THE ACQUISITION OF *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DISEASE: THE ROLE OF NUTRITIONAL CARE

BY

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DISSERTATION

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ABSTRACT

In 2013, the Centers for Disease Control and Prevention (CDC) classified *Clostridium difficile*, the bacterium responsible for *Clostridium difficile*-associated disease (CDAD), as one of the three most threatening microorganisms to human health. *C. difficile* has outpaced methicillin-resistant *Staphylococcus aureus* as the most common healthcare pathogen, and is currently the leading cause of antibiotic-associated diarrhea and gastroenteritis-related deaths in the United States. Advances in research, national campaigns for healthcare safety, mandated disease reporting, legislation for hospital accountability, and the creation of new antibiotics have all proven ineffective. The current treatment standards, which have been utilized since the discovery of *C. difficile* pathogenesis in pseudomembranous colitis in the 1970's, have shown waning effectiveness and may actually increase disease recurrence and treatment failure. Interest in non-antibiotic alternatives, the evolving understanding of the microbiome as a critical defense against pathogens, and the efficacy of oral nutrition supplements in hospitalized populations with gastrointestinal disease suggest that targeted nutritional therapy may provide a clinical benefit.

The intestinal microbiota can be modified by diet, and is critical for immunity, metabolism, synthesis of vitamins and other bioactive substances, and resistance against pathogens; the microbiota prevents pathogen adherence directly through physical competition, and indirectly through the proliferation of anti-inflammatory and antibiotic-like substances. Any event that disturbs the microbiome may allow opportunistic pathogens, like *C. difficile*, to adhere, colonize, and produce disease. Therefore, the ability to protect or reestablish the microbiota could have vast therapeutic implications.

Preliminary data suggest that nutritional status may relate directly to CDAD susceptibility. CDAD patients show markedly reduced gastrointestinal microbial diversity, which may promote pathological colonization and disease recurrence. Possible methods of repopulating the GI tract with healthful bacteria include fecal microbiota transplants and the administration of probiotics or prebiotics. The recent popularity of fecal transplants for treatment of GI infections is promising, but the process is expensive, unregulated, and aesthetically unappealing. Similarly, the ingestion of probiotics has generally shown potential in patients with recurrent CDAD, but premature degradation in the upper GI tract can be problematic, and caution is advised for use in critically ill or immunocompromised patients. Although prebiotics have the unique advantage of being well-tolerated, stable, commercially available, and easily incorporated into the diet, clinical studies in this area have been diverse, small, and scarce. Improvements in human studies have been attributed to measurable elevations in butyrate, an anti-inflammatory short-chain fatty acid byproduct of prebiotic fermentation, as well as an attenuated pro-inflammatory cytokine response.

The first aim of this research was to summarize the existing clinical literature regarding prebiotic administration and CDAD with a systematic review. The systematic review search identified five studies, yet only three were suitable for inclusion in a meta-analysis. Studies were heterogenous, but appeared to slightly, though not significantly, favor prebiotics, as 35/374 (9.36%) supplemented patients experienced CDAD compared to 64/393 (16.28%) patients in the control groups (OR 0.43, P=0.05). Neither side effects nor mortality differed between treatments, and further research is needed to determine whether prebiotics may provide a clinical benefit for either current or potential CDAD patients.

We next evaluated medical records from Carle Foundation Hospital (CFH) in two separate time periods to identify risk factors for CDAD and to determine whether malnutrition was related to CDAD prevalence and patient outcomes. A month-long preliminary study identified six risk factors (advanced age, admission from another healthcare unit or facility, recent hospitalization, and a history of diarrhea or documented CDAD diagnosis within the previous year) correlated with CDAD prevalence. These risk factors were then used to separate 1,277 patients from 2014, and then 973 patients from 2016, into high-risk groups for primary studies. Initial analysis revealed that advanced age, previous diarrhea, previous CDAD, malnutrition, nutrition consultation requests, and admission from a healthcare facility were individually associated with CDAD diagnosis in both 2014 and 2016. However, when multiple regression analysis was used to identify predictor variables for CDAD, only previous CDAD (OR 111.49, P<0.0001), age ≥65 years (OR 0.43, P=0.004), nutrition consultation requests (OR 1.70, P=0.04), and BMI (OR 0.96, P=0.02) retained significance in 2014, and only previous CDAD (OR 52.95, P<0.0001) and nutrition consultation requests (OR 1.96, P=0.004) in 2016. Although malnutrition was not independently associated with CDAD, we believe that it may more accurately mirror critical overlooked factors, such as frailty or comorbidity. CDAD prevalence did not change between 2014 and 2016 (18.6% vs 17.7%, P=0.57), although both malnutrition (10.4% vs 14.7%, P=0.002) and mortality (14.9% vs 18.9%, P=0.01) increased within the same time period. While our retrospective studies showed many consistencies between the two years and appeared to successfully identify high-risk patients within our sample, data restrictions prevented assessment of disease severity. Both the prevalence of malnutrition and CDAD were unusually low in comparison with national averages for hospitalized patients. Although the exclusive use of a high-risk group prevents comparison with current literature, we

intend to use the results of these projects to better direct interventions to prevent CDAD in at-risk individuals, and thereby mitigate bacterial transmission and lessen the overall CDAD disease burden.

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CHAPTER 1: INTRODUCTION

Clostridium difficile-associated disease (CDAD) is a debilitating illness with immense personal, political, medical, and financial implications. Hospital billing records indicate that over 9% of CDAD-coded hospital visits result in death compared to 2% for all non-CDAD admissions (Walters and Zuckerbraun, 2014). Following the emergence of the epidemic strain, "toxinotype III, restriction endonuclease analysis group BI, North American pulsed-field gel electrophoresis type NAP1, and polymerase-chain-reaction (PCR) type 027" (NAP1/B1/027), in Pittsburgh in 2001, CDAD prevalence, severity, and mortality have escalated dramatically. In 2001, *C. difficile*-related hospitalizations accounted for 5.6 per 1,000 discharges, which more than doubled to 12.7 per 1,000 patient discharges by 2011 (Steiner et al., 2014). Preliminary data predicted that CDAD hospitalizations would reach 14.2 per 1,000 patient discharges by 2013, which matched the prevalence provided by the CDC (CDC, 2016), but overestimated the approximation of 13.7 per 1,000 patient discharges from the Healthcare Cost and Utilization Project (HCUP) (HCUP, 2018). The most recent data released from the HCUP and CDC estimate CDAD prevalence to be 14.2 and 14.9 per 1,000 patient discharges, respectively (CDC, 2017; HCUP, 2018).

Similarly, mortality attributable to CDAD has increased five-fold, from 2,675 deaths per year in 1999-2000 to 14,368 deaths per year in 2006-2007 (Hall et al., 2012). Results from 2015 indicate that approximately 453,000 new infections arise annually, 29,300 cases result in death within 30 days of diagnosis, and half are directly attributable to CDAD (Lessa et al., 2015).

The cost of CDAD treatment in the U.S. ranges from $6,000-9,000 per uncomplicated case, with a conservative national annual estimate of $1-1.6 billion (Scott, 2009). However, some

sources have cited annual estimates as high as $4.8 billion for acute care facilities alone (Dubberke and Olsen, 2012). The presence of conflicting reports is common in infectious disease research, and is usually a product of discrepancies in design, location, strain, magnitude, and preferences. Analysis of the Premier Hospital Database, representing three years of patient records across 477 diverse U.S. acute care hospitals, revealed that costs for CDAD patients were over 40% higher than the average calculated for case-matched controls (Magee et al., 2015). The diagnosis added $7,286 per case to CDAD-related costs, even after adjusting for diagnosis-related groups (DRG), demographics, and hospital characteristics. These data, which only included expenses incurred during the index admission, did not capture the cost of significant readmission at 30, 60, and 90 days by CDAD patients, and thereby underestimate the total healthcare burden.

In 2009, the U.S. Department of Health and Human Services (HHS) developed an "action plan" for combatting seven of the most prominent healthcare-associated infection (HAI) measures, which included an ambitious 30% reduction in both CDAD hospitalizations and hospital-onset CDAD by 2013 (HHS, 2013). A progress assessment from 2012 indicated that results were not on track to meet expectations, and included a 17% increase in CDAD hospitalizations and a mere 2% decrease in hospital-onset CDAD. Although hospital-onset CDAD decreased by 8% from 2011 to 2014, the 4% increase observed from 2013-2014 mitigated preliminary progress, and may arguably signal an ongoing upward trend. The current version of the HHS Action Plan reiterates the same objective with a new timeline, and projects a 30% reduction in CDAD from the 2015 baseline by 2020.

Impending changes in legislation, healthcare, and demographics make the failure to contain CDAD uniquely problematic. Although it has yet to be adopted as a non-reimbursable

diagnosis, CDAD-related healthcare penalties are planned for FY2017 through Value-Based Purchasing (VBP), established by the 2011 Patient Protection and Affordable Care Act, and the Hospital Acquired Condition Reduction Program (HACRP) in 2015 (2010). VBP, a pay-for-performance initiative, issues payment to acute care hospitals based on quality and patient satisfaction, whereas HACRP withholds Medicare reimbursement from hospitals with the highest infection rates. These imminent Medicare revisions will have inordinate financial implications in regard to the anticipated shift in the national age structure. The incidence of CDAD is nearly nine times higher in patients over the age of 65, and 93% of CDAD deaths occur among patients in this age group (Kochanek, 2011). Elderly persons are expected to comprise over 20% of the U.S. population by the year 2030, and illnesses that disproportionately affect Medicare beneficiaries will likely demand unprecedented resources and care (Ortman et al., 2014).

C. difficile pathogenicity

As many HAIs have subsided with concomitant advances in research, patient education, and improved quality care, CDAD rates have remained high, with infections arising from both endemic and epidemic strains (Yakob et al., 2015). *C. difficile* is a resilient and effective pathogen that disproportionately targets vulnerable individuals. Advanced age, antibiotic use, recent healthcare facility exposure, and a history of diarrhea or CDAD within the previous year all increase CDAD risk (Cooper et al., 2013) **(Table 1.1**).

CDAD acquisition requires both a contaminated environment and a susceptible, compromised host. Most frequently, host colonization is precipitated by oral antibiotic use, which displaces the protective intestinal microbiota and permits the adherence of opportunistic pathogens. Following ingestion of *C. difficile* endospores, bacteria germinate in the small

intestine, and adhere to epithelial cells and elaborate inflammatory and destructive toxins in the colon. Infected patients may experience a range of symptoms from watery diarrhea to fulminant colitis and sepsis. Paradoxically, antibiotic regimens, which facilitate *C. difficile* colonization and infection, are the current treatment standard. The Infectious Disease Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) guidelines recommend restricting antimicrobial use to curtail CDAD risk (Cohen et al., 2010). For patients with active CDAD, IDSA/SHEA advises discontinuation of the inciting antibiotic, followed by administration of either metronidazole, vancomycin, or fidaxomycin. Treatment failures and recurrences are common, with a systematic review of over 7,000 patients reporting means of 22.3 and 22.1%, respectively (Vardakas et al., 2012).

The heightened recognition of antimicrobial resistance throughout most of the world has reinforced pre-existing concerns about excessive antibiotic use. The World Health Organization (WHO) currently urges antibiotic stewardship, regulation, and "innovation, research, and development" for alternative options (WHO, 2015). Several non-antibiotic therapies have shown promise in preliminary studies, and recent estimates indicate that 30-50% of antibiotics prescribed within hospitals and over 50% of those prescribed by outpatient facilities are either unnecessary or inappropriate (Lessa et al., 2015). Impaired gut colonization resistance is a hallmark of antibiotic treatment, and although these medications may interrupt vegetative *C. difficile*, their elimination of microbial diversity could promote future pathological colonization (Pérez-Cobas et al., 2014). Furthermore, even very specific and narrow-spectrum antibiotics may not eliminate the persistence of *C. difficile* spores. Some studies have suggested that antibiotic treatment is the main cause of CDAD recurrence, a phenomenon observed in approximately one

in five successfully treated individuals, an estimated 83,000 first-time episodes each year (Shields et al., 2015).

The inability to regulate primary CDAD infection and recurrence highlights the importance of disease prevention over management. Prophylactic therapies that systematically reinforce or restore commensal-mediated pathogen resistance may prove to be more valuable than additional microbial depletion. Furthermore, microbiota manipulation through pharmacological or dietary means provides an intuitive alternative to standard antibiotic treatment and represents an important mechanism through which nutrition directly impacts disease risk and development.

Introduction to malnutrition

The concept of "iatrogenic malnutrition" was publicized by the 1974 article, "The Skeleton in the Hospital Closet*"* (Butterworth). The issues that Charles Butterworth mentioned more than 40 years ago—deficiencies in nutrition education, the depersonalization of healthcare, and an inability to translate basic research into clinical improvements—still resonate with modern medicine. Malnutrition is severely underdiagnosed in hospitals due to a lack of education among medical staff and underutilized or unavailable dietetic resources (Gout et al., 2009).

Approximately 40% of all patients in acute care facilities and between 29-61% of elderly patients are affected by malnutrition (Corish and Kennedy, 2000). In developed countries, one in three patients are malnourished upon hospital admission (Barker et al., 2011); two-thirds of these patients will experience a further decline in nutritional status throughout the hospital stay due to illness, medical complications, and poor provision or access to food (Somanchi et al., 2011). Among patients who are adequately nourished upon admission, one-third will become malnourished during hospitalization (Braunschweig et al., 2000). The onset and exacerbation of

malnutrition during hospitalization have complex physiological, emotional, and social etiologies. Weight loss and reduced lean body mass can reflect the heightened metabolic demands and unique psychological pressures associated with inflammation, isolation, and inactivity (Ahmed and Haboubi, 2010); anorexia and anhedonia may arise from polypharmacy, a lack of social support, environmental instability, depression, or anxiety (Jansson et al., 2007). Malnutrition is inextricably linked to many aspects of physical and mental health. It is, therefore, critical to view the identification and treatment of iatrogenic malnutrition as an interdisciplinary issue.

Definitions and diagnosis

The diagnostic characteristics for adult malnutrition recently established by the American Society for Parenteral and Enteral Nutrition/Academy of Nutrition and Dietetics (ASPEN/AND) consensus statement include insufficient energy intake, weight loss, loss of muscle mass, loss of subcutaneous fat, localized or generalized fluid accumulation (edema), and decreased functional status (White et al., 2012). Although the term "malnutrition" technically describes any nutritional imbalance, it will be used synonymously with undernutrition for the purpose of this review.

