Study of Ciprofloxacin Degradation Potential of Human Gut Microbiota of Depression Patients

By

Ihtisham Ul Haq

Department of Microbiology

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad

2023

Study of Ciprofloxacin Degradation Potential of Human Gut Microbiota of Depression Patients

A thesis submitted in the partial fulfillment of the requirements for the degree of

Master of Philosophy

In

Microbiology

By

Ihtisham Ul Haq

Department of Microbiology

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad

2023

Declaration

The material and information contained in this thesis are my original work that was carried out at the Laboratory for Microbial Food Safety and Nutrition, Department of Microbiology, Quaid-I-Azam University, Islamabad, Pakistan. I have not previously presented any part of this work elsewhere for any other degree.

Ihtisham Ul Haq

Dedication

Every challenging work needs self-effort as well as guidance of elders especially those who are very close to our hearts.

My humble effort I dedicate to my sweet and loving.

Father, Mother, Brother

And

Sister

Whose affection, love, encouragement, and prayers of day and night make me able to get such success and honor.

Along all the hardworking and respected

Teachers

Certificate

This thesis submitted by **Ihtisham Ul Haq** is accepted in its present form by the Department of Microbiology, Quaid-i-Azam University, Islamabad, in partial fulfillment of the requirements for the Degree of Master of Philosophy in Microbiology.

Supervisor

Prof. Dr. Muhammad Imran

External Examiner

Prof. Dr. Tayyaba Yasmin

Chairman

Prof. Dr. Naeem Ali

Dated: 5th December, 2023

Contents

List of Figures

List of Tables

ABSTRACT

The human body is considered a microbial reservoir that colonizes various surfaces in continuous contact with the external environment. The human digestive tract is also inhabited by microorganisms including fungi, bacteria, viruses, archaea, protozoan, and viruses. These complex microbial communities of the gut are involved in diverse physiological and developmental processes. In clinical depression, the functional and taxonomical status of the gut microbiota changes which leads to the dysbiotic gut. It also impacts the pharmacodynamics of frequently used antibiotics like ciprofloxacin. This study was conducted to evaluate the potential of ciprofloxacin degradation by the gut microbiota of clinically depressed patients. For this purpose, stool samples of clinically depressed patients with known gut microbial diversity were taken from the laboratory collection. For degradation, the microbiota of these subjects was suspended with 4 and 8 μg ml^{-1} of ciprofloxacin (CIP) in a basic salt medium (BSM) for one week. Spectrophotometry and HPLC were performed to check the CIP degradation, which revealed that the fecal microbiota has the potential to degrade CIP. The degradation levels at concentrations of 4 μg/ml and 8 μg/ml were observed to range from 6% to 90% and 10% to 60%, respectively. The variability in degradation capabilities among individuals, influenced by factors such as microbial diversity, prior antibiotic exposure, and environmental and host genetic factors. The sensitive assay of the ciprofloxacin aliquots that were taken at different intervals unveils that the gut microbiota of clinically patients have reduced ciprofloxacin potency. However, it was found that gut microbiota of these individuals did not transform the ciprofloxacin into compounds having higher antibacterial. Moreover, 16S rRNA amplicon sequencing showed that every individual fecal microbial profile has a unique pattern and personalized composition, which forms the basis for the personalized response of gut microbiota to ciprofloxacin. This study also helps in understanding the intricate association within microbial ecosystems of the human body, highlighting their potential for drug metabolism and therapeutic outcomes.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Collectively, the microbes that colonize the human gastrointestinal tract are called gut microbiota. These microorganisms include bacteria, fungi, viruses, archaea, protozoan, and viruses (Bhalodi *et al.,* 2019). Nowadays, scientists consider the human gut as a completely sophisticated organ, because of the implications for maintaining normal physiology and preventing several critical illnesses . While, the gut microbiome, is the combination of all microorganisms present in the digestive tract of humans along with their cumulative genomic contents (Cresci & Gail, 2019).

The human gut microbiota is highly linked with the overall well-being of an individual (Jandhyala *et al.,* 2015). Recent studies revealed that healthy gut microbiota is steadily implicated in nutrient acquisition, diverse metabolic pathways, immunity, biogenesis of different biologically active compounds like vitamins, neurotransmitters, amino acids, and lipids (Hou *et al.,* 2022), and nervous system development (Pilmis *et al.,* 2020).

Gut microbiota is very dynamic in nature and its stability is highly dependent upon diverse modulatory factors. These factors include internal and external, i.e. genetics, immune system topographical variation, lifestyle, probiotics and prebiotics interventions, consumption of antimicrobials and use of antibiotics (Anwar *et al.,* 2021), and ecological characteristics of gut microbiota, or the mutual dependency of microbiome and host (Pérez *et al.,* 2013). Together, all these factors are responsible for shaping and governing the impact of the gut microbiota on human physiology (Xu *et al.,* 2013).

Antimicrobials been used for the control of various types of bacterial growth since ancient times. After the discovery of the first antibiotics in 1928, Penicillin revolutionized the medical field in terms of curing bacterial infections. But in the last few decades, due to the increase in the unceasing consumption and irrational utilization of antibacterial compounds led to disruption of the equilibrium of the human gut's microbiome (Kumari *et al.,* 2022). Antibiotics are a class of antimicrobials that are administered in or onto the body to vanish (bactericide) or hinder the growth (bacteriostatic) of bacteria. Mainly, Antibiotics are used to kill or stop the growth of pathobionts, but unfortunately, they also interrupt the complex, symbiotic microbial community of our gastrointestinal tract. Nonetheless, the influence of antibiotics on the human microbiota is

contingent upon the antibiotic type and category, dosage, amalgamation, method of delivery, and duration of therapy (Zhang *et al.,* 2020).

The gut microbiota of human is a complex ecological niche containing few bacterial phyla as core microbiota i.e. *Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Fusobacteria, and Verrucomicrobia* (Dogra *et al.,* 2021). Antibiotic administration has an invincible impact on the intestinal microbiota leading to a state of constant turbulence, called dysbiosis or dysbacteriosis. This state is characterized by a decrease in the diversity of technologically important phyla and disruption of the stability and functions of gut microbiota. As a result, it leads to a change in the metabolic status of the body, decreases colonial resistance increases gut permeability, and provides an advantageous environment for the growth of pathobionts and the exchange of antibiotic resistance genes (Ramirez *et al.,* 2020).

Dysbacteriosis might be involved in the pathophysiology of diverse types of non-communicable diseases and the conversion of these diseases from acute to chronic forms. There is ample evidence in the literature on the fact gut dysbacteriosis is linked with the beginning and development of dozens of diseases, inclusive of persistent renal diseases (Vaziri *et al.,* 2013), Diabetes Mellitus (Di Daniele *et al.,* 2017), cardiovascular diseases (Yang *et al.,* 2015), autoimmune diseases (Leung *et al.,* 2014; Livanos *et al.,* 2016), inflammatory bowel syndrome and disease (Ghoshal *et al.,* 2012; Lepage *et al.,* 2011), non-alcoholic fatty liver diseases (Estes *et al.,* 2018), and mental health disorders (Vijay & Valdes, 2022).

Emerging research in the field of human microbiomes has revealed the crucial physiological functions of human intestinal microbial communities in maintaining neural hemostasis (Lach *et al.,* 2018). A substantial body of indications from clinical and pre-clinical studies has manifested an association between gut dysbacteriosis and depression (Jiang *et al.,* 2015; Steenbergen *et al.,* 2015). Scientific studies have demonstrated that antibiotics have the potential to alter human emotional states and may even contribute to the development of psychological conditions, such as depression (LaSalvia *et al.,* 2010; Lurie *et al.,* 2015). This finding was substantiated through diverse experiments conducted in a mouse model, which demonstrated that dysbiosis induced by antibiotics could contribute to the onset of depression (Jang *et al.,* 2018).

The trajectory and behavior of pharmaceuticals are frequently governed not just by the host's characteristics, but also by the presence of microbial communities within the gut. The gut

microbiome is acknowledged for its ability to impact drug metabolism, both directly and indirectly. Recent indications point to the reciprocal effects that drugs can exert on the composition and operation of the gut microbiome (Clarke *et al.,* 2019). Alterations in drug metabolism mediated by the microbiota and modifications in the gut microbiome influenced by drugs can yield positive or adverse consequences for the host (Enright *et al.,* 2016).

The intestinal microbiome equipped with the ability to directly metabolize drugs and also exert an indirect influence on the host's drug-metabolizing capacity (Haiser & Turnbaugh, 2013; Wilson & Nicholson, 2017). Although numerous drugs undergo microbial biotransformation and biodegradation, the precise microorganisms responsible for these processes often remain unidentified. Certain researchers endeavored to investigate the routes and enzymes engaged in the biotransformation and biodegradation of various drugs, such as ciprofloxacin. The mechanisms involved fluorine elimination via oxidative process, demethylation reaction (Amorim *et al.,* 2014), quinoline nucleus hydroxylation (Fang *et al.,* 2021), N-oxidation (Zhang *et al.,* 2022), N-acetylation, and removal of ethylene in the piperazine ring (Reis *et al.,* 2020). While the current literature revealed that different microbial enzymes are involved in these processes like aminoglycoside acetyltransferase (Robicsek *et al.,* 2006), glutamine synthetase (Kim *et al.,* 2011), Laccases and independent peroxidase (MiP) (Čvančarová, Moeder, Filipová, & Cajthaml, 2015), CYP450 enzymes (Jia *et al.,* 2018), Streptomyces ipomoeae's alkaline laccase (SilA) (Blánquez *et al.,* 2016), chloroperoxidase (Zhao *et al.,* 2017).

To the best of my knowledge, there are few studies conducted evaluating the potential of human gut microbiota to degrade antibiotics that are frequently used in health practices. In this study, we will explore the microbial capabilities of ciprofloxacin degradation.

Aim of the Study

The aim of this study was to evaluate the ciprofloxacin degradation potential of human gut microbiota of clinically depressed patients.

Objectives

The aim of my study was achieved by following objectives:

- To investigate the ciprofloxacin degradation potential of gut microbiota of depression patients.
- To establish a correlation between the degradation capability of gut microbiota for Ciprofloxacin and the microbial diversity of these patients.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Dating back to 400 B.C., Hippocrates noted, "Death sits in the bowels," and he also proclaimed, "Bad digestion is the root of all evil." These insights underscore the long-standing recognition by health practitioners of the pivotal role played by the gastrointestinal tract in human physiology (Myers & Hawrelak, 2004). In the last few decades, most of the research endeavors have focused on the impact of gastrointestinal pathogens and their pathogenesis. But now there is an adequate shift in the direction of research studies toward exploring the ramifications of commensal microorganisms on the human gut and physiology.

2.1. Overview of the Human Gut Microbiota

2.1.1. Humans as a Repository of Microorganisms

Mostly multicellular organisms inclusive of humans' harbor in close association with microorganisms in or outside of the body. The human body is dwelled by a diverse number of bacteria, fungi, protozoans, viruses, archaea, and unicellular eukaryotes. The microbial aggregations that are living in a symbiotic relationship with their host, are called microbiota (Kunz *et al.,* 2009). In microbiota, the widely studied and well-characterized microorganisms are bacteria as compared to fungi, viruses, protozoans, and other unicellular eukaryotes. The most frequently used but abandoned narration stated that the human body comprises a bacterial count of 10^{14} which is ten times more than human cells. While Sender et al. reassessed this estimation that the enumeration of bacterial cells associated with the human body is approximately the same as human cells, i.e. 40 trillion (Sender *et al.,* 2016).

These microscopic forms of life colonize different body surfaces like respiratory pathways, the urogenital tract, skin, the buccal cavity, and most crucial, the gastrointestinal tract (Goel *et al.,* 2014), where the microorganisms flourish, colonize and develop complex microbial communities. A major portion of human microbiota (more than 70%) is colonized in the gastrointestinal tract, prominently the colon. The human gut is one of the largest organs for microbial colonization with an estimated surface area of 200 m^2 which is equal to the surface of a tennis court (Rao & Johncy, 2022). Furthermore, the human gut is a preferable site for microbial colonization due to the abundance of nutrients.

2.1.2. Who are those tiny creatures?

Majorly, the human gut microbiota is comprised of strictly anaerobic bacteria which overpopulates the facultative anaerobic and aerobic bacteria by a magnitude of two or three times (Sekirov *et al.,* 2010). A group of scientists reported that the gut microbiota of healthy humans comprised 8 bacterial phyla, 18 families, 23 classes, 59 genera, and 109 species. The large segment of gut microbiota was made up of the members of *Firmicutes, Actinobacteria, and Bacteroidetes* were present in a proportion of 63 (40%), 32 (20%), and 31 (19.7%) respectively. 20.3% of *Clostridia* were present in the phylum of *Firmicutes* followed by 18.5% of *Bacteroidia*, 16.6% of *Bifidobacteriales*, 14% of *Enterobacterales* and 14% of *Lactobacillale* (King *et al.,* 2019). While recently Mikhail et al. reported that the human intestine contains more than 800 species that weigh approximately 1 to 2 kg (Syromyatnikov *et al.,* 2022).

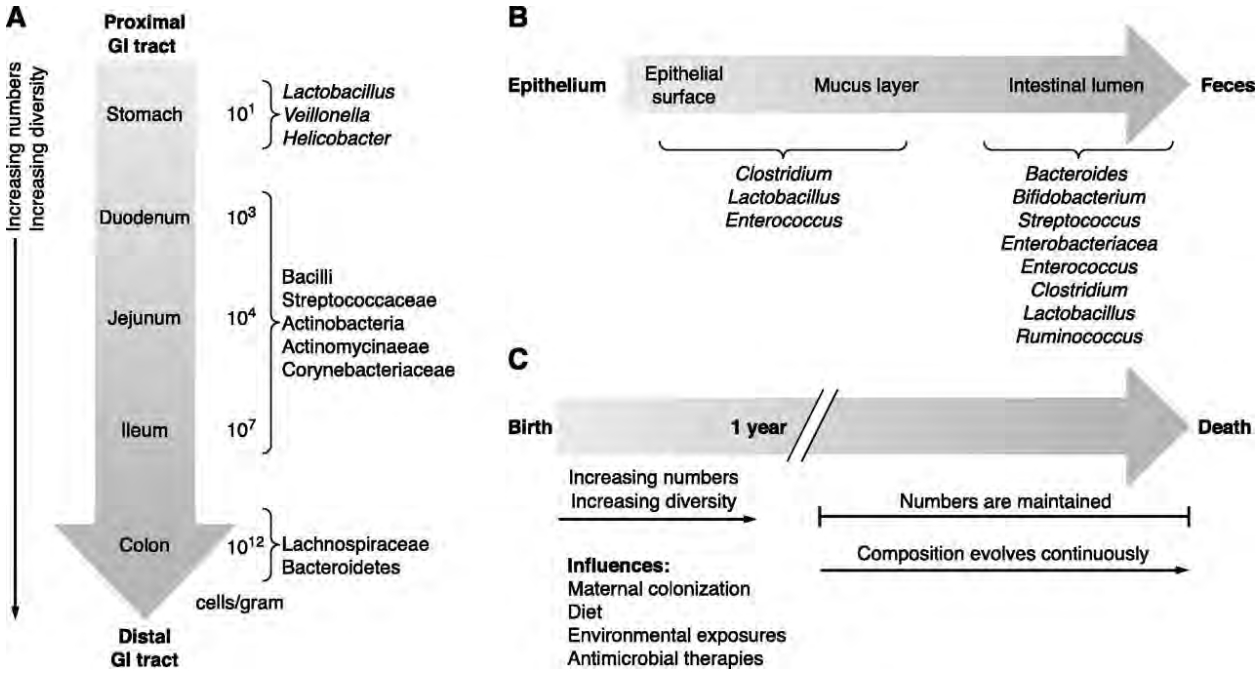

Figure 1. ***This figure explains the positional and chronological variation in the microbiological profile of the human intestine*** (Sekirov *et al.,* 2010)**.**

[A). Fluctuation in the microbial richness and abundance across the linear dimension of the human intestine. B). Fluctuation in the microbial richness across the diameter of the gastrointestinal tract. C). Chronological perspective of microbial colonization, community development, maintenance, and factors affecting microbial diversity and abundance].

