HIGH SCHOOL EDITION
Journal of Student Research
Volume 11 Issue 4 (2022)

Investigating the Role of Prescription Drugs on the Gut Microbiome

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ABSTRACT

The effect of prescription drugs on gut microbiome is a relevant and under-researched issue in the modern medicine-dependent world. The gut plays an important role in body function, and dysbiosis can influence the development of conditions such as obesity, diabetes, irritable bowel syndrome, inflammatory bowel disease, allergic reactions and many other human diseases. In this study we systematically organize and compare different commonly used prescription drugs. Studies based on murine and human subjects were analyzed on each of the three following classes: opioids, antidepressants, and marijuana to understand the role that these play on the microbiome. Opioids and antidepressants were shown to significantly change and modulate the microbial composition found in the gut that is associated with disease. Marijuana demonstrated the most conflicting results out of all three classes and needs further investigation. This information is significant for patients that are currently using these drugs as well as physicians to understand the role that these drugs play inadvertently, and to inform physicians to be more cautious when prescribing these drugs.

Introduction

The definition of the microbiome is the wide range of microbes found within a given environment, and this is also composed of each individual organisms' genetic material (Hills et al., 2019). The main focus of this paper will be surrounding the gastrointestinal tract, but many researchers are beginning to realize that there are very few sterile areas that do contain a microbiome. Within the gut microbiome, there are over 200 different prevalent microorganisms that consist of bacteria, viruses, and fungi (Hills et al., 2019). The dominant gut microbial phyla found are *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria,* and *Verrucomicrobia.* There are many roles that each of these bacterial communities play. *Firmicutes* break down food, and metabolites in the gut that digestion enzymes cannot digest such as carbohydrates. *Clostridia* is well known for playing immunological roles, and helps soften the effects of allergic reactions so it does not cause damage to the body. *Actinobacteria* aides with gut permeability and *Proteobacteria* helps retain redox balance in the gut. Many of these bacteria that are being found to play important roles are still being sequenced, and their biological functions are still being defined. These important bacteria can be changed based on dietary patterns, and environmental factors. The most well known and researched are how humans come out of the womb (natural vs. C. section), diet in early life (breast milk. Vs. formula), and antibiotic use. These microorganisms play influential roles as discussed above, and have formed a symbiotic relationship within us. Some of these roles are in physiology, homeostasis, development, immunity, and metabolism (Bull & Plummer, 2014). Therefore, when the gut is disrupted, this can prevent normal body functions and can influence the development of conditions such as obesity, diabetes, irritable bowel syndrome, inflammatory bowel disease, depression, cardiovascular disease, infection, and allergic reactions. For example, many bacteria prevent pathogen colonization by producing antimicrobial compounds, and outcompeting the pathogens for nutrients and sites of attachment on the gut epithelial lining, this is a concept known as *competitive-exclusion effect* (Bull &

Plummer, 2014). If this process were disrupted, pathogens would be allowed to enter the epithelial cells. However, diet and environment are not the only two factors that can lead to adverse effects on the gut. Many medical interventions that use non-antibiotic drugs can affect the diversity of the microbiome (Banerjee et al., 2016). However, this is a novel area of research. This is an issue that is being investigated because commonly prescribed drugs may adversely affect the gut microbiome diversity in patients. Before scientific discoveries can be made and implemented there are more studies that need to be done in order to understand this. A commonly prescribed drug that will be discussed in this paper are opioids to manage pain. Morphine is a widely used, pain-relieving drug and is isolated from opium poppy (Wang et al., 2018). This is a drug that is known for being very highly addictive and can also be deadly.

Statistics demonstrate that in the US 10% of the population has abused opioids in their lifetime (Juergens, 2014). The number of morphine addicts admitted to the emergency room increased by 106% between 2004-2008 (Juergens, 2014). There has been research that demonstrates that opioid use is commonly prescribed but has been known to cause many issues such as constipation, gateway to other drugs, and can work as an immunosuppressant.

Another commonly prescribed class of drugs in the US are antidepressants. In the US over 3.4 million people in 2019 were prescribed antidepressant medication (Elflein, 2020). There is research being done to analyze whether or not antidepressants have been found to disrupt the microbiome. The third class of drugs that will be discussed in this paper is medical marijuana. Medical marijuana has obtained over 3.6 million users once legalized (Rosenthal & Pipitone, 2020). Marijuana is derived from the plant cannabis sativa or cannabis indica.

