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PROBIOTICS LACTOBACILLUS PLANTARUM 299V AND BIFIDOBACTERIA B107 IN THE PREVENTION OF HOSPITAL ASSOCIATED INFECTIONS: A RETROSPECTIVE COHORT STUDY.

by

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Abstract

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Hospital acquired infections (HAI) are associated with increased mortality, morbidity and costs. Medical Intensive Care Unit (MICU) patients have increased risk for HAI. Probiotics may be beneficial in reducing HAI. A retrospective cohort study was completed to determine the effect of food form probiotics in the prevention of HAI among MICU patients. Patients received probiotic containing 50 billion colony forming units daily for periods of 24 hours to 93 days. Infection rates three years prior, eight months during and one year post probiotic implementation were collected and compared with incidence of HAI in the same patient population. Significant differences in the incidence of central line associated bloodstream infection, ventilator associated pneumonia and Clostridium *difficile* associated diarrhea (CDAD) were indicated between control one, the experimental group, and control two using Chi square analysis. Food form probiotics were determined to be effective at reducing rates of CDAD in MICU patients.

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CHAPTER 1: INTRODUCTION

Hospital acquired infections (HAI) are of great concern because such infections are associated with increased mortality, morbidity and financial costs (5). Ventilator associated pneumonia (VAP), Clostridium *difficile* associated diarrhea (CDAD), antibiotic associated diarrhea (AAD), and central line associated bloodstream infections (CLABSI) are the most costly HAI (42). Annually, 1.7 million HAIs are reported in United States hospitals and 100,000 deaths occur annually in patients with HAIs (14). HAIs are estimated to cost 17-20 billion dollars each year (14). Patients, particularly the elderly, in the Medical Intensive Care Unit (MICU) are at a higher risk for developing these HAI infections because they are typically sicker than their younger counterparts and require multiple interventions (25). These interventions include broad spectrum antibiotics, gastric acid suppressants, and other medications that alter gut mobility (25). This altered gut mobility may lead to decreased host defense and colonization by pathogenic bacteria, which in turn leads to increased risk of infection (5). Although many antibiotic therapies have been studied in decreasing the infection rate of VAP, *CDAD*, AAD, and CLABSI, there is an increase in antibiotic resistance to these infections and there are few new antibiotics available for use (34). Probiotics may be beneficial in optimizing host immune defenses and minimizing more dangerous bacteria species involved in HAI, but unfortunately, they have not been heavily researched in the prevention of VAP, CDAD, and CLABSI (34).

Probiotics are living microorganisms that provide benefits to the host when ingested (34). They are able to survive in the human gastrointestinal tract (GIT) because of their ability to tolerate acid and bile (4). Probiotics have three functions: suppression

of growth or epithelial binding/invasion by pathogenic bacteria; improving the intestinal barrier function, causing production of protective cytokines and modulation of the immune system, suppressing intestinal pro-inflammatory cytokines (3). Benefits include decreased susceptibility to pathogens and shortened duration of infection (4). Probiotics are beneficial for not only gastrointestinal disorders, but also for respiratory, urogenital, oral health and allergic diseases (4). Research has shown when digestive flora is lost due to infection it can be restored by implementing probiotics into the gut (4). Because of this, HAI, infection rates may be reduce by the addition of probiotics into the human GIT (4).

One infection that may be reduced by probiotics is VAP. Prolonged mechanical ventilation (MV) has been found to cause VAP in most ICU patients; however, some patients develop pneumonia in less than 48 hours after intubation (34). VAP rates are higher for patients in ICUs than in general medicine wards (34). VAP involves colonization of the aerodigestive tract with pathogenic bacteria (34). "VAP is defined as an inflammation of the lung parenchyma caused by infectious agents not present or incubating at the time MV was started" (34). Probiotics may be able to reduce the incidence of VAP through multiple effects that optimize host immune defenses (34).

CDAD may also be reduced through probiotic implementation. *Clostrdium difficile* is the leading cause of diarrhea in hospitalized patients. In 2002, it was estimated that this disease cost over 1 billion dollars annually in hospitalized patients (3). Diarrhea is also more common among patients in the ICU because of broad spectrum antibiotic use, leading to AAD (3). Clostridium *difficile* is a bacterium that spreads through the fecal oral route (25). It is most always related to the use of antibiotics, which is a reason

to find an alternate therapy to treat this diarrhea. Other risk factors include age, enteral feeding tube use, and prior hospitalization (25). Diarrhea results in loss of fluid and electrolytes which negatively impacts nutritional status, wound healing and skin integrity (26).

CLABSI is a central line associated blood stream infection, but probiotics have not been well studied in its' prevention. Mishandling of intravenous catheters is the largest cause of this infection (33). One key way to avoid this infection is through proper placement of catheters (33). However, if the infection has already developed, there is a possibility that probiotics could help in the healing process of the infection as well.

VAP, *CDAD*, and CLABSI all increase morbidity and mortality in critically ill patients (25,33,25). It is important to find treatments that lower morbidity and mortality rates to decrease length of stay and prolong the lives of patients. Probiotics have potential to be of benefit to patients in the MICU who have these infections. For this reason, a retrospective study was performed to establish what, if any, effect probiotics have on the infection rate of MICU patients with CDAD, VAP, and CLABSI.

