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THE USE OF LACTOBACILLUS IN THE TREATMENT OF CLOSTRIDIUM DIFFICILE INFECTION IN HOSPITALIZED ADULT PATIENTS

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THE USE OF *LACTOBACILLUS* IN THE TREATMENT OF *CLOSTRIDIUM DIFFICILE* INFECTION IN HOSPITALIZED ADULT PATIENTS

A thesis submitted in partial fulfillment of the requirements for the degree of Masters Science in Pharmaceutical Sciences at Virginia Commonwealth University.

by

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Clostridium Difficile Infection ..... CDI

Antibiotic-Associated Diarrhea ..... AAD

Lactobacillus rhamnosus GG ..... LGG

Clinical Resource Manager administrative database ..... CRM

University HealthSystem Consortium ..... UHC

International Classification of Diseases,
9th Revision, Clinical Modification ICD-9-CM

Colony-Forming Unit ..... CFU

Acquired Immune Deficiency Syndrome ..... AIDS

Randomized Controlled Trial ..... RCT

Odds Ratio ..... OR

Relative Risk ..... RR

Confidence Interval CI

Charlson Comorbidity Index CCI

Dartmouth-Manitoba version of the Charlson Comorbidity Index ..... DM-CCI

Intensive Care Unit ..... ICU

Case Mix Index ..... CMI

Abstract

THE USE OF *LACTOBACILLUS* IN THE TREATMENT OF C. *DIFFICILE*-ASSOCIATED DIARRHEA IN HOSPITALIZED ADULT PATIENTS

By Ali Alhammad, BPharm, MS

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science in Pharmaceutical Sciences at Virginia Commonwealth University.

Virginia Commonwealth University, 2009

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Objective

To describe the use of *Lactobacillus* by hospitalized patients and to examine its relationship with various *Clostridium difficile* infection (CDI) related outcomes.

Methods

The characteristics of *Lactobacillus* users and non-users and the initiation of *Lactobacillus* with respect to initiation of antibiotic therapy and CDI treatment were described using national hospital discharge database. The relationships between

Lactobacillus use and post-CDI length of stay, mortality, switch of CDI therapy, and readmission were analyzed.

Results

Lactobacillus users and non-users were different in most characteristics. Metronidazole and fluoroquinolones were the most frequently used antibiotics by *Lactobacillus* users. They were mainly CDI cases, used multiple antibiotics, extremely ill, and started *Lactobacillus* five or more days after initiation of antibiotics or CDI treatment. *Lactobacillus* use was associated with increased length of stay and switching of CDI therapy.

Conclusions

The true association between *Lactobacillus* use and CDI remains unclear. This study provides foundation for future research.

CHAPTER 1 INTRODUCTION

Background

Clostridium difficile is a gram-positive anaerobic spore-forming rod-shaped bacteria (bacillus). *C. difficile* produces two potent toxins: toxin A which is an enterotoxin, and toxin B which is a cytotoxin. Both are implicated in the pathogenesis of *C. difficile* infection (CDI). They act synergistically and are capable of damaging the human colonic epithelium.[1, 2]

The severity of CDI ranges from mild diarrhea to life-threatening conditions, such as pseudomembranous colitis and toxic megacolon.[3, 4] CDI is the leading identified type of nosocomial diarrhea.[5] In addition, it is implicated in 20% to 30% of patients with antibiotic-associated diarrhea (AAD) and in more than 90% of those with antibiotic-associated pseudomembranous colitis.[1, 2] CDI may result in an increased length of stay by three to seven days, as well as a 20% to 65% increase in the rate of subsequent infections. There may also be a two- to three-fold increase in mortality as a result of CDI.[6-8] In addition to these clinical consequences, the long-term costs attributed to CDI in the United States in 2003 were estimated to be between $897 million and $1.3 billion.[1, 2, 10, 11]

Current treatment strategies of CDI consist of discontinuing the offending antibiotic, if possible, and initiating antibacterial treatment, which could be either oral metronidazole or oral vancomycin. Unfortunately, these treatment strategies are associated

with a high recurrence rate of up to 28 %.[6, 12] Some evidence shows that oral teicoplanin may be a better choice than vancomycin in some cases, but this agent is not available in the United States.[13]

Probiotics, defined as live microbial dietary supplements that beneficially affect the host by improving intestinal microbial balance,[15] have been used as a way of restoring intestinal microflora. This acts as a protective barrier that resists the colonization of intestinal pathogens and consequently decreases the incidence and duration of antibiotic-associated diarrhea in general. Probiotics may also prevent CDI. They are relatively inexpensive, generally safe and well tolerated.[16]

There have been several studies reported in the last three decades looking at the effect of probiotics on the treatment of CDI.[7, 8, 18-26] These studies yielded contradictory results because of differences in study design, type and duration of probiotics therapy, differing doses and durations of antibiotic treatment. There are different types of probiotics that have variable efficacy in the prevention and treatment of CDI. *Lactobacillus rhamnosus* GG (LGG) and *Saccharomyces boulardii* were shown to be more effective than other probiotics. In addition, they have many other potential positive health effects.[24, 27, 28]

McFarland et al. studied the effect of adding *S. boulardii* to the regular treatment of CDI. Compared to the control group, they found that the *S. boulardii* group was significantly more likely to respond to antibiotic therapy and less likely to experience recurrence of diarrhea.[24] Surawicz et al. in similar study found that the high dose vancomycin group with *S. boulardii* demonstrated decreased frequency of CDI

recurrence.[22] Wullt et al. found no significant differences between the two groups in the cure rate of initial CDI or the recurrence of CDI.[20]

Objectives and Specific Aims

The main objective of this study is to describe the use of a blend of *Lactobacillus acidophilus* and *Lactobacillus helveticus* (Lactinex®, Becton-Dickson, Co.) by CDI patients.[29] The secondary objective is to examine the relationship between the use of *Lactobacillus* and various CDI related clinical outcomes.

The project has the following specific aims:

Specific Aim 1: Describe the demographic and clinical characteristics (age, gender, and race, CDI status, number of antibiotics received, transfer status, severity of illness, mortality, readmission, and overall length of stay) of patients who did and did not receive *Lactobacillus*.

Specific Aim 2: Describe the initiation of *Lactobacillus* with respect to initiation of initial antibiotic therapy among patients who were on *Lactobacillus*.

Specific Aim 3: Describe the initiation of *Lactobacillus* with respect to the initiation of CDI treatment among patients who were on *Lactobacillus* and had CDI.

Specific Aim 4: Among patients who had nosocomial CDI, examine the relationship between *Lactobacillus* use and:

a. Length of stay
b. Switch rate of CDI treatment (i.e., metronidazole to vancomycin or vice versa)
c. Readmission with CDI

d. Mortality

Significance

Based on review of published research, this study is the first descriptive study of the use of probiotics in CDI patients using a relatively large sample size. A meta-analysis by McFarland compared the efficacy of probiotics for the treatment of CDI based on the published randomized clinical trials (RCTs) in adult hospitalized patients. This meta-analysis concluded that probiotic therapy is effective in the treatment for CDI. The pooled relative risk from the six RCTs included was 0.59 (0.41, 0.85) for CDI in probiotics users. The heterogeneity of the included studies was not significant; however, the relatively small number of trials included could be a limiting factor in this meta-analysis.[18]

Several methodological issues have plagued prior research on the use of probiotics in CDI patients including small sample sizes, inappropriate or inadequate control groups, and lack of control for co-morbidities and other confounders.[18, 20, 22, 24, 25, 30, 31] Additionally, these studies have shown contradictory results of the effect of *Lactobacillus* on the treatment of CDI primarily because of insufficient power to detect significant differences due to differences in the study population, type and dose of probiotics given, or the duration of treatment.[18, 19]

This study seeks to describe the demographic and clinical characteristics of *Lactobacillus* users and non-users utilizing information from the Clinical Resource Manager (CRM) administrative database from the University HealthSystem Consortium (UHC) [Specific Aims 1-3]. This database has a large number of hospitalized patients from

participating teaching hospitals around the nation. Additionally, this study examines the relationship between the use of *Lactobacillus* by CDI patients and various related health outcomes [Specific Aim 4]. The findings of this study could be used to generate hypotheses for future studies.

CHAPTER 2 LITERATURE REVIEW

Antibiotic-Associated Diarrhea

Antibiotic-associated diarrhea (AAD) is defined as otherwise unexplained diarrhea that occurs in association with the administration of antibiotics.[32] Clindamycin, cephalosporins, broad-spectrum penicillins, and fluoroquinolones are more likely to cause the problem than other antibiotics, even though all antibiotics are implicated.[53] The rates of diarrhea associated with parenterally administered antibiotics, especially those with enterohepatic circulation, are similar to rates associated with orally administered agents.[33] The incidence of AAD in hospitalized adult patients could be (13–29%), or even up to 60% during hospital outbreaks, but is rare in an out-patient and ambulatory setting (<0.1%).[34, 35] The primary cause of AAD is the disruption of intestinal normal flora by antibiotics, which may lead to overgrowth of pathogens and colonization of the intestine. The most commonly diagnosed and potentially severe form of AAD is caused by *C. difficile*. This pathogen is implicated in 20% to 30% of patients with AAD, in 50% to 70% of those with antibiotic-associated colitis, and in nearly all cases of antibiotic-associated pseudomembranous colitis.[2,36] Besides *C. difficile* infection, other factors involved in AAD include overgrowth of other pathogens, impaired fecal fermentation, and changes in dietary fiber intake.[37, 38]

Pathogenesis of AAD

Figure 2.1 below shows the pathogenesis of AAD. The primary cause of AAD is the disruption of intestinal normal flora which acts as a protective barrier that resists the colonization of pathogens in the intestine.[36]

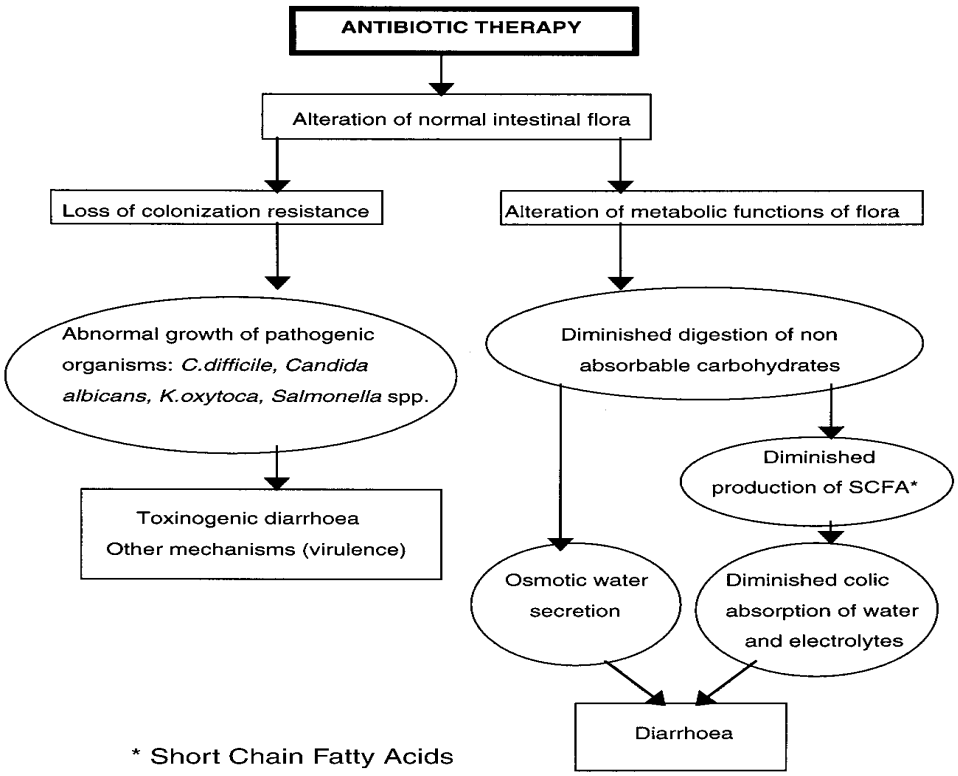

Figure 2.1. Pathogenesis of antibiotic associated diarrhea (Adapted from Ref. 36)

In addition, the disruption of intestinal normal flora diminishes its function of digestion of non-absorbable carbohydrates, normally metabolized by colonic bacteria as an energy source. The production of lactic acid and short-chain fatty acids by the anaerobic flora is decreased, which increases the osmotic pressure in the colon. That reduces absorption of water and electrolytes in the colon and results in osmotic diarrhea.[39]

Other factors potentially implicated in the pathogenesis of AAD are related to some antibiotic, such as penicillins, allergic and toxic effects on intestinal mucosa and to some antibiotic, such as macrolides, pharmacologic effects on intestinal motility. *C. difficile* is another important etiology of AAD.[28]

Clostridium Difficile **Infection**

Clostridium difficile infection (CDI) is most commonly associated with the exposure to antibiotics, especially cephalosporins, broad spectrum penicillins, clindamycin, and fluoroquinolones.[53] A small but increasing percentage of cases may also experience megacolon, perforation, colectomy, or death. Patients with CDI may have an increase in the length of stay by 8 to 36 days, a 20% to 65% increase in the rate of subsequent infections, as well as a two- to three-fold increase in mortality.[6-8, 42] In addition, CDI incurs substantially greater costs. [1, 2, 10, 11]

Epidemiology of CDI

C. difficile is the most common identified cause of nosocomial diarrhea. In a recent study, *C. difficile* was determined to be the causative agent of diarrhea in 19 out of 44 (43%) patients with nosocomial diarrhea.[40, 41] This pathogen is implicated in about 10% of cases of nosocomial diarrhea, 20% to 30% of patients with AAD, in 50% to 70% of those with antibiotic-associated colitis, and in nearly all cases of antibiotic-associated pseudomembranous colitis.[1, 2, 36] In 1995, Barbut et al. found that the prevalence of CDI in patients who were suspected of having nosocomial diarrhea was 35 out of 344 (10.2%).[42] In a similar retrospective study by Rohner et al. in 1998, the prevalence rate was 248 out of 2,531 (9.8%).[43] The clinical presentations of CDI include lower abdominal discomfort, diarrhea, colitis, and in severe cases can result in pseudomembranous colitis, toxic megacolon, or death.[44] About 3.2% of patients with CDI have complications requiring colectomy, while in 1%-2% of patients it results in death.[45, 46] However, this figures dramatically increases in severe cases. In 2001, Barbut found that among patients requiring a colectomy for toxic megacolon or perforation, the mortality rate was 35%-50%.[42]

In hospitals and long-term facilities, CDI is more common, with estimates of 25-60 cases per 100,000 occupied bed-days, compared to 7.7 cases per 100,000 person-years in the community.[47] A surveillance study in 2009 found similar results in Canadian hospitals with an overall incidence rate of nosocomial CDI for hospitalized adult patients of 4.6 cases per 1,000 patient admissions or 65 per 100,000 patient-days.[5] Among hospitalized children, a study by Kim et al. in the United Sates found that the annual incidence of CDI was 6.5 cases per 10,000 patient-days and 4.0 cases per 1,000 admissions.[48]

