Identification of Archaea Associated with Recovery from Antibiotic Exposure

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Abstract

The human gut microbiome is a complex system of microorganisms unique to each person. Balance in the gut microbiome is essential to maintaining host health. Dysbiosis, or a microbial imbalance, is especially concerning in the gut microbiome, as it can lead to neurological and immunological disorders, as well as poor cancer treatment response. Dysbiosis can be caused by many factors, but antibiotic usage is one of the main ones. Long-term antibiotic usage is linked to increased risk of infection and various types of diseases if the gut microbiome does not recover post-exposure. However, it is likely that there are certain normal microbiota of the human gut that can indicate whether a person's gut microbiome will recover. Bacteria make up many of these species, but there are fungi and archaea, also normal microbiota of the gut microbiome, that may similarly be associated with recovery. This study focuses on finding out if there are any archaeal species which are recovery-associated using Qiita processing software to compare alpha diversity over three timepoints and archaeal species count differences between recovers and non-recoverers. The results suggest that there are no species of archaea which are associated with recovery from antibiotic exposure due to insignificant differences in species counts between recoverers and non-recoverers.

Introduction

The human microbiome is a complex system of microorganisms that encompasses the entirety of the human body. From the species present in the oral cavity to those present on the skin, bacterial and fungal cells make up the majority of the human microbiome. Each person's microbiome is unique to themselves (Marchesi, J. R. et al 2016), and balance in a person's microbial community is necessary, as these microorganisms are believed to play important roles in maintaining host health, especially regarding energy and xenobiotic metabolism, and immune system stability (Marchesi, J. R., et al 2016).

Dysbiosis, or a microbial imbalance, is especially concerning in the gut microbiome. The gut microbiome is made up of the microbial communities in the gastrointestinal tract, including the stomach, large intestine, and small intestine. An imbalance of microbial species in the gut microbiome can be caused by either commensal species over-colonizing or pathogenic species colonizing in a niche normally taken by a commensal species (Donskey, C. J. 2004; Paterson, M. J., et al 2017), among other things. This imbalance has not only been shown to cause diseases of

the gut such as Crohn's disease, irritable bowel syndrome, and celiac's disease (Kostic, A. D., et al 2014), but it has been associated with immunological and neurological diseases (Gilbert, J. A., et al 2016) and poor response to cancer immunotherapy (Routy, B., et al 2018).

One of the many things that have been found to cause gut dysbiosis is the use of antibiotics. Antibiotics do, of course, help remove infections and pathogenic species, but they get rid of the useful microorganisms as well (Chng, K. R., et al 2020). This allows for empty niches in the gut microbiome that can be filled by other pathogenic species, antibiotic-resistant survivors of the original pathogen, or an overgrowth of a normal microbe (Donskey, C. J. 2004). Studies suggest that long-term effects of antibiotic usage may include increased risk of infection (Langdon, A., et al 2016), immunological diseases (Livanos, A. E., et al 2016), and metabolic diseases (Cox, L. M., et al 2015).

The role of bacteria in the gut microbiome has been extensively studied. 99% of the gut microbiome is thought to be made up of bacterial species, Bacteroidetes and Firmicutes being the most dominant phyla present (Qin, J., et al 2010). The fungal presence in the gut microbiome, on the other hand, is not nearly as well-defined or well-researched as the bacterial presence. Just under 280 fungal species have been identified as normal microbiota (Hamad, I., et al 2016), many of which are of the genus *Saccharomyces* or *Candida* (Hoffman, C., et al 2013). However, dysbiosis of the gut mycobiome has been associated with certain diseases. One study noted a decrease in fungal diversity in Type 2 diabetes mellitus (Al Bataineh, M. T., et al 2020). Another study noted an increase in pathogenic fungi in the gut microbiome in patients with bacterial keratitis (Jayasudha, R., et al 2018). Though under-researched, fungi in the gut microbiome play an important role in maintaining host health.

