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A diverse microbiome is essential to human health. There is no definition of a “standard” microbiome because every individual’s microbiome is impacted by their environment. The microbiome is relatively resistant to changes, which ensures that the individual continues to benefit from the protective properties of their distinct microbiome even after external factors temporarily impact community diversity. However, this is not always the case. Antibiotics and other modern disinfection techniques have decreased the natural diversity of microbiomes past the point of their resilience. Therefore, antibiotics should be used cautiously and alternative methods should be developed to reduce the detrimental effects of antibiotics on the normal microbiome. Genomic analyses will be increasingly important in these future developments intended to monitor and protect the diversity of the microbiome.

One of the main themes of Missing Microbes is that the microbiome is an important supplement to, and an essential component of the human immune system. Microbiota help optimize our metabolic processes, as well as provide protection against pathogens. Blaser argues that gut bacteria improve nutrition and efficiency of metabolism by breaking down nutrients that would be indigestible for a sterile human gut. (1) The microbiome assists the immune response because its composition has impacts on the efficiency of some medicines. Different “microbiome-medicine interplay(s)” determine the efficiency of drug metabolism and therefore the efficiency of the drug for the patient. (2) The microbiome can also protect us from pathogens in another, subtler, way. The existence of resident microflora protects surfaces of the body by competing with and sometimes excluding pathogens. As Antonopoulos puts it, “indigenous microbes form an ecological barrier that prevents the ingress of pathogenic microorganisms.” (3)

Sometimes this competition reduces the abundance of pathogens rather than eliminating them altogether. This is also beneficial, because colonization is harmless. (1) This competition allows pathogens to colonize the body in a low and manageable way. A low concentration of pathogens can even be beneficial, for example in the case of *Heliobacter pylori*. It was found in 1983 that *H. pylori* was concomitant, or naturally occurring, in 50% of the sampled population. (4) A low concentration of *H. pylori* cells increases the number of lymphocytes present and active in the gut. The activity of the lymphocytes protects the individual against asthma attacks. (1) However, a high concentration of *H. pylori* is harmful to the host, since this organism was first linked to gastritis and peptic ulcers in its human host. Of the infected population, it was found that only 20% will be negatively affected by *H. pylori*. (4) Blaser calls this phenomenon the “dual nature of pathogens.” (1)

In fact, harmless colonization of pathogens contributes to the diversity of the microbiome without negative effects on the individual’s health because the beneficial species still dominate. This diversity is essential for the resilience of the microbiome against potential pathogens and drastic changes to the internal environment. (1) A diverse community allows for a multitude of functions and mechanisms that could potentially be used to counteract the selective pressure. A diverse microbiome is beneficial not only for optimization of metabolic processes and protection against pathogenic infections, but also for future immune responses.

For these reasons, the microbial genome and its interactions have been considered just as important, or even more important than the host genome. Grice calls the microbial genome “an essential but largely ignored overlay.” (4) Furthermore, Carroll argues that “the inability of organisms to survive independently or to maintain normal health is a strong indication of coevolved mutualism.” (5) This suggests that human health is irrevocably linked and dependent on the microbial genome to function properly.

Given that the microbiome is so essential to human health, research should be carried out to improve our knowledge of this complex and largely unexplored system. One area of interest is the diversity and distribution of populations in an individual’s microbiome. By comparing genomes of different microbial strains present in different individuals, Benson *et al.* found that “intrapersonal variation is lower than interpersonal variation.” (1, 6) This means that an individual has a unique microbiome whose populations are more similar to each other than to species found in another individual’s microbiome. However, this is not the only trend in variation of individual microbiomes.

The environment, diet, and genome of the host are all factors that impact the resident microbe population, creating a microbiome that “can be as unique as a fingerprint.” (2) Chance also plays a role in the diversity and structure of the microbiome, since interactions between resident and transient microflora are not predictable. In competitive interactions, there are many stochastic factors such as limiting resources and “chance colonization events” that make the prediction of population behavior difficult. (2) This results in “significant inter-individual variation… caused by the accumulated effects of genetic and environmental influences on the gut microbial community.” (7)

It has been seen that many factors influence the microbiome, causing a unique composition for an individual. This identity is not only unique, but also a self-regulating system that preserves an equilibrium. After a temporary change in environment, diet, or gut health, the microbiome is usually able to recover to its original state. This was seen in Dr. Libusha Kelly’s talks on the human microbiome, where patients recovered after a move abroad and a stomach bug. (27 October 2017) Once a default population has formed, it is relatively stable to resistance. Grice claims that microbiota “converge toward an adultlike profile during the first year of life.” (4)