Effects of malnutrition

Malnourished patients often require more intensive care than their adequately nourished counterparts and risk severe injury due to fatigue, muscle wasting, and impaired physical capacity; hospital-associated deconditioning, a functional decline associated with acute hospitalization, almost exclusively affects malnourished individuals. A 2014 study revealed that 88% of elderly inpatients with hospital-associated deconditioning had a malnutrition diagnosis, while the remaining 12% were considered at-risk (Wakabayashi and Sashika, 2014). Expectedly, malnourished patients are likely to incur longer hospitalizations and accrue higher treatment costs (Correia and Campos, 2003; Lim et al., 2012). Even patients at-risk for malnutrition are

subject to extended stays and higher costs (Chima et al., 1997; Thomas et al., 2016). Remarkably, these extra personal and financial resources are not correlated with better outcomes. Retrospective data from 25 different hospitals detected elevated costs ($228 vs $138), mortality (12.4% vs 4.7%), complications (27% vs 17%), and length of stay (LOS) (16.7 days vs 10.1 days) for malnourished patients (Correia and Waitzberg, 2003). Similarly, a large Singapore case-control hospital study concluded that malnutrition was associated with a two-day increased LOS, 24% higher treatment costs, a 200% increase in the likelihood of unplanned 15-day readmission, and a four-fold and three-fold rise in mortality after one and three years, respectively (Lim et al., 2012). Less pronounced disparities were reported in an analysis of the 3,122-patient 2010 Australasian Nutrition Care Day Survey, which controlled for age, gender, diagnosis, and disease severity. A 50% increase in LOS and doubled mortality rates were observed for malnourished individuals, though readmission rates were only modestly increased (Agarwal et al., 2013).

The importance of nutritional care in ensuring positive outcomes is exemplified by the successes of clinical supplementation trials. Interventions have improved quality of life and decreased mortality, complications, and costs. A Cochrane database review revealed that oral protein and energy supplements reduce mortality risk among older undernourished people (RR 0.79) and decrease complications (RR 0.86) (Milne et al., 2009). A meta-analysis of 11 randomized controlled trials demonstrated lower mortality in patients receiving oral nutrition supplements (OR 0.61), while an analysis of seven trials confirmed reduced complications in supplemented groups (OR 0.31) (Stratton, 2003).

Malnutrition, infection, and immunity

Within the last few decades, model diseases like tuberculosis and HIV have highlighted the complex interdependence among infection, malnutrition, and immunity. Although CDAD and other diarrheal infections have a well-documented history of causing malnutrition (Guerrant et al., 2008), we hypothesize that the reverse scenario is equally plausible; modifications in gut microbiota and the immunological aberrations that characterize malnutrition may promote pathological colonization and illness, especially in susceptible, high-risk individuals.

Reciprocity between malnutrition and disease is not a particularly novel concept; the 1968 publication, *Interactions of Nutrition and Infection*, published by the WHO, recounts numerous studies detailing the relationship among malnutrition, bacterial infection, and diarrheal diseases (Scrimshaw, 1968). Excerpts from cited research affirm that diarrhea is more prevalent in malnourished children (Garcia Erazo, 1960), susceptibility to intestinal pathogens is increased by nutritional deficiency (McKenzie, 1940), and morbidity is three times greater and mortality is seven times greater in malnourished children than in those who were adequately nourished (Oropeza, 1963).

CDAD and malnutrition disproportionately affect the same groups, including hospital patients, long-term care facility residents, and elderly or immunocompromised individuals. Advanced age, one of the most substantial risk factors for CDAD development, is independently associated with several physiological manifestations of malnutrition, including hypochlorhydria, slowed intestinal transit, gut-leakiness, and tempered immunity. Furthermore, hospitalized elderly are often at greater risk due to weakened masticatory ability, altered sensory perception, sarcopenia, polypharmacy, dehydration and electrolyte imbalance, and disability (Brownie, 2006).

The composition of microbial communities within the gut vary with age, weight, exercise, drug use, diet, and health. In general, genetic diversity has been correlated with health, whereas lower diversity is observed in many disease states (Scott et al., 2015). Elderly hospital patients exhibit elevated proportions of bacteroides and lower bacterial diversity, total bacteria counts, bifidobacteria, and clostridium cluster IV when compared to healthy young controls (Zwielehner et al., 2009), and a reduced Firmicutes to Bacteroidetes ratio is observed among elderly individuals and those who have experienced recent weight loss (Ley et al., 2006). Increased risk among the elderly and negligible risk in infants may be associated with microbiome modification throughout the life cycle. Many studies have shown a correlation between nosocomial infections and malnutrition in this age group (Gamaletsou et al., 2012; Paillaud et al., 2005; Potter et al., 1995; Rothan-Tondeur et al., 2003), and notable research by Schneider et al. (2004) confirmed that even moderate malnutrition is an independent risk factor for nosocomial infection.

The mechanisms through which malnutrition promotes gastrointestinal infection involve the compromised efficacy of the gut-immune barrier and significant modifications in gastric pH, immune function, gut architecture, and microbial communities. In the stomach, reduced acid output enables the passage of pathogens into the small intestine (Gilman et al., 1988), which is likely responsible for the elevated CDAD risk observed among patients taking proton pump inhibitors (Deshpande et al., 2012). Lymphopenia may further impair host defenses and inhibit bacterial clearance as malnutrition reduces intestinal weight, particularly in gut-associated lymphoid tissue (Cole, 1999). Macroscopic physical remodeling and intestinal adaptation, including mucosal atrophy, tight junction impairment, and loss of barrier function (Shaw et al., 2012) increase gut permeability and bacterial translocation (Van Der Hulst et al., 1998).

Pathogen adherence and colonization are facilitated by the depletion of commensal microbiota, as well as restricted intestinal motility and prolonged transit time, which are both products of low enteral intake.

Correlational studies between malnutrition and CDAD

While many articles list malnutrition as a risk factor for CDAD development (Lo Vecchio and Zacur, 2012), research to support this claim is limited. Similar risk factors, as well as the observation that patients with CDAD are often malnourished, provide evidence for association, but have yet to establish directionality. An extensive review of nearly 900,000 patients determined that previous weight loss or malnutrition tripled the odds of acquiring *C. difficile* enterocolitis (Fry et al., 2010). This correlation has been corroborated by others, and was initially observed in a retrospective analysis of 172 Veterans Affairs hospitals in 2001 (Buchner and Sonnenberg, 2001). Another retrospective evaluation of 65 patients with CDAD reported a mean weight loss of 9% upon admission; the authors suggested that the weight loss constituted malnutrition and, therefore, concluded that CDAD patients are often malnourished (Barlow and Howell, 2010). Malnutrition may negatively affect prognosis for CDAD patients, perhaps independently of previous illness and comorbidities. In a pilot study from the United Kingdom, 54% of CDAD patients were classified as malnourished (defined as a MUST score of 2) at the time of diagnosis, and exhibited increased mortality and LOS compared to controls (Wong et al., 2009). Preliminary data from a later study yielded similar results, as CDAD patients had a greater rate of malnutrition at admission, higher mortality, more antibiotic exposure, and a longer LOS than uninfected patients (Monge et al., 2013).

In contrast to the studies that addressed protein-energy malnutrition and weight loss, recent work has acknowledged a novel relationship between CDAD and vitamin D status. In

2010, vitamin D deficiency was associated with elevated costs for CDAD patients (Youssef et al., 2010). Outpatients and inpatients with low levels of serum 25(OH)D accrued 1.5 and 5.7 times the cost of infected controls, respectively, which suggests that nutritional inadequacy exacerbates resource utilization among CDAD patients. Furthermore, Wang et al. (2014) reported that patients with low vitamin D levels were nearly five times more likely to maintain infection, and CDAD resolution was independently predicted by normal vitamin D levels. In this study, low vitamin D levels, as well as advanced age, were shown to independently predict disease recurrence. Retrospective analyses have identified an inverse relationship between vitamin D status prior to hospital admission and hospital-onset CDAD (Quraishi et al., 2015). The association was previously documented in a study of inpatients with Inflammatory Bowel Disease, which measured lower vitamin D levels among both CDAD patients and case-related deaths (Ananthakrishnan et al., 2014). In a computed tomography study, severe disease, represented by colitis, was more common in patients with low 25(OH)D3 (van der Wilden et al., 2015). While these vitamin D studies have strengthened the correlation between malnutrition and CDAD, causality remains indeterminable due to the preliminary, correlational, and retrospective nature of the available evidence.

Conclusion

CDAD and malnutrition are critical concerns for hospitals and significantly contribute to morbidity, mortality, and healthcare costs. To date, the role of nutritional status in CDAD susceptibility and severity remains unclear. Prior research limitations include the low detection of malnutrition among hospital patients, inconsistencies in nutritional screening and assessment, the recent emergence of *C. difficile* as a prominent healthcare pathogen, and the inability to

obtain comprehensive and accurate data. The completion of large-scale, well-controlled prospective trials is needed to determine causation.

In clinical studies, nutritional supplementation has consistently reduced costs, complications, and mortality among high-risk hospitalized populations. Susceptibility to infection in malnourished patients may be attributed to immune system impairment, aberrations in gut microbiota, and increased environmental exposure to opportunistic pathogens. Accordingly, therapies that strengthen immunity, reduce inflammation, reinforce microbiota, or minimize LOS are of particular interest. Enhanced antimicrobial vigilance throughout the world and the evolving appreciation for the protective role of commensal bacteria emphasize the importance of non-antibiotic treatment alternatives, while high CDAD recurrence rates highlight the need for disease prevention rather than management. A nutrition-based solution, including supplementation, improved dietetic resources, and the assurance of adequate provisions during hospitalization may improve patient outcomes and provide a practical, safe, and cost-effective alternative for CDAD prevention.

TABLE

Table 1.1 Risk factors associated with *Clostridium difficile*-associated disease (CDAD)

Advanced age (≥ 65 yr)
Admission from another healthcare facility (includes nursing home, jail, etc.)
Hospitalization within 90 days of current hospitalization
Antibiotic use within 90 days of current hospitalization
Clinical documentation of diarrhea within one year of current hospitalization
Positive CDAD diagnosis within one year of current hospitalization

Risk factors adapted from Cooper et al., 2013.

CHAPTER 2: LITERATURE REVIEW

Malnutrition in hospital patients

The 2010 data from HCUP reported that 3.2% of hospital discharges in the United States, or 1,248,680 patients, had malnutrition diagnoses. A comprehensive analysis by Corkins et al. (2014) sought to determine the validity of these diagnoses and examined commonalities among patients. Patients diagnosed with malnutrition, compared to patients who were not diagnosed, tended to be older (64.8 years vs 47.8 years; $P<0.001$), male (53.1% female vs 57.8% male; $P<0.001$), below the 50th percentile for income (57.9% vs 55.0%; $P=0.004$), and list Medicare as their primary payer ($P<0.001$). Whites made up the vast majority of both groups, and differences in racial composition were observed between diagnosed and non-diagnosed groups ($P<0.001$).

Malnourished patients were more likely to be discharged from private, for-profit hospitals ($P=0.002$), Southern or Midwestern hospitals ($P=0.003$), and hospitals within a professional network (71.4% vs 66.5%; $P = 0.005$). Expectedly, and in accordance with other data, patients who received malnutrition diagnoses experienced an extended length of stay (LOS) (12.6+0.5 days vs 4.4+1 days; $P<0.001$), accrued higher costs ($26,944 vs $9,485; $P<0.001$), were more likely to enter the hospital emergently (80% vs 60%; $P<0.001$), but less likely to have a routine discharge (28.8% vs 71.2%; $P<0.001$). Importantly, malnourished patients were two times as likely to be discharged to home health care (19.8% vs 10.4%; $P<0.001$), and five times more likely to be discharged to death (8.8% vs 5.7%; $P<0.001$).

Patients with malnutrition diagnoses experienced 27 of 29 comorbidities more frequently than undiagnosed patients. Some comorbidities were expected for the diagnosis (weight loss, fluid and electrolyte disorders, deficiency anemia), some malnutrition diagnoses could likely be

attributed to a comorbidity or its treatment (diabetes, lymphoma, metastatic cancer, AIDS, alcohol abuse, liver disease, peptic ulcer disease), and the etiology for others was unclear, or suggested a more malicious possibility (hypertension, chronic pulmonary disease, renal failure, congestive heart failure, neurological disorders, depression, hypothyroidism, coagulopathy, peripheral vascular disease, metastatic cancer, paralysis, psychosis, valvular disease, pulmonary circulation disease, rheumatoid arthritis). Predictably, patients with the malnutrition diagnosis had twice as many International Classification of Diseases, Ninth Revision (ICD-9) codes listed in their charts than patients without a malnutrition diagnosis (14.8 codes vs 7.9; $P<0.001$). Surprisingly, parenteral (PN) or enteral (EN) nutrition were only provided to 13.4% of patients with a malnutrition diagnosis, with 8.9% receiving PN, 5.2% receiving EN, and 0.7% receiving both PN and EN.

The low number of patients with a malnutrition diagnosis is consistent with other reports, which indicate that this condition is vastly under-diagnosed among hospital patients nationwide. Although cause and effect cannot be determined in this study, these data suggest that malnourished individuals are high risk patients and, in general, much sicker than their counterparts. The listing of Medicare as the primary payer for the malnourished group is significant, as these patients accounted for three times the cost and LOS of the undiagnosed group. Elevated costs and LOS are often required for malnourished patients, and will be discussed in a future section. Another commonality among malnourished patients is increased mortality, which is also represented by these data. It would be remiss to presume that nutritional status is the only contributor to the observed disparities between groups, especially when measured parameters imply the possibility of socioeconomic or racial bias; essentially, these data are valuable, as they illustrate the connection among malnutrition, immunity, healthcare delivery,

and lifestyle, and serve as a reminder that health is a multifaceted outcome. The difference in the prevalence of malnutrition diagnoses among hospital types and geographical regions underlies the need for education, diagnostic consistency, and action. The increase in patients coded for malnutrition in recent years, coupled with 91.7% of the malnourished group compared to 0.6% of the other group experiencing weight loss, suggests that malnourished patients were probably diagnosed correctly, and that errors are most likely from omission.

Nutrition screening and assessment

Laboratory-based methods of nutritional assessment are considered archaic by some, and the objective assessment of nutritional status has been historically problematic. The variability in malnutrition prevalence throughout the world greatly reflects not only inherent geographical, cultural, genetic, and socioeconomic differences, but also the need for mandatory healthcare screening, updated methodology, and universally accepted standards. The ASPEN/AND and the European Society for Clinical Nutrition and Metabolism (ESPEN) consensus statements criticize earlier methods, particularly the applicability of biochemical parameters for nutritional assessment (Cederholm et al., 2015). Serum protein measurements such as albumin, prealbumin, transferrin, retinol binding protein, C-reactive protein, and creatinine have not yielded consistent, accurate, and useful results in clinical practice (Bresnahan and Tanumihardjo, 2014), and may more accurately assess the presence of inflammation than malnutrition (Banh, 2006). The use of serum biomarkers for diagnosing malnutrition is particularly confounding for individuals with known comorbid infectious or inflammatory conditions, as low-level inflammation, immune senescence, and regular medication use likely exacerbate the potential for interpretive errors, particularly in geriatric populations. While the use of WBC, albumin, and creatinine measurements have fallen out of favor for nutritional diagnoses, they serve as valid predictors of

CDAD-related disease severity and mortality (Bloomfield et al., 2012; Henrich et al., 2009; Moshkowitz et al., 2007).