All three categories of these drugs have different chemical properties as will be shown in the table at the end of this paper, so there are various different ways that each of these drugs could affect the microbiome or target a specific strain and this also could be concentration dependent. This project not only will be informative and will analyze differences in microbiome composition when exposed to these different classes of medical interventions, but will also inform patients and doctors to help them make the right decisions.

Methods

The experiments examined in this article are from the years 2011-2022 in order to keep research more recent and relevant. Evidence of the effects of non-antibiotic drugs on gut microbiomes will be compiled from both human and rodent data. The subjects chosen, the methods used to administer the drugs, the amount and type of drugs, how long the experiment spanned, and the experimental methods will all be detailed. The changes in microbiome diversity will also be noted and summarized in a table.

Analysis Part 1: Opioids

The first drug that will be analyzed is a specific type of opioid known as morphine. This is one of the most common opioids prescribed in healthcare, and there is evidence that this disrupts the microbiome which will be supported by multiple papers that are discussed below.

There has been research that opioid use is commonly prescribed but has been known to cause many issues within the gastrointestinal issues, as demonstrated in the Banerjee lab, they demonstrate for the first time that morphine fosters significant gut microbial dysbiosis through disrupting cholesterol and bile acid metabolism (2016). Cholesterol and bile acid metabolism are important processes in the gastrointestinal gut and are important for the facilitation of lipids, and absorb cholesterol and bile acid through the digestive tract.

To evaluate this, morphine was administered into the mice, and then the fecal matter was collected and extracted 72 hours later through genomic sequencing (Banerjee et al., 2016). When analyzing the genomic data there was not a huge shift in alpha diversity but there was a significant change in beta diversity. For example, in the fecal samples, *Firmicutes* had higher abundance and there was a reduction of *Bacteroidetes*. This ratio change has been

linked to evidence for inflammation, and inflammatory bowel disease (Banerjee et al., 2016). There were also other elevated levels of bacteria found in these samples such as *Staphylococcaceae, Enterococcaceae, Bacilliceae, Streptococcaceae,* and *Erysipelotrichaceae.*

Not only was this disruption seen but there was also an imbalance of cholesterol and bile acids. To evaluate whether there were changes to this they used WT and TL2KO mice (these are mice that lack TL2, TL2 is a very influential protein that is found in the immune system, and blocks the efflux of cholesterol into the macrophage). These mice were implanted with a placebo, or a morphine pellet. The specific genomic sequencing that was done on these mice's fecal samples was mass spectrometry to measure the abundance of target bile acids, and lipids (Banerjee et al., 2016).

There was a high level of coprostanol which is a compound that has been found to decrease the amount of cholesterol consumption and is linked to cardiovascular disease. Ursodeoxycholate was significantly downregulated which is a bile acid that prevents cirrhosis of the liver and is linked to liver disease (Luketic & Sanyal, 1994).

The next lab that also investigated this idea was the Wang lab. This lab demonstrated that morphine causes a shift in metabolism, and a shift in the microbiome. This is a short-term model however, and only accounts for six days of morphine treatment. Fecal samples were collected from the control mice; these pellet treatments included placebo, naltrexone (a drug that is prescribed to those recovering from opioid addiction), morphine, and morphine plus naltrexone (Wang et al., 2018). Principal coordinate analysis was used in order to examine, and determine the microbial composition differences in all four groups of mice. There was an increase of different microbial groups such as *Flavobacterium, Enterococcus, Fusobacterium, Sutterella,* and *Clostridium.* Enhanced ratios of these bacterial communities cause issues with regulation of the cell cycle which typically indicates cancer formation (Wang et al., 2018). Expression profiling of species-specific 16srRNA genes, through quantitative real-time PCR demonstrated an increase in *Enterococcus faecalis* throughout post treatment, and this amplified over 100 times greater in the morphine group than in the other three groups on day 3 (Wang et al., 2018).

Fecal samples were collected over the next four days, and liquid chromatography-mass spectrometry were analyzed for their metabolite profile. This test demonstrated that the morphine-treated mice had a different metabolite profile compared to the placebo-treated mice (Wang et al., 2018). Some of the alterations in the metabolite profile included deoxycholic acid, and phosphatidylethanolamines. Increases in these metabolites are linked to obesity, diabetes, and cardiovascular disease (Wentworth et al., 2016).