During the current decade, there has been a dramatic increase in the incidence and severity of CDI in healthcare settings. Based on the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) code for CDI (008.45), to measure CDI prevalence, McDonald et al. found that the proportion of hospital discharges with CDI code increased from 0.37% in 2000 to 0.51% in 2003 for an estimated 178,000 CDI cases in patients discharged from short stay hospitals in 2003.[49] These clinically relevant changes are temporally associated with the emergence of a hypervirulent strain of *C. difficile* that has now become widely disseminated.[50]

CDI Impact on the Healthcare System

A recent study by Marya et al. found that there was a 23% annual increase in CDI hospitalizations in the 6-year period from 2000 through 2005. Also, the absolute number of CDI hospitalizations more than doubled in almost all age groups as shown in Table 2.1 or Figure 2.2.[51]

Table 2.1 – Absolute numbers of adult hospitalizations with *Clostridium difficile*, by age group, United States, 2000–2005

Hospitalizations	2000	2001	2002	2003	2004	2005
18–44 y	14,738	15,001	18,747	19,393	22,168	25,662
45–64 y	28,280	29,527	39,421	43,290	50,898	61,757
65–84 y	69,018	74,010	98,148	105,404	122,875	147,675
>85 y	22,325	25,194	31,899	35,363	43,341	56,209
All adult	134,361	143,732	188,215	203,450	239,282	291,303

(Source: Ref. 51)

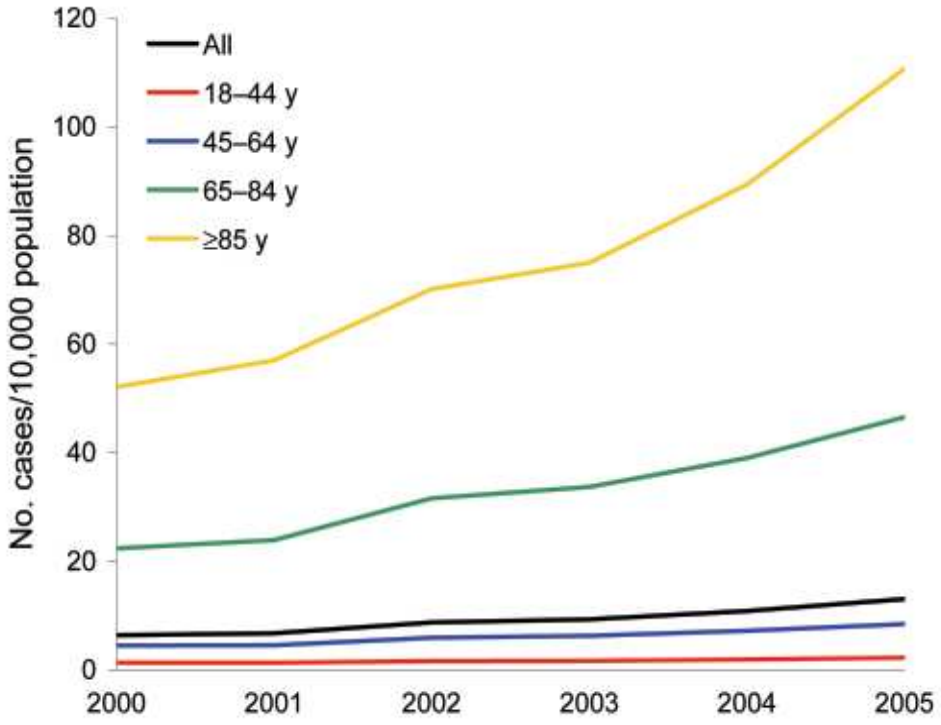

Figure 2.2 – Changes in the age-specific *Clostridium difficile*–associated disease incidence rate per 10,000 population in the United States, 2000–2005. (Source: Ref. 51)

The length of hospital stay for patients with CDI was found to increase by 8 days among adult inpatients and 36 days in geriatric patients.[42] McFarland et al. found that costs are particularly high for patients with recurrent CDI due to the long duration of the disease, the costs involved in diagnosis, treatment, hospitalizations, and recurrent treatments.[2] They also found that while the average cost of the first episode was $1,914, the average cost for subsequent episodes was $3,103 totaling on average to $10,970 for patients with multiple episodes. Kyne et al. performed an analysis of CDI-attributable costs using a cohort study design and found it to be $3,669 (95% confidence interval [CI] $1,126 to $7,024) per episode. Another recent study using a retrospective cohort study design found that CDI was associated with excess costs of $3,240 ($P < 0.001$; increase in cost, 33%) and with $5,042 (95% CI $3,797 to $6,481; increase in cost, 53%) attributable inpatient costs over 180 days.[11]

Risk Factors for CDI

Major risk factors for CDI include antibiotic exposure, hospitalization or admission to a long-term care facility, and advanced age.[52, 53] Other risk factors of recurrent CDI are increasing age, increased severity of underlying disease, and low serum antibody response to toxin A.[45,53] The use of gastric acid-inhibitors, especially proton pump inhibitors (PPI), has also been proposed as a possible risk factor.[54] In a recent case-control study by Aseeri et al. in 2008, PPI use was associated with an increased risk of CDI (OR = 3.6, 95% CI 1.7 to 8.3; $P < 0.001$).[55]

C. difficile spores can be transmitted through the fecal-oral route in the public; however, the spores are more prevalent in hospital and long-term care facilities. Transmission can occur through infected patients, contaminated surfaces in hospitals, or through personnel whose hands are contaminated by the bacteria.[45] In general, exposure to the healthcare setting also increases the risk of developing CDI. Within the hospital setting, patients admitted to an intensive care unit and those having a prolonged hospital stay are more likely to become infected with *C. difficile*.[53]

Exposure to almost any antibiotic can predispose a patient to a *C. difficile* infection. Historically, it is known that clindamycin, cephalosporins, and certain broad spectrum penicillins were most commonly associated with CDI.[53] Although most of the published literature identifies antibiotic use and hospitalization as the primary risk factors for CDI, there is some evidence to the contrary. A recent review article by Thomas et al. identifies several biases in articles reporting a relation between antibiotic use and CDI;[56] while a study by Wilcox found that only 50% of *C. difficile* cases had taken antibiotics in the previous month and only 32% had been hospitalized in the previous 6 months.

Advanced age also has been found to be a significant risk factor for the CDI. Patients older than 65 years have more than 15-fold greater chance of developing CDI compared with younger patients.[12, 57] Immunosuppressant and chemotherapy have also described as risk factors for CDI; however, this hypothesis has been questioned by others.[53]

Diagnosis of CDI

The diagnosis of CDI is generally based on the detection of *C. difficile* toxins (A, B, or both) in the stool. Usually only symptomatic patients are tested using diarrheal stool specimens as there is no value of testing stool samples of asymptomatic patients unless an outbreak is being investigated.[45] Culture of the stool for the *C. difficile* bacteria is another sensitive test; however, not all *C. difficile* strains produce toxin and the test may be positive for a non-toxicogenic strain. This test is not used routinely except in some research studies since it may require 48 hours for a culture to become positive.[59]

Tissue culture assay for cytotoxicity of toxin B is considered the "gold standard" for diagnosing CDI with a sensitivity of around 80%-100% and a specificity of 99%. Because of the sensitivity of the test, it is not usually necessary to test multiple stool samples.[45, 47, 59]

Many clinical laboratories use the enzyme-linked immunosorbent assay (ELISA) method, which can be performed more quickly than culture assay, usually within hours. This assay can detect either toxin A or B in stool and is very specific but less sensitive. This essay has been reported to have false-negative rate of up to 40%; however, it has the ability to identify cases of toxin A negative/toxin B positive. [45, 60, 61] Polymerase chain reaction test is another method for diagnosis of CDI and has been reported to have a very high sensitivity but poor specificity due to difficulty in distinguishing between asymptotic carriage and symptomatic infection.[47, 62]

Direct endoscopic visualization of the colonic mucosa can be useful in making the diagnosis of CDI. To avoid possible colonic perforation with this technique, it has been

reserved for patients with severe disease and negative laboratory results for rapid diagnosis.[47, 59]

Standard Treatment of CDI

Current treatment strategies of CDI consist of discontinuing, if possible, the offending antibiotic. This is sufficient for mild cases of the disease. For more severe cases, CDI is normally treated with oral metronidazole (250 mg four times a day or 500 mg three times a day for 10-14 days) or oral vancomycin (125 mg four times a day for 10-14 days). For severe cases, oral vancomycin is recommended as the first line therapy. Finally, for severe CDI cases with complication, vancomycin 500 mg orally or via nasogastric tube 4 times per day and/or intravenous metronidazole 500–750 mg every 8 hours is recommended.[45] In moderate cases metronidazole is the preferred initial choice because of its low price and to reduce the use of vancomycin to avoid increased resistance of *Enterococci* species.[45] These treatment strategies are associated with a recurrence rate of up to 28% in patients 3 to 28 days after the antibiotic has been discontinued.[6, 12] Some evidence shows that oral teicoplanin, which is not available in the United States, may be a better choice than vancomycin in some cases.[13] For patients not responding to antibiotics, surgical intervention may be required when colonic perforation or toxic megacolon is suspected.[45]

Probiotics

Probiotics, defined as live microbial dietary supplements that beneficially affect the host by improving intestinal microbial balance,[15] have been used as a way of restoring intestinal microflora. This acts as a protective barrier that resists the colonization of intestinal pathogens and consequently decreases the incidence and duration of AAD in general. Probiotics may also prevent and treat CDI. They are relatively inexpensive, generally safe, and well tolerated.[16] There are different types of probiotics that have been used for the prevention and treatment of CDI, including various strains of *Lactobacillus* (*Lactobacillus GG, Lactobacillus rhamnosus, Lactobacillus casei,* and *Lactobacillus plantarum 299v*) and the yeast *Saccharomyces* (*Saccharomyces boulardi* and *Saccharomyces cerevisiae*). *Lactobacillus GG* and *Saccharomyces boulardii* were shown to be more effective and more commonly used than other probiotics.[63, 64] In addition, probiotics have many other potential positive health effects.[24, 27, 28]

Mechanism of Action

As mentioned before, disturbance of the normal colonic flora prompts colonization of *C. difficile*.[45] Probiotics may prevent or treat the colonization by pathogens through restoring the equilibrium in the altered gastrointestinal normal flora.[65] Several potential mechanisms of action have been suggested by which probiotics can promote gastrointestinal health. Probiotics competitively inhibit pathogen adherence to colonic epithelial and mucosal cells. This keeps the tight junction proteins intact and prevents both uptake of intact macromolecules and translocation of organisms to the mesenteric lymph

nodes. Also, probiotics can enhance production and secretion of anti-inflammatory cytokines, including interleukin-10. Stimulation of secretory immunoglobin A and other immunoglobins by the immune system is another proposed mechanism of action.[66] In addition, probiotics may enzymatically modify toxin receptors and compete with pathogens for nutrients.[45, 56, 67]

Pharmacokinetics of Probiotics

The beneficial effects of probiotics depend on their ability to protect the active constituents, such as enzymes, against gastric acidity and deliver them to the colon. The survival of probiotics in the gastrointestinal tract differs widely depending on their intrinsic resistance, host factors, and the formulation used, Figure 2.4.[68]

Pharmacokinetic studies of *S. boulardii* and *Lactobacillus acidophilus* in healthy volunteers have shown that it reaches steady state levels of 1×10^8 colony-forming units (CFU)/gram after three days of oral dosing with 0.5gram (1×10^{10} CFU/g) twice a day.[69] The amount of probiotics in the colon declines rapidly after cessation of dosing.

As part of a randomized study on *S. boulardii* for the treatment of recurrent CDI, Elmer et al. measured *S. boulardii* concentrations at various times in the stool samples of patients. Patients in the intervention group received 1gram of lyophilized *S. boulardii* per day containing about 10×10^9 CFUs along with either vancomycin or metronidazole. Of the 50 intervention group patients, 41 (82%) had detectable stool concentrations of *S. boulardii* ranging from 1.5×10^3 to 6.2×10^7 CFUs per gram. Furthermore, they found *S. boulardii* concentrations were higher in patients who did not have a recurrence of CDI ($1 \times$

10^6 CFU per gram compared to 1.5×10^4 CFU per gram). These differences were not explained by age, gender, or antibiotic type/dose. The same study found that *S. boulardii* was cleared by 94% of patients by the third day after treatment was discontinued.[69]

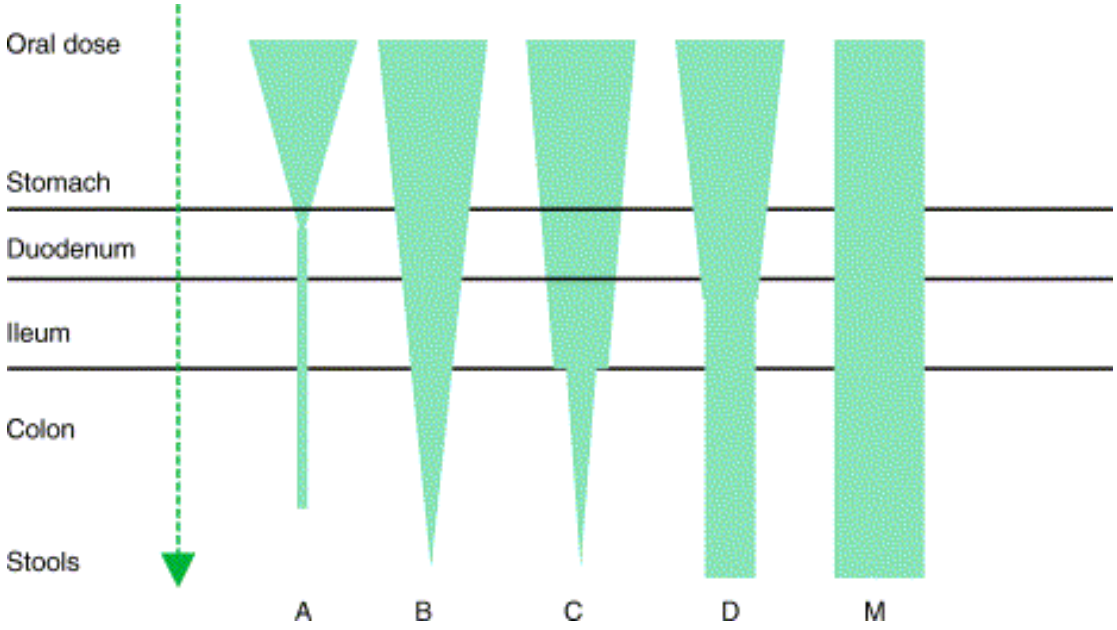

Figure 2.3 – Classification of probiotics according to their resistance in the gastrointestinal tract

