The Future of Microbiomes in Clinical Medicine: Diagnosis, Prediction, Prophylaxis, Treatment

Granny: what does it mean:
'take care of your microbiome and it will take care of you'?

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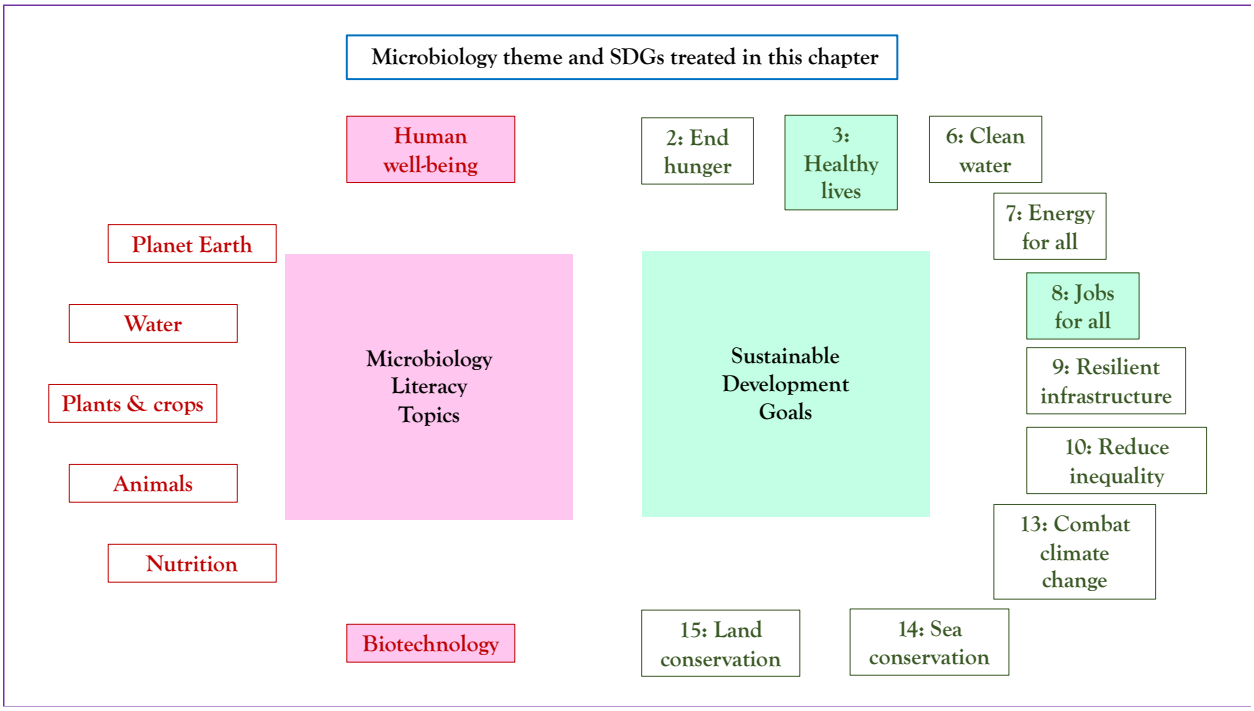
Storyline

Over the past 20 years, researchers have uncovered endless ways in which microbiota are associated with host health and behavior. The microbiota throughout the body - gut, oral cavity, nasal cavity, skin, vagina - all function in a delicate balance aiding in digestion, pathogen deterrence, and general host **homeostasis** – maintenance of its healthy equilibrium. Microbial **dysbiosis** - a shift away from healthy microbial composition - is also implicated in several conditions and diseases, ranging from obesity to cancer to behavioral and mental health disorders, like stress and Alzheimer's disease. There is constant bi-directional communication between the microbiota and the host, and the microbiota both drives health complications in the host and responds to them. Thus, the microbiome already does and will continue to play a role in health and health care best practices. Here we highlight the anticipated role of the microbiome and microbiome-driven applications in medicine in the coming years.

The Microbiology and Societal Context

The microbiology: microbiome; diagnosis and diagnostics; precision (personalised) medicine; health prediction; prophylaxis; therapy; pharmacomicrobiomics; pro-, pre-, post-biotics. *Sustainability issues:* health; economy and employment (biotechnology).