The last study that will be analyzed is from the Acharya lab, which investigated how chronic opioid use could be disrupting the microbiome, and causing gut dysbiosis to those that have other underlying conditions such as cirrhosis (2016). In this study there were 200 patients and were split up into two groups which were opioid use, or those with placebos. This was over a 6-month period (Acharya et al., 2016). Methods that were used to analyze this analysis were stool microbiota composition, specifically using multi-tag sequencing (a parallel sequencing method that gives higher resolution than the traditional sanger sequencing method), and PiCrust (phylogenetic investigation of communities package in python). Acharya found that there was a significant increase in gut dysbiosis, and that there was a significant increase in *Bacteroidetes.* The PiCrust analysis also revealed that there was an increase in aromatic amino acids, and endotoxin production. Increase in production of aromatic amino acids has been linked to liver disease (Tajiri & Shimizu, 2013). Summary table of all this information is found below.

Summary

Species	Results	Drug type	Normal function of bacterial strain in microbiome
Staphylococcaceae	Increased	Morphine	Commensal typically found in on the human epithelial skin
Enterococcaceae	Increased	Morphine	Opportunistic–normally commensal, but under right conditions can be pathogenic
Bacillaceae	Increased	Morphine	Germination of other species in the intestine and regulating intestinal conditions
Streptococcaceae	Increased	Morphine	Plays a role in the production of lactic acid
Erysipelotrichaceae	Increased	Morphine	Some species found to be pathogenic; increased abundance with colorectal cancer, altered levels in inflammation gastrointestinal diseases, correlation with high cholesterol metabolites
Flavobacterium	Increased	Morphine	Most are harmless, but some are opportunistic or true pathogenic
Fusobacterium	Increased	Morphine	Commensal found on mucous membranes of upper respiratory and gastrointestinal tracts
Sutterella	Increased	Morphine	Function unclear, suspected to be commensal and play a role in pathogenesis of IBD
Clostridium	Increased	Morphine	Ferment nutrients such as carbohydrates and proteins; some are pathogenic and can cause deadly diseases such as tetanus, botulism, and gas gangrene
Clostridiales XIV	Significantly decreased	Variety of opioids	Commensal, associated with intestinal immune homeostasis, resistance to intestinal inflammation and allergies; patients with IBD are found to have lower abundance
Bifidobacterium	Increased	Variety of opioids	Beneficial bacteria associated with good health and sometimes used as probiotic therapy; low levels associated with metabolic, immune, and intestinal diseases
Parasutterella	Decreased	Variety of opioids	Associated with various health outcomes, increased abundance found to decrease kyuneric acid (end product of tryptophan metabolism)
Peptostreptococcaceae	Increased	Variety of opioids	Abundance of certain species associated with colorectal cancer

Species	Results	Drug type	Normal function of bacterial strain in microbiome
Lachnospiraceae	Decreased	Variety of opioids	Impact on host physiology is inconsistent across various studies, produces beneficial metabolites, but has been associated with NAFLD, IBD, CKD, MSS, and the onset of obesity and diabetes
Bacteroidaceae	Decreased	Variety of opioids	This has been known to play an immense role in pathogen protection, and supplying nutrients to other symbiotic microbes that could be beneficial for the human body
Ruminococcaceae	Significantly decreased	Variety of opioids	One of the most abundant bacterial groups in healthy adults, lower abundance associated with IBD

Analysis Part 2: Antidepressants

The next section of the analysis will highlight antidepressants and their effects on microbial communities. The compounds that will be discussed are fluoxetine, escitalopram, venlafaxine, duloxetine, desipramine, phenelzine, bupropion, and aripiprazole. Fluoxetine is an antidepressant that belongs to a group of medications known as selective serotonin reuptake inhibitors (SSRIs). Escitalopram is a drug that is used for treating anxiety and depression. Venlafaxine is a drug that is used for treating depression, social anxiety, and panic disorder, and belongs to the same drug group as fluoxetine. Duloxetine is a drug that is used to treat depression and anxiety, and fibromyalgia. Phenelzine is an irreversible monoamine oxidase inhibitor that targets depression and anxiety. Aripiprazole is an antipsychotic.