Using data from a previous study (Raymond, F., et al), this study aims to use the microbiome-analysis tool Qiita (Gonzalez, A., et al 2018) to determine whether there are fungal species that are correlated with gut microbiome health and recovery from antibiotics.

Methods and Materials

Data Accession: The data used in this study was obtained from the European Nucleotide Archive from the study accession number PRJEB8094. It was originally collected for the study "The initial state of the human gut microbiome determines its reshaping by antibiotics"

(Raymond, F., et al 2015), and was found via the study "Metagenome-wide association analysis identifies microbial determinants of post-antibiotic ecological recovery in the gut" (Chng, K. R., et al 2020). The data was formatted for shotgun metagenomics, and the original study analyzed fecal samples from healthy individuals after administering antibiotics. Information about the data is located in Table 1.

Upload to Qiita: The data was uploaded by the Qiita Support Team under the title "The initial state of the human gut microbiome determines its reshaping by antibiotics" and study ID 13508. 72 samples were uploaded.

BIOM Preparation: Preparation of the samples into usable BIOMS was performed by the Qiita Support Team. A preparation file was uploaded for each sample under the category of Metagenomic preparations. Each preparation was trimmed using the Atropos v1.1.24 processing command and then sorted into taxonomic prediction groups using the Woltka v0.1.1 processing command. This processing produced 6 BIOMS for each preparation.

Creation of Analysis: The BIOM entitled 'Taxonomic Predictions-species' created during the BIOM preparation was added to an analysis called "The initial state of the human gut microbiome determines its reshaping by antibiotics." This created a new artifact called dflt_name (BIOM).

Identification of Gut Microbiota: The dflt_name (BIOM) artifact was processed using the command 'Visualize taxonomy with interactive bar plot [barplot],' creating a new artifact entitled 'visualization (q2_visualization).' The bar plot was then filtered by taxonomic level, producing a kingdom-level bar plot.

Diversity Tests: An alpha diversity test was run on dflt_name (BIOM) artifact. The alpha diversity test run was Simpson's Index test. The Simpson's Index is a diversity test that accounts for number and relative abundance of species present in a community, and uses the formula $D = 1 - (\frac{\Sigma n(n-1)}{N(N-1)})$ where n equals the total number of organisms of a species and N equals the total number of organisms of all species. The artifact was processed using the command 'alpha diversity [alpha].'

Classification into Recoverers and Non-recoverers: Recoverers and non-recoverers were determined by their post-antibiotic gut microbial diversity using the Simpson's Index alpha diversity test results. The test was used to compare pre-exposure microbial diversity and post-exposure microbial diversity, in that recoverers had a similar or higher post-exposure

microbial diversity to pre-exposure microbial diversity, and non-recoverers had a lower microbial diversity post-exposure than pre-exposure.

Identification of Recovery-Associated Microbiota: Separate analyses were done on the non-recoverer samples and the recoverer samples. Each analysis had its own artifact. Both artifacts were processed using the command 'Visualize taxonomy with interactive bar plot [barplot],' and a bar plot showing the species taxonomic level was generated. The archaeal features were specifically looked at, and a two-tailed student-t test was performed to define the difference between recoverer and non-recoverer archaeal features with the top five species in each group for the three time points (Day 0, Day 7, Day 90). The hypotheses associated with this test are

H_0: $\mu_1=\mu_2$

H_A: $\mu_1\neq\mu_2$

Where μ_1 is equal to the mean of the recoverer data and μ_2 is equal to the mean of the non-recoverer data. The alpha level used was 0.1.

Results

The Gut Microbiota— Kingdom

Only two out of seven kingdoms were represented within the samples, Bacteria and Archaea. Out of those two kingdoms, between 99% and 100% of the taxonomic predictions for the samples were of the kingdom Bacteria. Only 0% to 1% of the taxonomic predictions were of the kingdom Archaea. None of the samples contained any microbiota of the kingdom Fungi. This data is shown in Figure 1. Figure 1 also shows the number of Archaea features found in each sample to differentiate it from the Bacteria, with the highest count being 26164 in sample P19E0, and many samples showing no archaeal features.

