THE EFFECTS OF VANCOMYCIN ON INDUCED LACTOBACILLI IN THE LEWIS RAT

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Introduction

Probiotics are live microorganisms that are similar to beneficial microorganisms found in the human gut. They are often known as lactic acid bacteria and are normally consumed in the form of yogurt. Most often the bacteria will come from two groups, *Lactobacillus* and *Bifidobacterium*. These groups are normally found in the intestine. There are different species within each group, but specifically lactobacilli will be examined. Lactobacilli are normally found in the human intestine and vagina. Antibiotics are produced by or derived from certain bacteria, fungi, or organisms and can destroy or inhibit the growth of other microorganisms. They are used in the prevention and treatment of infectious diseases. Vancomycin is a bactericidal antibiotic used in the treatment of antibiotic-associated colitis and endocarditis. In this study, we will be examining the effects of vancomycin on the induced growth of lactobacilli in the Lewis male rat. In the animal model of rheumatoid arthritis, vancomycin was shown to reduce the disease scores of adjuvant-induced arthritis in the Lewis male rat [1]. In the same study following treatment of vancomycin, there was a noted increase in the lactobacilli but no other increases as compared with the controls.

For this study, induction of lactobacilli will be achieved with the use of Bene-Bac Pet powder. Bene-Bac Pet powder contains live, naturally occurring microorganisms and is recommended anytime animals experience changing nutritional or environmental conditions. These conditions include birth, post
surgery, weaning, worming, traveling, and antibiotic therapy [2]. There is a guaranteed 20 million colony forming units (CFU) per gram of viable lactic acid microorganisms. This powder will be used to insure that there will be lactobacilli growth occurring outside of the naturally occurring microorganisms. Interest in the interaction among vancomycin and lactobacilli arose from previously examining immunocomplexes and their relation to arthritis. The results of the study performed by Nieuwenhuis et al., 2000, seemed very interesting to repeat and see if their results were definite. The focus of this study will be to examine the ability of lactobacilli to grow (and recover) in the intestinal tract during and after treatment of the antibiotic vancomycin. Lactobacilli counts are expected to decrease due to vancomycin treatment since lactobacilli are gram positive and vancomycin is used to treat gram-positive bacterial infection. Recently it has been shown that during treatment of certain infections such as S. aureus, C. difficile, or L. casei, there would be a reoccurrence of the infections following the vancomycin treatment. Over the years of using vancomycin as a general treatment of diseases, many bacteria have become resistant to vancomycin. It is believed that lactobacilli are one of those resistant groups. It has been reported that 25 strains of lactobacilli isolated were resistant to vancomycin [3]. Lewis rats may possess a naturally occurring resistant strain. The results of the rats in the experimental group receiving treatments of both vancomycin and Bene-Bac Pet powder will be used to determine if these lactobacilli are in fact resistant to vancomycin. It is expected that the treatment of Bene-Bac Pet powder with vancomycin will prevent a decrease in lactobacilli counts.
and the treatment of Bene-Bac Pet powder without vancomycin will maintain lactobacilli counts if it is a non-resistant strain.

Hypothesis: Following the induction of lactobacilli using Bene-Bac Pet powder and the treatment of the antibiotic vancomycin, there will be a decrease in the growth of lactobacilli in the fecal matter of the male Lewis rat.
Literature Review

Lactic acid bacteria (LAB) consist of heterogeneous group of gram-positive bacteria, whose main fermentation product from carbohydrate is lactate [4]. An important characteristic used in the differentiation of the LAB genera is the mode of glucose fermentation under standard conditions. Due to these conditions, LAB can be divided into two groups: the homofermentative, converting glucose almost to lactic acid and the heterofermentative, fermenting glucose to lactic acid, ethanol/acetic acid, and CO$_2$ [5]. They are strictly anaerobic but are able to grow with or without the presence of oxygen. The LAB group consists of a number of bacterial genera: *Lactobacillus*, *Lactococcus*, *Carnobacterium*, *Enterococcus*, *Lactosphaera*, *Leuconostoc*, *Melissococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, *Weissella* and *Microbacterium*, *Bifidobacterium and Propionibacterium* [6].

Microbial cultures have been used for thousands of years in food and alcoholic fermentations, and have been examined for many years for their ability to prevent and cure various diseases. Interest in the beneficial effects of lactic acid bacteria began with Russian scientist, E. Mechnikoff, who proposed in 1908 that the extended longevity of the Balkan people could be attributed to their practice of ingesting fermented milk products [4]. He believed that gastrointestinal disturbances occur by intestinal growth of putrefactive microbes, and that lactic
acid bacteria could minimize or prevent the harmful effects of these microbes. Lactic acid bacteria were first isolated from milk and have been since found in foods and fermented products such as meat, milk products, vegetables, beverages and bakery products [6]. They continued to be used as a preservative in foods as well as a flavoring and texture agent. LAB such as *Lactobacillus lactis* and *Streptococcus thermophilus* inhibit food spoilage and the growth of pathogenic bacteria, preserving the nutritive qualities of raw food material for an extended shelf life [6]. Through research numerous strains of LAB have been used in a wide range of food products for human and animal nutrition. Their importance is extremely high and they continue to evolve as more and more strains are identified. LAB also produces acetaldehyde, hydrogen peroxide, diacetyl, carbon dioxide, polysaccharides and bacteriocins, some of which may act as antimicrobials [6].

Lactic acid bacteria are regarded as a major group of probiotics. Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. The term “probiotic” was initially used in the 1960s and comes from a Greek word meaning “for life” [7]. They are often referred to as “friendly bacteria” or “good bacteria.” The majority of probiotics belong to the genera *Bifidobacterium* and *Lactobacillus*, and the most effective strains have proved to be those of human origin [8]. These strains are usually found in the human intestine and urogenital tract and are the most studied intestinal bacteria. The interest in probiotics and their uses has grown significantly over the years. Scientists have been examining probiotics and their effects on illnesses for many years. They are similar to bacteria found naturally in the human gut. Probiotics
cooperatively maintain a delicate balance between the GI tract and immune system [9]. Figure 1 describes probiotics and their effect on health [9]. The taxonomy and nomenclature of probiotic LAB is still changing. It is no longer based only on morphological, physiologic, and biochemical criteria, but also on molecular-based phenotypic and genomic characteristics [10]. Probiotics are available to consumers in numerous forms. They can be contained in capsules, tablets, packets, or powders or are found most commonly in yogurt or dairy drinks. They have also been used in complementary and alternative medicine (CAM) [11]. In order for probiotics to be successful, they must be able to withstand passage through the GI tract, colonize and reproduce in the gut, attach and adhere to the intestinal epithelium, and stabilize the balance of the gut flora [7].

