Our microbes, our health: Current research on the human microbiome

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Executive summary

It is increasingly recognised that the trillions of microbes[1] that live on us and in us have a profound impact on multiple aspects of health. Changes in these microbial communities and their environmental contexts – the microbiomes – have been implicated in a wide range of metabolic, immune-related and brain-related conditions. Importantly, this emerging understanding has underpinned new approaches to treatment, including the successful use of faecal transplants to prevent *Clostridioides* difficile gut infections after antibiotic treatment.

In May 2022, the Academy of Medical Sciences and the Royal Netherlands Academy of Arts and Sciences held a joint symposium to discuss recent progress in microbiome research and its application to health, and how the field might move forward in the future.

Presentations focused on experimental studies examining the 'cross-talk' between microbes and the host, the role of diet in shaping the gut microbiome, and factors affecting the colonisation of the gut and the establishment of stable microbial communities. Talks also examined how disruption of the human microbiome could be involved in a range of conditions, including delayed infant development, autoimmune diseases such as type 1 diabetes, and the immune dysregulation seen in sepsis, and how a deeper understanding of the microbiome is underpinning the development of new therapies for metabolic and other diseases.

Discussions highlighted the fact that the field is **still in its infancy**. Although much progress has been made, many gaps in knowledge still exist. Given some high-profile success stories, there has been a rush to translate findings into the clinical arena. Although this could deliver innovative medical advances, there is also a risk that the field could be overhyped, and failures could undermine public trust in this novel form of treatment. Many of the associations between particular bacterial genera or species and human health are context-specific leading to divergent claims in the literature.[2]

These concerns highlight the importance of continuing to study **the role of the microbiome in health and disease**, to generate a deeper understanding of the microbiome and its contribution to different disease states. Currently, many clinical studies adopt a 'black box' mentality to the use of microbiome therapies, with little consideration of the specific mechanisms by which they might be achieving a therapeutic effect. A deeper mechanistic understanding of microbiome–host interactions will support the use of more refined therapeutic strategies based on specific strains or combinations of beneficial bacteria, or pharmaceutical interventions that achieve the same effect.

Of particular importance is the need to distinguish **correlation and causation**. Disturbances to the microbiome have been observed in multiple conditions, yet it is not always clear whether these are directly contributing to disease or are a consequence of some other more fundamental disease process. This has important implications for the likely success of a microbiome-based intervention.

Alongside **additional clinical studies**, including rigorously designed randomised controlled trials, participants recognised the need for further fundamental research and pre-clinical studies, for example using bioreactor-based 'artificial gut' models. Animal models may also yield valuable information, although it was acknowledged that significant differences between humans and experimental animals, such as mice, limit the generalisability of findings, particularly in areas such as gut–brain signalling.

1. R., Fuchs, S., Milo, R., 2016. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. Cell 164:337e340
2. Inger-Englar, T., Barlow, G., & Mathur, R. (2018). Obesity, diabetes, and the gut microbiome: an updated review. Expert Review of Gastroenterology & Hepatology. doi:10.1080/17474124.2019.1543023

It was also noted that the **complexity of microbiome–host interactions** present a particular challenge. Although microbial communities are relatively stable, they are dynamic over time, evolve during the life course, and involve constant cross-talk between microbial species and with host cells in the context of interindividual variation. Studying components of this system in isolation runs the risk of missing important interactions within a complex system. By drawing upon related fields, such as ecology and evolutionary theory and environmental microbiology, research may be able to generate a more holistic understanding to the microbiome and its role in human health, underpinning more rationally designed interventions.

This is an exciting time in microbiome research, with multiple clinical trials now underway. International collaborations will help to provide new insights into the role of the microbiome in health and disease, driving forward translation into the clinic.

Introduction

The human body is home to countless bacteria, the numbers of which likely exceed the numbers of host cells. Many of these bacteria have traditionally been seen as harmless commensals, but in recent years it has become increasingly clear that their presence has significant implications for our health. Disruptions to the microbiome have been identified in a wide range of conditions and multiple studies have been launched to manipulate the microbiome as a therapeutic strategy. Nevertheless, many aspects of the relationship between the human body and its associated microbiota remain poorly understood, and how the microbiome can best be targeted therapeutically remains unclear.

In May 2022, the UK Academy of Medical Sciences and the Royal Netherlands Academy of Arts and Sciences held a joint meeting in Amsterdam, The Netherlands, to highlight recent research on the human microbiome and to discuss the prospects for new treatments or prevention of disease based on manipulation of the microbiome. This report provides a summary of presentations and some of the key themes emerging from discussions.

Setting the scene

Following welcome addresses from **Professor Jos van der Meer**, Chair of the Section of Medicine KNAW, and **Professor Tom Solomon**, Vice-President (International) at the Academy of Medical Sciences, **Professor Elaine Holmes**, Professor of Chemical Biology, Imperial College, UK, highlighted some of the major advances in knowledge over the past decade. The introduction illustrated the many areas of human health influenced by the microbiome.[3]

The microbiome came to prominence through studies demonstrating differences in the microbiome of lean and obese mice[4], pointing to impacts on **host metabolism**. Further studies have highlighted the nutritional importance of the microbiome, including recovery from malnutrition.