Note: Some ingested probiotics are rapidly destroyed in the stomach (A), whereas others survive better beyond the stomach but are destroyed by bile (B) or by the endogenous flora (C). Some probiotics have a high survival through the gastro intestinal tract (D), close to that of a marker (M). (Source: Ref. 68)

Probiotics for Treatment of CDI

There have been several studies reported in the last three decades looking at the effect of probiotics, including *Lactobacillus* spp and *Saccharomyces* spp on the treatment of CDI. [7, 8, 18-26, 63, 64] These studies may have shown contradictory results because of insufficient power to detect significant differences due to differences in the study population, type and dose of probiotics given, or the duration of treatment.[18, 19]

In a letter to the Lancet in 1987, Gorbach et al. described the successful treatment of five patients with recurrent CDI using *Lactobacillus* GG. All patients (n = 5) had multiple recurrences within a 10-day period after antibiotic therapy. Following treatment with *Lactobacillus* GG there was no recurrence for periods ranging from four months to four years.[72] A study by Surawicz et al. reported cessation of CDI in 11/13 (84.6%) patients treated with *S. boulardii.*[23] Kimmey et al published a case report of a 67-year-old woman using *S. boulardii* to treat and prevent recurrent CDI. Over 8 months the patient experienced 8 CDI recurrences. The patient was treated with a 4 week course of vancomycin. After failing to resolve the diarrhea, *S. boulardii* was added with another vancomycin regimen until semi-formed stools developed. She took the probiotic for total of about 90 days.[73]

In a case study, Pakyz reported a use of *Lactobacillus* in the treatment of a recurrent CDI case in an 87-year-old resident of a long-term care facility. Two weeks after discharge from a hospital, the patient was readmitted for altered mental status, fever, and diarrhea. The patient was positive for *C. difficile* stool culture so she was placed on oral metronidazole and *Lactobacillus.* After five days of therapy, patient showed no minimal

improvement in diarrheal symptoms and was switched to oral vancomycin and *Lactobacillus*. Symptoms of diarrhea disappeared after 14 days of therapy.[74]

Table 2.2 summarizes six RCTs that evaluated the effect of different types of probiotics in the treatment of CDI or recurrent CDI. In a randomized, double-blind, placebo-controlled study, McFarland et al. studied 124 patients with active diarrhea and a positive result from at least one *C. difficile* assay (culture, toxin A, or toxin B) who were treated with vancomycin and/or metronidazole.[24] The intervention group (n = 57) was randomly selected to receive a lyophilized 500mg capsule of *S. boulardii* twice daily and the control group (n = 67) to placebo for 4 weeks. Approximately half (n = 60) of the patients had at least one prior CDI. Patients were excluded if they had AIDS or if they were immunosuppressed secondary to chemotherapy within the past three months. CDI recurrence rates were calculated for 4 weeks after discontinuing the *S. boulardii*. Overall the treatment failure rate was 26.3% in the *S. boulardii* group versus 44.8% in the placebo group ($P = 0.05$). In the patients with history of CDI, 34.6% of the treatment group failed therapy versus 64.7% of placebo patients (P = 0.04). Compared to the control, those in the treatment group were significantly more likely to experience cessation of diarrhea (Relative Risk [RR] = 1.33; 95% CI 1.02 to 1.74) and were significantly less likely to experience recurrence of diarrhea after cessation of antibiotic therapy (RR = 0.59; 95% CI 0.35 to 0.98). Although the treatment group was small, these results suggested that patients experiencing at least one recurrent episode of CDI may benefit from *S. boulardii* treatment. The main conclusion of the authors was that there was a statistically significant beneficial effect of *S. boulardii* on recurrent CDI, particularly among patients who have had at least

one prior episode of CDI. One of the limitations of this study was not accounting for severity of illness.[50]

Surawicz et al. studied 168 adult patients with recurrent CDI, defined as one or more previous episodes of diarrhea that had a positive *C. difficile* assay and initial response to antibiotic treatment.[22] Patients were divided into three treatment groups: high dose oral vancomycin (2 g/day; n = 32), oral low dose vancomycin (500 mg/day; n = 85), or oral metronidazole (1 g/day; n = 53), all for 10 day. The oral high dose vancomycin group was randomized to receive either *S. boulardii* 500 mg twice daily (n = 18) or placebo (n = 14) for 28 days starting on day 7 of the 10 day course of antibiotic. They were followed for a total of two months. No information about the other two groups was provided. In the high dose oral vancomycin treatment group, a 16.7% recurrence rate versus a 50% recurrence rate in the placebo group (P = 0.05; RR = 0.33; 95% CI 0.10 to 1.06). *S. boulardii* did not significantly decrease the recurrence rate in either the vancomycin 500 mg daily group or the metronidazole group. The authors concluded that there was a beneficial effect of *S. boulardii* in patients treated with high dose vancomycin even though this is in a small group of 32 patients. This was surprising, however, because these patients had more serious manifestations of CDI than other sub-groups and the confidence interval was very wide.[22]

In 2003, Wullt et al. in a multi-center randomized double-blind placebo controlled study examined 21 adult patients with recurrent CDI. Patients were included if they had ongoing diarrhea and a positive *C. difficile* toxin assay within six days of enrollment or if they had CDI within the previous two months and were not being treated with a list of

drugs including vancomycin and metronidazole at the time of enrollment. Patients were randomized to receive oral metronidazole 400 mg three times daily for 10 days in combination with either fruit drink containing *Lactobacillus plantarum 299v* once daily (n = 12) or placebo (n = 9) for 38 days. A total of only 21 patients completed the study across the nine centers over the two-year period. No statistically significant differences between the two groups were seen in the cure rate of initial CDI (RR = 0.93; 95% CI 0.73 to 1.19), recurrence of CDI (RR = 0.55; 95% CI 0.22 to 1.35), or cure rate by stool assay (RR = 0.75; 95% CI 0.41 to 1.36). The numbers of patients involved in this study were too small to allow any strong conclusions. The authors' concluded that probiotics either counteract the pathogenesis of *C. difficile* or have a positive overall impact on the microflora that prevents clinical recurrence; however, they did not provide any results to support that conclusion.[26]

Lawrence et al. conducted a randomized double-blind placebo controlled pilot study in a total of 15 adult patients with recurrent CDI. Patients were included if they had ongoing diarrhea and a positive *C. difficile* toxin assay and history of CDI in the preceding year. Exclusion criteria included critical or terminal illness, compromised immunity, more than five days of CDI treatment, and recent probiotic use. Patients were randomized to receive either 40 mg lyophilized *Lactobacillus* GG twice daily (n = 8) or placebo (n = 7) for 28 days in addition to the CDI treatment and were followed for 60 days. A total of three (37.5 %) cases of RCDI were observed in the *Lactobacillus* arm and one (14.3 %) in the placebo arm (RR = 2.6; 95 % CI 0.3 to 19.9). No conclusion was provided since the study

was not powered to detect a difference in outcomes for interest; that also resulted in a very wide confidence interval. The study was well designed but had a very small sample size.[30]

Table 2.2 – Description of reviewed studies

Study	No. and Type of Subjects	Probiotic	Probiotic Dose / Day	Treatment Duration	Follow-Up	Probiotic-Treated Group		Control Group	
						Cured (%)	Failed	Cured (%)	Failed
McFarland LV 1994	124 Adults with CDI or RCDI	SB + V/M	2×10^{10}	4 wk	4 wk	42 (73.68)	15	37 (55.22)	30
Surawicz CM 2000	32 Adults with RCDI	SB + V	2×10^{10}	4 wk	4 wk	15 (83.33)	3	7 (50.00)	7
Pochapin M 2000	25 Adults with CDI or RCDI	LGG + V or M	N/R	3 wk	0	7 (63.63)	4	9 (64.28)	5
Wullt M 2003	20 Adults with RCDI	LP 299v + M	5×10^{10}	38 days	0	7 (63.63)	4	3 (33.33)	6
Plummer S 2004	138 Inpatients varied ages	LABB, no V or M	2×10^{10}	20 days	0	67 (97.10)	2	63 (91.30)	6
Lawrence SJ 2005	15 Adults with RCDI	LGG + V or M	6×10^{11}	3 wk	4 wk	5 (62.50)	3	6 (85.71)	1

CDI: *Clostridium difficile* infection; RCDI: Recurrent *Clostridium difficile* infection; SB: *Saccharomyces boulardii*; LGG: *Lactobacillus rhamnosus* GG; LP: *Lactobacillus plantarum* 299v; V: Vancomycin; M: Metronidazole, LABB =*Lactobacillus acidophilus* and *Bifidobacterium bifidum;*N/R: Not reported. (Source: Reference 18)

A meta-analysis by McFarland compared the efficacy of probiotics for the treatment of CDI based on the published RCTs (n = 6) in adult hospitalized patients, Table 2.2. This meta-analysis concluded that probiotic therapy is effective in the treatment of CDI. The pooled relative risk (Figure 2.4) for CDI associated with probiotic use from the six RCTs included was 0.59 (95% CI 0.41 to 0.85). The heterogeneity of the included studies was not significant; however, the relatively small number of trials included could be a limiting factor in this meta-analysis.[18]

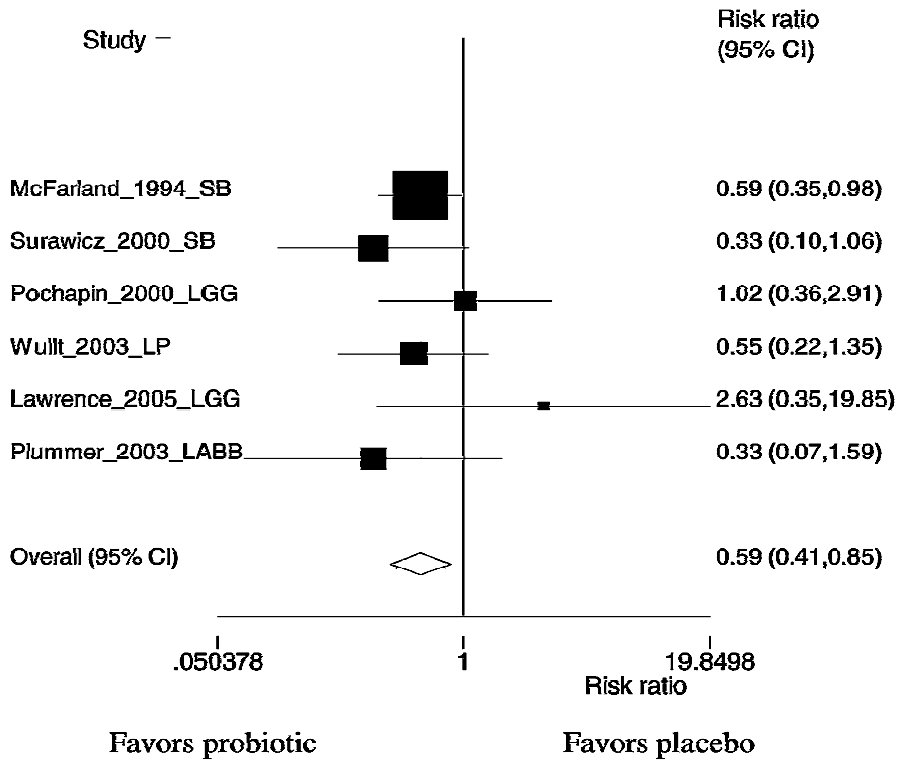

Figure 2.4 – Forest Plot of six randomized controlled trials of probiotics for the treatment of *Clostridium difficile* Infection. (Source: Ref. 18)

CHAPTER 3 METHODOLOGY

Data Source

The data used in this study were obtained from the Clinical Resource Manager (CRM) program developed and maintained by the University HealthSystem Consortium (UHC; www.uhc.edu). UHC is an alliance of 102 academic medical centers representing approximately 90% of the nation's non-profit academic medical centers. UHC provides programs and services to improve clinical, operational, and patient safety performance.[75] The CRM database program brings together data from a subset of participating hospitals, with the actual number of hospitals in the database varying by the year. The information in the database is obtained from various sources including patient encounters, billing information, transactional data, as well as discharge summaries to provide standardized information on in-hospital resource utilization and patient outcomes.[76] The drug use data in the CRM has been previously validated.[77]

Study Design

The study was composed of two general parts: descriptive and analytical. First, the demographic and clinical characteristics of *Lactobacillus* users and non-users were described (Specific Aim 1) using cross sectional study design. Among probiotic users, the initiation date of probiotics was described with respect to the initial antibiotic therapy

initiation date (Specific Aim 2) and CDI treatment initiation date (Specific Aim 3). The second part of this study is a retrospective cohort study (Specific Aim 4). A cohort of all nosocomial CDI patients in the CRM database was reviewed for certain CDI-related outcomes of interest. The relationship between the use of *Lactobacillus* and switch rate of CDI treatment, readmission for CDI, mortality, and post-CDI-diagnosis length of stay were examined. This study was approved by the Institutional Review Board at Virginia Commonwealth University.

Study Sample

The data for this project were derived from a separate study designed to examine the risk of CDI associated with various antibiotic agents.[9] It examined all patients having an ICD-9-CM code for CDI (008.45) and two controls for each case. Controls were randomly drawn from the same hospital and quarter of discharge as the cases. *Lactobacillus* may be considered a non-formulary agent at some institutions. This can result in unreported usage of the agent. Only those hospitals reporting *Lactobacillus* usage were included in this study. For the current study, data were obtained from hospitals participating in the CRM during the period from July 1, 2003, to December 31, 2005, representing 2.5 years in the study period. The analysis was limited to that period because it has relatively high number hospitals who consistently report *Lactobacillus* use and large number of CDI patients. The data included the following information for each patient: demographic characteristics, detailed antibiotics use (e.g., name, start date, end date, and length of therapy), admission and discharge dates, total length of stay, the All Patients

Refined-Diagnosis Related Group (APR-DRG) severity of illness category,[78] and all discharge diagnosis codes. All data were obtained directly from UHC.

Patients included in the analysis were 18 years of age or older and discharged from one of the participating hospitals during the study period. Based on unique patient identification numbers and admission dates, cases of recurrent CDI (readmission) were able to be identified.

Analysis Variables

For the first part of the study, users and non-users of *Lactobacillus* were described with respect to various patient demographic characteristics, CDI status (i.e., no CDI, nosocomial CDI, and non-nosocomial CDI). *Lactobacillus* users were also described with respect to when *Lactobacillus* was started in relation to antibiotic therapy and CDI treatment. Post-CDI-diagnosis length of stay, switch rate of CDI treatment (i.e., switching from vancomycin to metronidazole or vice versa), readmission for CDI, and mortality, are the outcome variables for the second phase.

The primary independent variable of interest for the models in the second part of the study was the use of *Lactobacillus*. Many potential confounding variables were included in the analysis. These included sex, race, transfer from outside hospital status, switch of CDI treatment status, severity of illness, comorbidity, and mortality.

