantitative assessment of the likely degree of intake.
4. *Risk characterization*
   integration of hazard identification and characterization and exposure assessment data into an estimate of adverse effects that may occur in each population, including concomitant uncertainties.

Risk Assessment of foods and chemicals incorporating the gut microbiome aims to provide answers about food- and chemical-induced changes to the human gut microbiome and, on the other hand, the impact of the gut microbiome on nutrients and chemicals. The goal is to establish a causal relationship between these interactions and adverse health effects in the host-induced

changes to the human gut microbiome and, on the other hand, the impact of the gut microbiome on nutrients and chemicals. The goal is to identify a causal relationship between these interactions and adverse health effects in the host (5).

Many molecular mechanisms are likely to underlie microbiome interactions. Food is a major source of precursors for the production of gut microbiota metabolites, and such metabolites can interact with the host and the microbiota itself. For example, the idea that the gut microbiome may contribute to host metabolism is deeply rooted in the field of drug metabolism, for which it is critical to study how the activities encoded by the human microbiome influence the dose of toxicologically active chemicals at the final target site (tissue, cell, or molecule).

How the human microbiome modulates the pharmacokinetics and metabolism of all substances (dietary, chemical, and environmental) needs to be brought up to date with how the microbiome modulates drugs and xenobiotics. The human microbiome might modulate the exposure-response relationship through a few general mechanisms (2,6):

- *Direct effect of a substance on the human microbiome*
  If exposure to a chemical, environmental, dietary or any other factor causes a perturbation in the microbiome, that perturbation could have distinct effects on the host. It is also conceivable that changes induced by chemical-environmental exposures could alter the microbiome's ability to metabolize chemicals.
- *Altered epithelial barrier function*
  Epithelial barriers form the interface between many host tissues and the external environment. For example, intimate bidirectional interactions between the gut microbiota and epithelial cells are now well established: the gut microbiota composition and activity modulate the intestinal epithelium structure and function and vice versa. The ability to regulate epithelial permeability and integrity has important implications for environmental chemical absorption, transport, and excretion.
- *Direct chemical transformation*
  The gut microbiome has a remarkable ability to metabolize a wide variety of environmental chemicals, showcasing its role as a significant player in the transformation of these substances. This metabolic capability is facilitated by specific enzymatic families, including azoreductases, nitroreductases, β-glucuronidases, sulphatases, and β-lyases, which enable the gut bacteria to catalyse diverse chemical reactions such as reduction, hydrolysis, and deacetylation.
- *Transformation of host-generated metabolites*
  For example, deconjugation reactions by intestinal β-glucuronidases promote some drug metabolites' reabsorption, potentially altering their pharmacokinetic profiles, toxicity, or efficacy. Since a wide range of environmental chemicals could be subject to elimination by β-glucuronidation, this mechanism may be more common than we can now appreciate.
- *Altered expression of metabolic enzymes and host tissue pathways*
  Recent studies have shown that the gut microbiota can regulate host genes involved in chemical transport and metabolism. However, further research is needed to understand the mechanisms by which the gut microbiome and its products interact with host nuclear receptors and whether similar processes can alter the expression of other types of host gene pathways involved in toxicity.

Interesting examples of research into the microbiome interaction with xenobiotics and the consequences for metabolism and health are studies on dietary exposure to mycotoxins in animals, where the effects of these substances on the composition and abundance of the microbiome and on animal performance have been investigated (7,8).

Microbiological Risk Assessment (MRA) is important in ensuring food safety by identifying and managing potential hazards associated with microbial contamination. The human gut microbiome interacts with ingested foods and, similarly, foods possess their own unique microbiota, influencing the overall microbial ecology. Recognizing this intricate interplay is essential for a comprehensive MRA, as these microbial communities can impact the safety and quality of food products. The interactions between the gut microbiome and microbiological food contaminants are complex and include microbial interactions and competition, and fermentation processes. The food microbiome can enhance the resilience of a food product against potential contamination events. Consequently, MRA should consider the overall health of the microbiome to predict and mitigate the risks associated with disruptions to these ecosystems. The microbiome is becoming an important part of MRA for food safety. The complex interactions, competition, and symbiotic relationships within microbial communities directly influence food products' safety, quality, and resilience.

Microbial community interactions should be considered within and between humans, plants, and animals from a One Health perspective, including microbiomes as elements of the so-called next-generation Risk Assessment (9) (Figure 1).

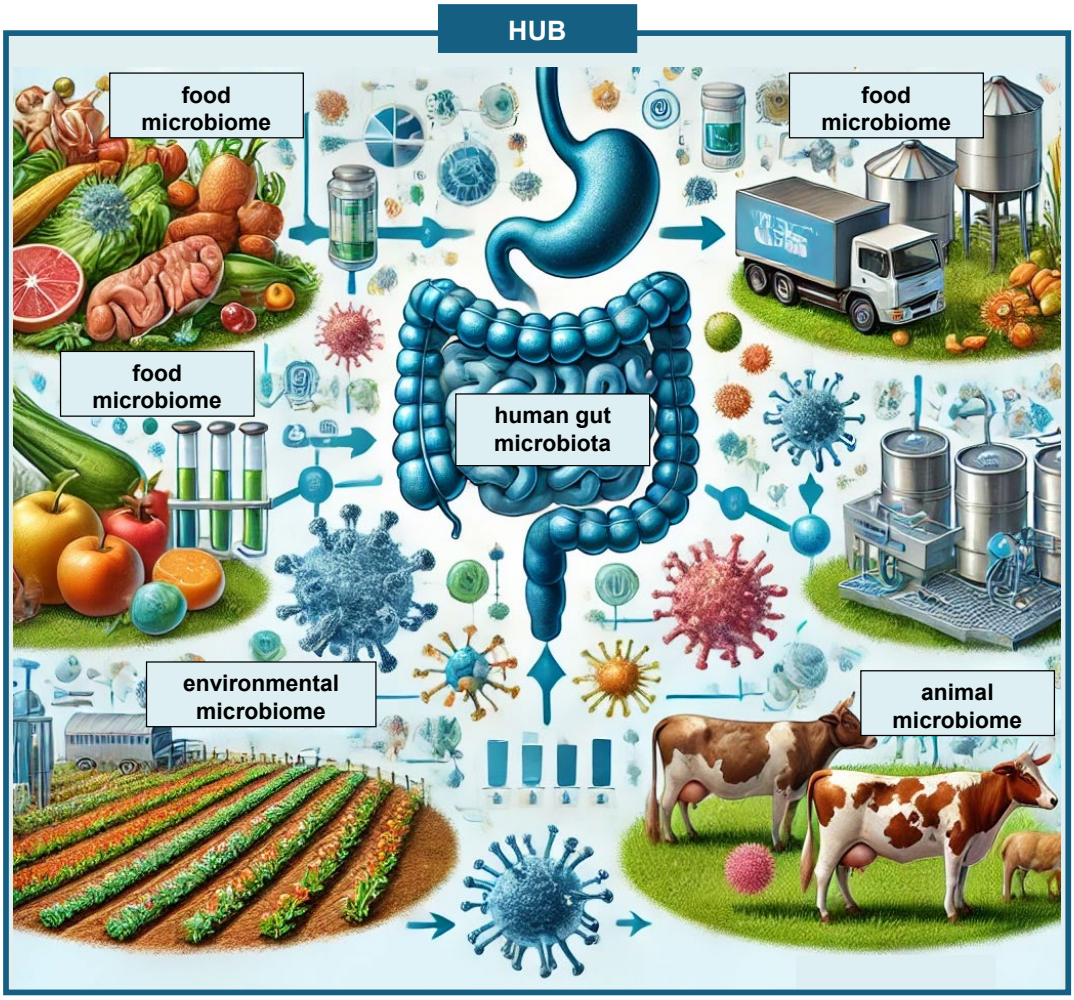

Figure 1. Flow and integration of microbiome information from diverse sources (human gut, food, animals, and the environment) into a central hub for food safety risk assessment

Figure 1 illustrates the flow and integration of microbiome information from diverse sources – human gut, foods, animals, and the environment – into a central hub for food safety risk assessment, which represents the European Food Safety Authority (EFSA) or any other body in charge of risk assessment. The interconnected sections highlight the multi-faceted nature of microbiome analysis, with each sector contributing critical data. The central hub visualizes how these inputs are converted into data (epidemiological, biological, genomic data) to feed into a comprehensive model that aids in assessing and mitigating food safety risks.

This perspective defines an integrated, unifying approach to balancing and optimizing the health of people, animals, and the environment through transdisciplinary cooperation (5, 10).

The main task of EFSA is to assess the risks to human and animal health and the environment from food and feed production-related contaminants. The growing importance of the microbiome and its interactions with metabolism and immunity poses serious questions on how this information should be incorporated into formal RA activities (11,12).

In particular, two central questions were proposed by EFSA (11):

- How to assess the impact of the various substances evaluated by EFSA on microbiomes?
- How to evaluate the impact of microbiomes on human, animal, and environmental health?

In 2023, the Italian National Focal Point of EFSA – considering the EFSA activities on microbiome capacity building started in 2020 and expected to continue in the next years – promoted an initiative involving the national research institutes entitled of scientific cooperation with EFSA (named "competent organizations") according to the art. 36 of the Regulation (EU) 178/2002 in order to appraise the state of the art in the field and to provide recommendations on the feasibility of taking microbiomes into account in human/domestic animal risk assessment. Such feasibility is also related to the advanced data analysis techniques that are increasingly implemented in food safety and Risk Assessment research (13). A list of the competent organizations classified for competence and country is available from an ad hoc webpage of the EFSA site.

The challenge for the risk assessors posed by the complexity of xenobiotic-microbiome interactions and the multitude of potential health consequences for humans and animals directly or indirectly associated with them requires the involvement of different competencies and disciplines, including human and veterinary medicine, food production and environmental health. Moreover, it implies implementing sophisticated methods and tools for analysing microbiome composition and its variation, like Next-Generation Sequencing (NGS) methods and innovative bioinformatic tools for data analysis, considering that such methods and tools are still at an early stage of development (14).

During the preliminary meetings among the national experts, four sectors of interest for exploring the relevance of the microbiome in Risk Assessment were identified: humans, animals, food, and the environment. For each sector, an in-depth bibliographic search was proposed to define the state of knowledge on the microbiome, the models and methods used for its study, and its effects on the different fields of interest.

This work is divided into two main parts, each addressing critical aspects of the microbiome's impact on human health and risk assessment:

- *Part A - Impact of the host microbiome on human health*
  This part aims to analyse the risks associated with the impact of the microbiome on human health, with a particular focus on the implications arising from exposure to various substances. In this section, the role of the microbiome in human health is examined, highlighting how alterations in its composition can influence susceptibility to diseases and metabolic disorders. The mechanisms through which intestinal microorganisms interact with chemical substances, modifying their toxicity and bioavailability, are also considered.

– *Part B - Microbiomes in animals, food and environment: integrated vision and approach*
In this part, the work provides an integrated view that explores the interactions between the microbiome, nutrition, animal health, risk assessment methodologies, and the environment. This section aims to evaluate how dietary practices, and environmental conditions can influence microbiome health and, consequently, human health. Through a multidisciplinary approach based on risk assessment, this work intends to offer useful insights for developing management and intervention strategies that can mitigate the potential negative effects of substances on the microbiome and public health.

References

1. Dekaboruah E, Suryavanshi MV, Chettri D, Verma AK. Human microbiome: an academic update on human body site specific surveillance and its possible role. *Arch Microbiol.* 2020;202(8):2147–67.
2. Committee on Advancing Understanding of the Implications of Environmental-Chemical Interactions with the Human Microbiome, Board on Environmental Studies and Toxicology, Board on Life Sciences, Division on Earth and Life Studies, National Academies of Sciences, Engineering, and Medicine. *Environmental Chemicals, the Human Microbiome, and Health Risk: A Research Strategy*. Washington, DC: National Academies Press; 2018. https://doi.org/10.17226/24960.
3. Hou K, Wu ZX, Chen XY, Wang JQ, Zhang D, Xiao C, *et al.* Microbiota in health and diseases. Signal Transduct Target Ther. 23 aprile 2022;7(1):135.
4. US EPA. *Conducting a human health risk assessment*. Washington, WA: U.S. Environmental Protection Agency; 2025. Available from: https://www.epa.gov/risk/conducting-human-health-risk-assessment; last visited January 2025.
5. Ampatzoglou A, Gruszecka-Kosowska A, Torres-Sánchez A, López-Moreno A, Cerk K, Ortiz P, *et al.* Incorporating the gut microbiome in the risk assessment of xenobiotics and identifying beneficial components for One Health. *Front Microbiol* 2022;13:article 872583. doi: 10.3389/fmicb.2022.872583
6. Lindell AE, Zimmermann-Kogadeeva M, Patil KR. Multimodal interactions of drugs, natural compounds and pollutants with the gut microbiota. *Nat Rev Microbiol.* 2022;20(7):431–43.
7. Zeebone YY, Bóta B, Halas V, Libisch B, Olasz F, Papp P, *et al.* Gut-faecal microbial and health-marker response to dietary fumonisins in weaned pigs. *Toxins*. 2023;15(5):328.
8. Yu S, Jia B, Lin H, Zhang S, Yu D, Liu N, *et al.* Effects of fumonisin B and hydrolyzed fumonisin B on growth and intestinal microbiota in broilers. *Toxins*. 2022;14(3):163.
9. Gruszecka-Kosowska A, Ampatzoglou A, Aguilera-Gómez M. Microbiota analysis for risk assessment of xenobiotics: cumulative xenobiotic exposure and impact on human gut microbiota under One Health approach. *EFSA J.* 2022;20(S2): e200916.
10. Cocolin L, Mataragas M, Bourdichon F, Doulgeraki A, Pilet MF, Jagadeesan B, *et al.* Next generation microbiological risk assessment meta-omics: The next need for integration. *Int J Food Microbiol*. 2018; 287:10–7.
11. Cerk K, Aguilera-Gómez M. Microbiota analysis for risk assessment: evaluation of hazardous dietary substances and its potential role on the gut microbiome variability and dysbiosis. *EFSA J.* 2022;20(S1):e200404.
12. Merten C, Schoonjans R, Di Gioia D, Peláez C, Sanz Y, Maurici D, *et al.* Editorial: Exploring the need to include microbiomes into EFSA's scientific assessments. *EFSA J.* 2020;18(6):e18061.
13. Carmody RN, Bisanz JE. Roles of the gut microbiome in weight management. *Nat Rev Microbiol*. 2023;21(8):535–50.
14. Walker AW, Hoyles L. Human microbiome myths and misconceptions. *Nat Microbiol*. 2023;8(8):1392–6.