However, this is not always the case. Some modern treatments are so effective that they can change the composition of the microbiome permanently. Broad-spectrum antibiotics target cell processes in both pathogenic and benign cells. Therefore, these medications can destroy the balance of existing populations and decrease overall diversity. Antonopoulos *et al.* found “a persistent, significant decrease in overall species richness in the gut community” after antibiotic treatment. (3) Because of the link between the natural microbiome and health, these changes can inhibit the possible immune responses of the host. A decrease in diversity limits the resilience and protective abilities of the microbiome because there is less functional diversity present to create an immune response. Furthermore, “pathogens can exploit the reduced competitiveness of a community disturbed by antibiotics, thereby establishing themselves in the host.” (2) Like invasive species, pathogens could take advantage of the environment and make a niche for themselves. Antibiotics can disturb the natural distribution and dynamics of the microbiome past its point of resilience.

Treatments are especially likely to cause long term changes if they are administered in the early stages of life, while the microbial signature is still developing. (1) Without an equilibrium to return to, an infant microbiome is especially vulnerable to long term changes. Therefore, antibiotic usage for babies should be even more carefully regulated.

The risk of the permanent alteration of the microbiome is compounded with modern approaches to medicine, where all microbes are pathogens and sterilization as the ideal. Hospitals use a variety of disinfectants and sterilization techniques to prevent cross contamination between patients, reduce the absolute number of microbes, and eliminate the spread of nosocomial diseases. However, these techniques provide opportunistic pathogens an environment, whether that is a hospital surface or human organ, that lacks competition from existing and transient microflora. While sterilization is efficient at reducing the probability of infection, there is still a chance that microbes can contaminate the space. If and when that happens, the invaders, benign or pathogenic, are primed to take full advantage of the resources and lack of competition. Still, intense sterilization is seen as the gold standard. Furthermore, *H. pylori* has been seen in many patients without negative effects, as mentioned previously, but is classified as a definite carcinogen by the World Health Organization. (8) The standards for sterilization and the view of microbes are binary, but factors such as Blaser’s dual nature of pathogens mean that these ideals are far from perfect. Instead, these techniques have the potential to interrupt the dynamics of a natural and beneficial microbiome.

Another modern approach that impacts the microbiome is the Caesarian section. C-section births have decreased the diversity of the microbiome and changed its composition. Infants are receiving random microbes from the air and hospital, instead of receiving beneficial microbes from the birth canal that have been selected by their mother and her ancestors for many generations. (1) The resulting microbiome is not as beneficial for the infant and does not protect them as well as an inherited community would. Furthermore, its composition and level of diversity may differ significantly from the mother’s microbiome. The acquisition of random strains from the environment can also disrupt the calibration of the developing microbiome, leaving its host vulnerable to invasive strains.

Furthermore, with the increasing frequency of C-section births, the mean microbiome of the entire population is shifting from its original state. The human genome itself has been collected from enough individuals to form a reference genome. Now that the microbiome is seen as a complement to the human genome, it makes sense that a similar template microbiome is established. The reference microbiome of the U.S. population was compared with the reference microbiome for an isolated population of Venezuelan natives. The diversity of the American microbiome was much lower than that of the Venezuelan population. (1, 9) The disadvantages of a low level of diversity on human health are compounded in a population compared to an individual, which relates to the concept of “modern plagues” of Blaser’s subtitle.

Because of antibiotics and other modern alterations of the natural microbiome, the selection pressures on the microbiome are weaker than they were in the past. Therefore, even more treatments are needed for individuals who lack this natural form of defense. This weakens the selection pressure even more, causing a cycle where the human population becomes increasingly dependent on antibiotics. It is a well-known challenge that incomplete treatments and the misuse of antibiotics in general are contributing to the development of antibiotic resistant strains. However, now there is a new reason to use caution that has to do with the individual patient’s future, rather than the future of the strain.

With knowledge this new side effect, it is possible that prescriptions and overuse may decrease. Instead of seeing antibiotic resistance as a long-term and impersonal warning, patients may pay more attention to the risk of impairing their microbiome for the rest of their life. This new motivation, along with education about the risks of antibiotics in general, is essential to the success of efforts to prevent misuse.