A huge range of metabolites are produced by microbes in the gut, influencing not just local tissues but also other organ systems. A notable discovery has been the impact of the gut microbiome on the **brain**, including a wide range of mood, neuropsychiatric and neurological disorders[5]. Considerable attention has also been given to the **vaginal microbiome**, and its important role in 'seeding' the gut microbiome of newborns[6] although recent work points towards an even more important role of the maternal **faecal microbiome** in this process.[7]

Research has shown that microbial populations are generally **structurally stable but unique to individuals**[8]. Studies of the gut microbiome have been facilitated by development of a **human intestinal tract chip**[9], contributing to a flourishing of **metagenomic** studies – analysis of the full diversity of bacterial populations using genetic tools, without any need for culturing of individual bacteria.[10]

Research has also been aided by the development of 'artificial guts', **bioreactor-based model systems** in which complex bacterial populations can be propagated, investigated and manipulated for extended periods[11]. As well as metagenomic approaches examining changes to the composition of bacterial populations, these kinds of set up can also be used to analyse production of bacterial metabolites. Several groups around the world have developed systems for profiling microbial metabolites, generally using mass spectrometry, and how they change over time.

3. Mousa WK, Chehadeh F, Husband S. Recent Advances in Understanding the Structure and Function of the Human Microbiome. Front Microbiol. 2022;13:825338. doi: 10.3389/fmicb.2022.825338.
4. Turnbaugh PJ, Ley RE, Mahowald MA et al. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027-31. doi: 10.1038/nature05414.
5. Margolis KG, Cryan JF, Mayer EA. The Microbiota-Gut-Brain Axis: From Motility to Mood. Gastroenterology. 2021 Apr;160(5):1486-1501. doi: 10.1053/j.gastro.2020.10.066.
6. Dominguez-Bello MG, Costello EK, Contreras M et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci USA. 2010;107(26):11971-5. doi: 10.1073/pnas.1002601107.
7. Korpela K, Helve O, Kolho KL et al. Maternal Faecal Microbiota Transplantation in Cesarean-Born Infants Rapidly Restores Normal Gut Microbial Development: A Proof-of-Concept Study. Cell. 2020;183(2):324-334.e5. doi: 10.1016/j.cell.2020.08.047.
8. Zoetendal EG, Akkermans AD, De Vos WM. Temperature gradient gel electrophoresis analysis of 16S rRNA from human faecal samples reveals stable and host-specific communities of active bacteria. Appl Environ Microbiol. 1998;64(10):3854-9. doi: 10.1128/AEM.64.10.3854-3859.1998.
9. Rajilić-Stojanović M, Heilig HG, Molenaar D et al. Development and application of the human intestinal tract chip, a phylogenetic microarray: analysis of universally conserved phylotypes in the abundant microbiota of young and elderly adults. Environ Microbiol. 2009;11(7):1736-51. doi: 10.1111/j.1462-2920.2009.01900.x.
10. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Antolin M, Artiguenave F, Blottiere H, Borruel N, Bruls T, Casellas F, Chervaux C, Cultrone A, Delorme C, Denariaz G, Dervyn R, Forte M, Friss C, van de Guchte M, Guedon E, Haimet F, Jamet A, Juste C, Kaci G, Kleerebezem M, Knol J, Kristensen M, Layec S, Le Roux K, Leclerc M, Maguin E, Melo Minardi R, Oozeer R, Rescigno M, Sanchez N, Tims S, Torrejon T, Varela E, de Vos W, Winogradsky Y, Zoetendal E, Bork P, Ehrlich SD, Wang J. (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010 Mar 4;464(7285):59-65
11. Macfarlane GT, Macfarlane S, Gibson GR. Validation of a Three-Stage Compound Continuous Culture System for Investigating the Effect of Retention Time on the Ecology and Metabolism of Bacteria in the Human Colon. Microb Ecol. 1998;35(2):180-7. doi: 10.1007/s002489900072.

Diet is a critical factor regulating the make-up of the human microbiome and its impact on the host[12]. Many metabolically active compounds produced by the human microbiota have been identified, including **short-chain fatty acids**, which appear to play a pivotal role in maintenance of gut health and may have multiple other beneficial effects.

The wide range of host systems influenced by the microbiome suggests that microbial abnormalities could have multiple **clinical implications**. Its influence on metabolism has implications in areas such as obesity and metabolic diseases; its modulation of the immune system could affect autoimmune and inflammatory disease; and maternal microbiome transmission could make vital contributions to infant health and development. The microbiome has even been found to influence the response to medicines.

A multitude of potential interventions can be envisaged to modulate the microbiome to promote good health. Significant progress has been made in the use of **faecal microbial therapy**, particularly to prevent recurrent *Clostridioides difficile* infections that has grown to standard care medical practice[13]. Multiple groups are experimenting with probiotic interventions or prebiotic nutritional interventions to manipulate bacterial population growth in favourable directions, with the potential for personalised interventions that reflect the precise make up of an individual's microbiome. As well as the gut microbiome, other microbial communities may be targetable, including the vaginal microbiome to improve pregnancy outcomes.

One innovative idea a participant proposed is to **'weaponise' the microbiome**, so that it forms an active defence against pathogen colonisation – an idea inspired by turkey vultures, which are able to survive on decaying carrion because of the defensive properties of their intestinal bacteria.[14]

12. Flint HJ, Duncan SH, Louis P. The impact of nutrition on intestinal bacterial communities. Curr Opin Microbiol. 2017;38:59-65. doi: 10.1016/j.mib.2017.04.005.
13. Van Nood E, A Vrieze, M Nieuwdorp, S Fuentes, EG Zoetendal, WM de Vos, CE Visser, EJ Kuijper, JFWM Bartelsman, JGP Tijssen, P Speelman, MGW Dijkgraaf & JJ Keller (2013) Duodenal infusion of donor feces for recurrent *Clostridium difficile*. New Engl J Med 368:407-15.
14. Zepeda Mendoza ML, Roggenbuck M, Manzano Vargas K et al. Protective role of the vulture facial skin and gut microbiomes aid adaptation to scavenging. Acta Vet Scand. 2018;60(1):61. doi: 10.1186/s13028-018-0415-3.