CHAPTER 2: LITERATURE REVIEW

Probiotics are an emerging area of research because of the effect they may have on hospital associated infections (HAI). Probiotics are, as defined by the World Health Organization, "viable microorganisms that, when ingested in a sufficient amount can be beneficial for health" (4). A microorganism is considered a probiotic if it can survive through the stomach's bile and acid and reach the intestines to be beneficial (4). There are many different strains and species of probiotics, all of which have varying effects on intestinal microbiota. The most common are Lactobacillus and Bifidobacteria species (4). This literature review will focus on probiotics' effect on Ventilator Associated Pneumonia (VAP), Antibiotic Associated Diarrhea (AAD), Clostridium *difficile* Associated Diarrhea (CDAD), and Central Line Associated Blood Stream Infection (CLABSI). Each of these HAI are costly and increase patient risk for mortality (4,42). In 2007, it was estimated that two-thirds of deaths from HAIs were caused by catheter associated blood stream infections (CABSI) (also known as CLABSI) and VAP, and these two infections are five times more deadly than any other HAI (42). VAP cases that are preventable range from 2.19 billion to 3.17 billion dollars annually (42). Probiotics have been shown to be effective in many different infections that are hospital associated (4).

Ventilator Associated Pneumonia

VAP occurs in 30% of patients on mechanical ventilation (5). VAP is defined as nosocomial pneumonia that usually develops after 48 hours of endotracheal intubation

(5). VAP is diagnosed either clinically or microbiologically. Microbiologically, the bacteria isolated most in VAP are gram negative organisms, but gram positive organisms are occasionally seen (5). The isolated organisms are directly related to hospital admission (5). Those patients in the ICU are at higher risk of getting VAP because they are more likely to require intubation (5). Once a patient is hospitalized, the oropharynx can become colonized by bacteria, and if a patient aspirates them, they enter the respiratory tract (37). If the airways become colonized by the bacteria, and it takes over natural host defenses, VAP occurs (37). Currently prevention mechanisms include avoiding intubation if possible, reducing time of mechanical ventilation, or using non invasive ventilation (37).

The studies using probiotics for reduction of VAP have had mixed results. In 2010, a study on prophylaxis of VAP using probiotics was published by Barraud et al and no clear recommendations were made (5). Patients were included if they were expected to be on ventilation for at least 2 days. This study used Ergyphilus® capsules that contained Lactobacillus *rhamnosus* GG, Lactobacillus *casei*, Lactobacillus *acidolphus*, and Bifidobacterium *bifidum*. All patients received the same enteral nutrition and the demographics of both the placebo and treatment group were similar. In this study, VAP was defined as, "a new and persistent infiltrate on chest radiograph associated with at least one of the following: purulent tracheal secretions, temperature 38.3˚C or higher, and a leukocyte count of 10,000 lL-1 or higher; and (b) positive quantitative cultures of distal pulmonary secretions obtained from bronchoalveolar lavage" (5). A total of 167 patients participated in the study done between 2006 and 2008, for 28 days. Mortality rates were not significantly different in the probiotic group (25.3%) and the placebo group (23.7%).

A decline in catheter related blood stream infections was noted in the probiotic group (1.84% vs 6.78%). However, probiotics were not shown to reduce VAP. This study also found that when probiotics were given to non severe sepsis patients, there was a higher risk of death. This was most likely due to poor hygiene practices leading to patients being exposed to other bacteria aside from the probiotics. Fortunately, there were no reported side effects caused by the probiotics in this study which is reason for their implementation. Ultimately the researchers concluded that probiotics should not be used in the critically ill patient because protective effects could not be determined and uncertainties still remain. Although this study did not find probiotics to be preventative against VAP, another study using ventilated patients did find that probiotic *Lactobacillus rhamnosus GG* reduced VAP (5, 35).

Morrow et al. conducted a prospective randomized double blind placebo controlled trial of the effect of probiotics in 146 mechanically ventilated patients (35). All participants required intubation for at least 72 hours and antibiotics were given at investigator discretion. The primary outcome was the reduction of VAP and the secondary outcome was the reduction of CDAD. In this study, *Lactobacillus rhamnosus* GG was selected and it was administered at 2×5^{10} CFU. The results showed that in this high risk population, probiotics were beneficial in preventing VAP. In the study, 33 out of 70 patients in the placebo group were clinically diagnosed with VAP and 17/68 in the treatment group were diagnosed. "Although VAP caused by gram-positive organisms did not differ between groups (12.8 vs. 5.8%; P50.16), cases of VAP caused by gram–negative organisms were dramatically different (22.8 vs. 8.8%; P , 0.02)" (35). The secondary outcomes of the study found that probiotics led to a delay in onset of

microbiologically confirmed VAP and reduced rates of CDAD. In the placebo group 18.6% of participants developed CDAD while only 5.8% in the treatment group developed the infection.