2.1.3. Where are they thriving?

The distribution and variety of gut microbiota exhibit variability throughout the human gastrointestinal tract. This variation arises from factors linked to the distinct anatomical and physiological circumstances within different segments of the gastrointestinal tract. Enumeration of bacterial cells within the human intestinal tract follows a pattern, ranging from 10^1 to 10^3 bacteria per gram in the stomach and duodenum, progressing to 10^4 to 10^7 bacterial cells per gram in the jejunum and ileum, and culminating at 10^{11} to 10^{12} bacterial cells per gram in the colon (Sinha & Anirban, 2020) (As shown in Fig. 1.). Another group of scientists demonstrated by comparing the tissue specimens from small intestine and colon that different types of bacteria were colonized at different anatomical sites in the gut of human. Specimens from the small intestine were found to be abundant in the *Bacilli* class of the *Firmicutes* and *Actinobacteria*. In contrast, in the colonic specimens, higher numbers of *Bacteroidetes* and the *Lachnospiraceae* family of the *Firmicutes* were found (Frank *et al.,* 2007). Moreover, there is also a great difference in microbiota from the epithelial surface to the intestinal lumen. The epithelial cells of the intestine are masked up from the intestinal lumen by a very thick physio-chemically complex mucus layer. The microbial communities present in close association with intestinal epithelium and mucus layer deviate greatly from the microbiota found in the intestinal lumen (Fig. 1B). Swidsinski et al found that many bacterial species of intestinal lumen microbiota are limited to the luminal space of the intestine. These bacteria include *Bacteroides*, *Bifidobacterium*, *Streptococcus*, *Enterococcus*, *Clostridium*, *Lactobacillus,* and *Ruminococcus.* While, only *Clostridium*, *Lactobacillus,* and *Enterococcus* were found to exist in the mucus layer and intestinal crypts of the small intestine (Swidsinski *et al.,* 2005).

2.1.4. What is their origin?

The establishment of microbiota inside the human gastrointestinal tract starts instantly at the time of delivery (Fig. 1C). The neonates take the first inoculum for the establishment of gut microbiota when they expose to vaginal microbiota while Parturition (D'Argenio & Salvatore, 2015). Studies reported a strong association that vaginal exposure of neonates to microbes short after birth can impact the development of the gut microbiota because of the similarity index of the intestinal microbiota of neonates and the vaginal microbiota of their mothers (Chong, Bloomfield, & O'Sullivan, 2018). Unlike the vaginal mode of delivery, neonates born by cesarean section acquire a different microbiota than their mother's vaginal microbiota. This is

because a cesarean section exposes neonates to skin microbiota, resulting in the development of gut microbiota more similar to their mothers' skin microbiota (Mueller *et al.,* 2019). Moreover, in the initial developmental stages of the gut microbiota of humans, it is comparatively simple, unstable, and fluctuates more from individual to individual and over time. However, the gut microbiota of children stabilizes after one year and resembles that of a young individual (Derrien *et al.,* 2019). It is surmised that the initial inoculum for the establishment of gut microbiota is responsible for determining the shape and composition of the intestinal microbiota. For example, few studies are evident on the association of initial exposure and determining the fate of gut microbiota. A scientific investigation has revealed that in animal models such as mice, there exists a significant correlation between the microbiota of offspring and that of their mother (Ley *et al.,* 2005). Another study has revealed that the monozygotic and dizygotic twins have shown comparable similarities in their gut microbiota to that of their siblings. These outcomes point out that the maternal microbiota, shared by both twins, played a more persuasive role in shaping their adult gut microbiota compared to their genetic variations (Zoetendal *et al.,* 2001). Though all this scientific evidence depicts the idea that maternal microbiota is crucial for the establishment of the gut microbiota of their offspring, the involvement of numerous confusing variables undermines the possibility of reaching a conclusive statement about this topic. For instance, it is very complicated to consider the impact of dietary habits on the human microbiota. Conversely, mouse experiments are conducted within highly controlled environments, minimizing microbial exposure apart from their fellow littermates and parental figures. Therefore, additional investigation is imperative to definitively establish the impact of maternal transmission on shaping the gut microbiota composition of their progeny.

2.1.5. How do they get chosen?

Along with the intestinal microbiota of the mother, several other influencing factors have been reported to influence microbial aggregation in the gastrointestinal tract of humans (Fig. 1C). Multiple scientific investigations have demonstrated that the host's genetic makeup can influence the gut microbiota's composition. For instance, in the context of mouse models, a remarkable difference exists between the microbial profiles of genetically obese mice and their genetically lean counterparts (Turnbaugh *et al.,* 2006). Furthermore, a genetic mutation affecting high-density lipoprotein (apolipoprotein A-I) in mice has the potential to result in a modified intestinal microbiota (Zhang *et al.,* 2010). While these investigations suggest a potential relationship

between host genetics and gut microbiota, it's important to recognize that any influence is likely to be indirect, operating through the broader mechanisms of host metabolism.

Scientific studies have provided insights into how dietary habits directly impact gut microbiota composition. Consuming a typical Western diet has been shown to alter the microbiota in a way that promotes obesity and weight gain (Bell, 2015). Conversely, adopting specific dietary interventions for weight loss has been proven to positively influence the composition of gut microbiota, promoting better health (Rinninella *et al.,* 2019).

Despite the ample modulating factors that can impact the microbial abundance and richness in the intestinal tract, it might be wondering that the diversity of the gut microbiota is significantly stable at the Phylum level. Although, most of the individuals share a core microbiota in which the dominating phyla are conserved. But the relative richness and abundance can vary in all individuals (Turner & Patricia, 2018). In contrast, when the bacterial genus and species were checked in the human intestinal microbiota, major changes were found (Hopkins *et al.,* 2002).

2.2. Host Physiology and Role of Gut Microbiota

Multiple experimental studies have illustrated the concept that the host and its indigenous microbiota have evolved in tandem. Moreover, when the microbiota are transferred from one host species to another, the transplanted community undergoes adaptations to resemble the native microbiota of the recipient host (Rawls *et al.,* 2006). The intestinal bacteria species possess great potential for adaptation to their environment and one another, resembling a form of "microevolution" that closely mirrors the evolutionary processes seen in our species on a larger scale (Mazzoleni, 2013).

The potential for adaptation is not surprising when considering the wide array of bacterial genera and species that play integral roles in various aspects of the host's physiology typical intestinal development (Fig. 2). Since few decades, there has been a huge intensification in research studies on gut microbiota related, with significant improvement made towards setting up identity of microorganisms, microbial groups or microbial metabolites which contributing to several perspectives of physiology of the host. Alongside this, the microbiota has been found to interact with host factors affecting various aspects of development and maturation. Though, a sufficient amount of scientific studies focused on investigating specific intestinal microbial contributors to host physiology was mostly done in gnotobiotic animal models in association with various bacterial members of dominant intestinal microbial phyla (e.g. *Bacteroides thetaiotaomicron,*

Bacteroides fragilis, Lactobacillus spp) or exposed to particular microbial elements like polysaccharide A (PSA) and lipopolysaccharide (LPS) and (Duncan *et al.,* 2007). Consequently, the uncovering of these microbial species or molecules contributing to a specific host structure or function suggests their capacity to provide that contribution, but it does not necessarily designate them as the primary microbe or molecule responsible for it in a host with a full-fledged microbial community.

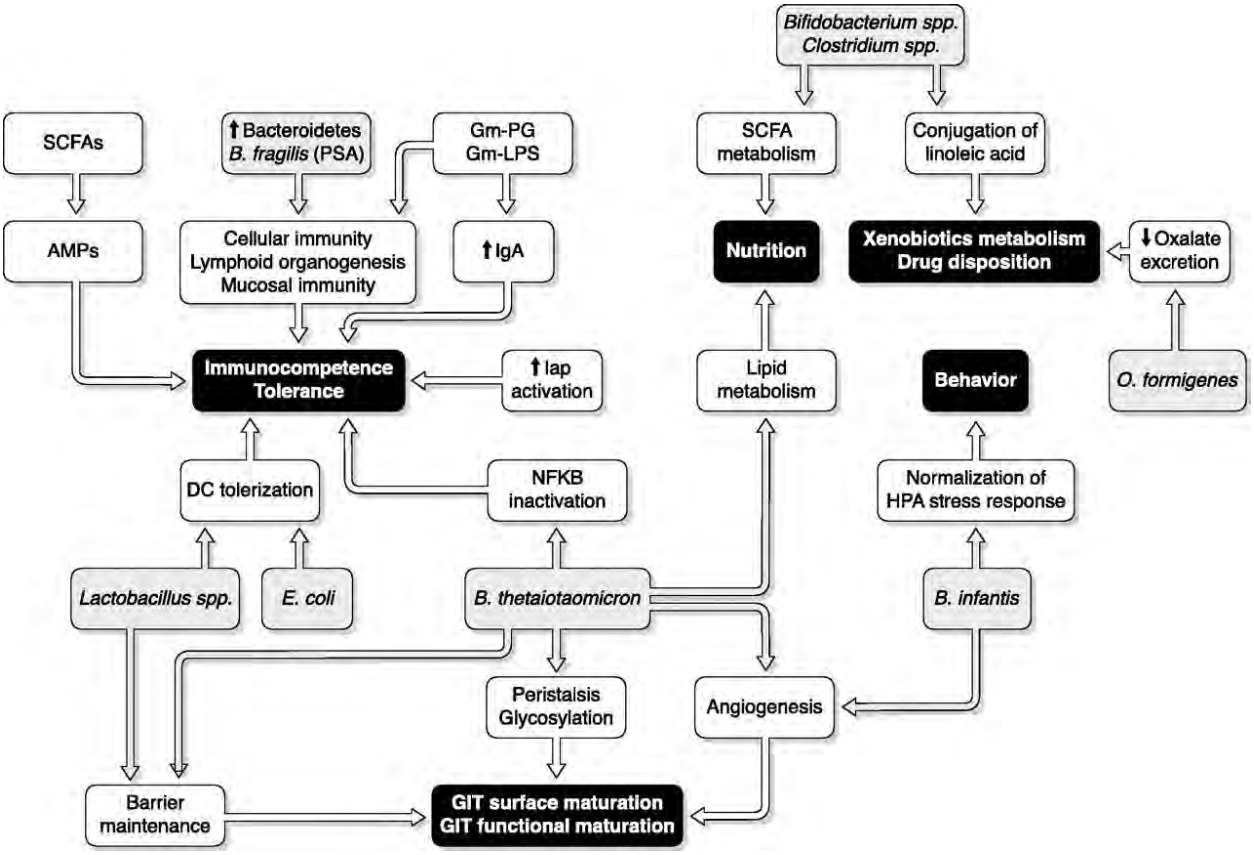

Figure 2. ***Host Physiology and Gut Microbiota*** (Sekirov *et al.,* 2010)

(This figure shows the complex microbial communities of the gut are involved in normal host physiology. Different microbiota-mediated secretion of metabolites can impact normal host development by diverse intricate mechanisms. In the above figure, the regions highlighted with grey show microbial members of the gut producing different components or metabolites. The regions highlighted in the white show the impact of gut microbiota on the cellular or organ level. The black-colored square boxes show the impacted host phenotypes).

Furthermore, due to the limitations in the existing methods for culturing the anaerobic microbial community of the intestines, fastidious, non-culturable microorganisms or those with highly mutual dependency on each other (Kuramitsu *et al.,* 2007). This confines our understanding on

the involvement of specific intestinal microbial species in a variety of host functions or processes to a small subset of microorganisms that are currently isolatable and culturable. However, the modern and accurate molecular techniques heightened our apprehension about the association of microbial activities with host physiology and significantly inflated the knowledge in this field of research.

2.2.1. Role in Immunoregulation

The higher significance of intestinal microbiota in the normal establishment of the immune system can be well acknowledged from investigations of microbiota-free (lacking microbes) animal models. These animals grow in the complete absence of microbiota, which leads to the development of an impaired immune system in terms of cellular profile, immune cell secretory compounds, compromised local and systemic lymphoid organs like spleen and lymph nodes in different body parts, Peyer's patches hypoplasticity, and a higher count of immature undifferentiated lymphoid follicles (Iweala *et al.,* 2006; Macpherson *et al.,* 2004). There is also a downfall in the antibody-secreting cells of the immune system and secretory antibodies in plasma. In addition to all these changes, microbiota-free animals also experience a disturbed pattern of cytokine profiles and weakened development of oral tolerance (Ishikawa *et al.,* 2008).
The main function of intestinal microbiota in the modulation of mucosal immune development is not astonishing since the gut mucous membrane depicts the major interaction site with the antigenic target of the exogenous source and a thick blanket of intestinal microbiota, which provides a huge quantity of antigens to the native immune cells of the intestine which activating the pattern recognition receptors of the native immune epithelial cell (Rakoff & Seth 2008).

A. Maturation and development of mucosal immunity

Microbiota-free animals display a significant immune deficiency, notably due to the failure of differentiation CD41 T-cell populations. However, this deficiency can be fully remedied by the intervention of microbial components of gut microbiota like PSA derived from *Bacteroides fragilis*. In a comprehensive set of experiments conducted by a group of scientists (Mazmanian *et al.,* 2005), who demonstrated that either administering gnotobiotic mice with B. fragilis or administering bacterial-derived components orally as PSA led to the differentiation of CD41 T cells and the restoration of lymphoid organs like spleen. This restoration was found to be dependent on the recognition of microbial-derived components by dendritic cells (DCs), followed by their exhibition to immature T lymphocytes in mesenteric lymph nodes (MLNs).

Furthermore, the Th2 cytokine profile observed in gnotobiotic animals can be reversed through bacterial polysaccharides like PSA treatment, which involves the Interleukin-12 and signal transducer and activator of transcription 4 (STAT4) signaling pathway. Thus, a single structural component from a common gut microbiota member can induce host immune maturation on both local and systemic levels, affecting molecular, cellular, and organ processes. The findings from these high-quality experiments shed light on the important role of gut microbiota in modulating the immune system (Mazmanian *et al.,* 2005).

B. Immunotolerance development in the intestinal mucosa

To ensure the harmonious coexistence of the gut and the dense bacterial blanket atop it, it is essential to avoid triggering an overwhelming immune response, both locally and throughout the body. This can be achieved by physically segregating microbial cells from host cells (Ahluwalia, Bani Magnusson, K, Öhman, & gastroenterology, 2017), modifying the antigenic properties of the gut microbiota to reduce their immunogenicity, or influencing the local host immune response towards a state of tolerance (Isolauri *et al.,* 2002).

C. Governing the ecosystem of gut microbes

Maintaining a balanced and healthy composition of gut microbes is crucial for supporting the overall health and well-being of the host. However, an excessive proliferation of bacterial communities can lead to a range of harmful conditions. To counteract this, the host employs various strategies to mitigate such unfavorable outcomes (Macfarlane, 2014). Plasma cells inhibiting the gut mucosa release secretory IgA antibodies that coat the gut microbial member and permit control of resident microbial members (Bunker *et al.,* 2017).

2.2.2. Role of gut microbiota in protection against pathogens

Gut microbiota employs various strategies for infection prevention by providing a physical barrier to external pathogenic bacteria and acting as a protective shield for its host. These include occupying the intestinal surface area to prevent the colonization of pathogens (Kitamoto *et al.,* 2016), ecological competition by utilization of nutrients (Cornforth & Foster, 2013), and secretion of diverse classes of antimicrobial compounds (Garcia-Gutierrez *et al.,* 2019).

2.2.3. Maintaining the structure and functions of the gastrointestinal tract

Recent studies have explored various facets of host-microbial interactions that facilitate the physiological and anatomical maturation of the gastrointestinal tract (GIT). This maturation

involves the development of effective gastrointestinal motility (Hou *et al.,* 2022), as well as the establishment of adequate surface area and blood supply for nutrient absorption (Sommer & Bäckhed, 2013). Moreover, the GIT must have suitable attachment sites to support the native intestinal bacterial communities while preventing the systemic dissemination of exogenous antigens from food and microbiota sources. Finally, the gut must maintain its homeostasis and exhibit regenerative capabilities in the event of injury.

2.2.4. Extra-intestinal roles of gut microbiota

The vital function of a well-balanced gut microbiota extends beyond the confines of the gastrointestinal tract. Contrarily, it also has a variety of physiological roles outside of the gut in different metabolic pathways and organs which was demonstrated by the dysbiotic gut in microbiota-free animal models, pointing toward vital roles of native intestinal microbes to their normal progression and hemostasis.

2.2.5. Role in cardiovascular functionality

A microbiota-free animal (GA) that grow without intestinal microbiota lead to diverse type of cardiovascular abnormalities (Aron *et al.,* 2016). The cardiac productivity of these animal models is much lowered than those with healthy intestinal microbiota. Moreover, germ-free axenic mice models on starvation are poorly utilizing the same amount of nutrients for energy acquisition for cardiac metabolic activities and increase heart biomass in comparison with those animal models with healthy gut microbiota .