In healthcare settings, nutritional status is evaluated through patient screening and assessment. The initial screening process identifies patients at risk for malnutrition and determines whether further comprehensive assessment is warranted. A 2005 review identified over 70 published nutrition screening tools and reviewed 35 after exempting tools that required complex anthropometry, significant biochemical or intake analysis, or were designed for aged populations (Green and Watson, 2005). Due to the plurality of acceptable methods, selection of the proper tool requires a fairly nuanced appreciation of their differences. Some tools are designed to verify nutritional status or identify patients who would potentially benefit from nutritional intervention, while others specifically predict risk, resource utilization, or patient outcomes. The limits and categories used for analysis, as well as the addition and weighting of non-nutritional items, are specific to each tool, and may, therefore, inconsistently approximate risk, even among identical patients (Elia and Stratton, 2012). The lack of a gold standard and low concurrent validity among tests complicates the screening process, both in terms of clinical care and nutrition-based research.

Nutritional assessment requires information about patient history, food intake, weight, and, occasionally, height and body composition. Analysis of one or more of these factors is not always possible, and criteria that evaluate changes over time are ineffectual without proper foresight and baseline documentation. Among elderly patients, age-related physical and cognitive constraints may impair assessment (Oliveira et al., 2009). Naturally occurring spinal compression, the presence of disabilities and illnesses that prevent upright posturing, inherent memory impairments, and degenerative disease processes can obscure or prevent the appropriate

interpretation of necessary parameters. In addition to physical confounders, technical and human errors or limitations can interfere with the collection of information relevant to treatment and research.

Biomedical research often relies on observational data or convenience samples, especially in preliminary phases or when interventional trials are unethical or unfeasible. This type of study is inexpensive, expedient, informative, and poses minimal risk, but is dependent on consistent, objective, and thorough records. Retrospective nutritional studies may utilize unconventional surrogate criteria, including the amount of weight lost, a body mass index calculation, basic anthropometry, and subjective patient accounts to infer a deficiency diagnosis when records do not explicitly address nutritional status. In summary, screening discrepancies, interpretation errors, and unavailable or inaccurate information may contribute to patient misclassification, provider uncertainty, and contradictory data.

The diagnosis and documentation of malnutrition are important in terms of patient care, as well as cost recovery. The diagnosis by physicians and subsequent grouping of patients into a Diagnosis Related Group (DRG) for insurance or Medicare reimbursement purposes may cite an additional complication or comorbidity (CC) or major complication or comorbidity (MCC) for additional payment when a qualifying secondary diagnosis is present (Lowry et al., 2015). Malnutrition, depending on severity, is either a MCC (kwashiorkor, nutritional marasmus, severe protein-calorie malnutrition) or CC (other or unspecified protein-calorie nutrition, and cachexia, wasting due to chronic illness). In many cases, as long as malnutrition is adequately documented, an institution can provide treatment, and not be held accountable for related expenses. Although malnutrition is vastly underdiagnosed, the abuse of malnutrition coding, most frequently as a

MCC, reflects a sincere need to accurately document cases and follow through with appropriate interventions.

CDAD acquisition and onset

For some adults and most infants, *C. difficile* is a normal component of the gut microbiota. Approximately 3% of healthy adults and up to 60-70% of newborns and infants are asymptomatic *C. difficile* carriers (Ghose, 2013). For hospitalized adults, 8% are estimated to carry *C. difficile* upon admission (Zacharioudakis et al., 2015). All colonized individuals serve as bacterial reservoirs and facilitate pathogen transmission. CDAD is typically acquired through accidental ingestion of endospores either within a contaminated environment, or through contact with contaminated people, and endospores have even been recovered in samples of hospital food (Koo, 2012). Endospores are uniquely resilient, as they resist ethanol degradation, desiccation, and can persist on hard surfaces for up to five months (Gerding et al., 2008). In addition to being resistant to conventional disinfection techniques, bacteria are inadvertently spread by visitors, patients, and hospital personnel. The anaerobic, spore-forming bacteria are transmitted via the fecal-oral route. After endospores bypass the acidic stomach, they germinate into vegetative bacteria in the small intestine. The two toxins traditionally produced by *C. difficile*, toxin A and toxin B, promote the release of inflammatory cytokines and initiate tissue destruction through disruption of the actin cytoskeleton and disturbance of epithelial cell tight junctions in the colon (Carter et al., 2010).

The onset of CDAD typically occurs after 48 hours of hospitalization, and a healthcare-associated case may not appear until up to four weeks following discharge. Healthcare-associated infections can have either a healthcare or community onset; only 24% of all CDAD cases occur during hospitalization, while the vast majority occur among patients who were

recently discharged (Lessa et al., 2015). The release of undiagnosed patients, unsuccessfully treated patients, and asymptomatic carriers into the community, including outpatient settings and long-term care facilities, has resulted in the emergence of community-associated cases (Gupta and Khanna, 2014). Infection data for 2014 were comprehensively modeled with demographic, epidemiologic, and economic factors to emulate a "societal perspective" of CDAD burden, which predicted a community-associated CDAD cost of $725 million (13.3% of the predicted total) (Desai et al., 2016).

A retrospective study of 400 hospital patients with CDAD reported that 46.1% and 33.3% of cases were expected to have originated in long-term care facilities and the community, respectively (Garg et al., 2013). The remaining 20.6% were classified as "hospital-acquired". Patient classification was determined by where each individual lived prior to admission, i.e., either a long-term care facility or the community, and patients were considered to have hospital-acquired CDAD if they exhibited symptoms and had a positive *C. difficile* test no earlier than three days after admission. Numbers in this case are likely skewed away from hospital-acquired cases, as it was defined as having been acquired at least three days after admission, and the study design excluded all patients with an overnight hospital stay within the last three months or with a history of antibiotics within eight weeks leading up to admission. Regardless of the composition of groups, these data are significant, as they show that both long-term care facilities and the community are important transmission zones. Additionally, only 18.2% of the presenting complaints were diarrheal, the primary symptom associated with CDAD. Instead, patients most commonly reported abdominal pain, fever, or altered mental state. Patients in the hospital-acquired group accounted for the majority of intensive care visits and deaths, which comprised 24.4% and 6.2% of the subgroup. Results suggest that CDAD from long-term care facilities and

the community may present without standard signs, and may follow a less severe clinical course. Another caveat not mentioned by authors is that higher mortality in the hospital-acquired group is not entirely unpredicted, as hospital acquired CDAD, by definition, takes at least 48 hours to develop and, therefore, could not be the primary admitting diagnosis.

CDAD in long-term care facilities

The reason for the high prevalence of CDAD in long-term care facilities is a combination of excessive antibiotic use and environmental contamination. Up to 90% of hospitalized patients, 42% of nursing home residents, and 25% of hospice patients, receive antibiotics within the last few weeks of life (Juthani-Mehta, 2015). In very elderly and dying patients, antibiotics are regularly given as a life-extending intervention, even for patients without clinical symptoms suggestive of a bacterial infection. The problem of transmission in long-term care facilities cannot be underestimated. In a Cleveland, OH long-term care facility, the majority of residents tested positive for *C. difficile* (Riggs et al., 2007). Excluding the 18 control patients with active CDAD, 51% of the remaining 68 patients were found to be asymptomatic carriers, testing positive for bacterial colonization, but without experiencing symptoms. Among the carriers, 37% carried especially virulent, epidemic strains. Environmental contamination was high, and within patient rooms, positive cultures were found for 78%, 59%, and 24% of patients with active CDAD, carriers, and non-carriers, respectively. Skin contamination, similarly, was found for 78%, 61%, and 19% of CDAD patients, carriers, and non-carriers, respectively. Within the six-month follow-up period, 20% of the asymptomatic carriers had developed CDAD. This study is significant for two reasons; it shows that *C. difficile* is easily transmissible by touch and environmental contamination, and that asymptomatic carriers may still acquire CDAD, likely due to infection by a strain other than the one they were initially colonized with.

CDAD in the community

Another atypical environment for *C. difficile* transmission is within the community. Although community-associated CDAD accounts for only 150,000 out of nearly a half million cases, most of these infected patients report indirect healthcare exposure. In a study of 984 documented community-associated CDAD cases, 82% of respondents reported at least one visit to an outpatient healthcare setting in the 12 weeks prior to diagnosis (Chitnis et al., 2013). This finding suggests that at least some community-associated CDAD may still be outpatient healthcare related, particularly since eradicating *C. difficile* spores often requires noxious cleaning agents and methods rarely used outside of hospitals. While community-associated CDAD is less likely to result in recurrence and mortality than the healthcare-associated type, it remains an important avenue for disease transmission.

CDAD in hospitals

Reports from the Agency for Healthcare Research and Quality indicated that healthcare-associated CDAD affected eight out of every 1000 (0.80%) hospital discharges in 2008 (Lucado et al., 2012) and 13.5 out of every 1000 (1.35%) hospital discharges in 2012 (Steiner et al., 2014). A 2015 meta-analysis of 19 studies estimated that the risk of infection in non-colonized patients was 3.4% (Zacharioudakis et al., 2015). Infection risk was 5.9 times higher in patients who were colonized with *C. difficile* upon hospital admission than non-colonized patients, which refutes the 1998 study by Shim et al. that reported a protective carrier status for further *C. difficile* diarrhea (Shim et al., 1998). The pervasiveness of *C. difficile* endospores within healthcare environments leave the sickest and highest risk individuals remarkably vulnerable to infection. Beyond traditional risk factors for CDAD, the literature has disputed the validity of many others, including gender, race, LOS, proton pump inhibitor use, recent gastrointestinal

surgery, malnutrition, and the presence of severe comorbid conditions (Lo Vecchio and Zacur, 2012).

Risk Assessment

A thorough review of the literature revealed many methods of assessing CDAD risk. Among the risk assessment tools were Horn's index, the Waterlow assessment score, and the Ratio of white cell count on the day of the positive *C. difficile* toxin test to two days previously, as well as the Urea, White cell count and Albumin on the day of the positive *C. difficile* toxin test (RUWA) system, among others. These indices generate a score based upon an individual's basic laboratory values or medical history, which then is used to estimate the likelihood that the individual will acquire the disease.

Horn's index ranks a patient on a scale of increasing disease severity, and requires that a qualified clinician rate the severity of the underlying disease, giving the patient a score of 1 for single mild illness, 2 for more severe illness but when uncomplicated recovery is expected, 3 for major illness or complications or multiple conditions requiring treatment, or 4 for a catastrophic illness that may lead to death (Arora et al., 2011). This high-throughput model is based upon the expectations of a disease course, and is highly dependent upon the observer. The Waterlow score, a screening tool initially created for the prediction of pressure ulcer formation, requires data for several parameters that are unlikely to be noted in a chart, including tactile evaluation of skin integrity, food consumption, and a five point mobility scale (Tanner et al., 2010). Unlike the other tools, the RUWA system relies exclusively on laboratory testing conducted at the time of laboratory diagnosis, and then again two days later (Drew and Boyle, 2009).

The primary issue with these methods is that patients may be infected before they are identified. Infection can occur at any time, and symptoms may not appear for up to four weeks

following infection. In a study with 247 CDAD positive cases, 148 (60%) were classified as healthcare-associated with hospital onset, an additional 77 patients had developed CDAD within 30 days of discharge, and the remaining 22 patients were diagnosed within 60 days following hospital discharge (Dubberke et al., 2009). The community-onset healthcare-associated CDAD rate after both 30 days (2.4/1,000 patient-days, $P<0.01$), and 60 days (2.6/1,000 patient-days, $P<0.01$) post-discharge, were significantly higher than the healthcare-associated hospital-onset CDAD rate (1.6 cases/1,000 patient-days). Infection rates between patients assessed 30 days vs 60 days after discharge did not differ ($P=0.31$).

Early intervention prevents the further transmission of bacteria among other patients and the introduction of bacteria into the community following hospital discharge. Cooper et al. (2013) introduced a risk factor-based system into the hospital's electronic medical record (EMR) software to help identify patients with a predisposition to CDAD. The screening tool was based on a prospective list of risk factors, which was created using the hospital's data, combined with elements from CDAD literature. From the list of possible risk factors, regression identified four significant risk factors for CDAD, including the presence of diarrhea ($P<0.0001$), prior CDAD ($P<0.0001$), multiple antibiotic use ($P<0.0001$), and admission from another health care facility ($P<0.0001$). The positive predictive value, or proportion of true positives, and negative predictive value, or proportion of true negatives, were calculated as 4.9 and 99.9%, respectively. Similarly, sensitivity, or the ability to detect all diseased individuals, and specificity, the ability to detect all non-diseased individuals, was 91.6 and 87.0%, respectively. The model identified 92.9% of CDAD cases within their initial study period (Cooper et al., 2013).

Prognosis

The clinical presentation of CDAD is highly variable, and ranges from severe diarrhea to toxic megacolon and fatal sepsis. The course and severity of the disease ultimately determines a patient's prognosis. Basic laboratory analysis may be used to stratify disease severity, as leukocytosis, hypoalbuminemia, and elevated creatinine and C-reactive protein have been correlated with worse outcomes (Pépin et al., 2004). In a retrospective review conducted by Henrich et al. (2009), the maximum leukocyte count was higher in patients with severe CDAD, and more patients with severe disease had minimum albumin <2.5g/dL (OR 3.4), maximum leukocyte count >20,000 cells/mL (OR 2.8), and maximum creatinine >2.0 mg/dL (OR 2.5). Although low albumin, elevated leukocytes, and high creatinine were associated with severe disease in this study, a positive computed tomography (CT) scan for intestinal inflammation was the most reliable marker (OR 13.5).

Serum markers may not only reflect disease severity, but may also predict mortality. In elderly patients with Pseudomembranous colitis, an aggressive clinical manifestation of CDAD, hypoalbuminemia (P=0.02) and increased white blood cell (WBC) count (P=0.009) were associated with high mortality (Moshkowitz et al., 2007). A 2012 systematic review conducted by Bloomfield et al. (2012) concluded that risk markers for CDAD mortality included WBC, serum creatinine, and serum albumin, but not other commonly considered variables such as hematocrit or diarrheal severity. Importantly, many methodologies and therapies that once were considered the gold standard are no longer effective due to treatment failures, disease recurrence, and concerns about antibiotic resistance.

