CHAPTER I

INTRODUCTION

1.1 Contextual Background

Physicians and other healthcare providers have long posited, even in absence of substantial scientific research, that one of the benefits of probiotics is to stimulate or replenish the gut microbes destroyed by antibiotics during antibacterial therapy. Historically, in the presence of only meager independent scientific (as opposed to trade) research supporting such, probiotics became widely accepted and, today, are being widely recommended by healthcare providers, and self-prescribed by patients and consumers.

Currently, research into this field of probiotics is intensifying. Studies on the beneficial effect in antibiotic-associated diarrhea and Clostridium difficile (*C. difficile* and *C. diff*) have proven favorable. There is a growing body of scientific evidence establishing the links between probiotics and physical health, probiotics and mental health, the beneficial effects of probiotics in certain diseases, the role of probiotics in the gut epithelium, the relationship of probiotics to the human microbiota, and more.

However, to date, there are no standardized protocol or even medically approved guidelines for the administration of probiotics, especially in the combined use with antibiotics. There are some general recommendations, but no prescriptive endorsements. Should medicine continue down this unchecked path regarding probiotic use? What are the possible consequences if medical oversight regarding the use of probiotics remains status quo?

All drugs, it is duly noted, have side effects. And, although probiotics are not classified as drugs (as yet), they certainly produce or induce certain drug-like effects. Should we not consider that probiotics, too, may have some undesirable side effects? If so, what are these adverse effects?

1.2 Aim and Objective

The main aim of this research is to ascertain the current medical practices in the use of probiotics during antibiotic treatment, and to assess collateral issues of such, such as any adverse effects of probiotic use and the role that the probiotic might play in antimicrobial resistance. This research will delve into other associated matters such as, clinical epidemiology, MD simulation, mathematical models and the journey of the probiotic through the alimentary canal. These topics serve as a foundation for the main objectives of this research which are to:

- explore probiotic use as a burgeoning phenomenon worldwide,
- explore the growing use of probiotics during antibiotic therapy,
- report on current practices in the use of probiotics during antibiotic treatment,
- investigate any decrease in efficacy of the antibiotic during concurrent probiotic use,
- understand the main factors contributing to the sluggish research and development of probiotics,
- research both the potential beneficial effects and the possible adverse effects of probiotic use in humans,
- confront conceivable epidemiological consequences of unregulated probiotic consumption, and

- recommend the establishment of medical guidelines for the use of probiotics– particularly during combined treatment with antibiotics.

These foregoing objectives lead to Section 1.3 wherein the research questions are proposed.

1.3 Research Questions

The following sections will not only provide specific research questions (Section 1.3.2), but place the origin of these questions in the background from which they were generated and developed (Section 1.3.1).

1.3.1 Background to the Research Questions

What really happens when probiotics are taken with antibiotics? This is a fundamental question. Certainly, augmenting antibiotic treatment with probiotics seems beneficial, perhaps even prudent. But could ingesting probiotics during antibiotic treatment effectively reduce the efficacy of the antibiotics? This question has some merit. After all, while the antibiotic is attacking the pathogenic bacteria, it is also causing collateral damage by destroying the native, health-giving flora of the gut. So, if the intrinsic flora of the gut is stimulated to replenish (through the ingestion of probiotics), would this not add more “target” microbes that the antibiotic (capped in a limited amounts per dose) would pursue to annihilate? And, with the microbes replenishing subsequent to each probiotic dose, would this not dilute the efforts of the antibiotic to seek and destroy its designated target, the pathogenic microbes it was ingested to destroy?

This question was a door; and by entering that door, many more doors to many more rooms of research regarding probiotics and antibiotics were opened. To answer the fundamental question, many more questions arose and those questions, in turn, needed to be explored and answered. One simple probiotic capsule led to one question, and that simple question lead to a complex, interconnected journey.

1.3.2 Research Questions Stated

The research questions are as follows:

- Are probiotics medically recommended during antibiotic therapy?
- Are there any medical (scientifically-proven) guidelines for probiotic use with antibiotic treatment?? If so, what are the guidelines?
- When taken concurrently, do probiotics diminish the efficacy of antibiotics?
- When taken concurrently, do antibiotics diminish efficacy of probiotics?
- What are the adverse effects of probiotic use?
- Can probiotics confer (or contribute to) antimicrobial resistance?

1.4 Problem Statement

There exist numerous challenges associated with understanding probiotics, in particular when they are used concurrently with antimicrobial agents. Probiotics are of many strains and potencies, and certain strains of probiotics can act and respond in characteristic ways when acting alone and in differing ways when used in combination with other strains. Their actions can vary depending on the qualities of the combinations concocted.

As yet, there are no medically-endorsed guidelines for the use of probiotics with antibiotics. And since manufacturers of probiotics are careful not declare any allied treatment for illness or disease regarding the probiotics, these same probiotics are, therefore, not under the auspices of the FDA. As such, it is difficult to track probiotic use and challenging to control the quality and composition of probiotics. The discovery of probiotics is over 100 years old; however, the consumer interest and trade promotion has far outpaced scientific research. But this is all beginning to change. Scientific research into the varied facets of probiotics is gaining traction and intensifying. For this reason even the findings and opinions set forth herein can be quickly eclipsed with the immediate publication of more current research.

1.5 Rationale of the Research

Probiotics, whether as food, such as yogurt and kefir; as additives in food; as nutritional supplements; or even as part of a medical regime, are known and established worldwide. Probiotics exert a dynamic influence on the human microbiome and are linked not only to physical health but to mental health. Research continues to uncover more links between probiotics and health. But in the more than one hundred years since their discovery, relatively little has been explored and understood about probiotics. Less yet is known (or being researched) about any possible adverse effects of probiotics on the individual; or, from an epidemiological standpoint, on society as a whole. Certain strains of probiotics exhibit antibiotic resistance (see Section 2.4.2, Table 2.1).

Currently, probiotics can be purchased and consumed by anyone with a willingness and financial ability to do so; no permission is needed, no medical guidelines offered. It would seem

that, with all of the apparent wide-ranging effects of probiotics, some caution should be exercised; and the fundamental research questions, presented in Section 1.3.2, answered.

CHAPTER II

IMPLICATIONS DRAWN FROM CURRENT PRACTICES IN THE USE OF PROBIOTICS DURING ANTIBIOTIC TREATMENT

2.1 Abstract

As mentioned in Section 1.1, physicians and other healthcare providers have long posited, even in the absence of supporting scientific research, that one of the functions of probiotics is to substitute or replenish the gut microbes destroyed by antibiotics during antibacterial therapy. Over time, mostly due to observation and based on anecdotal evidence, it became widely accepted that probiotics have a beneficial effect on the human body in reducing, or even eliminating, certain adverse effects and consequences of antibiotic treatment; as well as supporting the natural intestinal flora that may have been disrupted during the use of antibiotics. Much current research supports these prior hypotheses and practices that probiotic use is beneficial during antibiotic therapy in limiting antibiotic-associated diarrhea (AAD); in ameliorating disruptions of the epithelium of the lower intestine tract due to Clostridium difficile infections (CDI); and eliminating yeast (candida) infestation secondary to antibiotic therapy.

Although there are no scientifically-documented or medically-endorsed guidelines for the administration of probiotics during a course of antibiotics, especially in the timing of the doses of each group (the antibiotic and the probiotic), the general consensus among researchers and physicians is that it is better to stagger the doses of the antibiotic and the probiotic to enhance efficacy. It is suggested to take the probiotic 2-6 hours after the antibiotic dose throughout the course of antibiotic treatment, and continue with the probiotic 7-10 days after ending the

antibiotic regime. It is also helpful to take probiotics before beginning antibiotic therapy, if possible.

In this review it is also noted that more research is needed on the interaction of antibiotics and probiotics during combined treatment due to concerns about antibiotic resistance which may be conferred by way of certain strains of probiotics, and approved medical guidelines for the combined use of specialized probiotics and specific antibiotics need to be developed. Several interesting and relevant research topics relating to probiotic use during antibiotic therapy are offered. And two new medical acronyms, **CAP** and **hAMR**, are suggested to improve memorability and to better describe, and facilitate the reference to, combined antibiotic-probiotic use and antimicrobial resistance via horizontal gene transfer. **CAP** stands for combined antibiotic-probiotic; **hAMR** stands for antimicrobial resistance (AMR) via horizontal gene transfer (HGT).

These two new acronyms will be utilized in several chapters: CAP, Chapters 2, 4. 5, 11; hAMR, Chapters 2, 7, 8, 9, 11.

2.2 Keywords

antibiotics; diarrhea; dysbiosis; genetic stability; immunological; intestinal flora; microbiota; pathogenicity; probiotics; tight junctions; toxigenicity

2.3 Abbreviations

AAD: antibiotic-associated diarrhea; CDC: Center For Disease Control and Prevention; CDI: Clostridium difficile infection; CAP: combined antibiotic-probiotic; ECM: Albert Einstein

College of Medicine; FDA: Food and Drug Administration; hAMR: AMR via HGT); JAMA: Journal of the American Medical Association; JPH: Journal of Probiotics and Health; NCBI: National Center for Biotechnology Information; RAND: Research and Development Corporation

2.4 Premise

Clinical pharmacology is the study of drugs in humans. It is underpinned by the basic science of pharmacology with added focus on the application of pharmacological principles and methods in the real world [1]. Clinical epidemiology is the study of the determinants and effects of clinical decisions [2].

2.4.1 Supposition in Support of Using Probiotics

Historically, in the presence of only meager independent scientific research supporting such, probiotics have become widely accepted and are now being recommended by healthcare providers and being self-prescribed by patients and consumers. In antibiotic-associated diarrhea (AAD), probiotics may seem harmless while providing some benefits. They may be deemed "for" (pro) the "life" (biotic) of the microflora as the name, probiotic, suggests. But it must kept in mind that health supplements may not always elicit the claims that their name may infer. All drugs, it is noted, have side effects. Should we not consider that probiotics, too, have side effects? If so, what are these side effects?

Research into the beneficial effect of probiotics in AAD and Clostridium difficile (*C. difficile* and *C. diff*) is not limited to the United States. Research into the use of probiotics during antibiotic therapy for these conditions has been reported on in India, Pakistan, China, Latin

America and throughout Europe; thus making probiotic use a global phenomenon [3,4,5,6,7,8]. As a result, the incidence of combined antibiotic-probiotic (CAP) use is increasing worldwide. But what about the side effects of CAP use? What if, in some way(s), probiotics cause harm? Do probiotics originate or activate deleterious metabolic activities in the host? What about their potential for pathogenicity or toxigenicity? Can probiotics confer antibiotic resistance to elements of the human microbiome?

2.4.2 Resistant Strains of Probiotics

In the bright lights surrounding this assumed natural microbiome savior, the probiotic, medicine seems to have shied away from investigating the probiotic's possible dark side. Antibiotic resistance determinants have been identified and characterized in *Lactobacillus*, *Bifidobacterium* and the probiotic *Bacillus* [9,10]. If the incidence of CAP use is accelerating globally, and if probiotics can confer antibiotic resistance to the human microbiota, a forthcoming epidemic of enormous proportions could now be brewing. This should be of grave concern to those in the fields of clinical pharmacology and clinical epidemiology. Table 2.1 illustrates probiotic strains with antibiotic resistant determinants that have been identified.

Table 2.1 Antibiotic resistance determinants identified and characterized in lactobacilli, bifidobacteria, and probiotic *Bacillus* strains

Gene(s)	Resistance	Mechanism	Location
Lactobacillus			
blaZ	β-Lactams	Antibiotic hydrolysis	–
vat(E)	Quinupristin–dalfopristin	Antibiotic acetylation	–
Cat	Chloramphenicol	Antibiotic acetylation	Plasmid
msrC	MLS	Efflux	–
mef(A)	Macrolide	Efflux	–
aac(6′)-*aph*(2″), *ant*(6), *aph*(3′)-IIIa	Aminoglycoside	Enzymatic modification	–
erm(B), *erm*(C), *erm*(T), *erm*(LF), *erm*(GT), *erm*(A)	MLS	Ribosomal methylation	Plasmid, transposon, chromosome
tet(W), *tet*(M), *tet*(S), *tet*(O), *tet*(Q), *tet*(36), *tet*(Z), *tet*(W/O), *tet*(O/W/32/O/W/O)	Tetracycline	Ribosomal protection	Plasmid, transposon, chromosome
tet(K)/*tet*(L)	Tetracycline	Efflux	Plasmid
Bifidobacterium			
erm(X)	MLS	Ribosomal methylation	Transposon
tet(W), tet(M), *tet*(O), *tet*(W/32/O), *tet*(O/W)	Tetracycline	Ribosomal protection	Chromosome
tet(L)	Tetracycline	Efflux	Chromosome
Bacillus			
aaD2	Aminoglycoside	Antibiotic adenylation	Chromosome
erm(34)	MLS	Ribosomal protection	Chromosome
BCL-1	β-lactams	Antibiotic hydrolysis	Chromosome
cat(Bcl)	Chloramphenicol	Antibiotic acetylation	Chromosome

2.5 Introduction: Supposition for Probiotic Use During Antibiotic Treatment

Probiotics are currently recommended by many healthcare providers (homeopathic, naturopathic and allopathic alike) for use during antibiotic treatment. It is difficult to ascertain the number of patients self-prescribing probiotics while taking antibiotics, but it is certain that the number is considerable and continuing to increase. However, to date, there are no identified protocol or medically-approved guidelines for the prescription and administration of probiotics in conjunction with antibiotic therapy. The main supposition in support of CAP use is that while antibiotics kill the "bad" bacteria in the body, they also destroy the "good" bacteria in the body. It is put forth that probiotics help support the good bacteria; and, therefore, should be used during antibiotic therapy not only to maintain the normal, health-promoting balance of intrinsic bacteria in the intestine; but to avoid or lessen the unwanted side effects of antibiotic therapy, such as abdominal pain, flatulence, diarrhea and candida infestation [11].

2.5.1 What the Sparse Statistics and Studies Reveal

There are no circulating statistics on how many physicians prescribe, or even suggest, taking probiotics with antibiotics. In 2001, a study of physician practices regarding probiotics reported that the majority of the participating physicians did not prescribe probiotics during antibiotic treatment; 88% of them felt that research is needed for probiotics' concurrent use with antibiotics, and that medical guidelines for probiotics use should be established [12]. This was a small survey of only 66 physicians in Nova Scotia. *Clinical Use of Probiotic: A Survey of Physicians'Beliefs and Practice Patterns* reported that "peer practice patterns" influenced the group of physicians prescribing probiotics; whereas, the group of physicians that did not

prescribe probiotics cited the lack of evidenced-based research for doing so [13]. This was also a small survey limited to 27 physicians at Danville Regional Medical Center in Danville, Virginia. Additional research findings in this survey are interesting, as follows:

> Those [physicians] who used probiotics were significantly more likely to agree that probiotics have clinically beneficial effects ($p < 0.017$) and pose minimal risk ($p < 0.003$) than those who don't use probiotics (n = 12, 44.4%). Physicians using probiotics were also less likely to agree that more clinical evidence is needed to support the benefits of probiotics for their specialty ($p < 0.012$), and more likely to indicate "peer practice patterns" ($p < 0.032$) as prompting their use, whereas those not using probiotics were more likely to choose "original research ii i articles" ($p < 0.006$) as a source of information that would potentially change their practice with regard to probiotics. . . . Physicians' beliefs regarding the use of probiotics differ between those who recommend their use in clinical practice and those who do not. Physicians not using probiotics feel that more evidence-based research is needed to support their use in clinical practice [13].

2.5.2 Critical Missing Questions

What is missing from this research, however, are questions and responses from the antibiotic-prescribing group of physicians on the frequency of recommending probiotics during antibiotic treatment (as 32% of the physicians surveyed reported prescribing antibiotics). No data was sought from this group regarding combined use of probiotics during antibiotic therapy.

More often than a physician, it may well be a pharmacist that suggests probiotics for the patient when the patient is filling their antibiotic prescription. According to an article written by Scott, a pharmacist:

> There's reasonably good evidence that probiotics, when taken with antibiotics, will reduce the risk of antibiotic-associated diarrhea. There is still much to be learned about how these products can be used most effectively, which means it's unlikely we will see routine use recommended for some time. However, the reassuring lack of side effects, and potential to reduce potentially serious complications of antibiotic treatment, suggests that probiotics may become a valuable addition to antibiotic therapy [14].

Within this article are more suggestions and assumptions and, noticeably, less science-based conclusions. The article "suggest[s] that probiotics may become a valuable addition to antibiotic therapy." And, without investigating the possible (long-term) side effects, it notes "the reassuring lack of side effects ... ."

A pharmacist may make recommendations when asked by the patient about probiotics; or in the case where a patient expresses concern about antibiotic side effects, such as abdominal discomfort or bowel disturbance; or the pharmacist may simply take the initiative to explain to the patient the potential benefits of taking a probiotic during antibiotic therapy, such as supporting natural gut flora and reducing abdominal discomfort. There are no reliable statistics indicating how many times probiotics are taken with a specific course of antibiotics when the probiotics are self-prescribed by a patient undergoing antibiotic therapy. Therefore, it is not possible, at this time, to determine how many times a "course" of probiotics has been taken

concurrently with a course of antibiotics on a one-to-one basis. If the incidence and prevalence of CAP use was better documented, we could better assess outcomes of CAP use, and the need to develop more specific and scientific guidelines for its administration.

2.5.3 Absence of Medical Guidelines for CAP (Combined Antibiotic-Probiotic) Use

As yet, there are no sanctioned medical prescription guidelines for CAP treatment. Most current practices are based upon anecdotal experiences, inferences, opinions and peer practice patterns. Conclusions regarding their use are drawn based on a general understanding of how the probiotic and antibiotic each act independently, but not based on a wide body of scientific research on how probiotics and antibiotics react together in the human body.

2.6 Discussion

In 2015, the Center for Disease Control and Prevention (CDC) reported that outpatient antibiotic prescriptions in the United States in 2013 were 268.6 million prescriptions which, at the time, was the equivalent of 849 antibiotic prescriptions per 1,000 people [15]. However, there was (and is) no published data on how many times probiotics may have been prescribed by the physician for concurrent use with the antibiotic regime; nor is there any available data, to date, that indicates how many times a pharmacist may have suggested probiotics to be taken along with antibiotics; nor is there any information on how many patients subsequently purchased the probiotics, if recommended by a physician; nor is there any report on how many of those patients eventually followed through on taking the probiotic with the antibiotic; nor is there any statistic on the rate of patients self-prescribing probiotics during their physician-prescribed antibiotic

therapy. These are important and fundamental data to explore and understand. From such data, further research could develop to investigate not only the efficacy of CAP treatment; but the efficacy of particular strains, combinations and potencies of probiotics with different types of antibiotics. This is a fertile area for future research; for example, to create an analog to ascertain how many of those 268.8 million outpatient antibiotic prescriptions were recommended probiotics, how many followed through with the recommendations, and what the outcomes were. If the outcomes were favorable, this could eventually lead to the development of new, customized drugs that contain both probiotics and antibiotics to augment or replace each standalone antibiotic of today.

2.6.1 Tracking Antibiotic and Probiotic Use

We can track antibiotic use with some certainty as in the aforementioned CDC statistics, but it is difficult to do so for probiotics. It is also challenging to quantify probiotic use because– unlike antibiotics which usually come in the form of capsules or pills–the term, probiotic, refers to a wide range of dietary supplements; such as tablets, capsules, powders, lozenges and gums; and come in foods, such as yogurt and other fermented products. The tracking of such data is further impaired as probiotics are not covered by health insurance [16]. If probiotics were covered by health insurance, then it would be easier to track CAP use by studying such data obtained from the insurance industry. But since probiotics are not covered by health insurance, many people would be as likely to purchase a probiotic from their neighborhood health food store or specialty vitamin shop as they would from the medical pharmacy; and, even at the pharmacy, most probiotics are over-the-counter or are not accounted for by a pharmacist. This

further undermines the gathering of statistics on how many people do, in fact, take probiotics with their antibiotics. The patient may get their prescription antibiotics from one source, a pharmacy; and they would likely get their probiotics from a different source, a vitamin shop or over-the-counter. There is currently no way to track these statistics. And without insurance reimbursement for probiotics, many low-income patients and those with less discretionary income would likely opt out of probiotic use even if it was recommended by their healthcare provider. This would eliminate one entire population group–the low income group–from the study; and eliminate from the study, the evaluation of the associated factor of poor diet which, oftentimes, accompanies low income lifestyles. Another research suggestion would be to conduct surveys of physicians (and other healthcare providers and pharmacists) regarding the frequency of concurrent probiotic and antibiotic treatment, and/or surveys or questionnaires from retailers of probiotics on the consumer use of probiotics with antibiotics. This would be an important first step: to determine the rate of CAP use. This would certainly aid in more meaningful discourse regarding such; and support the need for the establishment of medical guidelines regarding CAP use, including contraindications.

One of the few probiotic statistics that we do have available to examine is sales figures. In 2014, the University of Berkeley reported that the annual global sales of all probiotic products were expected to hit $42 billion by 2016 [17]. Of course, comparing 268.6 million outpatient antibiotic prescriptions with $42 billion in probiotic sales to extrapolate the incidence or prevalence of CAP use is inherently flawed. Such formulation does not work. However, we can safely draw one supposition from these numbers: that there are lots of antibiotics being prescribed (849 antibiotic prescriptions per 1000 people in the U.S.) and lots of probiotics being

purchased ($42 billion worth) and consumed; and, most likely–*at the least*–a small percentage of the amounts of each are, undoubtedly, being taken concurrently. It is estimated that the rate of CAP use will continue to increase. Therein lies the basis of the need for better understanding of the interactions of probiotics with antibiotics in treating illness and disease, and the need for better guidelines for their concurrent use.

2.6.2 Historical Probiotic Use Supported and Stimulated by Trade Studies

Twenty, ten, or even five years ago, a patient might simply stumble into a local health food store, with a stomachache that came on during a course of antibiotics, and buy and gobble some probiotics (along with their antibiotics) in the hope they were gaining some benefit–that they were reseeding the unseen, magical microbiota within–and relieving their gastric dilemma. Doctors and patients, alike, had few ways of really knowing how, and if, those two forces–the antibiotic and the probiotic–were working together to bring about a better result. The reasons for taking the probiotic during antibiotic therapy were, most often, anecdotal or based on the best research available at the time which was, oftentimes, biased (or data-skewed or manipulated) as it was sponsored by the manufacturers of the probiotics, the data interpreted by the probiotic company's employees or hired research consultants, and those findings echoed by the retailers of their products and potions. With more current, independent and scientific research available, we can now begin to trust further in some of the proclaimed benefits of using probiotics with antibiotics; we can also start to piece together some general guidelines for the more efficacious use of CAP treatment.

2.6.3 Complications from Antibiotic Use

Antibiotic use can commonly result in the development of gastrointestinal disease. This can range from mild diarrhea to severe colitis. In the adult population, AAD occurs in 5-35% of patients taking antibiotics. The severity depends upon the specific type of antibiotic, the health of the patient and exposure to and type of pathogens. The pathogenesis occurs through the disturbance of the intrinsic microbiota resulting in pathogen overgrowth or metabolic imbalances [21]. In children over the age of two, the prevalence of AAD is 11%. A study found that probiotics reduce the risk of AAD in children; and for every 7-10 patients, one less would develop AAD [22]. A RAND study found that probiotics reduced the risk of AAD by 42 percent [23]. Combined treatments of antibiotics and probiotics have been used successfully in post-operative Chron's disease and, less so, in secondary pancreatic infections [24,25]. But these are unique conditions and special cases; outcomes may not readily transfer to other conditions with differing pathophysiologies. From these trials and studies, one can draw inferences, but no conclusions, regarding CAP use for other conditions. In all of these foregoing studies, not one study noted how and when the probiotics were administered.

2.6.4 Growing Body of Scientific Evidence in Support of Probiotic Use

Presently, there is growing body of scientific evidence establishing the links between probiotics and physical health, probiotics and mental health, the beneficial effects of probiotics in certain diseases, the role of probiotics in the gut epithelial tight junctions and leaky gut, the relationship of probiotics to the human microbiota, and more [18,19,20]. The catalogue of

research regarding probiotics (live bacteria) and prebiotics (specialized fiber that promotes the growth of beneficial microorganisms in the intestines) continues to expand. Today, there are publications and journals dedicated solely to research regarding probiotics. This is all very promising for the many challenges that lie ahead in local and global healthcare objectives and initiatives.

2.6.5 Warning Sirens of Probiotic Use

However, a note of caution needs to be sounded. It is easy to become distracted when considering the many beneficial effects and applications of probiotics. It is easy for the mind to divagate when contemplating the vastness and pervasiveness of the human microbiome and its effect on human physiology, immunology and psychology; and its profound implications in maintaining health and curing disease. Therefore, it should be brought to mind to be vigilant, and to examine the possible (and potential for) adverse effects of probiotics; and recognize the need for science-based guidelines for CAP use.

2.6.6 The Need for Guidelines in Clinical Pharmacology and Concern in Clinical Epidemiology

In addition to establishing the need in clinical pharmacology for defined medical guidelines for probiotic use (especially when taken in combination with antibiotics) and of sounding an alarm in clinical epidemiology concerning the conference of antibiotic resistance to the human microbiota by way of the probiotic, the final facet of this chapter is to identify some basic guidelines–that are being used today–in CAP treatment; and in doing so, call out the need

for further research in this regard, and the need to establish science-based guidelines (including contraindications) for the concurrent use of probiotics with antibiotics.

2.7 Summary of and Expansion Upon Previous Points

To summarize and expand upon several previous points: the use of probiotics is considered a means to boost and restore the beneficial microbes in the human gut; and the use of probiotics to prevent vaginal infections, following antibiotic therapy, is worthwhile if the antimicrobial drug adversely affects the vaginal microbiota (and there is some evidence to support this application) [26]. As antibiotics disrupt the intrinsic intestinal flora, the candida can rapidly infiltrate and spread with the gut.

2.7.1 The Beneficial Mechanisms of Probiotics in the Human Gut

Probiotics that stimulate the natural flora cause it to compete for space within the gut; thus having a positive effects on the immunological system which, when enhanced, can counter the candida. Yeast proliferation in the gut can have deleterious effects on the tight junctions resulting in leaking gut syndrome [27]. Probiotics may alter the local environment making it less hospital to candida. The reduction of candida responds well to strains of acidophilus and bifidus [28]. In all cases, yeast or bacteria, the appropriate strains and dosages of probiotics are critical. More in depth research regarding strains and dosages is beyond the scope of this research and is left for further investigation. Probiotics with strains like *Lactobacillus rhamnosus* and *Saccharomyces boulardii* help in preventing diarrhea and different infections that may arise as a result of using antibiotics.

Current research supports that probiotics do–in some ways and in certain conditions, such as AAD, CDI, Chron's disease and secondary pancreatitis–benefit patients that are taking antibiotics or have recently completed a course of antibiotics. How should these patients take–or physicians prescribe–the probiotics with antibiotic treatment? In addition, how should comparatively healthy people–who have been prescribed antibiotics for much less severe conditions (such as a mild streptococcus infection, skin, eye or ear infection, or urinary tract infection) and who want to mitigate abdominal distress and enhance their intestinal flora–take probiotics; how and when and for how long should they take probiotics? The consensus of opinion in this regard will be laid out in Sections 2.8, and a summary of such will be outlined in Section 2.9.