Host–bacterium interactions

Dr Patrick Varga-Weisz, Senior Lecturer, School of Life Sciences, University of Essex, UK, noted that microbiomes outside the gut can have a significant impact on health. In mouse models of malaria, for example, the host lung microbiota promotes malaria-associated acute respiratory distress syndrome, which leads to death in susceptible mouse strains.[15]

One key question relates to the mechanisms by which the microbiome affects host physiology. Microbiota release multiple bioactive compounds that can signal to host cells, and one possibility is that this signalling drives **epigenetic changes** in host cells, altering gene expression and the activities and properties of these cells.

One of the most common epigenetic processes in **histone acetylation**. Enzymes known as histone acetyltransferases (HATs) and histone deacetylases (HDACs) add and remove acetyl groups, respectively, controlling the access of regulatory proteins to genes and regulating gene activity. In general, histone acetylation leads to increased levels of gene expression.

Another recently discovered control mechanism is based on **crotonylation**, addition of a small chain fatty acid (crotonate) to specific amino acids in histones. Using antibiotic treatments to manipulate the host microbiome, Patrick Varga-Weisz and colleagues have found that microbiota depletion leads to changes in the expression of multiple host genes, but these changes are more likely to be mediated by histone crotonylation than acetylation.[16] This in turn appears to be mediated through the production of short chain fatty acids, which influence crotonylation by modulating histone deacetylase activity.[17]

Recently, Patrick Varga-Weisz and colleagues have begun to explore cell-type-specific responses within the gut, using single-cell RNA sequencing techniques to distinguish different populations of cells within the gut epithelium. These studies are suggesting that different types of cells respond differently to microbiota-derived metabolites, hinting at even greater complexity in the interactions between the microbiome and host cells.

The research of **Dr Julie McDonald**, Lecturer, Department of Life Sciences, Imperial College London, UK, has focused on the factors that underpin successful colonisation of the gut. Although bacterial populations are generally stable, they do change over the life-course and can be perturbed by many other influences. Antibiotic treatments, for example, can devastate bacterial populations, after which recolonisation occurs and equilibrium is restored.

This question is central to the success of **faecal microbiota transplant (FMT) as a new therapy**. A general goal is to move from faecal samples to carefully defined species combinations cultured in conditions consistent with medical use (also known as synthetic microbial consortia), or to use pharmaceutical interventions to stimulate the growth of specific beneficial microbes. These more refined approaches will depend on a good understanding of the factors influencing successful colonisation.

Multiple microbial activities and metabolites, as well as host products, can affect colonisation potential. These include short chain fatty acids generated by enzymatic breakdown of ingested fibre, bile acids produced by the liver and their derivatives generated by microbial modification, bactericidal compounds released by some microbiota and phage (bacterial viruses) that can propagate through bacterial populations in the gut.

15. Host lung microbiota promotes malaria-associated acute respiratory distress syndrome. Debanjan Mukherjee, Ângelo Ferreira Chora, Jean-Christophe Lone, Ricardo S. Ramiro, Birte Blankenhaus, Karine Serre, Mário Ramirez, Isabel Gordo, Marc Veldhoen, Patrick Varga-Weisz2 and Maria M. Mota Nature Communications, in press.

16. Fellows R, Varga-Weisz P. Chromatin dynamics and histone modifications in intestinal microbiota-host crosstalk. Mol Metab. 2020;38:100925. doi: 10.1016/j.molmet.2019.12.005.

17. Fellows R, Denizot J, Stellato C et al. Microbiota derived short chain fatty acids promote histone crotonylation in the colon through histone deacetylases. Nat Commun. 2018;9(1):105. doi: 10.1038/s41467-017-02651-5.

To explore the possible role of specific compounds in regulating colonisation, Dr McDonald and colleagues have used an artificial gut model (bioreactor) connected to multiple analytical technologies, to assess changes in metabolite production, gene expression and other readouts.[18] Focusing on FMT to control *C. difficile*, the team's hypothesis has been that antibiotic treatment eliminates the bacteria that generate metabolites inhibiting *C. difficile* colonisation, and that production of these inhibitory metabolites is restored by FMT.

The Imperial team has been able to simulate the effects of antibiotic use and *C. difficile* proliferation in this model system, and demonstrated the inhibitory effect of faecal microbial therapy, which reduced *C. difficile* counts by more than 90%. Notably, levels of one particular metabolite, **valerate**, were highly depleted following antibiotic treatment but restored by FMT[19]. Valerate levels were significantly higher in samples from healthy donors than those from donors with *C. difficile* infections, and valerate was found to inhibit *C. difficile* growth *in vitro*.

Recently, Dr McDonald has also begun to focus on **multidrug-resistant (MDR)** microorganisms. Many of the most clinically important MDR bacteria, such as vancomycin-resistant enterococci (VRE) and carbapenem-resistant Enterobacteriaceae (CRE), live in the gut, where they act as a reservoir of organisms with the potential to establish hard-to-treat systemic infections. Using the bioreactor model, Dr McDonald and colleagues have created models for exploring the colonisation of CRE and VRE after antibiotic treatment and its potential inhibition by FMT.[20]

18. Guzman-Rodriguez M, McDonald JAK, Hyde R et al. Using bioreactors to study the effects of drugs on the human microbiota. Methods. 2018 Oct 1;149:31-41. doi: 10.1016/j.ymeth.2018.08.003.