*Lactobacillus* is the largest of the genera included in lactic acid bacteria containing 148 species. Being heterogeneous, their range involves a large variety of phenotypic, biochemical, and physiological properties [5]. Many lactobacilli are associated with food and feed fermentation, mainly because they contribute to raw-material preservation due to acidification, but also because of their capacity to contribute to product characteristics such as flavor and texture. They are generally the most acid-tolerant of the LAB and will terminate lactic fermentations such as silage and vegetable fermentations [5]. In a study by Vaarala, Gram-negative bacteria have been tested in clinical trials examining their immunological effects but were deemed unsafe. Instead, Gram-positive bacteria such as lactobacilli offer a much safer means [12]. Microbes isolated from human and animal intestines and selected as probiotics include species of the genera *Lactobacillus, Bifidobacterium*
and *Enterococcus*. As mentioned before, *Lactobacilli* along with *Bifidobacterium* are two of the most commonly studied species. These strains in combination have been studied to examine their effects as treatment of childhood constipation [13]. The effects of lactobacilli have been explored beyond the human and animal intestine. The natural habitat of lactobacilli ranges from dairy, meat and plant material fermentations to the oral cavity, and the genital and gastrointestinal tracts of humans and animals [14]. Consumption of products containing probiotic lactobacilli has been shown to reduce risk and oral carriage of mutan streptococci and suggests a potential for oral lactobacilli to be used as probiotics for oral health [15]. In this study presented by Koll et al., 2008, it was demonstrated that several human oral lactobacilli possess good functional probiotic properties such as antimicrobial activity against oral pathogens as well as high tolerance of environmental stress factors making them suitable as potential probiotics for oral health.

Probiotics have been used for the prevention and treatment of various medical conditions and to support general wellness [7]. Illnesses associated with the gastrointestinal tract have been a common target of probiotics, mainly due to their ability to restore gut flora. Their usage has benefited the treatment of Crohn's disease, ulcerative colitis, irritable bowel syndrome (IBS), and *Helicobacter pylori* infections. Another mechanism studied concerning the action of probiotics involves immunomodulation. Animal studies have found that some probiotic strains augment the immune response by stimulating the phagocytic activity of lymphocytes and macrophages [7].
There is evidence from many studies that probiotics can be very effective in the treatment of acute diarrhea, especially in children with rotavirus infection. If these organisms are capable of surviving for a significant length of time in the stomach, they are then useful in treating gastric disorders [16]. There is interest in their use in gastrointestinal disorders such as constipation. Probiotics produce lactic, acetic and other acids resulting in a lowering of pH in the colon. A lower pH enhances peristalsis of the colon and decreases colonic transit time, which is beneficial in the treatment of constipation [13]. It is also assumed that probiotics soften the stools by stimulating water and electrolyte secretion. Studies have found that probiotics have a beneficial effect on atopic eczema. There was a 50% reduction in the frequency of atopic eczema during the first two years of children’s lives given probiotics compared with placebo [7]. These treatments were given to the children’s mothers (who had a family history of atopic disease) two to four weeks before their expected delivery dates.

Inflammatory diseases of the digestive tract include ulcerative colitis, Crohn’s disease, and pouchitis. These diseases manifest with rectal bleeding, diarrhea, abdominal pain, and weight loss associated with inflammation of the colon [17]. In a study demonstrated by Kumar et al, 2008, probiotics were used in experimental systems to modulate cytokine production, to strengthen the mucosal barrier and to reduce inflammation. These mechanisms of course which contribute to the therapeutic efficacy in treating colitis [18]. Treatments aimed at reducing bacterial concentrations in the gastrointestinal tract such as surgical bypass or antibiotic administration can lead to remission in some patients with inflammatory bowel
diseases [19]. Probiotics recently have appeared to be the choice of treatment and prevention of inflammatory bowel diseases, providing an appealing alternative to the use of antibiotics [4]. One of the most commonly used antibiotics is vancomycin.

In the 1950s, antibiotic resistance to penicillin was becoming a problem, and there was a need to find new agents with activity toward resistant isolates. The development of vancomycin began with the isolation of vancomycin-producing *Streptomyces orientalis* from a sample of dirt sent to scientists at Eli Lilly and Co. by a missionary in Borneo [20]. In the review by Dr. Levine, 2008, he states, “Because of the absence of an effective alternative for infections caused by β-lactamase-producing and other drug-resistant Gram-positive organisms, vancomycin was rapidly approved for general use.” Vancomycin, a narrow-spectrum antibiotic primarily active against Gram-positive bacteria, has been available to researchers and clinicians to treat human infections for more than half a century [20]. One of the many diseases that was and is still today treated with vancomycin is *Clostridium difficile*-associated disease.

*Clostridium difficile*, an anaerobic, spore-forming, gram-positive rod, is the enteric pathogen most frequently identified in patients with antibiotic-associated, nosocomially-acquired diarrhea [21]. This disease and associated toxin production results in colitis, most severely in the distal colon and rectum, which may result in pseudomembranous colitis. It most frequently occurs in the health care setting such as hospitals and long-term care facilities [21]. Onset can occur during antibiotic therapy. Antibiotics are usually delivered orally, intravenously, or rectally.
Antibiotic treatment alters the normal colonic flora and enables the germination of *C. difficile* spores acquired through fecal-oral transmission. The spores are acid resistant and survive passage through the stomach [22]. Another option of treatment involves the antibiotic metronidazole. Metronidazole is the first line treatment for *C. difficile*. Oral vancomycin for 10 days is recommended for more severe *C. difficile* cases, in metronidazole treatment failure, or when metronidazole cannot be used. Oral vancomycin is not absorbed in the gastrointestinal tract and is eliminated unchanged in the feces [21]. Although vancomycin is one of the most generally used antibiotics, it is very expensive and it would be more difficult to access for outpatients.

Vancomycin is also a risk factor for colonization and infection with vancomycin-resistant enterococci [22]. Diarrhea is the most common GI side effect of antibiotic therapy associated with *C. difficile* infections in adults and children [10]. The major complication of antibiotic treatment of *C. difficile* has been relapse, which is seen in about 20% of patients treated with metronidazole or vancomycin [23]. The future of vancomycin will depend on whether clinicians use it properly where its use should be limited to cases of confirmed multidrug-resistant infections and truly vancomycin-susceptible strains [20].
Outline of Project

Hypothesis: Following the induction of lactobacilli and the treatment of the antibiotic vancomycin, there will be a decrease in the growth of lactobacilli in the fecal matter of the male Lewis rat.