A probiotic must act against pathogens by mechanisms that are different from those of antibiotics, for instance by competition. They should also be non-pathogenic. In addition, probiotics must act rapidly and survive the challenges of the concurrent antibiotics, gastric acid and bile. Antibiotics kill the beneficial bacteria in the gastrointestinal system exposing the body to attacks by harmful pathogens like candida yeast. Yeast can quickly dominate the small intestine after completing the antibiotic regime resulting into candidiasis [10]. As such, it is advisable to take probiotics prior to, during and after completing the antibiotic dose. This helps maintain the balance in the digestive system and aid in reducing dysbiosis.

2.7.2 The Adverse Actions of Antibiotics in the Human Gut

Antibiotics kill microbes in the digestive system, reducing competition and creating a suitable environment for fast-growing candida to fill the gap. Use of probiotics during antibiotic

treatment slows the growth of candida by filling the gastrointestinal system with beneficial microbes. Probiotics reduce the side effects of antibiotics since they promote intrinsic microbe balance in the gut [10]. Antibiotics may kill good bacteria despite killing bad bacteria in the gastrointestinal system which may lead to diarrhea. Probiotics promote microbial balance altered by infections and antibiotics. Certain food products, like brands of miso and yogurts, have strains of probiotic bacteria added to them [30].

2.7.3 Studies on CAP (Combined Antibiotic-Probiotic) Use

A study done at the Einstein College of Medicine (ECM) supports the use of probiotics during antibiotic treatment. ECM scientists reported: “that up to one in five people stop taking their entire course of antibiotics due to diarrhea.” The ECM scientists reviewed the medical literature and found seven, high-quality studies in which probiotics were administered to people. The researchers concluded that the studies support the use of probiotics for avoiding diarrhea resulting from antibiotic use or from gastrointestinal viral or bacterial infections. In addition, the probiotics used in these studies were found to rarely cause adverse effects, even in children. "With the level of evidence that probiotics work and the large safety margins for them, we see no good reason not to prescribe probiotics when prescribing antibiotics," says Dr. Benjamin Kligler, a coauthor of the study and associate professor of clinical family and social medicine at ECM. Dr. Kligler also notes that the effects of probiotic doses are short-lived, so they should be taken throughout a course of antibiotic therapy. Probiotics will not diminish the effectiveness of antibiotics, he adds [30].

2.7.4 The Role of Probiotics in CAP (Combined Antibiotic-Probiotic) Use

Probiotics augment the role of antibiotics in the prevention and management of various microbial infections. Probiotics interfere with the invasion and adhesion of disease-causing microorganisms. In addition, they help in preventing bacteria from attacking cells that are already exposed, and also protect the gastrointestinal epithelium from further invasion. *Lactobacillus rhamnosus* increases the number of immunoglobulin secreting cells in the intestinal mucosa by stimulating interferon release. However, it is advisable to administer the antibiotic and the probiotic at different times. This is due to the fact that the antibiotic may reduce the efficacy of the probiotic microorganisms. One should continue taking the probiotic for a period of one week after completing the antibiotic dose.

2.8 Current Practice in Prescribing Probiotics During Antibiotic Treatment

Some physicians recommend the use of probiotics 6 hours after taking an antibiotic dose. Moreover, it is advisable to continue taking the probiotics for a period of ten days after the antibiotics are stopped. Some of the adverse effects of the antibiotics are counteracted by probiotics, for instance upsets in the stomach caused by the loss of lactobacillus from the gastrointestinal system. Other physicians believe it is advisable to difference the probiotic dose 4 hours after the intake of the antibiotic [31].

Current studies have shown that the combined use of probiotics with antibiotics can help reduce the unwanted side effects of the antibiotic therapy; in particular, gastric disorders and diarrhea, and candida infestation. To maximize the use of the probiotics, most researchers suggest staggering the doses; that is, taking the probiotic 2-6 hours after the antibiotic dose, and

continuing with the probiotic 7-10 days after ending the antibiotic treatment. It is also helpful to take probiotics before beginning antibiotic therapy, if possible [11,32].

2.8.1 Probiotics: Beware of Substandard Quality

Probiotics are not considered drugs (since they are not marketed as a cure for any medical condition, illness or disease); thus their manufacture is not subject to the strict guidelines and scrutiny of the FDA, nor the quality controls consistent with those of the pharmaceutical industry. Therefore, the quality and effectiveness of different brands and different strains of probiotics can vary widely. So, it is advised to investigate probiotic products thoroughly before prescribing or consuming them in order to maximize positive outcomes and minimize negative outcomes [33].

2.8.2 Probiotics: Beware of Contraindications

It is the opinion of some researchers and physicians that antibiotics do not adversely effect the potency of probiotics (if taken at different times) and that antibiotics fair well in the presence of probiotics without their efficacy being compromised. Nevertheless, caution must be observed as probiotics have side effects to the body, such as infections in people with poor immunity. These infections are sometimes resistant to antibiotic therapy [34]. Some patients suffering from Crohn's disease, an autoimmune disease, have shown unpleasant outcomes after probiotic administration [16]. (This is different from the aforementioned studies, cited in Section 2.6.3, regarding Chron's; wherein it was the post-surgical application of CAP treatment that was

deemed beneficial in some cases.) In addition, according to some researchers, probiotics can cause faster metabolism of drugs, such as sulfasalazine causing higher quantities of them in the body [35].

2.8.3 Probiotics: Beware of Genetic Instability, Pathogenicity and Toxigenicity

Genetically modified probiotics increase the mortality rate of patients suffering from pancreatic diseases, such as acute pancreatitis (as differentiated from secondary pancreatitis) [36]. According to an NCBI report, *Safety Assessment of Probiotics for Human Use*:

> Genetic stability of the probiotic over time, deleterious metabolic activities, and the potential for pathogenicity or toxigenicity must be assessed depending on the characteristics of the genus and species of the microbe being used. Immunological effects must be considered, especially in certain vulnerable populations, including infants with undeveloped immune function [37]

These are some concerns regarding probiotic use that warrant further investigation. In addition, more research needs to be done on the pharmacokinetics of CAP use.

2.9 Conclusion; Forward-Looking Research

Medical protocol for the appropriate use of particular strains of probiotics with different types of antibiotics is a long-term, but valuable, project. For the time being, the use of high-quality probiotics during antibiotic therapy; staggering the probiotic 4-6 hours after taking the antibiotic; continuing in this way throughout the course of antibiotic therapy; and continuing for

7-10 days after the completion of the antibiotic regime, especially in those groups prone to candida and diarrhea, is deemed helpful by many researchers and practitioners.

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CHAPTER III

METHODOLOGY: UNDERSTANDING THE EFFECTS OF PROBIOTICS ON THE HUMAN GUT

3.1 Abstract

Over the last several decades, scientists have begun to recognize the importance of microbiota in the human body. The microbiome is now considered to be a separate organ. However, while research has been conducted on microbiota, there are still many areas in which little is known. Despite this lack of information, researchers now understand that an imbalance in the types of species of microbiota can lead to a variety of symptoms and conditions. One method that can correct these imbalances is the administration of probiotics. An overview of the different types of probiotics will be provided as well a narrative of the probiotic's journey from ingestion to colonization, along with a description of the harsh conditions of the alimentary canal in which the probiotic must survive and thrive. The factors required for survival and colonization will be explained. Five broad categories of the mechanisms of probiotic effects will be described; these encompass: nutritional benefits, neuromodulatory effects, promotion of epithelial cell homeostasis, modulation of intestinal immune function, and blockage of pathogenic bacteria. While much is now known about the nature and function of probiotics, there are still many unknowns; as such, further research is needed in order to fully understand the effects of treatment with probiotics.

3.2 Keywords

amino acid decarboylation-antiporter reactions, Bacteriodetes, fecal transplant, Firmicutes, gut-brain axis, human gut microbiome, immunity, microbiota, mucin, neuromodulation, probiotics, tolerogenic transcriptomic

3.3 Abbreviations

BSH: bile salt hydrolase; COG: Clusters of Orthologus Groups; GABA: gamma-Aminbutyric acid; IgA: immunoglobulin A

3.4 Introduction: Historical Overview

Historically, when discussing digestion and the digestive system, the vast majority of gut research was focused on the biochemical pathways. The bulk of textbooks focused on the biochemical reactions which occurred in the digestive tract. The focus was primarily on how food was digested and how other organs coordinated in the digestion process. There was little consideration and discussion of the presence of bacteria in the gut. Until recently, most of textbooks did not include a detailed description of the microbiota. In the last several decades, however, there has been a surge in research which has led to a better understanding of the important role that gut bacteria play in human health. Now, the bacteria of the gut are often considered as a separate organ. While the mechanisms of host and microbiota interactions are still being unravelled, it is now understood that certain types of bacteria are important for optimal health and the prevention of disease. The gut microbiota play an important role in determining metabolic rate and in regulation of immunity [1].

3.4.1 The Two Divisions of Microbiota: Firmicutes and Bacteriodetes

With respect to metabolic rate, an imbalance in the composition of the microbiota has been shown to be linked to obesity. Previous research has identified that, in obese individuals, there is a distinct difference in the composition of microbiotia. In humans, the two predominant types of beneficial bacteria in the gut are the Firmicutes and the Bacteriodetes. Individuals of normal body weight have higher levels of Bacteroidetes bacteria than obese individuals [2]. In fact, a woman of normal body weight gained a significant amount of weight after undergoing a fecal transplant from an obese individual [3]. As such, researchers are now investigating the possibility of using fecal transplant as a method of weight loss in obese individuals. (Careful consideration of any donor for a fecal transplant must be conducted to prevent the occurrence of adverse side effects, such as obesity.) The microbiota also play a role in the metabolism of foods. The gut microflora are able to help in the breakdown of complex carbohydrates [4].

3.4.2 Definition and Development of Microbiota

By definition, the human gut microflora consists of all of the microorganisms which inhabit the digestive tract, including both bacteria and other microorganisms such as yeast. Early in life, there are several major factors which can influence the composition of the microflora. Before birth, the prenatal gut is void of any bacteria. Immediately after birth, however, colonization of the gut begins. The mode of birth, vaginal or caesarean, alters the composition of the gut microflora. Throughout the first years of development, a child's microbiota is changing. It becomes relatively stable between the ages of 3 to 5 years. Thereafter, while the microbiota is relatively stable, it can be altered by treatment with antibiotics. The microbiota can also be

altered through long-term dietary changes, by way of bacterial infections or as a consequence of surgery. Any major changes in the microbiota can result in an imbalance which can lead to disease or health complications [1].

3.4.3 The Unique Structure of the Gastrointestinal System: Folding

The human gastrointestinal system has a large amount of surface area which is in contact with the environment; this is due to folding. This amount of surface area is required to ensure that nutrients are maximally extracted throughout the digestive process. The total surface area of the gastrointestinal system is thought to be between 150 to 200m^2. In contrast, the surface of the skin is around 2m^2. In the gastrointestinal tract, the increase in surface area is obtained, first, by the formation of circular folds and, then, by the folding of the intestinal villi also known as the epithelium. These villi are then further folded into microvilli. In order to ensure proper lubrication, the goblet cells (which are found in the epithelial lining) produce mucus. Because of the high level of mucus and the high level of nutrients, the gastrointestinal tract provides optimal conditions for colonization of microorganisms. It is estimated that within this tract there are around 1x 10^{14} bacteria. This means that in the human body, there are approximately 10 times more bacterial cells than eukaryotic cells [5].

3.4.4 Modulation of the Microbiota Via Probiotics

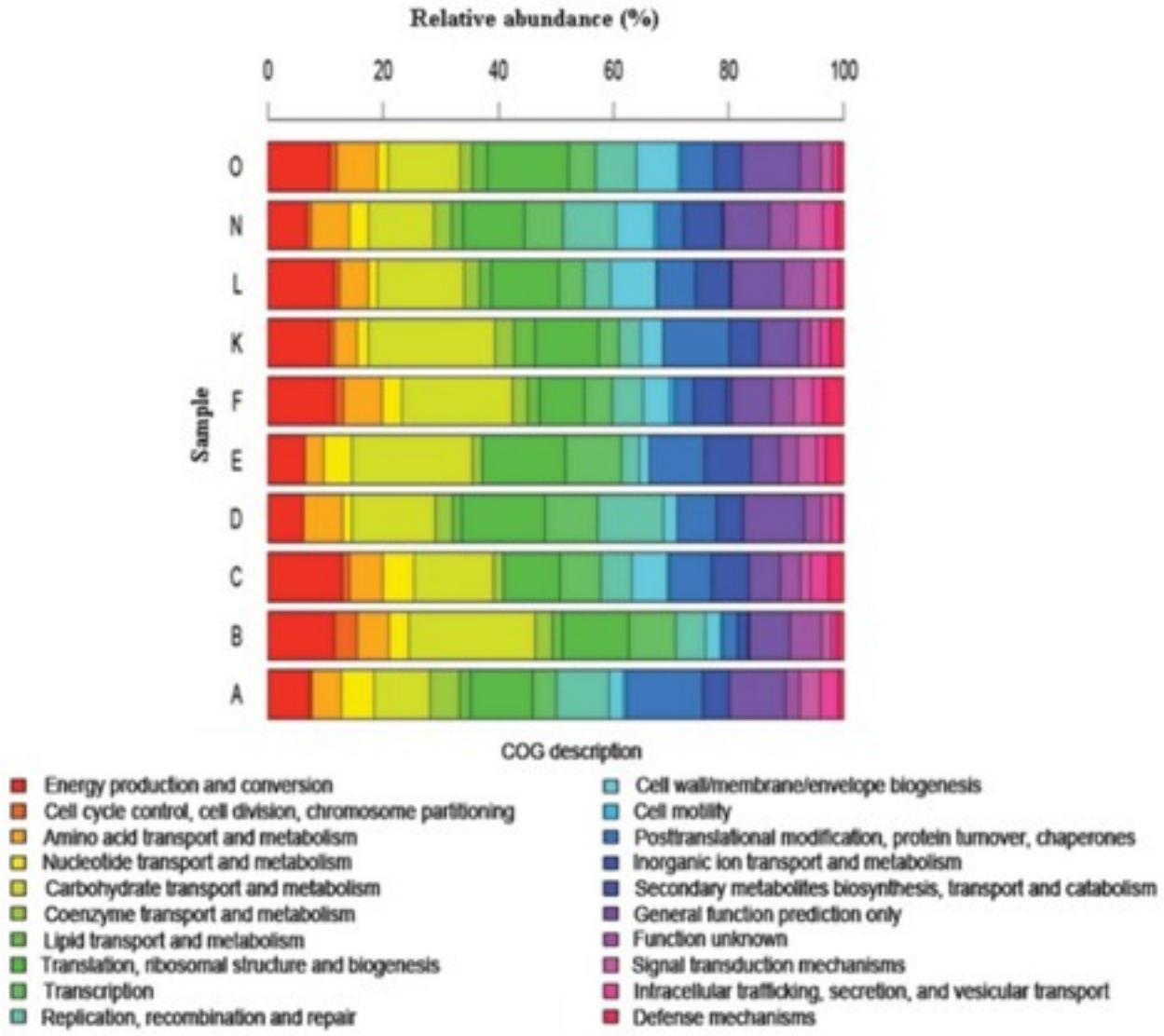
The importance of the gut microbiota and its impact on the health of individuals has begun to be recognized. Ways to modulate the composition of the microbiota are being

investigated. One method of altering the composition is through the use of probiotics. Probiotics, by definition, are live yeast and bacteria which are thought to be beneficial for digestive health. These probiotics are ingested and then colonize the gut [6]. Following is an overview of the current research surrounding probiotics; in particular, how probiotics survive the digestive system and ultimately colonize the digestive tract, as well the specific mechanisms of action of probiotics on modulating human health.

3.5 Discussion

There are many different types of bacteria which make up the gut microbiota. As mentioned earlier, the two predominant types of beneficial bacteria are the Firmicutes and the Bacteriodetes. Proteobacteria and Actinobacteria are also considered to be dominant groups, but are found in smaller amounts. Fusobacteria and Verrucomicrobia species are also found, but these two phyla of bacteria are not considered to be of the four dominant phyla [7]. Within the gastrointestinal tract, the microbiota are involved in a variety of different roles. Two of these roles are immunity and metabolism. A recent metatranscriptomic analysis of bacteria found in human feces determined that certain transcripts produced by bacteria could alter host function. The results of this transcriptomic analysis are shown in Table 3.1.

Table 3.1. Overview of a metatranscriptomic analysis of 10 separate fecal samples. Each color represents a separate cluster of orthologus group (COG)



3.5.1 Variation in Genes (and of Function) of the Microflora

All of the transcripts produced by the bacteria were assigned to Clusters of Orthologus Groups (COG). These categories were determined by comparing the sequence similarity of the bacterial transcript to that of other organisms in order to determine its function. Across the 10 different samples there is individual variation in the types of genes provided by the bacteria. In total, 20 different COGs were determined. The categories of genes which were most abundant included genes for energy production and conversion, coenzyme transport and metabolism, translation, ribosomal structure and biogenesis, and inorganic ion transport and metabolism. Of these main categories, the most variation in the number of transcripts was for carbohydrate transport and metabolism, and also in energy production and conversation category.

This variation in genes is important as it highlights the need for caution when treating individuals with probiotics. Due to the variation in the types of genes, a probiotic combination which works for one individual may not be effective in another individual. One of the dangers of probiotics is that there are many types of preparations available which have not been tested rigorously. Therefore, it is possible that adverse effects may occur from these untested probiotics [8].

3.5.2 Variances Among Probiotic Species

It has been identified that imbalances in the composition of microflora species in the human gut can lead to the development of health problems. Subsequently, it has been proposed that probiotics can be used to help alter the composition of the gut microflora and repair

imbalances. Not all probiotic species are the same, however. There are several different groups of probiotics which have distinct functions and disparate abilities to survive the gastrointestinal tract. While the type of species used for contrasting probiotic preparations are indeed different, the journey which all probiotics take, after oral ingestion, is similar for all types of probiotics.

3.6 The Journey of Probiotics Through the Alimentary Canal

Probiotics are taken orally. Therefore, in order to colonize the gut, they must first travel within the body. The alimentary canal is considered the entire pathway through which food passes through the body starting at the mouth until its exit at the anus. It is relevant to review the process of digestion relative to probiotics, and what happens to probiotics as they travel through the alimentary canal. The conditions in the route of travel are harsh. A segmental review of this process will allow for recognizing the essential components and mechanisms of action of probiotics [9].

After intake, the probiotics–if in the form of food like yogurt–begin the process of digestion in the mouth. In the mouth, the probiotic-containing food is mixed with saliva, and starches, if present, begin to be digested. The food is then swallowed and travels via the esophagus to the stomach. If the probiotic is in encapsulated form, the probiotic (for all intents and purposes) bypasses the digestive actions in the mouth, is swallowed and (as with the probiotic food) begins its travel through the esophagus and enters the stomach where it is mixed with stomach acid, also known as digestive juice. Here proteins begin to break down. The highly acidic mixture is then neutralized as it enters the small intestine where it is mixed with secretions from the liver and pancreas [9]. The walls of the small intestine absorb the digested nutrients into

the bloodstream where they are transported throughout the body. After the small intestine, waste products are processed and passed within the large intestine where they are concentrated and excreted out of the body as stool. Throughout this process, as probiotics travel along the alimentary canal, they may be deposited in the small intestine or colon where they can colonize [9,10].

3.7 Major Types and Classifications of Probiotics; Indications in Illness and Disease, and Associated Risks

There are many different types of probiotics available. The major types of probiotics can be divided into four main categories: yeast, Gram-negative bacteria, Gram-positive bacteria, and combination regimens. A summary of the common types of probiotics and their associated benefits in human disease are shown in Table 3.2.

Table 3.2 Selected organisms that are used as probiotic agents

Probiotic	Human disease in which benefit is shown	Animal model in which benefit is shown
Yeast		
Saccharomyces boulardii	*Clostridium difficile* infection[96,98]	*Citrobacter rodentium*-induced colitis[57]
Gram-negative bacteria		
Escherichia coli Nissle 1917	NA	DSS-induced colitis[59]
Gram-positive bacteria		
Bifidobacteria bifidum	NA	Rat model of necrotizing enterocolitis[91]
Bifidobacteria infantis	IBS[29]	NA
Lactobacillus rhamnosus GG (used with lactoferrin)	Sepsis in very low birth weight infants[88]	NA
Lactococcus lactis (engineered to produce IL-10 or trefoil factors)	Crohn's disease[121]	DSS-induced colitis and IL-10$^{-/-}$ mice (spontaneous IBD)[120,122]
Lactobacillus plantarum 299v	Antibiotic-associated diarrhea[100]	IL-10$^{-/-}$ mice (spontaneous IBD)[71]
Lactobacillus acidophilus	NA	Visceral hyperalgesia[40] and *C. rodentium*-induced colitis[57]
Lactobacillus rhamnosus	Pediatric antibiotic-associated diarrhea[101]	–
Lactobacillus casei	NA	DNBS-induced colitis[65]
Bacillus polyfermenticus	NA	DSS-induced colitis and TNBS-induced colitis[68]
Combination regimens		
Lactobacillus rhamnosus GG combined with *Bifidobacterium lactis*	Bacterial infections[10]	NA
Lactobacillus rhamnosus combined with *Lactobacillus helveticus*	NA	*C. rodentium*-induced colitis,[56,118] chronic stress,[39] and early life stress[36]
VSL#3 (*Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidocacterium infantis* and *Streptococcus thermophilus*)	Pouchitis and pediatric ulcerative colitis[76,77]	DSS-induced colitis,[60] IL-10$^{-/-}$ mice (spontaneous IBD; DNA only),[72] and SAMP mouse model of spontaneous IBD[73]

Abbreviations: DNBS, dinitrobenzene sulfonic acid; DSS, dextran sodium sulfate; IL-10, interleukin 10; NA, not available; TNBS, trinitrobenzene sulfonic acid.

The yeast category contains only one species *Saccharomyces boulardii*. It has been used primarily to treat *Clostridium difficille* infection [6]. Similar to yeast, the Gram-negative bacteria category also contains only one species *Escherichia coli* Nissile 1917 which is primarily used to treat inflammatory bowel diseases, such as ulcerative colitis [11].

Of the four categories, the Gram-positive bacteria category is the largest category. Within this category, the two main genera are *Lactobacillus* and *Bifidobacteria*; however, *Bacillus*

polyfermenticus has also been used as a probiotic. *Lactobacillus* species can be used for the treatment of low birth weight infant sepsis, pediatric ulcerative colitis, Chron's disease and antibiotic-associated diarrhea. In contrast, *Bifidobacteria* is primarily used for the treatment of inflammatory bowel syndrome [5].

The final category of probiotics is that of combination regimens. To increase health benefits, probiotics are combined together [6]. Typical combinations include multiple species of *Lactobacillus* or *Bifidobacteria* or a combination of species of *Lactobacillus and Bifidobacteria* [12]. An example of a combination of probiotics which is used to treat ulcerative colitis is VSL#3. VSL#3 is a combination which contains 8 different probiotic bacteria: *Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus bulgaricus,* and *Streptococcus thermophilus.* Previous studies have shown that treatment with VSL#3 can result in remission of ulcerative colitis [13]. One of the main modes of action of this probiotic combination is increased production of mucus from epithelial cells of the colon. As ulcerative colitis is the formation of ulcers in the colon, increased mucus production can help to reduce existing ulcers and prevent the formation of new ulcers [14].

In order to ensure maximum efficacy, it is necessary to fully understand the role that each type of bacteria plays in human health. While there are many different types of probiotics on the market, not all of the strains have been thoroughly evaluated. Depending on the imbalance which is present in a person, it is important that the appropriate type of probiotic be selected. If the wrong type of probiotic is administered, there may be no effect at all, or it could lead to negative effects on the health of the individual. Therefore, it is incumbent to have a fundamental

understanding of the mechanisms of action of the varying types, strains and combinations of probiotics.

3.8 Overview: Mechanism of Action of Viable Probiotics

The first step for a probiotic being beneficial to humans is that it must survive the gastrointestinal tract, and colonize. It is important to note that individuals must continuously take probiotics in order to maintain adequate levels of those strains in their gastrointestinal tract. After cessation of probiotics, most strains of probiotics are no longer detectable in fecal samples after 1 to 4 weeks. As such, if using probiotics to treat a particular health condition, patients must continue to take probiotics indefinitely [10].

There are several different traits which are important to help increase the chance of survival and colonization of probiotics; these include tolerance to acid conditions and tolerance to high levels of bile salt and the effect on genes which enhance attachment [15]. It is important that they are able to survive a highly acidic environment as the probiotic must travel through the stomach. In 2004-2005, two studies were published which identified genes associated with acid tolerance in *Lactobacillus acidophilus*. In *Lactobacillus acidophilus* it was found that there were nine two-component systems. One of the most effective ways known to respond to changes in the environment is to utilize a two-component system. These systems regulate signal transduction through the use of phosphorylation at a response regulator. The “dual nature” of this system allows for a rapid response to changes in the external environment. A higher number of systems is likely to indicate a higher capacity of the bacteria to survive extreme environmental changes [16].

3.8.1 Viability of Probiotics in the Extreme pH of the Stomach

One of the main categories of genes identified was that for the coding of amino acid decarboylation-antiporter reactions. This system is one of the main systems which is involved in the regulation and maintenance of pH. Bacterial species that are able to encode highly active versions of this system are more likely to be able to survive the journey through the highly acidic stomach [17].

3.8.2 Viability of Probiotics in the Presence of Bile Salts

Probiotic bacteria must also be able to withstand environments which are rich in bile salts. Certain species of bacteria can be extremely sensitive to high concentrations of bile salt due to the variety of different components in the cell walls of bacteria [15]. Two genes (lp_0237 and lp_0775) have been identified in *Lactobacillus plantarum* which are induced only in the presence of high concentrations of bile salt. As such, if a bacteria is unable to activate genes to modulate changes in the concentration of bile salt in the environment, they are not likely to survive [18].

3.8.3 Viability of Probiotics Due to Adhesive Properties

While survival is important and required for the species to make it to the colonization site, probiotic bacteria are only beneficial to humans if they are able to colonize somewhere in the gastrointestinal tract [15]. Determining the factors which help influence adhesion is critical to developing superior probiotics. It is challenging to investigate the mechanisms of attachment of these species due to the difficulty in culturing probiotic bacteria. Research on the types of components required for attachment is limited. There have, however, been several studies

conducted which have shed light on the components required for attachment. Research has shown that exported proteins are an important factor in determining the attachment of probiotic bacteria to the host [19]. In vitro experiments using a human colon epithelial cell line found that probiotic bacteria do indeed have exported proteins bonded to their cell wall. The presence of these proteins increases their ability to attach to the epithelial cells. All of the species of probiotics investigated contained at least one adhesive protein; *Lactobacilus plantarum* F1 contained two adhesive proteins [20]. Further research is needed in order to determine exactly the type of components which are required for attachment.