Comorbidity was measured using the Dartmouth-Manitoba version of the Charlson Comorbidity Index (DM-CCI), which is an adaptation of the original Charlson Comorbidity Index (CCI) to utilize the ICD-9-CM codes from administrative data.[58, 79] The

CCI is a list of 19 medical conditions which were selected and weighted based on the strength of their association with mortality. The comorbidity score is the total sum of weights assigned to each condition. The DM-CCI was selected as a measure of comorbidity because it is based on an ICD-9-CM coding system, which is the same coding system used in our database. DM-CCI has been validated in a study, by Ghali et al. comparing it two other comorbidity indexes.[17]

Methods

As mentioned previously, this study has two general parts: a descriptive part (Specific Aims 1-3) and an analytical part (Specific Aim 4). Throughout the study CDI cases are described as nosocomial or non-nosocomial. The nosocomial cases must meet two criteria, the ICD-9-CM code for CDI and starting CDI treatment (metronidazole or vancomycin) on or after day five of hospitalization. Those CDI cases starting treatment before day five were considered non-nosocomial cases.[77] Based on prior research, switching of CDI treatment, readmission for CDI, mortality, and length of stay were selected as the outcomes of interest. These variables are not only represent the morbidity and mortality of patients but also measure the impact of CDI to healthcare system. The exposure variable of interest is the use of a blend of *Lactobacillus acidophilus* and *Lactobacillus helveticus* (Lactinex®).

For the first aim, all patients receiving *Lactobacillus* were compared to those not receiving *Lactobacillus*. The demographic characteristics (age, gender, and race) and the clinical characteristics (CDI status, number of antibiotics received, transfer status, severity

of illness, mortality, readmission, and overall length of stay) were described. Analytical weights were used in this descriptive piece to take into account the way in which the controls were sampled. These weights are described in the next section. The top 5 ICD-9-CM discharge diagnosis codes for *Lactobacillus* users and non-users in the total sample, CDI cases (both nosocomial and non-nosocomial), and non-CDI cases were identified. The number and percentage of patients who had the code and the description of the code were reported. Also, the distribution of antibiotics used by study patients was described. Antibiotics were classified into seventeen different classes or individual drugs: penicillins, penicillinase-resistant penicillins, broad spectrum penicillins, 1st generation cephalosporins, 2nd generation cephalosporins, 3rd and 4th generation cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, macrolides, tetracyclines, trimethoprim/sulfamethoxazole, other sulfonamides, clindamycin, vancomycin, metronidazole, and miscellaneous antibiotics. The specific agents in each class are provided in Table 3.1. For each class of antibiotics the frequency and percentage were reported.

Table 3.1 – Antibiotic agent classifications

Antibiotic class	Antibiotic agents
Penicillins	Penicillin G, penicillin V, amoxicillin, amoxicillin/clavulanate potassium, ampicillin, ampicillin/sulbactam
Penicillinase-resistant penicillins	Cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin
Extended-spectrum penicillins	Carbenicillin disodium, carbenicillin indanyl sodium, mezlocillin, piperacillin, ticarcillin, ticarcillin/clavulanate, piperacillin/tazobactam
1st generation cephalosporins	Cefadroxil, cefazolin, cephalexin, cephalothin, cephapirin, cephradine
2nd generation cephalosporins	Cefaclor, cefprozil, cefonicid, cefotetan,cefoxitin, cefuroxime, loracarbef, cefditoren, cefamanadole
3rd and 4th generation cephalosporins	Cefixime, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, cefpodoxime, ceftriaxone, cefmetazole, cefdinir, ceftibuten, cefepime
Carbapenems	Imipenem/cilastatin, ertapenem, meropenem
Aminoglycosides	Amikacin, gentamicin, kanamycin, netilmicin, streptomycin sulfate, tobramycin, neomycin
Fluoroquinolones	Lomefloxacin, norfloxacin , ofloxacin, moxifloxacin, gatifloxacin, ciprofloxacin, levofloxacin, sparfloxacin, trovafloxacin
Macrolides	Azithromycin dehydrate, clarithromycin, erythromycin, troleandomycin, dirithromycin
Sulfonamide combinations	Sulfamethoxazole/trimethoprim
Other sulfonamides	Sulfadiazine, sulfamethoxazole, sulfasalazine, sulfisoxazole
Tetracyclines	Demeclocycline, doxycycline hyclate, minocycline, oxytetracycline, tetracycline

(cont.)

Table 3.1 (continued)

Lincosamides	Clindamycin
Glycopeptides	Vancomycin
Imidazoles	Metronidazole
Miscellaneous antibiotics	Aztreonam, colistimethate, methenamine hippurate, methenamine mandelate, metronidazole, moxalactam, polymyxin b sulfate, spectinomycin, trimethoprim, vancomycin, bacitracin, linezolid, daptomycin, fosfomycin, trimetrexate glucuronate, quinupristin/dalfopristin, chloramphenicol, tigecycline, telithromycin, furazolidone, nitrofurantoin, nitrofurantoin, macrocrystals, rifaximin

For the second aim, the day on which *Lactobacillus* was initiated with respect to antibiotic therapy initiation dates were described among patients who were on antibiotics and *Lactobacillus*. The frequency and percentage of patients who started *Lactobacillus* at each initiation day were reported. The percentages were also reported by CDI status.

For the third aim, the day *Lactobacillus* was initiated with respect to CDI treatment initiation dates were described among CDI patients who were using *Lactobacillus*. The frequency and percentage of patients who started *Lactobacillus* at each initiation day were reported. The percentages were also reported by CDI status.

For the fourth aim, the sample included all nosocomial CDI patients and the exposure variable of interest is *Lactobacillus* use. The crude (un-adjusted) relationship between *Lactobacillus* use and the following CDI related outcomes were calculated: switch rate of CDI treatment, readmission for CDI, mortality, and post-CDI-diagnosis length of stay. The adjusted relationships between *Lactobacillus* use and the same outcomes of interest were examined and reported while adjusting for various potential confounders, such as age, sex, race, transfer from outside hospital status, switch of CDI treatment status, severity of illness, intensive care unit (ICU) days, Charlson score, and mortality. Comorbidity and other potential confounding variables were accounted for in the analysis for more accurate assessment of the relationship. For assessment of crude and adjusted relationship between *Lactobacillus* use and switch rate of CDI treatment only those who used *Lactobacillus* before diagnosis were considered in the analysis

Statistical Analysis

Descriptive statistics including the mean and standard deviation or median and 25th and 75th percentiles for continuous variable or proportion with 95% confidence intervals for categorical variables were calculated for all demographic and clinical characteristics measured. For the comparison of continuous variables that were approximately normally distributed, the Student's t-test was used. For non-normally distributed variables, the Wilcoxon rank sum test was used. Fisher's exact test was used for categorical data. The relationship between *Lactobacillus* use and each of categorical outcome variables (switch rate of CDI treatment, readmission for CDI, and mortality) were examined with a multiple logistic regression model. A linear regression model was built for the examination of the relationship between *Lactobacillus* use and length of stay. Odds ratios from the logistic regression model and their 95% confidence intervals were reported for all categorical variables. From the linear regression model, the regression coefficients and 95% confidence intervals were reported. All potential confounders were accounted for in the data analysis to assure a more accurate assessment of the relationship between *Lactobacillus* and the selected outcome variables. For all analyses, statistical significance was determined using two sided Type I error level of 5%. Statistical analyses were conducted using Stata/SE version 10.0 (Stata Corporation, College Station, TX).

In the original dataset, all patients with an ICD-9-CM code for CDI were obtained from the CRM database within the study period. The non-CDI patients were sampled so that there were two of these non-CDI drawn from the same quarter and hospital as each CDI patient. To account for this random sampling approach, a set of analytical weights

were derived. These weights are equal to the inverse of the probability of being sampled. Since all patients with a CDI diagnosis code were sampled, their weight was equal to 1. For the controls, the weights were equal to the inverse of twice number of cases identified for a given hospital and quarter divided by the total number of discharges for that quarter less twice the number of cases. This weight can be represented as shown in the following equation where w_i is the weight for patient i, c_j is the number of cases from hospital j, and d_j is the number of adult discharges for hospital j.

$$w_i = \left(\frac{2c_j}{d_j - 2c_j} \right)^{-1}$$

These weights were applied when performing the descriptive analysis for Specific Aim 1.

CHAPTER 4: RESULTS

Hospital Characteristics

A description of the general characteristics of participating hospitals in the study sample are presented in Tables 4.1 and 4.2. The data used in this study represent 31 teaching hospitals from six different regions throughout the nation. About half of the hospitals were from the Midwestern or Southeastern region (22.58% each). The other half were mainly from Mid-Atlantic or Mid-Continent regions (19.35% each). Only two hospitals were from New England region, 6.45%. The capacity of these hospitals range from 156 to 805 beds (mean = 513.97, SD = 153.57). About 70% of the hospitals have a capacity range from 300 to 600 beds. All of the participating hospitals had a case mix index (CMI) over one ranging from 1.46 to 2.13, (mean = 1.81, SD = 0.16). That means these hospitals treat sicker patients and therefore, their adjusted cost per patient or per day is more than the average reimbursement by Medicaid.[14]

Table 4.1 – Hospital characteristics

Hospital Characteristic	No. Hospitals (%)
Region	
Midwestern	7 (22.58%)
Southeastern	7 (22.58%)
Mid-Atlantic	6 (19.35%)
Mid-Continent	6 (19.35%)
Western	3 (9.68%)
New England	2 (6.45%)
Bed size category	
1 to 199	1 (3.23%)
200 to 299	0 (0.00%)
300 to 499	12 (38.71%)
500 to 599	10 (32.26%)
600 to 699	4 (12.90%)
More than 700	4 (12.90%)
Bed size	
Mean (SD)	513.97 (153.57)
Median (25^{th}, 75^{th} percentile)	533 (396, 614)
Case mix index	
Mean (SD)	1.81 (0.16)
Median (25^{th}, 75^{th} percentile)	1.83 (1.68, 1.92)

SD: standard deviation

Study Population

The total population of this study, as shown in figure 4.1, was 35,670 patients. The cases were 8,968 (25%) and the remaining 26,703 (75%) were controls who had no CDI. Almost half of the cases were non-nosocomial cases 4,428 (12%) and the other half were nosocomial cases 4,540 (13%). There were 480 *Lactobacillus* users majority of them had CDI (237 (49%) had non-nosocomial CDI and 202 (42%) had nosocomial CDI). After applying the weight there were 2,564 *Lactobacillus* users majority had no CDI (Figure 4.2).

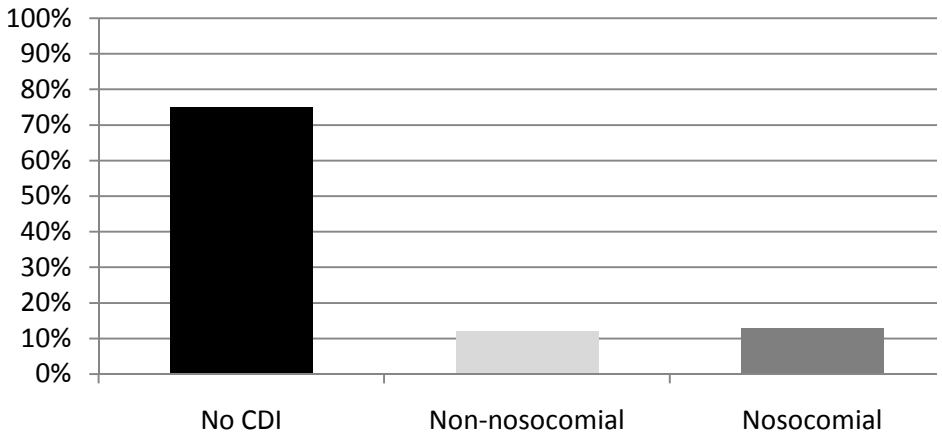

Figure 4.1 – CDI status in overall study sample

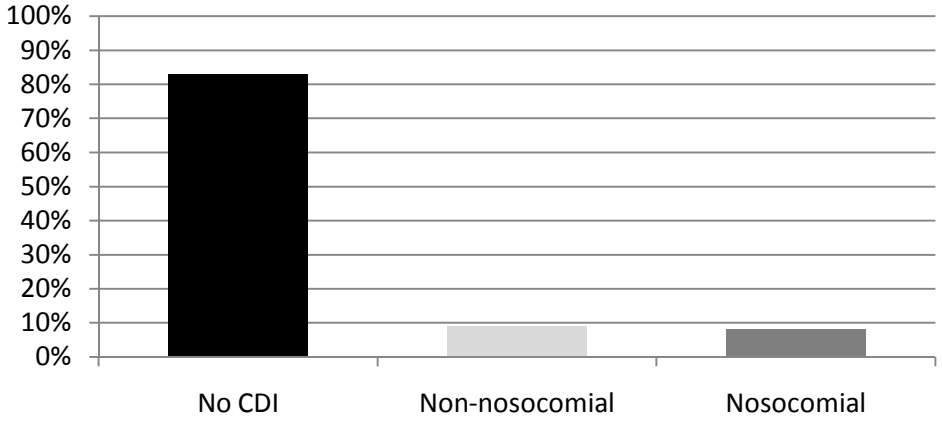

Figure 4.2 – CDI status within *Lactobacillus* users [weighted percentages]

Patients Demographic and Clinical Characteristics

Of the 480 *Lactobacillus* users, 439 had CDI (237 (54%) had non-nosocomial CDI and 202 (46%) had nosocomial CDI). The average duration of use was not significantly different among non-nosocomial and nosocomial cases, 9.55 days (SD = 16.66) and 11.29 days (SD = 18.10), respectively (Table 4.2).

The unweighted and weighted detailed description of the demographic and clinical characteristics of *Lactobacillus* users and non-users are listed below (Table 4.3 and Table 4.4, respectively). Users of *Lactobacillus* were significantly older that non-users with average ages of about 61.1 years (SD = 18.5) and 53.8 years (SD = 18.98), respectively. Both groups were almost equally distributed between male and female. The majority of both groups were Caucasian or African American. Among *Lactobacillus* users, 375 (78.13%) were Caucasian and 54 (11.25%) were African American. In the non-users, 21,806 (61.97%) were Caucasian and 7,597 (21.59%) were African American. There were no big differences in race distribution after applying the weight. Also, almost all *Lactobacillus* users 439 (91.46%).had CDI with 237 (49.38%) users having non-nosocomial CDI and 202 (42.08%) having nosocomial CDI. Of the non-users there were 4,191 (11.91%) non-nosocomial CDI cases and 4,338 (12.33%) nosocomial CDI cases (Table 4.3 and Figures 4.3). Patents with no CDI [2,125 (82.88%)] were the majority after applying the weights (Table 4.4 and Figure 4.4). With respect to the number of antibiotics received, over 60% of the *Lactobacillus* users had four or more antibiotics. In the non-users, almost 28% received no antibiotics and almost 25% received only one antibiotic (Table 4.3 and Figure 4.5). After applying the analytical weights, the percentage of

Lactobacillus users who did not use any antibiotics increased from 0.42% to 7.53%, and those who used four or more antibiotics decreased from 61.88% to 55.38%. For non-users the percentage of those who did not use any antibiotics increased from 27.68% to 36.38% and those who used four or more antibiotics decreased from 19.47% to 8.15% (Table 4.4 and Figure 4.6). A majority of the users had an APR-DRG severity of illness category of major (35.83%) or extreme (53.33%). Oppositely, the non-users were mainly minor (24.97%) or major (33.25%) severity of illness category (Table 4.3 and Figure 4.7). After applying the analytical weights, the percentage of users who were moderately ill increased from 10.42% to 22.12% and those who were extremely ill decreased from 53.33% to 45.16%. For non-users the percentage those who were moderately ill increased from 24.97% to 32.37% and those who were extremely ill decreased from 15.72% to 6.37% (Table 4.4 and Figure 4.8). Unlike the non-users, almost one third of *Lactobacillus* users were readmitted for CDI. The average length of stay was significantly higher for users (mean = 27.64, SD = 39.07; median = 17) than non-users (mean = 8.63, SD = 13.71; median = 4) (P <0.001). After applying the analytical weights, the mean length of stay for both users and non-users decreased from 27.64 days to 24.53 days and from 8.63 days to 5.44 days; respectively. Generally, there were minor differences between weighted and um-weighted statistics.