The Lewis male rats will be used to determine if there is a significant decrease in the growth of lactobacilli following treatments of the lactobacilli inducing Bene-Bac Pet powder and the antibiotic vancomycin. The rats will be separated into four groups with three rats in each group. All groups will be housed separately in the Physiology animal care facility. A project study was previously performed using corn syrup as the first control group and in both experimental groups in order to deter the taste of vancomycin. In the groups mentioned, rats were given 200 microliters of corn syrup via feeding needle once a day for 10 days. The project study’s second control group and experimental groups were the same as the experimental study with the exception of the corn syrup being used. Following the completion of the project study, it was then decided to perform the study again but with modifications. The experimental study was performed with the same animals as in the project study. In the first control group, rats will be fed via a feeding needle 200 microliters of dextrose/maltodextrin mixture once a day for 10 days. The second control group of rats will be fed 0.25 g of oral Bene-Bac powder once per day via feeding needle for 10 days. In the first experimental group, rats will be fed ~65 mg/kg of vancomycin once per day for 10 days along with 200 microliters of dextrose/maltodextrin to deter taste. In the second experimental groups, rats will
be fed 0.25 g of oral Bene-Bac powder once per day via feeding needle two hours prior to receiving ~65 mg/kg vancomycin along with 200 microliters of dextrose/maltodextrin to deter taste for 10 days. Fecal samples will be obtained from the rat prior to initial treatment and then once every three days during the experimental period and two days after the conclusion of the experimental procedure (n=5 samples per rat). The identification of *Lactobacillus* will be done by microbiological culture methods.
Materials and Methods

Animals

Lewis male rats were used in the project study and the experimental study. In the animal model of rheumatoid arthritis, vancomycin was shown to reduce the disease scores of adjuvant-induced arthritis in the Lewis male rat [1]. Animals used for the current study were in the range of 0.41-0.64 kg and were over 1 yr. in age. All rats were given normal food and water during the duration of the study. They were paired and housed in Ball State University animal facilities with approval. Three rats were used for each control and experimental group.

Fecal Sample Acquisition

Fecal samples were obtained from the rat prior to initial treatment and then once every three days during the experimental period and two days after the conclusion of the experimental procedure (n=5 samples per rat). The samples were obtained by holding the rat by the tail and waiting for the rat to defecate. To allow the rat to relax, it was placed on a flat surface with the rectum near the edge of a sterile Petri dish. The back of the rat was gently stroked to allow for the acquisition of fecal matter. The fecal samples were weighed. The same amount (approximately 0.10 g) was obtained from each rat. Immediately after weighing the sample, it was placed in sterile 990 microliters of 0.9% saline solution to prevent desiccation. The proportion of fecal sample to 0.9% saline solution was ~0.10 g/990 microliters. At the end of the experiment, rats were returned to the colony.
**Plating and Culturing of Fecal Matter**

The fresh fecal samples were homogenized within 0.9% sterile saline solution using sterile pipette tips and then vortexed to guarantee complete mixing. The samples were centrifuged at room temperature and 3000 rpm for 5 minutes. The resulting supernatant was used as a starting solution to make a 1:100 serial dilution. To create the 1:100 serial dilution 10 microliters of the starting solution was placed into 990 microliters of 0.9% sterile saline solution. In the project study, the 1:100 samples were not diluted. In the experimental study, each 1:100 sample was diluted 1:10,000 and 1:1,000,000 in the 0.9% sterile saline solution. In the project study, the solutions were vortexed thoroughly and 50 microliters were spread plated on de Man Rogosa Sharpe agar (MRS) plates. In the experimental study, the solution was vortexed thoroughly and 50 microliters was spread plated either on de Man Rogosa Sharpe agar (MRS) or tomato juice agar (TJA). Each dilution of every sample was plated on two MRS agar plates and the 1:100 dilutions were plated on two TJA plates. The MRS and TJA agar was selected for the growth of Lactobacillus spp. or Bifidobacterium spp. when grown under anaerobic chamber (GasPak EZ anaerobic chamber) along with GasPak Anaerobic Sachets. All of the inoculated plates and anaerobic growth chambers were placed in an incubator at 37 degrees Celsius and 10% CO2 for a total of 48 hours. After incubation, the plates were removed and the number of resulting colonies counted. The maximum number of colonies counted was 400 after which plates were deemed too many to count. After the number of colonies per plate will be determined, the bacteria per mL were determined using the following equation:
Gram Staining

Gram staining was performed to confirm growth of *Lactobacillus* species on selective media. Using a sterile pipette tip and 10 microliters of 0.9% sterile saline solution, individual colonies were smeared on a sterile glass slide and allowed to dry completely. Following the fixing of the sample to the glass slide, the staining protocol was followed according to Microbial Life directions to determine Gram-positive or Gram-negative bacteria [22].

Dextrose/Maltodextrin Feeding

In control group #1 and both experimental groups, rats were given a mixture of dextrose/maltodextrin. This mixture contained 7.5 g of dextrose and 2.5 g of maltodextrin. For each subject, .22 g of this mixture was added to 200 microliters of deionized water and fed to each rat via feeding needle. Dextrose/Maltodextrin was used due to the fact they are found in the Bene-Bac Pet powder as a substrate to ensure consumption. Dextrose is also found in lactobacilli MRS agar and broth and supplies nitrogen, carbon, and other elements necessary for growth. This mixture was used as a carrier for all other groups that were not given Bene-Bac Pet powder and to deter the taste for those given vancomycin.

Bene-Bac Feeding

Bene-Bac is a concentrated source of live naturally occurring digestive bacteria found in the intestinal tract. Its ingredients are LAB including 10 million colony forming units per gram (*Lactobacillus fermentum, Enterococcus faecium,*...
*Lactobacillus plantarum, Lactobacillus acidophilus*. It is available in powder or gel form. Bene-Bac Pet powder contains live, naturally occurring microorganisms and is recommended anytime animals experience changing nutritional or environmental conditions. These conditions include birth, post surgery, weaning, worming, traveling, and antibiotic therapy [2]. In order to make the liquid solution, ~1 g of Bene-Bac powder was added to 800 microliters of deionized water. Bene-Bac Pet powder was used as positive control to test against vancomycin. Due to Bene-Bac Pet powder and vancomycin being given to the second experimental group, another control group (control group #2) was made to examine the effects of Bene-Bac Pet powder on its own. The rats were fed via feeding needle.