3.9 Overview: Functions of Probiotics

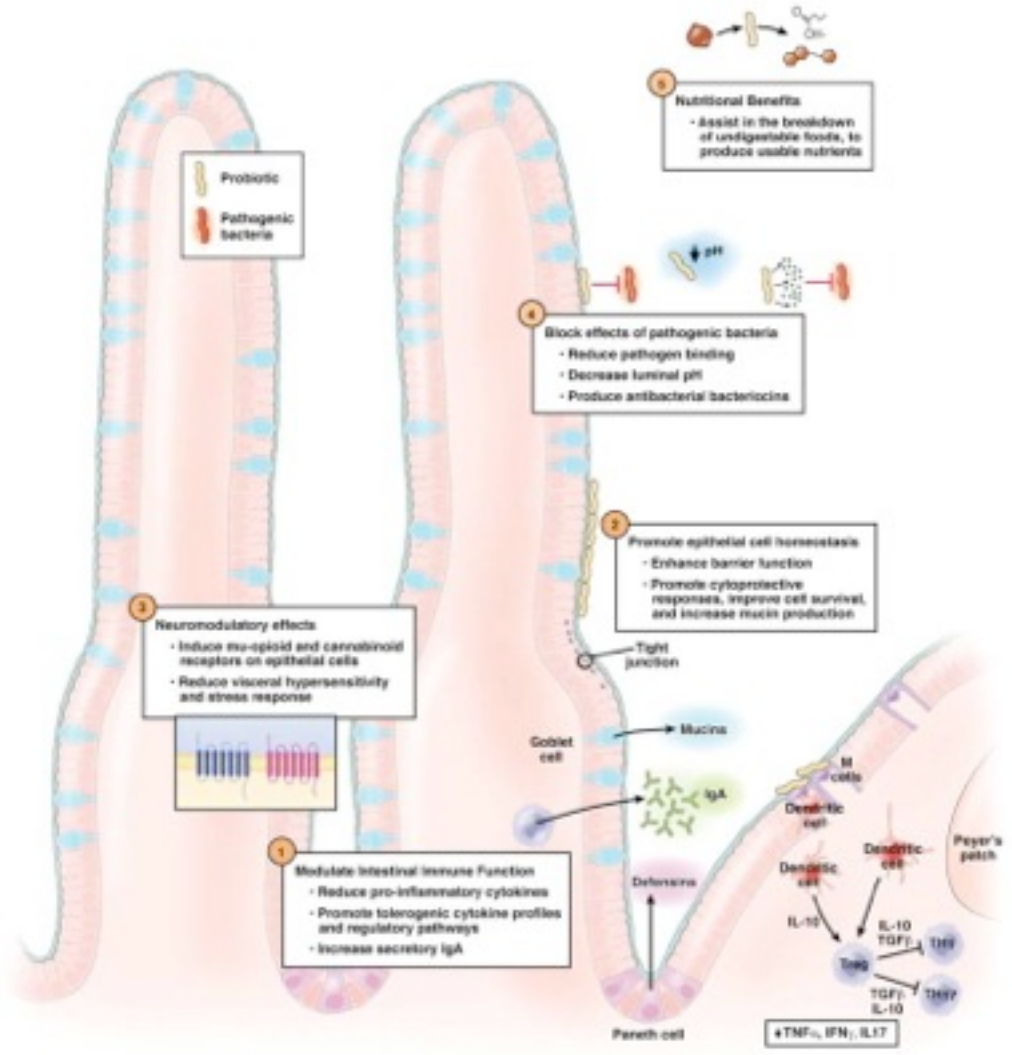
After colonization, the mechanistic functions of probiotics can be divided into five broad categories:

- nutritional benefits,
- neuromodulatory effects,
- promotion of epithelial cell homeostasis,
- modulation of intestinal immune function, and
- blockage of pathogenic bacteria [10].

A summary of the five main categories of mechanistic functions of probiotics is shown in Figure 3.1. While there may be other classification systems for characterizing the mechanisms of

probiotics (because of the wide range of species), a broad category system, such as depicted in Figure 3.1, is a simple and useful method of classification.

Figure 3.1 Illustration of the five (5) functions of successfully colonized probiotic bacteria.



3.9.1 Nutritional Benefits (and Digestion)

Probiotic species are able to assist the host with digestion by breaking down otherwise indigestible food products, thereby producing products which are usable by the host. An example of how probiotics can provide nutritional benefits is that of individuals with lactose intolerance.

Individuals with lactose intolerance are unable to metabolize lactose into glucose and galactose due to a deficiency in beta-galactosidase. Beta-galactosidase is the enzyme which is required to break lactose into glucose and galactose. Individuals with lactose intolerance typically experience undesirable symptoms after ingesting lactose. These symptoms include diarrhea, flatulence and abdominal discomfort. Bacteria are able to produce beta-galactosidase; however, the amount which is produced differs depending on the species. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus* produce higher levels of beta-galactosidase. Ingestion of these two types of probiotics has been shown to relieve symptoms of lactose intolerance [21].

Probiotics also exert an effect on bile salts. Bile salts are used to breakdown large globules of fat which can then be digested by the body. Bile salts are stored in the gallbladder and released into the small intestine. Bile salts are then reabsorbed and recycled for further use. Research has shown that up to 5% of bile salts, each day, are modified by the microbiota through a process known as deconjugation. The deconjugation of bile salts is an important first step which leads to other modifications of the salt. Certain species of microbiota are able to produce an enzyme known as bile salt hydrolase (BSH). This enzyme cleaves the amide bond of the salt resulting in free bile salts which can be used in other processes. Probiotic species *Lactobacillus* and *Bifidobacterium* have been shown to produce BSH. Increased levels of these strains of probiotics can help increase the availability of bile salts and improve the digestion of lipids [22].

3.9.2 Neuromodulatory Effects

Supplementation of certain probiotics can lead to increased levels of cannabinoid and mu-opioid receptors on epithelial cells of the intestinal tract. Increased levels of these receptors are important as they are involved in the regulation of appetite, mood and pain. Certain probiotics (including VSL#3) have been shown to cause a reduction in appetite [23]. This is likely due to the increased production of these receptors. Probiotics can reduce the stress response and visceral hypersensitivity. In a double-blind placebo study published in 2008, it was found that a probiotic combination of *Lactobacillus acidophilus* Rosell-52 and *Bifidobacterium longum* Rosell-175 led to a reduction in stress-related gastrointestinal problems. Participants receiving the probiotic combination had significantly lower levels of constipation, nausea, vomiting and abdominal pain over the group receiving the placebo [24].

The gastrointestinal tract of humans is highly innervated and forms a network known as the enteric nervous system. The main function of this system is to regulate the responses of the gut and mediate communication between the central nervous system and the gut. This communication system is commonly referred to as the gut-brain axis. Administration of probiotics has been shown to have a positive effect on the gut-brain axis leading to a reduction in psychological symptoms, such as anxiety. Anxiety can result from low levels of serotonin and GABA. Both serotonin and GABA can be supplied by probiotic bacteria [8].

3.9.3 Promotion of Epithelial Cell Homeostasis

Probiotics can help in the promotion of homeostasis in epithelial cells. The main method of action is through enhancement of barrier function and promotion of protective responses. These are accomplished by increasing mucin production and improving cell survival. Mucin is either membrane-bound or mobile outside the cell. Decreased mucin production is one of the main culprits in the vast majority of gastrointestinal disorders. Decreased levels of mucin production can lead to inflammation. Certain formulations of probiotics are able to reduce and reverse this inflammatory damage by stimulating the production of mucin. The reduction of the adhesion of pathogenic bacteria species is an added benefit of increased mucin production [25].

3.9.4 Modulation of Intestinal Immune Function

Probiotics modulate the function of gut immunity. This occurs primarily through three separate mechanisms: the reduction of proinflammatory cytokines, the promotion of tolerogenic regulatory pathways, and increased secretion of IgA.

3.9.4a Reduction of Proinflammatory Cytokines

Increased levels of probiotics are able to reduce the levels of proinflammatory cytokines as was demonstrated in a recent study conducted in type 2 diabetic patients with nonalcoholic fatty liver disease. Treatment with the Symbiter combination probiotic for 30 days led to a significant reduction in the level of plasma proinflammatory cytokines [26].

3.9.4b Promotion of Tolerogenic Regulatory Pathways

The second mechanism of immune function modulation is the promotion of tolerogenic regulatory pathways. Administration of probiotic bacteria leads to an upregulation in anti-inflammatory cytokines. This upregulation of anti-inflammatory cytokines leads to the promotion of an anti-inflammatory environment resulting in a reduction in cell damage and a reduction in stress responses [27].

3.9.4c Increased Secretion of IgA

The third mechanism of immune-function modulation is increased secretion of immunoglobulin A (IgA). IgA is an antibody found in the body which plays a significant role in the modulation of the immune system and in maintaining the health and function of mucus membranes. The vast majority of IgA in the body is produced by the mucosal membranes. Oftentimes, in gastrointestinal diseases, there is damage to the mucosal membranes which results in decreased mucin production. This coincides with decreased production of IgA. Treatment with probiotics, such as *Lactobacillus*, can increase levels of IgA, thereby improving intestinal health in diseased patients [28].

3.9.5 Blockage of Pathogenic Bacteria

Probiotics are able to reduce the colonization of pathogenic bacteria. One of the benefits of increased mucin production is decreased adherence of pathogenic bacteria. Besides increased

mucin production, alteration of luminal pH and production of bacteriocins also reduce pathogenic bacteria. Probiotic bacteria, specifically probiotics which produce lactic acid such as *Lactobacillis*, are able to decrease the pH of the lumen. This decrease in pH is a direct result of increased production of lactic acid. This decrease in pH exhibits an antimicrobial effect on pathogenic bacteria, and prevents them from adhering [29]. Bacteriocins are antimicrobial substances which are produced by probiotic species. These compounds are produced by one strain of bacteria, and prevent other closely related strains of bacteria from colonizing. They are considered to have a dual role; to be both antibiotic and probiotic. One of the many benefits of ingesting probiotics is that they will colonize the digestive tract and will produce bacteriocins which will prevent pathogenic species in the same phyla from binding. However, because bacteriocins only function against closely related species, ingestion of a combination of probiotics species is necessary to receive maximum antibiotic effect [30].

3.10 Conclusion

Probiotics can provide health benefits to individuals. Because they are living organisms and due to their unique characteristics, the distinct nature of probiotics can help correct imbalances in the human body which may occur due to a disruption to the natural, intrinsic microbiota. There are four main categories of probiotics, but the majority of current probiotics are Gram-positive bacteria. There is a lack of studies evaluating the efficacy of the vast majority of available probiotic products. While there are exceptions, such as VSL#3, many of the available probiotics have not been checked to ensure that they contain the stated number of live

bacteria [6,13,14]. Many of the strains used in probiotic combinations have not been validated. Therefore, a considerable amount of research must be conducted in order to ascertain the validity of these claims.

Despite this lack of research, the probiotics which have been studied have generally been shown to be beneficial to humans. Both species of *Lactobacillus* and *Bifidobacteria* have been shown to provide benefits to individuals with gastrointestinal diseases, such as irritable bowel disease and ulcerative colitis. While probiotics have been shown to have benefits in isolation, most studies have shown that combinations of probiotics have a higher number of benefits than treatment with a single probiotic species alone. This in a large part is due to the differences in composition of the microbiota between individuals. Unless sequencing is conducted, it is possible that the individual may already have high levels of bacteria similar to that being provided. Providing a combination of probiotics can help to increase the likelihood that the patient will see a positive effect [6].

One main limitation of probiotics is that, in order to continue to see benefits, the individual must continually take probiotics. Stoppage of the probiotic results in removal of the probiotic from the gastrointestinal tract after several weeks [10].

There are five separate modes of action for the effects of probiotic bacteria: nutritional benefits, neuromodulatory effects, promotion of epithelial cell homeostasis, modulation of intestinal immune function, and blockage of pathogenic bacteria. It is likely that with further research, there may be more categories identified [6,10]. While there has been a considerable increase in the number of studies conducted investigating the effectiveness of probiotics, many

areas of how probiotics function remain unclear. More research must be conducted in order to more fully comprehend the mechanisms and effects that probiotics exert on their human hosts. It is elemental to ascertain any adverse effects of probiotic use. Mutagenicity and toxigenicity of probiotics need to be addressed; and the probiotic's potential to contribute to antibiotic resistance is a consequential issue demanding further investigation.

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Figures

1. Reproduced from Ciorbra et al (2012) A gastroenterologist's guide to probiotics. *Clinical Gastroenterology and Hepatology* 2012 10, 960-968 DOI: (10.1016/j.cgh.2012.03.024). Retrieved online from: http://www.cghjournal.org/article/S1542-3565(12)00369-2/abstract

CHAPTER IV

THE COMPUTATIONAL MICROSCOPE IN MOLECULAR BIOLOGY: APPLICATIONS IN INVESTIGATING ANTIBIOTIC RESISTANCE IN CAP (COMBINED ANTIBIOTIC-PROBIOTIC) TREATMENT

4.1 Abstract

For the later part of the 20th century, researchers were limited in how they could observe biomolecules; thus, the data they gleaned was limited. Crystallography and other techniques of that era provided tremendous advantages over their predecessors. However, these techniques–which were advanced at the time–were limited to mostly static views of biomolecules [1]. Although the information gained through these techniques was valuable in terms of biomolecular identity, its limitations in spatial and temporal scales barred an investigation into the more important dynamic properties of these biomolecules, particularly on an atomic level.

It is the dynamic characteristics of these biomolecules that determines their functions. Recent advances in medical technology have allowed researchers to observe these structures in spatial and temporal scales. The computational microscope allows observations–through molecular dynamic (MD) simulations–that were nearly impossible around the turn of the 21st century; those calculations took an inordinate amount of time, were limited by computing power, or some were, indeed, impossible. With this advanced technology in medicine, it is now possible and practical to investigate these new frontiers [2]. One such frontier is investigating antibiotic resistance due combined antibiotic-probiotic (CAP) therapy.

4.2 Keywords

advanced technology, antibiotic resistance, computational microscope, crystallography, MD simulation, microbiome, probiotic, spatial, temporal

4.3 Abbreviations

AAD: antibiotic-associated diarrhea; CAP: combined antibiotic-probiotic; MD: molecular dynamics

4.4 Introduction: Limitations on Researching the Heretofore Hidden Realm of the Gut

Until recently, biomolecular mechanisms, at spatial and temporal scales, have been difficult to observe experimentally. This has resulted in the slow progress in understanding how microscopic living organisms function and interact at the cellular and atomic level. One such organism is the probiotic, an integral factor in treating human dysbiosis and maintaining intrinsic microbial balance and normal physiology in the human gut. Probiotics have become recognized as playing a beneficial role in gut homeostasis. They are being more frequently prescribed by physicians for use in conditions such as antibiotic-associated diarrhea (AAD) [3]. They are being used by an increasing number of consumers. However, relatively little is known about how they act and interact on a cellular level with elements of the human microbiome, not just in health but in disease. Perhaps of even more importance is that little is known–or being investigated–about any possible harmful side effects of probiotics in certain applications, such as CAP use in

patients. The incidence of probiotic use during antibiotic treatment is increasing and may soon be considered “the standard of care” when prescribing antibiotics.

But what is really happening when these two forces meet, the antibiotic and the probiotic? Do they cooperate, or do they clash? If the latter, what may be the spoils of such a “war” that plays out on the battlefield of the intestinal flora on the plains of the human gut when probiotics and antibiotics are consumed together? Surprisingly, we do not know much about this; and specific research on this subject is notably sparse. Yet, ingesting probiotics in some form or another, during a course of antibiotics, is becoming commonplace. How can we better understand this interaction? What advanced medical technologies can help uncover these secrets?

4.5 Discussion

The sluggish development in understanding how the probiotic acts in isolation and how probiotics and antibiotics react together (in harmony or disharmony)–if they act on each other at all–in the human microbiome is certainly not due to the lack of interest in probiotics.

4.5.1 Consumer Interest in Probiotics Outpaces Scientific Basis

In the United States alone the probiotic industry was on track to hit $42 billion in 2016 [4]. Production and sales of probiotic products are at a all-time high in the United States, and will likely mover higher. But why has the interest in probiotics far outpaced the scientific understanding of their use; and, more importantly, why has interest in (and use of) this “friendly” bacteria far outpaced investigation into conceivable and perhaps inherently “unfriendly” or

inimical corollaries to their use? It is due, in part, to the limitations in the technology of systematically investigating the probiotic, as well as the antibiotic, at spacial and temporal scales that would provide practical data. It is partly due, then, to the deferred development of medical technology for this field of research.

4.5.2 Technological Limitations in Understanding Microorganisms

The following analogy may help make the foregoing “deferred development”statement more clear. Imagine watching a live football match, a world-class ballet, the annual wildebeest migration, the breaching of a humpback whale or the simple act of someone walking across a street. And imagine taking taking still photographs as the event unfolds, and also taking a video of that same event. Which media–the still image or the video–would be more realistic, more representative? Which would render more practical data, especially considering that the video can be paused and analyzed? Obviously, the video would provide exponentially more data. The video is simply a series of still images taken at a much shorter time interval. Medical technologies of the later half of the 20th century were limited in the quantity, quality and practicality of information they could provide regarding microorganisms. These technologies acted more like taking a snapshot with too much time in between images; not enough time to better understand the actions and reactions of elements of the microbiota. They could not provide sufficient information on how a probiotic may react to or interact with an antibiotic, and vice versa. This is a fundamental reason why progress had been delayed. It was due, in part, to the relatively slow evolution of medical technology in this field over the last century.

4.5.3 Comparative Evolution of Two Breakthrough Discoveries

How deferred has this progress been? Let us take a brief walk back through time, to the discovery of the probiotic. The year was 1907. The place was Bulgaria. The person was Elie Metchnikoff, Russian scientist and Nobel Prize winner of the Pasteur Institute in Paris. Metchnikoff's curiosity regarding the longevity of villagers living in the Causcasus Mountains led to this discovery. The villagers drank a fermented yogurt drink on a daily basis; a drink that he discovered contained a probiotic, called *Lactobacillus bulgaricus,* which improved their health and may have helped in their longevity [5]. To put this date in context, four years earlier, Orville and Wilbur Wright–one a high school graduate, one a high school dropout–working out of a bicycle repair shop, made the first controlled, sustained flight of a powered, heavier-than-air aircraft on December 17, 1903 four miles south of Kitty Hawk, North Carolina, USA [6]. Almost unimaginable advances have been made with the discovery of those two high school-educated inventors. A lot happened in aviation during the next 100 years; now, commercial flights, space flights, and soon, perhaps, commercial space flights will be the norm. By comparison, very little has happened with the discovery of that Nobel prize winning researcher. Very little has changed in probiotics during that 100+ years; except now, it seems, probiotics are flying off the shelves of natural food stores, vitamins shops and over-the-counter. Basically, all that has changed is how probiotics are produced, packaged, promoted and consumed; and this has all been due, in part, to technological limitations for research and development. While aviation technology took off, ways to investigate and understand probiotics stalled–until now.

4.5.4 The Computation Microscope; Molecular Dynamic (MD) Simulation

Let us continue our walk, this time in the present, and perhaps take a peek into the future of advanced technology in medicine. In the 2012 *Annual Review of Biophysics*, Dror, et al. wrote:

> Molecular dynamics simulations capture the behavior of biological macromolecules in full atomic detail, but their computational demands, combined with the challenge of appropriately modeling the relevant physics, have historically restricted their length and accuracy. Dramatic recent improvements in achievable simulation speed and the underlying physical models have enabled atomic-level simulations on timescales as long as milliseconds that capture key biochemical processes such as protein folding, drug binding, membrane transport, and the conformational changes critical to protein function. Such simulation may serve as a computational microscope, revealing biomolecular mechanisms at spatial and temporal scales that are difficult to observe experimentally [1,2].

State-of-the-art atomic level biomolecular simulation is a super microscope that will allow researchers to better investigate and understand the probiotic and reveal more of its mysteries and its interactions with other biomolecules and drugs [7]. This should allow medicine to maximize the life-enhancing and beneficial effects of probiotics while avoiding or minimizing any adverse effects.

Crystallography and other techniques of the past fifty years have given scientists advantages in studying structural biology, such as proteins and nucleic acids. However, these molecules are protean in nature and their motions and configurational changes are crucial to their functions. These earlier methods of analysis, although valuable, are limited in the information that they can provide. They provide static properties of biomolecules from which inferences can be drawn; but little in the way of observing the dynamics of how they may interact with other biomolecules, how a biomolecule would interact with a hormone or drug, or how a particular probiotic may interact with a particular antibiotic. Simulation technology can bridge this research gulf, but simulation has been limited by computational expense and the headwind of developing appropriate physical models. Molecular dynamics (MD) has made notable progress in addressing these limitations over the past few years. Whereas, it used to take months to do complex simulations (with extreme simulations being unable to perform), now the timescales have improved by several orders of magnitude. In addition, recent improvements in MD simulation have far outpaced Moore's law (see Table 4.1) [1,2]. The technical reasons for these advances are beyond the scope and intent of this chapter. However, to abridge the technical reasons: the innovations in hardware, software and algorithms are contributive.

MD simulation can be used as a tool in molecular biology to observe conformational changes, membrane transport, protein folding and ligand binding. Recent advances in MD simulation methodology include accessing longer timescales, enhanced sampling and coarse-graining, and improving force field accuracy (see Table 4.1 and Table 4.2) [1,2].

4.5.5 Accessing Longer Timescales

Table 4.1 Illustrates the recent advances allowing longer timescales in MD simulation–far outpacing Moore's Law.

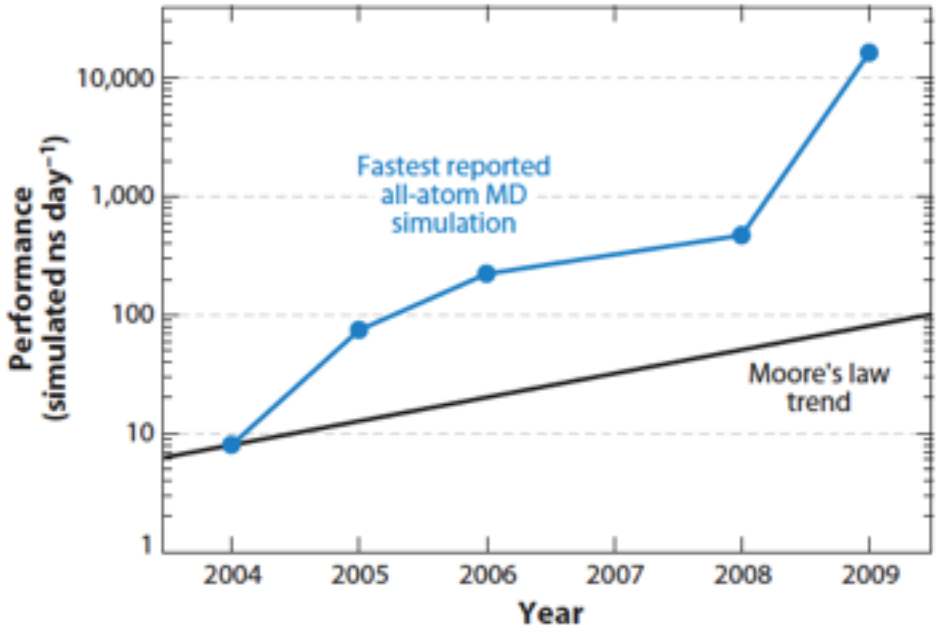


Table 4.2 Longest reported all-atom molecular dynamics simulations from 2006 to 2009. Demonstrates how far MD simulation technology has come in accessing longer timescales.

Year	Length (μs)	Protein	Platform	Reference
2006	2	Rhodopsin	Blue Gene/L[a]	54
2007	2	Villin HP-35	GROMACS[b]	22
2008	10	WW domain	NAMD[b]	27
2009	1,031	BPTI	Anton	82

[a]This simulation used IBM's Blue Matter software.
[b]These simulations were performed on a commodity computer cluster with the specified software.

4.5.6 Enhanced Sampling and Course-Grading

Enhanced sampling and course-grading are methods that enhance MD simulation by circumventing communication jams in enhanced sampling by running numerous short simulations in parallel, and by sacrificing some accuracy for the sake of expediency in the case of the course-grading.

4.5.7 Improving Force Field Accuracy

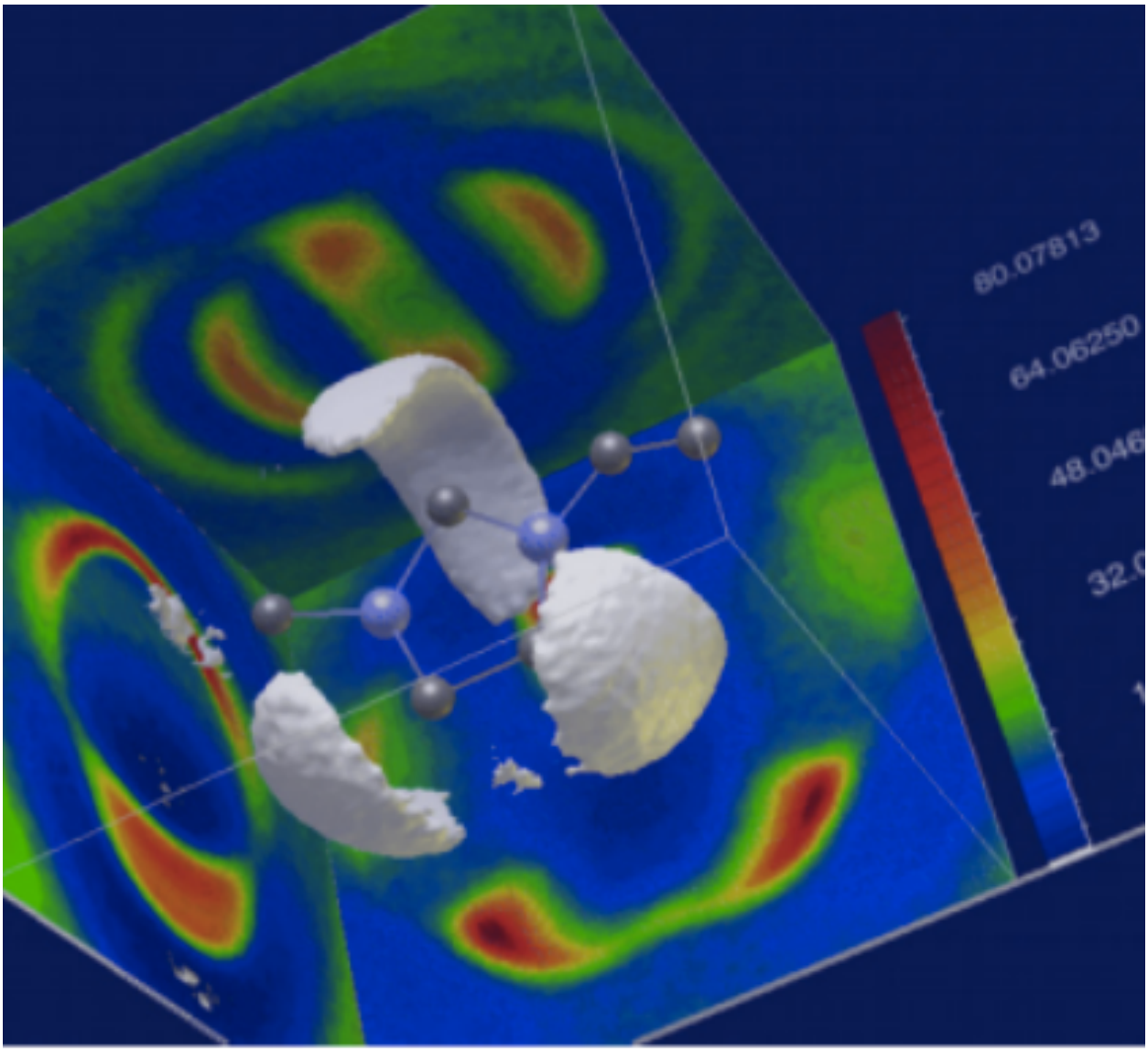
According to researcher M.A. Gonzalez: “A force field is a mathematical expression describing the dependence of the energy of a system on the coordinates of its particles [9].” Force fields are fundamental in MD simulation. Force fields are fickle, however, in that they may prove adequate in shorter simulations; they may be inadequate in scrutinizing longer timescales. Although primary force field models remain in use, they have been adjusted and criteria added.