There was small missing data (2.85%) in the race variable which was added to the "other" category. Also, there was negligible missing data (< 0.01%) in the gender variable. Other variables have no missing data. Three observations were dropped because their diagnosis dates were after their discharge dates.

Table 4.2 – Description of *Lactobacillus* usage by CDI patients

***Lactobacillus* Usage**	**Non-nosocomial CDI**	**Nosocomial CDI**	**P-value**
Count (%)	237 (53.99)	202 (46.01)	
Mean (SD)	9.55 (16.66)	11.29 (18.10)	0.2965
Median (25^{th}, 75^{th} percentile)	6 (2, 11)	6 (3, 12)	0.2276

SD: Standard deviation; CDI: *Clostridium difficile* infection.

Table 4.3 – Patient demographic and clinical characteristic of *Lactobacillus* users and non-users [Unweighted statistics]

	Lactobacillus		
Variables	**Users[a] (n = 480)**	**Non-users[b] (n = 35,190)**	**P-value**
Age in years [Mean (SD)]	61.09 (18.15)	53.78 (18.98)	< 0.001
Gender[c] [No. (%)]			0.166
Male	248 (51.67%)	19,294 (54.83%)	
Female	232 (48.33%)	15,892 (45.16%)	
Race [No. (%)]			< 0.001
Caucasian	375 (78.13%)	21,806 (61.97%)	
African American	54 (11.25%)	7,597 (21.59%)	
Hispanic	23 (4.79%)	2,415 (6.86%)	
American Indian/Eskimo	1 (0.21%)	170 (0.48%)	
Asian	3 (0.63%)	550 (1.56%)	
Other	12 (2.50%)	1,646 (4.68%)	
Unknown	12 (2.50%)	1,006 (2.86%)	
CDI status [No. (%)]			< 0.001
No CDI	41 (8.54%)	26,661 (75.76%)	
Non-nosocomial CDI	237 (49.38%)	4,191 (11.91%)	
Nosocomial CDI	202 (42.08%)	4,338 (12.33%)	
Number of antibiotics received [No. (%)]			< 0.001
None	2 (0.42%)	9,741 (27.68%)	
One	35 (7.29%)	8,679 (24.66%)	
Two	60 (12.50%)	5,929 (16.85%)	
Three	86 (17.92%)	3,988 (11.33%)	
Four or more	297 (61.88%)	6,853 (19.47%)	
Transferred from an outside hospital [No. (%)]			< 0.001
No	458 (95.42%)	34,984 (99.41%)	
Yes	22 (4.58%)	206 (0.59%)	
Severity of Illness[d] [No. (%)]			< 0.001
Minor	2 (0.42%)	8,788 (24.97%)	
Moderate	50 (10.42%)	11,701 (33.25%)	
Major	172 (35.83%)	9,162 (26.04%)	
Extreme	256 (53.33%)	5,533 (15.72%)	

(cont.)

Table 4.4 (continued)

Mortality [No. (%)]			< 0.001
No	438 (91.25%)	33,826 (96.12%)	
Yes	42 (8.75%)	1,364 (3.88%)	
Readmission [No. (%)]			< 0.001
No	345 (71.88%)	31,890 (90.62%)	
Yes	135 (28.13%)	3,300 (9.38%)	
Length of stay[e]			
Mean (SD)	27.64 (39.07)	8.63 (13.71)	<0.001
Median (25th, 75th percentile)	17 (9, 32)	4 (2, 9)	<0.001

SD: Standard deviation; CDI: *Clostridium difficile* infection.
[a]All probiotics users; [b]All probiotics non-users; [c]There are four non-users with unknown gender; [d]There were six non-users with no severity of illness specified; [e]Overall length of stay.

Table 4.4 – Patient demographic and clinical characteristic of *Lactobacillus* users and non-users [Weighted statistics]

	Lactobacillus		
Variables	**Users[a] (n = 2,500)**	**Non-users[b] (n = 1,386,538)**	**P-value**
Age-year [Mean (SD)]	58.92 (18.85)	51.88 (18.99)	< 0.001
Gender[c] [No. (%)]			0.2130
Male	1404 (54.74%)	600,000 (43.21%)	
Female	1161 (45.26%)	790,000 (56.79%)	
Race [No. (%)]			0.0011
White	2188 (85.30%)	850,000 (61.67%)	
African American	235.9 (9.20%)	290,000 (20.87%)	
Hispanic	70.84 (2.76%)	110,000 (7.82%)	
American Indian/Eskimo	1 (0.04%)	8,332 (0.60%)	
Asian	3 (0.12%)	22,000 (1.61%)	
Other	53.74 (2.10%)	65,000 (4.70%)	
Unknown	12 (0.47%)	38,000 (2.73%)	
CDI status [No. (%)]			< 0.001
No CDI	2125 (82.88%)	1,400,000 (99.38%)	
Nosocomial CDI	237 (9.24%)	4191 (0.30%)	
Non-nosocomial CDI	202 (7.88%)	4338 (0.31%)	
Number of antibiotics received [No. (%)]			< 0.001
None	193 (7.53%)	500,000 (36.38%)	
One	157.5 (6.14%)	410,000 (29.30%)	
Two	456.2 (17.79%)	240,000 (17.04%)	
Three	337.4 (13.16%)	130,000 (9.13%)	
Four or more	1420 (55.38%)	110,000 (8.15%)	
Transferred from an outside hospital [No. (%)]			< 0.001
No	2542 (99.14%)	1,400,000 (99.98%)	
Yes	22 (0.86%)	206 (0.02%)	
Severity of Illness [No. (%)]			< 0.001
Minor	2 (< 0.0018%)	450,000 (32.37%)	
Moderate	567.3 (22.12%)	550,000 (39.71%)	
Major	836.9 (32.64%)	300,000 (21.53%)	
Extreme	1158 (45.16%)	88,000 (6.37%)	

(cont.)

Table 4.4 (continued)

Mortality [No. (%)]			< 0.001
No	2219 (86.52%)	1,400,000 (97.70%)	
Yes	345.6 (13.48%)	32,000 (2.30%)	
Readmission [No. (%)]			< 0.001
No	2161(84.29%)	1,300,000 (95.79%)	
Yes	403 (15.71%)	58,000 (4.21%)	
Length of stay [d]			
Mean (SD)	24.53 (27.53)	5.44 (7.733)	< 0.001
Median (25^{th}, 75^{th} percentile)	19 (7, 31)	3 (2, 6)	<0.001

SD: Standard deviation; CDI: *Clostridium difficile* infection.
[a]All estimated probiotics users; [b]All estimated probiotics non-users; [c]There are four missing observations with missing/unknown gender; [d]Overall length of stay.

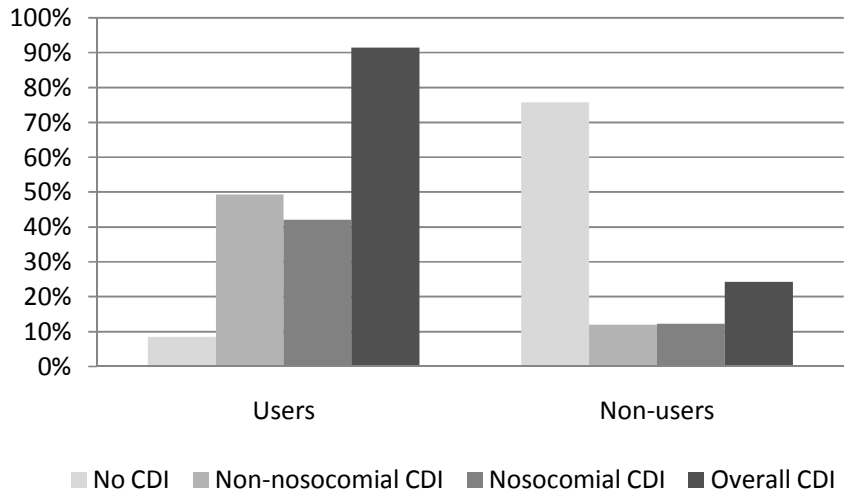

Figure 4.3 – Unweighted CDI status of *Lactobacillus* users and non-users

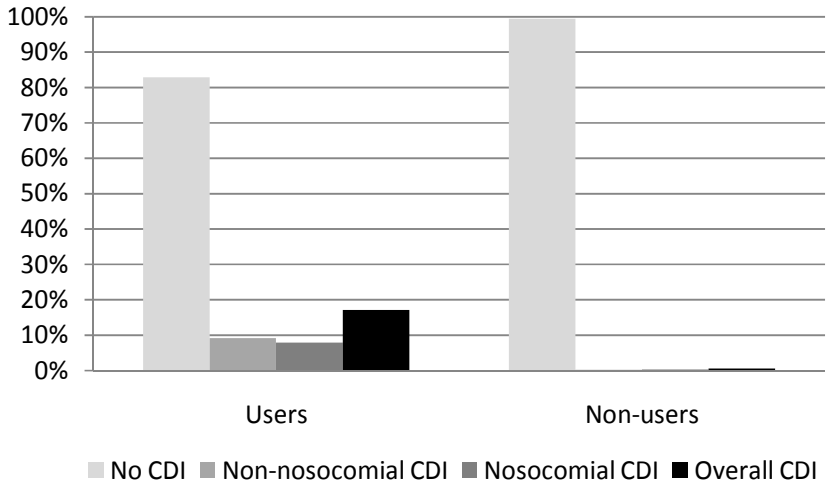

Figure 4.4 – Weighted CDI status of *Lactobacillus* users and non-users

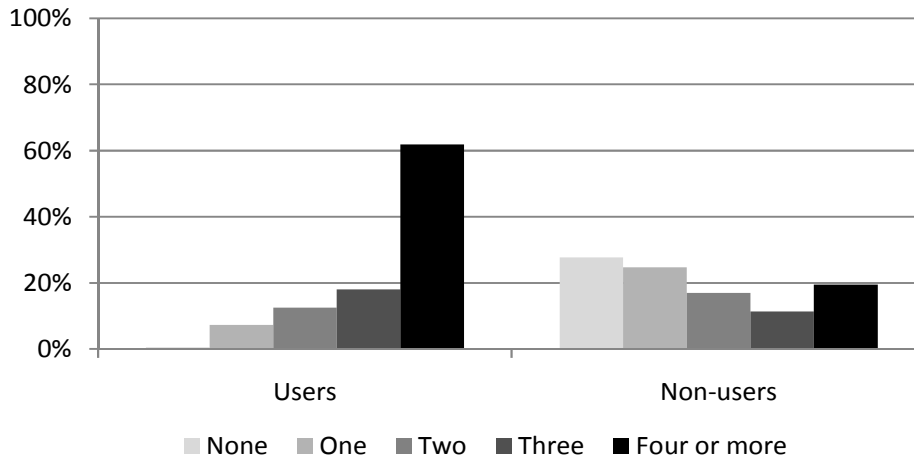

Figure 4.5 – Unweighted number of antibiotics received by *Lactobacillus* users and non-users

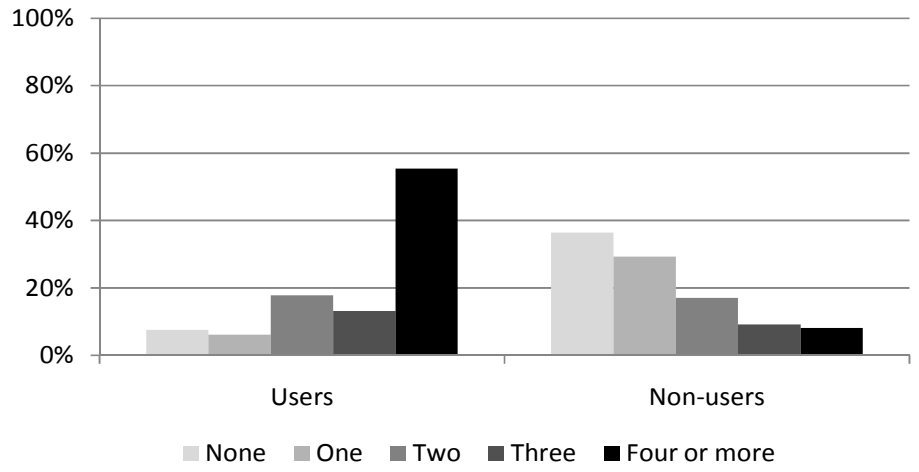

Figure 4.6 – Weighted number of antibiotics received by *Lactobacillus* users and non-users

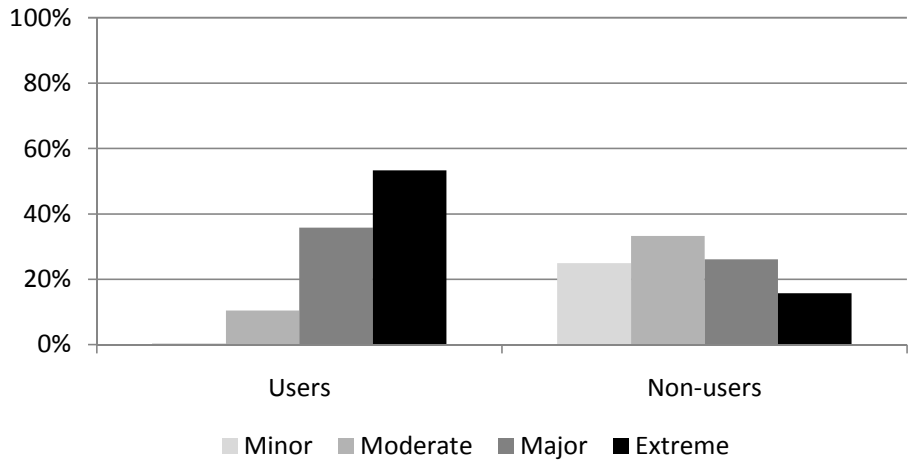

Figure 4.7 – Unweighted severity of illness of *Lactobacillus* users and non-users

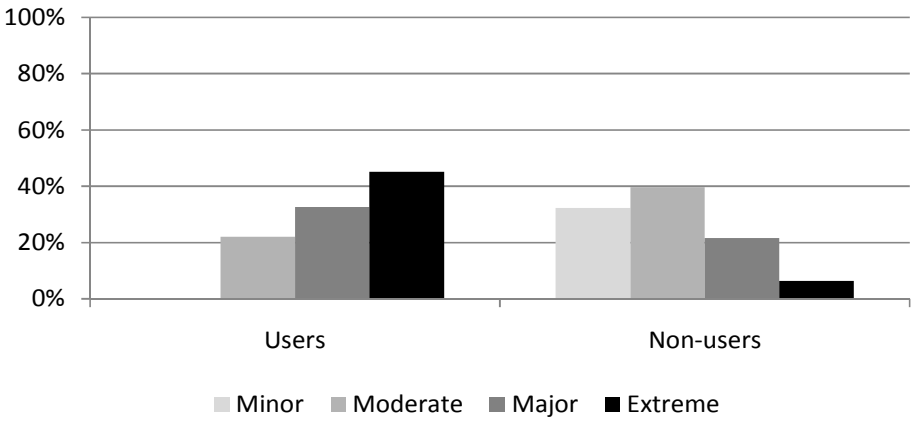

Figure 4.8 – Weighted severity of illness of *Lactobacillus* users and non-users

Top 5 Diagnoses Codes

In the total sample, CDI and unspecified essential hypertension were the first and second most frequent diagnosis codes among *Lactobacillus* users: 443 (7.0%) and149 (2.4%), respectively. The same codes, but in an alternate order, were the highest among non-users; 11,838 (3.7%) and 8,622 (2.8%). Other top 5 diagnosis codes for users and non-users in the overall sample were listed in Table 4.5.