The Future of Microbiomes in Clinic Medicine: the Microbiology

1. ***Diagnosis.*** To date, the microbiome, or more accurately, microbial **dysbiosis**, has been implicated in a range of human diseases and conditions, and researchers agree that microbial activity associated with **metabolite** production and breakdown can drive disease pathologies. Researchers are now actively striving to determine how specific bacterial taxa and metabolite profiles relate to metabolic pathway regulation and human health.

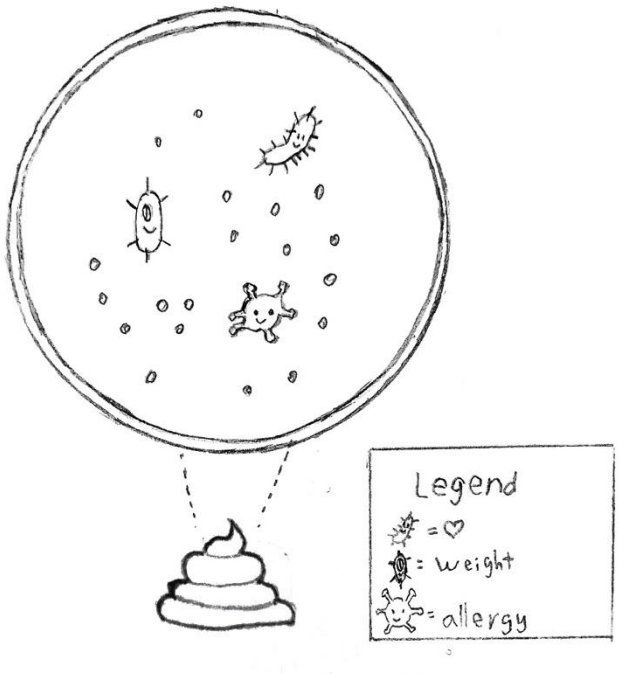
a. Biomarkers. Using state of the art tools, **metagenomics**, **metabolomics**, and the like, researchers are starting to pinpoint specific bacteria or metabolites in various diseases' onsets and **pathogeneses**. The natural extension of bacteria- or metabolite-focused studies is to identify relevant **biomarkers** - biological microbes/molecules found in body fluids or tissues that are correlated with specific health issues - for disease diagnosis. Specifically, unique microbial signatures have been found in blood and tissues of cancer patients, allowing differentiation of not only sick from healthy individuals, but also of different cancer types among sick individuals. Microbiome-derived biomarkers in saliva can also be used diagnostically: e.g., two bacterial species identified in the oral microbiome of pancreatic cancer patients can differentiate these individuals from healthy, age-matched individuals with extremely high accuracy. Metabolite profiles are also relevant diagnostic tools with applications already seen for cancers and Alzheimer's disease.

b. Screening. The use of non-specific microbiota screening by general practitioners and family physicians could serve as a tool at regular check-up appointments to identify hidden pathologies. Or a microbiome **panel** - a test to screen for the presence of a specific set of bacteria - could be offered, similar to how genetic testing is used to identify an individual's risk for genetic disorders or a couple's risk of passing genetic mutations on to their offspring.

General microbial or metabolic testing could be a relatively inexpensive way to screen for diseases that once required specialist visits and invasive or expensive procedures. Mail-in testing could also greatly increase healthcare access in less-serviced areas, not only improving individual well-

being but also reducing strain on an already fragile healthcare system. While specific panels of biomarkers for a range of diseases have already been identified, in the next 15 years, we anticipate general screening gaining momentum in non-specialty medical settings.

2. ***Prediction.***



Researchers have already demonstrated the applicability of using microbiota composition and metabolite profiles in diagnosing diseases, but impressively, they have also demonstrated its utility in predicting future disease onset, even before diagnosis is possible using current medical best practices, as demonstrated for gestational diabetes, coronary artery disease and celiac disease (intolerance of glutens).

a. Machine learning. Researchers are now trying to use machine learning (ML) tools to predict disease onset based on pre-dysbiosis, microbial imbalances that may serve as precursors driving specific diseases, and other patient characteristics (e.g., weight, age, family history. etc.). Thus, as with the described diagnostic measures above, we expect models with microbial or metabolite profile inputs to serve as relatively non-invasive clinical tools to predict future health complications in the coming decade.

b. Modelling. Machine learning models can include microbial features (specific bacteria) and their abundance as well as additional biomarkers – metabolites, cytokines, hormones – genes and other risk factors (historical, environmental and behavioural) to predict disease onset. The addition of microbial data, specific bacteria known to be enriched or depleted in a given disease state, improves models, when compared to typical demographic-based prediction methods and medical guidelines in use today. Research is exploding in the field of host–microbiome interactions, and thus the range of diseases and disorders that can be predicted will continue to grow. Modelling efforts could also be combined with the above-described microbial screening panels to not only diagnose patients but also predict future maladies, providing opportunities for early intervention.