4.6 Conclusion

MD simulation in all of its complexity can be simply termed, a computational microscope. The new generation computational microscope with the recent enhancements in MD stimulation are allowing scientists and medical researchers to observe the actions and interactions of biomolecules on the atomic level in spacial and temporal scales of several orders of magnitude over its predecessors. This enhanced simulation of the computational microscope may herald a new era in drug and protein design. Scientists will be able to study drug interactions on scales unimagined at the turn of the 21st century. One very practical application would be to

run enhanced MD simulations to investigate the interaction of specific probiotics with specific antibiotics to foretell of any long-term hazards of antibiotic resistance in humans [8]. See *The Magic of MD Simulation* (Figure 4.1).

Figure 4.1. Spatial radial distribution showing the distribution of Br anions around the imidazolium cation 1-ethyl-3methylimidazolium



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Figures

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CHAPTER V

COMBINING ANTIBIOTICS AND PROBIOTICS

5.1 Abstract

Antibiotics are widely used despite their adverse effects, sometime severe, on the human intestinal flora. Probiotics have become a popular answer to some of the side effects of antibiotics. Thus, it should be considered how antibiotics and probiotics interact with each other. While, in many cases, antibiotics and probiotics can be used together, further research should be performed to grasp and mitigate any potential problems. It is possible, with additional understanding, that probiotics could replace antibiotics for certain conditions. Probiotics have effects (both good and bad) on other medications and vaccines as well. Perhaps these beneficial effects could lead to furthering the possibility of probiotic use with other medicines in the future.

5.2 Keywords

antibiotics, efficacy, microbiome, pH, probiotics, vaccines

5.3 Abbreviations

AAD: antibiotic-associated diarrhea; CAP: combined antibiotic-probiotic

5.4 Introduction: The Effects Antibiotics and Probiotics Have on Each Other

The focus of most reviews and studies about antibiotics and probiotics are in relation to the effects that are experienced by the host; whether with regard to the immune system, the human microbiome, the brain, the bladder, or one of the other many systems that antibiotics and probiotics affect. However, in this review, the effects that will be considered are those that antibiotics and probiotics have on each other. This facet has not been considered much, other than brief mentions in clinical research or reviews of other topics. Considering the intrinsic properties that both antibiotics and probiotics have, it seems compelling to study and discern how those properties interact. Once that is determined, it would be constructive to speculate on ways for these effects to be mitigated or enhanced, depending whether or not the effect is negative or positive, respectively. It is also necessary to take into account the effect of probiotics on other medications. Antibiotic drug interactions are well-known and have been extensively studied. It is sensible that drug interaction studies should take place with probiotic strains (and combinations thereof), especially if probiotics become more prominent in the medical management of illness or disease.

5.5 Discussion

While some of the combined effects appear obvious, the theories provided herein depict how subsequent experiments could influence combined antibiotic-probiotic (CAP) use. It is important to consider any negative effects that antibiotics and probiotics might have on each other. Some unforeseen adverse effects might require emergency treatment or long-term observation and care. This is also true of any plausible drug interactions that probiotics may have

with other medications. Positive drug interactions should be considered for further experimentation.

5.5.1 The Effects of Antibiotics on Probiotics

Antibiotics serve the purpose of destroying bacteria in order to fight infection; however, antibiotics are not capable of acting with much discretion: both invading bacteria and useful bacteria will be destroyed [1]. Probiotics are living organisms, and are most often a strain of bacteria, which means that probiotics will not be immune to the destructive nature of the antibiotics [1].

If antibiotics can destroy probiotics, how should they be taken concurrently? It is all dependent on proper timing, the (type of) antibiotic being used, the (strain of) probiotic being used, and for how long the course of antibiotics has been prescribed [1]. Antibiotics and probiotics can be taken concurrently as long as there is at least two hours between the dose of antibiotics and the dose of probiotics, and the antibiotics must be administered first [1]. Some healthcare professionals prescribe a high, but safe, dose after the course of antibiotics has been completed to assist the intestinal microbiome recover more quickly from the dysbiosis caused by the antibiotic use [1].

There are many who say that using probiotics alongside antibiotics is the safest course of medical management when prescribing antibiotics. There are a few studies stating that taking antibiotics should only be done in life-threatening conditions, and should not be prescribed as widely as currently seen[1]. While limiting the prescription of antibiotics could be beneficial (in light of antibiotic resistance), it would be a radical reduction if the use of antibiotics was limited

to only critical conditions. A better course of action would be to experiment with certain probiotic strains that could possibly replace certain antibiotics. This would be a practical remedy, and would reduce the number of dysbiosis cases caused by antibiotics.

5.5.2 The Effects of Probiotics on Antibiotics

Recently, probiotics have become more mainstream. The benefits of probiotics are often lauded as the most effective way to do deal with antibiotic-associated diarrhea (AAD), and are the key to returning perturbed intestinal microbiota to a healthy state [2]. Probiotics have several effects on the intestinal microbiome: regulating intestinal motility, releasing of intestine-protecting metabolites, assisting with mucus production, and lowering intestinal pH [3]. It is the lowering of the intestinal pH that allows suppression of pathogenic bacteria, and gives the beneficial bacteria time to replenish allowing the intestinal microbiome to return to homeostasis [4].

The lowering of intestinal pH is an important function of probiotics. Antibiotics can be pH sensitive [5]. The situation is complex as there are several factors that determine pH sensitivity levels. How does an antibiotic react in the presence of certain bacterial strains? Does an antibiotic/bacterial combination work better in a lower pH or a higher pH? Certain antibiotics are diminished in the upward change in pH, while they are enhanced by a fall in pH [6].

The fact that probiotics can have an effect on antibiotics is not cause for concern. Since some antibiotics work better against certain bacteria at a lower pH, it would be beneficial to determine what combinations (of probiotics strains) work best at lowering pH; thus, the efficacy of the antibiotic could be expanded rather than retracted. This is, however, a daunting task as

there are numerous strains of probiotics to consider. There are several strains that are considered common, such as *Bifidobacterium*, *Lactobacillus,* and *Bacillus* [7]. There are also some yeast and fungi probiotics, but those are not seen as frequently as bacterial probiotics. There are vastly more strains of bacterial probiotics [7]. Experimentation could be performed with regard to modifying probiotics by genetic engineering to alter the adverse effects that should be avoided, and/or to remove the pH sensitivities from certain antibiotics.

5.5.3 The Effects of Probiotics on Other Medications

Antibiotics are not the only medication that can be affected by probiotics. While this area of research is considerably underdeveloped, there are some medications, on record, that have drug interactions with probiotics.

5.5.3a Probiotics, Vitamin K and Warfarin

Probiotics have an effect on the ability of the intestinal microbiome to absorb and/or process certain nutrients, such as vitamin K. Vitamin K antagonists, like Warfarin, would be directly affected by this ability being heightened or lessened [7]. If a treatment plan was in place that included both Warfarin and probiotics, it would be necessary to closely monitor the level of Warfarin in the patient's blood as it would be detrimental for it to be off in either direction, too low or too high. Also, if antibiotics were added to that said treatment plan, it might be necessary to increase the dose of probiotics as some antibiotics can cause vitamin K deficiency. Vitamin K deficiency can become quite severe, and lead to hemorrhaging [7].

5.5.3b Probiotics, Antifungals, Immunosuppressants and Chemotherapeutic Agents

Antifungal medications can also be affected. Any probiotic formula containing *S. boulardii* cannot be used in tandem with oral antifungals [7]. It should also be noted that using any type of probiotic while taking immunosuppressants or chemotherapeutic agents is risky because there is a chance the probiotic could cause pathogenic colonization in the host, or cause an infection [7]. As probiotic experimentation moves forward, it can be expected that more interactions will be discovered.

5.5.3c Probiotics and Vaccines

While probiotics can have detrimental drug interactions, they can also be beneficial to certain pharmaceuticals, such as vaccines. Probiotics can complement vaccines, especially those that affect mucosal immunization [8]. More specifically, the vaccine-specific IgG, IgG1 and IgG3 are greatly enhanced by the use of probiotics as an assistive to the delivery of the vaccine [8]. Not everyone reacts the same way to probiotics because of the individual's unique intestinal microbiome. In a study by Rizzardini et al., the vaccine enhancement with two probiotic strains (*Bifidobacterium animalis ssp. lactis*, BB-12® and *Lactobacillus paracasei ssp. paracasei*, L. casei 431®) was not detrimental. Nearly all study participants showed an increase in vaccine effectiveness [9]. This particular study used a common influenza vaccine [9]. The following two tables (Table 5.1 and Table 5.2) show results of additional tests on the effect of probiotics on immune response to mucosally and parenterally administered vaccines in adults.

Table 5.1 Effect of probiotics on immune response to mucosally administered vaccines in adults.

type of vaccine(s)	probiotic(s), location	study design	outcomes
oral cholera vaccine Dukoral®	five *Lactobacillus* strains, two *Bifidobacterium* strains, France	healthy adults assigned to one of seven probiotics (n = 9 for each) or placebo (n = 20) for 21 days; vaccination on days 7 and 14	significantly higher vaccine-specific serum IgG antibodies with *B. lactis* Bl-04 and *L. acidophilus* La-14 (p = 0.01)
oral *Salmonella* Typhi Ty21a vaccine	*Lactobacillus* GG or *Lactococcus lactis*, Finland	healthy adult volunteers receiving LGG (n = 10), *L. lactis* (n = 10) or placebo (n = 9) for seven days; vaccination on days 1, 3 and 5	no difference in vaccine-specific IgA, IgG or IgM antibody secreting cells, Trend for higher vaccine-specific IgA
OPV 1–3	*Lactobacillus* GG, *Lactobacillus paracasei*, Germany	healthy males given LGG (n = 21), *L. paracasei* (n = 21) or no (n = 22) for five weeks; vaccination on day 8	significant increase in neutralizing antibodies and poliovirus-specific IgA titre (p < 0.036)
nasal attenuated trivalent influenza vaccine for 2007/2008	LGG and inulin, USA	healthy adults given probiotic (n = 21) or placebo (n = 21) for 28 days after vaccination	increased seroprotection rate to the H3N2 strain at day 28 (p = 0.048), but not to the H1N1 or B strain no effect on seroconversion at day 56

Table 5.2 Effect of probiotics on immune response on parenterally administered vaccines in adults.

vaccine(s)	probiotics, location	study design	outcomes
parenteral inactivated trivalent influenza vaccine for 2004/2005	*Lactobacillus fermentum*, Spain	healthy adults given probiotic (n = 25) or placebo (n = 25) for four weeks; vaccination on day 14	probiotic increased vaccine-specific IgA antibodies post-vaccination (p < 0.05), influenza-like illnesses lower for five months
parenteral attenuated trivalent influenza vaccine for 2008/2009	*Bifidobacterium lactis* (BB-12®) or *Lactobacillus paracasei*, Italy	healthy adults given probiotic (n = 53 for BB-12®, n = 56 for *L. casei* 431®) or placebo (n = 102) for six weeks; vaccination at week 2	increase in vaccine-specific IgG antibodies (p < 0.001), vaccine-specific secretory IgA antibody in saliva in BB-12® p = 0.035 and *L. casei* p = 0.017
parenteral trivalent influenza vaccine and PCV-23	*Lactobacillus paracasei* and prebiotic, Chile	elderly subjects (greater than or equal to 70 years) given either probiotic and prebiotic (n = 30) for six months or no supplement (n = 30); vaccination after four months	no effect on antibody response to vaccines, NK activity increases lower incidence of infection after 12 months, in particular respiratory illnesses (p = 0.034)
parenteral trivalent influenza vaccine 2004–2005, 2006–2007	*Lactobacillus paracasei*, France	pilot study: probiotic (n = 44) or placebo (n = 42) for seven weeks main study: probiotic (n = 113) or placebo (n = 109) for 13 weeks. vaccination after four weeks	higher seroconversion rate for B strain in main study at three, six and nine weeks post-vaccination in probiotic versus placebo group (p = 0.02)
parenteral trivalent influenza vaccine 2006–2007	*Lactobacillus plantarum*, Spain	elderly (65–85 years) given two doses of probiotic or placebo (n = 20 each) three months AFTER vaccination for three months	increased influenza-specific IgA (p = 0.008) and IgG (p = 0.023) antibodies
parenteral trivalent influenza vaccine	MoLac, heat-killed *Lactobacillus casei*, Japan	elderly given probiotic (n = 8) or placebo (n = 7) for 12 weeks	no difference

5.6 Conclusion; Probiotics and the Future

Probiotics have a prodigious future potential, but it is necessary to ensure that proper testing is performed, and regulations put in place to protect patients. Strain identification is a priority. It is essential that there is a standard testing method in place. Results need to be uniform and reproducible [10]. Considering the effects that antibiotics and probiotics have on each other (and their growing combined use), it seems evident to consider CAP investigation a priority. Also to be considered are the effects of probiotics on other medications. These steps will enjoin probiotics as an adjunct to future medical prescriptions.

As said, the research on antibiotic-to-probiotic effects, probiotic-to-antibiotic effects, and probiotic-to-medication effects should be investigated in more depth. Further studies might reveal additional benefits or detriments that could shape the way forward more specifically, more dramatically and more quickly. The vaccine-specific effects of probiotics should be tested on more vaccines with more strains of probiotics to confirm if enhanced immunity is, indeed, a general property of probiotics, or if is it only specific to certain probiotics.

It would be a boon if research went further in the testing of replacing some antibiotics with specific strains of probiotics, where possible; thus, putting less stress on the patient and eliminating a cause of dysbiosis.

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CHAPTER VI

COMPARISON OF THE HUMAN MICROBIOME AFTER ANTIBIOTIC TREATMENT: WITHOUT PROBIOTIC SUPPLEMENTATION; WITH PROBIOTIC SUPPLEMENTATION

6.1 Abstract

This comparison evaluates the human microbiome and how it is affected by antibiotics alone and antibiotics combined with probiotics. General background of the human microbiome is provided for a frame of reference as well as different aspects of the microbiome defined. From this basis, the effects of antibiotics, both positive and negative, are evaluated. The human microbiome is complex, and any alteration to it only compounds that complexity. Probiotics are discussed in detail proceeding with an understanding of the human microbiome and the role played by probiotics on that microbiome, The effects of probiotics on the microbiome are evaluated.

Probiotics are capable of several pathways of treatment–especially because probiotics can be genetically engineered to be more effective and more precise. Probiotics are capable of communicating with different types of cells, thus many treatment options should be available. Investigating antibiotics and probiotics separately allows for a more detailed analysis of how using antibiotics and probiotics together will affect the human microbiome. It is important to note that this investigation and discussion is limited to the actual clinical use of probiotics, and does not contain references from general “public knowledge” or anecdotal evidence. Finally, the concept of target-specific treatment and further research ideas are provided.

6.2 Keywords

antibiotics, archaea, dysbiosis, genera, human microbiome, pathogen colonization, probiotics, taxa, taxon, vitamins

6.3 Abbreviations

AAD: antibiotic-associated diarrhea; GI: gastrointestinal; IBD: inflammatory bowel disease

6.4 Introduction: The Unique-Unique Microbiome and Its Functions

Individuals within the human population share many things in common, and it might be expected that the human microbiome would be one of those shared traits. However, studies have shown that there is a uniqueness to the microbiome of each individual [1]. Each individual has unique traits that comprise the configuration of the microbiome; no taxa are universally found in subjects' microbiomes [1]. In fact, the host has a direct influence on the composition of the human microbiome [2]. The microbiome is unique among organ systems in the human body, and it is unique to each individual; thus, it is characterized herein as being unique-unique.

The importance of the human microbiome to a person's health continues to expand as medical research moves forward. Not only does it play a necessary role in regulating inflammation and balancing immunity, but it plays a critical role in fat storage and metabolism. This could have major implications in obesity and type 2 diabetes [3]. Research also shows that the intestinal microbiome works with the brain in a highly complicated system of interactions

with the enteric nervous system, the autonomic nervous system and pituitary hormones [3]. Thus, the microbiome has the ability to affect nerve pathways and function as well as behavior [3]. Individuals also use a healthy microbiome for proper nutrition, and to resist colonization by foreign pathogens [4]. This same microbiome is also responsible for the production of essential vitamins, such as vitamin B12, folic acid and vitamin K [2]. Dysbiosis of the microbiome can result in malnutrition, and the development of such is impacted by the perturbation of the microbiome.

The human microbiome is comprised predominantly of bacteria, but it also has archaea species in the composition of flora [2]. Studies have shown that an individual's microbiome carries an average of 540,000 microbial genes, and fluctuations of these genes results in changes in human health [3]. Accounting for all possible human intestinal microbiota, there is an estimated 3.3 million microbial genes that could possibly be present. The human genome has roughly 150 times less genes than that [2]. According to estimates, there are at least one thousand species level phylotypes that can be found in the intestinal microbiome [2]. The metabolic interaction between the host and the microbes is beneficial to both parties [2].

There are several physiochemical factors that influence the configuration of the human microbiome, such as pH, nutrient supplies, intestinal motility, host secretions, an undamaged ileocecal valve, and redox potential [2]. Disturbing this complex system of gastrointestinal flora can cause a multitude of conditions from chronic disorders to infectious diseases [5]. It is speculated that dysbiosis is directly involved in inflammatory bowel disease (IBD), obesity, colorectal cancer, celiac disease, necrotizing enterocolitis and pouchitis [2].

Antibiotics are known to have a negative effect on the human microbiome, so it is essential to mitigate these effects whenever possible.

6.5 Discussion

Antibiotics effect on the intestinal flora is considered as well as how probiotics affect this system. The effects of antibiotics are evaluated, and the significance of those effects are discussed. The concerns should be tempered by the need antibiotics fill. It should be noted that some antibiotics may have already lost usefulness for many people due to the aggressive dysbiosis those antibiotics cause.

The effects of the probiotics are evaluated, and it is discussed whether or not those effects merit the use of probiotics in conjunction with antibiotics. Not all probiotics are created equal, and probiotic compounds allow for numerous variable configurations. It is necessary to consider which probiotics have the greatest viability as well as what combinations of probiotics are viable as components of compounds. Also, there is a need to address regulations and standard formulae, and education of healthcare professionals.

6.5.1 The Effects of Antibiotics on the Human Microbiome

The use of antibiotics for the elimination and suppression of pathogens is a time-tested and verified method in certain medical conditions [6]. Antibiotics, while beneficial in that they can destroy pathogens, cannot be limited in scope: they also target the intestinal microbiome [2]. This results in dysbiosis of the microbiome which leads to causatum, such as antibiotic-

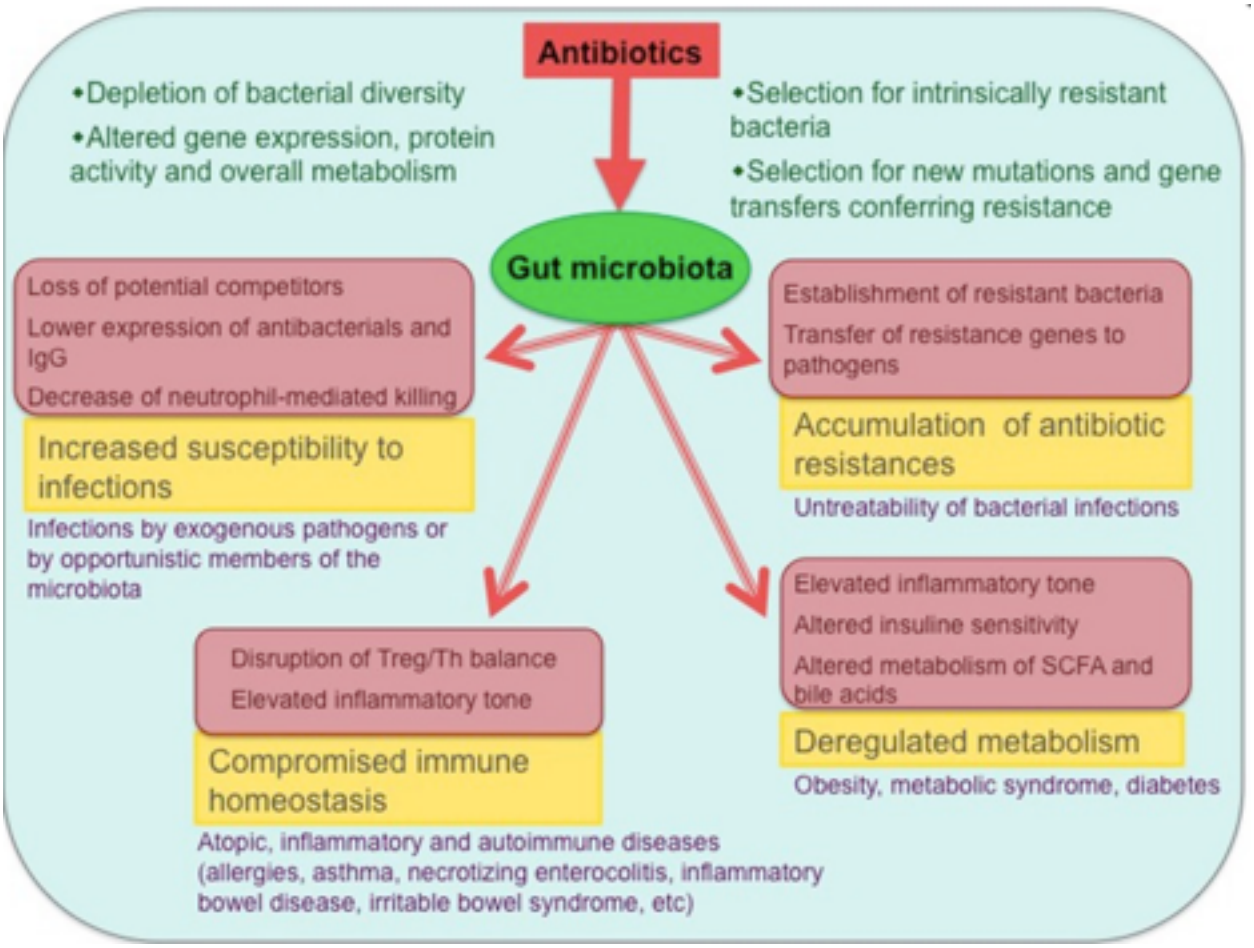
associated diarrhea (AAD) and a proliferation of *Clostridium difficil*e [2]. Most often the *Coriobacteriaceae, Bifidobateriaceae, Oxalobacterceae, Eubacteriaceae, Veillonellaceaem* and *Pasteurellaceae* bacterial families are decreased significantly when the microbiome is exposed to antibiotics [7]. While the dysbiosis of the intestinal microbiome is often temporary, studies have shown that long-term dysbiosis has become more common, especially with frequent antibiotic use [2]. The type of antibiotic used for pathogen suppression directly affects the length of time the intestinal microbiome requires to rebound from the antibiotic use [5]. Considerable numbers of antibiotics can permanently alter the healthy microbiome by depleting taxa [8].

Another matter associated with widespread antibiotic use is a resultant increase in antibiotic resistance inside of the microbiome [2]. Antibiotic resistance in pathogens is estimated to be the cause of 50,000 deaths in the United States and Europe every year. This suggests that antibiotics might become completely ineffective treatments by 2050 [8].

Some of the effects of antibiotics on the gut microbiota are depicted in more detail in Figure 6.1. Some listed effects are:

- depletion of bacterial diversity;
- altered gene expression, protein activity and overall metabolism;
- increased susceptibility to infections;
- compromised immune homeostasis;
- accumulation of antibiotic resistance; and
- deregulated metabolism.

Figure 6.1 Antibiotic effects on the gut microbiota and associated health problems.



Colonization resistance is a key function of the native gut microbiome, and this resistance is the ability to prevent pathogen colonization within the microbiome [9]. It is not specifically known why antibiotics weaken the colonization resistance of the native microbiota, but it is postulated that this is a reflection of how the ecology, immunology, and metabolism changes in response to the presence of those antibiotics [9]. In addition to colonization resistance, the human microbiome is also responsible for the generation of antimicrobial composites and the maintenance of the gastrointestinal (GI) motor and sensory functions and the mucosal immune barrier [2]. Interference by antibiotics can cause the microbiome to ineffectively interact with

the host's immune system which results in immunological disorders [8]. There are many different types of antibiotics derived from various natural sources which directly affect what side effects occur and what flora are affected.

6.5.2 The Effects of Probiotics on the Human Microbiome

Probiotics are capable of manipulating the microbiome composition within the intestinal tract [2]. Two of the most common probiotic genera are *Lactobacillus* bacteria and *Bifidobacterium* bacteria which are used as effective probiotics due to the large quantity of species and strains [2]. Probiotics are effective because they can act directly within the intestinal microbiome, are capable of influencing the mucosal immune system and barrier function, and have a positive effect on systemic immunity and other organs outside of the GI tract [2]. Additionally, probiotics can code sensors for biomarkers of specific pathogens and, in essence, "sense and destroy" pathogens specifically without damaging other natural flora of the microbiome [8]. Studies have also shown that modification of the microbiome can allow therapeutic manipulation of immune disorders in the host [10]. The use of probiotics also shows viability in the upregulation of anti-inflammatory factors, and in the suppression of proinflammatory factors [6]. This is particularly effective with regard to autoimmune disorders and attempting to mitigate the inflammation. However, probiotics have shown little impact or benefit to an already healthy intestinal microbiome [5]. Probiotics are most effective in helping the human microbiome recover from a reduction or disruption of the normal, native flora.