19. McDonald JAK, Mullish BH, Pechlivanis A et al. Inhibiting Growth of Clostridioides difficile by Restoring Valerate, Produced by the Intestinal Microbiota. Gastroenterology. 2018;155(5):1495-1507.e15. doi: 10.1053/j.gastro.2018.07.014.

20. Ghani R, Mullish BH, McDonald JAK et al. Disease Prevention Not Decolonization: A Model for Faecal Microbiota Transplantation in Patients Colonized With Multidrug-resistant Organisms. Clin Infect Dis. 2021;72(8):1444-1447. doi: 10.1093/cid/ciaa948.

Neonatal development

Dr Clara Belzer, Associate Professor, Department of Agrotechnology and Food Sciences, Wageningen University & Research, highlighted the many important roles played by gut microbiota across the life-course. These include degradation of indigestible fibre, production of vitamins, generation of short chain fatty acids, stimulation of immune responses and protection against pathogens. The microbiome is therefore critical to health, and this is particularly true in the first 1000 days of life – a critical time window in human development with long-term implications for health and development.

However, many factors can affect the microbiome of young infants, including gestational age, delivery route, the origins of the first colonising microbes and weaning. In particular, delivery by **caesarean section** has a significant impact on the infant microbiome. Compared to vaginal delivery, it leads to lower microbial diversity, delayed maturation of the microbiome, and lower levels of beneficial bacteria such as *Bifidobacterium*. Although there are distinct differences in the microbiome of vaginally-born versus caesarean section born infants, with lack of durable *Bacteroides* colonization observed within the first few weeks of birth associated with caesarean section[21], the microbiome stabilizes over the first few months to three years after birth.[22] However, these early microbiomic abnormalities may contribute to an increased risk of multiple conditions associated with caesarean delivery, including allergies, asthma, type 1 diabetes and obesity.[23]

Notably, maternal FMT has been investigated as a way to 'seed' maternal microbial populations in the guts of babies born by caesarean section. This approach has been found to normalise the microbiome[24] (although, it is not yet clear if it has longer-term impacts on health).

Of particular importance to neonates are bacterial species that metabolise **human milk oligosaccharides**. Although the third most abundant component of human milk, these are not digested by host enzymes – their main role appears to be to stimulate the growth of beneficial bacteria in the infant gut. Human milk contains a diverse mix of oligosaccharides, which are metabolised by enzymes from a range of microbial species.

Other factors affecting the infant microbiome include pre-term birth, which delays maturation of the microbiome, potentially affecting infant development, while respiratory support may also affect colonisation. Bacterial colonisation of the mucosal layer of the gut may be of particular importance, and there is growing interest in the use of nutritional strategies to promote the colonisation of beneficial bacteria. In the innovative 'Mum to Bum' cohort study, the digestion of human milk oligosaccharides is being compared in pre-term and full-term babies, using samples from mothers, stomach contents and faecal matter to assess any correlations between their digestion and infant growth and development.[25]

21. Mitchell et al Call Reports Medicine 2020
22. Yatsunenko et al Nature. 2012;486:222–227, .Yassour et al Sci. Trans. Med 2016
23. Sandall J, Tribe RM, Avery L et al. Short-term and long-term effects of caesarean section on the health of women and children. Lancet. 2018;392(10155):1349-1357. doi: 10.1016/S0140-6736(18)31930-5.
24. Korpela K, Helve O, Kolho KL et al. Maternal Faecal Microbiota Transplantation in Cesarean-Born Infants Rapidly Restores Normal Gut Microbial Development: A Proof-of-Concept Study. Cell. 2020;183(2):324-334.e5. doi: 10.1016/j.cell.2020.08.047.
25. Henderickx JGE, d'Haens EJ, Hemels MAC et al. From Mum to Bum: An Observational Study Protocol to Follow Digestion of Human Milk Oligosaccharides and Glycoproteins from Mother to Preterm Infant. Nutrients. 2021;13(10):3430. doi: 10.3390/nu13103430.

Diet and the microbiome

As discussed by **Dr Petra Louis**, Senior Research Fellow, Microbiology Group, Rowett Institute, University of Aberdeen, UK, dietary intake is a key factor influencing the impact of the microbiome on health[26]. In particular, **dietary fibre** is known to stimulate gut microbial growth. Fibre consists of a complex mix of carbohydrates that are broken down by a wide variety of microbial enzymes in the gut. Consisting of plant cell wall and storage material, fibre comes in soluble and insoluble forms, and its breakdown is dependent on its biochemical as well as physicochemical properties, which may restrict access of catabolic enzymes to the chemical structures they act on.

Controlled human diet studies have examined processing of resistant starch (carbohydrate that passes through the small intestine and is digested by microbes in the large intestine). Excreted levels were low in all subjects except two, who appeared to be unable to digest it effectively. These individuals were found to lack a specific gut bacterium, *Ruminococcus bromii*[27]. This microbe organises a multi-enzyme complex known as the amylosome, which appears to be critical for its superior starch-degrading ability.[28]

This example illustrates the limitations of using genome sequence to infer function: the genome of *R. bromii* does not carry more starch-degrading enzymes than other starch-degrading microbes, so it is their arrangement into the amylosome complex that seems to provide the ability to degrade certain types of resistant starch. Furthermore, another gut microbe, *Coprococcus eutactus*, has glycoside hydrolase genes that are commonly associated with cellulose breakdown in other bacteria, but appear to be involved in beta-glucan breakdown in this organism, demonstrating that it can be difficult to infer function from gene sequence alone.[29] Furthermore, the properties of bacteria can vary according to the conditions in which they are living and they may produce different compounds, potentially with different effects on host health, from different substrates.