Alpha Diversity

The Simpson's Index alpha diversity test was run on Qiita to determine the overall microbial diversity of the samples. Scores in this index can range from 0 to 1, with 0 indicating low diversity and 1 indicating high diversity. On this particular test, the scores ranged from 0.577 at sample_title P4E0 to 0.978 at sample_title P19E0. The mean index score was 0.926, and the median index score was 0.939.

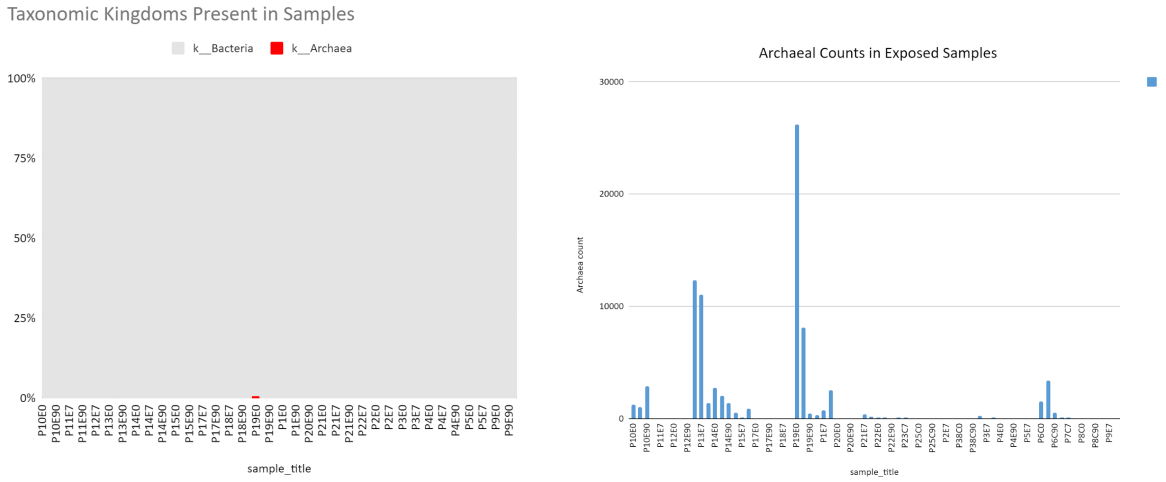

Figure 1: (Left) A bar plot of the taxonomic kingdoms present in all of the samples, exposed and not exposed. No fungi were detected in any of the samples. Within each sample, 99-100% of the microbiota found were of the kingdom Bacteria, and 0-1% of the microbiota found were of the kingdom Archaea. (Right) To highlight the miniscule presence of archaea while still giving a clearer image, a chart of how many archaeal features were detected in each sample. Many samples had no archaeal presence at all.

Recoverers and Non-recoverers

The individual sample results from the Simpson's Index alpha diversity tests were used to classify the samples into recoverers and non-recoverers. As each host had multiple experiments associated with it, the average index score across a set of experiments was used to determine the diversity at each time point. The samples which showed a lower post-exposure diversity score than the pre-exposure score (P1, P5, P15, P18, P19, P20) were classified as non-recoverers. These diversity scores were averaged to create Figure 2-a, which shows the decrease in microbial diversity post-exposure to antibiotics. The samples which showed either a similar post-exposure microbial diversity or a higher microbial diversity (P2, P9, P10, P11, P12, P13, P17, P21, P22) were classified as recoverers. These diversity scores were averaged to create Figure 2-b, showing an overall increase in diversity post-exposure to antibiotics. Three samples, P3, P4, and P14 were excluded from this analysis due to anomalous microbial diversity across the timepoints.