Recurrent CDAD

Disease recurrence is extremely common among patients who have been successfully treated for CDAD, and often follows a more expensive, aggressive, and indolent course than the

initial infection. It is characterized by the resolution of symptoms during treatment, followed by the reappearance of symptoms after treatment cessation. The term "recurrence" is used to describe both relapse and reinfection. A relapse refers to infection by the same strain of bacteria as the previous infection, whereas a reinfection involves colonization by a separate strain (Figueroa et al., 2012). It is not usually possible to clinically differentiate relapse and reinfection, but discernment is rarely warranted. Molecular typing of *C. difficile* is almost exclusively performed for research, although knowing the recurrence type may be helpful when treating a severe case. In a study of samples from ninety participants with recurrent CDAD, relapse (83.3%) was more common than reinfection (16.7%). All sample donors were adults, and most lived in the same area, which could account for the lack of reinfection diversity within the group. The time to relapse or reinfection is variable and, in this case, no significant difference was found (12.2 ± 6.4 days with relapse vs 14.7 ± 6.0 days with reinfection; P=0.18). Recurrence is usually seen within weeks, but may occur up to three months following the initial treatment. For administrative and surveillance purposes, the CDC's Emerging Infections Program Healthcare-Associated Infections Community Interface defines repeated CDAD episodes differently, as a time-based, rather than functional categorization; a duplicate is a positive specimen within two weeks of the last positive specimen, a recurrence is defined as a positive specimen between two to eight weeks of the last positive specimen, and any positive result beyond eight weeks of the previous positive result is considered a new case. This convenience classification was criticized in a surveillance study by Kamboj et al. (2011), as relapses constituted 65% of recurrences after eight weeks when assessed by PCR ribotyping.

When standard antibiotics were administered, recurrence affected 20-30% of patients (Cornely et al., 2012). For patients with more than one recurrence, the risk of an additional

episode was 40-60%. As previously indicated, colonization is not protective. The implications of recurrence should not be understated; a Canadian study reported that 11% of patients with recurrent infection experienced at least one serious CDAD complication, including shock, colectomy, megacolon, perforation, or death within 30 days of diagnosis (Pepin et al., 2006).

Treatment options

Antibiotics

Evidence suggests that the increased presence of *C. difficile* in the hospital, long-term care facilities, and the community is correlated with declining bacterial susceptibility to antimicrobial agents (Peláez et al., 2002). Even the gold standard antibiotics are not recommended for continuous use. Metronidazole, which was recommended as the first line of treatment for uncomplicated cases until 2018, is known to elicit neurotoxicity over time and was not approved by the U.S. Food and Drug Administration (FDA) for this use (Cohen et al., 2010). Vancomycin, which was primarily reserved for severe cases and infections that were refractory to treatment with metronidazole, was associated with recurrence rates as high as 50% (Louie et al., 2015). Ironically, new drugs and increasingly aggressive pulsed and tapered vancomycin protocols still are being tested for use in CDAD (McFarland et al., 2002; Nelson et al., 2017). Recent candidates, including fidaxomicin and cadazolid, delivered impressive results in preliminary clinical trials. A meta-analysis drawing from two recent clinical trials determined that fidaxomicin, although cost-prohibitive for many patients, was not inferior to vancomycin for curing CDAD, and associated with lower rates of recurrence for some *C. difficile* strains (Crook et al., 2012). It was approved by the FDA for use in CDAD patients in May 2011 (Venugopal and Johnson, 2012). Like fidaxomicin, cadazolid showed similar efficacy to vancomycin in

terms of clinical cure, but appeared more effective at preventing recurrence in a multi-center phase II trial (Louie et al., 2015).

It should be noted that many investigations into novel drugs have not been worthwhile. A 2011 systematic review declared that significant differences in efficacy were not found between vancomycin and several other candidate antibiotics, including metronidazole, fusidic acid, nitazoxanide, and rifaximin (Nelson et al., 2011). Moreover, clinical trials revealed unexpected safety concerns. Rifampin, when combined with metronidazole, delivered similar results to metronidazole alone for recurrence and time to symptom improvement. However, the trial ended in the death of nearly one-third of the patients in the rifampin combination group compared to just 5% in the metronidazole group (Lagrotteria et al., 2006). The study concluded that the cure rates for both treatments were unacceptably low, and that better alternatives are needed. Interestingly, this trial is one of the few to measure and report nonfatal adverse events, which occurred in approximately 40% of patients across both groups.

The 2017 update of "Antibiotic treatment for *Clostridium difficile*-associated diarrhoea in adults*"* from the *Cochrane Database of Systematic Reviews* features the addition of three drugs that were missing from the 2011 version (cadazolid, LFF517, and surotomycin) and data from studies that continue to be plagued by small size and a high risk of bias (Nelson et al., 2017). LFF517 exhibited higher clinical response and recurrence rates when compared to vancomycin, but was not well tolerated by nearly 30% of recipients (Maxwell-Scott and Goldenberg, 2017). Adverse effects were also seen in nearly 30% of surotomycin recipients. A phase II non-inferiority study showed reduced recurrence when compared to vancomycin, but was later discontinued after failing to meet two separate noninferiority criteria.

In addition to causing drug-related side effects, the use of antibiotics for CDAD may facilitate the transmission of drug-resistant pathogens. Both metronidazole and vancomycin have been shown to promote vancomycin-resistant enterococci (VRE) overgrowth (Al-Nassir et al., 2008), and studies have identified CDAD as a risk factor for VRE colonization (Rafferty et al., 1997; Zilberberg et al., 2014). Recently, investigators used a mathematical model to predict VRE prevalence after CDAD treatment, and reported that using a non-antibiotic treatment for 50% of CDAD cases produced a 18% relative reduction in institutional VRE (Grima et al., 2012). Antibiotic replacement may also temper the overall CDAD burden, and a similar model has estimated that a 30% reduction in broad-spectrum antibiotic use could reduce CDAD by 26% (Fridkin, 2014).

In February of 2018, an updated version of "Clinical Practice Guidelines for *Clostridium difficile"* was released by the IDSA/SHEA with new treatment recommendations, including the use of vancomycin for initial and non-severe cases and fidaxomicin for severe and recurrent CDAD (McDonald et al., 2018). A weak recommendation based high quality evidence was made for the use of metronidazole exclusively for initial, non-severe CDAD when vancomycin and fidaxomicin were unavailable. Two phase three fidaxomicin trials indicated non-inferiority compared to vancomycin for primary cure, but reported recurrence rates between 15-20%, a significant reduction over vancomycin (Cornely et al., 2012; Louie et al., 2011). Despite the favorable secondary outcome obtained by fidaxomycin, due to the exorbitant cost of the narrow-spectrum, minimally systemic drug, the practicality of this new recommendation remains questionable. The prices listed for a single course of metronidazole, vancomycin, and fidaxomicin were $22, $680, and $2,800, respectively (Surawicz et al., 2013).

Non-antibiotic alternatives

Several non-antibiotic therapies are currently being used for CDAD management. While surgical interventions are generally reserved for cases that cannot be otherwise controlled (e.g., sepsis, bowel perforation, toxic megacolon), new medical procedures, like fecal microbiota transplant (FMT), are becoming increasingly mainstream. FMT, which involves the transfer of a liquidized stool sample from a healthy donor into the GI tract of an ill patient, is a topic of great interest to the infectious disease community. Occasionally heralded as the new cure for recurrent CDAD, most studies, regardless of design, suggest efficacy. In a systematic review citing 36 studies, 87% of patients experienced post-transfer diarrheal resolution (Cammarota et al., 2014). The included studies were small in scale, ranging from 4-77 patients, included only one randomized controlled trial (RCT), and featured a variety of recipient transfer sites, bacterial counts, and transfer volumes. A comparison of efficacy across FMT, vancomycin treatment, and vancomycin treatment with bowel lavage, reported significantly higher resolution of diarrhea in the FMT group (80%) than either the vancomycin (31%) or vancomycin and lavage (23%) groups (van Nood et al., 2013). The unusually low resolution rates in the control groups was attributed to the extreme recurrence history within the sample; 35 of the 43 study participants had experienced more than one recurrence, which authors explained as the reluctance of patients with other options to receive fecal infusion. Similarly, another study reported that only 53% of the patients with refractory CDAD who underwent FMT responded that it would be their first treatment choice if their symptoms recurred, despite a 91% cure rate (Brandt et al., 2012).

Notably, the procedure is aesthetically unappealing, and long-term follow up and safety information is unavailable. In the aforementioned study, four patients developed “diseases of potential interest” following FMT that could not be definitively attributed to the procedure (Brandt et al., 2012). The primary contention with FMT is the fact that people cannot be sure of

what is being transferred. Variation in the intestinal microbiota has been experimentally linked to blood-brain barrier permeability (Braniste et al., 2014), insulin resistance (Udayappan et al., 2014), depression (Naseribafrouei et al., 2014), and, in one unexpected and well-publicized human transfer, even severe weight gain (Alang and Kelly, 2015). Fecal transplants have an all-or-nothing quality, and as with any unknown procedure, the certainty of achieving exclusively favorable outcomes cannot be guaranteed. In an article that echoes both fear and skepticism, authors wrote, “It is also worth noting that today's exceptionally healthy 25-year-old volunteer donor could develop major health problems in a decade” (Weil and Hohmann, 2015).

A 2016 metagenomic analysis evaluated three different groups (experimental FMT-transfer, sham FMT-transfer, and no intervention) three months after an FMT intervention to assess the fate of transferred material (Li et al., 2016). Interestingly, authors concluded that differences in transfer success across patients were dependent upon the compatibility of the donor and recipient microbiomes. In 80% of cases, the proportion of donor-specific species retained in recipient fecal samples was either less than or equal to the appearance of new species in the placebo and healthy groups. A resistance to introduced strains (39 ± 23%) and coexistence of donor and recipient strains (44 ± 14%) occurred. Highly variable transfer profiles were observed among recipients from a shared donor, and the presence of donor-specific, single-nucleotide variants (SNV) ranged from 12.0% to 55.6%. Effective transfer and dominance vs rejection of donor strains were not specific to a particular species, affected by strain abundance, or dependent upon clinical characteristics of the patients. The identification of SNPs prior to transfer and use of control groups to monitor and validate results were unique aspects of this work. The rejection of unshared strains and a lack of uniformity among responses emphasize the mutualistic nature between native and novel bacteria, as well as the need for tailored treatments.

Future obstacles for FMT include the identification and recruitment of healthy donors, development of an individualized treatment and standardized protocol, and the establishment of consistent regulatory criteria that align with the partial FDA withdrawal of "enforcement discretion". The relative success of FMT over conventional treatment implicates the microbiota in both disease acquisition and eradication.

In summary, host bacteria enhance immunity and provide a physical and biochemical barrier against opportunistic colonization; antibiotics, administered with the intention of restricting or destroying pathogenic bacteria, assert bacteriostatic or bactericidal effects on the adjacent microbiota. Taken together, disease recurrence following antibiotic treatment and subsequent bacterial clearance following FMT imply that the long-term efficacy of antibiotics may be dependent upon the host's ability to repopulate the GI tract. Therefore, targeted interventions that protect or reestablish commensal microbiota may have vast therapeutic implications.

Prophylaxis

Intestinal bacteria are vital to host health and are required for nutrient and drug metabolism, vitamin synthesis, immune regulation, and protection against pathogens. The microbiota are critical for GI maintenance and repair, and are easily supplemented by dietary means. The human GI tract represents over 1,000 bacterial species and approximately 95% of the bacterial microbiome (Lloyd-Price et al., 2016). At the individual level, bacterial structure is affected by genetics, environment, health, diet, age, and exposure to antimicrobial agents. Remarkably, seemingly innocuous foods and ingredients have been shown to favor pathogenic growth and disease; a recent article linked the FDA approval of the disaccharide, trehalose, as a food additive in the year 2000 to the rise of an epidemic *C. difficile* strain (Collins et al., 2018).

Two *C. difficile* strains were uniquely able to survive on low levels of environmental trehalose, and when mice fed a high-trehalose diet were infected with these strains, bacterial toxin production and mortality were greatly increased.

An estimated 150-170 species may predominate in the human colon at any given time yet, with few exceptions, commensal bacteria exhibit remarkable stability throughout the lifetime of their host (The Human Microbiome Consortium, 2012). The intestinal dysbiosis and reduction in bacterial diversity observed among CDAD patients suggests that restoration of the appropriate microbiota could yield favorable outcomes (Chang et al., 2008).

Probiotics and prebiotics are known to amplify non-pathogenic bacteria, promote microbial diversity, restrict pathogen adherence, and have been used anecdotally and experimentally for patients with various bowel disorders to reduce clinical symptoms and maintain remission. The ingestion of probiotics, or live microorganisms used to exert changes on the microbiome, has shown potential in patients with CDAD. However, premature degradation in the upper GI tract can be problematic, and caution is advised for use in critically ill or immunocompromised patients. Conversely, prebiotics have not been investigated as thoroughly, but may offer greater stability with lower risk and cost.

Probiotics

Research showing the effect of probiotics on *C. difficile*-related parameters has advanced significantly within the last decade. A 2008 Cochrane review of 336 patients across four studies determined that there was insufficient evidence to recommend probiotics as an adjunct or individual treatment for *C. difficile* (Pillai and Nelson, 2008). Among the low number of qualifying studies, a randomized placebo-controlled trial measuring outcomes in CDAD patients treated with antibiotics and either a placebo or *Saccharomyces boulardii*, showed potential for

probiotic therapy. The addition of *S. boulardii* significantly reduced recurrence in patients with recurrent CDAD, but did not prevent a first recurrence for patients with an initial CDAD episode (McFarland et al., 1994).

Five years later, an updated Cochrane review, which cited 31 studies with 4,492 total participants, concluded that moderate quality evidence indicated probiotic use for the prevention of *C. difficile* diarrhea (Goldenberg et al., 2013). The use of probiotics reduced *C. difficile* diarrhea by 64%, and similar results were reported in trials with either adults or children, low or high doses of probiotics, and across different probiotic species. A 20% reduction in risk of adverse events was calculated from 26 studies with 3,964 participants. Conversely, reports from 13 trials with 961 patients showed that the incidence of infection was not different between probiotic and control groups.

Similarly, a 2012 systematic review and meta-analysis of 20 trials with 3,818 patients revealed a 66% decrease in *C. difficile* diarrhea for patients receiving probiotics (RR 0.34; 95% CI 0.24 to 0.49) and studies administering multiple species showed larger effects (RR 0.25; 95% CI, 0.15 to 0.41) than those using one species (RR 0.50; 95% CI, 0.29 to 0.84) (Johnston et al., 2012). A reduction in adverse events was noted for patients in the probiotic group (9.3% with probiotics vs 12.6% of control; RR 0.82; 95% CI, 0.65 to 1.05). A 2016 systematic review (which included many of the same studies as the other reviews) with 7,957 patients across 26 RCTs also concluded that fewer patients treated with probiotics developed CDAD (1.5% probiotics vs 3.8% control, $P<0.001$), and added that *Lactobacillus* (RR 0.36, 95% CI 0.23 to 0.59; $P<0.001$), *Saccharomyces* (RR 0.42, 95% CI 0.212 to 0.80; $P=0.008$), and a mixture of probiotics (RR 0.42, 95% CI 0.26–0.66; $P<0.001$) all specifically reduced CDAD risk (Lau and Chamberlain, 2016). Neither study reported infection rates.