Bacterial populations in the gut therefore show great complexity, in terms of both high functional diversity across species and the adaptability of individual species. Furthermore, living conditions vary markedly through the gut, with pH levels, for example, varying along its length. Species may also vary in their requirements for particular vitamins. In addition, the presence of some species may be inhibited or promoted by the co-occurrence of others, for example if they rely on nutrients generated by a second species ('cross-feeding').[30]

These examples illustrate the importance of considering the wider ecology of the gut microenvironment. Microbial population dynamics will be sensitive to environmental conditions, as well as to cooperation between and competition with other microbial species.[31]

26. Flint HJ, Duncan SH, Louis P. The impact of nutrition on intestinal bacterial communities. Curr Opin Microbiol. 2017;38:59-65. doi: 10.1016/j.mib.2017.04.005.
27. Ze X, Duncan SH, Louis P, Flint HJ. Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon. ISME J. 2012 Aug;6(8):1535-43. doi: 10.1038/ismej.2012.4.
28. Ze X, Ben David Y, Laverde-Gomez JA et al. Unique Organization of Extracellular Amylases into Amylosomes in the Resistant Starch-Utilizing Human Colonic Firmicutes Bacterium Ruminococcus bromii. mBio. 2015;6(5):e01058-15. doi: 10.1128/mBio.01058-15.
29. Alessi AM, Gray V, Farquharson FM et al. β-Glucan is a major growth substrate for human gut bacteria related to Coprococcus eutactus. Environ Microbiol. 2020;22(6):2150-2164. doi: 10.1111/1462-2920.14977.
30. Soto-Martin EC, Warnke I, Farquharson FM et al. Vitamin Biosynthesis by Human Gut Butyrate-Producing Bacteria and Cross-Feeding in Synthetic Microbial Communities. mBio. 2020;11(4):e00886-20. doi: 10.1128/mBio.00886-20.
31. McDonald JE, Marchesi JR, Koskella B. Application of ecological and evolutionary theory to microbiome community dynamics across systems. Proc Biol Sci. 2020;287(1941):20202886. doi: 10.1098/rspb.2020.2886.

Sepsis and the immune system

The human gut is associated with an extensive mucosal immune system. As discussed by **Dr Joost Wiersinga**, Professor of Internal Medicine, and Translational Infectious Diseases, Amsterdam University Medical Centre, The Netherlands, by modulating the host immune system, gut microbiota could be contributing to multiple conditions that have an immune system component.

Although often lifesaving, antibiotics have a rapid and profound impact on the host microbiome, which typically takes months to return to normal after antibiotic treatment. There are indications that the extensive use of antibiotics in early life can have adverse longer-term health consequences, for example showing a strong association with the risk of inflammatory bowel disease.[32]

Non-antibiotic drugs have also been found to affect the microbiome – around a quarter of the 1000 drugs tested, including commonly used medications such as metformin, proton pump inhibitors and antipsychotics, inhibited one or more gut bacteria.[33] Potentially, this microbiomic impact could be contributing to the drug's therapeutic effects. However, there are also concerns that use of these drugs could induce antibiotic resistance.

Severe dysregulation of the immune system can lead to potentially fatal **sepsis**, characterised by multi-organ failure. Multiple changes to the microbiome are seen in critical illness, but the extent to which they are contributing to disease or are a consequence of widespread metabolic disruption remains unclear.

Nevertheless, it has been proposed that the microbiome may have an important role to play in protecting against serious bacterial infections, and interventions targeting the microbiome could be a way to restore systemic immunity or modulate immune system function in beneficial ways. To date, evidence from trials has been mixed. A large study in India found that a probiotic was highly protective against sepsis (although the contribution of the microbiome was not analysed)[34]. However, several other probiotic trials in critical care, for example to prevent ventilator-associated pneumonia[35], have generated negative findings.

Results from several FMT studies have been more positive. For example, faecal transplants were shown to be more effective than probiotics at reconstituting a healthy microbiome after antibiotic use[36]. Another encouraging example has been the use of faecal transplants to ameliorate graft-versus-host disease after haematopoietic stem cell transplants.[37] Such transplants are associated with extensive antibiotic use and loss of microbiome diversity, potentially leading to an exaggerated inflammatory response. This raises the prospect that faecal material from patients could be 'banked' before such treatment and then re-administered after treatment.