Morrow's study showed that probiotics can be beneficial in preventing VAP (35). It differed from the Barraud study, which could not conclude benefits, in the amount of probiotic administered and the type of probiotic. In the Morrow study, specifically Lactobacillus GG was used. Barraud et al. used a combination of three types of probiotics. Less CFU were used in the study that found reduction of VAP (5). Therefore, it is very possible that variations in the amount and type of probiotic impact the rates of VAP in critically ill patients as well as CDAD as evidenced by the two studies.

Antibiotic Associated Diarrhea and Clostridium *difficle* Associated Diarrhea

CDAD is a bacterium diarrhea caused by C.*difficile and C.difficile* is estimated to cause 10-25% of most AAD cases and annually, CDAD totals around 3.2 billion dollars to the health care industry in the US (22, 26). Once the bacterium is found in AAD, it becomes CDAD. Broad spectrum antibiotics are the main cause of AAD and CDAD, although advanced age and poor general condition also contribute (40). The standard treatment for *C. difficile* is discontinuation of the offending antibiotic and dietary maintenance, but these treatments are often ineffective in severe cases (40, 19). Even worse, about one in four patients who develop CDAD will relapse within 2 months (40, 19). Probiotics may reduce AAD and CDAD by suppressing pathogenic bacteria

colonization. While some studies have supported this claim, others could not draw conclusions (19).

Song et al. completed a study using probiotics on AAD that did not result significant findings (40). In 2010, the Journal of Korean Medical Sciences published their study on the effects of Lactobacillus (Lacidofil Cap®) on AAD. It was a prospective, randomized, double blind trial in patients with respiratory tract infections. Two-hundred and fourteen subjects passed screening but only 172 completed the trial. The subjects who were being given antibiotics were randomized into the placebo or Lactobacillus (Lacidofil Cap®) groups. Those participating in the study recorded their stool frequencies and consistency every day for 14 days. There were two potential outcomes, AAD-1 and AAD-2. AAD-1 was defined as, "loose or watery stools more than 3 times per day for at least 2 days within 14 days of enrollment" (40). AAD-2 consisted of, "loose or watery stools more than 2 times per day for at least 2 days within 14 days of enrollment" (40). Participants either received placebo or Lacidofil Cap. The Lacidofil Cap® contains, *L. rhamnosus* R0011, *L. acidophilus* R0052 bacterial culture (2×109 colony-forming units), maltodextrin, Mg stearate, and ascorbic acid. The placebo group received a tablet of maltodextrin, Mg stearate and ascorbic acid. Either the placebo or probiotic were given within 48 hours after the start of antibiotics. Each group received their specific capsule twice per day. The demographics and medical profiles of the subjects were similar in both groups. The antibiotics that were administered included cephalosporin, macrolides, fluoroquniolones, antituberculosis drugs, clindamycin and penicillin. There was no significance in the antibiotic selections. After analyzing the results, this study found that AAD-1 occurred in 3.9% of the study group and 7.2% in the

placebo group. AAD-2 occurred in 8.7% of the study group and 14.4% of the placebo group. Participants were only followed up for 2 weeks after treatment, but AAD has been shown to occur up to two months after antibiotic treatment. Although more people in the placebo group developed AAD, the study was limited by poor follow up. Therefore, probiotic use in AAD could not be definitively recommended based on the data.

The amount of probiotic needed to prevent AAD was not known when the previous study was completed, so another study, done by Gao et al. used a randomized double blind controlled trial to test different dosages of probiotics to determine what, if any, effect probiotics have on AAD and CDAD (18). Participants received either placebo, 50 billion CFU per day or 100 billion CFU per day. The CFU were in the form of Lactobacillus *acidolphus* and Lactobacillus *casei*. Each participant's respective dose was given within 36 hours of receiving antibiotics. The probiotic or placebo was received for 5 days and followed up for 21 days. Antibiotics that were administered included, clindamycin, penicillin, and cephalosporin. This study was conducted in older patients aged 50-70. Regardless of the treatment, each person received two pills to keep the study double blinded. The trial took place between January 2009 and March 2009 and found a distinct dose response relationship. Higher probiotic usage led to lower AAD. With CDAD, the dose response relationship was the same. Although higher doses of probiotics led to fewer incidences of CDAD and AAD, there were participants in each group who had AAD and CDAD. Twenty-three point eight (23.8) % of those in the placebo group were positive for CDAD, 9.4% who received 50 billion CFU were positive for CDAD, and only 1.2% who received 100 billion CFU were positive for CDAD. Both

doses of probiotic also led to shorter duration and symptoms of AAD and CDAD. This study only followed subjects for 21 days after treatment and AAD and CDAD can occur up to 8 weeks after antibiotic therapy. This study is only indicative of the dose effect of the certain strains that were used on CDAD and AAD. Therefore, other probiotics need to be studied to show their dosage requirements for AAD and CDAD.