One program that has been successful is the “Keep Antibiotics Working” campaign in France. (1, 10) The government’s goal in 2001 was to reduce overuse of antibiotics by educating both the general public and health care professionals. At the time, France had the highest antibiotic consumption in Europe, and the primary focus of the program was to decrease usage in children, whose level of usage was even higher than other groups. Their efforts were successful, as there was a 30% decrease in antibiotic use for children. (10) One reason why this program was so effective was because it targeted parents as well as doctors. (10) This was essential because one of the major causes in over-prescriptions is pressure from patients. A worried parent will not usually consider the disadvantages of prescribing antibiotics to their child because of the small probability of improvement is worth it. Therefore, the broad and multifaceted approach of educating the general public was key to the program’s success.

However, this multidimensional approach makes it difficult to expand this program to other nations, where antibiotic overuse is also a problem but the government does not have as many resources to spend on improving awareness. Therefore, other resource-effective methods are needed to solve the problems of antibiotic misuse and loss of microbiome diversity.

One method that should be considered is the development of narrow focus antibiotics. Currently, antibiotics target some specific process in the cell, and so are specific to that mechanism. However, when antibiotics target a certain mechanism that is present in many species, the specificity of the drug is not so narrow as it appears. Therefore, approaches with the same theme of specificity should be expanded to target specific species. Yao *et al.* claim that such specific medications must retain the diversity, abundance, and composition of the microbiome in addition to effectively removing pathogens. (11) The current method to increase drug specificity is to use combination drugs, or cocktails. (8) These are effective because the probability that a strain develops resistance to the cumulative effects of multiple drugs is lower than the probability of developing resistance to a single mechanism. However, the cocktail approach could potentially have large impacts on the microbiome because so many microbial mechanisms are being manipulated. Therefore, the development of novel narrow-spectrum drugs still needs to be a priority.

One example of a narrow-spectrum antibiotic is fidaxomicin. This medication is intended to treat *Clostridium difficile* infection (CDI). Eiland *et al.* found that fidaxomicin was an effective against 96% of the subjects tested. (12) Louie *et al.* found that fidaxomicin was just as effective as vancomycin, a broad-spectrum antibiotic that is usually used for a CDI diagnosis. (13) It was found that fidaxomicins’ efficiency was due to its inhibition of *C. difficile* spore production. (12, 13) The effect on the composition of the microbiome was measured using ribosomal RNA probes and quantitative real-time polymerase chain reaction. (13) QTPCR is a method used to quantify the relative abundancy of different species in a sample over time. Like PCR, it relies on the assumption that the larger the volume of polymerized genetic material, the larger the abundancy of the original species. Therefore, researchers observed the relative abundancies of different species using the distinct sizes and sequences of their ribosomal RNA. There were no significant changes to the microbiome composition in terms of absolute numbers or ratios of different species after fidaxomicin treatment. Therefore, fidaxomicin was found to be an effective narrow focus antibiotic. However, the main drawbacks are that the cost of this drug is extremely high, and the development of novel narrow-spectrum medications is time consuming and resource-intensive. (12) Therefore, parallel solutions are needed.

Another approach could be the development of vaccines that could decrease the need for antibiotics. Currently, vaccines reduce the need for antibiotics by creating antibodies in the immune system using an inactive form of the disease. The presence of memory antibodies reduces the severity of the infection if the vaccinated individual is infected later with the same strain. This reduced severity reduces the need for antibiotic use and reduces the total number of cases of the disease through herd immunity. (14) Vaccines have been incredibly effective, but are limited because they do not address the inevitable: microbial evolution. Every year, a new flu vaccine is developed to account for the evolution of the strain since the previous year. The efficiency of the vaccine depends on how closely it matches with the circulating strain of that year. If an individual is infected with an evolved strain, the vaccination does not help the immune response of the patient nor reduce the need for antibiotics. The design and development of the annual flu vaccine is becoming more and more difficult, since high selection pressure increases the number of parallel strains that may be circulating. This decreases the efficiency of the vaccine. Therefore, the vaccination approach should be updated to address the challenge of antibiotic resistance.

Vaccines should be adapted, much like antibiotics themselves, to be more specific. However, instead of targeting certain diseases, vaccines should go one step further and target certain antibiotic-susceptible strains. This could enhance and ensure the continued efficacy of antibiotics by “specifically targeting resistant alleles of a conserved protein or by targeting proteins uniquely present in resistant isolates.” (14) Like the development of new antibiotics, the discovery and design of new vaccines is slow and resource-intensive. Therefore, even more alternatives are required.