Gut Microbiota of Recoverers vs Non-recoverers

There were no fungi present in either group, recoverers or non-recoverers, however there were archaea present. As previous studies focused on bacterial presence, the archaeal presence was

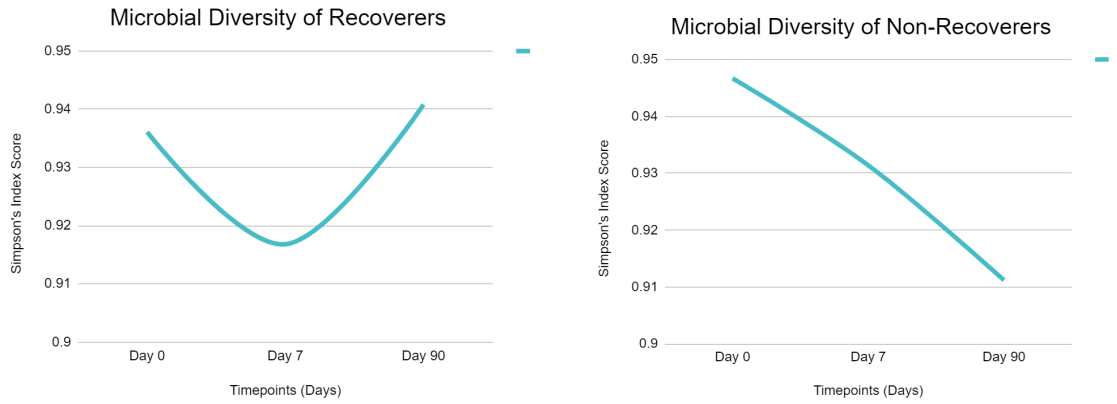

Figure 2: Graphs representing the Simpson's Index Scores for the samples over the three collection timepoints. The Simpson's Index Scores were averaged for each sample, sorted in recoverers and non-recoverers, and then averaged again to get the overall microbial diversity at each time point for both groups. As seen on the left, the recoverers showed a similar post-exposure diversity as pre-exposure, and the non-recoverers showed a lower post-exposure diversity than pre-exposure.

focused on to determine if there was a difference between the archaeal presence in recoverers and non-recoverers. The top two species found at each time point, in both recoverers and non-recoverers, were *Methanobrevibacter smithii* and *Methanobrevibacter smithii* CAG:186. With the exception of the *M. smithii* CAG:186 count in non-recoverers on Day 90, both species had counts in the thousands for all timepoints. The next three species generally differed by time point and recovery status. On Day 0, the next three species for recoverers were *Methanocorpusculaceae archaeon Phil4, Methanosarcina mazei*, and *Methanobrevibacter millerae*. The next three species for non-recoverers were *Methanosarcina mazei, Methanomicrobiales archaeon Methan_05,* and *Methanobrevibacter millerae*. On Day 7, the next three species for recoverers were *Methanosarcina mazei, Methanocorpusculaceae archaeon Phil4,* and *Methanobrevibacter millerae*. The next three species for non-recoverers were *Methanosarcina mazei, Methanomicrobiales archaeon Methan_05,* and *Methanocorpusculaceae archaeon Phil4*. On Day 90, the next three species for recoverers were *Methanosarcina mazei, Methanocorpusculaceae archaeon Phil4,* and *Sulfolobus acidocaldarius*. The next three species for non-recoverers were *Methanosarcina mazei, Methanomicrobiales archaeon Methan_05,* and *Methanocorpusculaceae archaeon Phil4*.

T-test

T-tests were performed on the mean count of the top five species in each group between recoverers and non-recoverers for all three time points. On Day 0, the species tested were *Methanobrevibacter smithii, Methanobrevibacter smithii CAG:186, Methanobrevibacter*

Species	Sum Recoverers: Day 0	Sum Non-Recoverers: Day 0	p-value
Methanobrevibacter smithii	$9.55*10^3$	$1.85*10^4$	0.55
Methanobrevibacter smithii CAG:186	$3.90*10^3$	$7.75*10^3$	0.55
Methanobrevibacter millerae	21.8	35.8	0.58
Methanosarcina mazei	73.0	$5.41*10^2$	0.39
Methanocorpusculaceae archaeon Phil4	87.6	25.4	0.52
Methanomicrobiales archaeon Methan_05	2.89	$1.00*10^2$	0.36