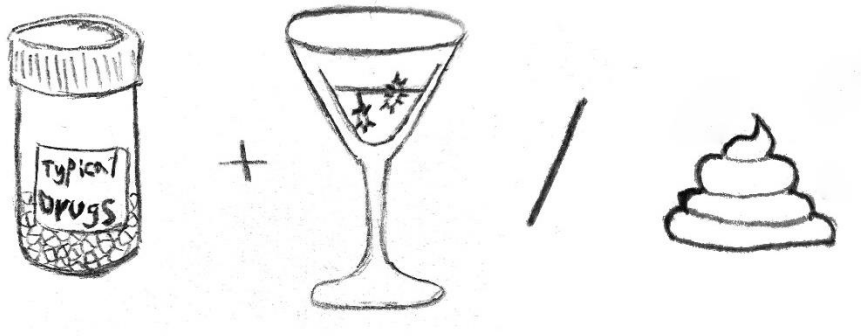
c. Host responses to treatments. Even more impressive, new research suggests that microbiota data can also be applied to predict host responsiveness to specific treatments, including those for cancers, *Clostridioides difficile* infection (associated with diarrhea and colitis), rheumatoid arthritis, bariatric surgery (for weight reduction) and inflammatory bowel diseases.

There can be considerable variation in how different patients respond to the same therapy regime. At the extremes of this variation, therapies can be either highly effective or ineffective, with those experiencing the latter being termed non-responders. In some cases, this variation may be due to variations of microbial metabolism in the gut of an orally-taken medicament, leading to different amounts of the active medicament entering the body. But the microbiome can also have indirect effects on both the uptake and activity of drugs, including the creation of drug metabolites with their own activities. Microbiome-mediated variations in therapy efficacy is usually related to the specific types of organisms that make up the gut microbiome, their abundances, and their interactions with one another, with the host gut environment, and the therapeutic agents.

In the case of rectal cancer, patient response to a given treatment was predicted with > 73% accuracy using 10 predictive microbial taxa differentially expressed between responders and non-responders. Additionally, a study of patients who did and did not respond to treatment for melanoma, a skin cancer, revealed both differentially abundant bacterial taxa and different **functionality profiles**, sets of molecular tasks the bacteria can perform, between the groups.

The microbiome is implicated in a range of diseases and host states, so it is only expected that it would be implicated in treatments as well. Accordingly, current research points to the utility of microbiota screening when planning treatment for a range of diseases. Further, a standardized ML model that accounts for patient characteristics, disease status, and microbiota composition could change the way we approach disease treatment. If patient responsiveness to treatments can be predicted from non-invasive faecal or saliva sampling, healthcare can be tailored to patient needs, both improving efficacy, and decreasing burdens – financial, mental and otherwise – associated with extended treatment plans and low success rates.

3. ***Treatment.***



We have described how microbiota and metabolite profiles can be used to diagnose diseases, predict future disease onset and even predict treatment success rates. It is only logical, then, that microbiota manipulation or supplementation can aid in treatment of a range of diseases: that is, specific modulation of the microbiome employed as a clinical intervention.

a. Pharmacomicrobiomics. The study of how drugs and host microbiota interact has come to be known as pharmacomicrobiomics. Pharmacomicrobiomics typically focuses on drug metabolism and treatment efficacy as they relate directly to the microbiota and indirectly to the metabolic processes driven by the microbiota. Early evidence in cancer models suggests that response to treatment can be improved through specific microbiota manipulation. In one study of melanoma in a rodent model, administration of several *Bifidobacterium* species increased treatment efficacy, and even when administered alone without therapy, these bacteria increased tumour control (i.e., improved host health). Similar results were demonstrated for another bacterium (*Bacteroides fragilis*) in a different study. Both studies are based on preliminary findings of differences in the microbiomes of responders and non-responders. From there, the researchers examined which bacteria were correlated with or caused the differences and from there, identified the functionally relevant bacterium to improve treatment. They showed how successful application of targeted treatment – here inoculation with specific bacterial strains – enhances treatment efficacy.