One particular probiotic, Florajen,® was tested in veterans for efficacy against AAD in a pilot study at the Madison, Wisconsin Veterans Administration (39). Florajen®, which contains the probiotic Lactobacillus *acidolphus*, was started on the same day the antibiotics were started. After exclusion, 23 veterans were randomized to treatment and 17 received placebo. One in the placebo group withdrew for his own reasons. The amount of probiotic per capsule was not verified, but each capsule was said to contain 20 billion CFU, and the treatment group received three doses per day. The study found no major differences in clinical outcomes between the two groups. In contrast, Hickson et al found promising effects of probiotics (22). Their study focused on older patient's response to probiotic bacteria while on antibiotics. All participants in the study were at least 50 years old. If they were on high risk antibiotics, (clindamycin, cephalosporins, or aminopenicillins) they were excluded. Within 48 hours of beginning antibiotic treatment, probiotics were given, and stopped one week after antibiotics were discontinued. A probiotic shake that contained, "Lactobacillus *casei* DN-114 001 (L casei imunitass) (1.0×108 colony forming units/ml), S *thermophilus* (1.0×108 cfu/ml), and L *bulgaricus* (1.0×107 cfu/ml)" was utilized. The placebo group received a sterilized milkshake. One hundred grams were consumed twice a day half an hour before and one to two hours after meals. Twenty-four samples were tested for Lactobacillus and the

mean count was 2.2 x 108 CFU/ml. After analysis, the study concluded that probiotics given twice daily for one week longer than antibiotic treatment is capable of preventing AAD and CDAD with 12% of patients on probiotics developing AAD and 34% of the control group developing AAD(P=.007). None of the participants in the treatment group tested positive for *C.difficile* while 17% of those on placebo tested positive. So, based on this study, with the proper length of administration of the probiotic, AAD and CDAD rates can be reduced. One draw-back of the study is that high risk antibiotics were not used.

Probiotics have also been studied in reducing the prevalence of diarrhea in children. *C.difficile* is the most prevalent bacteria that causes nosocomial diarrhea in children. *Lactobacillus GG* (LGG) is the strain that was tested in the prevention of CDAD in the study published in 2001 by The Journal of Pediatrics (41). Diarrhea was defined as 3 or more loose watery stools in a 24 hr period. LGG significantly reduced the risk of CDAD in this study. Relative risk in the placebo group was 33.3% and the treatment groups relative risk was 6.7%. Probiotics can be effective in children as well as adults, which shows there is benefit for their use over a wide spectrum of ages.

Central Line Associated Blood Stream Infection

Probiotics have been studied with AAD, CDAD, and VAP, but there is little research on the effects of probiotics on CLABSI prevention. CLABSI is defined as, "bacteremia with a recognized pathogen when the organism isolated from blood culture is not related to infection at another site; or isolation of a common skin contaminant from two or more blood cultures associated with signs and symptoms of infection (fever,

chills, or hypotension) that cannot be attributed to infection at another site" (33). The line can become infected from microorganisms from a person's own skin or from unwashed hands and other outside sources (33). CLABSI is very common in the ICU. Central lines are placed to provide life saving medical care and 250,000 infections occur in hospitalized patients in the U.S every year from them (30, 33). Twenty-five percent of those with central line infections die, which results in about 31,000 deaths annually in the United States (33). CLABSI costs range from 960 million to 18.2 billion dollars annually (42). Because multiple studies have shown probiotics to have no harm, and one study found probiotics effective in treating central catheter infection as a secondary measure, more studies should be conducted on probiotics and CLABSI. (27)

Lactobacillus *plantarum*

Specific bacteria strains found to be beneficial as probiotics are Lactobacillus and Bifidobacteria. Hutt et al in the Journal of Applied Microbiology studied antagonistic effects of select lactobacillus strains and bifidobacteria strains (23). The streak plate method was used to test lactobacilli and bifidobacteria strands against two forms of *E.coli, Salmonella enteric, Shigella sonnei, H. pylori* and *C.difficile*. The anti microbial activity of the strains were tested in broth and agar plates. The study found that Lactobacillus 299v had the strongest antagonistic property under microaerobic conditions. Lactobacillus 299v has been studied in the fore-mentioned conditions.

One study by McNaught et al. investigated *L. plantarum* 299v effect on gut barrier function, systemic inflammatory response syndrome (SIRS), and clinical outcomes in critically ill patients; and the impact of HAIs were reduced (32). Proviva was

given at 500 ml per day orally or 20 ml/hr continuous feed and 40 ml/2 hr bolus feed. "Proviva is an oatmeal and fruit drink containing 5 x107 colony forming units per ml of L. plantarum 299v" (31). Fifty-two patients were in the treatment group and 51 received placebo. The study found that, although *L. plantarum* 299v reduces the amplitude of SIRS, neither GI microflora nor clinical outcomes were affected. Based on this study, the use of probiotics in critically ill patients to improve gut function cannot be recommended. *L. plantarum* 299v may not have affected clinical outcomes in this set of critically ill patients, but it was shown to be effective in the reduction of the impact of nosocomial infections.