Species	Sum Recoverers: Day 7	Sum Non-Recoverers: Day 7	p-value
Methanobrevibacter smithii	$8.55*10^3$	$6.10*10^3$	0.96
Methanobrevibacter smithii CAG:186	$3.45*10^3$	$2.49*10^3$	0.95
Methanosarcina mazei	$4.53*10^2$	$1.47*10^2$	0.52
Methanocorpusculaceae archaeon Phil4	22.5	32.7	0.48
Methanomicrobiales archaeon Methan_05	0.750	$1.01*10^2$	0.36
Methanobrevibacter millerae	19.5	17.2	0.82

Species	Sum Recoverers: Day 90	Sum Non-Recoverers: Day 90	p-value
Methanobrevibacter smithii	$2.93*10^3$	$2.12*10^3$	0.94
Methanobrevibacter smithii CAG:186	$1.18*10^3$	$8.82*10^2$	0.92
Methanosarcina mazei	$1.92*10^2$	$8.11*10^2$	0.42
Methanocorpusculaceae archaeon Phil4	47.2	27.4	0.90
Methanomicrobiales archaeon Methan_05	11.0	31.2	0.47
Sulfolobus acidocaldarius	15.5	4.29	0.32

Figure 3: Charts showing the six species t-tests were performed on, the sum for recoverers and non-recoverers, and the p-values associated with those t-tests for each time point. For Day 0 (Top), the highest p-value was 0.58 with *Methanobrevibacter millerae* and the lowest was 0.36 with *Methanomicrobiales archaeon Methan_05*. For Day 7 (Middle), the highest p-value was 0.96 with *Methanobrevibacter smithii* and the lowest was 0.36 with *Methanomicrobiales archaeon Methan_05*. For Day 90 (Bottom), the highest p-value was 0.94 with *Methanobrevibacter smithii* and the lowest was 0.32 with *Sulfolobus acidocaldarius*.

millerae, Methanosarcina mazei, Methanocorpusculaceae archaeon Phil4, and *Methanomicrobiales archaeon Methan_05.* The respective p-values associated with the t-tests are 0.55, 0.55, 0.58, 0.39, 0.52, and 0.36. On Day 7, the species tested were *Methanobrevibacter smithii, Methanobrevibacter smithii CAG:186, Methanosarcina mazei, Methanocorpusculaceae archaeon Phil4, Methanomicrobiales archaeon Methan_05,* and *Methanobrevibacter millerae.* The respective p-values associated with the t-tests are 0.96, 0.95, 0.92, 0.46, 0.36, and 0.82. For Day 90, the species tested were *Methanobrevibacter smithii, Methanobrevibacter smithii CAG:186, Methanosarcina mazei, Methanocorpusculaceae archaeon Phil4, Methanomicrobiales archaeon Methan_05,* and *Sulfolobus acidocaldarius.* The respective p-values associated with the t-tests are 0.94, 0.92, 0.42, 0.90, 0.47, and 0.32.

Discussion

As expected, the majority of the microbiota present in the samples were bacteria. This is in line with previous research that has been done (Qin, J., et al 2010), where around 99% of the predicted microorganisms were bacteria. There was no detected fungal presence in any of the samples, which is what the study was originally intended to look for. This suggests that the hypothesis that there are fungal species associated with recovery from antibiotics is not supported by the data.

The absence of fungi is odd, considering that there were studies showing the impact of fungi on human health. There are a few potential explanations for this absence. The first is that fungi may not be as important to the gut microbiome as they seem. Perhaps the studies that have shown their impact are only basing it off of the population of a single group, where diet and environment might change the makeup of the microbiome. Another possibility is that there were fungal species, but they were unrecognizable to Qiita's software, either because the data was in an unreadable format or the software could not detect genes associated with fungi. A third explanation could be that the collection type for the data was mislabeled. The data should have been collected for a metagenomics study, which would be able to detect fungi, bacteria, and archaea. However, as many papers are, the collection could have been for 16S data. 16S sequencing refers to the 16S ribosomal RNA that only bacteria and some archaea have. Fungi have a similar type of rRNA, but not 16S specifically, and so would not be found using that method.