Perhaps the most compelling primary evidence for the use of probiotics for CDAD prevention comes from a 2007 randomized placebo-controlled trial of 135 patients receiving antibiotics (Hickson et al., 2007). Fewer patients in the probiotic group developed diarrhea than in the control group (12% with probiotics vs 34% with placebo; P=0.007), and none of the patients treated with probiotics developed CDAD (0% with probiotics vs 17% with placebo; P=0.001). Realistically, results may not be generalizable, as among the 1,760 eligible patients, 148 refused to participate in the trial, 1,167 were rejected for reasons involving confounding sources of diarrhea, recent antibiotic use, and informed consent, and another 310 were rejected for safety reasons, such as severe illness and immunosuppression; of the 135 patients participating in the trial, 22 (16%) were lost to follow-up. Unfortunately, serious limitations were present in many of the *C. difficile* and probiotic studies, including, but not limited to, non-generalizable or missing data, poor selection criteria, significant patient loss, and small sample size.

Probiotics are generally considered safe by the public (and many have obtained Generally Recognized as Safe, or GRAS, status), yet some trials have reported safety concerns involving their use in immunocompromised or severely ill populations (Besselink et al., 2008; Enache-Angoulvant and Hennequin, 2005). However, the estimated risk for developing infection from *S. boulardii* or lactobacillus species is one in 5.6 million users and less than one in one million users, respectively (Karpa, 2007). In conclusion, the literature is best summarized in the CDAD recommendations from the *American College of Gastroenterology*:

> There is limited evidence for the use of adjunct probiotics to decrease recurrences in patients with RCDI… Although there is moderate evidence that two probiotics (*L.*

rhamnosus GG and *S. boulardii*) decrease the incidence of antibiotic associated diarrhea, there is insufficient evidence that probiotics prevent CDI (Surawicz et al., 2013).

Synbiotics

A synbiotic is a combination of a probiotic and a prebiotic. The prebiotic is believed to enhance the survival of the probiotic and promote intestinal colonization. A study measuring symptomology and colonization of CDAD in a mouse model assigned animals to either a prebiotic, probiotic, or synbiotic (Kondepudi et al., 2014). Selected probiotics included *L. plantarum* F44, *L. paracasei* F8, *B. breve* 46, and *B. lactis* 8:8. Galactooligosaccharide (GOS), isomaltooligosaccharide (IMOS), and resistant starch (RS) were administered as prebiotics. All animals were given antibiotics, and after two days, half of the control mice became moribund and were sacrificed. No CDAD toxins were found in the synbiotic, probiotic, or prebiotic mice at the end of the experiment, whereas control mice expressed toxin throughout the study. Histopathology showed that all control mice, but only 17% of all treated mice exhibited cecal inflammation. Animals in the synbiotic group had higher bacterial counts than animals in the other groups, but all treatments provided protection against CDAD.

In a non-controlled retrospective analysis of 120 nursing home residents using antibiotics, chewable synbiotic tablets containing *S. boulardii* (7.5 billion cfu), *B. coagulans* (1 billion cfu), and 500 mg fructooligosaccharide (FOS) were provided twice a day beginning shortly after the onset of antibiotic treatment, and continuing for two weeks after antibiotic cessation (Spielholz, 2011). Antibiotic-associated diarrhea (AAD) did not occur in 95% of residents treated with antibiotics and synbiotics. Two residents were diagnosed with *C. difficile* infection, but only one was credited with having CDAD (tested positive for *C. difficile* and presented with symptoms of AAD). The method of *C. difficile* detection was not specified. No adverse effects were reported,

and the synbiotic was well tolerated overall, as mild gastrointestinal side effects were observed in less than 6% of residents. Inconsistent data and subject reporting, including unclear handling of non-compliant residents and drop-outs, as well as the removal of subjects for incomplete data sets, hindered the interpretation of evidence.

The uncontrolled design employed in this study is unfavorable from a research perspective, as it prevents the generalizability of data and cannot confirm efficacy without a proper control group for comparison. However, results indicate that the synbiotic is unlikely to cause significant harm, and the author may be able to use the unilateral intervention to help inform and improve in-house policies.

Synbiotics are an appealing option for supplementation, as they appear to combine the best of both probiotics and prebiotics. However, studies have reported that this assumption may not be accurate, and that the addition of certain probiotics can negate any benefit afforded by the prebiotic (Barnes et al., 2012; Madeo and Whitlick, 1999). Therefore, matching the appropriate type and dosage of the prebiotic and probiotic is imperative.

Prebiotics

Prebiotics are non-digestible substances that are selectively fermented by colonic microbiota. The most thoroughly studied prebiotics are fructoologosaccharides (FOS) and inulin. Other common types include galactooligosaccharides (GOS), xylooligosaccharides (XOS), and resistant starch (RS). Though not studied as extensively as probiotics, there is evidence, especially from in vitro and animal studies, that prebiotics could impair *C. difficile* colonization, growth, or virulence (Ambalam et al., 2015). Their ease of production and delivery, survival through inhospitable parts of the GI tract, and ability to maintain colonization resistance make prebiotic supplementation a promising avenue for future research.

The fermentation of dietary prebiotics yields several physiologically relevant products. Among them, short-chain fatty acids (SCFA) and lactic acid serve to decrease luminal pH, thereby providing an inhospitable colonic environment for exogenous microbe colonization. The primary SCFA include acetate, propionate, and butyrate. In particular, butyrate provides approximately 70% of the energy for the colonic mucosa, and mediates the immune response by inducing growth arrest and apoptosis (Carding et al., 2015). High SCFA concentration and low pH have been shown to regulate *C. difficile* in vitro (May et al., 1994).

Prebiotics are thought to exert protective action by acting as a substrate for either one or a limited number of beneficial bacteria, especially bifidobacteria and lactobacilli. In vitro studies have detailed the ability of bifidobacteria, lactobacilli, and bacteroides strains, isolated from stool samples of healthy volunteers, to prevent *C. difficile* growth (Rolfe et al., 1981), and bifidobacteria supernatants have proven effective at inhibiting *C. difficile* growth and preventing adherence to enterocytes (Trejo et al., 2006). A non-interventional clinical study concluded that the presence of indigenous lactobacilli was protective against CDAD development after measuring higher lactobacillus counts from fecal samples taken from *C. difficile* negative patients than *C. difficile* positive patients (Naaber et al., 1997); this may provide a direct connection between known prebiotic function and freedom from pathological infection and CDAD.

An in vitro study with human fecal samples from three healthy volunteers examined the results of clindamycin or vehicle treatment with supplementation with one of three experimental non-digestible oligosaccharide (NDO) preparations—galactooligosaccharide (GOS), fructooligosaccharides (FOS), or inulin (Hopkins and Macfarlane, 2003). Samples were tested at five time points over 48 hours. Fecal samples unaltered by clindamycin showed *C. difficile*

growth inhibition with NDO ($P<0.05$), which was paralleled by increased bifidobacteria counts ($P<0.01$). The reverse was seen in the clindamycin-treated groups, as NDO had no suppressive effect on *C. difficile* samples, which mostly contained slightly more bacteria than their respective controls. However, bifidobacteria concentrations were decreased by clindamycin, and further by NDO ($P<0.05$). NDO addition increased SCFA by 12 hours, and major fermentation products were acetate, propionate, and butyrate. As expected, addition of clindamycin to the samples decreased SCFA formation. The only toxin discovery was in the antibiotic and FOS group at 24 hours. In summary, NDO stimulated bifidobacteria growth and reduced *C. difficile*. Upon the addition of clindamycin, bifidobacteria were severely reduced, especially when incubated with NDO. Without antibiotic intervention, NDO improved colonization resistance, but the effects were not attributable to the action of bifidobacteria.

Several animal studies have examined CDAD-related outcomes after prebiotic feeding with FOS (Gaskins et al., 1996; Wolf et al., 1997). In a study measuring *C. difficile* diarrhea and toxin A production, mice were divided into control and antibiotic groups, fed either a control diet or the same diet supplemented with FOS, and all inoculated with *C. difficile* (Gaskins et al., 1996). After prebiotic feeding, toxin A titers were elevated only in the antibiotic-treated group with the control diet; both the antibiotic and control animals fed FOS-supplemented diets had normal toxin A titer. No difference in diarrheal severity was noted between the groups for animals receiving antibiotics, but non-antibiotic animals given the FOS-supplemented diet had lower diarrheal severity than animals consuming the control diet. Authors suggested that the FOS may directly bind toxins, leading to reduced toxin A titers, but that the protective effects of FOS may be dependent or increased by microbiota.

Another animal study of FOS supplementation in a CDAD model revealed that hamsters given FOS-supplemented drinking water had an increased mean survival time after inoculation with *C. difficile* (15 days with FOS vs 13.5 days with control; P<0.001) (Wolf et al., 1997). A subsequent 2x2x2 factorial hamster experiment provided either an antibiotic or a non-antibiotic control to animals receiving FOS-supplemented or control water, with or without inoculation. Results were similar to the previously mentioned mouse study, and showed that FOS supplementation improved outcomes for inoculated animals that did not receive antibiotics (14.5 days with FOS vs 13 days with control; P=0.07), but did not affect outcomes for inoculated animals that received antibiotics (19 days with FOS vs 20 days with control; P=0.7); like the in vitro Hopkins study, these data support the hypothesis that protection against *C. difficile* by FOS is dependent upon the existing microbiota. FOS increased survival of uninoculated animals that received antibiotics (19 days with FOS vs 17.5 days with control; P=0.02), and for uninoculated control animals (16.5 days with FOS vs 13.5 days with control; P=0.003). An interaction between antibiotics and inoculation was indicated. Hamsters that received antibiotics lived longer than those that were not treated, and in both hamster experiments, when all animals were pooled, FOS increased survival time compared to controls.

Interventional studies with prebiotics in human subjects

Although the use of prebiotics in clinical research is common, the majority of the existing evidence involves the supplementation of milk formula in newborns and infants. Unfortunately, since the efficacy of prebiotic supplementation is dependent upon the preexisting microbiota, insights gained by these studies are not compatible with the expectations of adult supplementation. Up to 70% of newborns and infants are colonized by *C. difficile*, but CDAD is rarely acquired (Ghose, 2013). It has been proposed that the lack of symptoms in colonized

infants is either due to increased protection afforded by Bifidobacteria (which represents 60-70% of the infant microbiome) (Arboleya et al., 2016), neutralization of the toxin by secretory IgA and oligosaccharides from breast milk, or a lack of functional *C. difficile* toxin receptor within the infant GI tract (Jangi and Lamont, 2010). While rare, CDAD can manifest in children (Schutze and Willoughby, 2013). A multicenter trial investigated the ability of inulin and FOS to prevent AAD and CDAD in children ranging in age from six months to 11 years (Szajewska et al., 2012). Participants received antibiotic treatment concurrently with either five grams of inulin and FOS or a maltodextrin placebo. Ninety-two children completed the study, no adverse effects were reported, and the prebiotic mixture was well tolerated. No significant differences were observed between the prebiotic and placebo groups in terms of the quantity of participants with diarrhea (6.7% with prebiotic vs 10.6% with placebo; RR 0.63; 95% CI 0.16 to 2.5), and the proportions of participants with AAD, both with and without *C. difficile* toxin (6.7% with prebiotic vs 8.5% with placebo; RR 0.78; 95% CI 0.19 to 3.3). This study showed no evidence for the use of inulin and FOS in the prevention of AAD, but the selection criteria were stringent, the number of participants with diarrhea was low (only seven participants between both groups), and the study was underpowered.

Publications involving prebiotic administration (either individually or with a probiotic) for prevention or treatment of *C. difficile* in adult human populations are limited to four full-length articles (Lewis et al., 2005a; Lewis et al., 2005b; Madeo and Whitlick, 1999; Spielholz, 2011) and two conference abstracts (Licht and Maltz, 2012; Rhim, 2008); results from the applicable interventions were analyzed in a systematic review, but the details of the individual studies will be introduced here.

Rhim, 2008

One of the conference abstracts considered for review was ultimately excluded due to the presence of a prognostic imbalance that confounded the study objectives (Rhim, 2008). A retrospective, case-control study of hospitalized patients with cirrhosis planned to use records obtained between January 2001 and March 2006 to confirm that lactulose ingestion could prevent *C. difficile* infection (CDI). A large sampling pool of 4,566 patients with decompensated cirrhosis were considered for the analysis. Exclusion criteria included the presence of diarrhea on admission and a recent positive *C. difficile* toxin assay. Within the cohort, cases of CDI were identified. CDI negative controls were randomly selected, but matched by age, sex, hospital location, and admission period. 35 CDI patients and 75 controls were selected and Chi-squared, t-tests, and conditional logistic regression were used for analysis. Hepatic encephalopathy (HE) was not selected as a covariate for the model, as it is highly related to lactulose exposure (the author introduces lactulose as a treatment for both HE and constipation). Results of their analysis suggested that lactulose decreased CDI, but needed to be confirmed in other populations.

Madeo, Whitlick, and Martin, 1999

The earliest preventive clinical CDAD and prebiotic study was an RCT that examined the ability of either a prebiotic (9.1 g FOS) or synbiotic (8.5 g FOS+ *L. acidophilus* and *B. bifidum*) to prevent AAD (including CDAD) in elderly hospital patients receiving oral antibiotics (Madeo and Whitlick, 1999). Sixty-six patients were blinded and randomized to receive either prebiotic, synbiotic, or control (9.1 g maltodextrin) granules once a day at the onset of antibiotic treatment. The intervention lasted for 15 days and patients were monitored for up to 10 additional days. Bowel action, frequency, and consistency were recorded by ward staff, and any diarrheal samples were examined for pathogens and *C. difficile* toxin. Patients who were discharged before the end of the study were sent home with granules and follow-up was completed by

telephone. Additionally, their physicians were contacted and asked to send stool samples if they developed diarrhea within two weeks.

AAD was only reported among six people in the study; four (13.3%) were from the synbiotic group and two (11.1%) were from the placebo group. No one in the prebiotic group acquired AAD, and differences were not significant. Despite mention of it in the study design, the results either failed to find a causative pathogen or *C. difficile* toxin, or neglected to report their results. Authors did not comment on adverse events.

Licht and Maltz, 2012

In a 190 patient retrospective case-control study, unspecified doses of antibiotics were administered with or without the addition of lactulose to determine whether lactulose was effective in preventing "C. diff" (Licht and Maltz, 2012). The duration of treatment was not specified, but fewer patients in the lactulose group developed "C. diff" (2.3% vs 9.7%; OR 0.22; P=0.05). Results were presented vaguely as an abstract, and adverse events were not reported. Interestingly, there was no mention of diarrhea beyond the title or what the lactulose was prescribed for, only that it is "commonly utilized in the management of constipation and hepatic encephalopathy". Furthermore, by excluding retrospective records of patients who received metronidazole or vancomycin (the primary treatments for CDAD), the authors likely removed many patients who were diagnosed and treated for the disease of interest during their hospital stay. Lastly, it seems unlikely that admissions to the medicine and surgery services of a large academic medical center would enroll only 87 subjects over the course of a year.