b. Patient stratification – precision (personalized) medicine. Once differences in responders and non-responders can be traced to the microbiota, treatments can be developed and applied in a **stratified** manner. This means that not all patients get the same treatment - microbially targeted therapies are only given when a patient profile suggests there would be a benefit. Pre-screening patients' microbiota to determine if they are good candidates for a certain treatment may become standard practice. It is no longer technologically complex to characterize an individual's microbiome with **16S rRNA gene sequence surveys** (a method of DNA sequencing to identify bacteria from patient samples), which are becoming less financially limiting. A purpose-built, rapid screening test with only a subset of bacterial species is still lacking but is not technologically out of reach.

c. Fecal microbiota transplant (FMT). As discussed above, bacterial inoculations can be highly customized, involving introduction of a single bacterial strain into patients prior to or concurrent with standard treatments. However, identifying specific bacteria suited to each of the many different therapeutics used to treat the wide range of complex human diseases remains a huge challenge. Researchers must identify bacteria that are differentially represented between patients and controls, isolate them, and test how they affect disease status, progression, and recovery - first in an animal model, and then in a human test. This is possible but often time prohibitive.

There is evidence, however, that total fecal microbiota transplant (FMT) from responders to non-responders, i.e. the transfer of essentially all microbes present in the gut, rather than just one or more specific ones, can also improve treatment outcomes. One study demonstrated that FMT from donor mice that had microbiota with antitumor effects to mice of the *same strain* but sourced from a *different facility* (with different microbiota that did not have antitumor effects) reduced tumour growth in recipients to a similar degree as a typical antibody treatment. Studies in **humanized mice** – those that received FMTs from human responders and non-responders – also show beneficial effects on treatment success following FMT from responders. A range of studies in humans are underway to determine the effects of FMT on patients who are non-responders to cancer treatments, and preliminary results from two pilot studies in humans revealed that FMT can increase responsiveness to immunotherapy. This offers early support for FMT rather than tediously isolating and testing effects of specific bacterial taxa.

d. Therapy side effects/adverse events. Beyond improving treatment outcomes, microbiota-based medicine could reduce unwanted effects of certain medicines. Medicines are

biologically-active substances that provoke metabolic responses in cells. The ideal medicine has its effect only on target cells, e.g. killing a pathogen, but most medicines also act on other cells and therefore have some unwanted side effects. This is encapsulated in the term 'selective toxicity' for agents used to kill cells, such as antibiotics used to kill pathogens or anti-cancer drugs used to kill cancer cells. Antibiotics usually have a high selective toxicity because pathogenic bacteria are very different from human cells, and do not affect them, but anticancer drugs usually have a low selective toxicity and significant side effects because cancer cells and normal cells are both human.

In several studies, researchers were able to predict, based on pretreatment microbiota, which patients would develop side-effects to treatment including colitis and weight gain or identify unique microbiota profiles between patients that did or did not develop side effects. Over the coming years, researchers should be able to exploit pharmacomicrobiomics not only to increase treatment success but also to reduce unwanted side effects of therapeutics, making treatments with potentially debilitating side effects in a subset of the population more palatable. Specifically, there is a general aversion to weight gain, so microbial cocktails could be prescribed together with treatments like antipsychotics, and even birth control, both of which are associated with weight gain, to increase reach and compliance.

e. Pro-, Pre- and Postbiotics. Faecal microbiota transplant is a blanket approach for altering the microbiota; a lot of bacteria are introduced, and the method is not without risks - pathogens could also be transferred along with the healthy bacteria that make up the total microbial community. **Probiotics**, **prebiotics** and **postbiotics** can offer better targeting - to specific bacteria and associated functionality - and may be safer than FMT. Once functionally relevant taxa and metabolites are identified from the sets of differentially abundant features that distinguish patients from healthy controls, bacterial or metabolite cocktails can be formulated in a disease-specific manner with the goal of regaining metabolic homeostasis. Further, patient profile – specific microbial or metabolite deficiencies both related to and independent of disease state – can be incorporated into models to tweak or supplement disease-specific cocktails. Such personalized approaches, that consider both disease state and specific patient characteristics, should not be too far off. As soon as models for disease prediction and treatment responsiveness are in use, taking modelling one step further towards personalized probiotic formulations is no longer a stretch.