2.2.6. Neurodevelopment and Gut Microbiota

The absence of gut microbiota (in germ-free axenic mice) can impact the neurodevelopment, leading to various abnormalities (Collins *et al.,* 2014). These can include malfunctioning of the hypothalamic-pituitary-adrenal (HPA) axis and reduced sensation of inflammatory pain. Lately, there has been a strong focus on the functions of the microbiota in influencing both central and peripheral neural mechanisms. These bidirectional communications are known as the "brain-gut microbiota axis". Disruptions in the this two way communication of gut and brain could lead to onset of different gastrointestinal conditions like Irritable gastrointestinal tract disorder due to atypical neurological activation of the neuronal system in the gut or even contribute to depression (Singh *et al.,* 2023).

2.2.7. Impact of gut microbiota on nutrition and metabolism

The intestinal microbiota of humans has versatile genetic composition in terms of different metabolite production as compared to the potential of humans (Sharifi *et al.,* 2020). The comprehensive analysis of the metabolic functions of our cells and the gut microbial community is termed metabonomics (Tuohy *et al.,* 2009). When it comes to metabolic processes essential for homeostasis, the microbiota plays a major role, surpassing our own relatively modest contributions (Zmora *et al.,* 2019).

2.2.8. The gut microbiota and Body Mass Index (BMI)

The germ-free axenic animal models usually require a huge concentration of calories to carry the normal metabolic process (Hooper *et al.,* 2002). This was confirmed by a study that the equal body mass of animals with healthy gut microbiota does not require that many calories for normal functioning. Furthermore, the gut microbiota of these animals actuated such pathways that amplified the caloric of the ingested foods (Krajmalnik *et al.,* 2012). Generally, these are achieved by one of two categories. Enhancing the digestion of normally indigestible oligosaccharides to extract additional calories and improving nutrient absorption and utilization through adjustments in the intestinal epithelium's absorptive capacity and nutrient metabolism (Nugent, 2005).

2.2.9. Drug Metabolism and Gut Microbiota

The various species of gut microbiota of human secrete a variety of metabolites that influence these metabolic profiles at different levels (Rowland *et al.,* 2018). These variations in gut microbiota from person to person and population to population, resulting in diversity in metabonomes, have been predicted to perform a role in the toxicities of commonly used drugs in different locations and populations (Tuohy *et al.,* 2009). The pharmaceutical industry's future toxicological studies will be influenced by our growing understanding of the gut microbiota's role in metabolizing xenobiotic compounds, including medications (Kang *et al.,* 2013). Furthermore, this insight will contribute to the progression of individualized healthcare. It is widely acknowledged that integrating pharmacogenetics is essential for optimizing drug development and administration. As our knowledge of the roles and structure of the microbiota deepens, there arises a necessity to broaden the scope of pharmacogenetics to encompass

pharmacometabonomics. This innovative approach would account for the collaborative impact of both host and microbiota metabolism on drug responses (Nicholson & Wilson, 2003).

2.3. What is gut dysbacteriosis?

Dysbacteriosis is defined as the variations in the compositions and/or functions in the ecological communities of microbes that they lose the potential to resist and recover (DeGruttola *et al.,* 2016). Dysbacteriosis is specific to the subject issue and circumstances. It can be caused by environmental and as well as host-related factors(Garcia-Cortes *et al.,* 2020).

2.3.1. Type of gut dysbacteriosis

The disturbance in microbial equilibrium that can cause gut dysbacteriosis in the intestinal ecological system may be due to the lack or inappropriate richness of important taxonomic species, altered metabolic functional profile, decreased alpha diversity, or higher number of pathogenic microbes (Bastard *et al.,* 2018). It is a state of distinctive microbial ecology. Gut dysbacteriosis is mainly divided into the following two groups.

A. Taxonomic gut dysbacteriosis

This type of gut dysbacteriosis is characterized by variation in the composition of microbes in terms of changes in the relative abundance and constituents of the microbial community, as well as disruptions in diversity and richness. This variation can lead to an elevated presence of pathobionts and a decrease in essential technological taxa, resulting in reduced alpha diversity.

The loss in microbial variety can be detected at various taxonomical levels, including phylum, class, genus, or even species level. For instance, in the context of obesity, there is a rise in the count of *Firmicutes* compared to the dominant phylum *Bacteroidetes*. The genus Firmicutes has huge potential for energy harvesting more(Kaoutari, Armougom, Gordon, Raoult, & Henrissat, 2013) proficiently from the dietary components which leads to an increase in the adipose tissues (Kaoutari *et al.,* 2013). While, in the case of IBD, there is a decreased diversity and number of *Firmicutes* and *Bacteroidetes* and an increased number of *Enterobacteriaceae* (Stojanov *et al.,* 2020). The reduced number of *Firmicutes* results in the lower production of butyric acid, however the higher abundance of *Enterobacteriaceae* results in a higher reduction of sulfate. This disturbance in the microbial ecology of the gut causes to intensify the gut permeability and inflammatory pathways (Koh *et al.,* 2016).

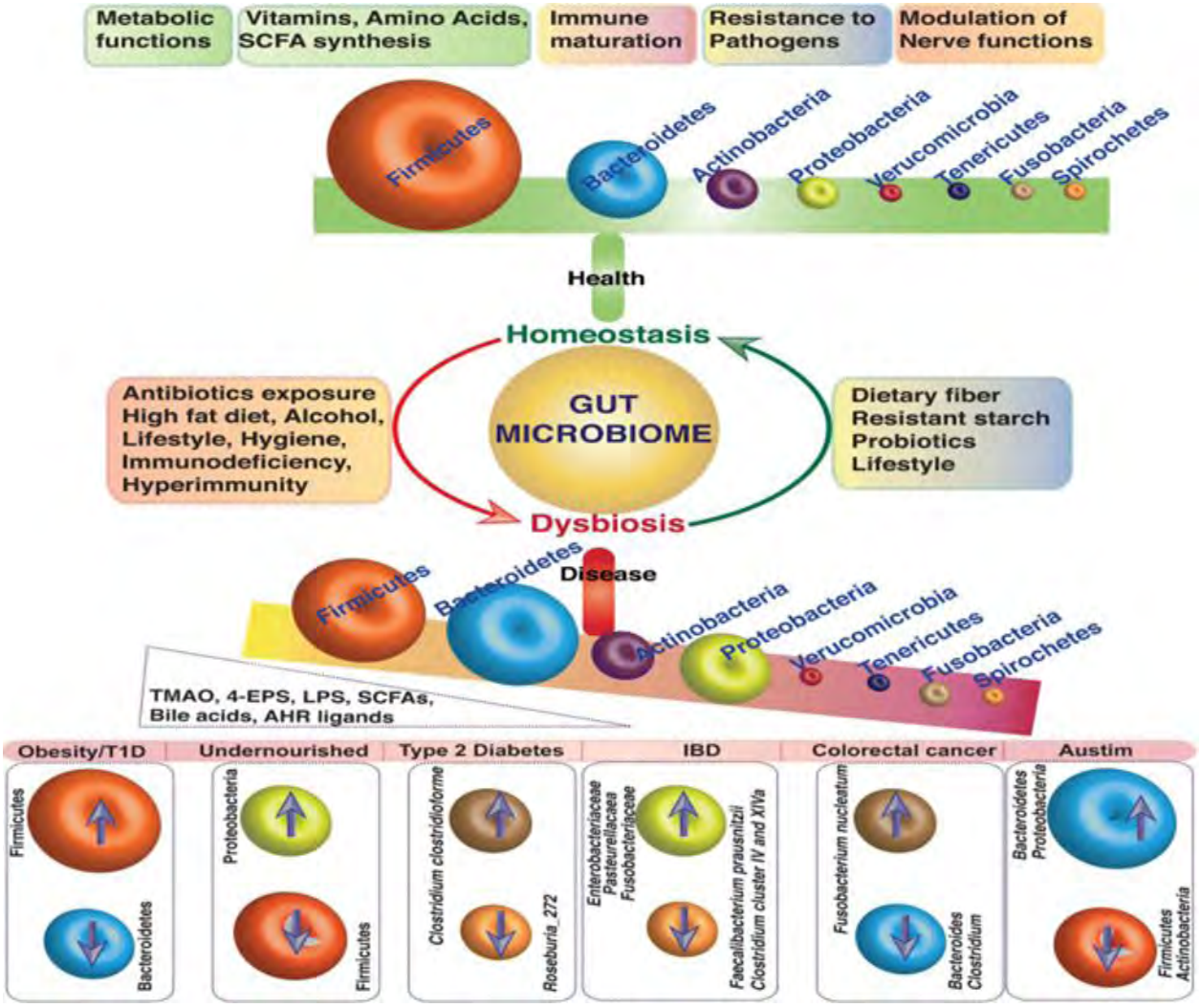

Figure3. ***Depicting the roles of eubiosis and dysbacteriosis of microbiota on the health and disease of humans*** (Sekirov *et al.*, 2010).

B. Functional gut dysbacteriosis

Functional dysbiosis does not display a reliable taxonomical indication of the microbiota, but it has a discrete variation in functional diversity due to variation in microbial genomes which is responsible for the production of different microbial metabolites. Moreover, in functional dysbacteriosis, there is a variation in types and quantity of the metabolites that are produced by the microbiota in the gut and blood of the host.

2.4. Antibiotics as the main player in gut dysbacteriosis

Various scientific experiments have consistently shown that antibiotic consumption plays a major role in gut microbiota dysbacteriosis, leading to both taxonomical and functional

imbalances (Liu *et al.,* 2020). The use of broad-spectrum antibiotics can impact up to 30% of the intestinal gut microbiota, resulting in a considerable reduction in alpha diversity (Haak *et al.,* 2019). After the cessation of antibiotic administration, the microbiota may show a degree of resiliency, enabling it to restore a composition similar to its original state (Yoon & Yoon, 2018). However, complete recovery to the initial state is often not achieved. Indeed, antibiotic-induced alterations to the microbiota can persist for extended periods, ranging from months to many years (Palleja *et al.,* 2018). Limited research has centered on investigating the impact of early antibiotic administration on the development of gut microbiota in newborns, despite the profound implications it may have on their long-term health. However, some studies have examined the gut microbiota of newborns who received antibiotic treatment in the initial days of life. The findings revealed significant effects within just one week and continued to show impacts up to two months after birth. Understanding the effects of early antibiotic exposure on gut microbiota development is crucial, as it can have far-reaching consequences on an individual's overall well-being throughout their life. Initial exposure to antibiotics in infants resulted in a decrease in microbiota diversity and significant changes in its alpha diversity, characterized by a reduction in *Bifidobacterium* and a notable rise in *Proteobacteria*. Furthermore, infants not directly treated with antibiotics but born to mothers who received antibiotics before delivery exhibited similar alterations in their microbiota, mirroring the changes observed in treated infants (Tanaka *et al.,* 2009).

The influence of antibiotics on the human gut microbiota has been comprehensively explored by employing advanced "omic" techniques for the last few decades. These techniques provide us with the opportunity for in-depth analysis and understanding of microbial communities (Franzosa *et al.,* 2015). By analyzing these experimental studies show that antibiotic administration not only unbalances the composition of bacterial communities but also influences the metatranscriptomics, metaproteomics, and overall metabolomics of the intestinal microbiota (Rojo *et al.,* 2017). These fluctuations can happen much more robustly compared to the replacement of taxa in the community (Pascale *et al.,* 2020). Several lines of scientific evidence have provided further confirmation that antibiotics cause rapid changes in the physiological state and functioning of the intestinal microbiota. When fecal samples were incubated with various antibiotics in laboratory settings, the fraction of gut microbiota cells with impaired membrane integrity, the active members of the intestinal microbiota shifted, and genes linked with antibiotic

resistance, stress response, and phage induction showed heightened expression (Maurice *et al.,* 2013).

2.5. Risk of Depression and Antibiotics

Clinical studies support the notion that antibiotics have the potential to change human emotional states and may even lead to psychological disorders, such as depression. The first recorded case of depression induced by antibiotics dates back to 2010 when a 75-year-old man, with no medical history of psychological disorder, encountered acute depression and tragically committed suicide after receiving antibiotics for infections at the surgical site following colorectal cancer surgery (LaSalvia *et al.,* 2010). Additional scientific investigations have provided further support to the notion that antibiotics could potentially lead to depression. A study utilizing a UK medical record database validated the link between antibiotics and an increased risk of depression and anxiety. Through these studies from 1995 to 2013, a team of researchers discovered that specific antibiotic administration, such as penicillin and quinolones, may slightly elevate the risk of experiencing depression and anxiety (Lurie *et al.,* 2015).

Initial investigations have revealed a correlation between depression and dysbacteriosis induced by antibiotics, highlighting the potential for certain antibiotic types and combinations to trigger depressive behaviors in mouse models. Moreover, a study involving C57 mice corroborated these findings by specifically demonstrating that ampicillin administration can lead to compromised psychological behaviors, ultimately manifesting as symptoms of depression and anxiety. To gauge anxiety-like tendencies in the mice, researchers employed the elevated plus maze (EPM), a well-established method for evaluating animal behavior. In this paradigm, a mouse's entry into an open arm signifies its normal exploratory behavior, while avoidance of the open arm is indicative of anxiety-like behavior. The results of the study revealed a notable decrease in the time mice spent in the open arms following a two-day intervention with 100 mg/kg ampicillin, providing compelling evidence that ampicillin administration can trigger anxiety-like behavior (Jang *et al.,* 2018).

After administering ciprofloxacin to mice, researchers came to know that there was a delayed entrance to the closed arm and a significant reduction in physical activities and investigative behavior during the maze test, predicting the emergence of depression-like behavior. Though, conflicting results have also been documented; for instance, neonatal BALB/c mice exposed to penicillin for six weeks displayed a clear rise in hostility rather than depression-like or anxiety-

like behaviors. The occurrence of such inconsistent results may be linked to the specific antibiotics used and the timing of their administration. While anxiety and depression-like behaviors were not precisely stated in this experiment, it is evident that antibiotic administration led to abnormal behaviors, hinting at a potential association between antibiotics and mental health issues (Leclercq *et al.,* 2017).

2.6. Biodegradation of antibiotics

Biodegradation is a very crucial process for the removal of environmental pollutants. Biodegradation is a biological procedure by which the structure of molecules, mostly carbon backbone, is transformed or disrupted into smaller fragments by living organisms (i.e. mostly microorganisms). Concerning environmental pollution, environmental microorganisms are very important for biodegradation. In mammals, the degradation of most of the xenobiotic compounds happens in their body (i.e. liver). Mostly biodegradation, but not in all scenarios, is conducive to obtaining full fudge degradation of complex molecules into simple inorganic products (mineralization). Mineralization is the complete degradation of pollutants into their inorganic compounds. The byproducts of mineralization depend on the composition of pollutants to be degraded, as some compounds convert into H_2O and CO_2, while other compounds yield by-products like S^{2-}(Sulphide), SO_4^{2-} (Sulphate), SO_3^{2-} (Sulphite), NH_3 (Ammonia), NO_2^- (Nitrite), NO_3^- (Nitrate), PO_4^{3-} (Phosphate), PO_3^{3-} (Phosphite), Cl^- (Chloride), and F^- (Fluoride). The members of Kingdom archaebacteria, eubacteria (bacteria), and eukaryotes (such as fungi, algae, yeasts, and protozoa) are very important for the mineralization of organic compounds (pollutants) in the environment (Reis *et al.,* 2020).

However, Heterotrophic bacteria have traditionally been deemed highly significant, but the importance of fungi is now gaining recognition. Although algae and cyanobacteria can facilitate certain bio-degradative processes, their overall role is likely limited in comparison. Natural compounds are easily degradable, and these microorganisms can degrade them. While xenobiotics, in most cases, are recalcitrant and non-biodegradable in the human body, biological treatment system, and environment. The organic pollutants are categorized as biodegradable, recalcitrant or persistent, and non-biodegradable (Knapp *et al.,* 2003; Van *et al.,* 1998).

2.6.1. Co-metabolism

Another very interesting concept, co-metabolism, is defined as the degradation of one compound that is highly dependent on the strict presence of another compound in that environment. This dictates a scenario in which a microbial community is only capable of degrading a compound in the presence of other compounds that are essential for the growth of that community. Moreover, in some cases, these facilitating compounds may be very specific while in others a diverse group of compounds may be required (Knapp *et al.,* 2003).