PART A
Impact of the host microbiome on human health

ROLE OF MICROBIAL METABOLITES

Teresa Zelante, Gianluca Vascelli
Department of Medicine and Surgery, University of Perugia, Perugia

The terrific expansion of therapeutic strategies based on the microbiome and more in detail on prebiotics, probiotics, and postbiotics implies profound changes in clinical Risk Assessment related to different microbiome translational aspects. It is estimated that microbes, directly or indirectly, may profoundly affect health homeostasis. With the advancement of technology toward NGS (Next Generation Sequencing), today we can understand in detail the composition of the microbiome in tissues where the biomass is exceptionally low and, most importantly, the functional aspects of the microbial components (1).

For several years, the functionality of the microbiome was neglected. Still, nowadays, microbial-derived molecules and metabolites impacting human health have been identified, and new studies are now focused on using small-molecule inhibitors that target specific gut microbial activities (2). High-throughput association studies have been performed, increasing the power of such analysis, and microbial/metabolite signatures are identified (3).

The introduction of the concept that microbiome components produce effects through molecules on the host tissues, gives rise to functional studies and research for causality more than studies based on adaptation of the host microbiome during host diseases (4). In this context, the faecal microbiota transplantation represents a clinical practice that proved the causality between 'healthy' microbiota and gut homeostasis in the condition of *Clostridiodes difficile* infection (5). Assessment of risks in this context has been performed, and patients are extensively informed about risks related to transplant, which rarely lead to the transfer of resistant bacteria or other side effects (5). Undoubtedly, it is still unobvious how the clinical practice of faecal transplantation is defined. In various countries, faecal transplantation is approached as a 'biological agent' in USA, 'human tissue' in Italy, 'medicinal product' in UK, and 'medical procedure' in Austria (5). The causality of the clinical effects due to faecal transplantation has been widely investigated and in part efficiency is due to metabolic products of amino acids, secondary bile acids, Short-Chain Fatty Acids (SCFAs). Xenobiotic Receptors (XR) represent only one class of many receptors able to interact with microbial-derived molecules as indole-derivatives, producing effects on the host immune system and, therefore, on the overall homeostasis. Other host receptors bind molecules deriving from diet fibres such as acetate, propionate, and butyrate, which are known as SCFAs. Receptors for bile acids include other XRs as G-protein coupled bile acid receptor 1, farnesoid X receptor (FXR), pregnane X receptor (PXR), vitamin D receptor (VDR), androstane receptor (CAR), liver X receptor (LXR). Those receptors also bind endogenous ligands, thus the interaction is based on a mutualistic relationship between the host and the microbiome, where metabolism at the interface leads to essential metabolic functions on both parties. The disruption of the mutualism brings indeed to a slew of diseases based on malfunctional gut tissue, nervous system, skin, cardiovascular system (6).

More recently, intratumor metabolic activity of the microbiome has been demonstrated in human cancer (7, 8). The nature of bacteria is mostly intracellular, and lipopolysaccharide (LPS) and lipoteichoic acid (LTA)-positive bacteria are present in both immune and cancer cells. The microbiome of breast tumours is richer and more diverse compared to other tumours. Metabolites produced by the intratumor microbiome are associated with specific tumours (9). Most importantly, the cancer microbiome differs among patients who are responders or non-responders

to immunotherapy. Using digital spatial profiling, other studies also showed that bacterial communities populate microniches with less vascularization, with recruitment of suppressor cells, promoting tumour progression (9).

In this regard, human melanoma has proved microbiome 'translocation' from the gut to the cancer tissue (10). Bender *et al.* (10) and Zelante *et al.* (11), demonstrated that T-cell cytotoxic activity is strongly enhanced by 3-IAld, a postbiotic produced by *Lactobacillus reuteri* which acts as agonist of the nuclear receptor AhR. A remarkably interesting translational approach has been considered because the combination of injection of *L. reuteri* and 3-IAld and anti-PD1 showed an increased effectiveness of immunotherapeutic protocols in a mouse melanoma model (10).

Mycobiome and not only general bacteria out from the microbiome have been identified in the cancer tissue. In particular, the enrichment of *Aspergillus sydowii* in lung adenocarcinoma impacts the tumour microenvironment since the activation of Dectin-1 by the fungal profiles shapes the immunity in the inner part of the tumour by promoting immune suppression. Thus, the bacterial and fungal fractions of the microbiome may impact human health by inducing T-cell exhaustion, which negatively affects tumour progression and patient outcome (12).

In conclusion:

1. High-throughput association studies now allow the identification of mechanistic effects in microbiome research.
2. The use of postbiotics in association with prebiotics or probiotics will require the definition of human clinical risk assessments.
3. Xenobiotic receptors represent an important novel target, being the most important system able to recognize microbial metabolites.
4. The modulation of xenobiotic receptors by the intratumor microbiome may describe novel mechanisms of cancer progression.

References

1. Alcazar M, Escribano J, Ferré N, Closa-Monasterolo R, Selma-Royo M, Feliu A, *et al.* Gut microbiota is associated with metabolic health in children with obesity. *Clin Nutr Edinb Scotl.* 2022;41(8):1680-8.
2. Kost C, Patil KR, Friedman J, Garcia SL, Ralser M. Metabolic exchanges are ubiquitous in natural microbial communities. *Nat Microbiol.* 2023;8(12):2244-52.
3. Merrick B, Allen L, Masirah MZN, Forbes B, Shawcross DL, Goldenberg SD. Regulation, risk and safety of Faecal Microbiota Transplant. *Infect Prev Pr.* 2020;2(3):100069.
4. Hsu CL, Schnabl B. The gut-liver axis and gut microbiota in health and liver disease. *Nat Rev Microbiol.* novembre 2023;21(11):719-33.
5. Galeano Nino JL, Wu H, LaCourse KD, Kempchinsky AG, Baryiames A, Barber B, *et al.* Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer. *Nature*. 2022;611(7937):810–7.
6. Nejman D, Livyatan I, Fuks G, Gavert N, Zwang Y, Geller LT, *et al.* The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science*. 2020;368(6494):973-80.
7. Livyatan I, Straussman R. A spatial perspective on bacteria in tumours. *Nature*. 2022;611(7937):674-5.
8. Bender MJ, McPherson AC, Phelps CM, Pandey SP, Laughlin CR, Shapira JH, *et al.* Dietary tryptophan metabolite released by intratumoral Lactobacillus reuteri facilitates immune checkpoint inhibitor treatment. *Cell*. 2023;186(9):1846-1862 e26.

9. Liu NN, Yi CX, Wei LQ, Zhou JA, Jiang T, Hu CC, *et al.* The intratumor mycobiome promotes lung cancer progression via myeloid-derived suppressor cells. *Cancer Cell.* 2023;41(11):1927-1944 e9.
10. Kundu P, Blacher E, Elinav E, Pettersson S. Our gut microbiome: the evolving inner self. *Cell*. 2017;171(7):1481-93.
11. Derrien M, Alvarez AS, de Vos WM. The gut microbiota in the first decade of life. *Trends Microbiol*. 2019;27(12):997-1010.
12. D'Amico F, Barone M, Brigidi P, Turroni S. Gut microbiota in relation to frailty and clinical outcomes. *Curr Opin Clin Nutr Metab Care*. 2023;26(3):219.

GUT MICROBIOTA AND AGEING

Silvia Turroni (a), Simone Rampelli (a), Claudio Franceschi (b, c)
(a) Department of Pharmacy and Biotechnology, University of Bologna, Bologna
(b) Department of Medical and Surgical Sciences, University of Bologna, Bologna
(c) Institute of Information Technologies, Mathematics and Mechanics, and Institute of Biogerontology, Lobachevsky State University, Nizhny Novgorod

Age, including changes in diet, lifestyle, geography and the exposome, has been identified as a major driver of gut microbiome variation (1). Indeed, the compositional and functional profile of the gut microbiome changes with age, following deterministic and predictable trajectories from infancy to adulthood and beyond.

Immediately after birth, a complex and dynamic interplay with environmental microbes begins, with maternal (and paternal) microbiomes and breast milk playing a major role in the assembly process.

From weaning, with the immune system's maturation and the gut mucosa's development, the microbiome begins to stabilize and converge towards the typical adult profile. Still, the exact period of establishment remains difficult to estimate, as it is closely linked to personal history.

To complicate matters further, the few studies available on childhood suggest that the gut microbiome may develop even more slowly than previously thought (2).

Another uncertain transition point is that from adulthood to old age, the gut microbiome begins to lose healthy features – especially the diversity and relative abundance of health-associated Short Chain Fatty Acids (SCFAs) producing taxa – while gaining detrimental traits, such as increased proportions of potential pathogens (3, 4). Such a configuration is closely linked to the physiological and lifestyle changes associated with the ageing process, such as tooth loss and alteration of taste and smell with changes in dietary habits (namely, lower fibre intake with the reduction in fibre-degrading bacteria), reduction in physical activity with a decrease in intestinal motility and greater opportunities for proliferation of opportunistic bacteria, "inflammageing" and "immunosenescence". It is thought to further contribute to age-related functional decline, including frailty syndrome (5).

However, the study of the gut microbiome of particularly long-lived individuals has highlighted some favourable characteristics that may represent potential markers of healthy ageing and longevity (6). Such characteristics include increased proportions of bifidobacteria (well-known probiotics), *Akkermansia* (a mucus-degrading bacterium, used as a postbiotic to treat obesity and related complications) and *Christensenella* (a potential heritable component of longevity), as well as maintaining the uniqueness of one's own microbiota profile.

In this regard, a study of over 9,000 individuals showed that the pattern of the gut microbiome, and in particular the loss of the individual microbial fingerprint, could predict reduced survival (7). Interestingly, geographically distant populations, such as Italians, Japanese, and Chinese, share the above characteristics, reinforcing their relevance as potential longevity signatures (6). On the other hand, as expected, there are some differences, probably related to differences in diet, lifestyle, environmental exposure and, last but not least, genetics.

Although the underlying molecular mechanisms are not known, *Akkermansia* and *Christensenella* possess genes capable of counteracting the formation of advanced glycation end products, which are known to be involved in ageing (8). Furthermore, maintaining a certain level of diversity and enrichment in microorganisms belonging to the *Christensenellaceae*, *Porphyromonadaceae* and *Rikenellaceae* families appears protective against cardiovascular and

metabolic disorders related to visceral fat in old age (9). Indeed, these characteristics have been associated with low levels of visceral adipose tissue and cardiovascular and renal risk factors, high levels of adiponectin (an anti-inflammatory cytokine), and low levels of potentially harmful circulating metabolites, such as branched-chain amino acids, fatty acids and bile acids, as well as healthy dietary habits (mainly higher fibre consumption), confirming the importance of diet as a tool for modulating the gut microbiota and supporting healthy ageing (10). In particular, the European NU-AGE ("New dietary strategies addressing the specific needs of the elderly population for healthy aging in Europe") project found that following a Mediterranean diet tailored for older people for one year was associated with increased proportions of bacteria related to reduced frailty, improved cognitive function and decreased levels of inflammatory markers (11). In addition, this diet resulted in potentially increased production of SCFAs, and potentially reduced levels of branched-chain fatty acids (generally associated with insulin resistance, diabetes and inflammation), bile acids and p-cresol (a microbial metabolite resulting from the metabolism of branched-chain amino acids, generally associated with adverse health outcomes).

From a functional point of view, an increase in microbial genes involved in the degradation of xenobiotics, such as those derived from industrial production, urban waste and various consumer products, has been observed with age (12). Similarly, ageing has been associated with an increased burden of antimicrobial resistance genes, particularly proteobacterial genes encoding multidrug efflux pumps (13). While this could be the result of an adaptive response of the gut microbiome to ageing in modern Western societies (including exposure to xenobiotics and antimicrobials), it also stresses the need to include the gut microbiome in risk prediction algorithms to improve Risk Assessment and to develop more effective, early personalized precision strategies to favour not only a eubiotic microbiome profile, but also an eubiotic microbiome trajectory across the lifespan.