In 2008, Klarin et al. found that *C.dificile* colonization was lower in participants, all on antibiotics, who received L.*plantarum* 299v than in a placebo group (24). A fermented oatmeal gruel containing 8 x108 CFU/ml of L. *plantarum* 299v was given to the study group. There were 22 participants in each group. The placebo group only received oatmeal gruel with lactic acid so the pH matched that of the placebo group. The antibiotics received were cephalosporines and carbapenems. Rectal fecal samples for *C. diff* and L. *plantarum* 299v were taken and cultured twice a week for the duration of the subjects stay in the ICU. Seventy-one fecal samples were analyzed in the *L. plantarum* group and none of the participants were found to have *C. difficile*. *C. difficile* was found in five fecal samples of the 80 analyzed in the placebo group. *L. plantarum* was seen in four participants in inclusion samples and after more samples were taken during the study, 18 out of 21 subjects had *L. plantarum* in the stool in the treatment group and only three in the placebo group. This study found 19% of the placebo group developed *C.difficile* bacteria while on antibiotics. *L.plantarum* recovery rate was high in the

treatment group which shows that it is viable through the GI tract and the dose given was adequate. The product was given in bolus feeds at six 100 ml doses at 12 hr intervals and after that, 50 ml twice per day. Because none of the patients who were receiving the *L.plantarum* 299v developed *C. difficile* bacterium, this study concludes that *L. plantarum* 299v may counteract or prevent colonization of *C. difficile* in patients on antibiotics.

L. plantarum 299v was also found to be beneficial in lowering the overall risk of developing loose or watery stools while on antibiotics in a Lonnermark et al study (26). Although loose watery stools were reported by participants, this did not meet the study definition of diarrhea. The study defined diarrhea as three loose or watery stools per day for two or more consecutive days. Because of this, it cannot be said that *L. plantarum* 299v reduces the risk of antibiotic associated diarrhea. The *L. plantarum* fruit drink contained 1010 CFU, much lower than most other studies. *C. difficile* bacteria were also analyzed in stool but no significance between the two groups were seen. Eighty individuals were in the treatment group and 83 received placebo in this double blinded placebo controlled trial. This study found no difference between the development of diarrhea between the two groups most likely due to inadequate dosage of *L. plantarum 299v.*

As stated previously, it is believed that those who develop CDAD are at higher risk of developing recurrent CDAD because intestinal flora loses its resistance to colonization by *C. difficile*. Another study done in Sweden focused on recurrent CDAD. Wultt et al conducted a prospective multicenter study and found no statistical significance in CDAD recurrence between the two groups (45). There were 21 people who were

included in the study and all had been diagnosed with *C. difficile* within the last two months before treatment. There were only 11 participants in the treatment group and recurrence of clinical symptoms was seen in four of them. Six of the nine in the placebo group had recurrent symptoms. Because the study was so small, no definite conclusions could be drawn.

L. plantarum in particular has also shown beneficial effects against VAP. A pilot study done by Klarin et al found benefit of using *L.plantarum* 299 on reducing the pathogenic bacteria in the oropharynx of intubated patients, which leads to VAP (24). Fifty patients who were critically ill were randomized to receive oral mechanical cleansing followed by application of .1% chlorhexidine (CHX) or an emulsion of *L.plantarum* 299. A total of 21 patients were analyzed in the placebo group and 23 in the probiotic group. Bacteria not present before the study were found in eight patients receiving *L.plantarum* 299 and 13 receiving CHX. With this, the study concludes that probiotics are safe to use and may be able to lower the rate of infection with harmful microbes, which would lead to reduction of VAP cases. However the difference in VAP was not conclusive in the study, one person in the treatment group contracted VAP and three in the control group. Probiotics would be better to use because CHX has side effects that include discoloration of teeth, burning sensation of the tongue, and irritation of the mucosa. CHX also shows little activity against gram negative bacteria, which are the main bacteria present in VAP. Probiotics, specifically L.*plantarum* strains, are stable and have not been shown to contribute to the development of antibiotic resistance strains as CHX does. "Besides offering a promising alternative to antiseptics like CHX, a probiotic that adheres to the oral mucosa will be able to counteract potentially pathogenic bacteria

24 hours a day, which is superior to the fairly short-term effect of orally applied chemical agents" (24). From this study, it is evident that L. plantarum 299 reduces VAP cases and is more appropriate for use than the CHX chemical.

Summary

Probiotics have promising effects in critically ill hospitalized patients. AAD, CDAD, and VAP have been decreased with probiotic use in certain studies. Elderly populations have also shown improved outcomes in hospital acquired infections with the use of probiotics. This is important when studying probiotics in veterans because the majority of them are older than 50. Although no differences in AAD/CDAD between treatment group and placebo group were seen in the Wisconsin veteran population, from the literature, it is clear that type and dose are very important in whether or not probiotics are able to prevent these nosocomial infections. In AAD and CDAD, the research for probiotics is promising, but longer follow up times of at least two months need to be implemented to be sure these diarrheas did not occur. *L. plantarum* 299v specifically has been proven to be effective in all of these infections with the exception of CLABSI. Although CLABSI has not been well studied, the nature of probiotics allows the belief that a positive outcome may occur in it as well. AAD, CDAD, VAP, and CLABSI are all important hospital acquired infections that are costly and increase mortality risk. Billions of dollars and thousands of lives can be saved annually if a method of prevention is implemented. Probiotics have the potential to prevent these infections, and therefore, future studies should be pursued.