In order for probiotics to become standard medical treatment, regulations for the study of probiotics and reporting of study results is needed [3]. Probiotic packaging is often inaccurate in listing the actual bacteria contained within the probiotic [5]. Probiotic strains have specific, individual properties, and need to be evaluated with precision. Even strains within the same species can have different effects on the intestinal microbiome [2]. Targeted approaches to combining various probiotic strains into mixtures opens up the opportunity for synergistic effects and improved efficacy. This also allows for more health benefits within one probiotic mixture [2]. Due to the advantages gained by genetic engineering, researchers are evaluating augmentation of the natural strains to design better products than the original strains [8]. This could streamline the recovery of the microbiome from dysbiosis. There needs to be more educational materials provided to healthcare providers in the correct use of probiotics, and documentation from regulatory authorities with regard to safety, use, and benefits of probiotics to the patient [3].

6.6 Conclusion; Using a Combination of Antibiotics and Probiotics

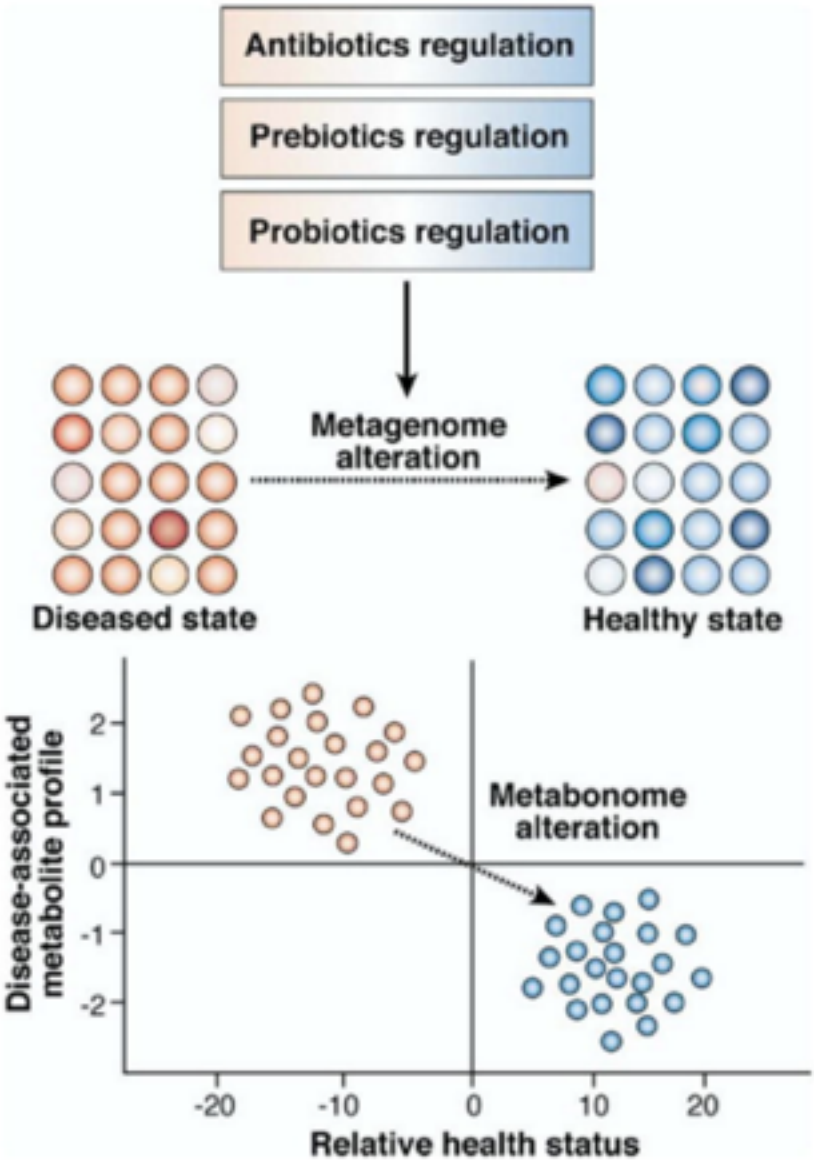
Probiotics create an avenue to communicate with many different types of cells [10]. Using probiotics can modulate the intestinal microbiome in a biological manner [2]. Mechanisms of beneficial probiotic action described, to date, include: facilitating adhesion to the intestinal-lumen interface, developing competition with pathogens for receptor binding, augmenting nutrition and colonization, enhancing the mucosal barrier function, promoting the innate and adaptive immune responses, stimulating the production of bacteriocins, and modulating cell kinetics. Further mechanisms of action are likely to be identified [11]. Probiotics help the

microbiome to induce heat shock protein and antimicrobial production, and can increase mucin production in the intestinal microbiome [10]. Probiotics also assist with the restoration of a healthy microbiome composition causing a counterbalance to the dysbiosis that will help to restore overall health [2]. It is important to note that probiotics and antibiotics need to be administered at least two hours apart to prevent the antibiotics from lessening the effect of the probiotics [12]. Otherwise, antibiotics would kill a majority of the bacteria in the probiotic compound rendering the probiotics minimally effective or completely ineffective.

The impact of antibiotics is affected by the initial composition of the intestinal microbiome. So, it would be an advantage to be able to target antibiotic treatments with the proper probiotic compound. In essence, this allows for patient-specific treatment that would mitigate complications before they arise [7]. The initial composition of the microbiome also has bearing on the capacity of the infectious agent to interact with and alter the microbiome [13]. Age, body mass index, gender and various additional metadata can be used to build correlating profiles of the initial composition of the microbiome [1].

Future experiments should focus on how the initial composition of the microbiome can be determined prior to antibiotic use. This will help track how certain initial compositions are affected by various antibiotics. Additional testing should occur to determine what strain of probiotics would best fit these initial compositions. Forming custom treatment plans based on initial composition and probiotic compatibility could gain medicine many more years wherein antibiotics remain a viable treatment option. Proceeding along this path may also result in a lessening amount of antibiotic resistant genes in the microbiome. New medical prescriptions could be created to better serve patients.

Figure 6.2 The gut microbiome as a therapeutic target.



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Figures

1. Reproduced from Francino, M.P., Antibiotics and the Human Gut Microbiome: Dysbioses and Accumulation of Resistances. Front Microbiol. 2015; 6: 1543. Published online 2016 Jan 12. Retrieved online from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4709861/

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CHAPTER VII

REVIEW OF ANTIMICROBIAL RESISTANCE PATHWAYS

7.1 Abstract

There are two primary pathways to developing antimicrobial resistance, each of which will be outlined in more detail later in this chapter. Understanding these pathways can help determine if probiotics, indeed, have the potential to participate in pathways to developing antimicrobial resistance in the human microbiome. Antibiotic resistance determinants have been identified and characterized in *Lactobacillus*, *Bifidobacterium* and the probiotic *Bacillus* [1,2]. Agar plate studies with a bacteria lawn of typical over-the-counter strains of probiotics (dietary supplements) layered with antibiotic disks have demonstrated a wide range of antibiotic resistance in various strains of probiotics. Therefore, it is safe to assume that probiotics can confer antibiotic resistance not only to other strains of probiotics, but also to intrinsic microbes of the human gut microbiome. It is incumbent on medical science to further investigate any consequences of combining probiotics and antibiotics, and to establish effective guidelines for their combined use; in particular, which strains of probiotics can be helpful with which classes of antibiotics in diminishing or eliminating adverse effects without potentiating antimicrobial resistance (AMR) in the human microbiome.

7.2 Keywords

acquired resistance, antimicrobial resistance, conjugation, horizontal gene transfer, intrinsic pathway, intrinsic resistance, microbiome, mutation, transduction, transformation

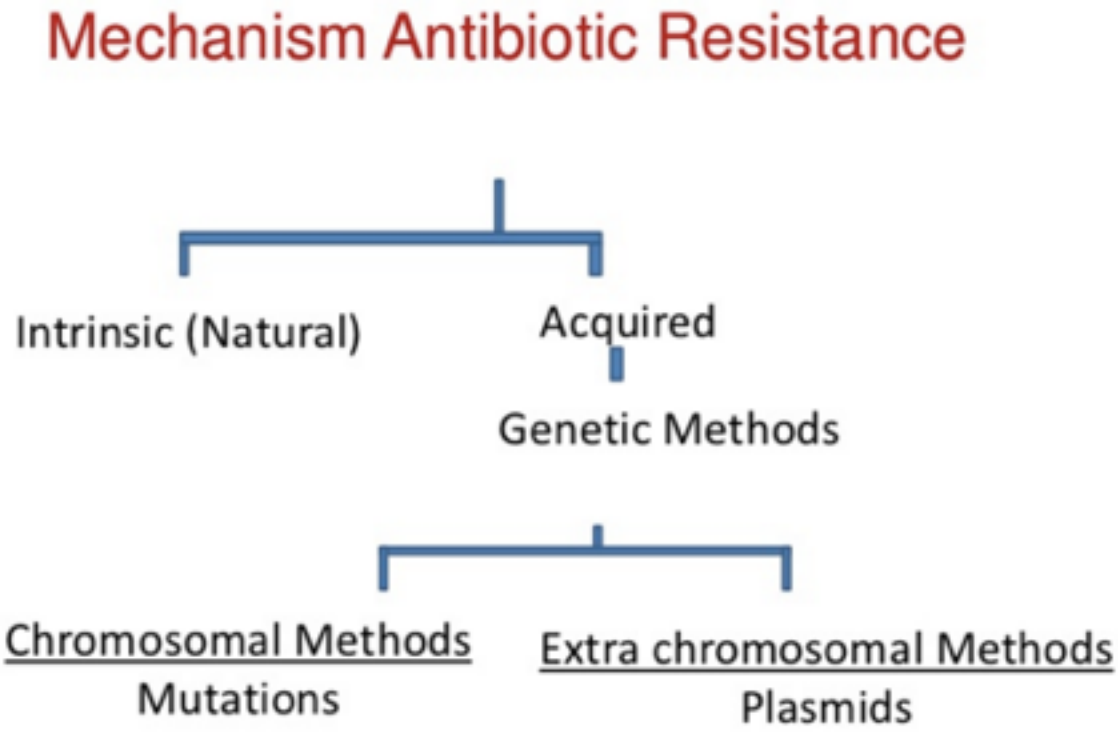
7.3 Abbreviations

AAD: antibiotic-associated diarrhea; AMR: antimicrobial resistance; hAMR: antimicrobial resistance via horizontal gene transfer (AMR via HGT); HGT: horizontal gene transfer; MD: molecular dynamic

7.4 Introduction: Review of Mechanisms in Developing Antimicrobial Resistance (AMR)

Before delving into the possible pathways for transference of antibiotic resistance from the probiotic to the microbiota of the human gut, it is deemed helpful to review the mechanisms of developing antimicrobial resistance (AMR). These are the various strategies that bacterial organisms utilize to contend with antimicrobial compounds. There are two distinct categories (pathways) of AMR: the “intrinsic pathway” and the “acquired pathway.” Surveying these two pathways will establish a foundation of understanding and augment percipience of the potential for horizontal gene transfer (HGT) in antimicrobial resistance (AMR) from the probiotic to the human gut microbiota. The descriptive term “horizontal” (as in horizontal gene transfer) in the context of AMR will be clarified in Section 7.9 and, therein, defined as a subset of the second pathway of acquired (as opposed to intrinsic or hereditary) AMR. Figure 7.1 illustrates antibiotic resistance pathways.

Figure 7.1 Antibiotic Resistance Pathways



7.5 The Intrinsic Pathway Defined

The first pathway of AMR to be discussed is “intrinsic resistance.” Intrinsic resistance is that pathway which is coded and expressed by most all–or all–strains of a bacterial species. In this sense, intrinsic resistance could be considered inherent resistance. Furthermore, intrinsic resistance is the innate ability of a bacterial species to resist activity of a particular antimicrobial agent through its inherent structural or functional characteristics which allow tolerance of a particular drug or antimicrobial class. This can also be called “insensitivity” since it occurs in organisms that have never been susceptible to that particular drug [3].

7.5.1 The Intrinsic Pathway Process

To survive in the presence of an antibiotic, bacterial organisms must be able to disrupt one or more of the essential steps required for the effective action of the antimicrobial agent. The intended modes of action of antibiotics may be counteracted by bacterial organisms via several different means. This may involve preventing antibiotic access into the bacterial cell or perhaps removal, or even degradation, of the active component of the antimicrobial agent. No single mechanism of resistance is considered responsible for the observed resistance in a bacterial organism. In fact, several different mechanisms may work together to confer resistance to a single antimicrobial agent [4].

7.5.2 The Intrinsic Pathway Mechanisms

Intrinsic resistance can occur due to the following mechanisms (singularly or in combination):

- preventing the antimicrobial agent from reaching its target by reducing its ability to penetrate the cell (inability of the drug to access the bacterial cell),
- expulsion of the antimicrobial agents from the cell via efflux pumps (expulsion of the drug by active exporters),
- inactivation of the antimicrobial agent via modification or degradation (the innate production of enzymes that inactivate the drug), and
- modification of the antimicrobial target within the bacteria (lack of affinity of the drug for the bacterial target).

Figure 7.2 Examples of mechanisms of antibiotic resistance

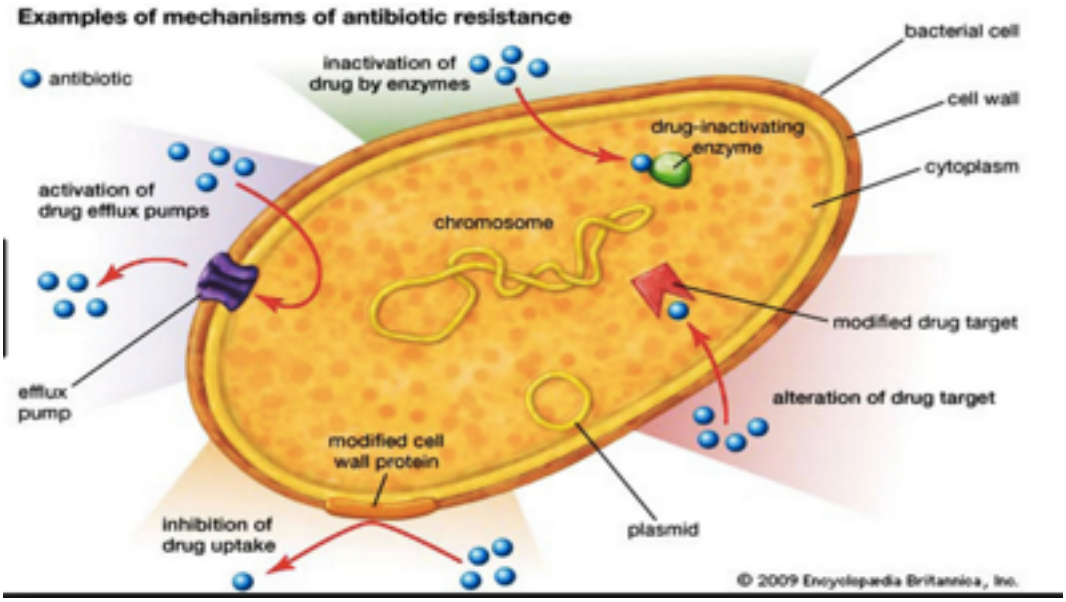


Table 7.1 Examples of intrinsic resistance and their respective mechanisms

ORGANISMS	NATURAL RESISTANCE AGAINST:	MECHANISM
Anaerobic bacteria	Aminoglycosides	Lack of oxidative metabolism to drive uptake of aminoglycosides
Aerobic bacteria	Metronidazole	Inability to anaerobically reduce drug to its active form
Gram-positive bacteria	Aztreonam (a beta-lactam)	Lack of penicillin binding proteins (PBPs) that bind and are inhibited by this beta lactam antibiotic
Gram-negative bacteria	Vancomycin	Lack of uptake resulting from inability of vancomycin to penetrate outer membrane
Klebsiella spp.	Ampicillin (a beta-lactam)	Production of enzymes (beta-lactamases) that destroy ampicillin before the drug can reach the PBP targets
Stenotrophomonas. maltophila	Imipenem (a beta-lactam)	Production of enzymes (beta lactamases) that destroy imipenem before the drug can reach the PBP targets.
Lactobacilli and *Leuconostoc*	Vancomycin	Lack of appropriate cell wall precursor target to allow vancomycin to bind and inhibit cell wall synthesis
Pseudomonas aeruginosa	Sulfonamides, trimethoprim, tetracycline, or chloramphenicol	Lack of uptake resulting from inability of antibiotics to achieve effective intracellular concentrations
Enterococci	Aminoglycosides	Lack of sufficient oxidative metabolism to drive uptake of aminoglycosides
	All cephalosporins	Lack of PBPs that effectively bind and are inhibited by these beta lactam antibiotics

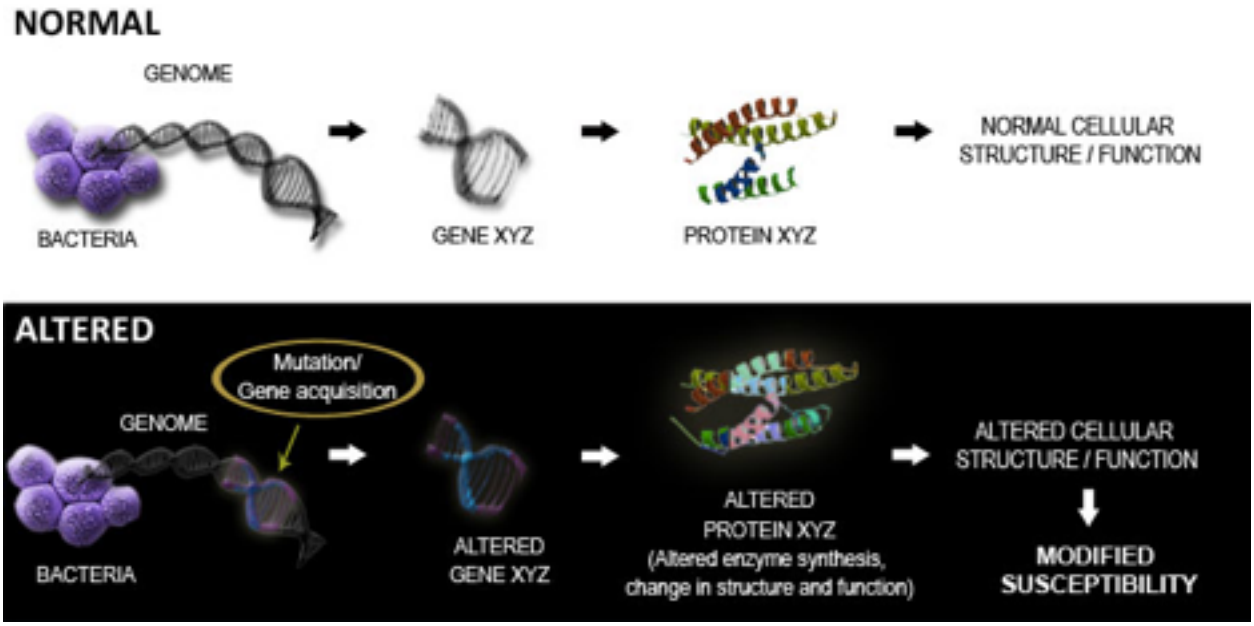
7.6 The Acquired Resistance Pathway Defined

The second pathway of AMR to be discussed is “acquired resistance.” Acquired resistance occurs through mutation or horizontal (from the environment) gene acquisition and may lead to a change in the nature of proteins expressed by the microbe. This can cause a change in the structure and, thus, the function of the microbe which can result in resistance to a specific antibiotic.

7.6.1 Acquired AMR, Subset I: Mutation

A mutation is a spontaneous change in the DNA sequence within the gene that may lead to a change in the trait which it codes for. Any change in a single base pair may lead to a corresponding change in one or more of the amino acids for which it codes. This can then change the enzyme or cell structure that consequently changes the affinity or effective activity of the targeted antimicrobials. These microbial changes can occur simply due to natural selection in replication aberrations (see Figure 7.3).

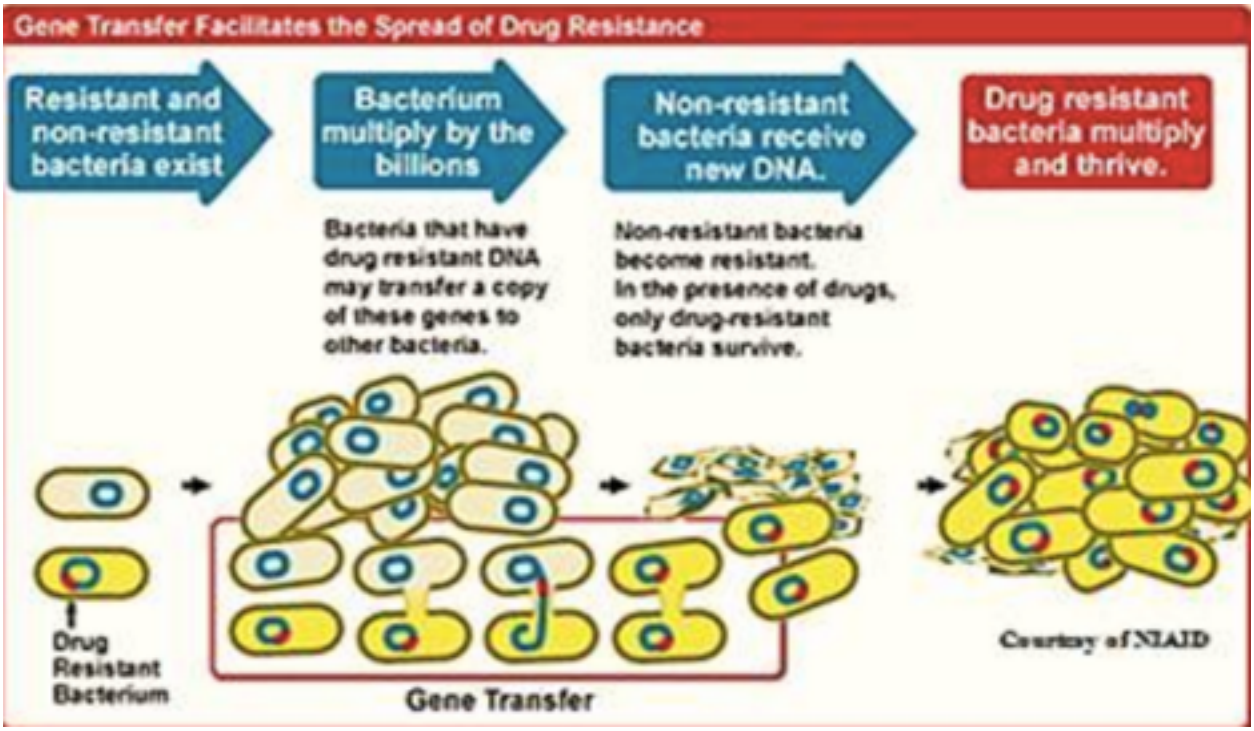
Figure 7.3 Normal and Mutation stages



In 2002, Gillespie reported: "For prokaryotes, there is a constant rate of spontaneous mutation of about 0.0033 mutations per DNA replication that is relatively uniform for a diverse spectrum of organisms. The mutation rate for individual genes varies significantly among and within genes [5]." In the dense and diverse environment of the human microbiome, mutations are extraordinary.

Mutations can also be spawned due to exogenous environmental factors. However, it is important to note that at this time, with limitations of understanding and in available technology, it is difficult to not only identify individual species in the human microbiota but to isolate and study them. Most can only be studied indirectly through their associated enzymes and other correlated pathways.

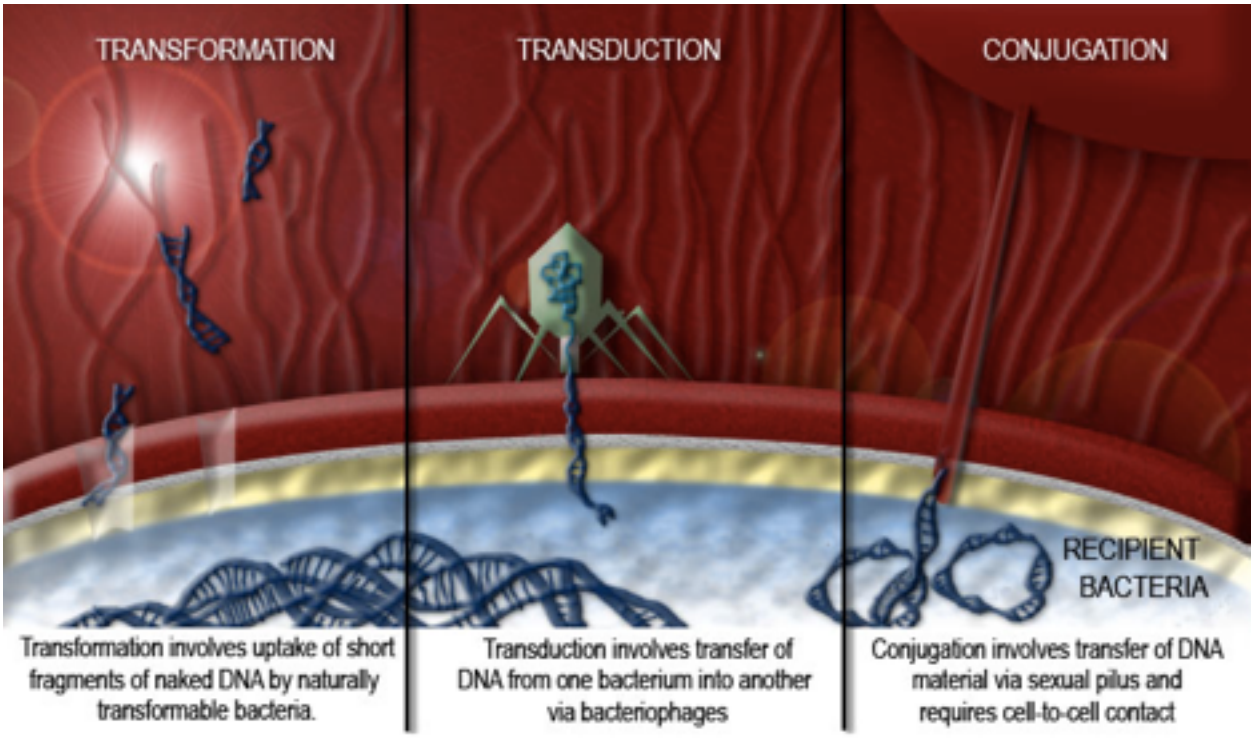
Figure 7.4 Gene Transfer of Drug Resistance



7.6.2 Acquired AMR, Subset II: Horizontal Gene Transfer (HGT)

Horizontal gene transfer (HGT) is another means by which AMR can be acquired. HGT is the process of swapping genetic material between coincident bacteria (and for the purposes of the hypothesis between probiotic and microbiota). HGT can occur by way of three main mechanisms: transformation, transduction or conjugation [6]. Figure 7.5 illustrates the three mechanisms of HGT.