In the CDI cases, CDI and unspecified essential hypertension, similar to the overall sample, were the first and second most frequent diagnosis codes among *Lactobacillus* users; 439 (7.5%) and 138 (2.8%) respectively. Exactly the same diagnosis codes were also the highest among non-users: 11,838 (3.7%) and 8,622 (2.8%), respectively. Other top 5 diagnosis codes for users and non-users among all CDI cases were listed in Table 4.6. CDI was the number one diagnosis in this group because the diagnosis code for CDI was used to identify CDI cases in participating hospitals for the original study.

In the non-CDI patients, volume depletion was the most frequent diagnosis code among *Lactobacillus* users (12 [2.4%]). Among *Lactobacillus* non-users in the non-CDI patient, essential hypertension with no complications was the most frequent diagnosis code (8,953 [4.6%]). Other top 5 diagnosis codes for users and non-users in the overall sample are listed in Table 4.7.

Table 4.5 – Top 5 diagnoses ICD-9-CM codes for *Lactobacillus* users and non-users in the total sample

Users codes		Non-users codes	
Diagnosis (code)	**No. (%)**	**Diagnosis (code)**	**No. (%)**
Intestinal infection with *Clostridium difficile* (008.45)	443 (7.00)	Essential hypertension, unspecified (401.9)	11,838 (3.86)
Essential hypertension, unspecified (401.9)	149 (2.35)	Intestinal infection with *Clostridium difficile* (008.45)	8,622 (2.81)
Urinary tract infection, site not specified (599.0)	144 (2.27)	Diabetes mellitus (unspecified type) without complications not stated as uncontrolled (250.00)	4,595 (1.50)
Volume depletion (276.5)	118 (1.86)	Coronary atherosclerosis of a native coronary artery (414.01)	4,143 (1.35)
Congestive heart failure, unspecified (428.0)	99 (1.56)	Esophageal reflux (530.81)	3,916 (1.28)

CDI: *Clostridium difficile* infection; N: Number of patients who have the code;Users: *Lactobacillus* users; Non-users: *Lactobacillus* non-users; ICD-9-CM: The International classification of diseases, 9th revision, clinical modification;

Table 4.6 – Top 5 diagnoses ICD-9-CM codes for *Lactobacillus* users and non-users among CDI patients

Users codes		Non-users codes	
Diagnosis (code)	**No. (%)**	**Diagnosis (code)**	**No. (%)**
Intestinal infection with *Clostridium difficile* (008.45)	439 (7.53)	Intestinal infection with *Clostridium difficile* (008.45)	8,552 (7.80)
Essential hypertension, unspecified (401.9)	138 (2.37)	Essential hypertension, unspecified (401.9)	2,885 (2.63)
Urinary tract infection, site not specified (599.0)	134 (2.30)	Urinary tract infection, site not specified (599.0)	1,936 (1.76)
Volume depletion (276.5)	106 (1.82)	Volume depletion (276.5)	1,683 (1.53)
Congestive heart failure, unspecified (428.0)	91 (1.56)	Congestive heart failure, unspecified (428.0)	1,517 (1.38)

CDI: *Clostridium difficile* infection; N: Number of patients who have the code; Users: *Lactobacillus* users; Non-users: *Lactobacillus* non-users; ICD-9-CM: The International classification of diseases, 9th revision, clinical modification.

Table 4.7 – Top 5 diagnoses ICD-9-CM codes for *Lactobacillus* users and non-users among non-CDI patients

Users		Non-users	
Diagnosis (code)	**No. (%)**	**Diagnosis (code)**	**No. (%)**
Volume depletion (276.5)	12 (2.40)	Essential hypertension with no complications (401.9)	8,953 (4.55)
Essential hypertension with no complications (401.9)	11 (2.20)	Diabetes mellitus (unspecified type) without complications not stated as uncontrolled (250.00)	3,352 (1.70)
Urinary tract infection, site not specified (599.0)	10 (2.00)	Coronary atherosclerosis of a native coronary artery (414.01)	3,128 (1.6)
Acute renal failure, unspecified (584.9)	9 (1.80)	Tobacco use disorder (305.1)	3,125 (1.59)
Congestive heart failure, unspecified (428.0)	8 (1.60)	Esophageal reflux (530.81)	3,039 (1.59)

CDI: *Clostridium difficile* infection; N: Number of patients who have the code; Users: *Lactobacillus* users; Non-users: *Lactobacillus* non-users; ICD-9-CM: The International classification of diseases, 9th revision, clinical modification.

Distribution of Antibiotic Use in *Lactobacillus* Users

Among antibiotic users, the frequency and percentage of those who were also on *Lactobacillus* and those who were not are described in Table 4.8 and Table 4.9. In the overall sample metronidazole (10,215 [28.64%]) and fluoroquinolones (9,532 [26.72%]), and 1st generation cephalosporins (8,883 [24.90%]) were the most commonly prescribed antibiotics. Other sulfonamides, tetracyclines, penicillinase-resistant penicillins were the least commonly prescribed antibiotics. After applying the weights (Table 4.9 and Figure 4.10) 1st generation cephalosporins (370,000 [26.54%]), fluoroquinolones (250,000 [18.16%]), and Penicillins (130,000 [9.58%]) were the most commonly prescribed antibiotics.

Before applying the analytical weights (Table 4.8 and Figure 4.9), among *Lactobacillus* users, metronidazole (431 [89.79%]), fluoroquinolones (289 [60.21%]), 3rd and 4th generation cephalosporins (171 [35.63%]), broad spectrum penicillins (167 [34.79%]), vancomycin (13; [27.08%]), and 1st generation cephalosporins (114 [23.75%]) were the most commonly prescribed antibiotics. The weighted frequencies (Table 4.9 and Figure 4.10) among *Lactobacillus* users were: fluoroquinolones (1,630 [63.57%]), metronidazole (1,294 [50.47%]), 1st generation cephalosporins (792 [30.88%]), 3rd and 4th generation cephalosporins (753 [29.36%]), broad spectrum penicillins (711 [27.72%]), macrolides (552; [21.54%]), and were the most commonly prescribed antibiotics.

Antibiotic prescribing trend in the *Lactobacillus* non-users was almost the same as the overall sample. Generally, tetracyclines and other sulfonamides were the least

prescribed antibiotics. Almost all *Lactobacillus* users (431 [89.79%]) used metronidazole mainly for the CDI treatment.

Table 4.8 – Distribution of antibiotic use in the total sample and in *Lactobacillus* users and non-users during total hospital stay [unweighted]

	Overall		Users		Non-users	
Antibiotics classes	**No.**	**%**	**No.**	**%**	**No.**	**%**
Penicillins	3,644	10.22	71	14.79	3,573	10.15
Penicillinase-resistant penicillins	550	1.54	23	4.79	527	1.50
Broad spectrum penicillins	4,337	12.16	167	34.79	4,170	11.85
1st gen. cephalosporins	8,883	24.90	114	23.75	8,769	24.92
2nd gen. cephalosporins	1,583	4.44	19	3.96	1,564	4.44
3rd & 4th gen. cephalosporins	5,327	14.93	171	35.63	5,156	14.65
Carbapenems	2,059	5.77	80	16.67	1,979	5.62
Aminoglycosides	3,530	9.90	82	17.08	3,448	9.80
Fluoroquinolones	9,532	26.72	289	60.21	9,243	26.27
Macrolides	2,467	6.92	85	17.71	2,382	6.77
Tetracyclines	420	1.18	10	2.08	410	1.17
Trimethoprim/Sulfamethoxazole	2,423	6.79	63	13.13	2,360	6.71
Other sulfonamides	199	0.56	0	0.00	199	0.57
Clindamycin	2,064	5.79	29	6.04	2,035	5.78
Vancomycin	1,068	3.03	130	27.08	1,198	3.36
Metronidazole	10,215	28.64	431	89.79	9,784	27.80
Miscellaneous	2,951	8.27	91	18.96	2,860	8.13

N: Number of users in each class of antibiotics; %: Percentage of users of each class of antibiotics to the total number of patients in the category.
Note: Percentage do not sum to 100 because patients may receive more than one class of antibiotics. An index for detail list of all antibiotics is provided in the appendix (A).

Table 4.9 – Distribution of antibiotic use in the total sample and in *Lactobacillus* users and non-users during total hospital stay [weighted]

	Overall		Users		Non-users	
Antibiotics classes	**No.**	**%**	**No.**	**%**	**No.**	**%**
Penicillins	130,000	9.58	375	14.63	130,000	9.58
Penicillinase-resistant penicillins*	16,000	1.18	222	8.65	16,000	1.17
Broad spectrum penicillins*	92,000	6.62	711	27.72	91,000	6.58
1st gen. cephalosporins	370,000	26.54	792	30.88	370,000	26.53
2nd gen. cephalosporins	66,000	4.74	218	8.52	66,000	4.74
3rd & 4th gen. cephalosporins*	120,000	8.69	753	29.36	120,000	8.65
Carbapenems*	28,000	2.03	428	16.68	28,000	2.00
Aminoglycosides*	110,000	7.61	453	17.67	110,000	7.60
Fluoroquinolones*	250,000	18.16	1,630	63.57	250,000	18.07
Macrolides*	72,000	5.15	552	21.54	71,000	5.12
Tetracyclines	13,000	0.96	99	3.86	13,000	0.95
Trimethoprim/Sulfamethoxazole*	60,000	4.34	370	14.41	60,000	4.33
Other sulfonamides	3858	0.28	0	0.00	3858	0.28
Clindamycin	73,000	5.24	128	4.98	73,000	5.24
Vancomycin*	2413	0.17	338	13.17	2076	0.15
Metronidazole*	85,000	6.15	1,294	50.47	84,000	6.07
Miscellaneous*	80,000	5.77	596	23.25	80,000	5.74

N: Number of users in each class of antibiotics; %: Percentage of users of each class of antibiotics to the total number of patients in the category.

Note: Percentage do not sum to 100 because patients may receive more than one class of antibiotics. An index for detail list of all antibiotics is provided in the appendix (A).

Distribution of Antibiotic Use and CDI status

Among antibiotic users, the frequency and percentage of non-CDI, non-nosocomial CDI, and nosocomial CDI patients are described in Table 4.10 and Table 4.11. Antibacterial usage in the non-CDI group was mostly1st generation cephalosporin (7,086 [26.54%]) and fluoroquinolones (4,746 [17.77%]) Vancomycin and tetracyclines were the least commonly prescribed antibiotics (Table 4.10 and Figure 4.10).

Before weighting (Table 4.10), metronidazole (4,227 [95.46%]), fluoroquinolones (2,107 [47.58%]), 3rd & 4th generation cephalosporins (1,214 [27.42%]), broad spectrum penicillins (1,022 [23.08%]), and vancomycin (715 [16.15%]) were the most commonly prescribed antibiotics among non-nosocomial CDI cases. After applying the weight (Table 4.11), the same antibiotics were most commonly prescribed. Other sulfonamides, penicillinase-resistant penicillins, and tetracyclines were the least prescribed antibiotics.

In the nosocomial CDI patients before weighting (Table 4.10), metronidazole (4,485 [98.79%]), fluoroquinolones (2,679 [59.01%]), 3rd & 4th generation cephalosporins (1,830 [40.31%]), broad spectrum penicillins (1,552 [34.19%]), and 1st generation cephalosporins (1,311 [28.88%]) were most commonly prescribed antibiotics. After applying the weight (Table 4.11), the same antibiotics were most commonly prescribed. Tetracyclines and other sulfonamides were the least prescribed antibiotics.

Generally tetracyclines and other sulfonamides were the least prescribed antibiotics. Almost all CDI cases used metronidazole, with (4,485 [98.59%]) of nosocomial cases and (4,227 [5.46%]) of non-nosocomial cases using the agent. This was most likely for CDI treatment.

Table 4.10– Distribution of antibiotic use in the non-CDI, non-nosocomial CDI, and nosocomial CDI patients [unweighted]

	Non-CDI		Non-nosocomial		Nosocomial	
Antibiotics classes	**No.**	**%**	**No.**	**%**	**No.**	**%**
Penicillins	2,449	9.17	477	10.77	718	15.81
Penicillinase-resistant penicillins	325	1.22	65	1.47	160	3.52
Broad spectrum penicillins	1,763	6.60	1,022	23.08	1,552	34.19
1st gen. cephalosporins	7,086	26.54	486	10.98	1,311	28.88
2nd gen. cephalosporins	1,149	4.30	109	2.46	325	7.16
3rd & 4th gen. cephalosporins	2,283	8.55	1,214	27.42	1,830	40.31
Carbapenems	570	2.13	519	11.72	970	21.37
Aminoglycosides	59.01	7.80	532	12.01	915	20.15
Fluoroquinolones	4,746	17.77	2,107	47.58	2,679	59.01
Macrolides	1,415	5.30	423	9.55	629	13.85
Tetracyclines	241	0.90	77	1.74	102	2.25
Trimethoprim/Sulfamethoxazole	1,139	4.27	511	11.54	773	17.03
Other sulfonamides	95	0.36	36	0.81	68	1.50
Clindamycin	1,361	5.10	214	4.83	489	10.77
Vancomycin	27	0.10	715	16.15	456	10.04
Metronidazole	1,503	5.63	4,227	95.46	4,485	98.79
Miscellaneous	1,588	5.95	498	11.25	865	19.05

N: Number of users in each class of antibiotics; %: Percentage of users of each class of antibiotics to the total number of patients in the category.