CHAPTER 3: METHODS

Research Design

In January 2011, the VAMC-Memphis performed a quality improvement study to determine the effect of food form probiotics in the prevention of Clostridium *difficile* infections, ventilator associated pneumonia, and central line associated bacteremia in Medicine Intensive Care Unit patients. From this, a trend in the data showed decreased rates of these hospital infections from the use of probiotics, but improvements could not be found to be definitively because of probiotic implementation. With the collected data from the quality improvement study, a retrospective cohort study was done on the change in infection rates before, during, and after the implementation of the food form probiotic. Infection rates three years prior to probiotic implementation will be analyzed (control 1), the eight months during probiotic implementation and one year post supplement (control 2). The supplement used was GoodBelly Big Shot 50®, which contains Lactobacillus 299v and Bifidobacteria b107. This particular supplement was chosen because it was food form, available in liquid form for easier flow through feeding tubes, and a smaller amount could be used with a large amount of colony forming units (CFU). Seventy-four individuals received 80 ml of probiotic containing 50 billion colony forming units once a day, and treatment time ranged from 24 hours to eight months.

Inclusion criteria during the quality improvement study:

- >18 years of age
- Expected to be hospitalized for at least 24 hrs

Exclusion Criteria during the quality improvement study:

- Immunocompromised
- History of organ transplantation
- Non functional GI tract
- Acute pancreatitis
- Receiving high dose steroids (>240 mg or equivalent of hydrocortisone)

Retrospective Cohort Study

Microsoft excel statistical analysis was used to analyze data. Specifically, chi square test and t-test were used to gather a valid explanation between the independent and dependent variables. Only patient information for those in the MICU who would have met the criteria for the quality initiative improvement was analyzed. A chart review was used to gather data. The independent variables being observed are patient demographics (age,sex), and if antibiotics were being administered. During the protocol time frame, extra independent variables include the duration of probiotic administration. The dependent variable for all three time periods is rate of infection. The independent variables in the three groups were compared to the rate of hospital associated infection during the specific time period. Then, each group's trends were compared to determine if confounding factors played a role in decreased rate of infection. The significance was measured at <.05. From this, it was determined if 50 billion CFU of Lactobacillus *plantarum* 299v and Bifidobacteria b107 are able to reduce infection rates in MICU patients hospitalized at the VAMC-Memphis.

CHAPTER 4: RESULTS

A total of 3,274 patients were admitted to or transferred in to the MICU at the VAMC-Memphis between January 1 2008 and March 31 2013. Of these, 1,935 were in control 1 (the pre quality improvement group), 391 were admitted during the quality improvement study group (QI), and 948 patients in control 2 (post quality improvement study). Of the 1935 charts from control 1 reviewed, 851 of those patients (44%) admitted did not meet improvement criteria and would not have received the food form probiotics. Of the QI group, 317 were ineligible (81%), and 503(53%) in control 2 would not have received probiotics. (Figure 1)

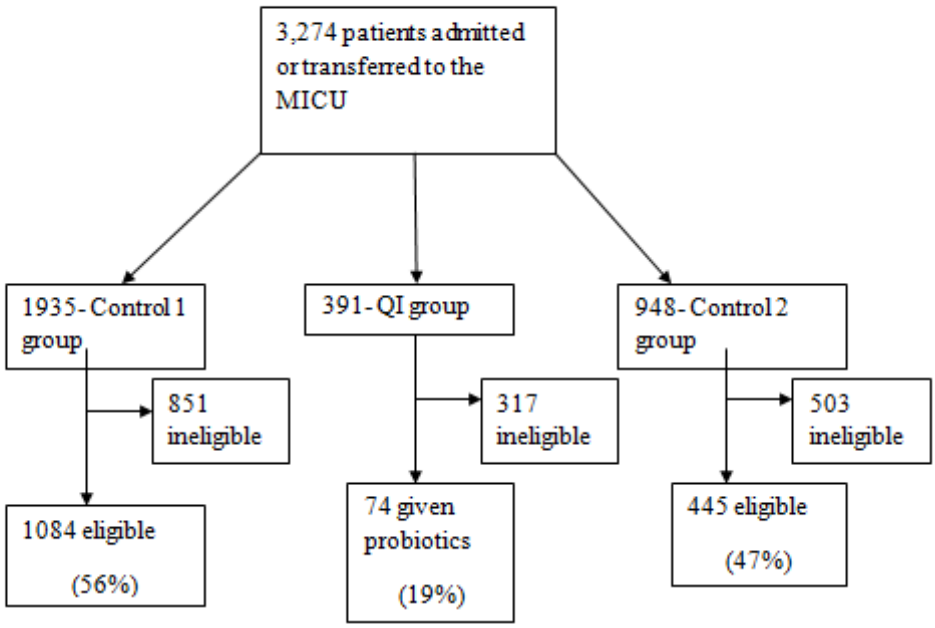

Figure 1. Study Participants.