Figure 7.5 Three mechanisms of HGT (horizontal gene transfer)



Conjugation requires cell-to-cell contact, usually via a pilus or pore that forms a channel which allows for the passage of plasmids. The main mechanism of horizontal transfer of plasmids is through conjugation [5]. Transformation occurs from the cell's desire to have greater diversity in order to respond more favorably to natural selection. It involves the uptake of short fragments of histone-free DNA (histones are the "spools" around which DNA winds). Transduction occurs when a bacteriophage (viruses that infect bacteria)–that has previously replicated in another bacterial cell–packages a portion of the host genome (donor) into the phage head and transfers the genes to another (recipient) bacterial cell [8].

Table 7.2 Examples of acquired resistance and their respective mechanisms

ACQUIRED RESISTANCE THROUGH:	RESISTANCE OBSERVED	MECHANISM INVOLVED
Mutations	*Mycobacterium tuberculosis* resistance to rifamycins	Point mutations in the rifampin-binding region of *rpoB*
	Resistance of many clinical isolates to fluoroquinolones	Predominantly mutation of the quinolone-resistance-determining-regiont (QRDR) of GyrA and ParC/GrlA
	E.coli, Hemophilius influenzae resistance to trimethoprim	Mutations in the chromosomal gene specifying dihydrofolate reductase
Horizontal gene transfer	*Staphylococcus aureus* resistance to methicillin (MRSA)	Via acquisition of mecA genes which is on a mobile genetic element called "staphylococcal cassette chromosome" (SCCmec) which codes for penicllin binding proteins (PBPs) that are not sensitive to ß-lactam inhibition
	Resistance of many pathogenic bacteria against sulfonamides	Mediated by the horizontal transfer of foreign *folP* genes or parts of it
	Enterococcus faecium and *E. faecalis* resistance to vancomycin	Via acquisition of one of two related gene clusters VanA and Van B, which code for enzymes that modify peptidoglycan precursor, reducing affinity to vancomycin.

7.7 Summary: Acquired Resistance Via Mutation Versus Acquired Resistance Via HGT

To summarize and simplify the understanding of this second pathway of AMR, acquired AMR, there are two subsets: mutation and HGT.

(Recall that microbes–bacteria–having **intrinsic** resistance are able to "resist" certain antimicrobial agents (one or more) due to the microbe's inherent mechanisms of structure and/or function. These resistant bacteria can be active in exporting antimicrobial agents that have entered them, they can be active in neutralizing the antimicrobial agent which has breeched the

bacterial wall, or they can simply “ignore” the antimicrobial agents which cannot penetrate the bacterial cell wall or affect the structure in any way.)

Microbes can acquire AMR through either of two subset pathways: mutation or HGT.

7.8 Mechanism of Mutation in Acquired Antimicrobial Resistance (AMR)

Mutations in a bacteria can and will occur spontaneously through the ongoing process of aberration and natural selection. These mutations can occur in deference to exogenous factors (simply occurring due to the benefit of rapid mitosis over time), or mutations can be stimulated due to exogenous factors.

7.9 Mechanism of Horizontal Gene Transfer (HGT) in Acquired Antimicrobial Resistance (AMR)

In contrast, HGT occurs by the exchange or swapping of genetic ingredients in the microbial “soup.” An antimicrobial resistant gene may become attached or spliced into the genome of a heretofore non-resistant bacteria making it now resistant to certain antimicrobial agents. It is important to note that although a gene may be identified as an antimicrobial resistant gene, it is, in fact, not just the identification alone but the expression of that resistance that translates into true antimicrobial resistance. If a particular antimicrobial resistant gene is not expressed in vivo, then it could be considered non-resistant (resistant but lacking expression; impotent). Thus, we need to concern ourselves with those bacteria (and probiotics) that can, in actuality, express antimicrobial resistance in vivo.

HGT is the most common cause of acquired AMR in the human gut flora. For the purposes of this research, and for simplification, it is proposed to use the acronym hAMR to denote AMR developed via the HGT pathway; as differentiated from mAMR via mutation in the acquired resistance pathway; in contrast to vAMR to denote vertical resistance via the intrinsic/ inherent pathway.

7.9.1 Review of the Factors in the Conference of hAMR

The human gastrointestinal tract provides an ideal combination of factors for antibiotic resistance genes to arise and spread through bacterial populations. One of these factors is high cell density. Other factors favoring spread of resistant infections include antibiotic exposure and subsequent selection followed by the innate ability for gene transfer through a variety of different mechanisms [9].

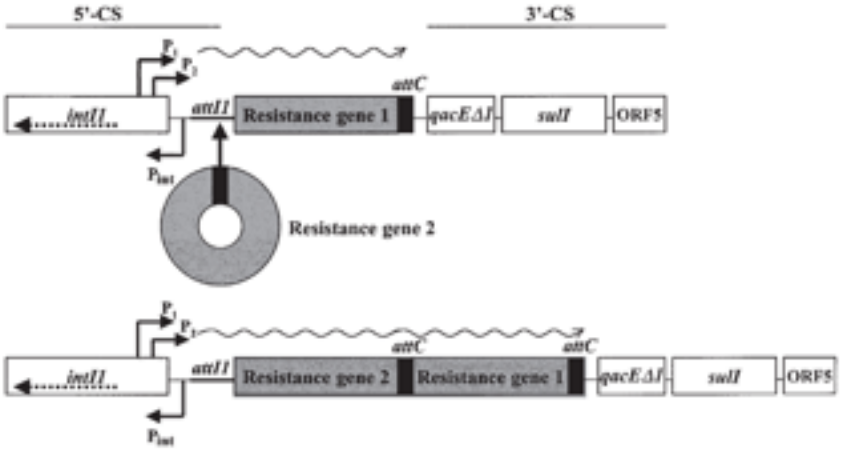
It has long been known that antibiotic resistance arises as a consequence of exposure to antibiotics. The resistant cells in a population have an advantage over sensitive cells when exposed to this strong selection. Therefore, the population becomes resistant to antibiotics. However, it is now known that antibiotics not only select for resistant populations through the clonal expansion of already resistant cells, but they also create them by inducing the horizontal transfer of resistance genes [10].

But how do these resistant microbes come about in hAMR? Integrons are mobile genetic elements that encode integrase and are capable of site-specific recombination and typically carry antibiotic resistance genes [11].

7.9.2 The Role of Integrons in hAMR

Resistance genes can be exchanged among bacterial populations [10,11]. Several mechanisms for the acquisition and dissemination of resistance determinants involve DNA exchange and, in this way, resistance genes can spread efficiently among bacterial populations from animals and humans [12,13]. The identification of specialized genetic structures responsible for the acquisition of resistance genes on horizontal gene vehicles represents an important discovery in our understanding of antibiotic resistance mechanisms. Naturally occurring gene expression elements, called integrons, have been described as a very efficient genetic mechanism by which bacteria can acquire resistance genes [14]. Integrons promote the capture of one or more gene cassettes within the same attachment site, thereby forming composite clusters of antibiotic resistance genes. Over the past few years, the analysis of many antibiotic resistance genes–identified in clinical and veterinary isolates of Gram negative organisms (particularly Enterobacteriaceae)–established the importance of integrons in the dissemination of resistance among bacterial pathogens from different geographical origins [12].

Figure 7.6 Carattoli's schematic representation of a class 1 integron and a model for gene cassette acquisition



The above schematic depicts the circularized gene cassette containing the resistance gene being inserted into the receptive site. Resistance gene cassettes inserted within the integron are indicated by grey boxes.

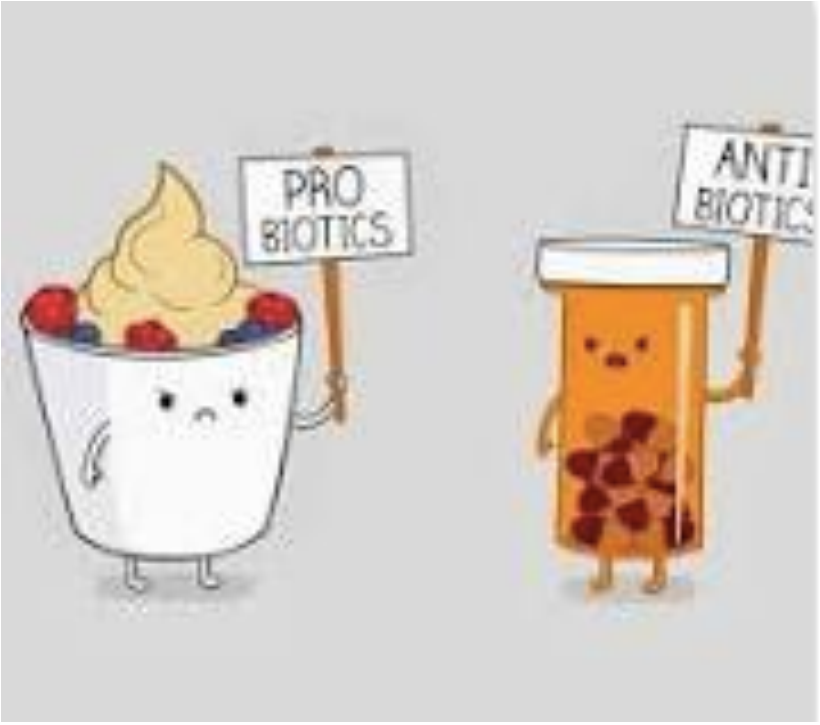
7.9.3 The Probiotic–hAMR Connection

Armed with a now known mechanism of hAMR via the integron, and with the understanding that the human microbiome is one the most densely populated open ecosystems in existence, and with the knowledge that in its "openness" the human microbiome is subject to hAMR from exogenous factors, we can safely conclude that (at least certain strains of) probiotics can in some way(s) contribute to hAMR in the human gut microbiome.

7.10 Studies of Antibiotic Resistance in Probiotics

It is fundamental to note the disparity between the way laypersons and consumers are influenced to view probiotics as a natural way to offset the adverse effects of antibiotics, and shuns the reality of what is really happening in the far reaches of the human gut. In many promotional ways (as in the following illustration), probiotics are depicted as a tasty, but helpful desert one consumes after having their intestinal flora torched by a course of antibiotics (see Figure 7.7).

Figure 7.7 The human microbiome's, "The Odd Couple"



But this nonthreatening depiction of the force of the determined probiotic against the force of the stubborn antibiotic strays quite far from the reality of this mix in the human gut as is reported as follows; according to William et al.:

> In recent years, the time-honored reputation of lactobacilli as promoters of gastrointestinal and female urogenital health has been qualified. This has occurred due to a rare association with human infection in the presence of certain predisposing factors and their potential to act as a source of undesirable antibiotic resistance determinants to other members of the indigenous microbiota. This necessitates greater caution in their selection for use in microbial adjunct nutrition and disease management (prophylaxis and therapy). **It was against this background that 46 *Lactobacillus* strains from human**

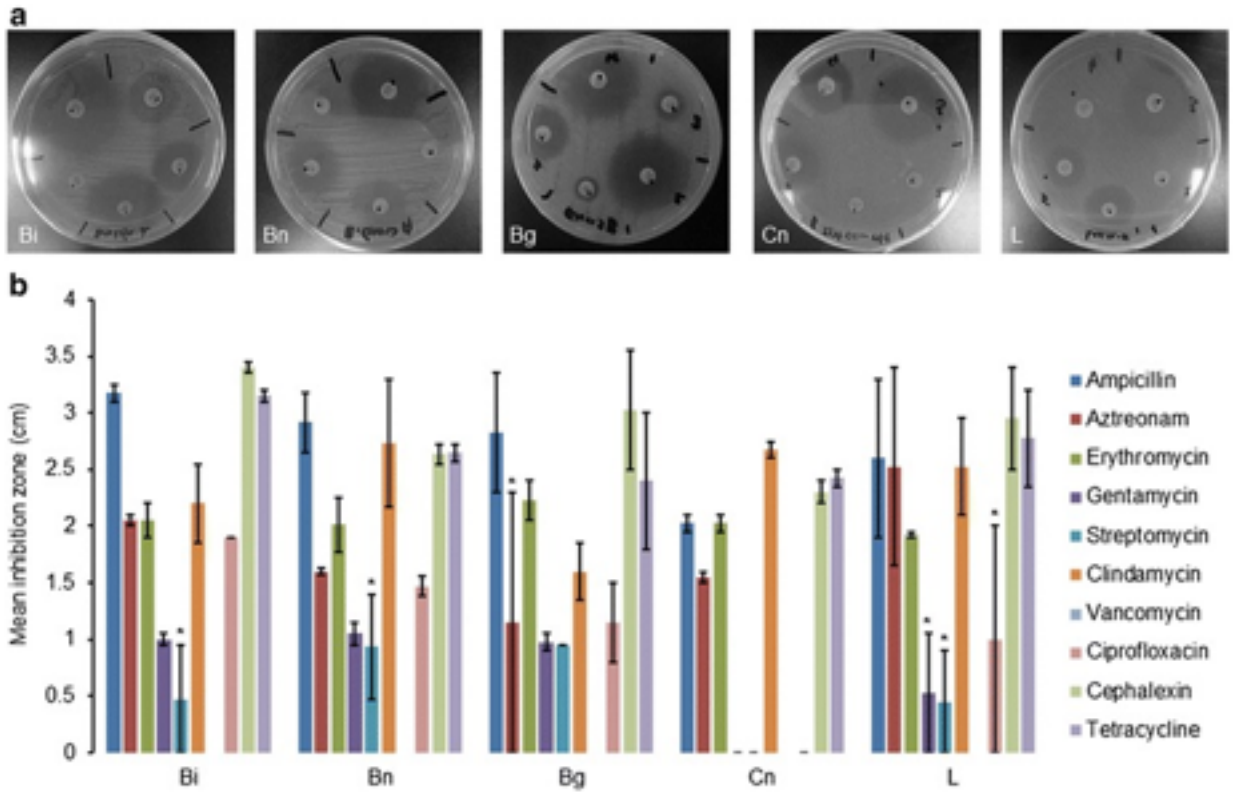
and dairy sources were assayed for susceptibility to 44 antibiotics. All strains were resistant to a group of 14 antibiotics, which included inhibitors of cell wall synthesis (cefoxitin [30 μg] and aztreonam [30 μg]), protein synthesis (amikacin [30 μg], gentamicin [10 μg], kanamycin [30 μg], and streptomycin [10 μg]), nucleic acid synthesis (norfloxacin [10 μg], nalidixic acid [30 μg], sulphamethoxazole [100 μg], trimethoprim [5 μg], co-trimoxazole [25 μg], and metronidazole [5 μg]), and cytoplasmic membrane function (polymyxin B [300 μg] and colistin sulphate [10 μg]). All strains were susceptible to tetracycline (30 μg), chloramphenicol (30 μg), and rifampicin (5 μg). Four human strains and one dairy strain exhibited atypical resistance to a penicillin, bacitracin (10 μg), and/or nitrofurantoin (300 μg). One human strain was also resistant to erythromycin (15 μg) and clindamycin (2 μg). These resistances may have been acquired due to antibiotic exposure in vivo, but conclusive evidence is lacking in this regard. Seven microorganism-drug combinations were evaluated for β-lactamase activity using synergy and nitrocefin tests. The absence of activity suggested that cell wall impermeability appeared responsible for β-lactam resistance. The occurrence of a minority of lactobacilli with undesirable, atypical resistance to certain antibiotics demonstrates that not all strains are suitable for use as probiotics or bacteriotherapeutic agents. The natural resistance of lactobacilli to a wide range of clinically important antibiotics may enable the development of antibiotic/probiotic combination therapies for such conditions as diarrhea, female urogenital tract infection, and infective endocarditis [15].

The following agar plate studies and corresponding color graphs (Figure 7.8) also show antibiotic resistance in strains of probiotics. This truth of the potential dangers of unregulated and unsupervised use of probiotics, especially with the concurrent intake of antibiotics, is in stark contrast to the commercial depiction as symbolized in Figure 7.7.

Figure 7.8 depicts the antibiotic susceptibility profile of probiotic bacteria in the dietary supplements. According to Wong, et al.:

(**a**) Representative MRS agar plates of bacteria lawn of Bi, Bn, Bg, Cn and L dietary supplements layered with antibiotic discs showing susceptibility towards multiple antibiotics as characterized by the presence of 'clear' inhibition zones. (**b**) Mean inhibition zones measured from the bacteria lawn of Bi, Bn, Bg, Cn and L dietary supplements layered with the respective antibiotic discs. Error bars represent standard error of the mean ($n \geq 2$) and (*) represents inhibition zone present in only certain batches of bacteria in the respective dietary supplement.

Figure 7.8 The antibiotic susceptibility profile of probiotic bacteria in the dietary supplements.



According to Stokes, et al.:

In the antibiotic resistant screening, clear inhibition zones of > 0.5 cm were measured from the bacteria lawn of isolates from all batches of brands Bi, Bn, Bg, Cn and L towards ampicillin, erythromycin, clindamycin, cephalexin and tetracycline antibiotics (b), suggesting no resistance towards these antibiotics. Meanwhile, inhibition zones detected in only certain batches of the probiotic products were that of brands Bi and Bn, Bg and L in the presence of the respective streptomycin, aztreonam and gentamycin, streptomycin and ciprofloxacin antibiotics (b). **This batch-to-batch variation implies that these resistances were not conferred by intrinsic genes but more likely a result of acquired mobile genetic elements by a transfer event and this is a concern because mobile elements such as plasmids and transposons can be transferred from one cell to another by conjugation. Indeed, this mechanism has**

been demonstrated *in vitro* where antibiotic resistant gene transferred from one *Lactobacillus* to another and more worryingly, also from *Lactobacilli* to other species including pathogenic strains such as*Staphylococcus* and vice versa. Streptomycin and gentamycin belong to the aminoglycosides and resistance towards this group of antibiotic may be conferred by intrinsic *Lactobacilli* aminoglycoside resistant genes *aac(6')-aph(2"), ant(6),* and *aph(3')-IIIa* respectively [16].

7.11 Summary and Conclusion

It is well beyond the scope of this chapter and research to delve into each strain of probiotic and its resistance to any specific class of antibiotics. The focus of this chapter has been to highlight that certain strains of probiotics (those that can be found in accessible dietary supplements) do exhibit resistance to certain types of antibiotics, and that they cannot only confer that resistance to other strains of probiotics but to constituents of the human microbiome. This, in and of itself, should sound a warning siren regarding the casual and unregulated practice of consumption of variable probiotics during antibiotic therapy.

In summary, probiotics have shown to be of benefit in reducing and, sometimes, eliminating adverse affects of antibiotic therapy. However, since probiotics also carry the potential for acquiring and conferring antibiotic resistance, medical guidelines should be established for the use of probiotics during antibiotic therapy; in particular, guidelines for the particular strain(s) that would be deemed effective (and not harmful) with a particular class or type of antibiotics. In the future, we may see different classes of antibiotics paired with different strains of probiotics to achieve the maximum benefit and effectiveness of both while mitigating the potential for conference of antimicrobial resistance to the human microbiome.

7.12 Footnote: Further Caution

It has been reported that probiotics “reseed” and stimulate the intrinsic flora of the human gut after constituents of this gut microbiome have been destroyed by antibiotic treatment. But this begs the question: does this repopulation of the intrinsic human gut bacteria in any way adversely affect the efficacy of the antibiotic treatment? After all, there is a definite (and finite) amount of antibiotic in each dose that is administered. It is presumed that this dose is adequate to destroy most of the target bacteria. It is also known that the antibiotic is diverted into causing collateral damage in destroying other bacteria, such as the intrinsic flora in the human gut. So the question is put forth: is it possible that by reseeding and stimulating the growth of the destroyed intrinsic bacteria, that the force of the antibiotic is further diverted and diminished by having to attack this regrowth? Is it possible that this “reseeding” and stimulation of the intrinsic gut flora diverts antibiotic potency leaving larger numbers of target bacteria not only to survive but to develop antibiotic resistance?

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CHAPTER VIII

CLINICAL EPIDEMIOLOGICAL CONSIDERATIONS OF AMR THROUGH THE PROBIOTIC PATHWAY

8.1 Abstract

There is an ever-diminishing development of new and effective antimicrobial agents (antibiotics). At the same time, there is an ever-growing resistance to existing antibiotics. From a clinical epidemiological standpoint, this scenario could be considered the proverbial “ticking time bomb” and a potential medical disaster of historical proportion which, if indeed it does play out, may cause all previously known plagues and epidemics to pale in comparison. This volatile combination of antimicrobial resistance (AMR) and lack of innovative antimicrobial agents could manifest as a healthcare armageddon for the human race. Unfortunately, the warning cries for the most part seem to have gone unnoticed or unheeded. Little is being done to avert this impending catastrophe. But some are taking notice, and taking action. This chapter reviews some of what is being done to bring this epidemiological issue to public and government awareness. It reports the initial regulatory steps that are being taken to change the status quo and to slow the momentum toward a “post-antibiotic era” wherein many illnesses, infections and diseases will no longer respond favorably to antibiotic treatment. Also included in this chapter is a fundamental review of the pathways to developing antimicrobial resistance, in particular horizontal gene transfer (HGT). It also includes the role that the current practices in the use of probiotics–particularly with antibiotic treatment–plays in antibiotic resistance. This chapter ends with a plea for the development of medical guidelines and drug formulations for the use of probiotics particularly when combined with antibiotics.

8.2 Keywords

antimicrobial resistance; drug resistance; horizontal gene transfer; microbiome; mutation; post-antibiotic era

8.3 Abbreviations

AMR: antimicrobial resistance; hAMR: antimicrobial resistance developed via horizontal gene transfer (AMR via HGT); HGT: horizontal gene transfer; MDR: multi-drug resistance; PAMTA: Preservation of Antibiotics for Medical Treatment Act

8.4 Introduction: AMR–A Global Phenomenon

On a less dramatic, albeit consistent, note; according to Penders, et al.:

> **Antimicrobial resistance (AMR) is worldwide one of the most important public health threats that we currently face.** AMR reduces clinical efficacy and increases treatment costs. Furthermore, AMR jeopardizes the achievements of modern medicine, since the success of interventions such as organ transplantation, cancer chemotherapy, and major surgery depends on effective antimicrobial agents for prevention and treatment of (nosocomial) infections. With a lack of novel antibiotics in the pipeline, the conservation of existing ones is crucial [1].

And from Hawkey, et al.:

> Antibiotic resistance is now a linked global problem. Dispersion of successful clones of multi-drug resistant (MDR) bacteria is common, often via the movement of people. **Local evolution of MDR bacteria is also important under the pressure of excessive antibiotic use, with horizontal gene transfer providing the means . . .** and the rapid dissemination of novel genes reflects their evolution under the selective pressure of antibiotic usage [2].

Consistent with the above theme, it is imperative that people minimize or restrict their exposure to those factors which can most readily contribute to AMR, such as dietary supplements, like probiotics, when taken in an uncontrolled, unchecked and unscientific manner.

8.5 Political Action Regarding AMR

Considering the long-term implications for the individual and the global healthcare system regarding AMR, several important studies and projects have been proposed and enacted to address this potential crisis.

8.5.1 National Academy of Sciences (NAS)

The National Academy of Sciences calculates that increased healthcare costs associated with antibiotic-resistant bacteria exceed $4 billion each year in the United States alone–a figure

that reflects the price of pharmaceuticals and longer hospital stays, but does not account for lost workdays, lost productivity or human suffering.

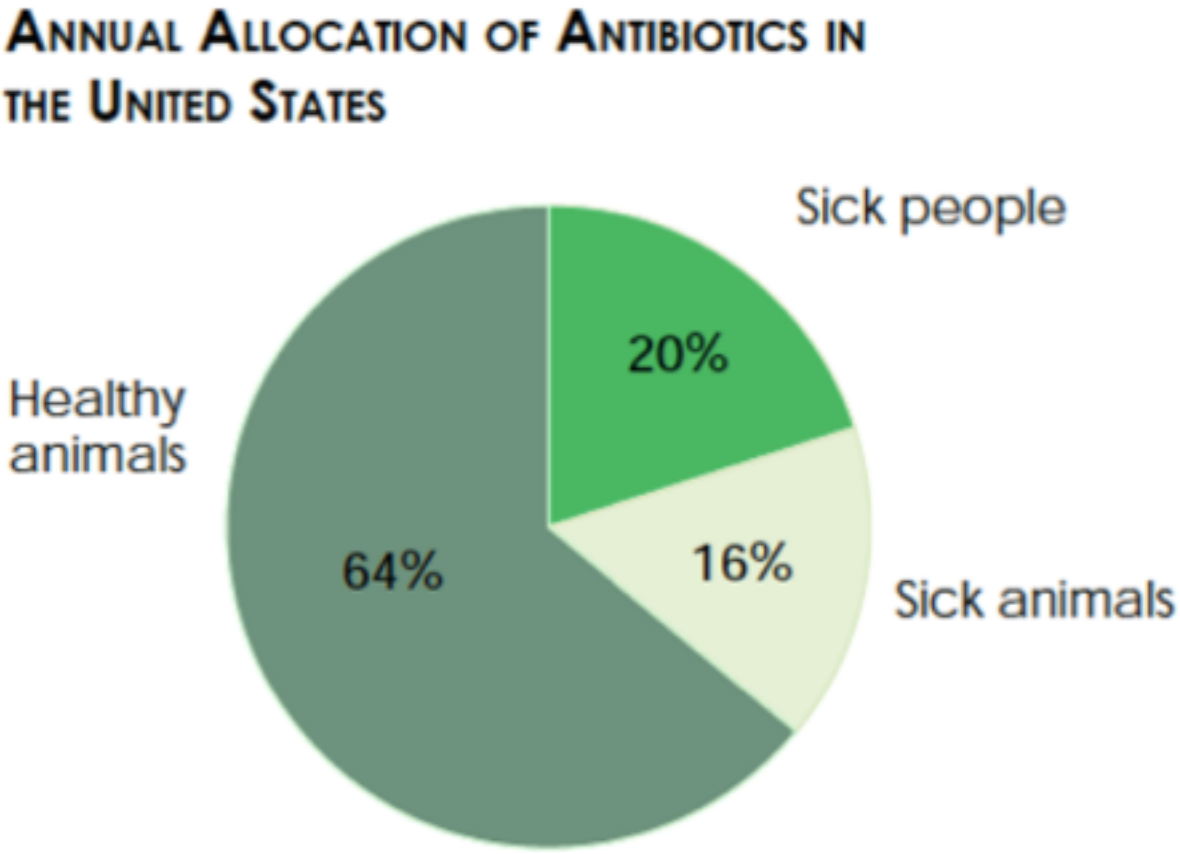
8.5.2 World Health Organization (WHO)

Dr. Margaret Chan, the Director-General of the World Health Organization, released a statement on World Health Day 2011 about the bleak future of treating bacterial infections if no steps are taken to slow the development of antibiotic resistant bacterial strains. She warned that "in the absence of urgent corrective and protective actions, **the world is heading toward a post-antibiotic era, in which many common infections will no longer have a cure and, once again, kill unabated** [3]."

8.5.3 Preservation of Antibiotics for Medical Treatment Act (PAMTA)

In 2011, an amendment to the Federal Food, Drug, and Cosmetic Act called the Preservation of Antibiotics for Medical Treatment (PAMTA), was introduced in the U.S. House of Representatives. The goal of the amendment was to create legislation that was focused on protecting the effectiveness of antibiotics used in treating human and animal diseases. It banned the use of seven classes of antibiotics–that are medically significant to humans–by the food animal industry, as well as restrict the use of many other antibiotics in animal feed [4].

