Jen Johnson BIOL 310 CW 2

A diverse microbiome is normal and essential to human health. There is no definition of a “standard” microbiome because every individual’s microbiome is impacted by their genome and their environment. An individual microbiome is relatively resistant to changes, which ensures that the host continues to benefit from the protective values of a diverse and individual microbiome. 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 the future developments and the protection of the microbiome.

One of the main ideas of “Missing Microbes” is that the microbiome is an important supplement to, and could 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 also provides protection against pathogens. The composition of the microbiome has impacts on the efficiency of some medicines. Different “microbiome-medicine interplay(s)” determine the efficiency of drug metabolism and therefore the effect of the drug on the patient. (2) Therefore, an individuals microbiome can both increase and decrease the efficiency of a treatment. The microbiome can also protect us from pathogens in another, more subtle, way. Just by existing, resident microflora protect the 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 at a low, manageable, and harmless way. A low concentration of pathogen 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 host against asthma attacks. However, a high concentration of *H. pylori* is harmful to the host, since its discovery was first linked to gastritis and peptic ulcers in its human host. Of the infected individuals, it was found that only 20% will be negatively affected by *H. pylori*. (5) Blaser calls this phenomenon the “dual nature of pathogens.” (1)

In fact, the presence of many (pathogenic) species in low numbers contributes to the diversity of the microbiome without negative effects on the state of health because the beneficial species still dominate. This diversity is essential for the resilience of the microbiome against potential pathogens. (1) A large diversity of bacteria means a large diversity of potential functions and mechanisms that could be used to eliminate the threat. A diverse microbiome is beneficial not only for optimization of metabolic processes and protection against pathogenic infections, but also for resilience in 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 for its proper functioning.

Given that the microbiome is so essential to human health, research should be carried out to improve our knowledge about this largely unexplored system. Genomic analyses could elucidate the diversity and distribution of populations in an individual’s microbiome. One method in bioinformatics used for analyzing and measuring the structure of a genome is the alignment of sequences to a reference genome. Indeed, by comparing genomes of different strains present in individuals, it has been shown that “intrapersonal variation is lower than interpersonal variation, as determined by 16S rRNA gene sequencing.” (6) This means that an individual has a unique microbiome whose populations are similar to each other in terms of their genes.

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.” (6) Chance also plays a role in the diversity and structure of the microbiome, since interactions between resident and transient microflora are not always 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. (6) This results in “significant inter-individual variation… from the accumulated effects of genetic and environmental influences on the gut microbial community.” (7)

Many factors influence the microbiome, causing a unique composition for an individual. However, this identity is not only unique, but also a system that self-regulates to preserve 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 (27 October 2017) on the human microbiome, where patients recovered after a move abroad and a stomach bug. 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. One study found “a persistent, significant decrease in overall species richness in the gut community” after antibiotic treatment. (8) Because of the link between the natural microbiome and health, these changes can have detrimental effects on the possible immune responses of the host. A decrease in diversity limits the possible immune responses afforded because there is less functional diversity present in the microbiome. Furthermore, “pathogens can exploit the reduced competitiveness of a community disturbed by antibiotics, thereby establishing themselves in the host.” (6) Like invasive species, pathogens could take advantage of the environment and make a niche for themselves. Antibiotics could 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. (cite) Without a stable equilibrium to return to, an infant microbiome is especially vulnerable to long term changes due to antimicrobial treatment. Therefore, antibiotic usage for babies should be even more carefully regulated case assessed for risks and benefits.

The risk of permanent altering of the microbiome is compounded with modern approaches to medicine, which view microbes as 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, lacking 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 above, but is classified as a definite carcinogen by the World Health Organization. Cite book? The standards for sterilization and the view of microbes are binary, but these effects of these ideals are far from absolute. Instead, these techniques have the potential to interrupt the dynamics of a natural and beneficial microbiome.

One example of a 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 from the hospital, instead of receiving beneficial microbes that have been selected by their mother and their ancestors for many generations. (1) The resulting microbiome is not as beneficial for the infant and does not do its job at protecting the immune system as well as an inherited community. Furthermore, its composition and level of diversity may differ significantly. This acquisition of random strains from the environment can also disrupt the equilibrium of the developing microbiome, leaving it vulnerable to invasive microbes.