Even though no fungi were detected, there were archaeal species detected. Only 0-1% of the detected microbiota were archaea per sample, but there was still a noticeable amount. As such, the study switched to focusing on archaeal association with recovery from antibiotics. Further reading suggested that the species *Methanobrevibacter smithii* has been identified as the most common in the gut (Miller, T. L., et al 1982). This is in line with the results of the study, as both the recoverers and non-recoverers showed high counts of *Methanobrevibacter smithii*, to the point that the most prevalent archaeal species was *M. smithii* for both.

There was no time point at which a significant difference between the archaeal counts of the top five species for recoverers and non-recoverers was found. On Day 0, pre-exposure, the lowest p-value was found with *Methanomicrobiales archaeon Methan_05*, which was the fourth most prevalent species for non-recoverers and unranked for recoverers, at 0.36. The rest of the p-values were higher. On Day 7, during the antibiotics course, the lowest p-value found was with *Methanomicrobiales archaeon Methan_05* as well. Similarly to Day 0, *M. archaeon Methan_05* was fourth for non-recoverers and unranked for recoverers. This is the case with Day 90 as well. This could potentially mean that the higher the level of *M. archaeon Methan_05*, the less likely the chances of recovery, but the p-values for all time points fall above the alpha level and so the difference is not significant. The p-value at Day 7 for *M. archaeon Methan_05* was 0.36, and the rest of the species were higher. On Day 90, the lowest p-value was found with *Sulfolobus acidocaldarius*, ranked fifth for recoverers and unranked for non-recoverers. This species was unranked for both groups at the rest of the time points, which could potentially indicate an association between recovery and *S. acidocaldarius*. However, the p-value was 0.32 and so the difference in this experiment was not significant. On Day 90, the rest of the p-values were greater than 0.32. As every p-value calculated, no matter the time point, was greater than 0.1, none of the differences were significant and the null hypothesis must be accepted. According to the results of this study, there likely are no archaea associated with recovery from antibiotics.

Though it would not affect the results, as they were excluded from the recoverer and non-recoverer groups for analysis, there were some anomalous samples. Most of the samples followed the pattern of having a relatively high pre-exposure diversity, a lower diversity during exposure, and then either regaining a high post-exposure diversity or dropping to a low post-exposure diversity. However, there were three samples which followed a different pattern of diversity. P3 had relatively low pre-exposure and post-exposure microbial diversity, but the

diversity jumped only during the antibiotics course, and by a score of around 0.03. P4 started with a fairly low diversity, and then the diversity jumped during exposure and continued to increase for the post-exposure diversity. P14 had a relatively low pre-exposure microbial diversity, but the diversity during the course and post-exposure were similar and relatively high. One explanation for this is that not all the samples were processed yet when this analysis was performed, and so it is possible that some samples that would've made these more normal were unable to be analyzed with the data. Another explanation may be that the hosts these samples came from were sick or otherwise had their gut microbiomes impacted prior to the antibiotics course, and as such were still recovering when the pre-exposure samples were taken.

Ideally, similar work will be done in the future. Based on the results of this experiment, next steps would be to study the complete absence of fungi from the samples, and to compare the archaeal presence within recoverer and non-recoverer groups between time points. The absence of fungi is strange, and studying the differences within recovery groups could show how the populations of archaea change during antibiotic exposure. Other future work to look into would be studying other data and seeing if fungi are present, and comparing those results to the results of this study to determine if non-fungal populations are affecting fungi levels.

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References

1. Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., ... & Mende, D. R. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *nature*, *464*(7285), 59-65.
2. Hamad, I., Raoult, D., & Bittar, F. (2016). Repertory of eukaryotes (eukaryome) in the human gastrointestinal tract: taxonomy and detection methods. *Parasite immunology*, *38*(1), 12-36.