Figure 8.1 *Annual allocation of antibiotics in the United States.* The Preservation of Antibiotics for Medical Treatment Act (PAMTA) will enhance drug safety standards to reduce the health risks associated with the widespread administration of antibiotics to healthy animals (adapted from FDA, 2009).



The foregoing statistics demonstrates the indiscriminate use of antibiotics in the food chain (healthy animals). This is to emphasize a major pathway to antibiotic resistance and the need to cull (eliminate) such unnecessary sources of antibiotic resistance facilitators, including probiotics which are being indiscriminately ingested by both sick and healthy humans.

8.6 The Genesis of AMR

The human gut microbiome is among the most densely populated microbial ecosystem on earth. While this microbiome exerts numerous healthy and beneficial functions, the high density of microorganisms within this ecosystem also facilitates horizontal transfer of antimicrobial resistant genes to potential pathogenic bacteria [1]

Antibiotics exert selective pressure on bacterial populations, killing susceptible bacteria while allowing strains with resistance to that particular antibiotic to survive and multiply. Traits for such resistance are then vertically passed on to offspring cells, subsequently creating a resistant population which can then spread and be further sources of resistance genes for other strains. Because resistance traits are not naturally eliminated or reversed, resistance to a variety of antibiotics may be accumulated over time. This can lead to strains with multiple drug resistance (MDR) which are more difficult to kill due to reduced treatment options [5].

8.6.1 Vertical Resistance Versus Horizontal Resistance

Vertical resistance is that resistance passed on directly from parent cell to offspring cell; in contrast to horizontal resistance which is acquired through the microbe's environment (e.g. resistant gene segments and other resources). Vertical is "top down" (intrinsic, hereditary); horizontal is "laterally" from local environment and neighboring genetic debris (acquired). Mutation also occurs in the acquired AMR pathway.

8.6.2 Interpretations of Vertical and Horizontal Resistance

Note that there are variations in the interpretation of “vertical” and “horizontal” in context of resistance in the field of botany versus the field of human microbiology; and even those within these respective fields may express varying interpretations of these two terms (vertical and horizontal). For the sake of maintaining focus on this topic, and to not become sidetracked by some very interesting but extraneous research, it seems judicious to apply the most common and frequent usage of these two distinct terms represented in current scientific literature and dialogue; this is, as mentioned previously: vertical (intrinsic/hereditary); horizontal (acquired).

8.7 Conclusion

The human gastrointestinal tract is an open system, which every day encounters a myriad of bacterial acquisitions originating from the environment (e.g., from food, water, soil, and other humans or animals) [6]. These incoming bacteria often harbor antibiotic resistance genes which can contribute to hAMR (HGT via AMR). Some of these encounters are incidental while some are intentional. It is incumbent on humans to eliminate or restrict these encounters (both incidental and intentional) whenever possible. The random, unscientific and–one might even say–reckless intake of probiotics (and probiotic-containing products); particularly while taking a course of antibiotics, without medical guidelines or supervision, may be a major causative factor for future eruptions of AMR.

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CHAPTER IX

DYNAMIC MATHEMATICAL MODEL OF MORTALITY WITH COFACTOR hAMR

9.1 Abstract

The importance of mathematical models of mortality with regard to antimicrobial resistance (AMR) cannot be overstated. In general, models help define the system being observed and assist the in the study of the influences of various components on the system. From these mathematical models, predictions can be made and observations and conclusions can be communicated succinctly. This chapter attempts to characterize and define factors that influence AMR in relation to death and disease. The mathematical model presented herein was developed from "scratch" and does not represent a final version; rather a mathematical model in progress. Section 9.4 provides a background and rationale to the development of the mathematical model, and Section 9.5.3 shares the revelation of the flawed first-generation model which spawned the latest, more accurate, second-generation version. This chapter concludes that AMR, through the pathway of horizontal gene transfer (HGT)–collectively known as hAMR (AMR via HGT)–can result in an increase in death and disease; and that through the intake of (untested and unregulated) probiotics, hAMR would likely increase, thereby increasing mortality. This current, second-generation mathematical model does not take into consideration any potential beneficial effects of probiotics in decreasing mortality due to its health benefits as this factor would be difficult to qualify and quantify at this time. Perhaps a later model will include such. This chapter depicts a simplistic working model–a baseline–to which future factors can be easily integrated.

9.2 Keywords

antibiotic resistance; horizontal gene transfer; human microbiome; mathematical model; mutation

9.3 Abbreviations

AMR: antimicrobial resistance; hAMR: antimicrobial resistance developed via horizontal gene transfer (AMR via HGT); HGT: horizontal gene transfer

9.4 Introduction; AMR as a Cofactor in Death and Disease

It is deemed helpful to briefly review certain core concepts presented in previous chapters, and use such as a springboard to the addition of new material.

It is established that the human microbiome is one of the most densely populated ecosystems known to man. It is further established that due to this density, the close proximity of cells in the human gut favors certain mechanisms of acquired AMR (i.e. mutation and hAMR). In addition, it is known that the human microbiome is an open system which means that, moment-to-moment, the human microbiome may be involved in encounters which can result in large populations of diverse cells becoming singularly resistant or cluster resistant to antibiotics; and therefore, we should be selective and restrict those encounters we have control over (e.g. the intentional but unregulated intake of probiotics). It is further established that AMR and the lack of novel antibiotics are two of the greatest threats to the human population and to many of the

medical advances that utilize antibiotics to augment these procedures, and to nosocomial infections.

In the most basic terms, resistance to antibiotics will result in more untreatable illnesses which will result in more disease, disability and death. This outcome will increase the cost of health care and tax the already overburdened healthcare system. It is difficult to place a value in terms of human suffering not only for the patient, but for family, friends, employer and society as a whole.

We can tell a literal tale of how AMR effects treatment outcomes in certain bacterial infections, such as "increased AMR increases death and disease (and disability)." It is more helpful and clear, however, to put a mathematical formula to the face of this crisis to readily recognize the effects of AMR on death and disease. This basic mathematical model can be utilized as a "drawing board" to build a more sophisticated version including other variables as they are realized and deemed consequential.

9.5 A Basic Mathematical Model of the Effects of AMR on Death and Disease

$$\sum Dd_{bi} \propto Pr_{bi} - (Tx^{ab} + SpRc); \text{ wherein } Tx^{ab} = k/rTx^{ab}$$

This basic mathematical model is geared toward epidemiology through the lens of medical mathematics. The concern is with AMR and its resultant effect on death and disease in the human population. To reiterate, this mathematical model is a working model, streamlined and

kept simple by design. This model is meant to be a baseline to which other factors may be incorporated. The foregoing mathematical model is deciphered as follows:

The sum total (∑) of death (D) and disease (d) due to a specific bacterial infection ($_{bi}$) is proportional to (∝) the prevalence (Pr) of that bacterial infection ($_{bi}$) minus (-) the successful antibiotic treatment of such bacterial infection (Tx^{ab}) minus [negative (-) times (x) positive (+) equals (=) negative (-)] the spontaneous, non-antibiotic treatment recovery (SpRc) from such bacterial infection.

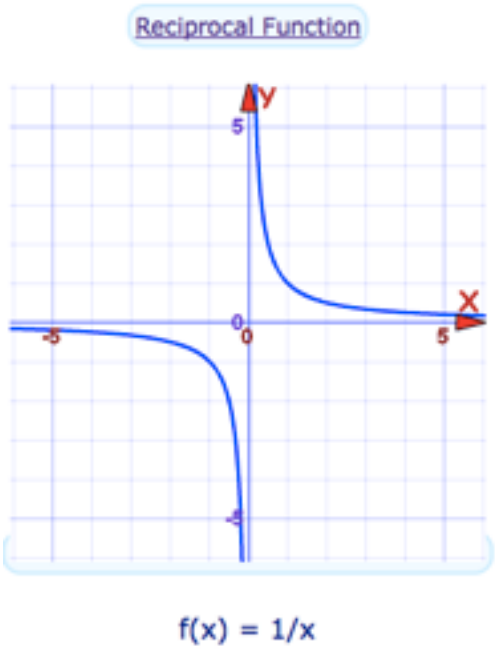
It is primal to note that Tx^{ab} is inversely proportional to the cases not cured due to AMR to such bacterial infection (rTx^{ab}); thus, in short, as rTx^{ab} increases, Tx^{ab} decreases.

Therefore, if given SpRc is constant, as resistance to antibiotic treatment (rTx^{ab}) increases, Tx^{ab} decreases, Pr_{bi} increases resulting in a total increase in Dd_{bi} (from said bacterial infection).

9.5.1 Model Depicting Inverse/Reciprocal Function

It is anticipated that the following graph (Figure 9.1) would represent the inverse relationship of Tx^{ab} to rTx^{ab} (see Figure 9.1).

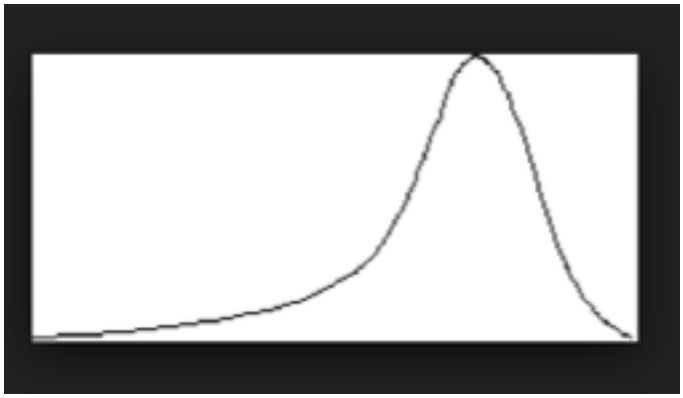
Figure 9.1 Graph of Reciprocal Function



9.5.2 Model Depicting Negative Skew

It is anticipated that the foregoing mathematica model, when depicted on a graph, would result in a negative skew as $\sum Dd_{bi}$ accelerates after reaching maximum rTx^{ab}. Time is on the X axis; rTx^{ab} on the Y axis (see Figure 9.2).

Figure 9.2 Graph of Negative Skew



9.5.3 The First Generation–Flawed–Mathematical Model

The mathematical model proposed in Section 9.5 is an outgrowth of the following original (but flawed) formula:

$$\sum \mathbf{Dd_{bi}} \propto \mathbf{Pr_{bi}} - [(\mathbf{Tx^{ab}} - \mathbf{rTx^{ab}}) - \mathbf{SpRc}]$$

This first generation mathematical model (depicting the epidemiological relationship of antibiotic resistance to death and disease) proved flawed due to the following:

> As rTx^{ab} increased Tx^{ab} would decrease. Therefore, with $Tx^{ab} - rTx^{ab}$, Tx^{ab} would be entered twice; once as a decreased Tx^{ab} due to rTx^{ab} and again reflected in the increased rTx^{ab}. This necessitated the reworking of the formula to show the inverse proportionality of Tx^{ab} and rTx^{ab}; allowing rTx^{ab} to be a factor of Tx^{ab}.

This flawed, first generation model is mentioned herein so the reader may better understand and appreciate the settled upon second-generation mathematical model. It purposes that the reader or future investigators do not travel the same, or similar, errant road of flawed logic.

9.6 Conclusion

With it proposed mathematically that any increase in hAMR would result in an increase in death and disease, it further obviates the need to evaluate the potential risks of transference of AMR through the ingestion of untested, unregulated and possibly hAMR-potentiating probiotics into the open system of the human microbiome. This is deemed so since probiotics are taken in such vast, unsupervised amounts throughout much of the world, and without much scientific research for their specific use. Probiotics are lacking in proven medical guidelines and criteria

for their use. These considerations alone (and there are more, such as probiotics in the food chain, food producing animals and food processing) trumpet the need for further scientific testing and outlining of medical protocol for probiotic use, especially when used with antibiotics.

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CHAPTER X

PROBIOTICS AND IMMUNITY

10.1 Abstract

Probiotic strains have unique, specific properties that allow them to be used for different types of treatments from short-term antibiotic-associated diarrhea (AAD) to long-term inflammatory disease. Probiotic strains, also, have several effects on the human immune system. The ability of the probiotic to affect the immune system in different ways allows for various types of diseases to be treated and managed. In addition to being used as part of a medical treatment plan for certain conditions in patients of all ages, probiotics can also be used to help promote a healthy immune system in infants and children. Probiotics play a role in the formation of the child's digestive system creating a better human microbiome. Studies have been conducted on the use of probiotics in allergic diseases, but there is insufficient evidence to prove probiotics for this specific use; however, it seems likely that probiotics can be of some help in allergic conditions. Further research is needed to fill in the gaps in the knowledge of how probiotic strains affect the human immune system. There are several types of probiotic studies that should be considered, especially those that could allow for stronger immune systems in pediatric patients.

10.2 Keywords

dysbiosis, human microbiome, immunity, irritable bowel, pathogen colonization, probiotics

10.3 Abbreviations

AAD: antibiotic-associated diarrhea; AGE: acute gastroenteritis; IBD: irritable bowel disease

10.4 Introduction: Probiotic Strains and Corresponding Immune Properties

Probiotics are living microorganisms that are beneficial to the host, and improve the host's intestinal microbiome to maintain intestinal homeostasis [1]. In order to be defined as a probiotic, a microorganism strain must be human in origin, safe for human consumption, be able to withstand both bile and acid, and have the capability of binding to the intestinal mucosa [2]. While there are a multitude of probiotic strains with a vast array of properties, the most commonly seen strains belong to genera *Bifidobacterium* and *Lactobacillus* [1]. *Bifidobacterium* and *Lactobacillus* are safe for human consumption, even for those who are immunocompromised, children, and infants [3]. Indeed, probiotics can assist the correct formation of the infantile immune system, providing a stronger intestinal microbiome [4]. Table 10.1 lists examples of the effects of probiotics on the immune system.

Table 10.1 Probiotic influence on various immune functions

Immune System Effect	Probiotic Organism
Increased phagocytosis capacity	*L. acidophilus La1* *L. casei* *B. lactis Bb12* *B. lactis HN019* *L. rhamnosus GG* *L. rhamnosus HN001*
Increased NK cell activity	*L. rhamnosus HN001* *B. lactis HN019* *L. casei (casei and dextran combination)*
Stimulation of IgA production	*B. bifidum* *L. acidophilus La1* *L. casei rhamnosus GG* *B. lactis Bb12*
Suppression of lymphocyte proliferation induction of apoptosis	*L. rhamnosus GG* *L. casei GG* *B. lactis* *L. acidophilus* *L. delbrueckii (bulgaricus)* *S. thermophiles* *L. paracasei* *E. coli Nissle 1917*
Increased cell-mediated immunity	*L. casei Shirota*

The current theory is that probiotics are capable of assisting a host fight the symptoms of human immunopathologies, such as inflammatory bowel disease (IBD) and certain atopic diseases [1]. It is also proposed that probiotics could be beneficial in preventing allergic diseases if the human microbiome is exposed to probiotics while still in immune development [5]. There is a need for more studies to be performed to help identify which strains are most beneficial as treatments for these disorders. Being able to assist with conditions, such as cancer and non-

alcoholic fatty liver disease, would open up treatment options for patients who already have limited choices for medical treatment.

The human immune system is comprised of a complex array of molecules and cells that interplay to provide protection against pathogenic microbes, such as bacteria, viruses, and parasites, which often contain antigens that induce an immune reaction [6]. Studies have shown that probiotics are capable of modulating the permeability of epithelial barriers, modifying the inflammatory potential of epithelial cells, providing competition against pathogens for intestinal mucosal colonization, and directly modifying the functioning of immune cells [1]. The interaction between the human microbiome and the mucosal immune system is key to the homeostasis of mucosal tissue, and provides protection against inflammatory diseases and infections at the mucosal level which is the frontline against pathogens invading the human body [1].

10.5 Discussion

Probiotics can be used for short periods or over long periods of time. What the probiotic is being used for and what the intended goal for that person is determines how long the person will need to have the probiotics. For example, recovering from a course of antibiotics would result in only a short course of probiotics; whereas, assisting a chronic inflammatory disease, like Crohn's, would require long-term use to have the desired effect. However, there are some conditions that would be difficult to determine treatment time. Repair of an individual's intestinal microbiome to help with an allergy could be lengthy if the microbiome is significantly damaged. A short course would only be needed if there is minimal damage to the intestinal flora. The type

of strain used could affect the length of time required. Certain strains can be used to treat the same conditions, but could cause different processes to be induced. In order for more research to be completed, it is necessary to categorize the strains into effective medical designations.

10.5.1 Short-Term Probiotic Use

The most common form of short-term probiotic use coincides with antibiotics. Taken together and during a period of recovery, the probiotics will the help the microbiome be returned to homeostasis [4]. Acute gastroenteritis (AGE) is also a common condition requiring short probiotic use, especially in pediatric patients [4].

Short-term use is intended to mitigate dysbiosis that has a known cause, such as antibiotics, or is a singularity that can be corrected by simply returning the intestinal microbiome to homeostasis. It does not require ongoing maintenance with supplemental probiotics. Restoring the perturbed microbiome promotes the establishment of stable immunoregulation and braking of inflammatory immune system reactions [7]. Further studies should be performed to evaluate other infections that could be treated by various strains of probiotics, especially common infections like bacterial sinus infections. This could alleviate the need for antibiotics for many of these sinus infections. Research in this regard has a positive lean as probiotics are effective at interacting with the mucosal immune system.

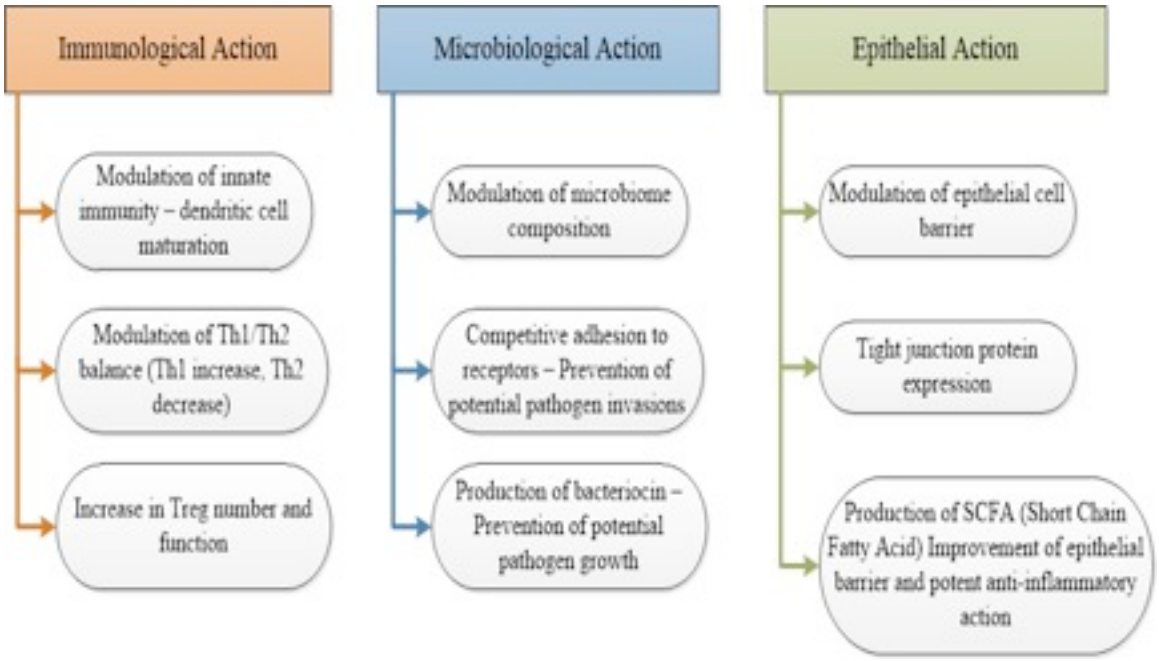
10.5.2 Long-Term Probiotic Use

Probiotics can be used in the long-term to help infants' and children's immune systems form, as well as the treatment of allergic diseases [5]. The intestinal microbiome is formed from

birth until reaching adulthood. Throughout this timeframe, various intestinal microbiota will colonize [6]. Using probiotics with infants and children will cause certain bacteria to colonize leading to a stronger collection of intestinal flora [6]. This process enables the development of a physical and immunological barrier between the environment and the host helping the microbiome remain in homeostasis [6]. Studies have shown that probiotics can reduce the severity of pediatric Crohn's disease. Adults only seem to benefit from the use of *Saccharomyces boulardii,* and it must be used in tandem with mesalamine [6].

Certain probiotics can also modulate the proinflammatory and anti-inflammatory cells. They will also modulate the activity of natural killer cells whose immunosurvelliance is key to preventing the formation of malignant tumors [6]. Probiotics are helpful to the child's developing system as well as the established adult microbiome. They are especially beneficial to the elderly as they help combat loss of immune defense caused by aging [6]. In addition to the effect that probiotics have on natural killer cells, they also affect the production of IgA which is needed to maintain intestinal humoral immunity [6]. Most detailed studies are showing a trend that is strain-specific, and has to do with a strain's colonization properties [6]. Figure 10.1 lists some effects of probiotics on the human microbiome.

Figure 10.1. Effects of probiotics on the intestinal microbiome



When it comes to allergic diseases, several studies have been conducted which have had mixed results. The results are trending towards the positive, but more research and testing needs to be performed to prove or disprove the probiotic's ability to aid in allergic diseases [6]. Probiotic strains have differing, unique properties, and the strains that are capable of having these effects need to identified.

Probiotics also show promise in the treatment of several long-term diseases. They have been shown to be effective as a treatment plan additions for the treatment of obesity, type 2 diabetes, insulin resistance syndrome, and non-alcoholic fatty liver disease [8]. The use of probiotics has been judged safe in patients with liver disease as long as the patient is not in end-stage liver cirrhosis [9].

10.6 Conclusion

Probiotics have varied effects on the human immune system through their interaction with the human microbiome, especially the mucosal barrier. By stimulating the immune system, the probiotic strains can be used to treat both short-term and long-term diseases. Various long-term diseases, such as inflammatory bowel disease and Type 2 diabetes, can be treated using probiotics. Whether on their own or as part of medical treatment plan, probiotics can help a person's immune system become more capable of coping with pathogens.

Probiotics have the ability to help in the formation of an infant's and a child's immune system by directing which microbiota become native to the microbiome. Also, probiotics have been shown effective in treating pediatric Crohn's disease.

There are so many benefits that probiotics offer. With so many strains and the ability to combine probiotics, and to pair them with other medications, it is essential that strains and their properties be identified. With identification, testing could be completed on many different types of illnesses as well as allergic disease. Studies would need to be long-term. Most diseases do not respond immediately to the administration of probiotics as probiotics take time to affect the intestinal microbiota. There needs to be ample time for the immune system reactions to be triggered.

Studies should also be performed to determine how well probiotics aid a newborn's development of their intestinal microbiota. There are a many possibilities for probiotic research in this area. Further studies could also lend some clarity on how probiotics affect allergic disease. Probiotics aiding in building a stronger immune system could reduce the amount of allergic disease.

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Tables

1. Reproduced from Salminen, S. J., Gueimonde, M., & Isolauri, E. (2005). Probiotics that modify disease risk. *The Journal Of Nutrition*, *135*(5), 1294-1298

Figures

1. Reproduced from: Kim, H., Kim, H. Y., Lee, S., Seo, J., Lee, E., & Hong, S. (2013). Clinical efficacy and mechanism of probiotics in allergic diseases. *Korean Journal of Pediatrics, 56*(9), 369-376. doi:10.3345/kjp.2013.56.9.369

CHAPTER XI

SUMMARY AND CONCLUSION

11.1 Summary

The following sections under Section 11.1 will cover the discovery of probiotics, early history of investigations and limitations thereof, current investigation mechanisms, characteristics of probiotics, functions of probiotics, health benefits and limitations of probiotic use, and any cause(s) for concern in the use of probiotics.

11.1.1 Early History of Probiotics Research

Probiotics have likely influenced human health and longevity for centuries, perhaps even thousands of years. The first reported discovery of probiotics was in Bulgaria in 1907 by Elie Metchnikoff, Russian scientist and Nobel Prize winner of the Pasteur Institute in Paris. Metchnikoff attributed the longevity of villagers living in the Causcasus Mountains to a fermented yogurt drink that they drank on a daily basis. The drink, Metchnikoff discovered, contained a probiotic called *Lactobacillus bulgaricus* which improved their health and may have helped in their longevity [1]. However, over the decades that followed (and for nearly 100 years), very little scientific investigation occurred regarding the health benefits of these probiotics for human consumption. This was due, in part, to the lack of interest in probiotics (and in natural health in general) and, to a greater degree, to the limitations in research apparatus.

11.1.2 Early Investigations of Probiotics as Medical Treatment

The beneficial effects of probiotics could be seen in case studies. Certain probiotics could relieve adverse effects caused by antibiotics. Antibiotic use can commonly result in the development of gastrointestinal disease. This can range from mild diarrhea to severe colitis. In the adult population, antibiotic-associated diarrhea (AAD) occurs in 5-35% of patients taking antibiotics. The severity depends upon the specific type of antibiotic, the health of the patient and exposure to and type of pathogens. The pathogenesis can occur through the disturbance of the intrinsic microbiota resulting in pathogen overgrowth or metabolic imbalances [2]. In children over the age of two, the prevalence of AAD is 11%. A study found that probiotics reduce the risk of AAD in children, and for every 7-10 patients one less would develop AAD [3]. A RAND study found that probiotics reduced the risk of AAD by 42 percent [4]. Combined treatments of antibiotics and probiotic have been used successfully in post-operative Chron's disease and, less so, in secondary pancreatic infections [5,6]. Many studies have shown the benefit of administering probiotics, but most could only provide inferences and conjecture as to why the probiotics provided benefit.

11.1.3 Limitations of Probiotic Research

Historically, when discussing digestion and the digestive system, the vast majority of gut research was focused on the biochemical pathways. The bulk of textbooks focused on the biochemical reactions which occurred in the digestive tract. Furthermore, the focus was primarily on how food was digested and how other organs coordinated with the digestive system. As such

there was little consideration and discussion of the presence of bacteria in the gut. Until recently, most textbooks did not include a detailed description of the microbiota. In the last several decades, however, there has been a surge in research which has led to an improved understanding of the important role that gut bacteria play in human health.

It was not until the advent of the computational microscope and molecular dynamic (MD) simulation that the actions, reactions and effects probiotics could be more thoroughly investigated and understood. State-of-the-art atomic level biomolecular simulation is the super microscope that allows researchers to better investigate and understand the probiotic and reveal its mysteries and its interactions with other biomolecules and drugs [7].

In order to ensure maximum efficacy, it is necessary to fully understand the role that each type of bacteria plays in human health. While there are many different types of probiotics on the market, not all of the strains have been thoroughly evaluated. Furthermore, depending on the imbalance which is present in a person, it is important that the appropriate type of probiotic be selected. If the wrong type of probiotic is administered, there may be no effect at all or it could lead to a negative effect on the health of the individual. Therefore, it is incumbent to have a fundamental understanding of the mechanisms of action of the varying types, strains and combinations of probiotics.