The mean age of the groups was 65.8 ± 12.2, 66.3 ± 12.5and 61 ±12.1 for control 1, QI, and controls 2 groups, respectively. There was no significant difference in age of the patients among the three groups (control 1- control 2, p= 0.278; control 1-QI, p=0.183; control 2-QI, p= 0.384). For antibiotic use, there was no significant difference between control 1 and control 2, p= 0.47. There were, however, significantly fewer patients eligible for probiotics receiving antibiotics in the QI group compared to control 1and control 2, p= <.001 for both. (Table 1) There were significantly more female patients in the control 2 group, p= <0.1. There were 1568 males total, 35 female included in the study.

Table 1. Demographics and characteristics

	Control 1 (n= 1084)	QI (n=74)	Control 2 (n= 445)	P value (PQI-QI)	P value (QI-POQI)	P value (PQI-POQI)
Age, mean ± SD, years	65.8 ± 12.2	66.3 ± 12.5	61 ± 12.1	0.183	0.384	0.278
Gender, M(F) % F	1067 (17) 1.6%	74 (0) 0%	427 (18) 4.2%	0.16	0.5	<.01
Antibiotics	322 (30%)	47 (12%)	127 (29%)	<0.1	<.01	0.47025945
Average LOP, days		7.29 ± 12.2				

PQI= Pre-quality improvement study, QI= quality improvement study, POQI = post quality improvement study. LOP= length of probiotics

Using chi square analysis, infection rates for CLAB and CDAD were significantly lower in the QI group. For CLAB, five patients in control 1(0.26%), zero in QI (0%), and one in control 2 (0.11%) developed the HAI, p= 0.03. CDAD infections were 11 (0.57%), two (0.51%), and eight (0.84%), for control 1, QI, and control 2, respectively (p= <0.05). With VAP, infection rate was significantly lower in the control 2 group; 11 control 1 (0.57%), 2 QI (0.51%), and 3 control 2(0.21%) developed the infection, p = 0.01. (Table 2)

Table 2. Infection Rates per incidence

Infection Type	Control 1 n=1935)	QI (n=391)	Control 2 (n= 948)	P value
VAP	11 (.57%)	2 (.51%)	3 (.32%)	.01
CLAB	5 (.26%)	0 (0%)	1 (.11%)	.03
CDAD	11 (.57%)	2 (.51%)	8 (.84%)	<.05

VAP= Ventilator Associated Pnemonia ; CLAB= Central Line Associated Bacteremia; CDAD= Clostridium difficile Associated Diarrhea

When infection rates per 1000 line and vent days were compared, there was no significant difference between the three groups. (Table 3)

Table 3. Infection Rates per line and vent day

Infection Type	Control 1 (n=1935)	QI (n=391)	Control 2 (n= 948)	P value
VAP, % per 1000 vent days	3.3	2.2	3.9	.786
CLAB, % per 1000 line days	1.02	0	0.19	.479

VAP= Ventilator Associated Pnemonia ; CLAB= Central Line Associated Bacteremia; CDAD= Clostridium difficile Associated Diarrhea

CHAPTER 5: DISCUSSION

Findings

This study was unable to determine how large an effect 50 billion CFU of Lactobacillus plantarum 299v and Bifidobacteria b107 given in food form had on HAI. Rates for CLAB were significantly lower in the QI group per incidence (infections per admit) but not per 1000 line days. At the current time, there are no other studies published regarding the effect of probiotics in CLAB prophylaxis. However, there have been research articles regarding the prevention of CLAB and based on findings from those studies, CLAB is a significant problem in the ICU.

There was no significant difference between VAP per 1000 vent days and percent infection per incidence was significantly lower in the control 2 group. This was similar to the results of the 2010 study on prophylaxis of VAP by Barraud et al which found insignificant mortality rates between the probiotic group who received Ergyphilus® capsules that contained 2 x 10^{10} CFU of Lactobacillus *rhamnosus* GG, Lactobacillus *casei*, Lactobacillus *acidolphus*, and Bifidobacterium *bifidum* (25.3%) and the placebo group (23.7%). However, it is important to note, that the post probiotics group had a lower infection rate, so the probiotics might have had some lasting effect through readmits. From research available, there have been no studies using food form probiotics for prophylaxis of VAP.

Rates of CDAD were significantly lower in the QI group, $p < .05$. In the Hickson et al study, a probiotic shake that contained, Lactobacillus *casei*, S *thermophilus*, and L

bulgaricus was used. None of the participants in that study in the treatment group tested positive for *C.difficile* while 17% of those on placebo tested positive.

Klarin et al determined L. *plantarum* 299v to counteract or prevent C.difficile in patients on antibiotics. This study used an oatmeal gruel food form probiotic. Studies have shown a dose dependent and time dependent relationship between probiotics and decreased HAI. Where 50 billion CFU were provided in this QI study, only 800 million CFU was given to patients in the 2008 study by Klarin et al and results still showed CDAD prophylaxis. Average probiotic administration in that study was 5.5 days. The average length of probiotics was 7.29 days in this QI study, ranging from one to 93 days. This helps confirm the findings of our study that the probiotics ingested will prevent CDAD.