However, one drawback of faecal transplants is the potential transfer of drug-resistant microorganisms to patients.[38] Alternative approaches include 'precision microbiome reconstitution', where key species mediating protection against specific pathogens are identified and used as probiotic therapeutics.[39]

32. Hviid A, Svanström H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. Gut. 2011 Jan;60(1):49-54. doi: 10.1136/gut.2010.219683.
33. Maier L, Pruteanu M, Kuhn M et al. Extensive impact of non-antibiotic drugs on human gut bacteria. Nature. 2018;555(7698):623-628. doi: 10.1038/nature25979.
34. Panigrahi P, Parida S, Nanda NC et al. A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature. 2017;548(7668):407-412. doi: 10.1038/nature23480.
35. Johnstone J, Meade M, Lauzier F et al. Effect of Probiotics on Incident Ventilator-Associated Pneumonia in Critically Ill Patients: A Randomized Clinical Trial. JAMA. 2021 Sep 21;326(11):1024-1033. doi: 10.1001/jama.2021.13355.
36. Suez J, Zmora N, Zilberman-Schapira G et al. Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT. Cell. 2018;174(6):1406-1423.e16. doi: 10.1016/j.cell.2018.08.047.
37. van Lier YF, Davids M, Haverkate NJE et al. Donor faecal microbiota transplantation ameliorates intestinal graft-versus-host disease in allogeneic hematopoietic cell transplant recipients. Sci Transl Med. 2020;12(556):eaaz8926. doi: 10.1126/scitranslmed.aaz8926.
38. DeFilipp Z, Bloom PP, Torres Soto M et al. Drug-Resistant E. coli Bacteremia Transmitted by Faecal MicrobiotaTransplant. N Engl J Med. 2019;381(21):2043-2050. doi: 10.1056/NEJMoa1910437.
39. Buffie CG, Bucci V, Stein RR et al. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. Nature. 2015;517(7533):205-8. doi: 10.1038/nature13828.

A further consideration relates to the development and evaluation of new antibiotics, where impacts on the microbiome could need to be monitored alongside efficacy and safety. A recent trial comparing temocillin and cefotaxime for complicated urinary tract infections, for example, examined both efficacy and effects on the gut microbiome, with temocillin found to be less disruptive.[40]

40. Edlund C, Ternhag A, Skoog Ståhlgren G et al. The clinical and microbiological efficacy of temocillin versus cefotaxime in adults with febrile urinary tract infection, and its effects on the intestinal microbiota: a randomised multicentre clinical trial in Sweden. Lancet Infect Dis. 2022;22(3):390-400. doi: 10.1016/S1473-3099(21)00407-2.

All hail mucus

One of the most promising microbiome-targeting interventions is derived from the recently discovered gut bacterium *Akkermansia muciniphila*. **Professor Willem de Vos**, Professor of Human Microbiomics, University of Helsinki, and Distinguished Professor at Wageningen University, The Netherlands, provided the background to its discovery and the current status of *A. muciniphila*-derived interventions.

The discovery of *A. muciniphila* followed studies suggesting that more degradation of mucus was occurring in the body than previously thought. Mucus consists of complex glycans with protective chemical 'caps'. Follow up led to the identification of an unusual mucus-degrading bacterium, *A. muciniphila*, which actually uses mucus as its primary substrate.[41]

A. muciniphila turned out to be very common, accounting for around 3% of the typical human microbiome – relatively high for a single species. It lives within the mucus layer lining the gut and signals across the gut wall to modulate immune responses. Notably from a health perspective, *A. muciniphila* levels were found to be low in people with conditions such as obesity and inflammatory bowel disease, suggesting that it might have a health-promoting function.

Further studies identified some of the immune processes modulated by *A. muciniphila*, as well as its effects on mucus formation and preservation of the gut's barrier function. Strikingly, it was found that pasteurised *A. muciniphila* cells also helped to preserve this barrier function.[42] Methods to culture *A. muciniphila* were developed and a proof-of-concept trial showed that *A. muciniphila* could be safely given to humans and led to a drop in weight and in the levels of inflammatory markers.[43]

Ultrastructural studies revealed that *A. muciniphila's* key mucus-degrading enzyme was located on pili, long filaments extending from the bacterial cell. This enzyme decaps mucus glycans, stimulating further mucus production. Furthermore, when tested on its own, an additional protein found on the pilus, known as **Amuc-1100**, was found protect against diet-induced obesity in mice and to improve gut barrier function.[44] This protein is heat-stable, potentially explaining why pasteurised *A. muciniphila* retains its beneficial properties. The structure of Amuc-1100 has been determined and studies have begun to identify how its structure and interactions with other proteins are linked to its biological function.

A. muciniphila may therefore be a critical 'gatekeeper' of mucosal integrity and gut barrier function. It is found in most mammals[45] and may be a protector of gut function and health across the animal kingdom.

41. Ottman N, Davids M, Suarez-Diez M et al. Genome-Scale Model and Omics Analysis of Metabolic Capacities of Akkermansia muciniphila Reveal a Preferential Mucin-Degrading Lifestyle. Appl Environ Microbiol. 2017;83(18):e01014-17. doi: 10.1128/AEM.01014-17.
42. Depommier C, Van Hul M, Everard A et al. Pasteurized Akkermansia muciniphila increases whole-body energy expenditure and faecal energy excretion in diet-induced obese mice. Gut Microbes. 2020;11(5):1231-1245. doi: 10.1080/19490976.2020.1737307.
43. Depommier C, Everard A, Druart C et al. Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat Med. 2019;25(7):1096-1103. doi: 10.1038/s41591-019-0495-2.
44. Plovier H, Everard A, Druart C et al. A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. Nat Med. 2017;23(1):107-113. doi: 10.1038/nm.4236.
45. Geerlings SY, Ouwerkerk JP, Koehorst JJ et al. Genomic convergence between Akkermansia muciniphila in different mammalian hosts. BMC Microbiol. 2021;21(1):298. doi: 10.1186/s12866-021-02360-6.

Faecal transplants and diabetes

The success of faecal transplant treatments, particularly for prevention of *C. difficile* infections, has led to great interest in their wider clinical application. As discussed by **Dr Nordin Hanssen**, internal medicine specialist and Principal Investigator, Amsterdam University Medical Centres, The Netherlands, one possible use may be in **type 1 diabetes**.