Another approach to decrease antibiotic overuse is probiotics, which are “live microorganisms that when administered in adequate numbers confer a health benefit on the host.” (1, 8) The challenge is that because of the wide diversity of microbiomes in a human population, it is difficult to know what probiotics are required by an individual and which supplement combination will be helpful. (1) Evidently, the quantification of a reference microbiome could assist in these types of treatments.

Technology should be used to address the challenge of defining a reference microbiome as well as monitoring individual microbiome diversity. Just as human genomic data is already helping researchers, microbial genomes have the potential of contributing crucial information for improving human health and medical approaches. This is part of the goal of the Human Microbiome Project. (1)

The first advantage of using technology to determine a reference microbiome is that automation is the only way to extract information from such large quantities of data. After tackling the human genome project, bioinformaticians saw that a limiting factor was computation time and information storage, both challenges linked to the volume of genomic data. This problem is seen again with the human microbiome project. Even though bacterial genomes are much smaller than the human genome, the diversity of species increases the amount of data exponentially. Technological approaches are essential in storing and processing this data.

In addition to simply storing data, technological approaches can contribute algorithms and methods to extract trends and distill patterns from the vast amount of information hidden in the microbiome. While there are a “potentially limitless number of microbial communities structures… (they) can be distilled into a finite number of types.” (1, 15) This could be implemented by using clustering algorithms. These approaches aim to eliminate noise caused by the “inherent baseline variability of the microbiota,” which could assist in the definition of a reference microbiome. (3)

The defining of a reference microbiome is similar in motivation to the defining of a human genome. Both could be applied to measure the “normalcy” of a genome. A standard baseline could be established so that changes in the microbiome induced by antibiotics can be detected and assessed. A measure of the level of diversity before and after treatment could act as an indicator or measure of the antibiotic’s effects on the natural microbiome. This is how microbiome diversity was measured in the Venezuela example mentioned previously. (9)

The isolation of genomic data from a certain species will also be helpful in developing targeted antibiotics and vaccines, methods that should be attempted to protect microbiome diversity. This is because certain genes or proteins could be identified in target pathogens, and their absence noted in the community as a whole. This would help the efficiency of identifying specific mechanistic targets. Furthermore, computer based methods can be used to “maximize molecular binding in a virtual environment.” (8) This could improve the efficiency of drug design and reduce the time and resources required for development.

Finally, the quantification of the microbiome could also be useful in determining causative factors between the microbiome composition and disease. Supervised machine learning algorithms could be used to link “life histories, behaviors, environments, and exposures” as well as features of the microbial signature with predisposition to diseases and the health of the patient. (15) This application could provide better motivation for immediate study of the microbiome than the other applications, since knowledge of risk factors could benefit patient care in the short term as well as in the long term.

The biggest challenges of the Human Microbiome Project are similar to the ones for the Human Genome Project. However, the larger scale of the microbiome complicates the core issues of working with genomic data. The first challenge in the immense intra-personal variation of the microbiome. We saw that a multitude of factors influence the microbiome, creating a unique microbial signature. Furthermore, an individual’s microbiome can shift over time due to significant changes in environment or health, even without the irreversible impact of antibiotics. Clustering algorithms can attempt to formulate a reference microbiome, but the results may not work as well as the reference genome because of the immense scale of variability. These simplified models may not reflect the full diversity of the microbiomes of a population. However, they still may provide enough information to assess antibiotics for their impacts on the microbiome. One way to address immense variability in data is to collect a multitude of samples from each species. The incorporation of multiple samples into the calculation of the mean microbiome would increase the accuracy of this template. This may seem like another complicating factor, but there are links between the diversity of one site and the diversity of another. Therefore, the collection of multiple samples may improve the mean microbiome as well as provide a more complete picture of the level of diversity of an individual microbiome.

The next challenge in microbiome analysis is that the microbial genomes themselves are also changing. A microbial genome can change quickly due quick replication rates and gene transfer via conjugation, transduction, and transformation. Therefore, reference genomes for bacteria are largely over-simplified. One approach is to update the template genome as new instances are added. Just as multiple samples will address the challenge of diversity, they could also assist in keeping the reference genome up to date.

Still, there will always be an arms race between antibiotics and the microbial genome. However, even limited knowledge of the target microbial genome may provide insights for developing targeted antibiotics and monitoring microbiome diversity. It has been seen that the microbiome is an essential factor contributing to human health. The diversity and variability of the microbiome make it a complex subject, which is why technological approaches are crucial. These approaches could help preserve microbiome diversity and improve modern medicine in the future.

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