2.6.2. Quinolones degradation

Quinolones are a class of antibiotics that target two bacterial enzymes involved in DNA replication and transcription, Topoisomerase II and IV (Hooper & David, 1999). Presently, the use of first-generation quinolones is largely discontinued in the medical field while fluoroquinolones and third-generation are extensively used in human treatment and less frequently in veterinary medicines (Hofacre *et al.,* 2013). These antibiotics are present in the environment as they are mostly excreted in their unaffected form and subsequently disseminated into soil and wastewater through human defecation and dung dispersal. Fluoroquinolones, specifically ciprofloxacin, have often been reported in the wastewater of hospitals, metropolitan wastewater treatment plants (WWTPs), and in surface waters, at concentrations of microgram per liter and nanogram per liter. Fluoroquinolones exhibit significantly high adsorption and absorption of organic materials. As a result, they are found in higher concentrations in the soil compared to the aquatic environment. Resistance to fluoroquinolones primarily arises through various mechanisms, including target modification (specifically DNA gyrase A), efflux pumps, and a more recently discovered process involving acetylation of the piperazine ring by aminoglycoside acetyltransferase (AAC(60)-Ib-cr) (Blair *et al.,* 2015).

2.6.3. Abiotic degradation of Fluoroquinolones

Photolysis and photo-catalytic processes induce substantial transformation of fluoroquinolones (Giri & Golder, 2019). When exposed to natural sunlight in water environments, fluoroquinolones exhibit a range of reactions including oxidative removal of the fluorine group from the compounds, piperazine ring oxidation, and consecutive degradation of the oxidized ring, as well as reductive defluorination and piperazine ring degradation (Baena-Nogueras *et al.,*

2017). These diverse photo-transformation reactions have been demonstrated to occur not only in water matrices but also in soil, potentially influenced by naturally occurring catalysts like manganese oxide (Reis *et al.,* 2020).

2.6.4. Pathways involved in the Biodegradation and biotransformation of Fluoroquinolones

As previously described fluoroquinolones are removed by the adsorption and absorption of organic matter in soil and easily degraded by light. But, they reveal that these pollutants are not easily degraded or resistant to microbial degradation of soil or activated sludge as demonstrated in the Closed bottle experiments (Al-Ahmad *et al.,* 1999), CO2 release tests, and $_{14}$C-labelled fluoroquinolones in soil (Gartiser *et al.,* 2007; Marengo *et al.,* 1997).

Fluoroquinolones are the second class of quinolones which is the most widely used and studied member among the quinolones. Fig. 4. Demonstrates the main pathways involved in the biotransformation and biodegradation of ciprofloxacin. These mechanisms involved fluorine elimination via oxidative process, demethylation reaction (Amorim *et al.,* 2014), quinoline nucleus hydroxylation (Fang *et al.,* 2021), N-oxidation (Zhang *et al.,* 2022), N-acetylation, and removal of ethylene in the piperazine ring. Furthermore, the first and fourth-generation quinolones are also bio-transformed by the pathways analogous to that of fluoroquinolones biodegradation (Reis *et al.,* 2020).

Among the mechanisms documented thus far, N-acetylation stands out as the prevailing pathway for fluoroquinolone transformation. This process can be facilitated by a wide array of bacterial and fungal strains (Li *et al.,* 2021; Manasfi *et al.,* 2020; Wetzstein *et al.,* 1997), and it is also a conventional (Pan *et al.,* 2018). Surprisingly, a modified form of aminoglycoside acetyltransferase (AAC (6′)-lb), which was originally associated with aminoglycoside resistance,

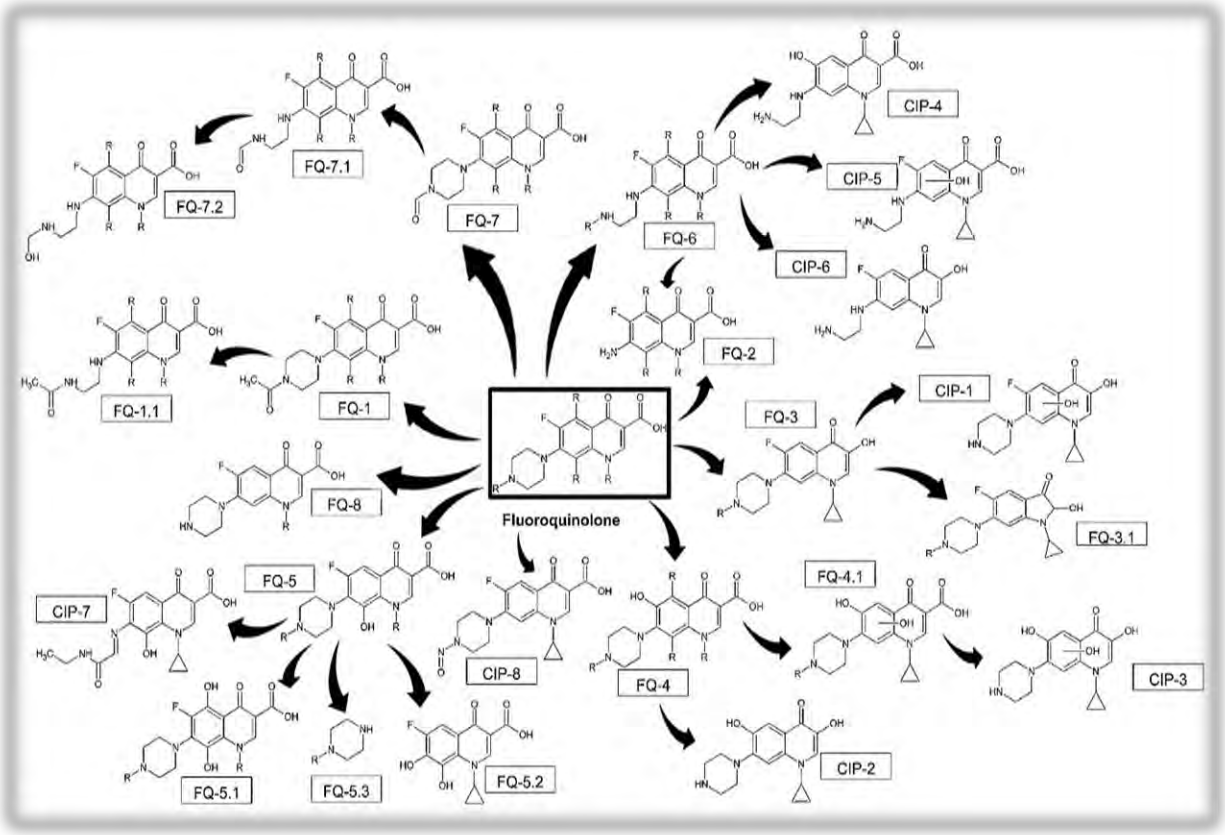

Figure 4. This figure demonstrates the mechanism/pathways involved in the biotransformation and biodegradation of 2nd generation quinolones by different species of bacteria and fungi (Sekirov *et al.,* 2010).

has been demonstrated to also possess the capability to N-acetylate ciprofloxacin. This discovery marks the first instance of an enzyme capable of modifying antibiotics from two distinct classes (Robicsek *et al.,* 2006). Most of the biotransformation processes reduce the antimicrobial activity of these antibiotics, however, sufficient information is not available on the specific enzymes and genes involved in the biotransformation and biodegradation (Parshikov, Sutherland, & Biotechnology, 2012). The FQ metabolites were only found for two or more fluoroquinolones and CIP metabolites were found only for ciprofloxacin (Reis *et al.,* 2020).

2.7. Microbial enzymes responsible for biodegradation and biotransformation of CIP and other FQ

The analyzed body of scientific information unveiled the fact that ciprofloxacin and other fluoroquinolones are competently degraded or transformed by different microbial species like fungal and bacterial. As the ciprofloxacin degraded products are shown in Table 2, the most frequent biotransformation pathway employed by different microbes is *N*-acetylation. An altered enzyme in *E. coli*, aminoglycoside acetyltransferase, is involved in the transformation of ciprofloxacin and norfloxacin into M1 and *N*-acetyl-norfloxacin (Robicsek *et al.,* 2006). Contrarily, in *microbacterim* sp, the process of *N*-acetylation of norfloxacin was catalyzed by glutamine synthetase. The aforementioned experiments showed that sometimes different enzymes are responsible for catalyzing the transformation pathways of the same substrate. Generally, the enzymes and pathways employed by microbes in the biotransformation and biodegradation of ciprofloxacin into different degraded products are mostly unidentified. To gain a deeper understanding of the intricate mechanisms at play, experiments involving whole-cell transformations were conducted. These experiments included the amalgamation of mediator and inhibitors for enzyme to enhance activity, both in-vivo and in-vitro. Additionally, in vitro assays were performed using crude or purified enzymes isolated from specific organisms. Moreover, the activity of different metabolites extracted from these microbes were checked in the lab. While exploring the biotransformation potential of these pharmaceuticals by means of fungi, Cvancarova et al. (Čvančarová *et al.,* 2015) noted minimal alterations in the synthesis of lignin-degrading enzymes Laccases, MnP, and manganese-independent peroxidase (MiP). Nevertheless, the statistical analysis through principal component analysis indicated a correlation between MnP and the transition of the three FQs, implying the potential involvement of this enzyme in the biotransformation process.

A study conducted by Jureczko *et al.,* (2021) observed a decline in ciprofloxacin concentration (at the start of 10 milligrams per Liter) within 20 hours of incubation utilizing purified Lac from *T. versicolor* . The addition of a Lac mediator led to enhanced removal of both ciprofloxacin and norfloxacin. To investigate the involvement of the CYP450 enzyme family in FQ biotransformation, a specific blocker (1-amino benzotriazole, ABT) was introduced to intact cell of *T. versicolor*. Significant blocking of the mechanism strongly suggested the participation of

the CYP450 enzyme family. As a result, both these enzyme systems are potentially involved in the biotransformation of fluoroquinolones by *T. versicolor*.

Another group of scientists had explored the degradation of various antibiotics simultaneously encompassing distinct categories (including fluoroquinolones, tetracyclines, and sulfonamides) utilizing a Membrane-based enzymatic reactor with immobilized Lac obtained from *T. versicolor*. Interestingly, deprived of a synergism of other enzymes, Lac application exhibited marginal effectiveness in eradicating fluoroquinolones. However, when combined with syringaldehyde as a mediator, substantial removal rates (ranging between 73% and 93% over 24 hours, with an initial concentration of 10 milligrams per Liter) were realized for specific fluoroquinolones. As a result, the potential contribution of Lac in FQ removal was suggested (Han *et al.,* 2023).

Likewise, findings from the research conducted by (Jia *et al.,* 2018), they indicated a notable impediment in the biodegradation of ciprofloxacin within sulfate-reducing bacteria sludge system (without oxygen) following the addition of ABT, a CYP450 inhibitor. Consequently, the authors posited the essential involvement of CYP450 enzymes in the process of ciprofloxacin biodegradation. Nonetheless, it is conceivable that the prevailing conditions were not entirely devoid of oxygen, considering that reactions like hydroxylation, facilitated by CYP450 enzymes, mandate the presence of molecular oxygen.

Table 1. Bacterial and Fungal CIP degradation Products.

Genus	Species	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19
Fungi	*D. squalens*	✓	✓	✓	✓			✓	✓											
	G. striatum				✓	☑				☑	☑	✓	☑	☑	✓	☑	✓		✓	
	I. lacteus		✓	✓	✓			✓	✓								✓			
	M. ramannianus	☑																		
	P. tigrinus	✓	✓	✓	✓			✓	✓											
	P. ostreatus	✓	✓	✓	✓			✓	✓											
	P. guepini																☑			
	T. versicolor	✓	✓	✓	✓	✓	✓	✓	✓								✓			
	T. viride																			☑
	X. longipes	☑	☑	☑	☑															
Bacteria	*Bradyrhizobium sp*					✓											✓			
	E. coli	✓																		
	L. portucalensis				✓						✓									
	M. frederiksbergense	☑																		
	M. gilvum	☑																☑		
	M. smegmatis	☑																		
	Rhodococcus sp				✓	✓			✓								✓			
	Thermus sp.		✓	✓	✓				✓								✓			

✓, CIP degradation products by MS analysis; ☑, CIP degradation products by NMR (Rusch *et al.,* 2019).

Another study examined the capability of Streptomyces ipomoeae's alkaline laccase (SilA) to convert ciprofloxacin and norfloxacin (both initially present at 50 milligrams per liter), with or without mediators. Notably high transformation rates (> 90% within 24 hours) were achieved using SilA in conjunction with acetosyringone as a mediator, strongly suggesting laccase involvement in the transformation mechanism (Blánquez *et al.,* 2016). Furthermore, a group of scientists scrutinized the breakdown of NOR in pharmaceutically contaminated water by utilizing a chloroperoxidase (CPO) extracted from the mycelium-forming fungus *Caldariomyces fumago*. They effectively validated this pharmaceutical could (starting concentration of 25.6

milligrams per liter) be efficiently degraded (82%) in just 25 minutes through CPO-facilitated oxidation (Zhao *et al.*, 2017). The literature analysis indicates that FQs are effectively eliminated using both intact cells and unrefined/processed enzymes. Certain biocatalysts (enzymes), including extracellular laccases and intracellular cytochrome P450 enzymes, have been suggested as potential contributors to the degradation of FQs.

CHAPTER 3

MATERIALS AND METHODS

3. Materials and Methods

This study was conducted at Laboratory for Microbial Food Safety and Nutrition, Department of Microbiology, Quid-I-Azam University, Islamabad, Pakistan. This research was a part of the doctoral research project led by Miss. Misbah Tabassum, where she carried out the study subject selection, fecal sample collection and analysis. The samples collected during her project were used in this study.

3.1. Study design

3.1.1. Selection of depression patient's fecal samples

In this study, seventeen (17) clinically depressed subjects from the psychiatry ward of Pak Red Crescent Hospital were enrolled.

3.2. Collection of Stool Samples

The inclusion criteria for the study comprised seventeen volunteers diagnosed with clinical depression, aged between 18 and 60 years. Within this group, there were ten male and seven female participants, denoted as subjects from IM-1 to IM-17. Among them, five individuals exhibited severe depression, with BDI scores ranging from 30 to 60, while another five demonstrated moderate depression, with BDI scores falling between 23 and 26. The remaining participants experienced mild depression, with BDI scores below 19.

These participants were carefully selected to exclude individuals with any prevailing gastrointestinal tract disease or recent antibiotic treatment within the preceding three months. Fecal samples were collected aseptically to avoid environmental contamination in stool containers filled with Buffered Glycerol Saline and RNA Later. The samples preserved in buffered glycerol saline and the RNA Later were stored for culture-dependent and metagenomic analysis, respectively (Reck *et al.,* 2015).

3.3. Microbiological Analysis of Stool Samples

The frozen fecal samples, preserved in buffered glycerol saline, were removed from the ultra-low temperature freezer (-80°C) and allowed to thaw under sterile conditions at room temperature. Following thawing, the stool samples were mixed thoroughly to obtain uniform distribution of

fecal microbiota. Then, 25 µl of the stool samples were aseptically introduced to 50 ml of tryptic soy broth (TSB) to facilitate enrichment. The culture was maintained at 37°C for 8 hours under anaerobic conditions, aiming to achieve an exponential growth phase for both strict and facultative anaerobic bacteria. To obtain the CFU/ml of the enriched bacterial culture, serial dilution was performed as mentioned above. The bacterial culture was diluted up to an eighth fold. After serial dilution, a known amount of bacterial broth culture was taken that contained 1 x 10^8 CFU mL^{-1} for centrifugation. The centrifugation was done at 6000 rpm at room temperature for 20 minutes. The supernatant was discarded, and the pellet was subjected to two rounds of washing using phosphate-buffered saline (PBS), followed by centrifugation. Then, the pellet was introduced to a basic salt medium. The bacterial pellets were re-suspended in the BSM at 37℃ for 7 days under anaerobic conditions with 4 µg/ml of the CIP, and the medium without bacteria was set as a negative control. Furthermore, another control was also used that contained just bacterial inoculum as positive control. The aliquots were collected from each experiment for further analysis at the interval of 1^{st}, 3^{rd} 5^{th} and 7^{th} day.

3.3.1. Preparation of Ciprofloxacin stock solution

Ciprofloxacin powder purchased from Sigma Aldrich (Darmstadt, Germany) was solubilized in deionized distal water to produce CIP stock solution (1mg/ml) and securely kept in amber bottles at – 20 °C. Subsequently, the prepared stock solution was employed at a concentration of 4 and 8 µg. mL^{-1} for the bacterial degradation experiment.