In conclusion:

- The human gut microbiome changes with age in relation to the personal exposome.
- The gut microbiome has the potential to promote healthy ageing and possibly longevity.
- RA should consider the gut microbiome profile and trajectory for improved preventive/therapeutic strategies.

References

1. Barone M, D'Amico F, Rampelli S, Brigidi P, Turroni S. Age-related diseases, therapies and gut microbiome: A new frontier for healthy aging. *Mech Ageing Dev*. 2022; 206:111711.
2. Biagi E, Nylund L, Candela M, Ostan R, Bucci L, Pini E, *et al.* Through Ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLOS ONE*. 2010;5(5): e10667.
3. Biagi E, Franceschi C, Rampelli S, Severgnini M, Ostan R, Turroni S, *et al.* Gut microbiota and extreme longevity. *Curr Biol CB*. 2016;26(11):1480–5.
4. Wilmanski T, Diener C, Rappaport N, Patwardhan S, Wiedrick J, Lapidus J, *et al.* Gut microbiome pattern reflects healthy ageing and predicts survival in humans. *Nat Metab*. 2021;3(2):274–86.
5. Danchin A. Bacteria in the ageing gut: did the taming of fire promote a long human lifespan? *Environ Microbiol.* 2018;20(6):1966–87.
6. Tavella T, Rampelli S, Guidarelli G, Bazzocchi A, Gasperini C, Pujos-Guillot E, *et al.* Elevated gut microbiome abundance of Christensenellaceae, Porphyromonadaceae and Rikenellaceae is associated with reduced visceral adipose tissue and healthier metabolic profile in Italian elderly. *Gut Microbes*. 2021;13(1):1–19.

7. Ghosh TS, Rampelli S, Jeffery IB, Santoro A, Neto M, Capri M, *et al.* Mediterranean diet intervention alters the gut microbiome in older people reducing frailty and improving health status: the NU-AGE 1-year dietary intervention across five European countries. *Gut*. 2020;69(7):1218–28.
8. Flanagan E, Lamport D, Brennan L, Burnet P, Calabrese V, Cunnane SC, *et al.* Nutrition and the ageing brain: Moving towards clinical applications. *Ageing Res Rev*. settembre 2020; 62:101079.
9. Tavella T, Turroni S, Brigidi P, Candela M, Rampelli S. The human gut resistome up to extreme longevity. *mSphere*. 2021;6(5): e0069121.
10. Rampelli S, Soverini M, D'Amico F, Barone M, Tavella T, Monti D, *et al.* Shotgun metagenomics of gut microbiota in humans with up to extreme longevity and the increasing role of xenobiotic degradation. *mSystems*. 2020;5(2): e00124-20.
11. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, *et al.* The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. *Microbiol Mol Biol Rev MMBR*. 2017;81(4): e00036-17.
12. Kapourchali FR, Cresci GAM. Early-life gut microbiome-the importance of maternal and infant factors in its establishment. *Nutr Clin Pract Off Publ Am Soc Parenter Enter Nutr*. 2020;35(3):386–405.
13. Simon DA, Kellermayer R. Disturbed pediatric gut microbiome maturation in the developmental origins of subsequent chronic disease. *J Pediatr Gastroenterol Nutr.* 2023;76(2):123–7.

GUT MICROBIOTA AND METABOLIC DISEASES IN PAEDIATRIC AGE

Elvira Verduci (a, b), Giulia Fiore (b, c), Martina Tosi (b, c)
(a) Metabolic Diseases Unit, Department of Paediatrics, V. Buzzi Children's Hospital, University of Milan
(b) Department of Health Sciences, University of Milan
(c) Department of Paediatrics, V. Buzzi Children's Hospital, University of Milan

During the developmental age, the gut microbiota evolves and is influenced by several environmental factors (1, 2). Indeed, this period is essential for its appropriate maturation and for the establishment of a multifarious relationship between the host and gut microbiota. On the contrary, when alterations in the homeostasis of the gut microbiota occur, children experience dysbiosis. This condition disrupts the dialogue between host and gut microbiota and has been found in several paediatric metabolic diseases. Dysbiosis is of great concern due to the potential immediate and long-term negative health consequences (3).

Among metabolic diseases, the role of the gut microbiota has been extensively studied in children with obesity. Gut microbiota is considered to be an aetiological factor influencing the risk of obesity, even in childhood, as it is actively involved in homeostasis and energy metabolism. The obesity-related gut microbiota has been classified as a microbiota with a high extractive capacity from human indigested substrates and typically characterised by an altered ratio of *Firmicutes* to *Bacteroidetes*, with the formers being higher than the latter (4,5). The increased extraction capacity from substrates, manifests itself thought higher levels of Short Chain Fatty Acids (SCFAs), this has been observed in multiple cohorts of both children (6,7) and adults with obesity (8, 9). Moreover, their gut microbiota is generally characterized by a low degree of biodiversity and enrichment in pathobiont bacteria, such as members of the family Enterobacteriaceae, as well as Erysipelotrichaceae and the sulphate reducer species *Bilophila wadsworthia* (4, 10-12).

It has been suggested that early life gut microbial patterns may influence future risk of overweight and obesity, as the abundance of the bifidobacteria population at 6 and 12 months is inversely correlated with overweight in 7-year-old children (2). Overall, the microbiota of children with obesity is characterised by lower alpha diversity (7), and current studies aim to evaluate possible interactions resulting from the metabolic status of the obese individual. In children the combination of unhealthy diets, low-grade inflammation and a dysbiotic, low-diverse and pro-inflammatory microbial layout may favour the onset of obesity (11). An intestinal microbial ecosystem high in proinflammatory Enterobacteriaceae and sulphate-reducing bacteria may consolidate the obesity-associated inflammation and insulin resistance (13). Interestingly, gut microbiota has been also characterized in children with obesity with the cooccurrence of metabolic alteration, also known as Metabolic Unhealthy Obesity (MUO). Among metabolically unhealthy children with obesity, the main complications are insulin resistance or impaired glucose metabolism, arterial hypertension, and/or dyslipidaemia. This metabolic status was shown to be characterized by a condition of dysbiosis, lower alpha diversity and lower richness when compared to children with metabolically healthy obesity. Specifically, gut microbiota was different at family and genus taxonomic levels, showing for example lower proportions of Christensenellaceae*, Lachnoclostridium* and *Akkermansia* (14-16).

It's also worth to underline that recent evidence supports the relationship between gut microbiota and Metabolic-Associated Fatty Liver Disease (MAFLD) (17). Although longitudinal studies on the paediatric population have showed that diet is one of the main drivers towards the development of an obesogenic microbiota, the extent of this impact and the mechanisms exerted by diet and dietary patterns have been poorly studied yet (18).

Inborn Errors of Metabolism (IEMs) are a group of inherited genetic disorders characterized by the alteration of metabolic pathways (19, 20). Thanks to Newborn Screening, many IEMs can be detected from the first days of life, permitting the early treatment, included the long-life dietary one. Nowadays, experts are evaluating IEMs' dietary management as one of the potential drivers in the reduction of gut microbial biodiversity and the promotion of gut dysbiosis (21, 22).

The strict control of Phenylalanine (Phe) intake and the replacement of natural protein foods with Phe-free or low in Phe protein substitutes in Phenylketonuria (PKU) have been demonstrated to be associated with lower microbial biodiversity and the depletion of some genera, i.e. *Firmicutes* (23), thus leading to less bacterial functions, because of their involvement in starch and sucrose metabolism, glycolysis and gluconeogenesis and amino acid biosynthesis.

Moreover, gut microbiota in PKU showed a decreased total content of SCFAs, and in particular, butyrate, because of the reduction of *Faecalibacterium* spp. and *Roseburia* spp., butyrate-producers' species (18). Recent studies concentrating on the consumption of glycomacropeptide (GMP)-based protein substitutes found an increase in beneficial bacteria such as *Agathobacter* and, in a subject-dependent manner, *Subdoligranulum* (24).

Further studies are required to evaluate the possible impact of Tryptophan metabolism, through gut microbiota, on cognitive and behavioural functions in IEMs (25).

A study on Glycogen Storage Diseases (GSD) showed a significant biodiversity reduction, an increase in the relative abundance of Enterobacteriaceae and Veillonellaceae families, a reduction of beneficial genera *Faecalibacterium* and *Oscillospira*, and an increase in faecal acetate and propionate (26).

Other studies highlighted the presence of intestinal dysbiosis, altered bile acids metabolism and changes of faecal SCFAs concentrations in GSD patients; these might be due to the disease itself or dietary management or medicines (27,28).

Few existing studies on Propionic Acidemia (PA) not only found a risk for gut dysbiosis but also an increase in *Proteobacteria* levels, and a decrease in butyrate-producing genera, such as *Roseburia* and *Faecalibacterium* (27).

In conclusion:

- In paediatric age, alterations in the composition of gut microbiota may contribute to the pathogenesis of several diseases, such as obesity, type 2 diabetes, and liver diseases. Moreover, the dietary treatment required for Inborn Errors of Metabolism (IEMs) has also been associated with gut microbiota alterations.
- As a dysbiotic state may contribute to an increased risk for NCDs, new dietary strategies to model the intestinal microbiota profile and offer an innovative approach to improve health outcomes are needed from early life.

References

1. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, *et al.* A core gut microbiome in obese and lean twins. *Nature*. 2009;457(7228):480–4.
2. Squillario M, Bonaretti C, La Valle A, Di Marco E, Piccolo G, Minuto N, *et al.* Gut-microbiota in children and adolescents with obesity: inferred functional analysis and machine-learning algorithms to classify microorganisms. *Sci Rep.* 2023;13(1):11294.

3. Rampelli S, Guenther K, Turroni S, Wolters M, Veidebaum T, Kourides Y, *et al.* pre-obese children's dysbiotic gut microbiome and unhealthy diets may predict the development of obesity. *Commun Biol*. 2018;1(1):1–11.
4. Borgo F, Verduci E, Riva A, Lassandro C, Riva E, Morace G, *et al.* Relative Abundance in Bacterial and Fungal Gut Microbes in Obese Children: A Case Control Study. *Child Obes Print*. 2017;13(1):78–84.
5. Riva A, Borgo F, Lassandro C, Verduci E, Morace G, Borghi E, *et al.* Pediatric obesity is associated with an altered gut microbiota and discordant shifts in Firmicutes populations. *Environ Microbiol*. 2017;19(1):95–105.
6. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, *et al.* Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut*. 2009;58(8):1091–103.
7. Di Profio E, Magenes VC, Fiore G, Agostinelli M, La Mendola A, Acunzo M, *et al.* Special diets in infants and children and impact on gut microbioma. *Nutrients*. 2022;14(15):3198.
8. European Society for Paediatric Gastroenterology Hepatology (ESPGHAN) and European Association for the Study of the Liver (EASL), on behalf of co-authors. Diagnosis of fatty liver in children should occur in parallel to investigation for other causes of liver disease. *Lancet Gastroenterol Hepatol*. 2023;8(7):598–600.
9. Verduci E, Carbone MT, Borghi E, Ottaviano E, Burlina A, Biasucci G. Nutrition, microbiota and role of gut-brain axis in subjects with Phenylketonuria (PKU): A Review. *Nutrients*. 2020;12(11):3319.
10. Colonetti K, Roesch LF, Schwartz IVD. The microbiome and inborn errors of metabolism: Why we should look carefully at their interplay? *Genet Mol Biol*. 2018;41(3):515–32.
11. Ferreira CR, Rahman S, Keller M, Zschocke J, ICIMD Advisory Group. An international classification of inherited metabolic disorders (ICIMD). *J Inherit Metab Dis*. 2021;44(1):164–77.
12. Montanari C, Parolisi S, Borghi E, Putignani L, Bassanini G, Zuvadelli J, *et al.* Dysbiosis, host metabolism, and non-communicable diseases: trialogue in the inborn errors of metabolism. *Front Physiol* 2021;12.
13. Bassanini G, Ceccarani C, Borgo F, Severgnini M, Rovelli V, Morace G, Verduci E, Borghi E. Phenylketonuria diet promotes shifts in firmicutes populations. *Front Cell Infect Microbiol*. 2019;9:101. doi: 10.3389/fcimb.2019.00101.
14. Carrizales-Sánchez AK, Tamez-Rivera O, Rodríguez-Gutiérrez NA, Elizondo-Montemayor L, Gradilla-Hernández MS, García-Rivas G, *et al.* Characterization of gut microbiota associated with metabolic syndrome and type-2 diabetes mellitus in Mexican pediatric subjects. *BMC Pediatr*. 2023;23(1):210.
15. Woo AYM, Aguilar Ramos MA, Narayan R, Richards-Corke KC, Wang ML, Sandoval-Espinola WJ, *et al.* Targeting the human gut microbiome with small-molecule inhibitors. *Nat Rev Chem*. 2023;7(5):319–39.
16. Thomas AM, Fidelle M, Routy B, Kroemer G, Wargo JA, Segata N, *et al.* Gut OncoMicrobiome Signatures (GOMS) as next-generation biomarkers for cancer immunotherapy. *Nat Rev Clin Oncol*. 2023;20(9):583–603.
17. Montanari C, Ceccarani C, Corsello A, Zuvadelli J, Ottaviano E, Dei Cas M, *et al.* Glycomacropeptide safety and its effect on gut microbiota in patients with phenylketonuria: a pilot study. *Nutrients*. 2022;14(9):1883.
18. Ceccarani C, Bassanini G, Montanari C, Casiraghi MC, Ottaviano E, Morace G, *et al.* Proteobacteria overgrowth and butyrate-producing taxa depletion in the gut microbiota of glycogen storage disease type 1 patients. *Metabolites*. 2020;10(4):133.