4. ***Prophylaxis.***

Beyond direct treatment consequences, personalized microbiota-based medicine could also be used to prevent disease onset prophylactically or improve quality of life or lifespan. Custom probiotics could abound – in response to findings from screening tests described above, to reduce weight, boost immunity during cold season, sharpen memory, improve fertility, aid in initial gut colonization and more. The microbiome is implicated in a range of developmental functions, diseases, mental disorders, and other host states. Even aging is associated with microbial dysbiosis, and FMT from young donor mice can counteract some of the aging phenotype observed in aged mice. There is also early evidence that centenarians have significantly different gut microbiota than elderly and young individuals with relevant differences in metabolic function, and marked microbial shifts were observed in the seven-month window prior to their passing. Thus, we might be able to increase longevity and healthspan through targeted microbiota supplementation. Following refinement of probiotic therapeutics based on emerging knowledge of beneficial bacteria in various health contexts, clinicians and nutritionists could prescribe specific formulas for patients with known microbial deficiencies or who wish to improve certain gut or even overall host functionality.

5. ***Fecal transplants, banking, and back-ups.*** As described above, microbiota manipulation, either via FMT or specific bacterial targets, can be used to treat or improve treatment of a range of diseases. Evidence of FMT to improve digestive function and reduce diarrhea dates back thousands of years to 4th century China and has historically been practiced in Africa as well. More contemporary research, some of which is described above, shows the utility of FMT in modern medicine, but the question of the optimal FMT donor is still hotly contested. Faeces are, by nature, non-sterile, and transfer of fecal matter from one individual to the another can have unwanted side-effects if fecal matter is not screened properly.

Thus, the option of **autologous FMT** (aFMT) is being explored. In this scenario, stool from the patient, which was collected prior to disease onset, can be used for FMT to reinstate a healthy gut microbiota. Support for application of aFMT is seen in a recent study demonstrating that aFMT can help obese participants maintain weight loss following weight-control diets. aFMT to treat disease, though, would require prior banking of faeces, something not common, at least in the year 2021. In the future, periodic faecal banking for future aFMT may become standard, or at least more common, like computer back-ups, just as cord blood banking at birth has started gaining traction to provide a life-long source of one's own stem cells for future medical needs.

Alternatively, donor banking may be more feasible for widespread application if sample screening, labelling, and storage methods are all uniform and rigorous. Once banked, samples could theoretically be characterized and classified to highlight which would most likely improve certain disease states, improve treatment responsiveness, reduce weight gain, and help with aging, even taking into account recipient characteristics. Down the line, we could even envision a world in which FMT from "star" donors might be used as probiotics are today - to improve general well-being and gut functionality, though as a one time or annual supplement rather than a daily one.

6. ***Conclusions.*** With the advent of next-generation sequencing (metagenomics), metabolomics, and other advanced techniques, specific functions can now more accurately be assigned to bacterial taxa, helping researchers to identify relevant target species for microbiota-based therapeutics. Bioinformatic pipelines and machine learning algorithms are constantly being refined to better integrate this wealth of omics data towards clinical application. The field of microbiota-

based diagnostics and personalized or condition-based microbiome-related therapeutics will likely rapidly develop. With the advancement of these technologies, we will probably also see an increase in non-essential therapeutics, including boutique and custom-mixed probiotics specifically suited to the recipients' **endogenous** microbiota and formulated to work on one or more quality-of-life targets. Overall, we can expect healthcare to benefit greatly from microbiome-enhanced clinical practices, both in the rarer cases of non-responders and for more widespread application in the general population in terms of screening towards early detection and intervention for a range of diseases. As the global population continues to age, researchers are constantly searching for new drugs and treatments; therapeutics, especially relatively inexpensive ones, associated with low risks (like faeces) will likely proliferate as independent treatments, as complementary treatments, and as preventatives.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 3. Improve health and reduce preventable disease and premature deaths.** The microbiome not only helps to break down the food we eat. It is also implicated in immune function, behavior, and overall well-being. We anticipate microbiome-targeted treatments, microbiome-assisted drugs, and microbiome-based diagnostics will proliferate in the next decade, increasing our ability to predict, diagnose, and treat a wide range of diseases.