Globally, type 1 diabetes is becoming increasingly common, at older ages as well as in children. It is often seen in combination with other auto-immune conditions, suggesting that it may reflect generalised immune over-activity to which insulin-producing beta-cells in the pancreas are particularly vulnerable.

Loss of beta-cells shows a biphasic pattern, with rapid cell loss followed by a period of a slower decline. The numbers of beta-cells retained varies markedly across patients. There is only limited correlation between beta-cell preservation and blood glucose control, suggesting that relatively large numbers of cells may need to be preserved to ensure effective blood glucose regulation.

There is some evidence of microbiome abnormalities in type 1 diabetes. The long-running TEDDY study, for example, has identified microbiome abnormalities associated with the development of type 1 diabetes in children[46], although they have less impact than genetic susceptibilities.

Notably, FMT was found to slow the decline of beta cell loss in recently diagnosed type 1 diabetes patients[47]. Curiously, patients' own faecal samples had a similar effect. A confirmatory placebo-controlled study is underway, using microbiome samples from patients with well-preserved beta-cell function despite a long history of type 1 diabetes.

As the TEDDY study has shown, bacterial products (metabolites) could have a protective effect in type 1 diabetes.[48] These and other results have helped to trigger a surge of interest in faecal transplants, with many trials registered to date. (It has also led to 'DIY' faecal transplants, promoted through YouTube videos…, a likely dangerous practice) The development of capsules containing freeze-dried faecal pellets could make FMT studies more feasible, although in the long run the use of selected cultured species is likely to be the preferred treatment option once the therapeutically most useful species are identified.

46. Stewart CJ, Ajami NJ, O'Brien JL et al. Temporal development of the gut microbiome in early childhood from the TEDDY study. Nature. 2018;562(7728):583-588. doi: 10.1038/s41586-018-0617-x.

47. de Groot P, Nikolic T, Pellegrini S et al. Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. Gut. 2021;70(1):92-105. doi: 10.1136/gutjnl-2020-322630.

48. Vatanen T, Franzosa EA, Schwager R et al. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. Nature. 2018;562(7728):589-594. doi: 10.1038/s41586-018-0620-2.

Discussion

The meeting heard of some of the exciting advances that have been made in microbiome research and their application in medicine. Despite some encouraging success stories, it was acknowledged that the field is still young and at only the beginning of its journey. While it is right to celebrate successes and to explore new therapeutic opportunities, there is also a risk that the field is overhyped and public trust is lost when the inevitable failures occur. *It was highlighted by Martijn Katan, Emeritus Professor of Nutrition at VU University Amsterdam that investigators tend to overstate the efficacy of microbiome interventions. e.g., effects on BMI have been uniformly nil in seven RCT's with 344 subjects.*

Participants stressed the great **complexity** of the microbiome and its interactions with the host. This is only beginning to be understood and many gaps in knowledge remain. It was suggested that clinical applications often take a 'black box' approach, experimenting with approaches such as FMT without a solid mechanistic understanding of how they might be having a therapeutic effect. While it may provide a fast way to medical practice as testified by the success of FMT in recurrent *C. difficile* infections, this makes it more difficult to identify reasons for success or failure and to use results to refine treatment.

Alongside clinical studies, including rigorous randomised controlled trials, it was therefore noted that further research with *in vitro* and animal models is required. **Bioreactor-based studies** have proven particularly useful; although they do not contain gut cells, the microbial populations appear to be a reasonable approximation of *in vivo* communities. Animal models provide more realistic *in vivo* environments, although there were concerns that significant differences between rodents and humans limited the applicability of results to humans, particularly in areas such as gut–brain signalling. **Organoids** may offer a new model combining the advantages of *in vitro* and *in vivo* systems, but they also have drawbacks, such as a lack of anoxic microbial microenvironments.

One key challenge identified was to effectively **distinguish correlation and causation**. Disturbances to the microbiome have been noted for a huge variety of health conditions. Often, however, these associations cannot distinguish between a causative role in disease and a consequence of some other critical dysfunction. This is a vital question, as targeting the microbiome is unlikely to be a therapeutically useful strategy if abnormalities are simply a result of a more fundamental disease process.

A related point is the need to consider whether observed changes are **biologically and clinically meaningful**. With the normal function of the microbiome not fully understood, it can be hard to be certain that observed changes have a biological or clinical consequence. Carefully designed mechanistic studies will help to reveal microbiome-related pathways that are integral to disease and help to identify situations when microbiome-directed interventions might have a reasonable chance of success.

The challenge of **complexity** has implications for the way that the microbiome is studied. It was noted that reductionist approaches, such as studying metabolites in isolation, could generate misleading results given that the microbiome and host form a complex system with multiple interacting components. Insights may need to be drawn from a wide range of fields, such as ecological and evolutionary theory and environmental microbiology, in order to provide a satisfactory holistic understanding of a complex system.

Nevertheless, it was agreed that this is an exciting time in microbiome research, with multiple clinical trials now underway. In the future, international and interdisciplinary collaborations are likely to play an important role in providing new insights into the role of the microbiome in health and disease and drive forward translation into the clinic.