19. Tims S, Marsaux C, Pinto A, Daly A, Karall D, Kuhn M, *et al.* Altered gut microbiome diversity and function in patients with propionic acidemia. *Mol Genet Metab*. 2022;137(3):308–22.
20. Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, *et al.* The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A*. 2004;101(44):15718–23.
21. Patterson E, Ryan PM, Cryan JF, Dinan TG, Ross RP, Fitzgerald GF, *et al.* Gut microbiota, obesity and diabetes. *Postgrad Med J*. 2016;92(1087):286–300.
22. Khan MJ, Gerasimidis K, Edwards CA, Shaikh MG. Role of gut microbiota in the aetiology of obesity: proposed mechanisms and review of the literature. *J Obes.* 2016; 2016:7353642.
23. Caesar R, Fåk F, Bäckhed F. Effects of gut microbiota on obesity and atherosclerosis via modulation of inflammation and lipid metabolism. *J Intern Med*. 2010;268(4):320–8.
24. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*. 2007;56(7):1761–72.
25. Kopp A, Buechler C, Neumeier M, Weigert J, Aslanidis C, Schölmerich J, *et al.* Innate immunity and adipocyte function: ligand-specific activation of multiple Toll-like receptors modulates cytokine, adipokine, and chemokine secretion in adipocytes. *Obes Silver Spring Md*. 2009;17(4):648–56.
26. Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, *et al.* Metagenomic analysis of the human distal gut microbiome. *Science*. 2006;312(5778):1355–9.
27. Haro C, Montes-Borrego M, Rangel-Zúñiga OA, Alcalá-Díaz JF, Gómez-Delgado F, Pérez-Martínez P, *et al.* Two healthy diets modulate gut microbial community improving insulin sensitivity in a human obese population. *J Clin Endocrinol Metab*. 2016;101(1):233–42.
28. Haro C, García-Carpintero S, Rangel-Zúñiga OA, Alcalá-Díaz JF, Landa BB, Clemente JC, *et al.* Consumption of two healthy dietary patterns restored microbiota dysbiosis in obese patients with metabolic dysfunction. *Mol Nutr Food Res*. 2017;61(12).

GUT MICROBIOTA AND OBESITY-RELATED METABOLIC DISEASES IN ADULT AGE

Giovanna Muscogiuri
Dipartimento di Medicina Clinica e Chirurgia, Università degli Studi di Napoli Federico II, Naples

Gut microbiota has been reported to be altered in metabolic diseases such as obesity and has a role in increasing energy harvesting from diet (1). Thus, gut microbiota is involved in fat storage through the formation of storable fat synthesis (2). In addition, compounds are produced at the gut microbiota site that, once absorbed, contribute through systemic circulation to the onset of obesity-related disease complications since they increase both tissue inflammatory damage and insulin resistance and, thus, the risk of developing type 2 diabetes (3).

A change in gut microbiota composition has been detected in obesity, and it has been associated with the ability to decrease metabolic energy consumption compared to the gut microbiota composition of lean people. This effect is based on the property of obesity-related gut microbiota to allow an efficient fermentation of indigestible carbohydrates into short-chain fatty acids, thus supplying an excess energy substrate to the host, which creates a favourable milieu for the onset of obesity (3). In addition, gut microbiota abnormalities may influence lipid metabolism, thus favouring the development of atheromatous plaque thanks to several inflammatory-dependent mechanisms (4). The main determinant seems to be Lipopolysaccharide (LPS), which is a component of the outer membrane of Gram-negative bacteria that can go through the gut wall in two ways, "passive" chylomicron-associated transport and "active" leakage through faulty tight junctions (leaky gut) (5). The binding of LPS to Toll-Like Receptors (TLRs) has been associated with an increase of macrophage infiltration in adipose tissue and the reduction of insulin sensitivity (6). The consequent upregulation of inflammatory signalling pathways could influence individuals' predisposition to metabolic syndrome and cardiovascular diseases. Gut microbiota composition, through its involvement in carbohydrates, amino acids, and fatty acid metabolism and in the modulation and development of host immunity, could also affect the development/ progress of inflammation in the liver, thus contributing to the onset of Non-alcoholic Fatty Liver Disease (NAFLD) (7). Based on this background, it can be assumed that acting on gut microbiota composition could be considered among the promising treatments for metabolic diseases. In this sense, following the Mediterranean diet for over 2 years has been reported to restore gut microbiota equilibrium, contributing to improving insulin resistance in subjects with obesity and coronary heart diseases (8,9).

Very Low-Calorie Ketogenic Diet (VLCKD)has been demonstrated to be another promising approach to restore a physiological gut microbiota composition in obesity (10).

Bariatric surgery, an antiobesity treatment reserved for subjects with severe obesity, is characterized by the removal of a portion of the stomach or decreasing food entry into the stomach using a gastric band, or by re-routing the remaining stomach "pouch" into the small intestine, has been reported to have an effect on gut microbiota composition. An increase in both *Gammaproteobacteria* (including Enterobacteriaceae) and Fusobacteriaceae, along with a proportional decrease in *Clostridiathus* content, has been detected in subjects with obesity undergoing gastric bypass (11). It has been hypothesized that these changes could be mediated by the reduced duration of gut wall exposure to food and the different pH distribution (11).

In conclusion:

- Gut microbiota composition could predispose to the onset of obesity and obesity-related metabolic disorders, including type 2 diabetes, NAFLD, metabolic syndrome, and cardiovascular disease.
- Obesity treatments such as nutritional approach and/or bariatric surgery modify the gut microbiota in ways that result in health benefits.
- Further studies are needed to better understand the reported associations between gut microbiota and obesity and to establish whether manipulation of the gut microbiota could provide potential therapeutic options for the prevention or treatment of human obesity.

References

1. Zambrano AK, Cadena-Ullauri S, Guevara-Ramírez P, Frias-Toral E, Ruiz-Pozo VA, Paz-Cruz E, *et al.* The impact of a very-low-calorie ketogenic diet in the gut microbiota composition in obesity. *Nutrients*. 13 giugno 2023;15(12):2728.
2. Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, *et al.* Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci U S A*. 17 febbraio 2009;106(7):2365–70.
3. Chen S, Luo S, Yan C. Gut microbiota implications for health and welfare in farm animals: a review. *Anim Basel*. 31 dicembre 2021;12(1).
4. Mulder IE, Schmidt B, Stokes CR, Lewis M, Bailey M, Aminov RI, *et al.* Environmentally acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces. *BMC Biol*. 20 novembre 2009; 7:79.
5. Van Gompel L, Luiken REC, Hansen RB, Munk P, Bouwknegt M, Heres L, *et al.* Description and determinants of the faecal resistome and microbiome of farmers and slaughterhouse workers: A metagenome-wide cross-sectional study. *Env Int.* ottobre 2020; 143:105939.
6. Mosites E, Sammons M, Otiang E, Eng A, Noecker C, Manor O, *et al.* Microbiome sharing between children, livestock and household surfaces in western Kenya. *PLoS ONE*. 2017;12(2): e0171017.
7. Reperant LA, Cornaglia G, Osterhaus AD. The importance of understanding the human-animal interface: from early hominins to global citizens. *Curr Top Microbiol Immunol*. 2013; 365:49–81.
8. Vestergaard DV, Holst GJ, Basinas I, Elholm G, Schlunssen V, Linneberg A, *et al.* Pig farmers' homes harbor more diverse airborne bacterial communities than pig stables or suburban homes. *Front Microbiol*. 2018; 9:870.
9. Oppliger A, Moreillon P, Charriere N, Giddey M, Morisset D, Sakwinska O. Antimicrobial resistance of Staphylococcus aureus strains acquired by pig farmers from pigs. *Appl Env Microbiol*. novembre 2012;78(22):8010–4.
10. Pehrsson EC, Tsukayama P, Patel S, Mejia-Bautista M, Sosa-Soto G, Navarrete KM, *et al.* Interconnected microbiomes and resistomes in low-income human habitats. *Nature*. 12 maggio 2016;533(7602):212–6.
11. Schultz Marcolla C, Sosa Alvarado C, Willing BP. Early life microbial exposure shapes subsequent animal health. *Can J Anim Sci*. 2019;99(4):661–77.

PART B
Microbiomes in animals, food and environment: integrated vision and approach

MICROBIOMES OF ANIMALS

Alberto Finamore (a), Paolo Trevisi (b), Diana Luise (b), Luigina Romani (c), Claudio Costantini (c), David Meo Zilio (d)
(a) Research Centre for Food and Nutrition, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Rome
(b) Department of Agricultural and Food Science, University of Bologna, Bologna
(c) Department of Medicine and Surgery, University of Perugia, Perugia
(d) Research Centre for Animal Production and Aquaculture, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Monterotondo, Rome

Animals

According to the One Health concept, human health and well-being are deeply associated with the health of other ecosystem components such as soil, plants, and animals.

A microbial ecosystem with a vast diversity is necessary for human health as well as for animal farms, in particular, gut microbiota is affected by many factors, including age, diet, and rearing system (1). In both, animals and humans, several factors impact the microbiome such as new diets, altered social structures, environmental exposure, different housing, and contact with wild animals (2). Human and animal interactions vary between populations, reflect industrialization, and involve not only direct contact but also the environment that they share and the consumption of animal products (3). Interactions among humans, animals, and the environment are the cause of microbes' movement between different host species (4, 5). Interaction of farm workers with livestock is associated with microbiome differences as well as work in industrial farms is well known to be a risk for zoonotic transmission and antimicrobial resistance through the transmission of pathogens (6).

In rural agricultural communities, animal faeces have been identified as a potential way for microbial gene transfer to human-associated microbes (7). As demonstrated, the interactions with livestock are also important sources of beneficial microbial exposure that may impact human health, for example, it has been shown that the microbiome of pig farmers increased in microbial diversity compared to that of urban people (8). Consuming animal meat and animal products may be a potential way for commensal and pathogenic microbial exposure for humans, as shown that the handling and consuming raw or undercooked meat is commonly associated with zoonotic infection as well as workers that handle meat are more likely to make contact with microbes of meat products and they may subject to a higher risk to pathogen exposure than others (4). Actual production practices in livestock have the purpose of reducing animal exposition to pathogens to avoid the reduction in productivity and animal and/or human health. However, all these practices, may inhibit the exposure of animals to commensal microbes and reduce the colonization of the gastrointestinal tract with healthy commensal microbes (9). Thus, considering the large differences in the various species of farmed animals, it is necessary to develop and apply specific strategies to consistently and efficiently manipulate the gut microbiota toward the establishment of stable, host-adapted, and species-specific microbiota that can promote animal health and performance of farmed animals. To this end, laboratory animal models can provide insight into the effects of microbiota manipulation and its risk assessment on host health.

Monogastric animals

Industrial production accounts for 55% and 71% of global pork and poultry production, respectively. According to the Food and Agricultural Organization (FAO) (10), the global demand for meat is projected to increase by 58% by 2050, with a significant portion of this demand coming from developing countries. In this framework, ensuring the safety and quality of the food chain is crucial to prevent the spread of foodborne pathogens during both the rearing and processing of animal products. Common foodborne pathogens associated with chicken, pork, and egg production and consumption include *Campylobacter jejuni*, *Salmonella typhimurium*, *Escherichia coli*, *Clostridioides perfringens* and *Staphylococcus aureus*.

Microbiome technology can significantly improve the monitoring and prevention of spread of these common foodborne pathogens both during the farming rearing system and during the animal product processing system (11, 12). Indeed, during the rearing system, the microbiome analysis of non-invasive matrix including faecal samples or environmental samples (litter, air, soils) can lead to a continue control and monitoring of the spread of potential pathogenic and foodborne pathogens both in poultry and pigs (13, 14).

The prompt detection of pathogens and knowledge of the microbial community in an animal's intestinal tract can aid in determining the most effective drug treatment and recovery strategy. Additionally, administering certain additives such as pre and probiotics, essential oils, and extracts, along with pharmacological treatment and an appropriate diet, can accelerate animal recovery and maintain intestinal health (15).

In addition to improving health and robustness, knowledge of the gut microbiota and its manipulation can enhance feed efficiency and conversions in pigs (16, 17), thereby increasing productivity and promoting a more environmentally friendly food animal industry. In addition to the sole culture-based methods, the use of metagenomics approach can lead to the detection of new relevant species (opportunistic pathogens including *Corynebacterium, Neisseria, Helicobacter, Enterobacter, Klebsiella,* and *Pseudomonas*) or group of taxa relevant for food security as well as the detection of relevant virus and fungi (11, 18). Finally, the inclusion of this technology can help in detecting and controlling the spread of new antimicrobial resistance phenomena in the environment derived by monogastric livestock animals (11). Indeed, it is well known that part of the antibiotic resistance is spread from livestock animal to human by meat but the majority of it is due to the use of pigs' manure in the environments.