Potential Implications for Decisions

1. ***Governmental Policies and recommendations***
    a. Implications for insurance policies: prescreening, biases
    b. Large-scale health planning for future population disease onset
    c. Information-driven vs doctor-driven medicine and the balance between them

Pupil Participation

1. ***Class discussion of why we would/would not want to use predictive methods for (i) future disease onset and (ii) treatment efficacy***
    a. What does a patient do with this information?
    b. Do we need microbiome counsellors like we have genetic counsellors?
    c. What does an insurance company do with this information
    d. What about the cost-prohibitivenss of custom treatments - do we not try anything if a model assumes a patient will be a non-responder?
    e. Should these tests be required to increase health care planning abilities in light of the constantly aging population?
    f. What experimental treatments seem useful and what is going too far? How should these be regulated - like probiotics (generally safe) or drugs (testing and approval)
        i. A Multivitamin-like FMT/probiotic cocktail for overall wellbeing?
        ii. Anti-aging cocktails?
        iii. Weight-loss cocktails?
        iv. Cognitive enhancers for children? Teens? adults?

g. Is it better to have a donor bank with "star" donors or use aFMT?
    i. Should donors be the same age as recipients?
    ii. The same sex?
    iii. The same ethnicity?
    iv. How often should we bank feces for aFMT?
    v. Who would be liable in case of infection?

2. Pupil stakeholder awareness

a. Increased access to medical care, ease of diagnosis
b. Early insight regarding future health outcomes - both diagnosis and treatment success
c. The decision to use predictive tools: pros and cons of knowing about potential disease onset vs. diagnosis of an active condition

Glossary

autologous fecal microbiota transplant (aFMT): an FMT in which the donor is also the recipient (also: self FMT) - this is a way of reducing risks associated with colonization by foreign microbiota.
16S rRNA gene sequence survey: a DNA sequencing tool to identify bacteria at the genus (and sometimes species) level focusing on a specific region of the bacterial genome (the 16S rRNA gene) that is found in all bacterial taxa but is different enough to differentiate between them.
Bifidobacterium: a genus of bacteria in the phylum Actinobacteria that is commonly found in the human gut microbiota. It is thought to be a beneficial taxa with anti-inflammatory properties and there are a number of *Bifidobacterium* probiotics on the market.
biomarker: a biological molecule found in blood and other body fluids or tissues.
dysbiosis: a microbial imbalance or shift away from healthy microbial composition.
endogenous: substances (here, bacteria) that originate within a system (here, the host).
functionality profile: a set of molecular tasks that the microbiota perform.
healthspan: refers to the number of *healthy* years one lives (as opposed to lifespan, which is the total number of years on lives).
homeostasis: a stable equilibrium of internal state or condition.
humanized mice: mice that have a human-like microbiota, typically as a result of fecal microbiota transplant (FMT).
inoculation: introduction of or infection by a particular organism (here, the introduction of a specific bacterial strain to a host).
machine learning: the use of computer algorithms to identify or predict some state (here, disease onset) through experience and by use of available data. A method within the field of artificial intelligence.
metabolites: vitamins, antioxidants, short chain fatty acids and other by-products of metabolism.
metabolomics: the study of chemical processes involving metabolites - here, a method used to identify metabolites in a sample and compare metabolite profiles between samples.
metagenomics: the study of genomic material from a given sample - typically through whole genome sequencing.
panel: a set of targets examined - a panel of diseases or microbes whose presence within a sample is being tested.

pathogenesis: the way in which a disease progresses or develops.
pharmacomicrobiomics: how drug metabolism and treatment efficacy relate directly to the microbiota and indirectly to the metabolic processes driven by the microbiota.
postbiotics: beneficial metabolite by-products of bacteria that can be given as a supplement instead of supplementing with the actual bacteria.
prebiotics: a substrate that is selectively utilized by host microorganisms conferring a health benefit.
probiotics: live microorganisms which when administered in adequate amounts confer a health benefit on the host.
prophylactic: a method or treatment that can protect against or prevent disease occurrence and spread
stratified: applying a treatment, analysis, study, etc. in partitions such that subpopulations within an overall population are the focal unit

Authors' Note: *Much of the text has been adapted from the article written for a special issue of Microbial Biotechnology: Turjeman S and Koren O. (2021). Using the microbiome in clinical practice. Microbial Biotechnology 15: 129-134.*
Image credit: Lihi Bahar