**Vancomycin Feeding**

In the animal model of rheumatoid arthritis, vancomycin was shown to reduce the disease scores of adjuvant-induced arthritis in the Lewis male rat [1]. The researchers also noted an increase in the lactobacilli but no other increases as compared with the controls. To examine the effects on vancomycin on the intestinal bacteria, rats in both experimental groups were fed ~65 mg/kg of the vancomycin solution via feeding needle with the exception of the second experimental group that received the antibiotic two hours after administration of the Bene-Bac solution. Vancomycin solution was made by adding 1 g of vancomycin to 7.5 ml of deionized water.
Results

The most widely used probiotics include lactic acid bacteria, specifically *Lactobacillus* and *Bifidobacterium* species. Probiotics exert their beneficial effects through various mechanisms, including lowering intestinal pH, decreasing colonization and invasion by pathogenic organisms, and modifying the host immune response [4]. Vancomycin has been used for more than half a century and has been effective against Gram-positive bacteria when treating human infections. Numerous studies have been performed to test the effectiveness of vancomycin as well as the ability of the body to recover after such a treatment. A retrospective study (1993-2003) study of the epidemiology of *Clostridium difficile* colitis confirmed there had been an increase in the severity of this infection and showed that the use of vancomycin as primary treatment was associated with a lower frequency of progression to complicated colitis and/or death within 30 days after diagnosis, compared with metronidazole [23]. For this study, Lewis male rats were used and through their fecal matter it was confirmed that probiotic species such as lactobacilli were present in the intestinal tract. Throughout the course of the experiment, it was noted that rats in the experimental groups as well as control #2 had much softer feces. It is assumed that this is due to the probiotic as well as the antibiotic effects. It is also assumed that probiotics soften the stools by stimulating water and electrolyte secretion [13]. On day 3 of the project study, plating had to be repeated due to the counts of all the samples equaling zero. The second data values (counted 9/30/09) were used. In the project study, for control group #1, there was a slight increase in fecal bacteria but growth started to decline over the course of the
experiment (Figure 2a). In control group #2, there was increase in lactobacilli, which also declined but increased after the conclusion of the study (Figure 2b). For the experimental groups, experimental group #1, which received just vancomycin, followed a pattern similar to Bene-Bac control group #2; demonstrating an increase in gram positive bacteria followed by an initial decrease and an increase upon conclusion of the experiment (Figure 2c). However, vancomycin counts were greater than Bene-Bac and significantly greater by day 6 (see Table 6). Experimental group #2, Bene-Bac with Vancomycin, displayed counts similar to the Bene-Bac control group #2 with as drastic a decrease in counts at day 9 (Figure 2d). A comparison of all groups is available in Figure 2e.

Confirmation of gram-positive bacteria was aided through the use of MRS and TJA plates that promotes the growth of Lactobacillus (table 3). Observation of the colonies and the Gram-staining procedure confirmed Gram-positive bacteria. Pictures were taken of the slides and compared to other images in order to confirm the presence of Lactobacillus and Streptococcus [24](Figure 4).

**Experimental study**

In the experimental study, both control groups reached peak counts by day 6 (Figure 3a-b). However, the Bene-Bac group maintained counts and it remained fairly steady over the course of the experiment and after the final feeding of Bene-Bac powder solution (Figure 3b). In the experimental groups, there was a further increase in lactobacilli in the fecal matter of the Lewis male rat during the duration of the 10-day feeding trial (Figure 3c-d). With vancomycin, experimental group #1
maintaining this increase even after the last collection of fecal matter two days after
the feeding of the vancomycin solutions (Figure 3c), while the amount of lactobacilli
dropped slightly in the second experimental group (Figures 3d). Figure 3e provides
a comparison of all four groups.

In many cases, at the 1:100 serial dilutions, plates were shown to have over
growth. Diluting samples did lower counts and was able to show elimination of
increased counts (Figure 3f-g). In both experimental groups, there was a greater
increase in the growth of bacteria. This data could be supported by the fact that
experimental group #1 received the Bene-Bac powder solution alone that in turn,
greatly induced the growth lactobacilli due to its ingredients. Therefore, in
experimental group #2, it could be assumed that although the group received the
Bene-Bac solution initially, once they received the antibiotic treatment of
vancomycin, there would be a significant decrease in the growth of lactobacilli. This
would also be assumed for the group that received vancomycin alone. However, for
each group, the data shows there was an increase. It appears as though the
vancomycin does not inhibit the growth of LAB and has a positive interaction both
with and without the Bene-Bac solution on the growth of LAB in the intestinal tract
of the Lewis male rat. Confirmation of gram-positive bacteria was achieved using
gram stain for the Experimental study (Figure 5).

It should also be noted that the there were differences in the average final
weights of the rats in each group. The average weight loss of each group is as
follows: (1) control #1 = 4.7 g, (2) control #2 = ~25.3 g, (3) experimental #1 = ~22.8
g and (4) experimental #2 = ~37.8. The data appears to show that with the treatment of the antibiotic vancomycin, the rats showed a greater weight loss. With these overall results, it appears that vancomycin does in fact aid in the induction of lactobacilli in the digestive tract. The Bene-Bac Pet powder was given in order to induce lactobacilli growth and as an aid in the antibiotic treatment of the vancomycin. The treatment of both Bene-Bac powder and vancomycin did increase the growth of lactobacilli but not as great as experimental group #1. The lactobacilli appeared to have remained steady in the gastrointestinal tract.
Discussion

Scientific understanding of probiotics and their effectiveness in preventing illnesses is an ever-growing study. Lactic acid bacteria have been the focus of studies since the beginning of the 20th century. Their benefits include: improving intestinal tract health, enhancing the immune system, synthesizing and enhancing the bioavailability of nutrients, reducing symptoms of lactose intolerance, decreasing the prevalence of allergy in susceptible individuals, and reducing risks of cancers. It has been shown that fecal levels of specific strains of lactic acid bacteria increase after ingestion [4]. There are numerous studies being conducted around the world on examining what other benefits are available from their use.

Vancomycin has been the generally used antibiotic in the treatment of such illnesses as C. difficile and the emerging Staphylococcus aureus (MRSA). It has been studied that recent treatments of vancomycin have begun to fail due to the presence of vancomycin-resistant subpopulations of MRSA strains that seem to be vancomycin susceptible [20].

The focus of this study was to examine the ability of lactobacilli to recover and grow in the intestinal tract after treatment of the antibiotic vancomycin. The Lewis male rat was used in the induced lactobacilli model. The biological and clinical importance of bacteria resident in the gut is becoming increasingly recognized. The human gut microflora may be considered as very relevant to an improvement in host health [8]. Though there are numerous studies examining how lactobacilli can survive in the intestinal tract, it is still difficult to prove if they actually can survive.
Lactobacilli have been shown to have beneficial effects in the alleviation of many human conditions. Lactobacilli capable of surviving in the stomach for extended periods of time will be more aciduric, ensuring not only that more cells survive gastric transit to reach the intestine, but also allowing for greater survival and shelf life in fermented dairy products [16].