The first study that will be analyzed was from the Lukić lab, this lab looked into multiple different antidepressants which were fluoxetine, escitalopram, venlafaxine, duloxetine, and desipramine in the mouse model (2019). Then, stool and behavioral tests were conducted 21 days later after intraperitoneal injection of the drugs. In acclimated behavior rooms, mice were tested for depressive-like behavior, which required tail suspension test, forced swim tests, and the sucrose preference test (Lukić et al., 2019). 1 hour after i.p. injection of antidepressants, 16s rRNA DNA was isolated and analyzed using the QIIME 1 pipeline for alpha diversity, community richness, community evenness, and beta diversity (Lukić et al., 2019). Linear discriminant analysis effect size method and permutational multivariate analysis of variance was done in order to identify differences in relative abundance of bacterial taxa. RNA was extracted from the medial prefrontal cortices in six samples, mRNA enrichment was done, and mRNA was sequenced and analyzed. QPCR was then used to confirm microbial species composition profiling (Lukić et al., 2019).

The alpha diversity analyses demonstrated that all antidepressants except desipramine reduced the richness of microbial communities. Beta diversity analyses demonstrated that fecal microbial communities had a higher beta diversity compared to the control group. All antidepressant groups demonstrated lower abundance in *Ruminococcus, Adlercreutzia*, and other undefined groups (Lukić et al., 2019). This lab then wanted to explore if *Ruminococcus flavefaciens* changed the effects of antidepressants and inhibited this drug. When *Ruminococcus* is present in high amounts the mice were less mobile and exhibited more depressive behavior as opposed to those that did not contain high amounts of *Ruminococcus*.

This is a very controversial paper because even though ruminococcus is able to inhibit duloxetine, this is also a very helpful bacteria in relieving constipation. Constipation is a typical gastrointestinal problem that occurs when patients are on antidepressants so further research will need to be evaluated to find a similar microbe to supplement, that doesn't inhibit duloxetine, but also helps with constipation (Lukić et al., 2019).

The second study that will be evaluated in this review is from the Chait lab. In this study there were six antidepressants that were tested to see if they had antimicrobial properties on various intestinal strains commonly found in the human gut (Chait et al., 2020). The six antidepressants which were Phenelzine, Venlafaxine, Citalopram, Desipramine, Bupropion, and Aripiprazole. The twelve intestinal strains that were evaluated came from these groups: *Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria,and Verrucomicrobia* . These strains were grown with oxidase to increase growth of anaerobic strains (Chait et al., 2020). For each strain, there were two plates, one with only medium, and then the other containing both medium, and the bacterial strain.

An agar-well diffusion test was performed. Each plate contains a single strain of the bacteria, and contains wells with varying concentrations of the antidepressants (Chait et al., 2020). The minimum inhibitory concentrations were found, and the samples were incubated for sixteen hours. Desipramine and aripiprazole had the strongest inhibitory effect out of all six drugs.

Desipramine had a strong inhibitory effect on 10 out of 12 intestinal strains that were tested (Chait et al., 2020). Viability of bacteria after antidepressant treatment was tested in terms of colony forming units, and expressed in logarithmic reductions. Desipramine and aripiprazole were able to completely inhibit *A. muciniphila* and *E. coli* specifically but aripiprazole had to be at a much higher dose than desipramine (Chait et al., 2020). Each bacterial strain such as *L. reuteri, C. leptum, B. fragilis, P. aeruginosa, L. casei, and B. animalis* were treated with desipramine which was able to inhibit replication but didn't completely inhibit growth (Chait et al., 2020).

The final study that will be analyzed in this section came from the Aydin lab. This was a study on type-1 diabetic rats who were tested for the effect of Reboxetine on the gut microbiome (Aydin). The rats were divided into four experimental groups in separate cages: saline-administered normoglycemic control group, saline-administered diabetic control group, RBX-treated normoglycemic group, and RBX-treated diabetic group (Aydin). 4 weeks post diabetes onset, RBX dissolved in saline was intragastrically administered for 14 days. Stool samples were collected on the fourteenth day, and DNA was isolated and sequenced.