Note: Percentage do not sum to 100 because patients may receive more than one class of antibiotics.

Table 4.11– Distribution of antibiotic use in the non-CDI, non-nosocomial CDI, and nosocomial CDI patients [weighted]

	Non-CDI		Non-nosocomial		Nosocomial	
Antibiotics classes	**No.**	**%**	**No.**	**%**	**No.**	**%**
Penicillins*	130,000	9.56	477	10.77	718	15.81
Penicillinase-resistant penicillins*	16,000	1.17	65	1.47	160	3.52
Broad spectrum penicillins*	89,000	6.47	1,022	23.08	1,552	34.19
1st gen. cephalosporins*	370,000	26.58	486	10.98	1,311	28.88
2nd gen. cephalosporins*	65,000	4.74	109	2.46	325	7.16
3rd & 4th gen. cephalosporins*	120,000	8.52	1,214	27.42	1,830	40.31
Carbapenems*	27,000	1.93	519	11.72	970	21.37
Aminoglycosides*	100,000	7.56	532	12.01	915	20.15
Fluoroquinolones*	250,000	17.93	2,107	47.58	2,679	59.01
Macrolides*	70,000	5.11	423	9.55	629	13.85
Tetracyclines*	13,000	0.95	77	1.74	102	2.25
Trimethoprim/Sulfamethoxazole*	59,000	4.28	511	11.54	773	17.03
Other sulfonamides*	3754	0.27	36	0.81	68	1.50
Clindamycin*	72,000	5.23	214	4.83	489	10.77
Vancomycin*	1242	0.09	715	16.15	456	10.04
Metronidazole*	77,000	5.56	4,227	95.46	4,485	98.79
Miscellaneous*	79,000	5.71	498	11.25	865	19.05

N: Number of users in each class of antibiotics; %: Percentage of users of each class of antibiotics to the total number of patients in the category.

Note: Percentage do not sum to 100 because patients may receive more than one class of antibiotics.

Description of Lactobacillus and Antibiotic Initiation Dates

Table 4.12 and Figure 4.9 show the description of the day on which *Lactobacillus* was initiated with respect to antibiotic initiation dates among non-CDI, non-nosocomial CDI, and nosocomial CDI patients. Over half of the non-CDI patients used *Lactobacillus* five or more days after initiation of antibiotic therapy and (11 [30.56%]) started *Lactobacillus* on or before the same day antibiotics were initiated. Similar to the non-CDI group, the majority of non-nosocomial CDI patients started *Lactobacillus* five or more days after antibiotic therapy was initiated; (86 [42.36%]). Also, the number of those who used it on the same day or one day after antibiotic therapy was initiated was relatively high at 36 (17.73%) and 25 (12.33%), respectively. In nosocomial CDI cases the majority (151 [80.32%]) started *Lactobacillus* five or more days after antibiotic therapy was initiated. In general, *Lactobacillus* was mostly initiated five days or more after antibiotic therapy was initiated (256 [59.95%]) or at the same day antibiotic therapy was initiated (59 [13.82%]).

Table 4.12 – Description of the day *Lactobacillus* was initiated with respect to antibiotic initiation date

Initiation of antibiotic therapy	No CDI (n=36)	Non-nosocomial CDI (n= 203)	Nosocomial CDI (n=188)	Total (n= 427)
	No. (%)	No. (%)	No. (%)	No. (%)
Before*	5 (13.89)	15 (7.39)	9 (4.79)	29 (6.79)
Same day	6 (16.67)	36 (17.73)	17 (9.04)	59 (13.82)
One day after	2 (5.56)	25 (12.33)	3 (1.60)	30 (7.03)
Two days after	0 (0.00)	13 (6.40)	2 (1.06)	15 (3.51)
Three days after	1 (2.78)	16 (7.88)	4 (2.13)	21 (4.92)
Four days after	3 (8.33)	12 (5.91)	2 (1.06)	17 (3.98)
Five or more days after	19 (52.77)	86 (42.36)	151 (80.32)	256 (59.95)

CDI: *Clostridium difficile* infection.
**Lactobacillus* has been initiated before antibiotics therapy was initiated.
Note: Only patients who used the *Lactobacillus* were included in this table. Total number of patients in this table is less than total *Lactobacillus* users since 53 patients used *Lactobacillus* but not antibiotics.

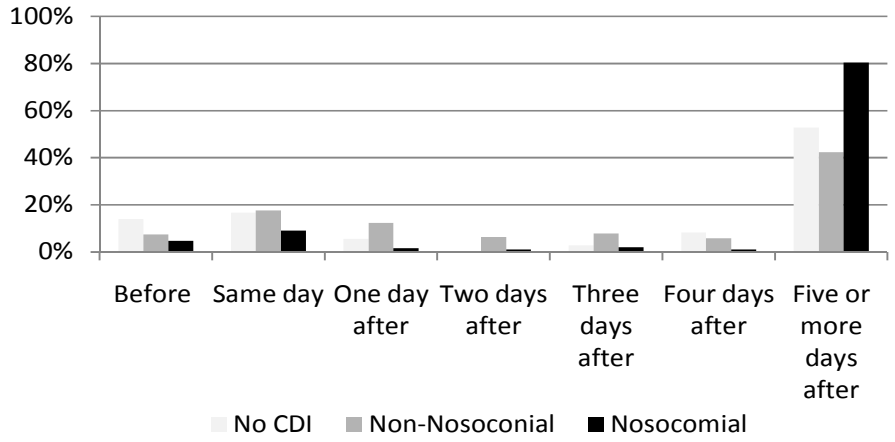

Figure 4.9 – Number of days between initiation of *Lactobacillus* and the start dates of antibiotics in overall sample

Description of *Lactobacillus* and CDI Treatment Initiation Dates

Table 4.13 and Figure 4.10 show the description of the day *Lactobacillus* was initiated with respect to CDI treatment (metronidazole or vancomycin) initiation dates among non-nosocomial and nosocomial CDI cases. Among the non-nosocomial cases, the majority started *Lactobacillus* five or more days after CDI treatment was initiated (95 [40.08%]). Also, those who started *Lactobacillus* on the same day or one day after CDI treatment was initiated were relatively high at (61 [25.74%]) and (27 [11.39%]), respectively. In the nosocomial CDI cases, the majority started *Lactobacillus* either before (4; [20.79%]) or on the same day (42 [20.79%]) that CDI treatment was initiated. Also, the percentage of those who started it five days or more after CDI treatment was initiated was high; (76 [37.62%]). In general, *Lactobacillus* was mostly initiated five days or more after CDI treatment was initiated (171 [38.95%]) or on the same day CDI treatment was imitated (103 [23.46%]).

Table 4.13 – Description of the day *Lactobacillus* was initiated with respect to CDI treatment initiation date

Initiation of CDI treatment	Non-nosocomial CDI (n= 237) No. (%)	Nosocomial CDI (n= 202) No. (%)	Total (n= 439) No. (%)
Before*	15 (6.33)	42 (20.79)	57 (12.98)
Same day	61 (25.74)	42 (20.79)	103 (23.46)
One day after	27 (11.39)	20 (9.90)	47 (10.71)
Two days after	19 (8.02)	8 (3.96)	27 (6.15)
Three days after	12 (5.06)	7 (3.47)	19 (4.33)
Four days after	8 (3.38)	7 (3.47)	15 (3.42)
Five or more days after	95 (40.08)	76 (37.62)	171 (38.95)

CDI: *Clostridium difficile* infection.
**Lactobacillus* has been initiated before antibiotics therapy was initiated.
Note: Only CDI patients who used the *Lactobacillus* were included in this table. Total number of patients in this table is less than *Lactobacillus* users since 39 patients who used *Lactobacillus* did not develop CDI.

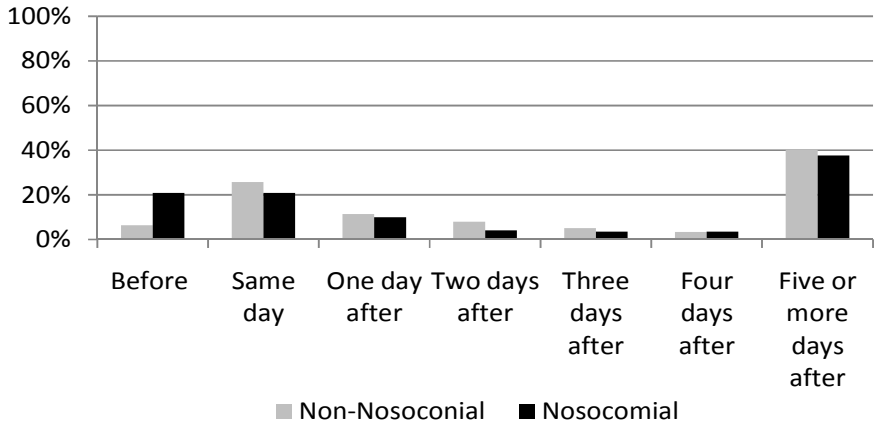

Figure 4.10 – Number of days between initiation of *Lactobacillus* and the start dates of CDI treatment in the cases

Crude Analysis of the Relationship Between *Lactobacillus* and CDI outcomes

The crude analysis of the relationship between the use of *Lactobacillus* and the outcomes of interest in nosocomial CDI patients is shown in Table 4.11. The crude analysis showed a significant relationship between *Lactobacillus* and post CDI diagnosis length of stay (β = 8.643; 95% CI 5.744 to 11.542; $P < 0.001$). Use of *Lactobacillus* is associated with an increase in post-CDI-diagnosis length of stay by approximately eight days. The crude analysis showed a significant protective effect of *Lactobacillus* use of the switch rate of CDI treatment (OR= 0.159; 95% CI 0.037 to 0.684; $P = 0.014$). Those who used *Lactobacillus* were about 6 times less likely to switch CDI therapy than non-users. There was no significant relationship between *Lactobacillus* use and readmission for CDI or mortality.

Table 4.14 – The crude relationships between CDI related outcomes and the use of *Lactobacillus*

Outcomes	Estimate[a] (SE)	P-value	95 % CI
Post diagnosis length of stay	8.643 (1.479)	< 0.001	(5.744, 11.542)
Switch rate[b]	0.159 (0.118)	0.014	(0.037, 0.684)
Readmission	1.310 (0.229)	0.124	(0.930, 1.845)
Mortality	0.946 (0.223)	0.822	(0.584, 1.533)

[a]Estimate for length of stay is regression coefficient and others are odds ratios; [b]Only patients who used *Lactobacillus* after CDI diagnosis were considered (440 patients).

Adjusted Analysis of the Relationship Between *Lactobacillus* and CDI Outcomes

The adjusted analysis of the relationship between *Lactobacillus* and the outcomes of interest in nosocomial CDI patients are shown in Tables 4.12 and 4.13. After adjusting for all available potential confounders in a logistic regression model, *Lactobacillus* use was still significantly associated with an increase in post-CDI-diagnosis length of stay (β =5.672; 95% CI 3.183 to 8.161; P < 0.001). The strength of the association, however, was reduced when compared to the crude analysis (from 8.643 days to 5.672 days). After adjustment, *Lactobacillus* use increases the post-diagnosis length of stay by about 5 days. For the assessment of the adjusted relationship between *Lactobacillus* use and mortality, severity of illness was re-categorized to two categories (minor/moderate, major/extreme) because of large differences in patients in those groups. After adjustment, *Lactobacillus* use was still significantly associated with a reduction in the likelihood of switching CDI treatment (OR= 0.166; 95% CI 0.037 to 0.740; *P* = 0.019). There was not a large difference in the strength of the association the crude and adjusted analysis. *Lactobacillus* use was not significantly associated with readmission for CDI (OR= 1.376; 95% CI 0.970 to 1.951; *P* 0.074) after adjusting for potential confounders in a logistic regression model. As in the crude relationship, *Lactobacillus* use was not significantly associated with mortality (OR= 0.736; 95% CI 0.510 to 1.063; *P* = 0.103) after adjusting for all available potential confounders in a logistic regression model. Detailed tables with the output of the four regression models used in this analysis are included in Appendix A

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Table 4.15 – Adjusted relationship between *Lactobacillus* use and length of stay and likelihood of switching CDI treatment

	Length of stay		Switch rate	
Independent variables	***β* (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Lactobacillus use	5.672 (3.183,8.161)	< 0.001	0.166 (0.037, 0.740)	0.019
Age (years)	-0.071 (-0.10, -0.04)	< 0.001	1.002 (0.983, 1.022)	0.841
Sex	-0.080 (-1.13, 0.97)	0.882	1.412 (0.723, 2.757)	0.313
Race:		0.350		0.054
Caucasian (Ref.)	—		—	
African American	0.624 (-0.754, 2.002)		4.097 (0.968, 17.335)	
Other	1.010 (-0.509, 2.529)		0.938 (0.403, 2.181)	
Transfer from outside hospital	1.532 (-1.952, 5.016)	0.389	0.953 (0.127, 7.151)	0.962
Switched CDI treatment	0.260 (-5.235, 5.755)	0.926	N/A	N/A
Severity of illness:		< 0.001		0.406
Minor/Moderate (Ref.)	—		—	
Major	1.935 (0.190, 3.679)		0.562 (0.159, 1.990)	
Extreme	6.809 (5.111, 8.506)		0.565 (0.162, 1.972)	
ICU-days	0.679 (0.642, 0.717)	< 0.001	1.015 (0.985, 1.046)	0.320
Charlson score	0.087 (-0.124, 0.298)	0.420	1.029 (0.897, 1.179)	0.686
Mortality	-0.009 (-1.844, 1.826)	0.993	0.839 (0.280, 2.513)	0.754
Constant	8.368 (2.459, 14.277)	0.006	N/A	

OR: Odds ratio; *β*: Regression coefficient; CI: Confidence interval; SOI: Severity of illness; ICU-days: Number of intensive care unit days; N/A: not applicable.