During the QI study, a hand washing protocol was also emphasized on in the ICU. This protocol continued after the probiotic study was discontinued. This could explain why there was a lower infection rate in the control 2 group for VAP. This could also explain why CLAB infection rates for the control 2 group were lower than control 1. One study completed by Harris et al in 2011 among Pediatric ICU patients found that improving hand hygiene, catheter care, and oral care reduced infections and saved the hospital 12 million dollars annually (21).

Of the 391 patients admitted to the MICU during the quality initiative study, 74 of them received probiotics (19%). Because less than 50% of patients received probiotics, it is difficult to say whether probiotics led to decreased infection rates or other factors, such as good hygiene practices, played a larger role. Although there were significantly more

female patients in the control 2 group than the control 1 group, there has been no research showing females to be at higher risk for HAI than males.

Significantly fewer patients during the QI time period eligible for probiotics received antibiotics. This could mean that probiotics decreased infections, which decreased the number of patients who needed antibiotics. Contrary to this, it could mean that the control 1 and control 2 group were sicker and at higher risk for developing infections than the QI group as it is widely known that broad spectrum antibiotic use is one of the main causes of C. *difficile* associated diarrhea.

Limitations

While using the CPRS charting system, some problems were encountered. Some admits had lengthy medical histories which crashed the system while attempting to load their medical records. If the data needed was more than 3000 charts back, it was not reviewed. For these patients, they were counted as not being candidates for probiotics. This is a potential issue because these patients were included in the reported infection rates, and there is no way to say for certain whether they met exclusion criteria or not. In the quality improvement study, high dose steroids were considered 240 mg of hydrocortisone or equivalent. Unfortunately, it was said that doctors determine what dose of steroid is high on a case by case basis. Because of this, all admits who received any type and amount of steroids were not included in the overall result analysis. This

also caused some inconsistency with admits that would have been eligible to receive probiotics.

Unfortunately, data connecting specific patients to specific infections was unavailable. This would have strengthened the study if it was known if people actually receiving probiotics developed the infection. It is also unknown what percent of the patients eligible for probiotics were on the vent or had central lines placed which limits the accuracy of rates reported.

Time was a major limiting factor in data collection. This retrospective review should be completed again with more data collectors and with more time for data collection. This way, more information can be gathered and more inquiries made to the infection control department at the VA requesting more data.

CHAPTER 6: CONCLUSION

From this retrospective cohort study, it was determined that Lactobacillus *plantarum* 299v and Bifidobacterium b107 in food form played a role in the prevention of hospital associated infections. Specifically CDAD infection rates were significantly lower in the group that received probiotics. This study does not suggest that probiotics reduce rates of VAP or CLAB. There was a significant difference between the groups in antibiotic administration and a larger emphasis placed on hand washing during the QI and control 2 group. Therefore, due to study limitations, differences between groups and other hospital interventions concurrently with this study, this study is unable to determine how large of a role probiotics play in the prevention of HAI. Further research using this data should be done. By extracting the 19 patients who received probiotics and evaluating them specifically for HAI, a more conclusive argument for the use of probiotics in HAI may be determined.

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Appendix A

The University of Memphis Institutional Review Board, FWA00006815, has reviewed and approved your submission in accordance with all applicable statuses and regulations as well as ethical principles.

PI NAME: Deidra Nelson
CO-PI:
PROJECT TITLE: Probiotics Lactobacillus plantarum 299v and Bifidobacteria b107 In the Prevention of Ventilator Associated Pneumonia, Clostridium difficile Associated Infections, Antibiotic Associated Diarrhea, and Central Line Associated Bloodstream Infection: A Retrospective Cohort Study
FACULTY ADVISOR NAME (if applicable): Margaret Williams
IRB ID: #2631
APPROVAL DATE: 4/9/2013
EXPIRATION DATE: 3/26/2014
LEVEL OF REVIEW: Expedited

Appendix B

DEPARTMENT OF VETERANS AFFAIRS
INSTITUTIONAL REVIEW BOARD
Memphis Veterans Affairs Medical Center
1030 Jefferson Avenue
Memphis, TN 38104

DATE:	February 28, 2014
TO:	Michelle Grabowski, BS Principal Investigator
FROM:	Timmy Edwards, Pharm.D. Memphis VAMC Institutional Review Board Chair
PROTOCOL TITLE:	[427141-5] Probiotics Lactobacillus plantarum 299v and Bifidobacteria b107 In the Prevention of Ventilator Associated Pneumonia, Clostridium difficile Associated Infections, Antibiotic Associated Diarrhea, and Central Line Associated Bloodstream Infection: A Retrospective Cohort Study
VAMC Tracking ID #:	
SUBMISSION TYPE:	Continuing Review/Progress Report
REVIEW TYPE:	Expedited Review
RISK DETERMINATION:	Minimal Risk
ACTION:	APPROVED
EFFECTIVE DATE:	February 25, 2014
PROTOCOL EXPIRATION DATE:	February 24, 2015