3.3.2. Preparation of basic salt medium (BSM)

The following table outlines the formulation of the mineral salt medium utilized in this investigation. The hydrogen ion concentration of the medium was adjusted to 7.0 with acidic solution, and the medium was sterilized at 121 °C for a duration of 20 minutes (Sekar *et al.,* 2011).

Table 2.*Composition of Basic Salt Medium (BSM).*

S. No	Chemical(s)	Chemical Formula	Amount (g/l)
1	Dipotassium hydrogen phosphate	K_2HPO_4	1.73
2	Potassium dihydrogen phosphate	KH_2PO_4	0.68
3	Magnesium Sulfate Heptahydrate	$MgSO_{4.7}H_2O$	0.1
4	Sodium chloride	NaCl	1
5	Ferrous sulfate heptahydrate	$FeSO_{4.7}H_2O$	0.03
6	Ammonium Nitrate	NH_4NO3	1
7	Calcium Chloride	$CaCL._2H_20$	0.02

3.4. Determination of Ciprofloxacin Degradation by spectrophotometry

The quantification of ciprofloxacin in control and experimental group was done via Perkin Elmer Precisely, Lambada 25 UV/VIS Spectrophotometer. For the preparation of standard curve, dilution with different ciprofloxacin concentrations were prepared from stock solution of 1mg/ml. The ciprofloxacin standard was screened at the wavelength (λ) of 200-700 nm and the best OD was obtained at wavelength (λ) max 330 nm. Ciprofloxacin standard curve was made using different concentrations of CIP (2-10 µg/ml).

For analysis of the degradation of ciprofloxacin through a spectrophotometer, 96-well plates were used. Each aliquot was tested in triplicate, with 200 µl of the aliquot added to each wells using a pipette. Before analysis, the aliquots were purified through microfiltration (using a 0.22 µm pore size) to eliminate bacterial biomass and unwanted residues.

The following formula are used for the determination of ciprofloxacin concentration in aliquots taken from BSM at different intervals as

$$Determination\ of\ ciprofloxacin\ concentration\ in\ aliqout = optocal\ denisty\ of\ aliquot\ at\ 330\ nm - \frac{0.045}{0.0425}$$

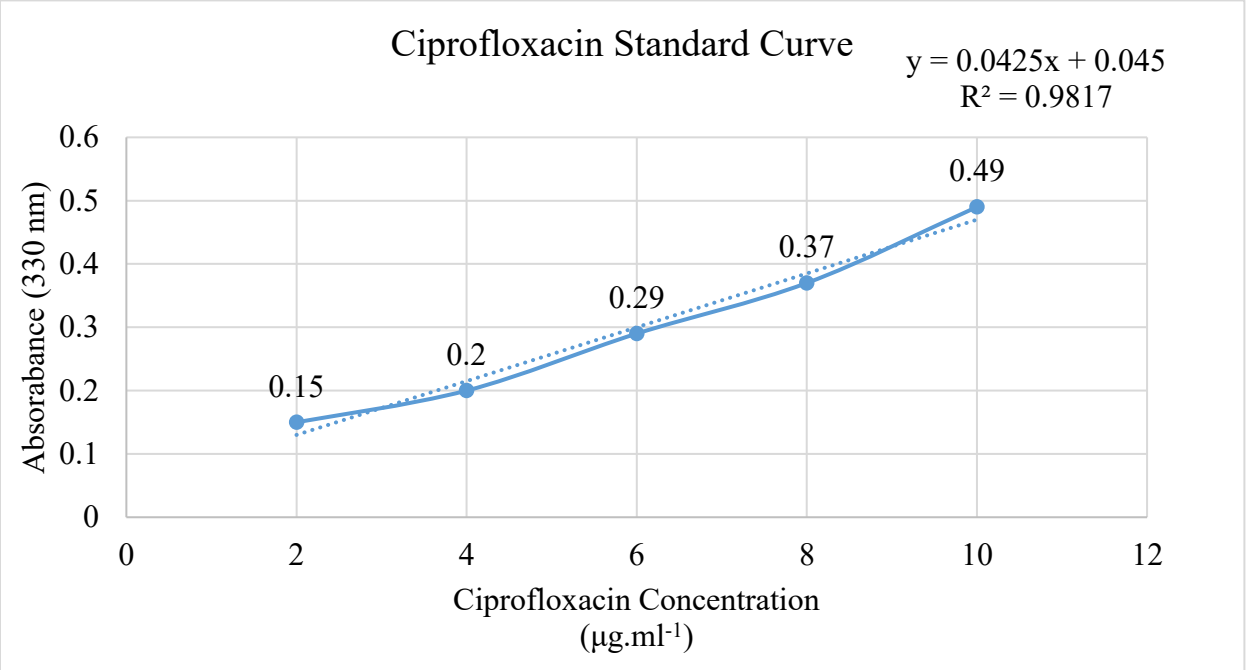

Figure 5. This figure depicts the standard curve of Ciprofloxacin.

3.5. Validation of Ciprofloxacin degradation by High-performance liquid chromatography (HPLC) of selective samples

The confirmation of spectrophotometric analysis and the determination of the final ciprofloxacin concentration in BSM post-fermentation were executed through HPLC employing a C18 column at a wavelength of 278 nm. The procedure encompassed the injection of a 25 µl liquid sample into the equipment, with a rate of flow of 1.5 mL min^{-1} and the temperature of the system maintained at 30 °C. The mobile phase composition consisted of a ratio of 87:13 for mobile phase A (0.05 mol L^{-1} phosphoric acid solution with pH adjusted to 2.4 using triethylamine) to mobile phase B (100% acetonitrile). The retention time corresponding to ciprofloxacin spanned around 1.56 minutes (Qu *et al.*, 2021).

The following formula are used for the determination of ciprofloxacin concentration in aliquots taken from BSM at different intervals as

$$Concentration\ of\ ciprofloxacin\ after\ degradation = area\ of\ the\ sample\ at\ retention\ time\ 1.56\ \frac{minutes}{area}\ of\ the\ standard * conentration\ of\ standard$$

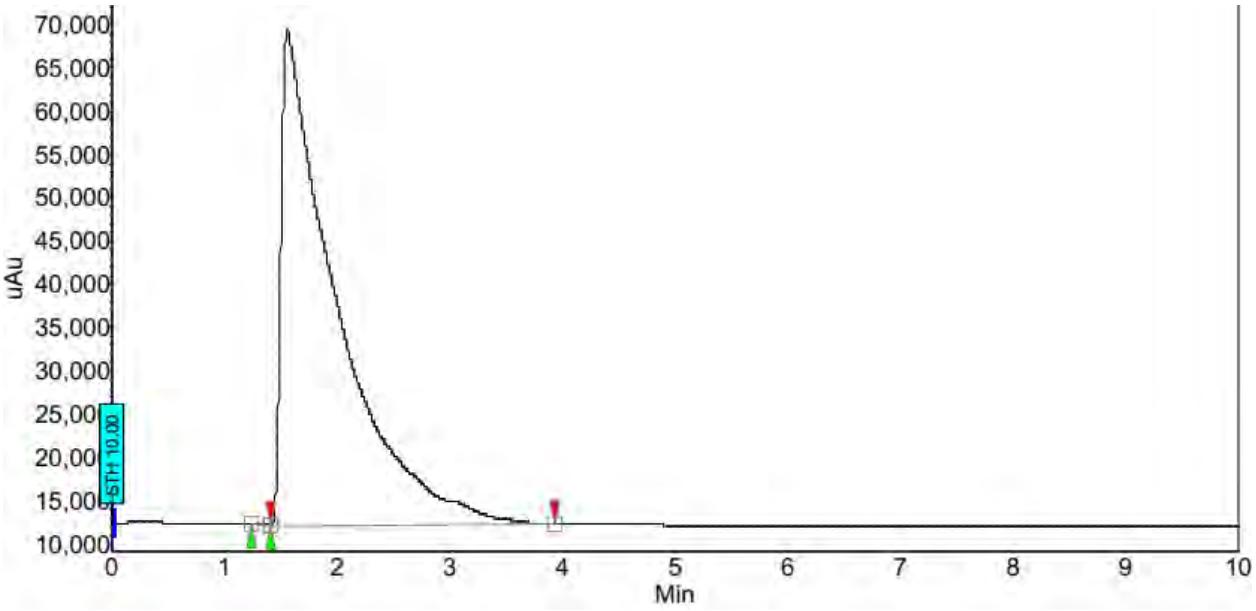

Figure 6. Chromatograph of the standard for ciprofloxacin

3.6. Determining the percentage antibacterial activity of ciprofloxacin after degradation by sensitive assay

The antibacterial potential of the ciprofloxacin after incubation with fecal microbiota was assessed against *Pseudomonas aeruginosa* (ATCC 27853), and *Bacillus subtilis* subsp. subtilis (ATCC 19659). This assay was conducted for the purpose to potency of ciprofloxacin at different intervals of incubation i.e. 1st, 3rd,5th, and 7th day (Dafale *et al.,* 2016). This assay was performed utilizing the disc diffusion method on Nutrient agar plates. The aliquots taken from both the experimental and control groups were evaluated at intervals of the 1st, 3rd, 5th, and 7th days of the experiment. These aliquots were purified to eliminate bacterial biomass and unwanted residues through microfiltration employing a microfilter (0.22 µm pore size). The resulting antimicrobial activity was subsequently compared to varying concentrations of ciprofloxacin (Thillaimaharani *et al.,* 2013).

The following formula that was used for calculation of percentage antimicrobial activity;

$$\%\, antimicrobial = antimicrobial\ of\ cip\ \frac{byproducts}{antimicrobial}\ of\ control * 100$$

3.7. Correlation of Ciprofloxacin degradation with Gut microbial diversity of clinically depressed patients

The basic statistical analysis was conducted by OriginPro 2021 (Version: 9.8.0.200). QIIME2 pipeline was used to conduct analysis of metagenomic data of 16S rRNA genes. To access liner relationship of the ciprofloxacin degradation with the gut microbial diversity of clinically depressed patients, Pearson correlation was conducted on the Operational taxonomic units (OUTs) of genus of each individual gut microbiota with the percent reduction in the ciprofloxacin after one week incubation by GraphPad Prism 9 (Version: 9.5.1).

CHAPTER 4
RESULTS

4. Results

4.1. Determination of Ciprofloxacin Degradation by Spectrophotometry

The valuation involved calculating the residual ciprofloxacin concentration at various time intervals following the incubation period. This was achieved by referencing a ciprofloxacin standard curve. The outcomes were examined through one metrics that the percentage of reduction.

The degradation of ciprofloxacin by fecal microbiota was determined by Spectrophotometer which showed that some patients' microbiotas have the capability to degrade ciprofloxacin at variable pattern among different individuals and concentrations. At 4 µg. ml^{-1} of ciprofloxacin, the IM-7 was the best degrader among all which degraded about 90% of ciprofloxacin within one

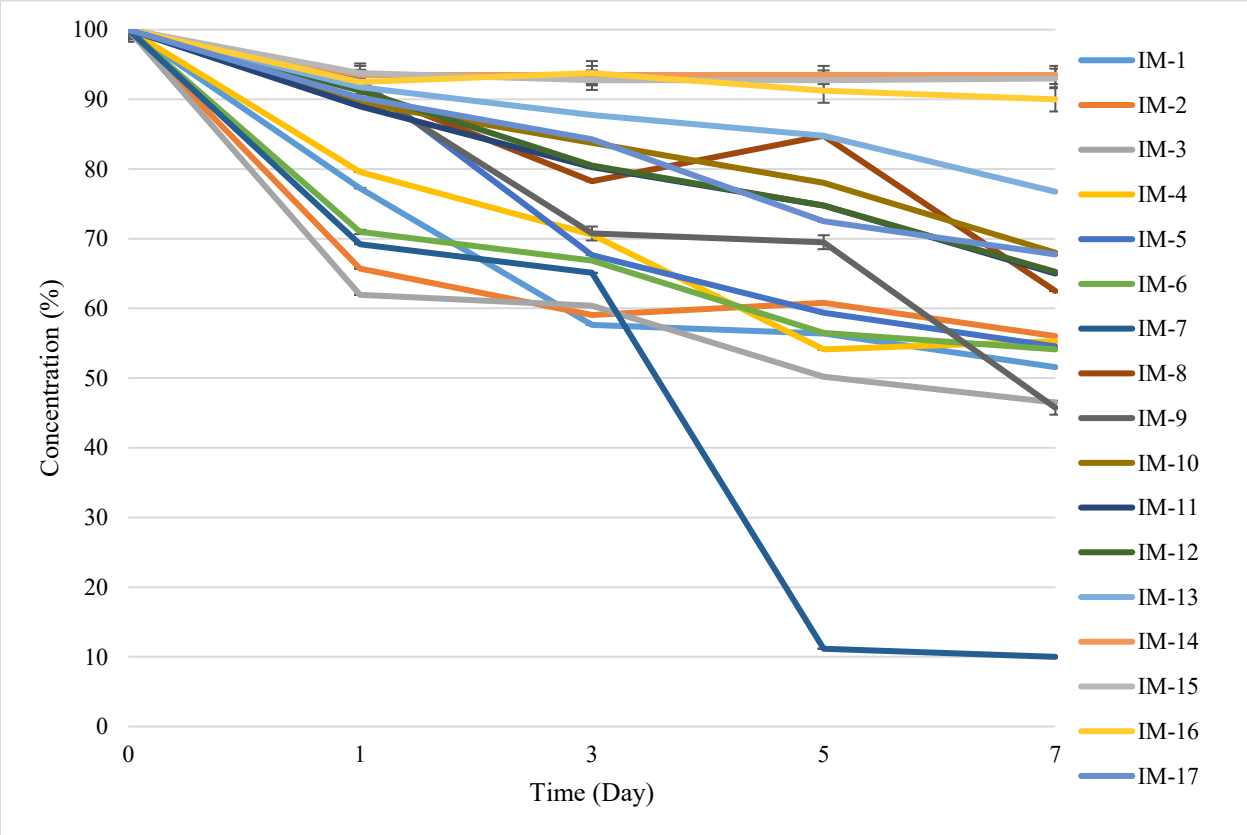

Figure 7. Depicting the degradation potential of IM-1 to IM-17 at 4 µg ml^{-1} Ciprofloxacin.

week. While the remaining samples also showed significant degradation ranging from 20% to 55%, except IM-13,14 and 15 showed the non-significant (lowest) degradation ranging from 6% to 10%.

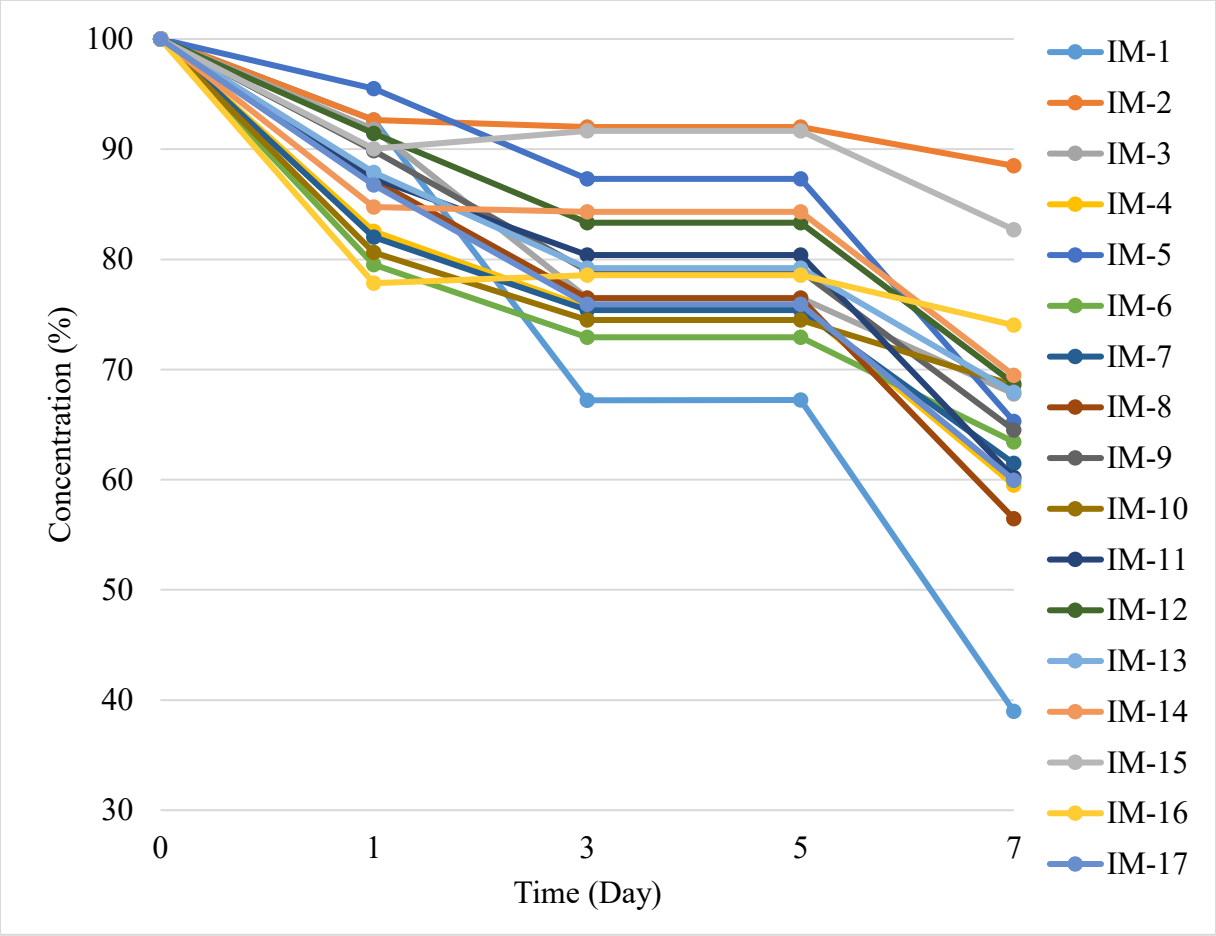

Figure 8. Depicting the degradation potential of IM-1 to IM-17 at 8 µg ml^{-1} Concentration.