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 in the Venezuelan population. (1) how did they measure diversity? The disadvantages of a low level of diversity on the health are compounded in a population compared to an individual, which relates to the concept of “modern plagues” of Blaser’s subtitle.

With the altering of the natural microbiome by antibiotics and other modern medical approaches, the selection pressures on the microbiome are weaker than they were in the past. Therefore, even more treatments are needed to combat diseases in patients who lack the natural defense of a microbiome. 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 overuse of antibiotics are contributing to the development of resistant strains that are even more difficult to treat. However, now there is a new reason to use caution, and it 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, parents may pay more attention to the impairment of their child’s microbiome for the rest of their life. In this way, this new motivation, along with education about the side effects of antibiotics in general, is essential in these efforts to decrease overuse.

One program that has been successful was the “Keep Antibiotics Working” campaign in France. (7) 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 a major goal of the program was to decrease usage in children, who had an even higher usage compared to other nations. Their efforts were successful, and there was a 30% decrease in antibiotic use in children. (7) One reason why this program was so effective was because it targeted parents as well as doctors. (cite) This was essential because one of the major causes in over-prescriptions is social pressure from patients. A worried parent will not want to consider the disadvantages of prescribing antibiotics to their child, even when the probability of antibiotics actually helping is slim. Therefore, this broad and multifaceted approach of educating the general public was key to the program’s success.

However, this multifaceted 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 their awareness efforts. Therefore, other resource-effective methods are needed to accompany the increasing awareness of the drawbacks to these miracle drugs.

One method that should be considered is the development of narrow focus antibiotics. Currently, antibiotics usually target some specific process in the cell, and so are specific to that mechanism. However, when antibiotics target a certain mechanism that is still 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. (8) One method of doing this is to use combination drugs, or cocktails. Cite book These are effective because the probability that a strain develops resistance to the combined effects of multiple drugs is lower than the probability of developing resistance to a single mechanism. This addresses the arms race challenge of antibiotic use. Does it address the challenge of retaining the composition of the natural microbiome?

One example of a narrow-spectrum antibiotic is fidaxomicin. This medication is intended to treat *Clostridium difficile* infection (CDI) and prevent *C. difficile*-associated diarrhea. Eiland *et al.* found that fidaxomicin was an effective against 96% of the subjects tested. (9) Louie *et al.* found that fidaxomicin was just as effective as vancomycin, a broad-spectrum antibiotic that is usually used against CDI. (10) It was found that fidaxomicins’ efficiency was due to its ability limit spore production of C. difficile as well as its decreased impact on the normal protective microbiota. (9, 10) The effect on the composition of the microbiome was measured using ribosomal RNA probes and quantitative real-time polymerase chain reaction. (10) 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, the researchers were able to observe 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 cost of this drug is extremely high, and the development of narrow-spectrum medications is time consuming and resource-intensive. (9) Therefore, parallel solutions are needed.

Another approach could be the development of vaccines that select for resistant strains. Currently, vaccines reduce the need for antibiotics by creating antibodies in the immune system using an inactive form of the disease. The creation 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 or indirect protection. (11) Vaccines have been incredibly effective at saving lives, but they do not solve the problem of antibiotic resistance. Cite book The efficiency of the vaccine depends on how closely it matches with circulating strain of that year. Every year, a new flu vaccine is developed to account for the evolution of the strain since the previous year. If an individual is infected with a different or evolved strain, the vaccination does not help the immune response. Cite. The design and development of the annual flu vaccine is becoming more and more difficult, since the high selection pressure increases the number of parallel strains that may be circulating, and decreases the efficiency of the vaccine. Therefore, the vaccination approach should be updated to account for the increasing problem of antibiotic resistance.

Vaccines should be adapted, much like antibiotic themselves, to be more specific. However, instead of targeting certain diseases, vaccines should go one step further and target certain strains. Vaccines could select for bacterial strains that are antibiotic susceptible, to enhance and ensure the continued efficacy of antibiotics. This could be done by “specifically targeting resistant alleles of a conserved protein or by targeting proteins uniquely present in resistant isolates.” (11) Like the development of new antibiotics, the discovery and design of new vaccines is slow and resource-intensive. Therefore, alternatives are required.