These scientific advancements in medical research also allow for in depth research into the unique properties of each strain of probiotic, and of combinations of probiotics; and how each (singularly or in combination) may interact with the human microflora, hormones, and

drugs such as antibiotics. This should allow medicine to maximize the probiotics' beneficial effects and avoid or minimize any adverse effects.

11.1.4 Characteristics of Probiotics

During their journey through the human alimentary canal, probiotics experience extremes in environment. They must survive the pH of the stomach and high concentration of bile salts in the small intestine. And, while survival is important and required for the species to make it to the colonization site, probiotic bacteria are only beneficial to humans if they are able to colonize somewhere in the gastrointestinal tract [8].

11.1.5 Functions of Probiotics

After colonization, the mechanistic functions of probiotics can be divided into five broad categories: nutritional benefits, neuromodulatory effects, promotion of epithelial cell homeostasis, modulation of intestinal immune function, and blockage of pathogenic bacteria [9].

11.1.6 Benefits and Limitations of Probiotics

Probiotics can provide health benefits to individuals. Because they are living organisms and due to their unique characteristics, probiotics can help correct imbalances in the human body which may occur due to a disruption to the natural, intrinsic microbiota. There are four main categories of probiotics, but the majority of the current probiotics are Gram-positive bacteria.

There is a lack of studies evaluating the efficacy of the vast majority of the available probiotic products. While there are exceptions, such as VSL#3, many of the available probiotics have not been checked to ensure that they contain the stated number of live bacteria [10,11,12]. Many of the strains used in probiotic combinations have not been validated. Therefore, a substantial amount of research must be conducted in order to determine the validity of these claims. One main limitation of probiotics is that, in order to continue to see benefits, the individual must continually take probiotics. Stoppage of the probiotic results in removal of the probiotic from the gastrointestinal tract after several weeks [9].

11.1.7 Causes for Concern and Caution in Probiotic Use

There are many prospects for probiotic use. Investigation into applications of individual strains and combination strains of probiotics for maintaining health and preventing and curing disease has gained traction, and is accelerating. It is more inspiring (and more profitable) to focus on the encouraging effects that probiotics have on human physiology, immunology and psychology. It is easier to focus solely on these favorable prospects for probiotics. Medicine should, however, exercise caution and consider the possible (and potential for) adverse effects in probiotics; and recognize the need for science-based guidelines for combined antibiotic-probiotic (CAP) use. All drugs, it is noted, have side effects. Although probiotics are not classified as drugs–in that they are not promoted for treatment of illness or disease–they do exhibit drug-like effects on human physiology. Therefore, should it not be considered that probiotics, too, have side effects? If so, what are these side effects?

Antibiotic resistance determinants have been identified and characterized in *Lactobacillus, Bifidobacterium* and the probiotic *Bacillus* [9, 13]. If CAP use is rising globally and if probiotics can confer antibiotic resistance to the human microbiota, an epidemic of profound proportion could now be brewing. As stated previously, this should be of grave concern to those in the fields of clinical pharmacology and clinical epidemiology.

It is also important to note that the quality and efficacy of different brands and different strains of probiotics can vary widely. So, it is advised to investigate probiotic products thoroughly before prescribing or consuming them in order to maximize positive outcomes and minimize negative outcomes [14].

Caution must be considered as probiotics have side effects to the body, such as infections in people with poor immunity. These infections are sometimes resistant to antibiotic therapy [15]. Some patients suffering from Crohn's disease, an autoimmune disease, have shown unpleasant outcomes after probiotic administration [16].

Genetically modified probiotics increase the mortality rate of patients suffering from pancreatic disease, such as acute pancreatitis (as differentiated from secondary pancreatitis) [17]. And, according to the NCBI report, *Safety Assessment of Probiotics for Human Use*: "There is concern for genetic stability of the probiotic over time, deleterious metabolic activities, and the potential for pathogenicity or toxigenicity. Immunological effects must be considered, especially in certain vulnerable populations, including infants with undeveloped immune function [18]."

11.2 Conclusion

What really happens when probiotics are taken with antibiotics? This question is apropos for much of the early research of probiotics was based on anecdotal evidence; and the beliefs and opinions derived from such remain with us today. Much of this early anecdotal evidence has been supported by current scientific research; but, still, research lags behind the application of probiotics and their use by consumers. There are no prescribed medical guidelines for the human use of probiotics, and most people are left on there own to sort out the scattered research and come to their own conclusions. This paper is, partly, an attempt to answer some of the basic questions and concerns regarding the use of probiotics; in particular, with CAP use. In the following sections of Section 11.2, each of the fundamental research questions listed in Section 1.4 will be addressed.

11.2.1 Are probiotics medically recommended during antibiotic therapy?

Probiotics are recommended across the spectrum of healthcare providers, but not inclusive of all providers in any group. Much current research supports probiotic use as being beneficial during antibiotic therapy, particularly in eliminating or limiting AAD, disruptions of the epithelium of the lower intestine tract due to Clostridium difficile infections (CDI), and even yeast (candida) infestation secondary to antibiotic therapy. Research on the use of probiotics during antibiotic therapy for these conditions has been reported on in India, Pakistan, China, Latin America and throughout Europe; thus making probiotic use a global phenomenon [19,20,21,22,23,24]. So, it can be seen that probiotics are being researched and, now,

recommended by physicians worldwide; albeit by a limited number of physicians for a limited number of indications and applications.

11.2.2 Are there any medical (scientifically-proven) guidelines for probiotic use with antibiotic treatment? If so, what are the guidelines?

To date, there are no medically-endorsed or science-based guidelines for CAP use. In a (relatively small) survey of 66 physicians in Nova Scotia. it was reported that “peer practice patterns” influenced the group of physicians prescribing probiotics, whereas the group of physicians that did not prescribe probiotics cite the lack of evidenced-based research for doing not doing so [25]. However, as more research becomes available in support of probiotic use, more physicians will likely recommend them.

Of those physicians that do recommend probiotics, many recommend the use of probiotics 6 hours after taking an antibiotic dose. Moreover, it is advisable to continue taking the probiotics for a period of 10 days after the antibiotics are stopped. Some physicians feel it is advisable to wait 4 hours after the antibiotic dose before taking the probiotic [26].

Current studies have shown that the CAP use can help reduce the unwanted side effects of the antibiotic therapy; in particular, gastric disorders and diarrhea, and candida infestation. To maximize the use of the probiotics, most researchers suggest staggering the doses; that is, taking the probiotic 2-6 hours after the antibiotic dose and continuing with the probiotic 7-10 days after ending the antibiotic treatment. It is also helpful to take probiotics before beginning antibiotic therapy, if possible [27,28].

Although these suggestions vary slightly, it is noted that the timing of the antibiotic and probiotic should be differentiated from 2-6 hours (with an average of 4 hours for intake of the probiotic after the antibiotic dose). However, caution must be observed when selecting the probiotic.

There is a lack of studies evaluating the efficacy of the vast majority of the available probiotic products. While there are exceptions, such as VSL#3, many of the available probiotics have not been checked to ensure that they contain the stated number of live bacteria [29,30,31]. Many of the strains used in probiotic combinations have not been validated. Therefore, a substantial amount of research must be conducted in order to determine the validity of these claims. The physician must perform their due diligence in selecting the appropriate probiotic for a particular condition or situation. Providing a combination of probiotics can help to increase the likelihood that the patient will see a positive effect [10].

It is important for the physician and patient to keep in mind is that one main limitation of probiotics is that, in order to continue to see benefits, the individual must continually take probiotics. Stoppage of the probiotic results in removal of the probiotic from the gastrointestinal tract after several weeks [9]. So, short-term use (7-10 days after the cessation of antibiotics) of probiotics is deemed effective for ameliorating certain undesirable side effects of antibiotic treatment; to continue to see benefit, the probiotics must be taken perpetually.

11.2.3 When taken concurrently, do probiotics diminish the efficacy of antibiotics?

Little to no research has been done in respect to probiotics effect on antibiotics. Most research is concerning the effect of both probiotic and antibiotics in treatment of certain conditions, and the beneficial effect when combined. The current opinion is that probiotics do not affect antibiotics, but they can have an effect on other drugs. The species of bacteria, *Lactobacillus acidophilus*, accelerates the rate at which the human body absorbs the drug sulfasalazine. While scientists have observed this, they do not know whether the interaction is harmful. Sulfasalazine treats ulcerative colitis, or colon inflammation. In addition, an autoimmune condition, such as celiac disease, that causes your immune system to attack your own tissues may require you to take immunosuppressant drugs. By suppressing your immune system, the medicine also makes you more susceptible to illnesses. In this case, probiotics may backfire and cause a bacterial or yeast infection [32].

A few researchers have stated that probiotics do not interfere with antibiotics. With that said, reasoning might indicate otherwise. Considering that a dose of antibiotics is finite, and that the antibiotics not only target pathogenic bacteria but the gut microflora as well; it would seem that by stimulating the regeneration of the intestinal microflora during a course of antibiotic treatment, one could be diminishing the amount of antibiotic available to target pathogenic bacteria by diverting the antibiotic to targeting the “regrowth” of the intestinal microflora. Could this result in decreased efficacy of the antibiotic in targeting and destroying the pathogenic bacteria? Whether this would prove true is a reasonable subject to explore in future research.

11.2.4 When taken concurrently, do antibiotics diminish the efficacy of probiotics?

When taken concurrently (around the same time), antibiotics would diminish the efficacy of the probiotic. This can occur through two pathways. First, the antibiotic would disrupt the work of the probiotic at the epithelium. Second, the antibiotic can destroy some strains of probiotic (in that they are bacteria); whereas, other strains may survive. In any event, the beneficial effects of probiotics can be disrupted by the intake of antibiotics.

11.2.5 What are the adverse effects of probiotic use?

As mentioned in Section 11.1.7:

> Caution must be considered as probiotics have side effects to the body, such as infections in people with poor immunity. These infections are sometimes resistant to antibiotic therapy [15]. Some patients suffering from Crohn's disease, an autoimmune disease, have shown unpleasant outcomes after probiotic administration [16].
>
> Genetically modified probiotics increase the mortality rate of patients suffering from pancreatic diseases, such as acute pancreatitis (as differentiated from secondary pancreatitis) [17]. And, according to the NCBI report, *Safety Assessment of Probiotics for Human Use*: "There is concern for genetic stability of the probiotic over time, deleterious metabolic activities, and the potential for pathogenicity or toxigenicity. Immunological effects must be considered, especially in certain vulnerable populations, including infants with undeveloped immune function [18]."

In order to ensure maximum efficacy, it is necessary to fully understand the role that each type of bacteria plays in human health. While there are many different types of probiotics on the market, not all of the strains have been thoroughly evaluated. Furthermore, depending on the imbalance which is present in a person, it is important that the appropriate type of probiotic be selected. If the wrong type of probiotic is administered, there may be no effect at all, or it could lead to a potentially negative effect on the health of the individual.

Also to be considered is the variation in genes in the microbiome when treating individuals with probiotics. Due to the variation in the types of genes, a probiotic combination which works for one individual may not be effective in another individual. One of the dangers of probiotics is that there are many types of preparations available which have not been tested rigorously. Therefore, it is possible that adverse effects may occur from these untested probiotics [33].

11.2.6 Can probiotics confer (or contribute to) antimicrobial resistance?

Antibiotic resistance determinants have been identified and characterized in *Lactobacillus, Bifidobacterium* and the probiotic *Bacillus* [25,34]. Agar plate studies with a bacterial lawn of typical over-the-counter strains of probiotics (dietary supplements) layered with antibiotic disks have demonstrated a wide range of antibiotic resistance in various strains of probiotics [35].

Acquired resistance occurs through mutation or horizontal gene transfer (HGT) and may lead to a change in the nature of proteins expressed by the microbe. This can cause a change in

the structure and thus the function of the microbe which can result in resistance to a specific antibiotic. HGT is a means by which antimicrobial resistance (AMR) can be acquired. HGT is the process of swapping genetic material between coincident bacteria (and for the purposes of this research, between probiotic and microbiota). HGT can occur by way of three main mechanisms: transformation, transduction or conjugation [36]. Resistance genes can be exchanged among bacterial populations [37,38]. Herein, hAMR is proposed as an acronym for AMR acquired via HGT.

Naturally occurring gene expression elements, called integrons, have been described as a very efficient genetic mechanism by which bacteria can acquire resistance genes [39]. Integrons promote the capture of one or more gene cassettes within the same attachment site, thereby forming composite clusters of antibiotic resistance genes.

According to Charteris et al.:

> In recent years, the time-honored reputation of lactobacilli as promoters of gastrointestinal and female urogenital health has been qualified. This has occurred due to a rare association with human infection in the presence of certain predisposing factors and their potential to act as a source of undesirable antibiotic resistance determinants to other members of the indigenous microbiota. This necessitates greater caution in their selection for use in microbial adjunct nutrition and disease management (prophylaxis and therapy) [40].

As reported in a study by Wong et al.:

> In antibiotic resistant screening . . . this batch-to-batch variation implies that these resistances were not conferred by intrinsic genes but more likely a result of acquired mobile genetic elements by a transfer event and this is a concern because mobile elements such as plasmids and transposons can be transferred from one cell to another by conjugation. Indeed, this mechanism has been demonstrated in vitro where antibiotic resistant gene transferred from one *Lactobacillus* to another and more worryingly, also from *Lactobacilli* to other species including pathogenic strains such as*Staphylococcus* and vice versa [35].

Armed with a known mechanism of hAMR via the integron; and with the understanding that the human microbiome is one the most densely populated open ecosystems in existence; and with the knowledge that in its “openness” the human microbiome is subject to hAMR from exogenous factors, we can safely conclude that (at least certain strains of) probiotics can in some way(s) contribute to AMR in the human gut microbiome.

As CAP use continues to grow globally and as probiotics have the potential to confer antibiotic resistance to human microbiota, an epidemic of significant proportion could now be brewing. To reiterate, this should be of grave concern to those in the fields of clinical pharmacology and clinical epidemiology.

11.3 Two New Acronym to Increase Memorability and Streamline Communication

The proper use of acronyms can benefit writers and readers, and speakers and listeners. First and foremost, among their benefits, is increased memorability [41]. With that in mind, two new acronyms were developed and introduced in this research: CAP which stands for combined antibiotic-probiotic; and hAMR which represents AMR via HGT.

11.3.1 CAP (Combined Antibiotic-Probiotic)

As more research is performed on probiotics, in particular probiotics administered with antibiotics, it seems relevant to have an acronym that refers to such. CAP has been proposed. In the not-to-distant future, it is opined, certain strains of probiotics will be packaged (or included in formulations) with antibiotics to target not only specific side effects of antibiotics, but to target treatment of specific conditions. Indeed, a few drug formulation like this are available in some markets today, mostly dealing with AAD. So as combined antibiotic-probiotic use becomes more widely known and utilized, the acronym CAP, for combined antibiotic-probiotic, may become commonplace in this specialized field.

11.3.2 hAMR (AMR Via HGT)

A long form way to define this term, hAMR, is antimicrobial resistance brought about through the pathway (mechanism) of horizontal gene transfer. In scientific literature, AMR commonly stands for antimicrobial resistance; HGT commonly for horizontal gene transfer. As medical technology improves and research becomes even more specific and detailed, there will

be even more discussion of AMR and HGT. Even now, these two terms are commonly mentioned together in the literature. It seems a rational next step to combine these two terms. The acronym, hAMR, has been used fluidly throughout this research and has facilitated the writing of the linked concepts. It is helpful to further delineate hAMR among other pathways of antimicrobial resistance: vertical (intrinsic/inherent) AMR: vAMR; and acquired AMR brought about by mutation: mAMR.

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CHAPTER XII

RECOMMENDATIONS FOR FUTURE RESEARCH

12.1 Background

It seems practical and prudent to consider future research suggestions in parallel to the research questions posed in Section 1.3.2. Following, therefore, will be presented each research questioned posed and comments concerning future research that may better, or more fully, answer those research questions.

12.2 Future Research Relative to Research Questions

In order to facilitate reading and review, the following subsections (12.2.1, 12.2.2, etc.) correspond directly with the subsections of the previous chapter (11.2.1, 11.2.2, etc.). Thus, the future research posed in this chapter will correspond directly with the answers to the research questions presented in Chapter XI.

12.2.1 Are probiotics medically recommended during antibiotic therapy?

This is the starting point off of which extensive research can develop. It is important to know how many (in numbers) or what percentage of physicians currently recommend probiotics during antibiotic treatment. Again, this is a fundamental starting point. Few studies like this have been performed. One such study from the Nova Scotia group was cited in Section 2.5.1; but as

mentioned, this was a small study of only 66 physicians from a unique geographical region. It is deemed helpful to have a larger sample from diverse regions participate in such studies.

Questionnaires could be developed and sent to physicians. Surveys of physicians in private, group or hospital practice could be gathered in different regions of a country (or in different countries). Surveys could include the following questions:

- Do you recommend probiotics when prescribing antibiotics?
- How often do you recommend probiotics when prescribing antibiotics?
- How "strong" are these recommendations? Do you recommend to all patients prescribed antibiotics or just some, such as those patients that ask about probiotics, those with a history of antibiotic-associated diarrhea, or those patients who seem to be "health conscious" and open to natural therapies?
- If a physician does recommend probiotics for patients who are prescribed antibiotics– what is the rationale for doing so (e.g. peer group practices, etc.)?
- If a physician does not recommend probiotics for patients who are prescribed antibiotics, what is the rationale for not doing so (e.g., lack of scientific evidence, etc.)
- Does the physician take any probiotics? Or vitamins? Or natural healthcare supplements?
- In the absence of regulated prescription guidelines, if the medical evidence strongly supported such, would the physician prescribe probiotics for a patient taking antibiotics?

These are just some of the survey questions that could be asked of physicians (and other healthcare providers) regarding their current practices with respect to antibiotics and probiotics.

12.2.2 Are there any medical (scientifically-proven) guidelines for probiotic use with antibiotic treatment? If so, what are the guidelines?

This is an enormous topic and all-encompassing project considering all of the different and specialized strains of probiotics and how they may benefit the many conditions of the human population. It would be wise to begin with the currently known conditions that probiotics have shown to be of benefit to, such as antibiotic-associated diarrhea (AAD). Research on this subject is scattered throughout the world. Much of the research is done in countries with high poverty levels, and on a population readily susceptible to intestinal diseases and other conditions requiring antibiotics.

It is suggested to perform tests on selected groups with the same condition but using different combinations and concentrations of probiotics. The conclusions could be most helpful in developing specific medical guidelines not only for the combined use of antibiotics and probiotics, but also in probiotic use alone.

This proposed research could, step-by-step, condition-by-condition, disease-by-disease slowly, but methodically, develop specific science-based guidelines for combined antibiotic-probiotic (CAP) use when the condition being investigated requires the administration of antibiotics. The results of the research need not be limited to how the probiotic may eliminate or

minimize the adverse effects of the antibiotic use. The results could also include if the probiotic and antibiotic combined had a better outcome than with the antibiotic alone.

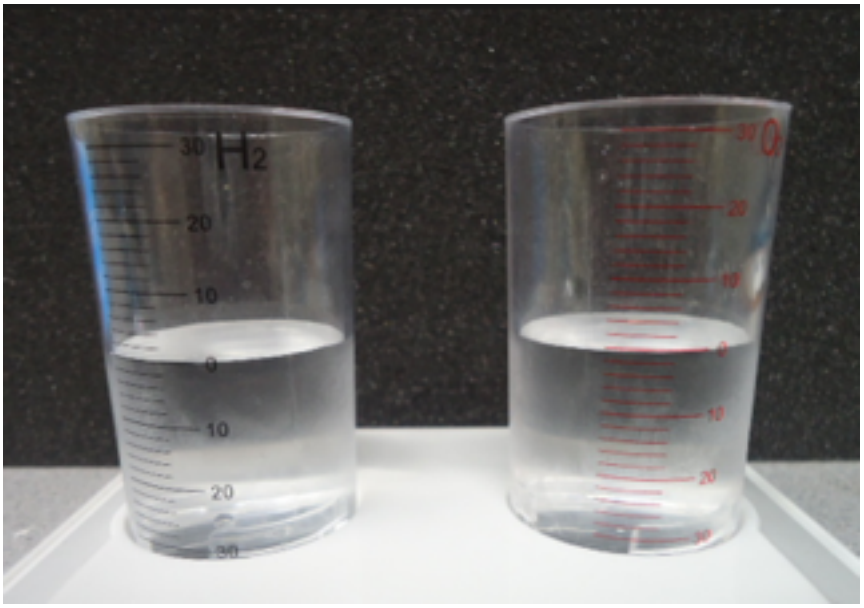
12.2.3 When taken concurrently, do probiotics diminish the efficacy of antibiotics?

The first tests that come to mind are simplistic; solution and agar plate experiments. To date, limited agar plate studies have been performed, but the results of such tests are most interesting and promising. Much more testing is needed with different strains of probiotics and different types of antibiotics. These proposed tests could be conducted on a lawn of antibiotics onto which probiotics are "sprinkled," or vice versa.

12.2.3a Solution Experiments

Mix probiotics with antibiotics in solution and observe/analyze what occurs. More specifically, have two beakers side-by-side both containing equal amounts of HCl at normal stomach pH (see Figure 12.1). In the left beaker, add a quantified amount of antibiotics; in the right beaker add the same amount of antibiotic (as added in the left beaker) plus a measured amount of probiotic. Observe and analyze the results.

Figure 12.1 Beakers for contrast studies

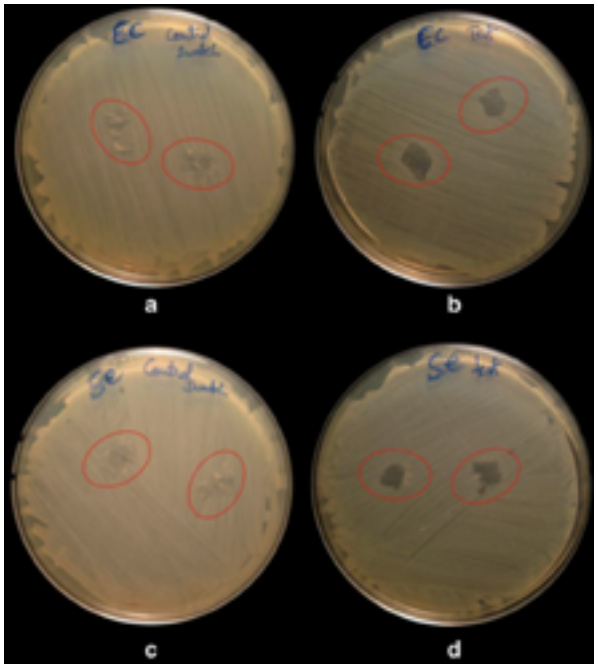


12.2.3b Agar Plate Experiments

Introduce antibiotics with probiotics on an agar plate and observe/analyze what occurs. More specifically, as in the color pie chart (see Figure 12.2)–which, for this purpose, represents an agar dish–the quarter area (represented in blue) would be where only a particular type of antibiotic is placed; the quarter area (represented in yellow) would be where only a particular strain of probiotic is placed; the quarter area (represented in green) would be where both antibiotic and probiotic are placed together; and, finally, the quarter area (represented in red) is where nothing was placed, remaining void. Inferences and hypothesis could be developed by observing the results of such testing. It may prove more practical and provide more accurate testing if four separate agar plates are used, rather than combining all elements on one agar plate (see Figure 12.3).

Figure 12.2 Color-coded pie chart representing agar plate quadrants for contrast studies;

Figure 12.3 Four (4) agar plates



12.2.4 When taken concurrently, do antibiotics diminish the efficacy of probiotics?

The same “beaker experiment” (as in Section 12.2.3a) could be performed to observe/ analyze more specifically how probiotics react when in the presence of antibiotics. To do so, add the measured probiotic (instead of the antibiotic) to the HCl solution in the left beaker; and add both the equal amount of probiotic (as is placed in the left beaker) and a quantified amount of antibiotic to the solution, together, in the beaker on the right side. Observe and analyze the results.

12.2.5 What are the adverse effects of probiotic use?

Two main pathways for future research of this question are a more detailed meta-analysis (than provided herein) and clinical trails.

12.2.5a Meta-Analysis

The first study recommended in this section would be a meta-analysis involving not only conditions in which probiotics are contraindicated (such as those people suffering compromised immune systems) but other conditions and situations; such as long-term use, or use with other drugs. This would involve a review of the literature in associated studies citing adverse effects of probiotic use.

12.2.5b Clinical Trials

The second study would involve clinical trials. Some situations that come to mind are intermittent or sporadic use of probiotics. (After all, it has been stated that probiotics should be taken perpetually to maintain their presence in the human gut.) So, what would be the results on a population of volunteers, wherein, one group ingested a strain or mix of probiotics "perpetually" (for a finite period of time until final analysis); a second group ingested the same probiotic strain or mix sporadically; and a third group "cycled" on and off at regular intervals. (It would be most helpful if all 3 groups had a similar microbiome makeup.) In this type of study it might be determined that probiotics have a negative effect on one or more of the groups. The findings would give an indication for more substantiated recommendations for probiotic use.

12.2.6 Can probiotics confer (or contribute to) antimicrobial resistance

This topic, in and of itself, is fodder for an entire dissertation. It would likely be structured as a meta-analysis and review of the relevant literature. Another collateral study would be more in-depth research into antimicrobial resistance profiles of individual strains of probiotics, and profiles of varying combinations of strains.

12.3 Final Word Regarding the Main Research Question

For the time being, the use of high-quality probiotics during and after antibiotic therapy; staggering the probiotic 4-6 hours after taking the antibiotic; continuing in this way throughout the course of antibiotic therapy; and continuing for 7-10 days after the completion of the antibiotic regime, especially in those groups prone to candida and diarrhea, is deemed helpful by many researchers and practitioners.

The research into, and the establishment of, standardized medical guidelines for the appropriate use of particular strains of probiotics with different types of antibiotics is a long-term and valuable project for the effective treatment of certain conditions and for the ongoing health benefit of the patient. Treatments should be patient-specific, taking into account as many variables as possible (e.g. age, gender, race, etc.); and, in particular, when technologically possible and practical, microbiome-specific treatment for each individual. Without standardized treatment protocol, the many benefits of probiotics for human use may remain unknown to, or be ignored by, the majority of physicians worldwide, to the detriment of the patient.

Figure

1. Reproduced from Fuel Cell Store. Retrieved from: http://www.fuelcellstore.com/30ml-hydrogen-storage-cylinders

2. Reproduced from Pie Chart. ClipArtBest.com. Retrieved online from: http://www.clipartbest.com/pie-chart

3. Reproduced from Images of agar plates with bacterial growth tested with polyester fabric. ResearchGate. Retrieved online from: https://www.researchgate.net/figure/262572931_fig10_Fig-10-Images-of-agar-plates-with-bacterial-growth-tested-with-polyester-fabric-a-E

APPENDIX

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