Lewis, Burmeister, and Brazier, 2005

The remaining two human CDAD studies were RCTs using high-dose oligofructose powder (12 g/day) and antibiotics. The first of the studies measured relapse of diarrhea following

diarrheal cessation, treatment failure, and *C. difficile* toxin in 142 consecutive inpatients with CDAD (Lewis et al., 2005a). Of 527 subjects identified as *C. difficile* toxin-positive, 142 participated in the study (93 declined, 186 could not provide consent, 51 were excluded due to diabetes, and 55 were excluded due to immunosuppression or GI disease), and five patients discontinued the powders after a few days (one oligofructose, four placebo), as they disliked the taste. The prebiotic powder or sucrose was given along with antibiotics beginning at the time of diagnosis, and ending 30 days after the cessation of diarrhea. Patients were assessed after 30 days (with termination of the prebiotic or placebo) and followed up with 30 days later.

Oligofructose supplementation reduced relapse (8.3% vs 34.3%; OR 0.17; P=0.0004), but not treatment failure (6.9% oligofructose vs 4.3% control; OR 1.67; P=0.5) or asymptomatic carriage at 30 days (43.1% vs 58.6%; OR 0.53; P=0.07) or 60 days (34.7% vs 48.6%; OR 0.6; P=0.1). Nineteen (13%) deaths were reported, but fatality rates for relapsing patients did not differ between groups (17% vs, 8.3%, OR 2.2, NNTH=12; P=0.6). *C. difficile* was not provided as a primary cause of death in any case.

The authors stated an intention-to-treat (ITT) analysis, yet evaluation of the results from terminal patients suggested classification by their last observation carried forward (although this is not discussed in the text). It is unclear whether the five patients who refused treatment after several days were dismissed from the study or included within the 142-patient data set, and data reporting among tables appeared to be inconsistent. *C. difficile* was not remeasured/confirmed in patient samples upon diarrhea recurrence, but this represents more of a methodological issue than author bias.

Lewis, Burmeister, Cohen, Brazier, and Awasthi, 2005

The other study published by the same primary author within the same year examined the efficacy of high-dose oligofructose (12 g/d) in preventing multiple types of AAD, including CDAD (Lewis et al., 2005b). Unlike the relapse study, results of this trial were not significant. A total of 116 patients (27%) experienced diarrhea, with similar results between groups; diarrhea was reported in 56/215 (26%) and 60/220 (27%) patients who received oligofructose and placebo, respectively (OR=1.0, NNTB=117, p=0.8). When stratified by severity, 36 patients in the prebiotic group presented with significant diarrhea, and another 20 patients experienced non-significant diarrhea vs 37 significant and 23 non-significant cases in the placebo group. Severe adverse events, including death among patients with significant CDAD, occurred in 2/19 (10.5%) patients in the oligofructose group. Exclusion of 15 patients from analysis (eight for GI bleeds and seven before treatment was provided) contradicted the stated ITT analysis.

The current evidence regarding the use of prebiotics for CDAD is extremely diverse, but most of the studies indicate that prebiotic administration improved outcomes within their respective study population. In order to fairly assess the available research, a systematic review was conducted and presented in the next chapter of this thesis. The initial hypothesis was, that with appropriate prebiotic selection, dosage, and treatment duration, as well as careful selection of the precipitating antibiotic (for high-risk patients), prebiotic supplementation may represent a safe and effective method of preventing infection in humans, and may reduce the need for further antibiotic use and microbial depletion. However, after summarizing research and pooling outcomes, where appropriate, the results of the systematic review were unable to either corroborate or disprove the hypothesis due to scarce, low-quality, and heterogeneous evidence.

Additionally, two separate retrospective chart reviews were conducted using inpatient records from Carle Foundation Hospital (CFH) to identify and validate specific risk factors for

CDAD and determine whether malnutrition was related to CDAD acquisition. The identification of at-risk patients is critical, but the lack of consensus surrounding many of the published risk factors makes generalization problematic. Historically, the precipitous rise in CDAD prevalence following outbreaks with epidemic strains illustrates the fact that infectivity and toxigenic potential vary greatly among different types of *C. difficile*, and contribute to regional, national, and global infection patterns. Risk may also be modified by regulated hospital practices, such as infectious disease surveillance, antibiotic stewardship programs, proper sanitation, and conscientious patient care.

The retrospective chart reviews represent data from two separate years, and will comprise the fourth and fifth chapters of this thesis. The initial review was conducted using data from 2014, and was separated into a preliminary and a primary study. The second chart review, conducted using 2016 data, was intended to validate the 2014 review and surveil regional CDAD, malnutrition, and mortality rates over time. The overarching goal of this research was to combine the knowledge obtained in the systematic review and both chart reviews into a clinical study to be conducted at CFH. The retrospective chart reviews were specifically designed to help identify which patients would benefit most from a nutrition intervention and facilitate the rapid and reliable recruitment of high risk patients upon admission, prior to potential *C. difficile* exposure during the hospital stay.

In summary, the most practical way to mitigate prospective disease burden is to protect people at risk. Prophylactic therapies may benefit the host by resisting pathogenic colonization, neutralizing enterotoxins and cytotoxins, strengthening an immune response, and either protecting or restoring endogenous colonic microbiota. The ability to evade opportunistic infection will lessen the individual disease burden and prevent transmission to others via passage

of spores. Hospital implementation of such a strategy could inhibit the spread of CDAD within the facility, reduce the need for antibiotic usage, and ultimately improve infection rates.

CHAPTER 3: USE OF PREBIOTICS FOR PREVENTION AND REDUCTION OF *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DISEASE IN ADULTS: A SYSTEMATIC REVIEW

ABSTRACT

The present systematic review collates research on the use of prebiotics for the prevention of *Clostridium difficile*-associated disease (CDAD). The literature search examined studies published up until May 2016 using the following search terms: *Clostridium difficile* infection, *Clostridium difficile* disease, diarrhea, prebiotics, fructans, lactulose, galacto-oligosacharides, and inulin. Five studies fullfilled the inclusion criteria, and evaluated prebiotic supplementation in adult patients at risk for CDAD. Prebiotics were administered during and after antibiotic treatment independently in four studies, and in conjunction with a probiotic (synbiotic) in two studies; pooled results examined prebiotic interventions either with or without the accompanying probiotic. Although prebiotics may safely and effectively decrease the incidence of CDAD among at-risk adults, insufficient evidence prevents the accurate assessment of clinical benefit.

Key words: *Clostridium difficile*, prebiotic, diarrhea, systematic review

INTRODUCTION

Clostridium difficile–associated disease (CDAD) most commonly occurs in elderly hospitalized patients with a recent history of antibiotic use, and is a significant cause of morbidity and mortality among hospitalized adults. CDAD is an opportunistic bacterial infection that requires the displacement of the normal colonic microbiota with subsequent pathogen ingestion, germination, colonization, and proliferation. In addition to recent hospitalization and

antibiotic use, risk factors for CDAD include advanced age, admission from a long-term care facility, length of hospital stay, previous diarrhea or CDAD, malnutrition, polypharmacy, and compromised immunity (Bignardi, 1998; Cooper et al., 2013; Klingler et al., 2000; Monge et al., 2011; Wong et al., 2009). Treatment is complicated by a high recurrence rate, caused by either reinfection or recolonization with residual bacterial spores (Figueroa et al., 2012; Wilcox et al., 1998). Recurrence and treatment failure are common with the existing antibiotic regimens and have been reported in 22% and 21% of patients, respectively (Vardakas et al., 2012). The precipitating role of antibiotic administration in CDAD development and the recent success of fecal microbiota transplants for animal and human subjects with recurrent CDAD reflect the importance of microbiome dysregulation in disease establishment, as well as the potential for symptom abatement through the restoration of normal gastrointestinal (GI) microbiota. For patients with CDAD, long term cure relies not only upon pathogen elimination, but the regeneration of colonization resistance.

The highly resilient and transmissible nature of *Clostridium difficile* (*C. difficile*) emphasizes the need for future therapies that focus on the prevention of CDAD, rather than the management of primary, recurrent, and treatment-resistant disease. Any therapy that aids the reinforcement and rapid recovery of endogenous GI defenses, primarily the microbiota, may prevent pathogenic colonization entirely, and thereby reduce CDAD-related morbidity, mortality, and costs, and create safer healthcare environments.

CDAD emerged as an international threat in the early 2000's when hypervirulent strains, most notably BI/NAP1/027, were detected throughout the United States, Canada, and Europe. Expectedly, data from this period reflect escalated incidence, severity, and mortality (See et al., 2014). In the United States, the rate of CDAD hospitalization per 1,000 discharged adults

increased from 5.6 in 2001 to 12.7 in 2011 (Steiner et al., 2014) and was projected to reach 14.2 in 2013; an estimate released by the CDC corroborated this timeline (CDC, 2016), yet the Healthcare Cost and Utilization Project (HCUP) did not confirm this peak prevalence until 2015 (HCUP, 2018). According to the CDC, in 2015, patients experienced an unprecedented 14.9 CDAD cases per 1,000 patient discharges (CDC, 2017).

Infection rates in Canada increased modestly from 4.7 to 5.4 nosocomial CDAD cases per 1,000 admissions from 2007 to 2011, but decreased to 3.6 in 2014. A recent survey of 95 Quebec hospitals estimated a nosocomial CDAD incidence of 6.8 per 10,000 patient-days from April 2014 to March 2015 (Garenc et al., 2016), whereas The European Centre for Disease Prevention and Control (ECDPC) reported that the United Kingdom, Finland, and Poland each described an incidence of greater than 10 per 10,000 patient-days (ECDPC, 2015). While CDAD rates appear lower in certain parts of Europe, Latin America, Asia, and Africa, it is unclear whether these estimates accurately reflect overall disease prevalence or regional variations in clinical detection and surveillance practices.

CDAD represents a significant social and economic burden. In 2009 alone, the U.S. and Canada spent $8.2 billion (Lucado et al., 2012) and over $281 million, respectively, on CDAD treatment (Levy, 2013). In Europe, the annual cost of CDAD has been estimated at €3000 million and is expected to double over the next four decades due to a growing elderly population (Kuijper et al., 2006). Remarkably, the extensive utilization of financial, medical, and personal resources has not fostered overtly positive outcomes; between 2000 and 2007, a U.S. study reported a five-fold elevation in mortality attributable to CDAD, from 2,675 to 14,368 deaths per year (Hall et al., 2012). Approximately 10% of CDAD cases prove fatal within 30 days of diagnosis (Lessa et al., 2015).

Clinical practice guidelines developed by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) recommend metronidazole for standard infections and vancomycin for severe or recurrent cases (Cohen et al., 2010). Experimental alternatives, including the newer antibiotics, fidaxomicin and rifaximin, as well as intravenous immunoglobulin therapy, fecal transplantation, and the administration of probiotics and prebiotics are under investigation (Brunser et al., 2006; Cocanour, 2011; Surawicz and Alexander, 2011). With growing concerns about antibiotic resistance and the inability of antibiotics to prevent recurrent infections, therapies that target the microbiome are becoming increasingly salient options. Prebiotics are selectively fermented ingredients that promote changes in the composition and activity of the gastrointestinal microbiota to confer benefits upon the host (Roberfroid, 2007). Examples include fructans, such as inulin, oligosaccharides, and fructooligosaccharides (FOS), as well as galactooligosacharides (GOS), and lactulose (Gibson et al., 2004). GOS is naturally found in human and cow milk (Tuohy et al., 2005), lactulose is a synthetic sugar produced by the alkaline isomerization of lactose (Licht et al., 2012), short-chain FOS (scFOS) is a synthetic food additive derived from sucrose, and fructans are found in a variety of vegetables including onions, chicory root, artichokes, and agave (Mancilla-Margalli and Lopez, 2006). The site and extent of intestinal fructan fermentation by luminal bacteria are affected by its molecular structure and degree of polymerization (DP), and short-chain and long-chain fructans are fermented in the proximal colon (Hegazi, 2013). An expanded definition of prebiotics was released in 2017 by the International Scientific Association for Probiotics and Prebiotics (ISAPP) that extends to non-carbohydrate substances and non-food categories; fermentation selectivity and health benefits in the selected host remain vital to classification (Gibson et al., 2017).

Prebiotics are known to confer health benefits through several complementary mechanisms. Within the colon, prebiotic fermentation yields short-chain fatty acids (SCFA), primarily acetate, propionate, and butyrate. SCFA production decreases luminal pH to inhibit the adherence and proliferation of pathogens (Hegazi, 2013; Ríos-Covián et al., 2016), while butyrate specifically serves as the primary energy source for colonocytes and affects both intestinal and systemic processes (Canani et al., 2011). In addition to preventing pathogenic colonization, prebiotics selectively enhance the growth or activity of beneficial microbiota, regulate host immunity, improve mineral bioavailability, and reinforce intestinal integrity (Khailova et al., 2009; Searle et al., 2010). The protective effects of prebiotics against CDAD have been demonstrated with animal and in vitro studies (Hegazi, 2013; Wolf et al., 1997).

Investigation into the therapeutic potential of prebiotics is further warranted by cost and safety data. Prebiotics are generally well-tolerated in both healthy and critically ill populations (Manzanares and Hardy, 2008). Furthermore, their presence in naturally occuring edible plants, widespread availability as dietary supplements, and integration into popular comestibles attests to their biochemical stability and marketability. Although the evidence is limited, prebiotics may be a practical, affordable, and effective option for the prevention and treatment of CDAD. The objective of this systematic review was to assess the efficacy of prebiotic therapy for the reduction and prevention of CDAD in adults receiving antibiotic treatment at risk for *C. difficile* infection.

METHODS

Search strategy

This study was conducted according to the procedures outlined by the Cochrane Collaboration for systematic reviews (Higgins and Green, 2011). A standard protocol for study

identification, inclusion, and data abstraction was developed and followed after establishment of the following study (Population, Intervention, Comparison, and Outcome [PICO]) question: "Can prebiotic consumption prevent or reduce the prevalence of CDAD in adults receiving antibiotic treatment?"

Multiple electronic databases, including Cochrane Central Library, PubMed/Medline, and Embase, were searched through May 2016 using the following terms, alone or in combination: *Clostridium difficile* infection, *Clostridium difficile*, diarrhea, prebiotics, fructans, lactulose, fructoologosaccharides, galactooligosaccharides, and inulin. The reference lists of review articles were reviewed to find additional articles not identified in the electronic database. Results were restricted to English, French, and Spanish language studies.