Another approach to decrease antibiotic overuse is probiotics. “Probiotics are live microorganisms that when administered in adequate numbers confer a health benefit on the host.” (5) The challenge here 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 combination will be effective.

We have seen that the measuring of a template microbiome in terms of composition/diversity/population as well as obtaining genomic data for individual species would be helpful in these advancements. What can be done? How can this be approached?

Technology should be used to address the challenge of defining a reference microbiome.

The first advantage of using technology to determine a reference microbiome is that technology is useful for large datasets. 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 will be essential in storing and processing this data.

In addition to simply storing data, technological approaches can contribute algorithms and patterns to abstract and distill 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.” (12) This could be implemented by using clustering algorithms and unsupervised machine learning. These approaches aim to eliminate noise and address the challenge of the “inherent baseline variability of the microbiota.” (3)

The goal of the human microbiome project is two fold: researchers need to distinguish intra and inter personal variation to define a reference microbiome, and be able to obtain genomic data from an individual species present in the community.

The defining of a reference microbiome is similar in motivation to the defining of a human genome. The equivalent could be applied to measure the “normalcy” of a microbiome. Clustering algorithms such as principle component analysis (as mentioned above) could be used to eliminate noise due to intra-personal variation and find significant trends in inter-personal variation. (3) A standard baseline could be established so that changes in the microbiome induced by antibiotics can be detected and assessed in terms of the risks of antibiotic usage. 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. We have seen this in the Venezuela example.

The isolation of genomic data from a certain species will be helpful in developing targeted antibiotics and vaccines, as mentioned above. This is because certain genes or proteins could be identified in the target pathogens, and their absence noted in the community as a whole. This would help the efficiency of identifying unique mechanistic targets of pathogens. Furthermore, computer based methods can be used to maximize molecular binding in a virtual environment. Cite book. This could improve the efficiency of drug design and reduce the time and resource intensive process.

The quantification of the microbiome could also be useful in determining causative factors between the microbiome composition and disease. Machine learning algorithms could be used to link “life histories, behaviors, environments, and exposures” with predisposition to diseases. (12) Therefore, technology can identify risk factors. This application could provide motivation for further research more directly than the other applications, since knowledge of risk factors could benefit patient care in the short term as well as the long term.

The biggest challenges that face the human microbiome project are similar to the ones that faced 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 “fingerprint” microbial signature. Furthermore, an individual’s microbiome can change over time due to significant changes in environment or health, even without the irreversible impact of antibiotics. Therefore, how can a reference microbiome even be possible?

These simplified models may not reflect the diversity of the microbiomes of a population, but still may provide enough information to assess antibiotics for their impacts on the microbiome. This is how the Venezuela paper dealt with generalized representations of the microbiome of a population. This paper also used a variety of samples to observe microbial diversity. (13) This is because the microbiome is both individual, temporal, and spatially unique. While this may seem like another complicating factor, there are links between the diversity of one site and the diversity of another. Therefore, the collection of multiple samples may provide more convincing evidence of the level of diversity of an individual microbiome.

The next challenge is that the microbial genomes themselves are also changing. A microbial genome can change quickly due fast replication rates and gene transfer via conjugation, transduction, and transformation. Therefore, reference genomes for bacteria are largely over-simplified. Will these templates provide useful to researchers interested in targeting specific and evolved strains in the field? How will the templates be updated? One approach is to update the template and reference genome as each new instance is added. This will take into consideration the many different levels of diversity present in a population. By comparing a bacterial genome to the mean of many samples of that species, the estimation of normalcy is much more accurate.

Still, there will always be an arms race between antibiotics and the microbial genome/bacteria. However, even limited (and outdated) knowledge of the target microbial genome may provide insights for targeted antibiotics. This is why technology is useful for observing the microbiome. It has been seen that the microbiome is an essential factor contributing to human health, which suggests that further research will be essential for figuring out this system. Already, there have been astounding findings, such as in the Venezuela study. They found “unprecedented of bacterial diversity”, which could translate into a stronger immune system. (13) Therefore, the study of the microbiome is in its infancy, and technology will allow analyses and great discoveries that will contribute to advancements in modern medicine.

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