Table 4.16 – Adjusted relationship between *Lactobacillus* use and readmission and mortality

	Readmission		Mortality	
Independent variables	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Lactobacillus use	1.376 (0.970, 1.951)	0.074	0.736 (0.510, 1.063)	0.103
Age (years)	0.998 (0.994, 1.003)	0.469	1.019 (1.014, 1.024)	<0.001
Sex	0.865 (0.738, 1.013)	0.072	0.910 (0.780, 1.063)	0.234
Race:		0.287		0.2421
Caucasian (Ref.)	—		—	
African American	1.086 (0.887, 1.330)		0.878 (0.717, 1.075)	
Other	0.870 (0.687, 1.101)		0.851 (0.669, 1.082)	
Transfer from outside hospital	0.601 (0.318, 1.138)	0.118	1.590 (1.089, 2.321)	0.016
Switched CDI treatment	1.560 (0.605, 4.021)	0.358	0.732 (0.408, 1.315)	0.297
Severity of illness:		0.591		<0.001
Minor/Moderate (Ref.)	—		—	
Major	1.062 (0.820, 1.375)		N/A*	
Extreme	1.155 (0.899, 1.486)		16.525 (7.364, 37.081)	
ICU-days	0.985 (0.978, 0.993)	< 0.001	1.032 (1.027, 1.036)	< 0.001
Charlson score	0.986 (0.955, 1.019)	0.405	1.119 (1.089, 1.150)	< 0.001
Mortality	0.393 (0.270, 0.571)	< 0.001	N/A	N/A
Constant	N/A		N/A	

OR: Odds ratio; CI: Confidence interval; SOI: Severity of illness; ICU-days: Number of intensive care unit days; N/A: not applicable.

*Severity of illness was re-categorized to two categories (minor/moderate, major/extreme) to reduce the variability.

CHAPTER 5: DISCUSSION

Incidence and severity of CDI has been continuously increasing in the United States over the last decade.[47] This is a debilitating illness with a high cost, especially among recurrent CDI cases. The literature shows some evidence of the beneficial effects of probiotics in treatment of CDI through its ability to restore the balance in the normal flora that have been disrupted by the prior use of antibiotics

This retrospective cohort study gives a detailed description of the characteristics of *Lactobacillus* users and non-users among CDI cases and non-cases. This is the first study to describe in detail probiotics users. This study found that almost all *Lactobacillus* users were patients with CDI indicating that the use of *Lactobacillus* may be mainly for treatment of CDI and not prevention of it. Also most all *Lactobacillus* users (89.16%) had either major or extreme severity of illness unlike the non-users who had mainly minor or moderate severity of illness. This also supports the conclusion that *Lactobacillus* is only prescribed for severe cases of CDI. As the severity of illness increased, the frequency of *Lactobacillus* use increased. The opposite relationship was true for the severity of illness and number of *Lactobacillus* non-users. Additionally, it was found that users were significantly different from non-users in age, number of antibiotics received, mortality, readmission, and length of stay. Users were significantly older (mean age = 61.09; 95% CI 59.46 to 62.71) than non users (mean age = 53.78; 95% CI53.85 to 53.98). As the number

of antibiotics received increased, the frequency of *Lactobacillus* use increased. The opposite relationship was true for the number of antibiotics received and number of *Lactobacillus* non-users. Readmission and length of stay were significantly higher for *Lactobacillus* users than non-users. All of these risk factors are associated with CDI and consequently with the use of *Lactobacillus* and are consistent with the reported risk factors in the literature[12, 57, 58] In both users and non-users, IDC-9-CM code (008.45) for CDI was among the top diagnosis codes in general because the sample was selected based on that code.

This study found that certain antibiotic classes (metronidazole, fluoroquinolones, 3rd and 4th generation cephalosporins, broad spectrum penicillins, vancomycin, and 1st generation cephalosporins) were used most frequently by *Lactobacillus* users. This supports the published literature that describes classes of antibiotics associated with CDI.[52, 53, 56] That could introduce bias in the ability of determining the effects of *Lactobacillus* on CDI related outcomes. The results are also consistent with prior research findings that hospital-acquired CDI is related to age, time spent in the hospital (before the diagnosis date), use of proton pump inhibitors, and gastrointestinal disease.

Analytical weights were used in the analysis to account for the random sampling approach in the original risk factor study. These weights allowed each individual to be representative of the actual number of patients in the UHC population. For that reason the there were large differences between weighted *Lactobacillus* users who were non-CDI patients (n = 2,125 [82.88%]) and unweighted *Lactobacillus* users non-CDI patients (41 [8.54%])

There was evidence of a statistically significant relationship between *Lactobacillus* use and post-CDI-diagnosis length of stay. *Lactobacillus* users were expected to have longer length of stay than non-users. However, the direction of these relationships was unexpected. This could be explained by the fact most of CDI cases were older and more severely ill than non-CDI patients. All of these factors may have driven the relationship in the unexpected direction even after accounting for it in a regression model. Also, there was a statistically significant relationship between *Lactobacillus* use and switch rate of CDI treatment. Non-users were about 6 times more likely to switch their CDI treatment than users. *Lactobacillus* use was not significantly associated with either readmission for CDI or mortality. Switch rate of CDI treatment was selected as an outcome variable because it could be used as a marker of CDI treatment failure. Patients who are cured on oral metronidazole usually switch to oral vancomycin. From the descriptive part of this study, it is clear that *Lactobacillus* was mainly prescribed for the sicker patients as a last resort treatment. However, literature shows that probiotics are better for prevention of CDI than treatment. This fact may also have reduced the ability of detecting the true relationship and may have driven it in the unexpected direction.

The large sample size used in this study theoretically allowed for a high power to detect the association between *Lactobacillus* use and the outcome variables of interest. Given the significant differences between *Lactobacillus* users and non-users, more complex models would need to be conducted with an observational data to estimate more appropriately the relationship between *Lactobacillus* use and the CDI-related outcomes.

Limitations

Even though the data used in this study came from different hospitals around the nation, there is a considerable variation in the number of participating hospitals each year. That variability may have introduced bias in that they may have had greatly different characteristics with regards to antibiotic and *Lactobacillus* use. Elimination of these hospitals would have greatly reduced the sample size. Considering that all the hospitals in this study were from academic healthcare centers, the finding may not be generalizable to other non-academic hospitals.

C. difficile is one of most commonly indentified causes of nosocomial diarrhea. It is certain that infection control practices such as gloves, hand washing, and disinfectant use may affect the rates of infection. The data used in this study do not include such information, and therefore it was not accounted for in the regression models used in the analysis.

Another potential limitation is the lack of laboratory information that might help in confirming the diagnosis of CDI. The CRM database was developed for administrative purposes and does not include laboratory results. Because of that limitation confirmation of ICD-9-CM codes with laboratory assay results for CDI was not done. Instead, cases were indentified based on the ICD-9-CM code (008.45) for CDI using a recently developed and validated definition by Schmiedeskamp et al.[77] Another potential limitation is that the validity of the drug usage data in the UHC CRM database has been examined in only one participating hospital.

Lactobacillus users and non-users were very different since they were not matched to each other. Instead, CDI cases and controls were matched on the hospital and quarter in which they were discharged. This variability between *Lactobacillus* users and non-users is an issue for the analytical parts, but not really for the descriptive piece. In this study *Lactobacillus* use was not classified as before or after-CDI-diagnosis since there was only small number of patients who used it before-CDI-diagnosis. That did not allow assessment of *Lactobacillus* use as a prevention therapy.

Another limitation is that the direction of switch of therapy was not measure. Switching from metronidazole to vancomycin or vancomycin to metronidazole was considered the same. However, that could have different meaning. Switching from metronidazole to vancomycin represents treatment failure while switching from vancomycin to metronidazole does not.

Future Directions

The descriptive findings from this study provide information on the general characteristics of *Lactobacillus* users that could be used as a foundation for generating hypothesis for future research in this field. The population of this study was adult hospitalized patients. A future descriptive study on hospitalized children could be conducted.

This study also attempted to identify the relationship between the use *Lactobacillus* and several CDI related outcomes. Due to the large differences between CDI cases and the controls the true relationships were difficult to identify. To overcome this problem,

modification in the design or the analysis of the study should be considered in the future studies. Matching on more variables in a retrospective or a prospective cohort or case control studies will reduce the variability and might better identify the true relationships between *Lactobacillus* use and various CDI related outcomes. If the there were significant relationship after a prospective study, a randomized clinical trial with sufficient sample size should be conducted for more accurate assessment of the true relationship. In addition, propensity score method could account for more variability between *Lactobacillus* users and non-users.

Conclusions

Lactobacillus users and non-users were different in most characteristics. *Lactobacillus* use by CDI cases was high and mainly started on the same day or before initiation of antibiotics. *Lactobacillus* use was associated with increased length of stay and switch of CDI therapy. Although this study describes the types of patients who are receiving *Lactobacillus*, the true association between *Lactobacillus* use and CDI-related outcomes remains unclear and further research is needed.

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Literature Cited

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Appendix A

Detailed Model Results

Table A.1 – Adjusted relationship between *Lactobacillus* use and post CDI diagnosis post length of stay

					95 % Confidence Intervals	
Independent variables	***β***	**SE**	**t**	**P-value**	**Lower**	**Upper**
Lactobacillus use	5.672	1.270	4.47	< 0.001	3.183	8.161
Age (year)	-0.071	0.016	-4.42	< 0.001	-0.102	-0.039
Sex	-0.080	0.535	-0.15	0.882	-1.129	0.970
Race:				0.350		
Caucasian (Ref.)	—	—	—		—	—
African American	0.624	0.703	0.89		-0.754	2.002
Other	1.010	0.775	1.30		-0.509	2.529
Transfer from outside hospital	1.532	1.777	0.86	0.389	-1.952	5.016
Switched CDI treatment	0.260	2.803	0.09	0.926	-5.235	5.755
Severity of illness:				< 0.001		
Minor/Moderate (Ref.)	—	—	—		—	—
Major	1.935	0.890	2.17		0.190	3.679
Extreme	6.809	0.866	7.86		5.111	8.506
ICU-days	0.679	0.019	35.57	< 0.001	0.642	0.717
Charlson score	0.087	0.108	0.81	0.420	-0.124	0.298
Mortality	-0.009	0.936	-0.01	0.993	-1.844	1.826
Constant	8.368	3.014	2.78	0.006	2.459	14.277

No. observations = 4,345; $F_{(12, 4,332)} = 148.05$; $p < 0.001$; $R^2 = 0.291$; Adjusted $R^2 = 0.289$

β: Regression coefficient; SE: Standard error; ICU-days: Number of intensive care unit days; CDI: *C. difficile* infection.

Table A.2 – Adjusted relationship between *Lactobacillus* use and the likelihood of switching CDI treatment

					95 % Confidence Intervals	
Independent variables	**OR**	**SE**	**Z**	**P-value**	**Lower**	**Upper**
Lactobacillus use	0.166	0.126	-2.350	0.019	0.037	0.740
Age (year)	1.002	0.010	0.200	0.841	0.983	1.022
Sex	1.412	0.482	1.010	0.313	0.723	2.757
Race:				0.054		
Caucasian (Ref.)	—	—	—		—	—
AfricanAmerican	4.097	3.015	1.920		0.968	17.335
Other	0.938	0.404	-0.150		0.403	2.181
Transfer from outside hospital	0.953	0.980	-0.050	0.962	0.127	7.151
Severity of illness:				0.406		
Minor/Moderate (Ref.)	—	—	—		—	—
Major	0.562	0.363	-0.890		0.159	1.990
Extreme	0.565	0.360	-0.900		0.162	1.972
ICU-days	1.015	0.016	0.990	0.320	0.985	1.046
Charlson score	1.029	0.072	0.400	0.686	0.897	1.179
Mortality	0.839	0.470	-0.310	0.754	0.280	2.513

No. observations = 4,186; Log likelihood = -209.99; Likelihood ratio χ^2 = 13.01; p = 0.292
OR: Odds ratio;CI: Confidence interval; SE: Standard error; ICU-days: Number of intensive care unit days; CDI: *C. difficile* infection.

Table A.3 – Adjusted relationship between *Lactobacillus* use and readmission

					95 % Confidence Intervals	
Independent variables	**OR**	**SE**	**Z**	**P-value**	**Lower**	**Upper**
Lactobacillus use	1.376	0.245	1.79	0.074	0.970	1.951
Age (year)	0.998	0.002	-0.72	0.469	0.994	1.003
Sex	0.865	0.070	-1.80	0.072	0.738	1.013
Race:				0.287		
Caucasian (Ref.)	—	—	—		—	—
African American	1.086	0.112	0.80		0.887	1.330
Other	0.870	0.105	-1.16		0.687	1.101
Transfer from outside hospital	0.601	0.196	-1.56	0.118	0.318	1.138
Switched CDI treatment	1.560	0.754	0.92	0.358	0.605	4.021
Severity of illness:				0.436		
Minor/Moderate (Ref.)	—	—	—		—	—
Major	1.062	0.140	0.46		0.820	1.375
Extreme	1.155	0.148	1.13		0.899	1.486
ICU-days	0.985	0.004	-3.91	< 0.001	0.978	0.993
Charlson score	0.986	0.016	-0.83	0.405	0.955	1.019
Mortality	0.393	0.075	-4.90	< 0.001	0.270	0.571

No. observations = 4,345; Log likelihood = -2020.44; Likelihood ratio $\chi^2 = 67.33$; $p < 0.001$
OR: Odds ratio; CI: Confidence interval; SE: Standard error; ICU-days: Number of intensive care unit days; CDI: *C. difficile* infection.

Table A.4 – Adjusted relationship between *Lactobacillus* use and mortality

					95 % Confidence Intervals	
Independent variables	**OR**	**SE**	**Z**	**P-value**	**Lower**	**Upper**
Lactobacillus use	0.736	0.138	-1.63	0.103	0.510	1.063
Age (year)	1.019	0.003	7.72	<0.001	1.014	1.024
Sex	0.910	0.072	-1.19	0.234	0.780	1.063
Race:				0.2421		
Caucasian (Ref.)	—	—	—		—	—
African American	0.878	0.091	-1.26		0.717	1.075
Other	0.851	0.104	-1.32		0.669	1.082
Transfer from outside hospital	1.590	0.307	2.40	0.016	1.089	2.321
Switched CDI treatment	0.732	0.219	-1.04	0.297	0.408	1.315
Severity of illness:				<0.001		
Minor/Moderate (Ref.)	—	—	—		—	—
Major /Extreme	16.525	6.814	6.80		7.364	37.081
ICU-days	1.032	0.002	13.68	<0.001	1.027	1.036
Charlson score	1.119	0.016	8.00	<0.001	1.089	1.150

No. observations = 4,345; Log likelihood = -2020.44; Likelihood ratio $\chi^2 = 67.33$; $p < 0.001$
OR: Odds ratio; CI: Confidence interval; SE: Standard error; ICU-days: Number of intensive care unit days; CDI: *C. difficile* infection.

Vita

Ali Alhammad was born in Hufof, Saudi Arabia on May 31, 1977. He was raised in Rumilah, Al Hassa in eastern province of Saudi Arabia. He is a Saudi resident currently living in Richmond, Virginia, with his wife, Wasilah Alhashim and his two daughters, Wala and Hawra.

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Aug. 2006 – Present	Virginia Commonwealth University, School of Pharmacy, Richmond, VA Masters of Sciences, Pharmacy Administration candidate, to be awarded May 2009
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Aug. 2000 – Dec. 2000	King Fahad Military Medical Complex (KFMMC), Dhahran, Kingdom of Saudi Arabia Internship trainee
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Aug. 2003 – March 2006	Grade-I hospital pharmacist, King Fahad Military Medical Complex (KFMMC), Dhahran, Kingdom of Saudi Arabia
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