3. Hoffmann, C., Dollive, S., Grunberg, S., Chen, J., Li, H., Wu, G. D., Lewis, J. D., & Bushman, F. D. (2013). Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. *PloS one*, *8*(6), e66019.
4. Raymond, F., Ouameur, A. A., Déraspe, M., Iqbal, N., Gingras, H., Dridi, B., ... & Frenette, J. (2016). The initial state of the human gut microbiome determines its reshaping by antibiotics. *The ISME journal*, *10*(3), 707-720.
5. Gonzalez, A., Navas-Molina, J. A., Kosciolek, T., McDonald, D., Vázquez-Baeza, Y., Ackermann, G., ... & Sanders, J. G. (2018). Qiita: rapid, web-enabled microbiome meta-analysis. *Nature methods*, *15*(10), 796-798.
6. Kostic, A. D., Xavier, R. J., & Gevers, D. (2014). The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology*, *146*(6), 1489-1499.
7. Chng, K. R., Ghosh, T. S., Tan, Y. H., Nandi, T., Lee, I. R., Ng, A. H. Q., ... & Barkham, T. (2020). Metagenome-wide association analysis identifies microbial determinants of post-antibiotic ecological recovery in the gut. *Nature Ecology & Evolution*, *4*(9), 1256-1267.
8. Al Bataineh, M. T., Dash, N. R., Lassen, P. B., Banimfreg, B. H., Nada, A. M., Belda, E., & Clément, K. (2020). Revealing links between gut microbiome and its fungal community in Type 2 Diabetes Mellitus among Emirati subjects: A pilot study. *Scientific reports*, *10*(1), 1-11.
9. Jayasudha, R., Chakravarthy, S. K., Prashanthi, G. S., Sharma, S., Garg, P., Murthy, S. I., & Shivaji, S. (2018). Alterations in gut bacterial and fungal microbiomes are associated with bacterial Keratitis, an inflammatory disease of the human eye. *Journal of biosciences*, *43*(5), 835-856.
10. Paterson, M. J., Oh, S., & Underhill, D. M. (2017). Host–microbe interactions: commensal fungi in the gut. *Current opinion in microbiology*, *40*, 131-137.
11. Donskey, C. J. (2004). The role of the intestinal tract as a reservoir and source for transmission of nosocomial pathogens. *Clinical infectious diseases*, *39*(2), 219-226.
12. Marchesi, J. R., Adams, D. H., Fava, F., Hermes, G. D., Hirschfield, G. M., Hold, G., ... & Thomas, L. V. (2016). The gut microbiota and host health: a new clinical frontier. *Gut*, *65*(2), 330-339.

13. Gilbert, J. A., Quinn, R. A., Debelius, J., Xu, Z. Z., Morton, J., Garg, N., ... & Knight, R. (2016). Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature*, *535*(7610), 94-103.
14. Routy, B., Le Chatelier, E., Derosa, L., Duong, C. P., Alou, M. T., Daillère, R., ... & Fidelle, M. (2018). Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. *Science*, *359*(6371), 91-97.
15. Livanos, A. E., Greiner, T. U., Vangay, P., Pathmasiri, W., Stewart, D., McRitchie, S., ... & Gao, Z. (2016). Antibiotic-mediated gut microbiome perturbation accelerates development of type 1 diabetes in mice. *Nature microbiology*, *1*(11), 1-13.
16. Cox, L. M., & Blaser, M. J. (2015). Antibiotics in early life and obesity. *Nature Reviews Endocrinology*, *11*(3), 182.
17. Langdon, A., Crook, N., & Dantas, G. (2016). The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. *Genome medicine*, *8*(1), 39.
18. Miller, T. L., Wolin, M. J., de Macario, E. C., & Macario, A. J. (1982). Isolation of Methanobrevibacter smithii from human feces. *Applied and environmental microbiology*, *43*(1), 227-232.