In the project study, all groups reached a peak in LAB counts by day 6 (Figure 2). Looking at the groups individually, there was a significant increase in LAB growth for both control group #1 (corn syrup) and experimental group #1 (vancomycin) by day 3 (Table 7). However, these groups were also significantly different from one another by day 3 with the vancomycin with a significantly greater degree of growth (Table 6). The experimental data of this study showed that there was an increase in the growth of LAB in the experimental groups as well as control group #2. Control #1 group also produced fecal matter containing lactobacilli. But this could be due to the fact that lactobacilli are naturally found in the gastrointestinal tract. In control group #2, the rats were fed the Bene-Bac powder solution that is made to induce the growth of lactobacilli species as well as others. The increased growth of bacteria was expected in this group. The amount of bacteria per ml was calculated for each plate of each sample. These results also helped to determine if there were lactobacilli present. Typical numbers of endogenous fecal lactobacilli in health range between $10^5$ and $10^8$ CFU (colony forming units) g of feces. In order to attain a fecal population level within this range could be considered as a minimum measure of gastrointestinal relevance for a
probiotic *Lactobacillus* strain [5]. In this study, all the results showed a range of $10^4$-$10^8$ range.

In the experimental study, all four groups were significantly different from each other by day 12 of the study (Table 8), however, the Bene-Bac/vancomycin failed to reach significance over the initial value (Table 9). The reason for this may be due to the higher LAB value at the beginning of the experiment since the group did reach the saturation count value during the experiment.

Control group #1 (dextrose/maltodextrin) and experimental #1 (vancomycin) were significantly different from one another by day 12. Control group #1 (dextrose/maltodextrin) and experimental group #2 (Bene-Bac/vancomycin) were significantly different from one another by day 3 and continued to decrease by the end. Control #1 and experimental #1 were significantly different from the other groups by day 6 when examining them individually (Table 8).

The p values of the serial dilutions were also performed (Tables 10 and 11). In the 1:10000 dilution groups, control group #1 and the experimental group #1 were significantly different from each other by day 9. Control #1 was also significantly different from experimental #2 by day 1 and continued to decrease over the experiment. Control #2 and experimental #2 were significantly different from each other by day 6 and continued to decrease. Looking at the groups individually, control #1 reached significance by day 3 and continued to decrease, experimental #1 and #2 both had significance by day 9.
In the 1:100000 serial dilutions (Tables 12 and 13), all four groups were significantly different by day 3 and both experimental groups were different from each other by day 6. There were no significant changes for each group for each day of the study. In the experimental groups, it was believed that the vancomycin would deter the growth and decrease the amount of bacteria in the fecal matter. Oral vancomycin was not found to be absorbed in the gastrointestinal tract and is eliminated unchanged in the feces. Due to this finding, it could be possible that vancomycin increased and helped the induction of lactobacilli already present in the intestine of the rat, especially in the group that received vancomycin alone. In the experimental #2 group, the rats received the Bene-Bac powder solution as well as the vancomycin solution. This group showed a steady increase in the lactobacilli growth. By observing the results of control group #1, it can be seen there was a moderate range of growth of the lactobacilli. The vancomycin group (experimental group #1) had the highest growth overall among all groups. These results agree with data found in the Nieuwenhuis et al study [1]. Having increased numbers in control #2 group and experimental #1 group, then it can be assumed that the last experimental group results with both Bene-Bac and vancomycin would have doubled, but the data shows that there was no apparent doubling of counts. Experimental group #2 shows a steady increase in the growth of lactobacilli and remained steady throughout the course of the experiment. After repeating the study both times, it appears as though the Lewis rat has a high rate of lactobacilli already present. This conclusion arose from the amount of lactobacilli present after the initial collections in both the project and experimental studies.
Since probiotics contain live microorganisms, concurrent administration of antibiotics could kill a large number of the organisms, reducing the efficacy of the *Lactobacillus* and *Bifidobacterium* species. Patients are instructed to separate administration of antibiotics from bacteria-derived probiotics for at least two hours [7]. The previous assumption of vancomycin being able to decrease the lactobacilli within the gastrointestinal tract was disproven showing that it had the ability to increase the growth. This may suggest that LAB in Lewis rats is a resistant strain. In a study by Johnson et al. it was believed that lactobacilli resistant to vancomycin are developing [3]. However, it appears that the vancomycin aids in the induction of lactobacilli and supports the study by Nieuwenhuis et al., 2000, where an increase of lactobacilli in the intestine of the Lewis rat was noted [1].

One of the most beneficial effects of probiotics is their ability to stabilize the balance of the gut flora. A probiotic can typically contain several billion microorganisms to increase the chance that adequate gut colonization can occur. The study of probiotics and their interaction within the gastrointestinal tract is one of importance within developing and even poorly developing countries. Lactic acid bacteria have benefitted our lives in numerous ways. Scientific research and trials conducted today are in hope of finding new ideas and uses for the LAB. Vancomycin is still an important antibiotic that has saved many lives. Its treatment towards illnesses is continually changing. The rise in vancomycin-resistant diseases has prompted more examination in the increased use of alternative medications [20]. In high-risk cases, vancomycin is still the most common treatment. The future of probiotics is limitless and expanding more. In order to achieve that goal, future
research needs to encompass better-designed clinical trials in larger populations and for longer durations. Only then will we be able to fully appreciate the possibilities of microorganisms and their abilities.

**Future Directions**

Future directions should include more plate samples of all the dilutions in the experiment. More samples of the serial dilutions should be plated so that it can be assured that the methods performed were done accurately. There also should be more media types to compare the induced lactobacilli growth with. If there were more media types, there would be an even more accurate identification of the bacteria found in the fecal matter. Being able to identify if vancomycin existed in the fecal samples would be able to show that vancomycin is completely eliminated unchanged in the feces; further proving that vancomycin decreased the lactobacilli growth in the fecal matter.
References


24. [http://serc.carleton.edu/microbelife/research_methods/microscopy/gramstaining](http://serc.carleton.edu/microbelife/research_methods/microscopy/gramstaining)

Figure 1. Probiotics flow chart.

Flow chart explaining the various health benefits from probiotics consumption. Probiotics are available to consumers in the forms of capsules, tablets, packets, or powders and are found most commonly in yogurt or dairy drinks. Figure modified from Probiotics by N. Williams [7].
Table 1. Project study set-up with 3 Lewis male rats in each group.

Project study set-up shows which rat group received Corn Syrup solution, Bene-Bac solution, and Vancomycin. With Experimental #1 and #2 groups, rats were given the vancomycin along with the dextrose/maltodextrin solution in order to deter the taste of the antibiotic. The Experimental #2 group however, was given the Bene-Bac powder solution two hours prior to receiving the vancomycin solution.