At 8 µg ml^{-1}, the best degradation was reported by IM-1 which is about 60% reduction. The remaining samples, IM-3,4,5,6,7,8,9,10,11,13,15 and 16, also showed significant reduction in the concentration of ciprofloxacin by 25% to 45%. While IM-2 and 14 showed the lowest reduction (10% to 15%) in ciprofloxacin.

4.2. Validation of Ciprofloxacin degradation by High-performance liquid chromatography (HPLC) of selective samples

High performance liquid chromatography (HPLC) has validated that gut microbiota of depression patients have the potential to degrade the Ciprofloxacin. The chromatographs showed that IM-1,4,6 and 7 degraded and reduced the concentration of ciprofloxacin from 4 µg. ml^{-1} to 1.56 µg. ml^{-1}, 1.62 µg. ml^{-1}, 0.5 µg. ml^{-1}, and 0.21 µg. ml^{-1}, respectively. While IM-3 reduced the initial concentration (4 µg. ml^{-1}) to 3.5 µg. ml^{-1}, was confirming that the fecal microbiota of IM-3

has lowest ciprofloxacin degradation potential con confirmed by Spectrophotometric analysis and HPLC.

Table 3. Depicting the concentration that was determined by Spectrophotometer and HPLC

S. No	Sample Type	Initial Concentration (µg/ml)	Final Concentration (µg/ml) by HPLC	Final Concentration (µg/ml) by Spectrophotometer
1	IM-1	4	1.56	2.25
2	IM-3	4	3.51	3.79
3	IM-5	4	1.62	2.18
4	IM-6	4	0.50	2.16
5	IM-7	4	0.21	0.91

4.3. Determining the percentage antibacterial activity of ciprofloxacin after degradation by sensitive assay

The antibacterial activity of ciprofloxacin aliquots that were taken from basic salt medium (BSM) at different intervals were evaluated against different pathogenic bacterial strains i.e. *Pseudomonas aeruginosa* (ATCC 27853), and *Bacillus subtilis* subsp. subtilis (ATCC 19659). The results showed that gut microbiota of these patients has the potential to degrade the ciprofloxacin. With the increase in the time of incubation, the zones of inhibition of ciprofloxacin were also decreasing as shown in the below table. Additionally, these results also predicted that fecal microbiota of these individuals upon degradation of ciprofloxacin yielded compounds that were possessing lower antibacterial activity. It is also worth mentioning that positive control consistently displayed significant antibacterial activity against pathogenic bacterial strains as compared to the experimental groups. Moreover, it is also seen that the pattern of antibacterial activity of the ciprofloxacin aliquots was different against the *Pseudomonas aeruginosa* (ATCC 27853), and *Bacillus subtilis* subsp. subtilis (ATCC 19659).

***Table 4. Showing the percentage antibacterial of ciprofloxacin against Pseudomonas aeruginosa* (ATCC 27853).**

Antibacterial activity of CIP degradation byproducts								
Zone of inhibition (%)								
Time Interval (Day)								
	4µg/ml				8µg/ml			
Subjects	1st	3rd	5th	7th	1st	3r	5th	7th
C	99	98	97	97	98	97	97	96
IM1	44	32	0	0	61	27	0	0
IM2	56	29	0	0	0	0	0	0
IM3	68	56	34	29	35	27	0	0
IM4	24	0	0	0	0	0	0	0
IM5	38	32	0	0	35	0	0	0
IM6	0	0	0	0	71	33	25	0
IM7	74	47	0	0	81	18	0	0
IM8	62	41	22	0	61	45	34	0
IM9	56	32	0	0	58	36	0	0
IM10	47	21	0	0	68	48	0	0
IM11	74	62	44	29	58	39	0	0
IM12	74	62	53	37	68	45	22	0
IM13	85	68	59	37	61	39	0	0
IM14	35	21	0	0	68	48	34	0
IM15	62	56	66	63	65	58	53	20
IM16	62	50	38	0	74	55	34	0

The nutrient agar was the chosen medium, with incubation set at a temperature of 37 ± 2 °C in an aerobic environment. The absence of a zone of inhibition is denoted by the digit '0', while numerical values indicate the percentage reduction in the zone of inhibition calculated from the control. *Pseudomonas aeruginosa (ATCC 27853)* served as the indicator pathogen in this experiment.

***Table 5. Showing the percentage antibacterial of ciprofloxacin against Bacillus subtilis subsp. subtilis* (ATCC 19659).**

Antibacterial activity of CIP degradation byproducts								
Zone of inhibition (%)								
Time Interval (Day)								
	4µg/ml				8µg/ml			
Subjects	1st	3rd	5th	7th	1st	3r	5th	7th
C	98	97	97	94	98	96	95	94
IM1	58	39	0	0	61	27	0	0
IM2	52	0	0	0	65	64	47	0
IM3	48	33	0	0	35	27	0	0
IM4	0	0	0	0	61	33	0	0
IM5	55	33	0	0	81	42	0	0
IM6	48	36	0	0	71	33	25	0
IM7	0	0	0	0	68	33	0	0
IM8	61	33	28	0	61	45	34	0
IM9	48	36	0	0	58	36	34	0
IM10	52	21	0	0	77	39	0	0
IM11	61	45	0	0	58	39	34	0
IM12	61	42	0	0	65	39	22	0
IM13	94	72	70	66	65	36	0	0
IM14	48	27	0	0	68	58	53	53
IM15	74	66	59	52	68	52	59	0
IM16	74	45	22	0	74	55	0	0

The nutrient agar was the chosen medium, with incubation set at a temperature of 37 ± 2 °C in an aerobic environment. The absence of a zone of inhibition is denoted by the digit '0', while numerical values indicate the percentage reduction in the zone of inhibition calculated from the control. *Bacillus subtilis* subsp. subtilis (ATCC 19659) served as the indicator pathogen in this experiment.

4.4. Establishing correlation of the gut microbiota at the phylum level with ciprofloxacin degradation potential of depression patients

The Pearson correlation showed that degradation at 4 µg/ml of ciprofloxacin was positively correlated with genus *Succinivibrio* and *Lachnospiraceae*. While negatively correlated with the genus *Lactobacillus, Romboutsia, Clostridium, Haemophilus, Sutterella, Veillonella, and Turicibacter*. At CIP concentration of 8 µg/ml of ciprofloxacin, the degradation was positively correlated with genus *Dialister, Dorea, Escherichia, Ruminococcus,* and *Monolobus*. While degradation was negatively correlated with genus *Prevotella* and *Agathobacter*.

The positive liner relationship of the percent reduction of ciprofloxacin and different microbial species at the mentioned concentration depicts that the concentration of the antibiotic decreases, the abundance or activity of these microbial species also decreases. This could be explained as the species being involved in the degradation of the ciprofloxacin or dependent upon the antibiotic. While a negative correlation could suggest that as the concentration of the antibiotic decreases, the abundance or activity of the microbial species increases. This might indicate that the species is not involved in the degradation of the antibiotic.

DISCUSSION

5. DISCUSSION

The human body is considered a microbial depot containing microscopic lives that belong to the Phylum bacteria, fungi, viruses, archaea, protozoan, and viruses (Bhalodi *et al.,* 2019). Generally, these microorganisms are present on every surface that is exposed to the external environment. Stable consortia of these microscopic lives are very important for homeostasis and normal physiological functions. It is also implicated in diverse pathways of metabolism and nutrition, immunity, biogenesis of different biologically active compounds, and nervous system development (Hou *et al.,* 2022; Pilmis *et al.,* 2020).

However, the trajectory and behavior of pharmaceuticals are frequently governed not just by the host's characteristics, but also by the presence of microbial communities within the digestive tract. The gut microbiome is acknowledged for its ability to impact drug metabolism, both directly and indirectly (Clarke *et al.,* 2019). Recent indications point to the reciprocal effects that drugs can exert on the composition and operation of the gut microbiome. Modifications in drug metabolism mediated by the microbiota and modifications in the gut microbiome influenced by drugs can yield positive or adverse consequences for the host (Enright *et al.,* 2016).

To accomplish these objectives, stool samples of clinically depressed patients were taken from the laboratory collection. The microbial diversity of these samples was already characterized by the 16s rRNA gene. For degradation, the microbiota of these subjects was suspended with 4 and 8 µg ml-1 of ciprofloxacin (CIP) in a basic salt medium (BSM) for one week. The aliquots were collected from each experiment for further analysis at the intervals of the 1st, 3rd, 5th, and 7th day of the experiment. To determine the degradation of ciprofloxacin in the experimental and control groups, the aliquots were analyzed by spectrophotometer and HPLC. To check the potency of the ciprofloxacin after one week with fecal microbiota of clinically depressed patients, the sensitive assay of each aliquot taken on the 1st, 3rd, 5th, and 7th day of the experiment was performed against two pathogenic strains i.e. *Pseudomonas aeruginosa* (ATCC 27853), and *Bacillus subtilis* subsp. subtilis (ATCC 19659) (Dafale *et al.,* 2016).

The spectrophotometric analysis of the aliquots taken at the mentioned interval revealed that the fecal microbiota of most samples exhibited a significant reduction in the concentration of CIP but not complete elimination. At 4 µg. ml^{-1} of ciprofloxacin, the IM-7 was the best degrader among all which degraded about 90% of ciprofloxacin within one week. While the remaining

samples also showed significant degradation ranging from 20% to 55%, except IM-14,15 and 16 showed the non-significant (lowest) degradation ranging from 6% to 10%. At 8 µg ml^{-1}, the best degradation was reported by IM-1 which is about 60% reduction. The remaining samples, IM-2,3,4,5,6,7,8,9,10,11,12,13,15 and 16 also showed significant reduction in the concentration of ciprofloxacin by 25% to 45%. While IM-1 and 14 showed the lowest reduction (10% to 15%) in ciprofloxacin. Gjonbalaj et al. (2020) conducted an experiment to investigate the role of commensal gut microbiota in the degradation of orally administered antibiotics. They discovered that these bacteria possess enzymatic machinery capable of degrading commonly used antibiotics (Gjonbalaj *et al.,* 2020).

The variability in the degradation potential of the gut microbiota at the individual level can be attributed to differences in microbial diversity, prior antibiotic exposure, environmental factors, and host genetics. It is well-established that each individual possesses a personalized intestinal microbial profile characterized by unique microbial patterns, each with distinct metabolic capabilities compared to others (Dethlefsen *et al.,* 2008; Liao *et al.,* 2016). Previous exposure of the gut microbiota to antibiotics can also impact its degradation abilities. Studies have reported that microbiotas previously exposed to higher concentrations of ciprofloxacin exhibit higher degradation capabilities (Liao *et al.,* 2016). Environmental factors such as diet, age, health status, and stress, as well as host genetics, can influence the composition and function of the gut microbiota. These factors ultimately impact the degradation of ciprofloxacin (Chang & Kao, 2019).

The incomplete biodegradation of CIP by the fecal microbiota in various subjects can be attributed to the limited microbial potential for the bioremediation of these antibiotics. This was confirmed by Liyanage *et al.,* who demonstrated that a considerable portion of antibiotics exhibited limited biodegradability by microorganisms. Consistent with patterns observed with various antibiotics, CIP demonstrates reduced biodegradation potential and a notable tendency for sorption in soil (Liyanage & Manage, 2018). The biodegradation of primary carbon source and microbial physiology were the two main reasons reported in the literature for the degradation potential of bacterial isolates or pure culture (Fischer *et al.,* 2014).

High performance liquid chromatography (HPLC) has validated that gut microbiota of depression patients have the potential to degrade the Ciprofloxacin. The chromatographs showed

that IM-1,4,6 and 7 degraded and reduced the concentration of ciprofloxacin from 4 µg. ml^{-1} to 1.56 µg. ml^{-1}, 1.62 µg. ml^{-1}, 0.5 µg. ml^{-1}, and 0.21 µg. ml^{-1}, respectively. While IM-3 reduced the initial concentration (4 µg. ml^{-1}) to 3.5 µg. ml^{-1}, was confirming that the fecal microbiota of IM-3 has lowest ciprofloxacin degradation potential con confirmed by Spectrophotometric analysis and HPLC. The same result was found in a study conducted by Souhila et al. that microbes significantly degrade the CIP in absence of any other carbon source (Souhila *et al.,* 2023).

The interpretation of the percentage antibacterial activity of ciprofloxacin after incubation with fecal microbiota at different interval against different pathogenic strains unveils that the gut microbiota of clinically individuals of transforming ciprofloxacin into products with lower or no antimicrobial activity compared to control. This deduction is confirmed by evaluating the potential for zones of inhibition formation of these degraded products. Among the CIP degradation products detected in the aliquots, the majority exhibited notably weak activity against ATCC pathogenic strains, including *Pseudomonas aeruginosa* (ATCC 27853), and *Bacillus subtilis* subsp. subtilis (ATCC 19659). Consequently, the zone formation of the control serves as evidence that neither photo-degradation nor disintegration through reactions with the constituents of the media has occurred. The exact molecular mechanism involved is yet to be explored. However, the possible reason cited in the literature could be the incomplete destruction of the piperazine ring and the addition of formyl or acetyl groups by different microbial processes. This leads to the production of degradation products with lower antibacterial activity (Rusch *et al.,* 2018).

The interpretation of the linear relationship between the ciprofloxacin degradation potential of the gut microbiota, in terms of percent reduction, and different members of the intestinal microbiota by Pearson correlation shows that some are involved in positive correlations, while others are in negative correlations. The positive correlation indicates that as the concentration of ciprofloxacin decreases, the activity of the microbial species also decreases. This suggests that these species may be involved in the degradation of ciprofloxacin or are dependent upon the antibiotic. However, the negative correlation indicates that the activity of microbial species increases with a decrease in ciprofloxacin concentration, suggesting that ciprofloxacin might be involved in the inhibition of these microbial species. Scientific studies are predicting that the microbiota associated with depression can influence the metabolism of various drugs, such as

antidepressants (Xu *et al.,* 2023). However, the use of antidepressants can alter the pattern of microbial profiles, leading to the induction of defensive mechanisms in bacteria, such as the production of reactive oxygen species (Chait *et al.,* 2020). These defensive strategies help the gut microbiota to survive against antidepressants. Consequently, these strategies exert selective pressure on the intestinal microbiota, potentially enhancing the emergence of antibiotic-resistant genes and promoting the transfer of antibiotic resistance genes (Rukavishnikov *et al.,* 2023). Eventually, these changes could influence the degradation of antibiotics, especially in terms of ciprofloxacin. However, this area of research is still under investigation and not fully explored; scientists are working to clarify it.

