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FOREWORD

A field is born

"I then most always saw, with great wonder, that in the said matter there were many very little living animalcules, very prettily a-moving."
— Antonie van Leeuwenhoek.

Despite being considered by many as a relatively modern field of research, the first descriptions of human-associated microbiota date back to the 1670s–1680s, when Antonie van Leeuwenhoek started using his newly developed, handcrafted microscopes. In a letter written to the Royal Society of London in 1683, he described and illustrated five different kinds of bacteria (although he called them animalcules at the time) present in his own mouth and that of others, and subsequently also compared his own oral and faecal microbiota, determining that there are differences between body sites as well as between health and disease. Some of the first direct observations of bacteria were of human-associated microbiota.

Fast-forward a couple of centuries and, in 1853, Joseph Leidy published a book entitled *A Flora and Fauna within Living Animals*, which some consider to be the origin of microbiota research. Then, the work of Pasteur, Metchnikoff, Koch, Escherich, Kendall and a few others, laid the foundations of how we understand host–microrganism interactions. Pasteur developed the germ theory of disease, but also thought that non-pathogenic micro-organisms might have an important role in normal human physiology; Metchnikoff believed that microbiota composition and its interactions with the host was essential for health; and Escherich was convinced that understanding the endogenous flora was essential for understanding the physiology of digestion and the pathology and therapy of intestinal disease. Sound familiar? The themes we explore in these 'Milestones in human microbiota research' largely brought to bear the hypotheses and early work of these microbiology giants, on the shoulders of which the field stands today.

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In 1890, Koch published his famous postulates, four criteria designed to establish a causative relationship between a microorganism and a disease, and during the first half of the twentieth century, microbiology became more focused on the identification of etiological agents of disease. This was also likely due to the fact that most bacterial pathogens can grow in the presence of oxygen, whereas most members of the gut microbiota cannot and thus could not be studied at the time. Alfred Nissle, a German physician, isolated the *Escherichia coli* Nissle 1917 strain — which remains a commonly used probiotic — in 1917. During World War I, when the first gut eukaryotic microorganisms and bacteriophages were also described, Nissle noticed that one soldier did not succumb to dysentery and thought he might have a protective microorganism in his gut. He isolated the strain and later showed that it antagonized other pathogens, establishing the concept of colonization resistance, whereby human-associated microorganisms prevent the establishment of pathogens in the same niche.

Despite these early insights, the field only took off in earnest once methods to culture anaerobic organisms were discovered in the 1940s and 1950s, when members of the microbiota were grown and studied in the laboratory. This is where we have chosen to start our timeline of milestones, as increasing numbers of researchers became interested in understanding the composition and function of the microbial communities that live on our different surfaces and how they change throughout our lives. The realization that much of the normal physiology of conventional laboratory mice was missing in germ-free mice, and could be reconstituted through colonization with bacteria obtained from faeces, enabled the first in vivo experiments. Comparisons of germ-free and colonised animals in the 1960s led to observations that predicted much of what has since been discovered using methodologies that enable more in-depth analyses. Despite advances in culturing microorganisms, it soon became apparent that there were gross discrepancies between the numbers of existing cells and how many could grow in the lab, what became known as the 'great plate count anomaly'. This key observation motivated the development of sequencing-based approaches to identify unculturable microorganisms, which were pioneered by Woese, Pace, Fox and others to study environmental microorganisms and subsequently adapted to the analysis of human-associated communities, providing an unprecedented view into their composition. A key step in popularising microbiota research, which got it into the mainstream news and made it a household concept, was the finding by the Gordon group, in 2006, that reconstituting mice with the microbial communities associated with a human disease state could transplant the phenotype to the animals. This opened the door to research trying to establish causal relationships between altered microbial communities and disease, which has become a cornerstone of the field.

Although the first use of faecal microbiota transplantation (FMT) in Western medicine was published in 1958 by Ben Eiseman and colleagues, who successfully treated four people suffering from pseudomembranous colitis (before *Clostridioides difficile* was the known cause), FMT was already used in ancient Chinese medicine. Fourth-century Chinese medical literature mentions its use, by Ge Hong among others, to treat food poisoning and severe diarrhoea. In the sixteenth century, Li Shizhen used oral administration of a 'soup' containing fresh, dry or fermented stool to treat abdominal diseases. In seventeenth century Europe, the Italian Fabrizio and the German Paullini documented the use of FMT, and the American microbiologist Stan Falkow candidly recalled his role in preparing first-generation poop pills to reconstitute the gut communities of surgical patients a year before Eiseman and colleagues published their work.

We recognize that an enormous body of work precedes each milestone that we have selected to highlight progress in this field. This foreword aims to pay homage to some of these microbiota pioneers. With this project, divided into 25 milestones, we want to highlight particular areas of research — both established and burgeoning — that have contributed to a better understanding of our microbial selves, as well as methodological advances that have propelled the field forward. We also want to highlight important but lesser known aspects of the field, such as the fact that our microbiota is not just composed of bacteria; that human-associated, health-promoting microbial communities exist on all bodily surfaces, not only our gut; and, importantly, that to have a complete picture of the functional capacity of our microbiota and its roles in human health, we need to look beyond the gut of white, Western populations.

We thank the many researchers from all corners of the field who have advised on the different aspects of this project, as well as those who have participated in the podcasts.
It is, of course, impossible to cover everything in a field as broad and diverse as this one, but we hope to have captured the major steps forward. In our attempt to summarise almost 350 years of research, we will have unavoidably missed important contributions and sincerely apologize for any unintended oversights. Although we have focussed these milestones on the study of human-associated microbiota, other vibrant research communities are trying to understand plant- and animal-associated, as well as environmental, microbial communities. We hope that this journey through history will be inspirational and we look forward to the exciting developments that are sure to come, ultimately aiming to harness our understanding of microbial communities to improve not only human health, but that of plants, animals and ecosystems.

Nonia Pariente, *Nature Microbiology*

FURTHER READING Savage, D. C. Microbial biota of the human intestine: a tribute to some pioneering scientists. *Curr. Issues Intest. Microbiol.* **2**, 1–15 (2001) | Finegold, S. M. A century of anaerobes: a look backward and a call to arms. *Clin. Infect. Dis.* **16**, 453–457 (1993) | Falk, P. G., Hooper, L. V., Midtvedt, T. & Gordon, J. I. Creating and maintaining the gastrointestinal ecosystem: what we know and need to know from gnotobiology. *Microbiol. Mol. Biol. Rev.* **62**, 1157–1170 (1998) | Leidy, J. *A Flora and Fauna Within Living Animals* (Smithsonian Institution, 1853).