<table>
<thead>
<tr>
<th></th>
<th>Corn Syrup 200 microliters</th>
<th>Bene-Bac Powder Solution 0.25 g</th>
<th>Vancomycin ~65 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control #1</td>
<td>+</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Control #2</td>
<td>_</td>
<td>+</td>
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</tr>
<tr>
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<td>+</td>
<td>_</td>
<td>+</td>
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<tr>
<td>Experimental #2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lewis Male Rats</td>
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<td>Bene-Bac Powder Solution</td>
<td>Vancomycin</td>
</tr>
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<td>-------------------------------</td>
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<td>------------</td>
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<tr>
<td></td>
<td>200 microliters</td>
<td>0.25 g</td>
<td>~65 mg/kg</td>
</tr>
<tr>
<td>Control #1</td>
<td>+</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Control #2</td>
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<tr>
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</tr>
<tr>
<td>Experimental #2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2. Experimental set-up with 3 Lewis male rats in each group.

Experiment set-up shows which rat group received Dextrose/Maltodextrin solution, Bene-Bac solution, and Vancomycin. With Experimental #1 and #2 groups, rats were given the vancomycin along with the dextrose/maltodextrin solution in order to deter the taste of the antibiotic. The Experimental #2 group however, was given the Bene-Bac powder solution two hours prior to receiving the vancomycin solution.
<table>
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<th>*9/30/09</th>
<th>10/1/09</th>
<th>10/3/09</th>
<th>10/5/09</th>
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</thead>
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<td>Control #2</td>
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<td>5.76 x 10^4</td>
<td>4.56 x 10^4</td>
<td>2.68 x 10^4</td>
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<tr>
<td>Experimental #1</td>
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<td><strong>8.0 x 10^4</strong></td>
<td><strong>8.0 x 10^4</strong></td>
<td>1.76 x 10^4</td>
<td>3.68 x 10^4</td>
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<td>Experimental #2</td>
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<td>7.58 x 10^4</td>
<td>5.7 x 10^4</td>
<td><strong>8.0 x 10^4</strong></td>
<td>2.8 x 10^4</td>
</tr>
</tbody>
</table>

*Samples collected on 9/27/09 were unable to be used due to the fact all counts amounted to zero. Solutions were plated again and placed in incubator. Counts were determined on 9/30/09.

** These numbers represent plates that had colony counts >400 and was given this automatic result.

**Table 3. Project study set showing number of bacteria/ml calculated after colony counts on MRS media.**

Data showing results of colony counts of fecal matter from all four groups for each collection date. Fifty microliters of solutions (1:100) were plated on MRS agar plates and placed in incubator for a total of 48 hours. After incubation plates were removed and the numbers of resulting colonies were counted (The maximum count was 400). The bacteria per mL were determined using the equation: 

\[
\text{#bacteria/mL} = \left(\frac{\text{dilution factor} \times \text{(#of colonies)}}{\text{amount plated}}\right)
\]

Lactobacilli were the main interest of the colony counts and were confirmed by their growth on the MRS agar plates and the gram staining procedure.
<table>
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<th>1/27/10</th>
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<th>2/2/10</th>
<th>2/5/10</th>
<th>2/8/10</th>
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<tbody>
<tr>
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<td>8.0 x 10^6</td>
<td>7.0 x 10^5</td>
<td>5.6 x 10^5</td>
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<tr>
<td>Control #2</td>
<td>1.0 x 10^5</td>
<td>4.0 x 10^4</td>
<td>6.12 x 10^6</td>
<td>2.0 x 10^6</td>
<td>2.34 x 10^6</td>
</tr>
<tr>
<td>Experimental #1</td>
<td>8.0 x 10^4</td>
<td>3.0 x 10^4</td>
<td>6.7 x 10^6</td>
<td>*8.0 x 10^6</td>
<td>3.1 x 10^5</td>
</tr>
<tr>
<td>Experimental #2</td>
<td>2.0 x 10^4</td>
<td>1.6 x 10^5</td>
<td>2.14 x 10^6</td>
<td>*8.0 x 10^6</td>
<td>1.16 x 10^6</td>
</tr>
</tbody>
</table>

*These numbers represent plates that had colony counts >400 and was given this automatic result

**Table 4. Experimental set showing number of bacteria/ml calculated after colony counts on MRS media.**

Data showing results of colony counts of fecal matter from all four groups for each collection date. Fifty microliters of solutions (1:100, 1:10000, 1:100000) were plated on MRS agar plates and placed in incubator for a total of 48 hours. After incubation plates were removed and the numbers of resulting colonies were counted (The maximum count was 400). The bacteria per mL were determined using the equation: #bacteria/mL = [(dilution factor) X (#of colonies)]/amount plated. Lactobacilli were the main interest of the colony counts and were confirmed by their growth on the MRS agar plates and the gram staining procedure.
Table 5. Experimental set showing number of bacteria/ml calculated after colony counts on TJA media.

Data showing results of colony counts of fecal matter from all four groups for each collection date. Fifty microliters of solutions (1:100) were plated on TJA agar plates and placed in incubator for a total of 48 hours. After incubation plates were removed and the numbers of resulting colonies were counted (The maximum count was 400). The bacteria per mL were determined using the equation: \#bacteria/mL = [(dilution factor) X (#of colonies)]/amount plated. Lactobacilli were the main interest of the colony counts and were confirmed by their growth on the TJA agar plates and the gram staining procedure.
Figures 2 A-B. *Project study set colony counts.*

Experimental figures depicting the growth of lactobacilli colonies over the course of the 12-day experimental study. Fecal samples were collected from each subject prior to initial treatment and then once every three days (n=5 samples per rat). Samples were cultured, plated, and then placed in incubator for 48 hours. After incubation, colonies present were counted and averaged. Figure A-Corn Syrup. Figure B-Bene-Bac Pet Powder. For day 3 of the project study plating was repeated since no data could be obtained from the first plating results (Counts = 0). These numbers listed are the second data values.
Figures 2 C-D. Project study set colony counts.

Experimental figures depicting the growth of lactobacilli colonies over the course of the 12-day experimental study. Fecal samples were collected from each subject prior to initial treatment and then once every three days (n=5 samples per rat). Samples were cultured, plated, and then placed in incubator for 48 hours. After incubation, colonies present were counted and averaged. Figure C-Vancomycin. Figure D-Bene-Bac Pet Powder/Vancomycin. For day 3 of the project study plating was repeated since no data could be obtained from the first plating results (Counts = 0). These numbers listed are the second data values.
Figure 2 E. Comparison graph of project study results.

Experimental figure depicting the averages of both control groups and experimental groups of the project study. Fecal samples were collected from each subject prior to initial treatment and then once every three days (n=5 samples per rat). Samples were cultured, plated, and then placed in incubator for 48 hours. After incubation, colonies present were counted and averaged. For day 3 of the project study plating was repeated since no data could be obtained from the first plating results (Counts = 0). These numbers listed are the second data values.
Figures 3 A-B. Experimental set colony counts.