Overall, the integration of metagenomics information on the microbiota with data on feeding, nutritional management, health parameters derived from precision feeding, and other omics information will benefit the development of a more ecological and secure monogastric rearing system in the future.

Ruminants

Ruminants contribute to sustainable global food security, playing a major role in the conversion of plants, some inedible for humans, into high-quality protein even from lands unsuitable for cropping or other human activities, across all over the world, under very different economic and social demographics. Thus, tools, methodologies, and systems to optimize protein yield and quality from ruminants strongly impact on food security. Ruminants house a complex rumen microbial community (that may equal or exceed host cell counts in number and is one of the most diverse gut ecosystems yet described in the animal kingdom) essential to digest fibre from the lignocellulosic part of plants, through microbial-mediated fermentation and to convert non-protein nitrogen into protein and amino acids. The other side of the coin is that rumen fermentations are responsible for environmental pollution (i.e., CH_4 emission and N excretion).

In addition, microbiome in ruminant influences also reproductive efficiency and health, including zoonosis (19) and rumen fermentation may adversely influence the nutritional value of ruminant end-products (milk and meat) (20). So, thoroughly understanding of the rumen key microorganisms and their activities is essential to manipulate rumen processes successfully, and the introduction and integration of metagenomic, transcriptomic, proteomic and metabolomic techniques is offering the greatest potential to do that. However, research effort on cultivation of microorganisms for in-depth studies and characterization is still needed and broader approach (beyond the prokaryotic population) should be considered to improve and optimize host-microbe-symbioses and to produce production-specific phenotypes and outputs (19).

It is well known that differences in terms of abundance (21), diversity (between and within taxa) and in particular functionality of rumen population are related to feed efficiency (22), impacting feed utilization, fibre digestibility and in turn protein synthesis.

We have different methods available to drive rumen activity and metabolism and to improve health, yield and quality: diet (forage and concentrates), feed additives (probiotics, prebiotics, phytobiotics, enzymes, etc.), genetics and management (e.g., early life intervention such as specific supplementation at weaning). Diet is one of the majors (23). The ban of antimicrobial growth promoters in animal production systems has led to an increasing interest in the use of plant extracts to manipulate the rumen (e.g., saponins, polyphenol compounds, essential oils, etc.); however, there are also some limitations, such as inconsistency or transient and adverse effects. Applying an integrative approach (i.e., systems biology) – encompassing nutritional management effects on rumen microbiota, tissue responses and production outcomes – will provide added value to nutritionists attempting to boost high quality animal production and to meet the global growing demand (24).

Murine model for risk assessment

Murine models have been extensively characterized for the effects of dietary and/or pharmacological interventions on the composition and function of the microbiota. The most obvious intervention with direct effects on the microbiota is represented by antibiotic usage. Whether broad- or narrow-spectrum, antibiotics unbalance the equilibrium among bacterial taxa and the shifts in microbiome composition may have repercussions on the well-being of the animal. An increased risk of infections is a common side effect of antibiotic usage, encompassing bacterial (25, 26), viral (27-29), and fungal (30) infections, with mechanisms ranging from reduced colonization resistance to impaired immunological pathways of tolerance. Deregulation of immune response is not limited to microbial infection, but extends to other external cues, with increased susceptibility to inhaled (31) and food (32) allergens. Finally, the effect of antibiotics on the microbiota may have consequences that go beyond the immune system, to include, among others, the cardiovascular (33) and the neurological (34) systems. Similar to antibiotics, different dietary regimens may unbalance the equilibrium among bacterial taxa by promoting the expansion of certain bacteria at the expense of others in a way that affect animal well-being. For instance, the adoption of a high-fat diet alters the composition of the microbiome to sustain inflammation and obesity (35,36) and unbalance the metabolism of the essential amino acid tryptophan at the host-microbial interface (37). Besides fats, an important component of the diet able to influence microbial composition and function is represented by fibres. Mammals are unable to digest dietary fibres, which are instead fermented by commensal bacteria to produce Short Chain Fatty Acids (SCFAs), such as acetate, propionate and butyrate, which play important physiological roles, such as working as primary energy source for colonocytes and inducing immune tolerance at the intestinal barrier (38).

These examples illustrate how microbial metabolites, such as tryptophan metabolites and SCFAs, play crucial physiological roles, and may be used as a proxy for animal well-being. As a corollary, monitoring microbial metabolites may inform on animal health and disease, and therapeutic targeting with pre-/ pro- and/or post-biotics may be envisioned to re-equilibrate microbial metabolites in pathological conditions. Extrapolation to farm animals of the results obtained in murine models may take the evaluation of microbial metabolites to the next step. Indeed, they may be used as markers for both animal health and risk assessment for human health in the ONE health perspective.

References

1. FAO. *Balanced feeding for improving livestock productivity – Increase in milk production and nutrient use efficiency and decrease in methane emission.* Rome: Food and Agriculture Organization of the United Nations; 2012. (FAO Animal Production and Health Paper No. 173)
2. Billington C, Kingsbury JM, Rivas L. Metagenomics approaches for improving food safety: a review. *J Food Prot*. 2022;85(3):448-64.
3. Kim H, Cho JH, Song M, Cho JH, Kim S, Kim ES, *et al.* Evaluating the prevalence of foodborne pathogens in livestock using metagenomics approach. *J Microbiol Biotechnol*. 2021;31(12):1701-8.
4. Hubbard LE, Givens CE, Griffin DW, Iwanowicz LR, Meyer MT, Kolpin DW. Poultry litter as potential source of pathogens and other contaminants in groundwater and surface water proximal to large-scale confined poultry feeding operations. *Sci Total Env*. 2020;735:139459.
5. Giles TA, Belkhiri A, Barrow PA, Foster N. Molecular approaches to the diagnosis and monitoring of production diseases in pigs. *Res Vet Sci*. 2017; 114:266–72.
6. Trevisi P, Luise D, Correa F, Bosi P. Timely control of gastrointestinal eubiosis: a strategic pillar of pig health. *Microorganisms*. 2021;9(2).
7. Aladhadh M. A Review of modern methods for the detection of foodborne pathogens. *Microorganisms*. 2023;11(5).
8. Newbold CJ, Ramos-Morales E. Review: Ruminal microbiome and microbial metabolome: effects of diet and ruminant host. *Animal*. 2020;14(S1): s78–86.
9. Myer PR, Clemmons BA, Schneider LG, Ault TB., Microbiomes in ruminant protein production and food security. *CAB Reviews* 2019;14(008):1-11 pages.
10. Guan LL, Nkrumah JD, Basarab JA, Moore SS. Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency. *FEMS Microbiol Lett*. novembre 2008;288(1):85–91.
11. Hungate RE. *The rumen and its microbes*. New York and London: Academic Press; 1966.
12. Loor JJ, Elolimy AA, McCann JC. Dietary impacts on rumen microbiota in beef and dairy production. *Anim Front*. 2016;6(3):22–9.
13. Henderson G, Cox F, Ganesh S, Jonker A, Young W, Global Rumen Census C, *et al.* Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Sci Rep.* 2015; 5:14567.
14. Tiwari P, Dwivedi R, Bansal M, Tripathi M, Dada R. Role of gut microbiota in neurological disorders and its therapeutic significance. *J Clin Med*. 2023;12(4).
15. Dessein R, Bauduin M, Grandjean T, Le Guern R, Figeac M, Beury D, *et al.* Antibiotic-related gut dysbiosis induces lung immunodepression and worsens lung infection in mice. *Crit Care*. 2020;24(1):611.

16. Theriot CM, Koenigsknecht MJ, Carlson PE, Hatton GE, Nelson AM, Li B, *et al.* Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to *Clostridium difficile* infection. *Nat Commun*. 2014; 5:3114.
17. Erttmann SF, Swacha P, Aung KM, Brindefalk B, Jiang H, Hartlova A, *et al.* The gut microbiota prime systemic antiviral immunity via the cGAS-STING-IFN-I axis. *Immunity*. 2022;55(5):847-861 e10.
18. Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, *et al.* Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proc Natl Acad Sci USA*. 2011;108(13):5354–9.
19. Sencio V, Machado MG, Trottein F. The lung-gut axis during viral respiratory infections: the impact of gut dysbiosis on secondary disease outcomes. *Mucosal Immunol*. 2021;14(2):296–304.
20. Costantini C, Nunzi E, Spolzino A, Palmieri M, Renga G, Zelante T, *et al.* Pharyngeal microbial signatures are predictive of the risk of fungal pneumonia in hematologic patients. *Infect Immun*. 1 2021;89(8): e0010521.
21. Borbet TC, Pawline MB, Zhang X, Wipperman MF, Reuter S, Maher T, *et al.* Influence of the early-life gut microbiota on the immune responses to an inhaled allergen. *Mucosal Immunol*. 2022;15(5):1000–11.
22. Zhang Q, Cheng L, Wang J, Hao M, Che H. Antibiotic-induced gut microbiota dysbiosis damages the intestinal barrier, increasing food allergy in adult mice. *Nutrients*. 2021;13(10).
23. Bayer F, Ascher S, Pontarollo G, Reinhardt C. Antibiotic treatment protocols and germ-free mouse models in vascular research. *Front Immunol*. 2019;10:2174.
24. Laurans L, Venteclef N, Haddad Y, Chajadine M, Alzaid F, Metghalchi S, *et al.* Genetic deficiency of indoleamine 2,3-dioxygenase promotes gut microbiota-mediated metabolic health. *Nat Med*. 2018;24(8):1113–20.
25. Kim KA, Gu W, Lee IA, Joh EH, Kim DH. High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. *PLoS ONE*. 2012;7(10):e47713.
26. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006;444(7122):1027–31.
27. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, *et al.* Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*. 2013;504(7480):446–50.
28. Banerjee S, van der Heijden MGA. Soil microbiomes and one health. *Nat Rev Microbiol*. 2023;21(1):6–20.
29. Rusiñol M, Hundesa A, Cárdenas-Youngs Y, Fernández-Bravo A, Pérez-Cataluña A, Moreno-Mesonero L, *et al.* Microbiological contamination of conventional and reclaimed irrigation water: Evaluation and management measures. *Sci Total Environ*. 2020; 710:136298.
30. Sharma M, Reynnells R. Importance of soil amendments: survival of bacterial pathogens in manure and compost used as organic fertilizers. *Microbiol Spectr*. 2016;4(4): 10.1128/microbiolspec.pfs-0010–2015.
31. Rapidel B, Ripoche A, Allinne C, Metay A, Deheuvels O, Lamanda N, *et al.* Analysis of ecosystem services trade-offs to design agroecosystems with perennial crops. *Agron Sustain Dev.* 2015;35(4):1373–90.
32. Manyi-Loh C, Mamphweli S, Meyer E, Okoh A. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules*. 2018;23(4):795.
33. Mann A, Nehra K, Rana JS, Dahiya T. Antibiotic resistance in agriculture: Perspectives on upcoming strategies to overcome upsurge in resistance. *Curr Res Microb Sci*. 2021; 2:100030.
34. Liao H, Li X, Yang Q, Bai Y, Cui P, Wen C, *et al.* Herbicide selection promotes antibiotic resistance in soil microbiomes. *Mol Biol Evol*. 2021;38(6):2337–50.

35. Krause S, Le Roux X, Niklaus PA, Van Bodegom PM, Lennon JT, Bertilsson S, Grossart HP, Philippot L, Bodelier PL. Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. *Front Microbiol.* 2014;5:251. doi: 10.3389/fmicb.2014.00251.

36. Maron PA, Mougel C, Ranjard L. Soil microbial diversity: Methodological strategy, spatial overview and functional interest. *C R Biol*. 2011;334(5):403–11.

37. FAO, ITPS, GSBI, CBD, EC. *State of knowledge of soil biodiversity - Status, challenges and potentialities: Report 2020*. Rome: Food and Agriculture Organization of the United Nations; 2020. https://doi.org/10.4060/cb1928en

38. Lo CC. Effect of pesticides on soil microbial community. *J Environ Sci Health Part B*. 2010;45(5):348–59.

MICROBIOME OF FOOD

Chiara Devirgiliis (a), Marianna Roselli (a), Alberto Finamore (a), Francesca De Filippis (b), Simone Guglielmetti (c)
(a) Research Centre for Food and Nutrition, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Rome
(b) Department of Agricultural Sciences, University of Naples Federico II, Naples
(c) Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan

Within the context of One Health approach, among the environmental microbial interactions that carry the potential to affect the human gut microbiome, and human health in turn, *via* the food chain, particular focus should be given to the food microbiome. This term refers to microbial communities and their entire "theatre of activity" in a specific food environment (1).

Indeed, a large subset of foods, including traditional and fermented ones, can act as vehicles of live environmental microbes between soil and human gut through plants and animals (soil-food-gut microbial flow).