Annexes

Annex 1: Agenda

Programme	
9.00	Registration, coffee and tea
9.30	**Jos van der Meer**, Chair of Section of Medicine KNAW and Professor of Internal Medicine, Radboud University and **Tom Solomon** Chair of Neurology and Director of the National Institute for Health Research Health Protection Research Unit in Emerging and Zoonotic Infections at the University of Liverpool and an Associate Pro-Vice-Chancellor in the Faculty of Health and Life Sciences – *Welcome to the symposium*
Session One	
9.40	**Willem de Vos**, Professor of Human Microbiomics at the University of Helsinki and Distinguished Professor at the Wageningen University and **Elaine Holmes**, Professor of Chemical Biology in the Department of Surgery and Cancer at Imperial College, in London, UK – *Introduction to the symposium*
9.50	**Clara Belzer**, Assistant Professor at the Laboratory of Microbiology, Department of Agrotechnology and Food Sciences at Wageningen University & Research – *The early-life microbiome*
10.20	**Julie McDonald**, Lecturer, Department of Life Sciences, Faculty of Natural Sciences, Imperial College London – *Microbiome and disease risk*
10.50	General discussion
Session Two	
11.10	**Petra Louis**, Senior Research Fellow, Microbiology Group, Rowett Institute, University of Aberdeen – *Diet and the microbiome*
11.40	**Patrick Varga Weisz**, Senior Lecturer, School of Life Sciences, University of Essex – *Chromatin dynamics and host-microbiome interaction in the gut*
12.10	**Joost Wiersinga**, Professor of Internal Medicine, and Translational Infectious Diseases, at the University of Amsterdam's Faculty of Medicine (AMC-UvA) – *The microbiome and immune programming*
12.40	General discussion
13.00	**Lunch**
Session Three	
14.30	**Willem de Vos**, Professor of Human Microbiomics at the University of Helsinki and Distinguished Professor at the Wageningen University – *Single microbes – case of Akkermansia*
15.00	**Nordin M. J. Hanssen**, Internal medicine specialist and Principal Investigator, at Amsterdam University Medical Centres – *Can the gut microbiome be used to treat autoimmune diabetes?*
15.30	General discussion
15.50	Final panel/general discussion – with overview of key messages to emerge from each session led by Willem de Vos
16.30	Conclusions
16.40	**Close**

Annex 2: Attendees

Maaike Alkema, University of Amsterdam

Quinten Augustijn, Amsterdam UMC

Clara Belzer, Wageningen University; Speaker

Isolde Besseling, Winclove Probiotics

Fien van Beveren, UMC Utrecht

Lena Ciric, UCL

Kathrin Cohen Kadosh, University of Surrey

Veerle Dam, Sensus

Wouter de Steenhuijsen Piters, UMCU/RIVM

Stan Driessen, Amsterdam UMC

Marina Fassarella, Wageningen University & Research

Coco Fuhri, Amsterdam UMC

Pablo Gallardo, University of Santiago

Aldo Grefhorst, Erasmus University Rotterdam

Nordin M.J. Hanssen, Amsterdam University Medical Centres; Speaker

Hermie Harmsen, UMCG

A.G. (Onno) Holleboom, Amsterdam UMC

Veera Houttu, Universiteit van Amsterdam

Ian Jones, Independent Science Writer

Martijn Katan, KNAW Member

Prokopis Konstanti, Wageningen University & Research

Sergey Konstantinov, Erasmus University Amsterdam

Nienke Koopman, University of Amsterdam

Vera Korenblik, Amsterdam UMC

Ed Kuijper, CMAT, LUMC

Lei Liu, University of Chicago

Luis Llanos, Wageningen University & Research

Petra Louis, University of Aberdeen

Anne Linde Mak, University of Amsterdam

Emma Martinez, FEAM

Julie McDonald, Imperial College London; Speaker

Jos van der Meer, KNAW & Radboud University; Speaker

Benjamin Mullish, Imperial College London

Mari-Lee Odendaal, National Institute for Public Health and the Environment

Nathalie Oldenburger, -

Cathleen Parsons, Oxford University

Raymond Pasman, SILS

Pieter Pekelharing, Microbiome Center

Ivonne Peugnet González, -

Niels Plomp, UMCG

Marie-Luise Puhlmann, Wageningen University & Research

Rachel Quinn, UK Academy of Medical Sciences

Anneleen Segers, Wageningen University & Research

Luc Sterkman, Caelus Health

Marieke de Swart, Wageningen University & Research

Charlotte Teunis, Guest Nordin Hanssen

Pim van Leeuwen, University of Amsterdam

Patrick Varga Weisz, University of Essex; Speaker

Elaine Vaughan, Unilever

Willem de Vos, University of Helsinki and Distinguished Professor at the Wageningen University; Speaker

Lisa Walters, UMCG

Catherine Wanjiku, UK Academy of Medical Sciences

Joost Wiersinga, University of Amsterdam; Speaker

Alexandra Zhernakova, University of Groningen

Piet Borst, KNAW Member

David Boverhoff, -

Stanley Brul, University of Amsterdam

Nazima Corne (nee Pathan), Imperial College London

Niharika Duggal, University of Birmingham

Vanessa Harris, Amsterdam UMC

Jannie Henderickx, LUMC

Elaine Holmes, Murdoch University/Imperial College London; Speaker

Jan Knol, Wageningen University & Research

Don Kruid, -

Bob Kullberg, Amsterdam UMC

Bob Pinedo, KNAW Member

Sander Rensen, Maastricht University

Coco Snethlage, NVOO

Tom Solomon, UK Academy of Medical Sciences

Guido Tytgat, Amsterdam UMC

Rob van Berkel, Rob van Berkel voeding en gezondheid

Carrie Wegh, Wageningen University & Research

Esmée van Rixel, Student University of Amsterdam

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