Experimental figures depicting the growth of lactobacilli colonies over the course of the 12-day experimental study. Fecal samples were collected from each subject prior to initial treatment and then once every three days (n=5 samples per rat). Samples were cultured, plated, and then placed in incubator for 48 hours. After incubation, colonies present were counted and averaged. Figure A-Dextrose/Maltodextrin. Figure B-Bene-Bac Pet Powder.
**Figure 3 C-D. Experimental set colony counts.**

Experimental figures depicting the growth of lactobacilli colonies over the course of the 12-day experimental study. Fecal samples were collected from each subject prior to initial treatment and then once every three days (n=5 samples per rat). Samples were cultured, plated, and then placed in incubator for 48 hours. After incubation, colonies present were counted and averaged. Figure C-Vancomycin. Figure D-Bene-Bac Pet Powder/Vancomycin.
Figure 3 E. Comparison graph of experimental study results.

Experimental figure depicting the averages of the 1:100 serial dilutions of both control groups and experimental groups of the experimental study. Fecal samples were collected from each subject prior to initial treatment and then once every three days (n=5 samples per rat). Samples were cultured, plated, and then placed in incubator for 48 hours. After incubation, colonies present were counted and averaged.
**Figure 3 F.** *Comparison graph of 1:10000 experimental study results.*

Experimental figure depicting the averages of the 1:10000 serial dilutions of both control groups and experimental groups of the experimental study. Fecal samples were collected from each subject prior to initial treatment and then once every three days (n=5 samples per rat). Samples were cultured, plated, and then placed in incubator for 48 hours. After incubation, colonies present were counted and averaged.
**Figure 3 G.** Comparison graph of experimental study results.

Experimental figure depicting the averages of the 1:100000 serial dilutions of both control groups and experimental groups of the experimental study. Fecal samples were collected from each subject prior to initial treatment and then once every three days (n=5 samples per rat). Samples were cultured, plated, and then placed in incubator for 48 hours. After incubation, colonies present were counted and averaged.
Figures 4 A-B. *Pictures taken of slides of project study gram-positive bacteria.*

Pictures showing gram-positive bacteria present in fecal samples. After culturing and plating, samples were spread and fixed on sterile glass slides. Gram staining was performed to identify lactobacilli species on the selective media. Pictures were taken microorganisms present. Figure A (Control #1) Corn Syrup. Figure B (Control #2) Bene-Bac Pet Powder.
Pictures showing gram-positive bacteria present in fecal samples. After culturing and plating, samples were spread and fixed on sterile glass slides. Gram staining was performed to identify lactobacilli species on the selective media. Pictures were taken of microorganisms present. Figure C (Experimental #1) Vancomycin. Figure D (Experimental #2) Bene-Bac Pet Powder/Vancomycin.
Figures 5 A-B. Pictures taken of slides of experimental study gram-positive bacteria.

Pictures showing gram-positive bacteria present in fecal samples. After culturing and plating, samples were spread and fixed on sterile glass slides. Gram staining was performed to identify lactobacilli species on the selective media. Pictures were taken microorganisms present. Figure A (Control #1) Dextrose/Maltodextrin. Figure B (Control #2) Bene-Bac Pet Powder.
Figures 5 C-D. Pictures taken of slides of study gram-positive bacteria.

Pictures showing gram-positive bacteria present in fecal samples. After culturing and plating, samples were spread and fixed on sterile glass slides. Gram staining was performed to identify lactobacilli species on the selective media. Pictures were taken of microorganisms present. Figure C (Experimental #1) Vancomycin. Figure D (Experimental #2) Bene-Bac Pet Powder/Vancomycin.
### Table 6. *P* values of both control and experimental groups in the project study.

After colony counts were performed, the numbers were averaged for each subject of each group. ANOVA was performed by comparing all groups as one and then comparing each group to one another in order to determine if there were any significant changes throughout the course of the study. For day 3 of the project study plating was repeated since no data could be obtained from the first plating results (Counts = 0). These numbers listed are the second data values.

<table>
<thead>
<tr>
<th>P values of Project Study (1:100)</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1,C2,E1,E2</td>
<td>0.544761</td>
<td>0.109364</td>
<td>0.013286*</td>
<td>0.348257</td>
<td>0.166389</td>
</tr>
<tr>
<td>C1 vs. C2</td>
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<td>0.0727</td>
<td>0.098575</td>
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<td>0.145273</td>
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<tr>
<td>C1 vs. E1</td>
<td>0.161743</td>
<td>0.009111*</td>
<td>1.3 x 10^{-6}</td>
<td>0.754228</td>
<td>0.019937*</td>
</tr>
<tr>
<td>C1 vs. E2</td>
<td>0.483371</td>
<td>0.21055</td>
<td>0.229168</td>
<td>0.274004</td>
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</tr>
<tr>
<td>C2 vs. E1</td>
<td>0.094026</td>
<td>0.120149</td>
<td>0.00397*</td>
<td>0.987521</td>
<td>0.621428</td>
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<tr>
<td>C2 vs. E2</td>
<td>0.387276</td>
<td>0.813093</td>
<td>0.653994</td>
<td>0.318088</td>
<td>0.946411</td>
</tr>
<tr>
<td>E1 vs. E2</td>
<td>0.870376</td>
<td>0.470385</td>
<td>0.124646</td>
<td>0.313902</td>
<td>0.507443</td>
</tr>
</tbody>
</table>

*Indicates significance related to *p* = 0.02
<table>
<thead>
<tr>
<th>P values of all 1:100 groups (p= 0.02)</th>
<th>Corn Syrup (Control #1)</th>
<th>Bene-Bac Pet Powder (Control #2)</th>
<th>Vancomycin (Experimental #1)</th>
<th>Bene-Bac Pet Powder/Vancomycin (Experimental #2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 vs. Day 3</td>
<td>0.008983*</td>
<td>0.031338</td>
<td>0.004069*</td>
<td>0.394509</td>
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<tr>
<td>Day 0 vs. Day 6</td>
<td>0.026106*</td>
<td>0.031074</td>
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</tr>
<tr>
<td>Day 0 vs. Day 9</td>
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<td>Day 0 vs. Day 12</td>
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*Indicates significance related to p = 0.02

Table 7. P values of both control and experimental groups in the project study.

After colony counts were performed, the numbers were averaged for each subject of each group. ANOVA was performed for each group according to each day in order to determine if possible what day any significant changes occurred throughout the course of the study. For day 3 of the project study plating was repeated since no data could be obtained from the first plating results (Counts = 0). These numbers listed are the second data values.
Table 8. P values of both control and experimental groups in the experimental study.

After colony counts were performed, the numbers were averaged for each subject of each group. ANOVA was performed by comparing all groups as one and then comparing each group to one another in order to determine if there were any significant changes throughout the course of the study.