Beta diversity analysis demonstrated that diabetic rats have a distinct microbiome compared to healthy rats, which was expected. However, RBX treatment significantly affected beta diversity in healthy and diabetic rates compared to the control. Relative taxa analysis reveals that RBX treatment reduced *Firmicutes* but increased *Bacteroides*, and *Proteobacteria* phyla (Aydin et al., 2021). In terms of families, *Prevotellaceae* increased, but *Lactobacillaceae* and *Clostridiaceae* decreased. Random forest analysis, this is a technique that assigns OTU's, and is a form of metagenomic sequencing that uses the 16sribosomal RNA to identify species in a particular sample, and fecal samples were analyzed in this study (Aydin et al., 2021). There was a total of 3782 OTU's identified even though there was not any significant difference on the species level there was a significant difference in diversity within the microbiome (Aydin et al., 2021). Unifrac analysis was able to identify the differences in the colonies found but was unable to identify the differences between which species were more commonly found in diabetic rats vs. the control rats when treated with RBX. When using the random forest analysis pipeline researchers discovered that *Fibrobacteres* and *Spirochaetes* were significantly more abundant in the diabetic fecal samples vs. the control fecal samples. When treated with RBX the fecal samples reduced *Firmicutes*, and increased *Proteobacteria and Bacteroidetes*. In both groups *Prevotellaceae* increased while *Lactobacillaceae* and *Clostridiaceae* both decreased with RBX treatment.

Summary

Species	Results	Drug type	Normal function of bacterial strain in microbiome
Ruminococcus	Inhibited	Fluoxetine, escitalopram, venlafaxine, duloxetine, and desipramine	Mutualistic, degrades complex polysaccharides to nutrients for host
Adlercreutzia	Inhibited	Fluoxetine, escitalopram, venlafaxine, duloxetine, and desipramine	This is important for converting daidzen to equol. This is a compound that helps with improving bone loss, and improving muscle function.
A. muciniphila	Inhibited replication	Desipramine	Degrades mucin, correlated with lower obesity, diabetes, inflammation, and metabolic disorders
E. coli	Inhibited replication	Desipramine	Compete with pathogens for nutrients and receptors, but some are pathogenic for diarrhea, dysentery, meningitis, and urinary tract infection
L. reuteri	Inhibited replication	Desipramine	Produce antimicrobial molecules, such as organic acids, ethanol, and reuterin, which inhibit colonization of pathogenic bacteria
C. leptum	Inhibited replication	Desipramine	One of the most significant in the gut microbiome, contains species that produce butyrate and are fibrolytic (can break down complex plant-based polysaccharides)
B. fragilis	Inhibited replication	Desipramine	This plays a large role in immune defense
P. aeruginosa	Inhibited replication	Desipramine	Opportunistic pathogen, can cause a variety of infections in humans
L. casei	Inhibited replication	Desipramine	Probiotics that help other bacteria that play a large role in immune defense to colonize
B. animalis	Inhibited replication	Desipramine	Probiotic with benefits in gastrointestinal health and immune function
Firmicutes	Decreased	RBX	Break down carbohydrates that the body struggles with such as starch
Bacteroides	Increased	RBX	Provide protection from other pathogens, and provide nutrients for other microbes

Species	Results	Drug type	Normal function of bacterial strain in microbiome
Proteobacteria	Increased	RBX	May play a role in colonization of strict anaerobes required for healthy gut function; contains several known pathogens, associated with metabolic diseases and IBD, common linking symptom is inflammation
Prevotellaceae	Increased	Control and RBX	Breakdown indigestible protein and carbohydrates
Lactobacillaceae	Decreased	RBX	Plays a role in intestinal permeability and the immune system and may inhibit growth and counteract translocation of harmful bacteria
Clostridiaceae	Decreased	RBX	Digestion of energy and proteins

Analysis Part 3: Marijuana

Another class of drugs that has been shown to affect microbiome diversity is medical marijuana. However, research on this drug is more recent compared to the other two sections, and the topic is very controversial. CBD (Cannibidol) and THC (tetrahydrocannabinol) are medical marijuana compounds that come from the plant cannabis sativa. This is a common drug that is used to treat anxiety, pain, nausea, and many other ailments. The first study comes from the Gorelick lab, mice were separated into four different groups: mice fed a healthy diet, mice fed a high fat cholesterol diet, mice fed HFCD with CBD, and mice fed with THC (Gorelick et al., 2022).

The animals were fed for seven weeks and then euthanized and their tissues were harvested (Gorelick et al., 2022). Metagenomic analysis was then done by analyzing the 16s ribosomal RNA gene. This is typically useful because all areas except 9 regions variables are highly conserved, so it is a useful target to help identify different species of bacteria (Fuks et al., 2018). Sequences with 97% similarly were assigned operational taxonomic units and relative abundance was based on OTU's. THC and CBD reduced *Deferribacteres* and *Firmicutes* abundance. When treated with CBD, *Clostridia*, *Ruminococcus*, *Bilophila*, and *Mucispirillum* abundance is increased (Gorelick et al., 2022). Many of these bacterial strains have benefits for the microbiome, and contribute to overall health.