Within the "soil-food-gut microbial flow", in addition to Fermented Foods (FFs), emphasis should also be given to the role of foods that are ingested raw as a vehicle of microorganisms. In this context, for instance, commercial ready-to-eat raw vegetables, which are increasingly consumed globally, harbour abundant and taxonomically rich populations of live bacteria, including Lactic Acid Bacteria (LAB), which can survive gastrointestinal transit, potentially contributing to shaping of the human gut microbiome (2, 3).

Based on the above reported considerations, we underline the importance of including food/gut microbiome interactions in risk assessment evaluation, given the ability/possibility of foodborne microbes to shape the gut microbiome and to provide additional enzymatic activities which can in turn affect host health.

Recently it has clearly emerged that the inclusion of the gut microbiome in food safety risk assessment is of utmost importance, given its connection with human health. It is also worth noting that the increasing understanding of the link between microbiomes and health requires a future mapping of their roles in scientific evaluation regulatory processes in order to understand their potential impact on health (4).

To date, there is no guidance or methodology arranged to define the structure and dynamics of environmental microbiomes and how they can be included in risk evaluation.

In light of One Health approach, in addition to gut microbiome, we therefore should also consider environmental microbiome interactions, that carry the potential to affect the human gut microbiome, and human health in turn, *via* the food chain.

In particular, food microbiome can greatly impact on gut microbiome. The term food microbiome refers to microbial communities and their entire "theatre of activity" in a specific food environment 1).

A large subset of foods, including traditional and fermented ones, can act as vehicles of live environmental microbes between soil and human gut through plants and animals (soil-food-gut microbial flow), often contributing food quality assets (e.g., health-associated microbial metabolites).

In the last decades, interest in FFs has risen, especially due to their contribution to a healthy gut microbiome mediated by some of the microorganisms with potential health-promoting features (5,6). The landscape of FFs is quite complex, comprising a wide array of foodstuffs mostly from dairy, meat and vegetable sources, characterized by distinct production processes

and consumption frequencies, often reflecting local resources and traditional dietary profiles (7). Fermented products have been recently defined by an expert panel of the International Scientific Association for Probiotics and Prebiotics (ISAPP) as "foods made through desired microbial growth and enzymatic conversions of food components" (8). It should be noted that not all the foods and beverages obtained through fermentation contain live microbes at the time of consumption, due to various processing steps, such as pasteurization, baking, smoking or filtering. Among fermented foods, dairy products, often untreated after fermentation, represent one of the most important sources of foodborne microbes ingested upon consumption, due to the high levels of live bacteria and yeasts. These microorganisms represent a complex consortium characterized by a high biodiversity in terms of microbial strains of environmental origin (9). FF microbes may overcome the barriers encountered during the gastrointestinal passage (low pH, bile salts, high concentration of digestive enzymes) and reach the gut. Within this context, an important role is played by the food matrix which could protect microorganisms during their journey from FFs to the gut (10).

Genomic studies highlighted that several LAB species commonly found in FFs harbour genes related to stress response in the gastrointestinal environment, as well as factors involved in the engraftment to the gut epithelium, such as exopolysaccharide production, mucus-binding proteins and pili, that may promote the persistence of these taxa in the gut (11). A recent clinical trial with a diet rich in FFs highlighted that gut microbial diversity and the number of shared taxa between FFs and gut microbiome increased during the intervention, and this was correlated with a decrease in some inflammatory markers (12). In addition, comparative genomics of LAB species reconstructed from gut and FF metagenomes demonstrated that LAB strains with high genomic similarity occur in both FFs and gut (13). Although data on FF consumption were not available, the authors speculated that FFs can be regarded as a possible source of LAB for the gut microbiome (13).

Although a definitive response about the gut colonization ability of FF microbiome does not exist, the consumption of FFs containing live microbes has been associated to several positive health outcomes, such as weight maintenance, cardiovascular disease and metabolic syndrome risk reduction, improvement of glucose and lipids levels, improvement of allergies, food intolerance and gut inflammatory diseases (5). Indeed, Marco *et al.* (14) recently suggested a classification of foods (including FFs) based on the content of live microbes and, based on this classification, estimated the intake of live microbes in the American population, demonstrating that higher intake is associated with lower levels of inflammatory markers, blood insulin, plasma glucose and systolic pressure (15). Indeed, ISAPP suggested that dietary guidelines should be revised to include a recommended daily dose of live microbes, since sufficient evidence exists on the beneficial health effects (16). However, as ISAPP pointed out, FFs have to be carefully differentiated from probiotics (17). The term probiotic can be used only when a strain-specific health benefit has been proven by a well-designed intervention study, as well as the determination of a dose-dependent effect to confer the claimed benefit (8), that does not apply to most of the FFs available on the market, although some microorganisms from FFs are evolutionarily highly related to probiotics, and can share the same molecular mechanisms responsible for health-promoting properties.

Nevertheless, the health benefits of FFs may not always be related to the intake of live microbes. Indeed, several FFs contain molecules arising from microbial metabolism that may confer health benefits if ingested, even when the microorganisms are dead. For example, Taylor *et al.* demonstrated that habitual consumers of FFs showed higher plasmatic levels of conjugated linoleic acid, which is suggested to have an anti-obesity and anti-atherogenic effect (18).

Indeed, microorganisms can also be intentionally ingested in large quantities through probiotic products, which contain well-defined and characterized "live microorganisms that, when

administered in adequate amounts, confer a health benefit on the host" (17). Probiotics, including both foods and supplements, fall within the scope of the general food law in the European Union (EU) and are supervised by the European Food Safety Authority (EFSA). On the contrary, products containing live microorganisms intended for preventing or curing specific diseases in sick or vulnerable people are regulated as biological drugs defined live biotherapeutic products, according to the European Pharmacopoeia (19).

EFSA is responsible for assessing potential safety issues related to the use of probiotics through a generic safety pre-assessment based on the assignment of the Qualified Presumption of Safety (QPS) status of specific microbial taxa (20), and through guidelines for the strain-specific assessment of the potential risks of dissemination of antibiotic resistance genes (21,22). Therefore, we suggest referring to specific documents published by EFSA and any corresponding regulations regarding risk assessment for probiotics.

Based on the above reported considerations, we underline the importance of including food/gut microbiome interactions in risk assessment evaluation, given the ability/possibility of foodborne microbes to shape the gut microbiome and to provide additional enzymatic activities which can in turn affect host health. Within the "soil-food-gut microbial flow", in addition to FFs, emphasis should also be given to the role of foods that are ingested raw as a vehicle of microorganisms. In this context, for instance, commercial ready-to-eat raw vegetables, which are increasingly consumed globally, harbour abundant and taxonomically rich populations of live bacteria, including LAB which can survive gastrointestinal transit, potentially contributing to shaping of the human gut microbiome (23,24).

Another aspect to consider in the risk assessment is the possibility that food associated microbes could act as reservoir of antibiotic resistance transmissible to gut microbes through Horizontal Gene Transfer (HGT). This issue is already being investigated by EFSA including the role that microbiomes could play in the wider environment as reservoirs for antimicrobial genes (4). However, the actual risk of transmission of antibiotic resistance genes from food to gut microbiomes is to date not clearly proven and more evidence-based data are needed (24).

Useful criteria that can be proposed for evaluating the possible effects related to the ingested foodborne microbes should be based on an in-depth analysis of the food microbiome, which is characterised by high microbial diversity, both at species- and strain-level. The diversity and function of food-associated microbes can now be examined in detail applying molecular and other -omic approaches. In recent years, the advances in molecular techniques and the reduction in High-Throughput Sequencing (HTS) costs boosted the application of metagenomics for studying food microbiome 25,26).

An important parameter to evaluate is represented by the colonization potential of foodborne microbes. Food microorganisms are temporary members of the gut microbiome and generally persist in the gut a few days following ingestion, constituting the so-called "transient microbiome" (27). Understanding the link between colonization ability of different food-related microbial species/strains and the potential to influence gut microbiota diversity, structure, and function requires the definition of standardized protocols to be applied. Since the food matrix can promote the survival of microorganisms along the GI tract, this aspect should be considered.

Colonization ability could also be related to the minimum dose of live microorganisms requested to positively impact on host health. The average viable microbial cell load in some of the most common FFs ranges between 10^6 and 10^9 cells/g or ml (27). Live bacterial counts of over 10^7 CFU/g were also reported for commercial ready-to-eat raw vegetables (23,28). However, to date there are few or no dietary guidelines recommending the optimal dose of live microorganisms that should be ingested. A clear quantification of the microbial content of foods, especially those obtained from spontaneous fermentation committed to autochthonous microbes present in the raw

material should be provided and collected into dietary databases, in order to estimate the dietary intake of live microbes better.

The evaluation of all these aspects will help to understand the role of food microbiome in risk assessment and to integrate it into EFSA regulatory framework.

References

1. Berg G, Rybakova D, Fischer D, Cernava T, Verges MC, Charles T, *et al.* Microbiome definition re-visited: old concepts and new challenges. *Microbiome.* 2020; 8:103. https://doi.org/10.1186/s40168-020-00875-0.
2. Marco ML, Heeney D, Binda S, Cifelli CJ, Cotter PD, Foligne B, *et al.* Health benefits of fermented foods: microbiota and beyond. *Curr Opin Biotechnol.* 2017;44:94-102. https://doi.org/10.1016/j.copbio.2016.11.010.
3. Roselli M, Natella F, Zinno P, Guantario B, Canali R, Schifano E, *et al.* Colonization ability and impact on human gut microbiota of foodborne microbes from traditional or probiotic-added fermented foods: a systematic review. *Front Nutr.* 2021;8:689084. https://doi.org/10.3389/fnut.2021.689084.
4. Şanlier N, Gökcen BB, Sezgin AC. Health benefits of fermented foods. *Crit Rev Food Sci Nutr.* 2019;59:506–27. https://doi.org/10.1080/10408398.2017.1383355.
5. Marco ML, Sanders ME, Ganzle M, Arrieta MC, Cotter PD, De Vuyst L, *et al.* The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods. *Nat Rev Gastroenterol Hepatol* 2021;18:196-208. https://doi.org/10.1038/s41575-020-00390-5.
6. Tamang JP, Cotter PD, Endo A, Han NS, Kort R, Liu SQ, *et al.* Fermented foods in a global age: East meets West. *Compr Rev Food Sci Food Saf.* 2020;19:184-217. https://doi.org/10.1111/1541-4337.12520.
7. Saxelin M, Lassig A, Karjalainen H, Tynkkynen S, Surakka A, Vapaatalo H, *et al.* Persistence of probiotic strains in the gastrointestinal tract when administered as capsules, yoghurt, or cheese. *Int J Food Microbiol.* 2010;144:293-300. https://doi.org/10.1016/j.ijfoodmicro.2010.10.009.
8. De Filippis F, Pasolli E, Ercolini D. The food-gut axis: lactic acid bacteria and their link to food, the gut microbiome and human health. *FEMS Microbiol Rev.* 2020;44:454–89. https://doi.org/10.1093/femsre/fuaa015.
9. Wastyk HC, Fragiadakis GK, Perelman D, Dahan D, Merrill BD, Yu FB, *et al.* Gut-microbiota-targeted diets modulate human immune status. *Cell.* 2021;184:4137-4153e14. https://doi.org/10.1016/j.cell.2021.06.019.
10. Pasolli E, De Filippis F, Mauriello IE, Cumbo F, Walsh AM, Leech J, *et al.* Large-scale genome-wide analysis links lactic acid bacteria from food with the gut microbiome. *Nat Commun.* 2020;11:2610. https://doi.org/10.1038/s41467-020-16438-8.
11. Mantegazza G, Duncan R, Telesca N, Gargari G, Perotti S, Riso P, *et al.* Lactic acid bacteria naturally associated with ready-to-eat rocket salad can survive the human gastrointestinal transit. *Food Microbiol.* 2024;118:104418. https://doi.org/10.1016/j.fm.2023.104418
12. Marco ML, Hutkins R, Hill C, Fulgoni VL, Cifelli CJ, Gahche J, *et al.* A Classification System for Defining and Estimating Dietary Intake of Live Microbes in US Adults and Children. *J Nutr.* 2022;152:1729-36. https://doi.org/10.1093/jn/nxac074.
13. Hill C, Tancredi DJ, Cifelli CJ, Slavin JL, Gahche J, Marco ML, *et al.* Positive Health outcomes associated with live microbe intake from foods, including fermented foods, assessed using the NHANES Database. *J Nutr.* 2023;153:1143-9. https://doi.org/10.1016/j.tjnut.2023.02.019.
14. Marco ML, Hill C, Hutkins R, Slavin J, Tancredi DJ, Merenstein D, *et al.* Should there be a recommended daily intake of microbes? *J Nutr.* 2020; 150:3061–7. https://doi.org/10.1093/jn/nxaa323.

15. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, *et al.* The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol.* 2014;11:506-14. https://doi.org/10.1038/nrgastro.2014.66.

16. Basak S, Duttaroy AK. Conjugated linoleic acid and its beneficial effects in obesity, cardiovascular disease, and cancer. *Nutrients* 2020;12:1913. https://doi.org/10.3390/nu12071913.

17. Franciosa G, Guida S, Gomez Miguel MJ, Von Hunolstein C. Live biotherapeutic products and their regulatory framework in Italy and Europe. *Ann Ist Super Sanita.* 2023 Jan-Mar;59(1):56-67. doi: 10.4415/ANN_23_01_09..

18. Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. Opinion of the Scientific Committee (Question No EFSA-Q-2005-293) Adopted on 19 November 2007. *EFSA Journal*. 2007;587:1-16. Available from: https://www.efsa.europa.eu/en/efsajournal/pub/587; last visited April 18, 2024).

19. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. *EFSA Journal*. 2012;10(6):2740. [10 pp.] doi:10.2903/j.efsa.2012.2740.

20. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R, Galobart J. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*. 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206.

21. Mantegazza G, Gargari G, Duncan R, Consalez F, Taverniti V, Riso P, *et al.* Ready-to-eat rocket salads as potential reservoir of bacteria for the human microbiome. *Microbiol Spectr.* 2023;11:e0297022. https://doi.org/10.1128/spectrum.02970-22.

22. Meier H, Spinner K, Crump L, Kuenzli E, Schuepbach G, Zinsstag J. State of knowledge on the acquisition, diversity, interspecies attribution and spread of antimicrobial resistance between humans, animals and the environment: a systematic review. *Antibiotics.* 2023;12:73. https://doi.org/10.3390/antibiotics12010073.

23. De Filippis F, Parente E, Ercolini D. Recent Past, Present, and Future of the Food Microbiome. *Annu Rev Food Sci Technol.* 2018;9:589-608. https://doi.org/10.1146/annurev-food-030117-012312.

24. Walsh AM, Leech J, Huttenhower C, Delhomme-Nguyen H, Crispie F, Chervaux C, *et al.* Integrated molecular approaches for fermented food microbiome research. *FEMS Microbiol Rev.* 2023;47. https://doi.org/10.1093/femsre/fuad001.

25. Merten C, Schoonjans R, Gioia DD, Peláez C, Sanz Y, Maurici D, *et al.* Editorial: Exploring the need to include microbiomes into EFSA's scientific assessments. *EFSA Journal* 2020;18:e18061. https://doi.org/10.2903/j.efsa.2020.e18061.

26. Rezac S, Kok CR, Heermann M, Hutkins R. Fermented foods as a dietary source of live organisms. *Front Microbiol.* 2018; 9:1785. https://doi.org/10.3389/fmicb.2018.01785.

27. Calonico C, Delfino V, Pesavento G, Mundo M, Lo Nostro A. Microbiological quality of ready-to-eat salads from processing plant to the consumers. *J Food Nutr Res.* 2019;7(6):427-34.

28. den Besten HMW, Amézquita A, Bover-Cid S, Dagnas S, Ellouze M, Guillou S, *et al.* Next generation of microbiological risk assessment: Potential of omics data for exposure assessment. Int *J Food Microbiol.* 2018;287:18-27. https://doi.org/10.1016/j.ijfoodmicro.2017.10.006.

MICROBIOME OF ENVIRONMENT

Luigi Orrù (a), Loredana Canfora (a), Riccardo Scotti (b), Simone Rampelli (c)
(a) Research Centre for Genomics & Bioinformatics, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Fiorenzuola d'Arda (PC)
(b) Research Centre for Vegetable and Ornamental Crops, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Pontecagnano (SA)
(c) Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Milan

Microorganisms in nature play a critical role in ensuring many ecosystems function that are crucial for maintaining a healthy environment, such as nutrient cycling, organic matter degradation, and pathogen control. Microbial communities respond strongly to environmental changes resulting from human activities, making them a potential indicator of their environmental impact. Therefore, microbiome analysis is a promising tool for environmental risk assessment. Furthermore, the microbiome approach following the One Health concept provides a method for addressing the interconnectedness of environmental health with animal and human health (1). From an environmental standpoint, a risk assessment should consider the use of microbiome and microbiota-based analysis to assess the potential adverse effects on ecological systems and human health caused by the release into the environment of products such as chemicals or biological entities. In this context, the term "biological entity" may include any living entities released into the environment, such as genetically modified organisms or soil microbial inoculants, but also pathogens spread by agricultural practices. Pathogens may be spread, for example, by organic soil amendments such as manure and compost (2) or irrigation (3).

Agroecosystems represent 38% of global land use (4), and agricultural activities result in several processes associated with different types of risks. The environmental impact of agriculture involves the use of pesticides and phytosanitary products and the amplification of antibiotic resistance. The increase in antimicrobial resistance of human pathogens related to the rising use of antibiotics in the agricultural sector becomes a critical area of concern (5). Antibiotics administered to farm animals are not fully metabolized and can be released into the environment with raw animal manure used for fertilization, as well as by irrigation with wastewater generated from farm activities (6). Furthermore, the increase in antibiotic resistance in microorganisms has also been associated with the use of other anthropogenic substances such as pesticides or herbicides (7).

The development of Next Generation Sequencing (NGS) has provided a culture-independent tool to characterize the response of microbial communities to environmental stimuli sourced from human activities. NGS technologies have been widely used in environmental analysis to characterise patterns and drivers of microbial community composition. However, although it has become very popular in ecological studies, investigating microbiomes based on NGS approaches must face several challenges to be included in risk evaluation. The temporal and spatial variation of microbiota is one of them. Environmental differences at local and regional spatial scales and temporal differences result in a range of microbial taxonomic variations. In soil, microbes show high fine-scale spatial heterogeneity with considerable variability in taxon abundance, even in similar paedogenic conditions (8,9). Furthermore, a portion of the microbial diversity detected can be in a dormant state or dead (10). In such a context, identifying a standard set of taxonomic indicators of environmental health status might not be easy to obtain. Metabarcoding approaches based on the sequencing of target genes, such as the bacterial ribosomal 16S gene, in this sense may not be the best option because simple taxonomic descriptions may not provide sufficient

information about the environmental health status of a site. It is also difficult to draw biological conclusions regarding risk assessment using alpha diversity measures such as Shannon or Simpson indexes obtained from metabarcoding data. Different pesticides, for example, can either stimulate or inhibit the growth of microorganisms (11). In our opinion, these observations suggest that alpha diversity indices should be used with caution for environmental risk assessment.

A possible approach to address this issue may be to consider functional biodiversity besides taxonomic biodiversity. Some methods to infer the community functional profile starting from the taxonomic composition have been developed. These approaches include tools such as *Phylogenetic Investigation of Communities by Reconstruction of Unobserved States* (PiCRUST) or *Taxonomy-based Functional Annotation* (Tax4Fun). However, the performance of these tools seems to be limited outside human samples (12), and their accuracy in environmental systems is a matter of debate (13).

Shotgun metagenomic sequencing allows the exploration of microbial functional diversity by identifying the entire set of functional genes displayed by microorganisms. Using this approach, the yardstick for assessing risk is not the number and kind of taxa but the impact on the functions shown by the microbial communities. The analysis can be done by estimating the abundance of functional genes. Another proposed approach is based on the analysis of the diversity of functional gene variants (13). With this approach, gene variants are considered a reflection of the diversity of microbial species (13). This last approach is of particular interest, considering that ecosystems are characterized by functional redundancy, which means that a function is carried out by multiple species (14). Functional redundancy has been associated with ecological resilience and stability (15). In this sense, functional redundancy could be a parameter to be considered for assessing the state of health of an environment. However, a function-focused perspective requires the identification of specific microbial functions that serve as proxy indicators of environmental health status. The selection of appropriate functional trait-related genes is critical in risk assessment and should be based on the sensitivity of genes and function to the environmental changes brought about by the release of anthropogenic products.

References

1. Banerjee S, van der Heijden MGA. Soil microbiomes and one health. *Nat Rev Microbiol.* 2023;21:6-20. https://doi.org/10.1038/s41579-022-00779-w.
2. Rusiñol M, Hundesa A, Cárdenas-Youngs Y, Fernández-Bravo A, Pérez-Cataluña A, Moreno-Mesonero L, *et al.* Microbiological contamination of conventional and reclaimed irrigation water: Evaluation and management measures. *Sci Total Environ.* 2020;710:136298. https://doi.org/10.1016/j.scitotenv.2019.136298.
3. Sharma M, Reynnells R. Importance of soil amendments: survival of bacterial pathogens in manure and compost used as organic fertilizers. *Microbiol Spectr.* 2016 Aug;4(4). doi: 10.1128/microbiolspec.PFS-0010-2015.
4. Rapidel B, Ripoche A, Allinne C, Metay A, Deheuvels O, Lamanda N, *et al.* Analysis of ecosystem services trade-offs to design agroecosystems with perennial crops. *Agron Sustain Dev.* 2015;35:1373–90. https://doi.org/10.1007/s13593-015-0317-y.
5. Manyi-Loh C, Mamphweli S, Meyer E, Okoh A. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules.* 2018;23:795. https://doi.org/10.3390/molecules23040795.
6. Mann A, Nehra K, Rana JS, Dahiya T. Antibiotic resistance in agriculture: perspectives on upcoming strategies to overcome upsurge in resistance. *Curr Res Microb Sci.* 2021;2:100030. https://doi.org/10.1016/j.crmicr.2021.100030.

7. Liao H, Li X, Yang Q, Bai Y, Cui P, Wen C, *et al.* Herbicide selection promotes antibiotic resistance in soil microbiomes. *Mol Biol Evol* 2021;38:2337-50. https://doi.org/10.1093/molbev/msab029.
8. Krause S, Le Roux X, Niklaus PA, Van Bodegom PM, Lennon JT, Bertilsson S, *et al.* Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. *Front Microbiol* 2014;5.
9. Maron P-A, Mougel C, Ranjard L. Soil microbial diversity: methodological strategy, spatial overview and functional interest. *Comptes Rendus Biologies.* 2011;334:403–11. https://doi.org/10.1016/j.crvi.2010.12.003.
10. FAO, ITPS, GSBI, CBD, EC. *State of knowledge of soil biodiversity - Status, challenges and potentialities: Report 2020*. Rome: Food and Agriculture Organization of the United Nations; 2020. https://doi.org/10.4060/cb1928en
11. Lo C-C. Effect of pesticides on soil microbial community. *J Environ Sci Health B.* 2010;45:348-59. https://doi.org/10.1080/03601231003799804.
12. Escalas A, Hale L, Voordeckers JW, Yang Y, Firestone MK, Alvarez-Cohen L, *et al.* Microbial functional diversity: from concepts to applications. *Ecol Evol.* 2019;9:12000–16. https://doi.org/10.1002/ece3.5670.
13. Biggs CR, Yeager LA, Bolser DG, Bonsell C, Dichiera AM, Hou Z, *et al.* Does functional redundancy affect ecological stability and resilience? A review and meta-analysis. *Ecosphere* 2020;11: e03184. https://doi.org/10.1002/ecs2.3184.
14. Jurburg SD, Salles JF. Functional redundancy and ecosystem function — the soil microbiota as a case study. In: Blanco J, LoY-H, Roy S (Ed.). *Biodiversity in ecosystems - Linking structure and function*, London: IntechOpen Limited; 2015. http://dx.doi.org/10.5772/58981.
15. Fischer FM, de Bello F. On the uniqueness of functional redundancy. *NPJ Biodivers*. 2023;2(1):23. doi: 10.1038/s44185-023-00029-z.

METHODOLOGICAL ASPECTS FOR THE ANALYSIS OF MICROBIOME

Emilia Nunzi (a), S. Rampelli (b), L. Romani (a), Silvia Turroni (b), Chiara Devirgiliis (c)
(a) Department of Medicine and Surgery, University of Perugia, Perugia
(b) Department of Pharmacy and Biotechnology, University of Bologna, Bologna
(c) Research Centre for Food and Nutrition, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Rome

Over the past two decades, Next-Generation Sequencing (NGS) technologies have significantly enhanced the ability to qualitatively and quantitatively characterize microbiomes in foods and food chains, allowing their mapping to be incorporated into regulatory scientific assessment processes (1-3). Many environmental and host-associated microbiomes are currently being explored using NGS to uncover their roles in health and disease (2). As mentioned earlier, due to their immense functional potential, microbiomes are strong candidates to play a central role in the holistic One Health framework. This framework addresses human, animal, plant, and environmental health from an integrated perspective.

The assessment of the compositional and functional profiles of microbial communities in microbiome studies is now routinely conducted using sequencing-based methods, including marker gene analysis (targeted amplicons), metagenomics (DNA), and metatranscriptomics (RNA), as well as other omics approaches like metaproteomics (proteins) and metabolomics (metabolites) (4, 5). Specifically, the gut microbiota (GM) has been shown to contribute significantly to regulating host health, including modulating metabolism, immune function, and influencing the activity and toxicity of xenobiotics (6, 7).

In the following sections, these methods will be briefly described, emphasizing their main characteristics, along with their advantages and disadvantages when applied to microbial communities in food and food chain Risk Assessment.