<table>
<thead>
<tr>
<th>P values of Experimental Study (1:100)</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
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<tr>
<td>C1,C2,E1,E2</td>
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<tr>
<td>C1 vs. E2</td>
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*Indicates significance related to p = 0.02
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<th>Bene-Bac Pet Powder (Control #2)</th>
<th>Vancomycin (Experimental #1)</th>
<th>Bene-Bac Pet Powder/Vancomycin (Experimental #2)</th>
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<tr>
<td>Day 0 vs. Day 9</td>
<td>0.169489</td>
<td>0.155099</td>
<td>1.3 x 10^{-6}*</td>
<td>0.000199*</td>
</tr>
<tr>
<td>Day 0 vs. Day 12</td>
<td>0.308494</td>
<td>0.243173</td>
<td>2.03 x 10^{-6}*</td>
<td>0.373901</td>
</tr>
</tbody>
</table>

*Indicates significance related to p = 0.02

**Table 9.** *P* values of both control and experimental groups in the experimental study.*

After colony counts were performed, the numbers were averaged for each subject of each group. ANOVA was performed for each group according to each day in order to determine if possible what day any significant changes occurred throughout the course of the study.
Table 10. *P values of both control and experimental groups 1:10000 serial dilutions in the experimental study.*

After colony counts were performed, the numbers were averaged for each subject of each group. ANOVA was performed by comparing all groups as one and then comparing each group to one another in order to determine if there were any significant changes throughout the course of the study.

<table>
<thead>
<tr>
<th>P values of Experimental Study (1:10000)</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1,C2,E1,E2</td>
<td>0.034759</td>
<td>0.470877</td>
<td>0.463426</td>
<td>2.68 $\times 10^{-10}$</td>
<td>0.398963</td>
</tr>
<tr>
<td>C1 vs. C2</td>
<td>0.003093</td>
<td>0.125956</td>
<td>0.565542</td>
<td>0.737748</td>
<td>0.275389</td>
</tr>
<tr>
<td>C1 vs. E1</td>
<td>0.231794</td>
<td>0.125846</td>
<td>0.499024</td>
<td>2.37 $\times 10^{-6}$*</td>
<td>0.249458</td>
</tr>
<tr>
<td>C1 vs. E2</td>
<td>0.003612*</td>
<td>0.591304</td>
<td>0.597603</td>
<td>2.37 $\times 10^{-6}$*</td>
<td>0.004142*</td>
</tr>
<tr>
<td>C2 vs. E1</td>
<td>0.370753</td>
<td>1</td>
<td>0.16666</td>
<td>5.91 $\times 10^{-6}$*</td>
<td>0.816599</td>
</tr>
<tr>
<td>C2 vs. E2</td>
<td>0.324387</td>
<td>0.355917</td>
<td>0.022854*</td>
<td>5.91 $\times 10^{-6}$*</td>
<td>0.851841</td>
</tr>
<tr>
<td>E1 vs. E2</td>
<td>0.405297</td>
<td>0.355908</td>
<td>0.110112</td>
<td>#NUM!</td>
<td>0.524044</td>
</tr>
</tbody>
</table>

*Indicates significance related to p = 0.02
<table>
<thead>
<tr>
<th>P values of all 1:10000 groups (p = 0.02)</th>
<th>Dextrose/Maltodextrin Group (Control #1)</th>
<th>Bene-Bac Pet Powder (Control #2)</th>
<th>Vancomycin (Experimental #1)</th>
<th>Bene-Bac Pet Powder/Vancomycin (Experimental #2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 vs. Day 3</td>
<td>0.013205*</td>
<td>0.228229</td>
<td>0.355743</td>
<td>0.397514</td>
</tr>
<tr>
<td>Day 0 vs. Day 6</td>
<td>0.767769</td>
<td>0.288701</td>
<td>0.042372</td>
<td>0.124567</td>
</tr>
<tr>
<td>Day 0 vs. Day 9</td>
<td>0.005345*</td>
<td>0.185993</td>
<td>0.007214*</td>
<td>7.85 x 10^{-8}</td>
</tr>
<tr>
<td>Day 0 vs. Day 12</td>
<td>0.005315*</td>
<td>0.123792</td>
<td>0.815155</td>
<td>0.00083*</td>
</tr>
</tbody>
</table>

*Indicates significance related to p = 0.02

**Table 11.** *P values of both control and experimental groups 1:10000 serial dilutions in the experimental study.*

After colony counts were performed, the numbers were averaged for each subject of each group. ANOVA was performed for each group according to each day in order to determine if possible what day any significant changes occurred throughout the course of the study.
<table>
<thead>
<tr>
<th>P values of Experimental Study (1:100000)</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1,C2,E1,E2</td>
<td>0.700566</td>
<td>0.020332*</td>
<td>0.162704</td>
<td>0.028495</td>
<td>0.087043</td>
</tr>
<tr>
<td>C1 vs. C2</td>
<td>0.045068</td>
<td>0.070415</td>
<td>0.455408</td>
<td>0.976681</td>
<td>0.249399</td>
</tr>
<tr>
<td>C1 vs. E1</td>
<td>0.953026</td>
<td>0.070415</td>
<td>0.416039</td>
<td>0.53645</td>
<td>0.812508</td>
</tr>
<tr>
<td>C1 vs. E2</td>
<td>0.956733</td>
<td>0.076505</td>
<td>0.318365</td>
<td>0.069447</td>
<td>0.030576</td>
</tr>
<tr>
<td>C2 vs. E1</td>
<td>0.357871</td>
<td>#NUM!</td>
<td>0.072466</td>
<td>0.491701</td>
<td>0.283268</td>
</tr>
<tr>
<td>C2 vs. E2</td>
<td>0.361069</td>
<td>0.373901</td>
<td>0.895606</td>
<td>0.066633</td>
<td>0.471792</td>
</tr>
<tr>
<td>E1 vs. E2</td>
<td>0.997305</td>
<td>0.373901</td>
<td>0.001884*</td>
<td>0.08802</td>
<td>0.036729</td>
</tr>
</tbody>
</table>

*Indicates significance related to p = 0.02

**Table 12.** P values of both control and experimental groups 1:1000000 serial dilutions in the experimental study.

After colony counts were performed, the numbers were averaged for each subject of each group. ANOVA was performed by comparing all groups as one and then comparing each group to one another in order to determine if there were any significant changes throughout the course of the study.
Table 13. *P values of both control and experimental groups 1:10000 serial dilutions in the experimental study.*

After colony counts were performed, the numbers were averaged for each subject of each group. ANOVA was performed for each group according to each day in order to determine if possible what day any significant changes occurred throughout the course of the study.