CONCLUSION

6. CONCLUSION

From this study, it is concluded that the gut microbiota of depressed patients has the potential to degrade ciprofloxacin at different concentrations such as 4 and 8 µg/ml which was confirmed by spectrophotometric and HPLC analysis. The observed degradation pattern varies among individuals and at different concentrations. It was also found that by increasing the incubation time (7 days), there is a reduction in the concentration of ciprofloxacin up to 90% at 4 µg/ml and 60% at 8 µg/ml. Evaluating the percentage antibacterial activity of ciprofloxacin aliquots taken at different intervals showed that the gut microbiota of clinically depressed patients might transform substances into forms exhibiting lower or no antimicrobial activity. Moreover, 16S rDNA amplicon sequencing showed that every individual fecal microbial profile has a unique pattern and personalized composition, which forms the basis for the personalized response of gut microbiota to ciprofloxacin.

FUTURE PERSPECTIVES

7. FUTURE PERSPECTIVES

- Incorporating metagenomics, metatranscriptomics, and metabolomics studies are needed to elucidate the precise degradation pathways of ciprofloxacin within the gut microbiota.
- Investigating the influence of individual variations in the gut microbiome on ciprofloxacin degradation can provide a deeper understanding of the factors contributing to variable degradation rates.
- Investigating the role of microbial consortia in ciprofloxacin degradation could uncover synergistic interactions among different microbial species.
- Studying how antibiotic degradation products influence the gut-brain axis could uncover potential connections between drug metabolism, microbial activity, and mental health.
- Inspecting how antibiotic degradation in the gut might affect the overall effectiveness of antibiotics in treating infections.
- Exploring the link between the antimicrobial activity of frequently used antipsychotics and emergence of antibiotic resistant genes in the gut microbiota.

REFERENCES

8. References

Uncategorized References

Ahluwalia, Bani Magnusson, K, M., Öhman, & gastroenterology, L. J. S. j. o. (2017). Mucosal immune system of the gastrointestinal tract: maintaining balance between the good and the bad. *52*(11), 1185-1193.

Al-Ahmad, A., Daschner, F., Kümmerer, K. J. A. o. e. c., & toxicology. (1999). Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of waste water bacteria. *37*, 158-163.

Al-Asmakh, M., & Zadjali, F. J. J. M. B. (2015). Use of germ-free animal models in microbiota-related research. *25*(10), 1583-1588.

Amorim, C. L., Moreira, I. S., Maia, A. S., Tiritan, M. E., Castro, P. M. J. A. m., & biotechnology. (2014). Biodegradation of ofloxacin, norfloxacin, and ciprofloxacin as single and mixed substrates by Labrys portucalensis F11. *98*, 3181-3190.

Anwar, H., Iftikhar, A., Muzaffar, H., Almatroudi, A., Allemailem, K. S., Navaid, S., . . . Khurshid, M. (2021). Biodiversity of Gut Microbiota: Impact of Various Host and Environmental Factors. *BioMed Research International, 2021*, 5575245. doi:10.1155/2021/5575245

Aron, W., Judith, & Clément, K. J. N. R. N. (2016). The gut microbiome, diet, and links to cardiometabolic and chronic disorders. *12*(3), 169-181.

Baena-Nogueras, R. M., González-Mazo, E., Lara-Martín, P. A. J. E. S., & Technology. (2017). Photolysis of antibiotics under simulated sunlight irradiation: identification of photoproducts by high-resolution mass spectrometry. *51*(6), 3148-3156.

Bastard, Q., Ward, T., Sidiropoulos, D., Hillmann, B. M., Chun, C. L., Sadowsky, M. J., . . . Montassier, E. J. S. r. (2018). Fecal microbiota transplantation reverses antibiotic and chemotherapy-induced gut dysbiosis in mice. *8*(1), 6219.

Bell, D. S. J. P. m. (2015). Changes seen in gut bacteria content and distribution with obesity: causation or association? *, 127*(8), 863-868.

Bhalodi, A. A., van Engelen, T. S. R., Virk, H. S., & Wiersinga, W. J. (2019). Impact of antimicrobial therapy on the gut microbiome. *J Antimicrob Chemother, 74*(Suppl 1), i6-i15. doi:10.1093/jac/dky530

Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. J. N. r. m. (2015). Molecular mechanisms of antibiotic resistance. *13*(1), 42-51.

Blánquez, A., Guillén, F., Rodríguez, J., Arias, M. E., Hernández, M. J. W. J. o. m., & Biotechnology. (2016). The degradation of two fluoroquinolone based antimicrobials by SilA, an alkaline laccase from Streptomyces ipomoeae. *32*, 1-8.

Bunker, J. J., Erickson, S. A., Flynn, T. M., Henry, C., Koval, J. C., Meisel, M., . . . Bendelac, A. J. S. (2017). Natural polyreactive IgA antibodies coat the intestinal microbiota. *358*(6361), eaan6619.

Chait, Y., Mottawea, W., Tompkins, T. A., & Hammami, R. J. S. r. (2020). Unravelling the antimicrobial action of antidepressants on gut commensal microbes. *10*(1), 17878.

Chang, C.-S., & Kao, C.-Y. J. J. o. b. s. (2019). Current understanding of the gut microbiota shaping mechanisms. *26*, 1-11.

Chong, C. Y. L., Bloomfield, F. H., & O'Sullivan, J. M. J. N. (2018). Factors affecting gastrointestinal microbiome development in neonates. *10*(3), 274.

Clarke, G., Sandhu, K. V., Griffin, B. T., Dinan, T. G., Cryan, J. F., & Hyland, N. P. J. P. r. (2019). Gut reactions: breaking down xenobiotic–microbiome interactions. *71*(2), 198-224.

Collins, J., Borojevic, R., Verdu, E., Huizinga, J., Ratcliffe, E. J. N., & Motility. (2014). Intestinal microbiota influence the early postnatal development of the enteric nervous system. *26*(1), 98-107.

Cornforth, D. M., & Foster, K. R. J. N. R. M. (2013). Competition sensing: the social side of bacterial stress responses. *11*(4), 285-293.

Cresci, & Gail, I., Kristin. (2019). Chapter 4 - Gut Microbiome. In M. L. Corrigan, K. Roberts, & E. Steiger (Eds.), *Adult Short Bowel Syndrome* (pp. 45-54): Academic Press.

Čvančarová, M., Moeder, M., Filipová, A., & Cajthaml, T. J. C. (2015). Biotransformation of fluoroquinolone antibiotics by ligninolytic fungi–metabolites, enzymes and residual antibacterial activity. *136*, 311-320.

D'Argenio, V., & Salvatore, F. J. C. c. a. (2015). The role of the gut microbiome in the healthy adult status. *451*, 97-102.

Dafale, N. A., Semwal, U. P., Rajput, R. K., & Singh, G. J. J. o. p. a. (2016). Selection of appropriate analytical tools to determine the potency and bioactivity of antibiotics and antibiotic resistance. *6*(4), 207-213.

Dalvie, D. K., Khosla, N. B., Navetta, K. A., Brighty, K. E. J. D. m., & disposition. (1996). Metabolism and excretion of trovafloxacin, a new quinolone antibiotic, in Sprague-Dawley rats and beagle dogs. Effect of bile duct cannulation on excretion pathways. *24*(11), 1231-1240.

DeGruttola, A. K., Low, D., Mizoguchi, A., & Mizoguchi, E. J. I. b. d. (2016). Current understanding of dysbiosis in disease in human and animal models. *22*(5), 1137-1150.

Derrien, M., Alvarez, A.-S., & de Vos, W. M. J. T. i. m. (2019). The gut microbiota in the first decade of life. *27*(12), 997-1010.

Dethlefsen, L., Huse, S., Sogin, M. L., & Relman, D. A. J. P. b. (2008). The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *6*(11), e280.

Di Daniele, N., Noce, A., Vidiri, M. F., Moriconi, E., Marrone, G., Annicchiarico-Petruzzelli, M., . . . De Lorenzo, A. J. O. (2017). Impact of Mediterranean diet on metabolic syndrome, cancer and longevity. *8*(5), 8947.

Dogra, S. K., Chung, C. K., Wang, D., Sakwinska, O., Colombo Mottaz, S., & Sprenger, N. J. M. (2021). Nurturing the early life gut microbiome and immune maturation for long term health. *9*(10), 2110.

Duncan, S. H., Louis, P., & Flint, H. J. J. L. i. a. m. (2007). Cultivable bacterial diversity from the human colon. *44*(4), 343-350.

Enright, E. F., Gahan, C. G., Joyce, S. A., Griffin, B. T. J. T. Y. j. o. b., & medicine. (2016). Focus: microbiome: the impact of the gut microbiota on drug metabolism and clinical outcome. *89*(3), 375.

Estes, C., Razavi, H., Loomba, R., Younossi, Z., & Sanyal, A. J. J. H. (2018). Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *67*(1), 123-133.

Fang, H., Oberoi, A. S., He, Z., Khanal, S. K., & Lu, H. J. W. R. (2021). Ciprofloxacin-degrading Paraclostridium sp. isolated from sulfate-reducing bacteria-enriched sludge: Optimization and mechanism. *191*, 116808.

Fischer, K., Majewsky, M. J. A. m., & biotechnology. (2014). Cometabolic degradation of organic wastewater micropollutants by activated sludge and sludge-inherent microorganisms. *98*, 6583-6597.

Frank, D. N., St. Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N., & Pace, N. R. J. P. o. t. n. a. o. s. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *104*(34), 13780-13785.

Franzosa, E. A., Hsu, T., Sirota-Madi, A., Shafquat, A., Abu-Ali, G., Morgan, X. C., & Huttenhower, C. J. N. R. M. (2015). Sequencing and beyond: integrating molecular'omics' for microbial community profiling. *13*(6), 360-372.

Garcia-Cortes, M., Robles-Diaz, M., Stephens, C., Ortega-Alonso, A., Lucena, M. I., & Andrade, R. J. J. A. o. T. (2020). Drug induced liver injury: an update. *94*, 3381-3407.

Garcia-Gutierrez, E., Mayer, M. J., Cotter, P. D., & Narbad, A. J. G. m. (2019). Gut microbiota as a source of novel antimicrobials. *10*(1), 1-21.

Gartiser, S., Urich, E., Alexy, R., & Kümmerer, K. J. C. (2007). Ultimate biodegradation and elimination of antibiotics in inherent tests. *67*(3), 604-613.

Ghoshal, U. C., Shukla, R., Ghoshal, U., Gwee, K.-A., Ng, S. C., & Quigley, E. M. J. I. j. o. i. (2012). The gut microbiota and irritable bowel syndrome: friend or foe? *, 2012*.

Giri, A. S., & Golder, A. K. J. J. o. E. S. (2019). Ciprofloxacin degradation in photo-Fenton and photo-catalytic processes: Degradation mechanisms and iron chelation. *80*, 82-92.

Gjonbalaj, M., Keith, J. W., Do, M. H., Hohl, T. M., Pamer, E. G., Becattini, S. J. I., & immunity. (2020). Antibiotic degradation by commensal microbes shields pathogens. *88*(4), 10.1128/iai. 00012-00020.

Goel, A., Gupta, M., Aggarwal, R. J. J. o. g., & hepatology. (2014). Gut microbiota and liver disease. *29*(6), 1139-1148.

Haak, B. W., Lankelma, J. M., Hugenholtz, F., Belzer, C., de Vos, W. M., & Wiersinga, W. J. J. J. o. A. C. (2019). Long-term impact of oral vancomycin, ciprofloxacin and metronidazole on the gut microbiota in healthy humans. *74*(3), 782-786.

Haiser, H. J., & Turnbaugh, P. J. J. P. r. (2013). Developing a metagenomic view of xenobiotic metabolism. *69*(1), 21-31.

Han, Z., Wang, H., Zheng, J., Wang, S., Yu, S., & Lu, L. J. E. R. (2023). Ultrafast synthesis of laccase-copper phosphate hybrid nanoflowers for efficient degradation of tetracycline antibiotics. *216*, 114690.

Hofacre, C. L., Fricke, J. A., & Inglis, T. J. A. t. i. v. m. (2013). Antimicrobial drug use in poultry. 569-587.

Hooper, & David, J. D. (1999). Mode of action of fluoroquinolones. *58*, 6-10.

Hooper, Midtvedt, T., & Gordon, J. I. J. A. r. o. n. (2002). How host-microbial interactions shape the nutrient environment of the mammalian intestine. *22*(1), 283-307.

Hopkins, M., Sharp, R., Macfarlane, G. J. D., & Disease, L. (2002). Variation in human intestinal microbiota with age. *34*, S12-S18.

Hou, K., Wu, Z.-X., Chen, X.-Y., Wang, J.-Q., Zhang, D., Xiao, C., . . . Chen, Z.-S. (2022). Microbiota in health and diseases. *Signal Transduction and Targeted Therapy, 7*(1), 135. doi:10.1038/s41392-022-00974-4

Ishikawa, H., Tanaka, K., Maeda, Y., Aiba, Y., Hata, A., Tsuji, N., . . . Matsumoto, T. J. o. C. a. E. I. (2008). Effect of intestinal microbiota on the induction of regulatory CD25+ CD4+ T cells. *153*(1), 127-135.

Isolauri, E., Kirjavainen, P., & Salminen, S. J. G. (2002). Probiotics: a role in the treatment of intestinal infection and inflammation? , *50*(suppl 3), iii54-iii59.

Iweala, I, O., Nagler, & R, C. (2006). Immune privilege in the gut: the establishment and maintenance of non-responsiveness to dietary antigens and commensal flora. *J Immunological reviews*

213(1), 82-100.

Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., & Nageshwar Reddy, D. (2015). Role of the normal gut microbiota. *World J Gastroenterol, 21*(29), 8787-8803. doi:10.3748/wjg.v21.i29.8787

Jang, H.-M., Lee, H.-J., Jang, S.-E., Han, M. J., & Kim, D.-H. J. M. I. (2018). Evidence for interplay among antibacterial-induced gut microbiota disturbance, neuro-inflammation, and anxiety in mice. *11*(5), 1386-1397.

Jia, Y., Khanal, S. K., Shu, H., Zhang, H., Chen, G.-H., & Lu, H. J. W. r. (2018). Ciprofloxacin degradation in anaerobic sulfate-reducing bacteria (SRB) sludge system: mechanism and pathways. *136*, 64-74.

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., . . . immunity. (2015). Altered fecal microbiota composition in patients with major depressive disorder. *48*, 186-194.

Jureczko, M., Przystaś, W., Krawczyk, T., Gonciarz, W., & Rudnicka, K. J. J. o. H. M. (2021). White-rot fungi-mediated biodegradation of cytostatic drugs-bleomycin and vincristine. *407*, 124632.

Kang, M. J., Kim, H. G., Kim, J. S., Oh, D. G., Um, Y. J., Seo, C. S., . . . toxicology. (2013). The effect of gut microbiota on drug metabolism. *9*(10), 1295-1308.

Kaoutari, A. E., Armougom, F., Gordon, J. I., Raoult, D., & Henrissat, B. J. N. R. M. (2013). The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *11*(7), 497-504.

Kim, K.-R., Owens, G., Kwon, S.-I., So, K.-H., Lee, D.-B., Ok, Y. S. J. W., Air,, & Pollution, S. (2011). Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. *214*, 163-174.

King, C. H., Desai, H., Sylvetsky, A. C., LoTempio, J., Ayanyan, S., Carrie, J., . . . Mazumder, R. (2019). Baseline human gut microbiota profile in healthy people and standard reporting template. *PLOS ONE, 14*(9), e0206484. doi:10.1371/journal.pone.0206484

Kitamoto, S., Nagao-Kitamoto, H., Kuffa, P., & Kamada, N. J. J. o. g. (2016). Regulation of virulence: the rise and fall of gastrointestinal pathogens. *51*, 195-205.

Knapp, J., Bromley-Challoner, K. J. H. o. w., & microbiology, w. (2003). Recalcitrant organic compounds. 559-595.

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. J. C. (2016). From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *165*(6), 1332-1345.

Krajmalnik, Brown, R., Ilhan, Z. E., Kang, D. W., & DiBaise, J. K. J. N. i. c. p. (2012). Effects of gut microbes on nutrient absorption and energy regulation. *27*(2), 201-214.

Kumari, R., Yadav, Y., Misra, R., Das, U., Das Adhikari, U., Malakar, P., & Dubey, G. P. (2022). Emerging frontiers of antibiotics use and their impacts on the human gut microbiome. *Microbiological Research, 263*, 127127. doi:https://doi.org/10.1016/j.micres.2022.127127

Kunz, C., Kuntz, S., & Rudloff, S. J. A. E. M. B. (2009). Intestinal flora. *639*, 67-79.