Sequencing-based methods

Marker gene analysis is a targeted technique based on PCR amplification of marker genes for bacteria (e.g., hypervariable regions of the 16S rRNA gene) and fungi (e.g., internal transcribed spacers) from mixed genomic DNA. It is the cheapest (and therefore the most widespread) sequencing method that deserves particular attention, as it currently enables good resolution in detecting Amplicon Sequence Variants (ASVs), even with short-read sequencing, thus managing to reveal also rare features. Since amplicon data are compositional in nature, appropriate analysis tools are claimed for managing biases (8). Unless the data are properly normalized, direct comparison of feature abundance between samples will be heavily affected by differences in sample library size, even when they have been sequenced using the same target gene. Moreover, comparison between different microbial populations (e.g., *Bacteria, Fungi and Archaea*) is not possible, as it is necessary to use different target genes, thus complicating the analysis of interactions between microbial populations that is relevant for several environments. The high microbial classification resolution provided by ASVs, together with high sequence quality, also allows for effective analysis with machine and deep learning techniques belonging to the so-called Predictive Metagenomic Profiling (PMP) (9,10). They make available information previews that

were previously the prerogative of metagenomics and metatranscriptomics alone, and that nowadays 16S rRNA amplicon sequencing can predict at a tenth of their cost.

Shotgun metagenomics is a quantitative technique based on the untargeted analysis of the whole DNA extracted from a given sample. It reveals the presence of all types of microbes present, such as eubacteria, archaebacteria, microeukaryotes like fungi, and viruses, and allows high-resolution taxonomic and functional characterization (11,12). This provides a comprehensive overview of the potential activities of the microbiome, for example identifying genes related to flavour and sensorial properties, potential health-related activities (e.g., biosynthesis of vitamins or beneficial metabolites, engraftment factors) or pathogenic traits (e.g., production of toxins, biogenic amines or other harmful metabolites, antibiotic resistance genes). Importantly, the absence of a Polymerase Chain Reaction (PCR) step also eliminates some of the biases associated with marker gene analysis. In particular, the recovery of Metagenome-Assembled Genomes (MAGs) of the most abundant species has become a common task in metagenomics studies (13). However, the following main drawbacks should be listed:

i) the cost, which is still prohibitive for large-scale studies;
ii) the amount of sequencing data, which requires servers with high computing power and a considerable amount of time;
iii) much more complex pipelines compared to marker gene analysis, to address the large heterogeneity of the data (11).

Enrichment of microbial DNA could help, facilitating identification by bioinformatics tools and the removal of reads from other sources (14).

Metatranscriptomics is an untargeted sequencing of mRNA isolated from a sample. It identifies transcribed genes as well as transcriptionally active microbes, including RNA-based viruses, thereby providing further insights into potential functional characteristics of the microbial community and allowing a closer look at expressed transcripts and active community members (discriminating them from dormant or dead ones). However, it requires relatively expensive, complex, and labour-intensive methods for sample storage and preparation, as it is subject to RNA instability and rapid degradation, and a great sequencing depth to avoid under-detection (15). Despite these practical difficulties, it is currently the most frequently applied technique to obtain quantitative information on the real activity of microorganisms in an ecosystem, although there are also challenges associated with bioinformatic analysis (16). Moreover, since gene expression is not causally related to a physiological response, mRNA analysis alone cannot be used as the only type of input information for Microbiological Risk Assessment (MRA), but must necessarily be integrated with marker gene analysis, metaproteomics and metabolomics. Therefore, the multi-omics approach is highly recommended to manage the necessary information to be considered in MRA.

Other omics (non-sequencing-based) methods

Metaproteomics evaluates the whole protein complement generated by microbes. By analysing phenotypical variations in different states, it could corroborate links between both genomic and transcriptomic data and biological functions (17).

Metabolomics detects and analyses small-weight metabolites by integrating chemistry, biochemistry and bioinformatics techniques, and relates the data to microbial phenotypic characteristics. Lipidomics (i.e., the study of lipid content) is probably the most common subfield.

For the pros and cons of metaproteomics and metabolomics, please see the relevant literature in the field (18, 19). Certainly, the type and quality of the sample are of outmost importance, as well as the technique used, for reliable and conclusive findings across studies. Standardization of

the different phases of analysis is therefore a necessity shared by all omics methods (based or not on sequencing). For the pros and cons of metaproteomics and metabolomics, please see the relevant literature in the field (18, 19). Certainly, the type and quality of the sample are of the outmost importance, as well as the technique used, for reliable and conclusive findings across studies. The standardization of the different phases of analysis is therefore a necessity shared by all omics methods (based or not on sequencing).

Microbiological risk assessment

The new analytical perspective offered by NGS methodologies outlines a profound change in the methods already applied by microbiologists to analyse the ecology and diversity in food. Linking properly the NGS results with metaproteomics and metabolomics could suggest new associations between genomic data and phenotypes. In this context, Microbiological Risk Assessment (MRA) can undoubtedly benefit from the revolution that NGS has brought to the analysis of food microbial ecology. However, the biggest challenge remains the establishment of widely accepted criteria and comparable methodologies for integrating omics data into MRA (9). In order to prevent foodborne diseases and outbreaks and limit the spread of pathogens along the food chain, food safety management systems are already in place that assess both hazard analysis and problematic control points. However, their control and effectiveness require in-depth a priori knowledge of the specific microorganisms present and their potential risks. It follows that the integration of NGS techniques, which provide for rigorous statistical processing and data interpretation (for a correct assessment of the biological significance of microbial communities along the food chain), may be particularly relevant for MRA purposes.

Next-generation sequencing and other omics approaches have enabled a major leap forward in the compositional and functional profiling of microbiomes.
MRA can significantly benefit from multi-omics, but the standardization of the various procedural steps is essential.

In the first approach, known as the metataxonomic amplicon-targeted method, genes useful for taxonomic identification are targeted through a PCR step, e.g.

The growing amount of information obtained by integrating multi-omics data to assess microbial diversity, composition, and functionality in food matrices, can be used to improve the reliability of mechanistic MRA models, not only in terms of monitoring abundance and virulence of pathogens, but also to explore the relationship between microbiomes and food components or other chemicals (7,20). It should be taken into account that each of techniques discussed above generates a large amount of data in a short time. It follows that correct storage, processing and interpretation of data governed by a transdisciplinary approach is necessary. Furthermore, since this amount of data is not yet directly usable by risk managers, computing power and advanced biostatistical skills are also needed for a correct administration of the information required to manage food risk appropriately.

References

1. Cocolin L, Ercolini D. Zooming into food-associated microbial consortia: a ‘cultural’ evolution. *Current Opinion in Food Science.* 2015; 2:43–50. https://doi.org/10.1016/j.cofs.2015.01.003.
2. Merten C, Schoonjans R, Di Gioia D, Peláez C, Sanz Y, Maurici D, *et al.* Editorial: Exploring the need to include microbiomes into EFSA’s scientific assessments. *EFSA Journal.* 2020;18:e18061. https://doi.org/10.2903/j.efsa.2020.e18061.

3. Rantsiou K, Kathariou S, Winkler A, Skandamis P, Saint-Cyr MJ, Rouzeau-Szynalski K, *et al.* Next generation microbiological risk assessment: opportunities of whole genome sequencing (WGS) for foodborne pathogen surveillance, source tracking and risk assessment. *Int J Food Microbiol.* 2018;287:3-9. https://doi.org/10.1016/j.ijfoodmicro.2017.11.007.
4. De Filippis F, Parente E, Ercolini D. Recent Past, Present, and Future of the Food Microbiome. *Annu Rev Food Sci Technol* 2018; 9:589–608. https://doi.org/10.1146/annurev-food-030117-012312.
5. Walsh AM, Leech J, Huttenhower C, Delhomme-Nguyen H, Crispie F, Chervaux C, *et al.* Integrated molecular approaches for fermented food microbiome research. *FEMS Microbiol Rev.* 2023;47. https://doi.org/10.1093/femsre/fuad001
6. de Vos WM, Tilg H, Van Hul M, Cani PD. Gut microbiome and health: mechanistic insights. *Gut.* 2022;71:1020-32. https://doi.org/10.1136/gutjnl-2021-326789.
7. Lindell AE, Zimmermann-Kogadeeva M, Patil KR. Multimodal interactions of drugs, natural compounds and pollutants with the gut microbiota. *Nat Rev Microbiol.* 2022;20:431–43. https://doi.org/10.1038/s41579-022-00681-5.
8. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome datasets are compositional: and this is not optional. *Front Microbiol.* 2017;8. https://doi.org/10.3389/fmicb.2017.02224.
9. Cocolin L, Mataragas M, Bourdichon F, Doulgeraki A, Pilet M-F, Jagadeesan B, *et al.* Next generation microbiological risk assessment meta-omics: The next need for integration. *Int J Food Microbiol*. 2018;287:10–7. https://doi.org/10.1016/j.ijfoodmicro.2017.11.008.
10. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, *et al.* PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol.* 2020;38:685-8. https://doi.org/10.1038/s41587-020-0548-6.
11. Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. Shotgun metagenomics, from sampling to analysis. *Nat Biotechnol.* 2017;35:833-44. https://doi.org/10.1038/nbt.3935.
12. Bowers RM, Kyrpides NC, Stepanauskas R, Harmon-Smith M, Doud D, Reddy TBK, *et al.* Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. *Nat Biotechnol.* 2017;35:725–31. https://doi.org/10.1038/nbt.3893.
13. Zhou Y, Liu M, Yang J. Recovering metagenome-assembled genomes from shotgun metagenomic sequencing data: Methods, applications, challenges, and opportunities. *Microbiol Res.* 2022;260:127023. https://doi.org/10.1016/j.micres.2022.127023.
14. Abu-Ali GS, Mehta RS, Lloyd-Price J, Mallick H, Branck T, Ivey KL, *et al.* Metatranscriptome of human faecal microbial communities in a cohort of adult men. *Nat Microbiol.* 2018;3:356–66. https://doi.org/10.1038/s41564-017-0084-4.
15. Shakya M, Lo C-C, Chain PSG. Advances and challenges in metatranscriptomic analysis. *Front Genet.* 2019;10. https://doi.org/10.3389/fgene.2019.00904
16. Lehmann R. From bedside to bench-practical considerations to avoid pre-analytical pitfalls and assess sample quality for high-resolution metabolomics and lipidomics analyses of body fluids. *Anal Bioanal Chem.* 2021;413:5567–85. https://doi.org/10.1007/s00216-021-03450-0.
17. Armengaud J. Metaproteomics to understand how microbiota function: The crystal ball predicts a promising future. *Environ Microbiol.* 2023;25:115–25. https://doi.org/10.1111/1462-2920.16238.
18. Walsh AM, Leech J, Huttenhower C, Delhomme-Nguyen H, Crispie F, Chervaux C, *et al.* Integrated molecular approaches for fermented food microbiome research. *FEMS Microbiol Rev.* 2023;47. https://doi.org/10.1093/femsre/fuad001.
19. De Filippis F, Parente E, Ercolini D. Recent past, present, and future of the food microbiome. *Annu Rev Food Sci Technol.* 2018;9:589–608. https://doi.org/10.1146/annurev-food-030117-012312.
20. den Besten HMW, Amézquita A, Bover-Cid S, Dagnas S, Ellouze M, Guillou S, *et al.* Next generation of microbiological risk assessment: Potential of omics data for exposure assessment. *Int J Food Microbiol.* 2018;287:18-27. https://doi.org/10.1016/j.ijfoodmicro.2017.10.006.

CONCLUSIONS

Annalisa Silenzi, Luca Busani, Roberta Masella
Centro di riferimento Medicina di Genere, Istituto Superiore di Sanità, Rome

Since microbial communities can act as sensitive indicators, offering insights into the impact of products such as chemicals or biological entities on ecosystems and human health, microbiome analysis represents a crucial tool for assessing environmental risks caused by human activities.

The limitations of traditional taxonomic biodiversity metrics, especially in the context of alpha diversity indices, suggest the need for a shift towards functional biodiversity in risk assessment. Shotgun metagenomic sequencing, focusing on functional genes and their variants, emerges as a promising method, considering functional redundancy as a potential parameter for evaluating the health of an environment.

To conclude, the importance of having a network of scientific institutions with high technical and analytical capacity to support risk assessment and making possible the inclusion of microbiome from human, animals, food and the environment can be summarized in these points:

- *Enhanced analytical precision*
  Scientific institutions with advanced technological and analytical capabilities offer the precision required to characterize microbiome diversity and functionality across complex ecosystems. Their expertise ensures the accurate identification of microbial hazards and beneficial organisms relevant to food safety.

- *Comprehensive data integration*
  Leveraging institution networks allows for the aggregation and synthesis of data from diverse microbiome sources – human, animal, food, and environmental – which is essential for a holistic understanding of microbial interactions and their impact on food safety risks.

- *Standardization and reproducibility*
  Institutions with high-capacity laboratories promote the standardization of microbiome research methods, ensuring consistent and reproducible data critical for robust risk assessment models and international food safety regulations.

- *Advanced predictive modelling*
  These networks can employ cutting-edge bioinformatics and machine learning tools to develop predictive models that assess microbiome-related risks, enabling proactive identification and mitigation of food safety threats.

- *Capacity for collaborative research*
  Scientific institution networks foster multidisciplinary collaboration, integrating microbiology, bioinformatics, toxicology, and environmental science expertise. This synergy strengthens the ability to address complex microbiome-mediated food safety challenges comprehensively.

Serie Rapporti ISTISAN
numero di gennaio 2025

Stampato in proprio
Servizio Comunicazione Scientifica – Istituto Superiore di Sanità

Roma, marzo 2025