In the Silvestri lab, mice were orally gavaged with CBD and fish oil (FO). These mice had colitis, and then FO was administered twice a day for eight to fourteen days (Silvestri). After the FO treatment, CBD was administered by oral gavage for 8 to 14 days. All animals were euthanized after this treatment with carbon dioxide gas (Silvestri et al., 2020). In order to determine changes in composition of the microbiome, fecal samples were taken from the mice, and DNA was extracted. After induction of colitis, the *Firmicutes* to *Bacteroidetes* ratio increased but not until after day fourteen (Silvestri et al., 2020). *Akkermansiaceae*, and *Tannerellaceae* abundance also increased. Several families were not modified based on induction of colitis, but some families were increased based on treatment of CBD (Silvestri et al., 2020). Those that increased in abundance from CBD treatment included: *Clostridiaceae*, *Defluvitaleaceae*, *Marinifilaceae*, and *Desulfovibrionaceae*. Those that decreased in abundance were *Anaerotruncus*, *Candidatus_Arthromitus*, *Odoribacter*, and *Tyzzerella_3*.

The third study that will be analyzed is from the Al-ghezi lab. The purpose of this study was to investigate the use of cannabinoid treatment during experimental autoimmune encephalitis, in order to determine how cannabinoid treatment affects the gut microbiome (Al-ghezi et al., 2019). In this experiment the researchers used six- to eight-week-old mice. EAE mice were injected with an intraperitoneal injection of THC+CDC, or the vehicle of DMSO. Then bacterial abundance and species composition was measured by 16srRNA analysis (Al-ghezi et al., 2019). This

lab also conducted fecal microbial microbial transplants, the way this was done was by doing cecal flushes which were harvested by an anaerobic chamber, and then suspended in sterile PBS. Each mouse was given antibiotics such as streptomycin and ampicillin by oral gavage (Al-ghezi et al., 2019). This was to make sure that the microbiome was completely depleted.

Beta diversity was depicted in a principal coordinate analysis and at the phylum level there were noticeable differences for example the EAE mice had significantly lower abundance of *Bacteroidetes* when compared to control mice (Al-ghezi et al., 2019). EAE mice treated with THC+CBD had higher abundance of *Firmicutes* as compared to the control and had a lower *Verrucomicrobia* species. *Proteobacteria* in the EAE mice treated with THC+CBD were significantly higher than the controls as well. This sequencing data was then validated using primers specific for each species (Al-ghezi et al., 2019).

Metabolite production was also altered in the EAE patients treated with CBD+THC than the control. Metabolomics were measured using PICRUST, a method that computationally measures metabolite change in different metabolic pathways. When comparing controls to EAE mice treated with THC+CBD, there was an increase in abundance of metabolites that were produced in the KEGG pathway. The KEGG pathway is a pathway that is involved in the secretion system that produces proteins involved in pathogenesis. In order to corroborate this data, brain lysates were extracted from these experimental groups and short chain fatty acid composition was analyzed (Al-ghezi et al., 2019). Compared to the controls, EAE treated with CBD+THC, had an increase in production in isobutyric acid, buyric, isovaleric, and valeric. These are important because these metabolites are important for production of LPS.

Summary

Species	Results	Drug type	Normal function of bacterial strain in microbiome
Deferribacteres	Decreased	THC and CBD	Preparing the gut for colonization by the anaerobes, and maintaining redox homeostasis
Firmicutes	Decreased	THC and CBD	Break down carbohydrates that the body struggles with such as starch
Clostridia	Increased	CBD	Digestion of energy and proteins
Ruminococcus	Increased	CBD	Mutualistic, degrades complex polysaccharides to nutrients for host
Bilophila	Increased	CBD	Commensal microorganism
Mucispirillum	Increased	CBD	Corrects defects in the intestinal epithelial barrier
Clostridiaceae	Increased	CBD	Digestion of energy and proteins
Defluviitaleaceae	Increased	CBD	Unknown