Kuramitsu, H. K., He, X., Lux, R., Anderson, M. H., Shi, W. J. M., & reviews, m. b. (2007). Interspecies interactions within oral microbial communities. *71*(4), 653-670.

Lach, G., Schellekens, H., Dinan, T. G., & Cryan, J. F. J. N. (2018). Anxiety, depression, and the microbiome: a role for gut peptides. *15*, 36-59.

LaSalvia, E. A., Domek, G. J., & Gitlin, D. F. J. G. h. p. (2010). Fluoroquinolone-induced suicidal ideation. *1*(32), 108-110.

Leclercq, S., Mian, F. M., Stanisz, A. M., Bindels, L. B., Cambier, E., Ben-Amram, H., . . . Bienenstock, J. J. N. c. (2017). Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. *8*(1), 15062.

Lepage, P., Häsler, R., Spehlmann, M. E., Rehman, A., Zvirbliene, A., Begun, A., . . . Raedler, A. J. G. (2011). Twin study indicates loss of interaction between microbiota and mucosa of patients with ulcerative colitis. *141*(1), 227-236.

Leung, D. Y., Guttman-Yassky, E. J. J. o. A., & Immunology, C. (2014). Deciphering the complexities of atopic dermatitis: shifting paradigms in treatment approaches. *134*(4), 769-779.

Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. J. P. o. t. n. a. o. s. (2005). Obesity alters gut microbial ecology. *102*(31), 11070-11075.

Li, X., Hou, Y., Li, Q., Gu, W., & Li, Y. J. J. o. E. M. (2021). Molecular design of high-efficacy and high drug safety Fluoroquinolones suitable for a variety of aerobic biodegradation bacteria. *299*, 113628.

Liao, X., Li, B., Zou, R., Dai, Y., Xie, S., Yuan, B. J. E. S., & Research, P. (2016). Biodegradation of antibiotic ciprofloxacin: pathways, influential factors, and bacterial community structure. *23*, 7911-7918.

Liu, Shan Gao, J., Zhu, M., Liu, K., & Zhang, H.-L. J. M. n. (2020). Gut microbiota and dysbiosis in Alzheimer's disease: implications for pathogenesis and treatment. *57*, 5026-5043.

Liu, Q., Luo, Y., Ke, X. J. E.-B. C., & Medicine, A. (2022). Interaction between the Gut Microbiota and Intestinal Motility. *2022*.

Livanos, A. E., Greiner, T. U., Vangay, P., Pathmasiri, W., Stewart, D., McRitchie, S., . . . Kim, S. J. N. m. (2016). Antibiotic-mediated gut microbiome perturbation accelerates development of type 1 diabetes in mice. *1*(11), 1-13.

Liyanage, G., & Manage, P. M. (2018). Removal of Ciprofloxacin (CIP) by bacteria isolated from hospital effluent water and identification of degradation pathways.

Lurie, I., Yang, Y.-X., Haynes, K., Mamtani, R., & Boursi, B. J. T. J. o. c. p. (2015). Antibiotic exposure and the risk for depression, anxiety, or psychosis: a nested case-control study. *76*(11), 825.

Macfarlane, S. J. E. m. (2014). Antibiotic treatments and microbes in the gut. *16*(4), 919-924.

Macpherson, J, A., Harris, & Immunology, N. L. J. N. R. (2004). Interactions between commensal intestinal bacteria and the immune system. *4*(6), 478-485.

Magalhaes, J. G., Tattoli, I., & Girardin, S. E. (2007). *The intestinal epithelial barrier: how to distinguish between the microbial flora and pathogens.* Paper presented at the Seminars in immunology.

Manasfi, R., Chiron, S., Montemurro, N., Perez, S., Brienza, M. J. E. S., & Research, P. (2020). Biodegradation of fluoroquinolone antibiotics and the climbazole fungicide by Trichoderma species. *27*, 23331-23341.

Marengo, J. R., Kok, R. A., O'Brien, K., Velagaleti, R. R., Stamm, J. M. J. E. T., & Journal, C. A. I. (1997). Aerobic biodegradation of (14C)-Sarafloxacin hydrochloride in soil. *16*(3), 462-471.

Maurice, C. F., Haiser, H. J., & Turnbaugh, P. J. J. C. (2013). Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *152*(1), 39-50.

Mazmanian, S. K., Liu, C. H., Tzianabos, A. O., & Kasper, D. L. J. C. (2005). An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *122*(1), 107-118.

Mazzoleni, I. (2013). *Architecture follows nature-biomimetic principles for innovative design* (Vol. 2): Crc Press.

Mueller, N. T., Hourigan, S. K., Hoffmann, D. E., Levy, L., von Rosenvinge, E. C., Chou, B., . . . Ethics. (2019). Bacterial baptism: scientific, medical, and regulatory issues raised by vaginal seeding of C-section-born babies. *47*(4), 568-578.

Myers, S. P., & Hawrelak, J. J. A. M. R. (2004). The causes of intestinal dysbiosis: a review. *9*(2), 180-197.

Nicholson, J. K., & Wilson, I. D. J. N. R. D. D. (2003). Understanding'global'systems biology: metabonomics and the continuum of metabolism. *2*(8), 668-676.

Nugent, A. P. J. N. B. (2005). Health properties of resistant starch. *30*(1), 27-54.

Ostaff, M. J., Stange, E. F., & Wehkamp, J. J. E. m. m. (2013). A ntimicrobial peptides and gut microbiota in homeostasis and pathology. *5*(10), 1465-1483.

Palleja, A., Mikkelsen, K. H., Forslund, S. K., Kashani, A., Allin, K. H., Nielsen, T., . . . Zhang, C. J. N. m. (2018). Recovery of gut microbiota of healthy adults following antibiotic exposure. *3*(11), 1255-1265.

Pan, L.-j., Li, J., Li, C.-x., Yu, G.-w., & Wang, Y. J. J. o. h. m. (2018). Study of ciprofloxacin biodegradation by a Thermus sp. isolated from pharmaceutical sludge. *343*, 59-67.

Parshikov, I. A., Sutherland, J. B. J. J. o. I. M., & Biotechnology. (2012). Microbial transformations of antimicrobial quinolones and related drugs. *39*(12), 1731-1740.

Pascale, A., Marchesi, N., Govoni, S., & Barbieri, A. J. P. R. (2020). Targeting the microbiota in pharmacology of psychiatric disorders. *157*, 104856.

Pérez, C., Elena, A., Artacho, A., Knecht, H., Ferrús, M. L., Friedrichs, A., . . . Gosalbes, M. J. (2013). Differential Effects of Antibiotic Therapy on the Structure and Function of Human Gut Microbiota. *PLOS ONE, 8*(11), e80201. doi:10.1371/journal.pone.0080201

Pilmis, B., Le Monnier, A., & Zahar, J.-R. (2020). Gut Microbiota, Antibiotic Therapy and Antimicrobial Resistance: A Narrative Review. *8*(2), 269.

Qu, C., Wu, Z., Pan, D., Cai, Z., & Liu, X. J. B. L. (2021). Characterization of Lactobacillus reuteri WQ-Y1 with the ciprofloxacin degradation ability. *43*, 855-864.

Rakoff, N., & Seth , M., R %J Mucosal immunology. (2008). Innate immune recognition of the indigenous microbial flora. *1*(1), S10-S14.

Ramirez, J., Guarner, F., Bustos Fernandez, L., Maruy, A., Sdepanian, V. L., Cohen, H. J. F. i. c., & microbiology, i. (2020). Antibiotics as major disruptors of gut microbiota. *10*, 572912.

Rao, A. A., & Johncy, S. J. C. (2022). Tennis Courts in the Human Body: A Review of the Misleading Metaphor in Medical Literature. *14*(1).

Rawls, J. F., Mahowald, M. A., Ley, R. E., & Gordon, J. I. J. C. (2006). Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. *127*(2), 423-433.

Reck, M., Tomasch, J., Deng, Z., Jarek, M., Husemann, P., Wagner-Döbler, I., & genomics, C. c. J. B. (2015). Stool metatranscriptomics: A technical guideline for mRNA stabilisation and isolation. *16*, 1-18.

Reis, A. C., Kolvenbach, B. A., Nunes, O. C., & Corvini, P. F. J. N. b. (2020). Biodegradation of antibiotics: the new resistance determinants–part II. *54*, 13-27.

Rinninella, E., Cintoni, M., Raoul, P., Lopetuso, L. R., Scaldaferri, F., Pulcini, G., . . . Mele, M. C. J. N. (2019). Food components and dietary habits: keys for a healthy gut microbiota composition. *11*(10), 2393.

Robicsek, A., Strahilevitz, J., Jacoby, G. A., Macielag, M., Abbanat, D., Hye Park, C., . . . Hooper, D. C. J. N. m. (2006). Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *12*(1), 83-88.

Rojo, D., Méndez-García, C., Raczkowska, B. A., Bargiela, R., Moya, A., Ferrer, M., & Barbas, C. J. F. m. r. (2017). Exploring the human microbiome from multiple perspectives: factors altering its composition and function. *41*(4), 453-478.

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., & Tuohy, K. J. E. j. o. n. (2018). Gut microbiota functions: metabolism of nutrients and other food components. *57*, 1-24.

Rukavishnikov, G., Leonova, L., Kasyanov, E., Leonov, V., Neznanov, N., & Mazo, G. J. F. i. B. N. (2023). Antimicrobial activity of antidepressants on normal gut microbiota: Results of the in vitro study. *17*, 1132127.

Rusch, M., Spielmeyer, A., Zorn, H., Hamscher, G. J. A. m., & biotechnology. (2018). Biotransformation of ciprofloxacin by Xylaria longipes: structure elucidation and residual antibacterial activity of metabolites. *102*, 8573-8584.

Rusch, M., Spielmeyer, A., Zorn, H., Hamscher, G. J. A. m., & biotechnology. (2019). Degradation and transformation of fluoroquinolones by microorganisms with special emphasis on ciprofloxacin. *103*, 6933-6948.

Sekar, S., Mahadevan, S., Kumar, S. S. D., Mandal, A. B. J. J. o. t. a., & calorimetry. (2011). Thermokinetic responses of the metabolic activity of Staphylococcus lentus cultivated in a glucose limited mineral salt medium. *104*(1), 149-155.

Sekirov, I., Russell, S. L., Antunes, L. C. M., & Finlay, B. B. (2010). Gut Microbiota in Health and Disease. *90*(3), 859-904. doi:10.1152/physrev.00045.2009

Sender, R., Fuchs, S., & Milo, R. J. C. (2016). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *164*(3), 337-340.

Sharifi, Rad, J., Rodrigues, C. F., Stojanović-Radić, Z., Dimitrijević, M., Aleksić, A., . . . Milton Prabu, S. J. M. (2020). Probiotics: versatile bioactive components in promoting human health. *56*(9), 433.

Singh, S. V., Ganguly, R., Jaiswal, K., Yadav, A. K., Kumar, R., & Pandey, A. K. J. W. J. o. C. C. (2023). Molecular signalling during cross talk between gut brain axis regulation and progression of irritable bowel syndrome: A comprehensive review. *11*(19), 4458.

Sinha, & Anirban, J. E. E. M. R. (2020). Gut Microbiome: The New Organ? *, 5*, 01-02.

Sommer, F., & Bäckhed, F. J. N. r. m. (2013). The gut microbiota—masters of host development and physiology. *11*(4), 227-238.

Souhila, S., Slimane, M., Martínez-Alcalá, I., & Erenler, R. J. E. J. o. B. (2023). Screening of Antibiotics Biodegradability from Wastewater. *82*(1), 70-85.

Steenbergen, L., Sellaro, R., van Hemert, S., Bosch, J. A., Colzato, L. S. J. B., behavior,, & immunity. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *48*, 258-264.

Stojanov, S., Berlec, A., & Štrukelj, B. J. M. (2020). The influence of probiotics on the firmicutes/bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. *8*(11), 1715.

Swidsinski, A., Loening-Baucke, V., Lochs, H., & Hale, L. P. J. W. j. o. g. W. (2005). Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. *11*(8), 1131.

Syromyatnikov, M., Nesterova, E., Gladkikh, M., Smirnova, Y., Gryaznova, M., & Popov, V. (2022). Characteristics of the Gut Bacterial Composition in People of Different Nationalities and Religions. *10*(9), 1866.

Tanaka, S., Kobayashi, T., Songjinda, P., Tateyama, A., Tsubouchi, M., Kiyohara, C., . . . Microbiology, M. (2009). Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. *56*(1), 80-87.

Thillaimaharani, K., Sharmila, K., Thangaraju, P., Karthick, M., Kalaiselvam, M. J. I. J. o. P. S., & Research. (2013). Studies on antimicrobial and antioxidant properties of oyster mushroom Pleurotus florida. *4*(4), 1540.

Tuohy, K. M., Gougoulias, C., Shen, Q., Walton, G., Fava, F., & Ramnani, P. J. C. p. d. (2009). Studying the human gut microbiota in the trans-omics era-focus on metagenomics and metabonomics. *15*(13), 1415-1427.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. J. n. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *444*(7122), 1027-1031.

Turner, & Patricia, V. J. A. m. e. m. (2018). The role of the gut microbiota on animal model reproducibility. *1*(2), 109-115.

Van, Agteren, M. H., Keuning, S., & Oosterhaven, J. (1998). *Handbook on biodegradation and biological treatment of hazardous organic compounds* (Vol. 2): Springer Science & Business Media.

Vaziri, N. D., Wong, J., Pahl, M., Piceno, Y. M., Yuan, J., DeSantis, T. Z., . . . Andersen, G. L. J. K. i. (2013). Chronic kidney disease alters intestinal microbial flora. *83*(2), 308-315.

Vijay, A., & Valdes, A. M. (2022). Role of the gut microbiome in chronic diseases: a narrative review. *European Journal of Clinical Nutrition, 76*(4), 489-501. doi:10.1038/s41430-021-00991-6

Wetzstein, H.-G., Schmeer, N., Karl, W. J. A., & Microbiology, E. (1997). Degradation of the fluoroquinolone enrofloxacin by the brown rot fungus Gloeophyllum striatum: identification of metabolites. *63*(11), 4272-4281.

Wilson, I. D., & Nicholson, J. K. J. T. R. (2017). Gut microbiome interactions with drug metabolism, efficacy, and toxicity. *179*, 204-222.

Xu, Xiaofei, X., Pingping, Ma, C., Tang, J., & Zhang, X. (2013). Gut microbiota, host health, and polysaccharides. *Biotechnology Advances, 31*(2), 318-337. doi:https://doi.org/10.1016/j.biotechadv.2012.12.009

Xu, Xie, Q., Kuang, W., & Dong, Z. J. N. (2023). Interactions Between Antidepressants and Intestinal Microbiota. *20*(2), 359-371.

Yang, T., Santisteban, M. M., Rodriguez, V., Li, E., Ahmari, N., Carvajal, J. M., . . . Zubcevic, J. J. h. (2015). Gut dysbiosis is linked to hypertension. *65*(6), 1331-1340.

Yoon, M. Y., & Yoon, S. S. J. Y. m. j. (2018). Disruption of the gut ecosystem by antibiotics. *59*(1), 4-12.

Zhang, Chenhong, Z., Menghui, Wang, S., Han, R., Cao, Y., Hua, W., . . . Wei, C. J. T. I. j. (2010). Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *4*(2), 232-241.

Zhang, Huiqun Quan, H., Yin, S., Sun, L., Lu, H. J. E. S., & Technology. (2022). Unraveling the Toxicity Associated with Ciprofloxacin Biodegradation in Biological Wastewater Treatment. *56*(22), 15941-15952.

Zhang, Yifei Gu, Y., Ren, H., Wang, S., Zhong, H., Zhao, X., . . . Wang, W. (2020). Gut microbiome-related effects of berberine and probiotics on type 2 diabetes (the PREMOTE study). *Nature Communications, 11*(1), 5015. doi:10.1038/s41467-020-18414-8

Zhao, R., Li, X., Hu, M., Li, S., Zhai, Q., Jiang, Y. J. B., & Engineering, B. (2017). Efficient enzymatic degradation used as pre-stage treatment for norfloxacin removal by activated sludge. *40*, 1261-1270.

Zmora, N., Suez, J., Elinav, E. J. N. r. G., & hepatology. (2019). You are what you eat: diet, health and the gut microbiota. *16*(1), 35-56.

Zoetendal, E. G., Akkermans, A. D., Akkermans-van Vliet, W. M., de Visser, J. A. G., de Vos, W. M. J. M. e. i. h., & disease. (2001). The host genotype affects the bacterial community in the human gastronintestinal tract. *13*(3), 129-134.