Inclusion and exclusion criteria

Studies that registered adult CDAD and administerd prebiotic therapies either alone or in combination with another treatment were eligible for inclusion. Adults were defined as individuals aged 18 years and older. Due to the fact that symptoms rarely occur without microbiota dispacement, it was critical to assess adults who had recently undergone antibiotic treatment, as it confers susceptibility to CDAD.

The primary outcome of interest was the prevalence of CDAD following prebiotic therapy. The diagnosis was confirmed by the presence of appropriate symptomology and coinciding evidence of *C. difficile* colonization in order to avoid the inclusion of unrelated GI illnesses and asymptomatic carriers. Reasons for exclusion included duplicate data, review articles, analysis from pediatric patients, and research protocols not associated with CDAD. Inclusion criteria were evaluated by three independent reviewers.

Study selection

Articles identified in the literature search were examined by two reviewers. After removal of duplicate titles and abstracts, the remaining articles were screened. Full text articles that met inclusion criteria were retained for data analysis. Articles that did not meet inclusion criteria were excluded, and disagreements were resolved by a third reviewer.

Data extraction

A data extraction form was developed and piloted jointly by the authors, after which data extraction was performed by two reviewers; disagreements were resolved by a third reviewer. Information regarding participants, study design, interventions, outcome measures, potential bias, limitations, and study quality was extracted.

Assessment of bias and quality

Two independent reviewers evaluated the studies for methodological quality, including multiple domains of selection, performance, detection, attrition, reporting, and other biases. If a risk of bias was unclear, attempts were made to clarify by contacting the senior study authors. Discrepancies in trial bias assessments between reviewers were resolved by consensus or third-party intervention. Information was summarized using RevMan software (version 5.3, Cochrane Collaboration).

Statistical analysis

Summary statistics, including therapeutic gain, (prebiotic event rate - placebo event rate), number needed to treat for an additional beneficial outcome (NNTB; NNTB = 1/[proportion benefiting for experimental intervention - proportion benefiting for control intervention]), number needed to treat for an additional harmful outcome (NNTH; NNTH = 1/[proportion benefiting for experimental intervention - proportion benefiting for control intervention), and odds ratio (OR; OR = [number of prebiotic-treated subjects experiencing event/number of event-

free prebiotic treated subjects]/[number of placebo-treated subjects experiencing event/number of event-free placebo-treated subjects]) were calculated as described by the Cochrane Collaboration in order to provide safety and efficacy comparisons between prebiotic and placebo groups. A meta-analysis was performed on pooled CDAD prevalence data, and heterogeneity among studies was calculated using the I^2 test.

RESULTS

Included studies

A total of 2,716 citations were identified, and 2,088 unique results remained after removal of duplicates. Analysis of titles and abstracts yielded 22 studies for full review. Five studies, including four full-text manuscripts and one conference abstract, met inclusion criteria (**Figure 3.1**). Three of the studies exclusively reported prebiotic administration (Lewis et al., 2005a; Lewis et al., 2005b; Licht and Maltz, 2012), one study included both a prebiotic and a synbiotic intervention (Madeo and Whitlick, 1999), and one study examined synbiotic intervention only (Spielholz, 2011).

Study and patient characteristics

The study durations, populations, and outcomes of interest from the included studies are represented in **Table 3.1**. The study designs included three randomized, controlled trials (RCTs) (Lewis et al., 2005a; Lewis et al., 2005b; Madeo and Whitlick, 1999), one uncontrolled trial (Spielholz, 2011), and one retrospective cohort study (Licht and Maltz, 2012). A total of 953 patients were included in the five studies. Patients were predominantly elderly, and except the control group selected by Licht and Maltz (2012), all groups recorded a mean age greater than 65 years. The gender distribution across groups was fairly equal, although three studies (Lewis et al., 2005a; Madeo and Whitlick, 1999; Spielholz, 2011) included more female patients than male

patients in all groups, and two studies (Lewis et al., 2005b; Licht and Maltz, 2012) included more females in the control group than the treatment group. All patients included in the five studies were prescribed an antibiotic either prior to or at the onset of prebiotic therapy.

Several variables, including the type of prebiotic being assessed, product dose, administration frequency, and method of delivery differed among the studies. The abstract did not report most of these items, and emailed requests for further information were not returned (Licht and Maltz, 2012). Three prebiotics were represented, including oligofructose (Lewis et al., 2005a; Lewis et al., 2005b), fructooligosaccharide (Madeo and Whitlick, 1999), and lactulose (Licht and Maltz, 2012), whereas the synbiotics, or combined prebiotic and probiotic, contained FOS and either *Sacchromyces boulardii* and *Bacillus coagulans* (Spielholz, 2011) or *Lactobacillus acidophilus* and *Bifidobacterium* (Madeo and Whitlick, 1999). Prebiotic dosage varied from one g/d (Spielholz, 2011) to 32.4 g/d (Madeo and Whitlick, 1999), and while supplementation occured during antibiotic administration in all cases, endpoints ranged from seven days following antibiotic cessation (Lewis et al., 2005b) to 30 days following symptom abatement (Lewis et al., 2005a). Two studies did not define a clear endpoint (Licht and Maltz, 2012; Madeo and Whitlick, 1999).

Risk of bias

The risk of bias for the included experimental studies is summarized in **Figure 3.2**. The risk of selection bias was generally low in the three RCTs (Lewis et al., 2005a; Lewis et al., 2005b; Madeo and Whitlick, 1999) except for a high risk in allocation concealment for a trial that contradicted an intention-to-treat (ITT) analysis by excluding patients after randomization (Lewis et al., 2005b). A low risk of bias was also observed for performance bias (blinding of participants and personnel), detection bias (blinding of outcome assessment), and other bias in

the RCTs (Lewis et al., 2005a; Lewis et al., 2005b; Madeo and Whitlick, 1999). The synbiotic intervention was not controlled and, therefore, possesses a high risk of selection, performance, or detection bias (Spielholz, 2011). The risk of attrition bias was either unclear (Lewis et al., 2005a; Madeo and Whitlick, 1999) or high (Lewis et al., 2005b) for all studies, and reflect the removal of patients during the study period (Lewis et al., 2005b) and unclear handling of non-compliant, early-discharge, or deceased patient data (Lewis et al., 2005a; Madeo and Whitlick, 1999) (Spielholz, 2011). An unclear (Lewis et al., 2005a; Lewis et al., 2005b) and high risk of bias (Madeo and Whitlick, 1999; Spielholz, 2011) were noted for selective reporting as well. Reporting bias was affected by inconsistencies in data presentation (Lewis et al., 2005a; Lewis et al., 2005b; Spielholz, 2011), and failure to remark on critical outcomes described in the protocol (Madeo and Whitlick, 1999).

Efficacy of prebiotics for CDAD prevention

The safety and efficacy of prebiotic therapy for all included studies is shown in **Table 3.2**. **Table 3.3** contains summary statistics for four studies (Lewis et al., 2005a; Lewis et al., 2005b; Licht and Maltz, 2012; Madeo and Whitlick, 1999), as the synbiotic study did not include a control group for comparative analysis (Spielholz, 2011). Notably, the single group cohort exhibited very low diarrhea (5%) and CDAD (0.8%) prevalence following preventive synbiotic therapy.

Among the three prebiotic studies that examined both the presence of diarrhea and *C. difficile* colonization (Lewis et al., 2005a; Lewis et al., 2005b; Licht and Maltz, 2012), pooled data measured CDAD in 99/767 (12.9%) patients. Prevalence was lower in the prebiotic group, as 35/374 (9.4%) supplemented patients experienced CDAD vs 64/393 (16.3%) of patients in the control groups (OR 0.5, 95% CI 0.3 to 0.8; P=0.005). The trial that evaluated separate prebiotic

and synbiotic therapies was excluded from these analyses as authors deviated from their protocol and did not disclose whether *C. difficile* was responsible for the diarrhea observed in the control and synbiotic groups (Madeo and Whitlick, 1999); however, the absence of diarrhea among patients receiving FOS is promising, albeit not statistically different from the placebo group (OR 0.2, 95% CI 0.01 to 4.00; P=0.3).

A meta-analysis of the three CDAD studies revealed a significant level of heterogeneity (I^2=64%) across the three studies, but the lower prevalence of CDAD among prebiotic patients was not retained when data were applied to a random effects model **(Figure 3.3).**

Adverse events

Two prebiotic studies did not report adverse events (Licht and Maltz, 2012; Madeo and Whitlick, 1999) and the uncontrolled trial confirmed synbiotic tolerability, with minor GI side effects reported by seven participants (Spielholz, 2011). The trials that reported severe adverse events did not identify *C. difficile* as the primary cause of death in any of the 25 cases (Lewis et al., 2005a; Lewis et al., 2005b). Fatality rates did not differ between groups (4.5% vs 4.1%, OR 1.1, NNTH=12; P=0.8), nor did defecatory frequency, abdominal pain, or bloating.

DISCUSSION

The present systematic review summarizes the results of five randomized and non-randomized studies that examined the efficacy and safety of prebiotic consumption for CDAD prevention among susceptible adults. Two studies did not report any adverse events (Licht and Maltz, 2012; Madeo and Whitlick, 1999), and two studies (Lewis et al., 2005a; Lewis et al., 2005b) did not indicate that any adverse events were related to the intervention, or differed between prebiotic and control groups. The decreased CDAD prevalence observed in the prebiotic

group was not statistically significant, and despite a lack of increased harm, any clinical benefit potentially afforded by prebiotics remains uncertain.

The authors of four studies (Lewis et al., 2005a; Licht and Maltz, 2012; Madeo and Whitlick, 1999; Spielholz, 2011) claimed that a prebiotic or synbiotic may be effective, but only two studies reported a significant benefit (Lewis et al., 2005a; Licht and Maltz, 2012). Although Spielholz et al. (Spielholz, 2011) showed a low prevalence of diarrhea among their unilaterally synbiotic-supplemented patients, the study could not confirm efficacy, as it did not employ an appropriate control group for comparison. Similarly, for the FOS and synbiotic study, which reported diarrhea in less than 10% of participants, the marginal incidence within the control group precludes the ability to validate the study objectives (Madeo and Whitlick, 1999). Notably, this study was published two years before the first hypervirulent NAP1 *C. difficile* outbreak in 2001, during which time disease prevalence was historically low (McDonald et al., 2006). In contrast to the other study by the same authors, which showed an attenuated relapse rate among oligofructose supplemented patients (Lewis et al., 2005a), the larger CDAD and antibiotic-associated diarrhea (AAD) trial was unique in its assertion that prebiotics may lack the efficacy to reduce AAD (Lewis et al., 2005b). In patients who were recently prescribed various broad-spectrum antibiotics, oligofructose was unable to prevent CDAD and AAD. The authors suggested that their exclusion of high-risk candidates (including those with gastrointestinal disease, impaired immunity, diabetes, or the inability to provide consent) was the most plausible explanation for the unexpectedly low CDAD incidence, and perhaps the lack of efficacy shown in this study.

Further explanations for the lack of measurable variation between groups include the possibility that the prescribed antibiotics abrogated any possible advantages afforded by the

prebiotic, or that the duration of prebiotic intervention was inadequate to produce a significant effect. Interestingly, the studies that reported specified medications showed efficacy with patients exclusively treated with metronidazole and vancomycin (Lewis et al., 2005a), but not broad spectrum antibiotics (Lewis et al., 2005b). The variety of broad spectrum antibiotics employed in this particular trial may have decreased the selectivity of FOS to stimulate the growth of beneficial bacteria by impairing the metabolism of the intestinal microbiota (Lewis et al., 2005b). The relationship between antibiotic type and CDAD development is unclear in the current literature due to the presence of conflicting reports.

The limitations of this systematic review include the small number and size of current studies and differences in design and outcome assessment. However, low data availability for this topic necessitated liberal inclusion criteria. Articles that were considered, although not ultimately selected for inclusion in this review, were eliminated due to the presence of perceived confounds or the exclusion of critical study parameters and, generally, did not reflect preferences in overall study design and quality (Rhim, 2008).

Methodological and clinical diversity contraindicated complete meta-analysis for all studies, as treatment durations, delivery methods, dosages, products, and trial populations varied substantially. The meta-analysis performed on the three studies that assessed CDAD (Lewis et al., 2005a; Lewis et al., 2005b; Licht and Maltz, 2012) detected moderate to substantial heterogeneity, which was not unexpected given the low number of entries and dissimilarities among interventions, but suggests that the observed inconsistencies cannot be attributed to chance.

Prebiotics enhance the growth of beneficial GI bacteria, reduce luminal pH, modulate immunity, and may restore a protective microbial phenotype within the colon (Slavin, 2013).

Restructuring the microbiota may directly prevent *C. difficile* adherence through colonization resistance, enhance endogenous protection mechanisms, or antagonize toxin production (Seekatz and Young, 2014). Reviews of existing literature attribute many of the potential benefits of prebiotic supplementation to its ability to increase healthful bacteria, most notably, bifidobacteria (Patel and Goyal, 2012; Slavin, 2013). In trials that performed fecal bacterial analysis, elevated bifidobacteria in stool cultures from both subsets of oligofructose-supplemented patients were observed, and may validate this theory (Lewis et al., 2005a; Lewis et al., 2005b). These results are in agreement with previously established research (Gibson et al., 1995; Rao, 2001).

While the general aims of newer therapies, including prebiotics, probiotics, and fecal microbiota transplants (FMT) are similar, the aversiveness, uncertainty, and costs associated with FMT are not compatible with preventive care. The dietary interventions are more sustainable, and despite differences in research quantity and quality, prebiotics and probiotics are comparable in terms of heterogeneity and positive symptom improvement (Goldenberg et al., 2013; Johnston et al., 2012). It appears that each possess different advantages; the fact that probiotics are ingested as live microorganisms confers a very specific contribution to the bacterial community, but severely complicates their processing and storage requirements, as they may be subjected to premature environmental or digestive degradation (Corona-Hernandez et al., 2013).

CONCLUSIONS

While the administration of prebiotics may be an effective and safe method of decreasing CDAD prevalence among adults receiving antibiotics, the scarcity of relevant publications in this field, methodological and clinical diversity across studies, and low quality of available evidence warrants further investigation. Future work may benefit from evaluating both prophylactic and therapeutic prebiotic options against other emerging intervention methods. Additional clinical

trials are needed to appropriately evaluate efficacy using multiple types of prebiotics, eludicate an optimal dosage and duration for intervention, and address critical issues involving antibiotic compatibility.