Species	Results	Drug type	Normal function of bacterial strain in microbiome
Marinifilaceae	In-creased	CBD	Prevents tissue inflammation, and interacts with the immune system
Desulfovibri-onaceae	In-creased	CBD	This plays an essential role in sulfur metabolism and utilizing metal ions for different biochemical reactions in the gut
Anaerotruncus	De-creased	CBD	Unknown
Candidatus_Ar-thromitus	De-creased	CBD	Can induce multiple adaptive immune responses in order to protect the body from pathogens
Odoribacter	De-creased	CBD	Modulates the host glucose levels, and maintains homeostasis
Tyzzerella_3	De-creased	CBD	Unknown
Firmicutes	In-creased	THC+CBD	Break down carbohydrates that the body struggles with such as starch
Verrucomicro-bia	De-creased	THC+CBD	Mucin degradation, and maintains glucose homeostasis
Proteobacteria	In-creased	THC+CBD	May play a role in colonization of strict anaerobes required for healthy gut function; contains several known pathogens, associated with metabolic diseases and IBD, common linking symptom is in-flammation

Future Directions

There is much evidence that many different prescription drugs from all different classes can affect the gut microbiome and this is becoming a new evolving area of research. Many of the drugs, when altering the microbiota, seem to have a negative impact on human health, and are associated with many different diseases. Certain bacterial species when the ratio is altered from normal abundance and this is typically associated with diseases. For example, irritable bowel syndrome (IBS) is commonly associated with increased *Firmicutes* to *Bacteroidetes* ratio, increased *Clostridia* and *Clostridiales* abundance, and decreased *Bacteroidia* and *Bacteroidales* abundance (Duan et al., 2019).

It is also associated with lower abundance of butyrate-producing bacteria (known to improve intestinal function) and *Methanobacteria*, and the increased abundance of *Proteobacteria* (Chong et al., 2019). Diabetes is also associated with an altered gut microbiome. One study found that gut microbiome changes often preceded development of Type 2 Diabetes, and they developed a predictive model that determines how likely an individual is to develop Type 2 Diabetes based on these alterations (Vals-Delgado et al., 2022).

Marijuana has been a highly controversial topic, because there are conflicting results on whether it has positive or negative effects on health. For example, Gorelick found that THC and CBD (separately) decreased *Firmicutes*, while Al-Ghezi found increased *Firmicutes* (higher abundance is associated with obesity and diabetes) with THC+CBD (combined) treatment. Therefore, this class of drugs, along with the mechanisms involved in changing the

gut microbiome should be further investigated. This is the most under researched area, and has only recently been investigated.

There will be a plethora of other experiments that can be tested in the future. For example, prescription drugs can be tested against their isomers in order to see how structural changes might alter effects on the gut microbiome. An example of this would be to look into heroine which is a drug that is derived from morphine, and see how it changes the microbiome. Understanding the dysbiosis that occurs under addiction, could help doctors understand how this dysbiosis could contribute to the changes in the dopamine receptor that is over expressed in addition, and if metabolites that are produced in the microbiome interact with this receptor. There is a huge movement spreading in the field about the connection between the gut-brain axis, and antidepressants.

Understanding the metabolites that are produced by microbes under these conditions of addiction, and depression could help us create drugs that target this, instead of destroying the microbiome as researchers are beginning to realize, especially with chronic use of antidepressants.

Understanding the effect of different prescription drugs on the mycobiome is also becoming a new area of research. Fungi are typically opportunistic pathogens; this means that most fungi do not typically cause disease unless under specific host niche conditions. Researchers are starting to see that there are many different fungi species that are found in our mycobiome, and understanding how these prescription drugs impact this system could give us clues as to why some people are more prone to being affected by fungal diseases vs. other people.

Another category that hasn't been looked into is how immunosuppressants affect the microbiome. Immunosuppressants are drugs that are used to inhibit the activity of the immune system. Since researchers are looking into how the microbiome plays a role in the immune system it would be interesting to see how the microbiome behavior and composition changes in the presence of these immune drugs. There is so much further research that needs to be done in this field, and understanding these different areas could change human health for future generations.

Conclusion

The effect of prescription drugs on gut microbiome is extremely relevant to the modern world, yet critically under-researched. The purpose of this article is to bring awareness to this emerging topic. Multiple studies, based on murine and human subjects, were tested with prescription drugs in the three following classes: opioids, antidepressants, and marijuana. This analyzes the changes of composition of the microbiome with exposure to different prescription drugs, and shares ideas of just a few future projects that could be done to explore this topic even further.

Acknowledgments

I would like to thank Heather Murdoch for supporting and advising me while writing this article.

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