FIGURES AND TABLES

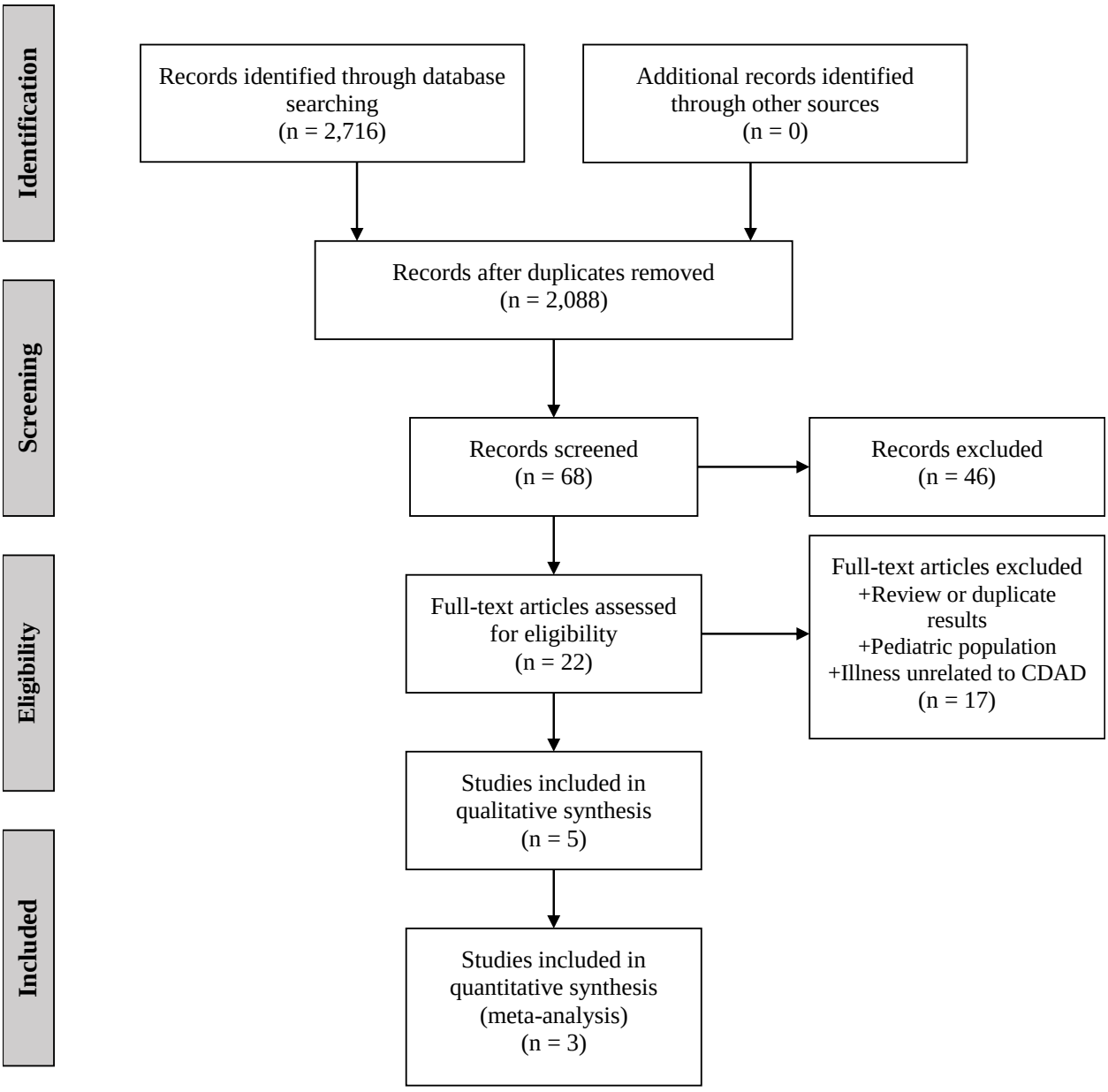

Figure 3.1. PRISMA Flow Diagram. The PRIMSA diagram describes the search and selection process applied during the systematic review.

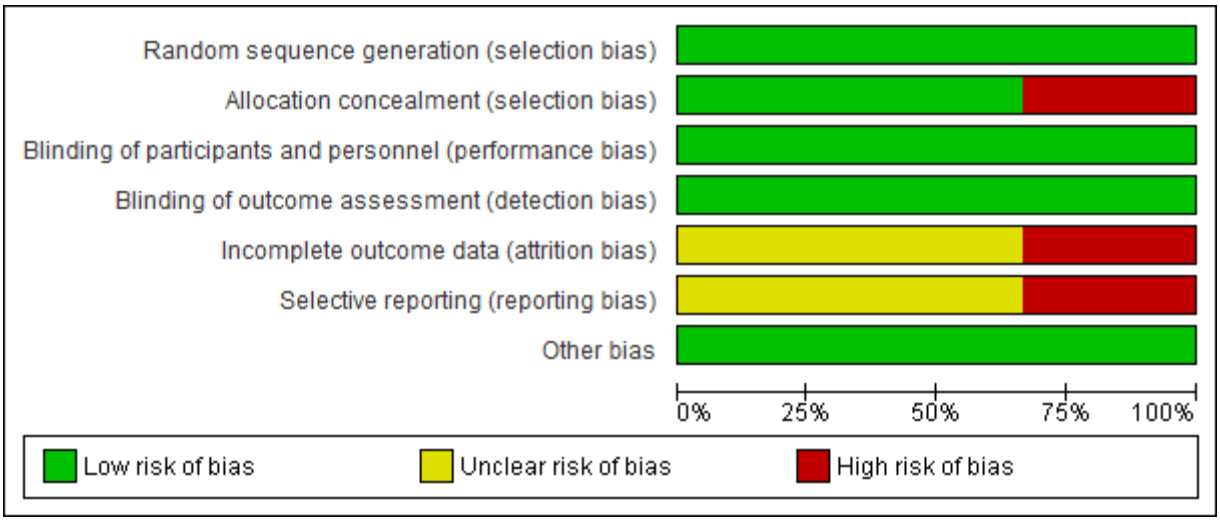

Figure 3.2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included experimental studies. Attrition bias and reporting bias were of particular concern in these studies.

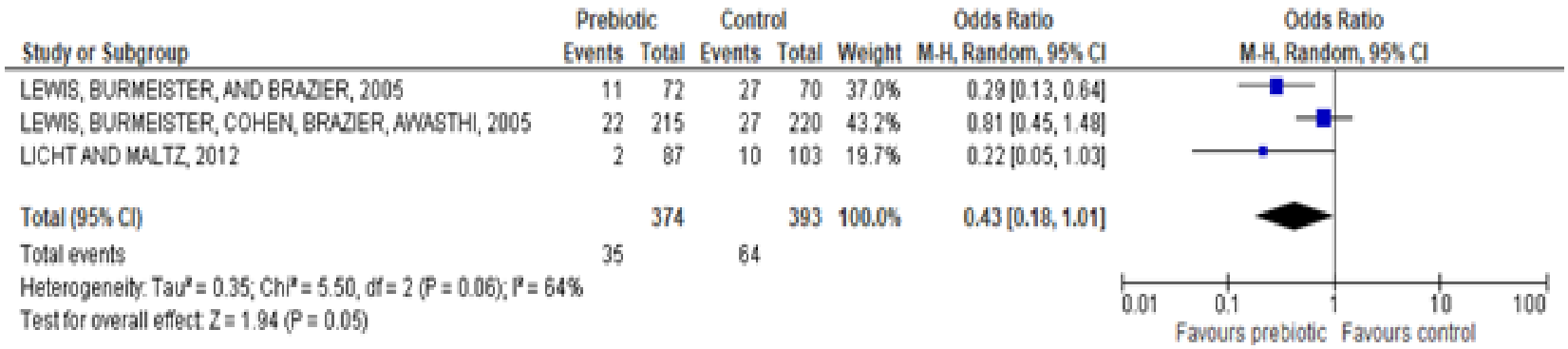

Figure 3.3. Forest plot of the three studies for which specific Clostridium difficile-associated disease (CDAD) outcome measures were included. Studies were heterogenous, as evidenced by the I2=64%, which describes the percentage of variation across studies that is due to heterogeneity rather than chance. The random effects model indicates that the odds ratio (OR) for prebiotic intervention is 0.43 with a 95% CI between 0.18 and 1.01, which is not statistically significant.

Table 3.1. Summary of studies included in the systematic review

Study	Patients (N, location)	Treatment Group				Control Group				Outcome(s)
		Participants (n)	Age Mean (range)	Gender (M/F)	Prebiotic	Participants (n)	Age Mean (range)	Gender (M/F)	Placebo	
Licht and Maltz, 2012	190, Large U.S. academic medical center	87	67 (-)	(46/41)	Lactulose (administered with antibiotics)	103	60 (-)	(50/53)	No placebo, only antibiotics	Incidence of *C. difficile* infection
Spielholz, 2011	120, 17 nursing homes	120	80 (40-96)	(43/77)	Synbiotic tablet- FOS (500 mg) + *S. boulardii* (7.5 billion cfu), *B. coagulans* (1 billion cfu). Administered b.i.d. after beginning antibiotic treatment and for 2 weeks following treatment	Control group was not used				1. Incidence of diarrhea 2. *C. difficile* infection 3. Adverse events and side effects
Lewis et al., 2005a	142, 3 UK hospitals	72	76 (65-84)	(31/41)	Oligofructose powder (12 g/day) administered concurrently with antibiotics after diagnosis and for 30 days after cessation of diarrhea	72	74 (63-80)	(28/41)	Sucrose powder (12 g/day) administered concurrently with antibiotics after diagnosis and for 30 days after cessation of diarrhea	1. Treatment failure 2. Relapse of diarrhea following diarrheal cessation 3. Stool culture for *C. difficile* 4. Death
Lewis et al., 2005b	435, 3 UK hospitals	215	76 (70-83)	(101-114)	Oligofructose powder (12 g/day) administered concurrently with antibiotic treatment and continued for 7 days following treatment	220	78 (72-84)	(112/108)	Sucrose powder (12 g/day) administered concurrently with antibiotic treatment and continued for 7 days following treatment	1. Incidence of diarrhea 2. *C. difficile* infection 3. Adverse events and side effects
Madeo et al., 1999	66, 6 inpatient medical elderly wards	FOS 18	FOS 82(-)	FOS (4/14)	Prebiotic granules- FOS (9.1g), fructose (9.1g), apricot powder (2g). Synbiotic granules-FOS (8.5 g), fructose (9.1 g), apricot powder (2 g) + *L. acidophilus*, *B. bifidum* (unspecified quantity). Administered q.d. upon onset of antibiotic treatment and continued for 15 days	18	90 (-)	(3/15)	Placebo granules-Fructose (9.1 g), maltodextrin (9.1 g), apricot powder (1.8 g). Administered q.d. upon onset of antibiotic treatment and continued for 15 days	Incidence of diarrhea
		FOS+ Probiotics 30	FOS+ Probiotics 81(-)	FOS+ Probiotics (11/19)						

Table 3.2. Summary outcomes (efficacy and safety) in patients treated with prebiotics against *C. difficile* colonization (infection)

Study (total treatment duration/study duration)	Treatment: patients with outcome/n (%)			Control: patients with outcome/n (%)		
	Incidence of diarrhea	**CD infection**	**Adverse events**	**Incidence of diarrhea**	**CD infection**	**Adverse events**
Licht and Maltz 2012 (treatment duration not indicated/1 year for patient recruitment)	-	2/87 (2.3)	-	-	10/103 (9.7)	-
Spielholz, 2011 (concurrently with antibiotics+2 weeks/2 month protocol implementation)	6/120 (5)	1/120 (0.8)	No adverse events reported GI side effects 7/120 (5.8)	-		
Lewis et al., 2005a (at diagnosis until 30 days after diarrhea cessation/follow up 30 and 60 days after diarrhea cessation; duration not specifically indicated)	Relapse 6/72 (8.3)	Culture-positive carrier 30 days 20/72 60 days 14/72	Death 9/72 (12.5)	Relapse 24/70 (34.3)	Culture-positive carrier 30 days 14/70 60 days 7/70	Death 10/70 (14.3)
	No resolution 5/72 (6.9)	Relapse 6/72 (8.3)	No difference between oligofructose and placebo in defecatory frequency, abdominal pain, or bloating	No resolution 3/70 (4.3)	Relapse 24/70 (34.3)	No difference between oligofructose and placebo in defecatory frequency, abdominal pain, or bloating
		No resolution 5/72 (6.9)			No resolution 3/70 (4.3)	
Lewis et al., 2005b (upon initiation of antibiotics until 7 days after antibiotic treatment/follow up two weeks after antibiotic treatment; duration not indicated)	56/215 (26.0)	22/215 (10.2)	Death 4/215 (1.9)	60/220 (27.3)	27/220 (12.3)	Death 2/220 (0.9)
Madeo et al., 1999 (15 days/2 months)	FOS 0/18 (0)	-	-	2/18 (11.1)	-	-
	FOS+ probiotics 4/30 (13.3)	-				

Abbreviations: Fructooligosaccharide (FOS), *Clostridium difficile* (CD), gastrointestinal (GI)

Table 3.3. Summary statistics for efficacy and safety outcomes of prebiotics against *C. difficile* infection

Study	Prebiotic vs. Control or placebo					
	Incidence of diarrhea		C. difficile infection		Death	
	OR	NNTB/NNTH	OR	NNTB/NNTH	OR	NNTB/NNTH
Licht and Maltz 2012	-	-	0.22 95% CI 0.05-1.03 P=0.054	NNTB 14	-	-
Lewis et al., 2005a	Relapse 0.17 95% CI 0.7-0.46 P=0.0004	Relapse NNTB 4	Relapse 0.17 95% CI 0.7-0.46 P=0.0004	Relapse NNTB 4	0.86 95% CI 0.33-2.26 P=0.75	NNTB 56
	No resolution 1.67 95% CI 0.38-7.26 P=0.50	No resolution NNTH 38	No resolution 1.67 95% CI 0.38-7.26 P=0.50	No resolution NNTH 38		
Lewis et al., 2005b	0.96 95% CI 0.63-1.47 P=0.84	NNTB 117	0.81 95% CI 0.45-1.48 P=0.50	NNTB 50	2.07 95% CI 0.37-11.4 P=0.41	NNTH 106
Madeo et al., 1999	FOS 0.18 95% CI 0.008-3.99 P=0.28	FOS NNTB 9	-	-	-	-
	FOS+ Probiotics 1.23 95% CI 0.20-7.51 P=0.82	FOS+ Probiotics NNTH 45				

Abbreviations: Number needed to treat (NNTB), number needed to harm (NNTH), and odds ratio (OR)

CHAPTER 4: IDENTIFYING RISK FACTORS AND MALNUTRITION IN PATIENTS AT HIGH-RISK FOR *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DISEASE: 2014 EVALUATION

ABSTRACT

Clostridium difficile-associated disease (CDAD) is a life-threatening illness that is primarily associated with healthcare delivery systems. Infection is precipitated by the disruption of gastrointestinal microbiota, subsequent loss of colonization resistance, and contact with resilient endogenous or exogenous bacterial spores. CDAD selectively affects elderly individuals with antibiotic and healthcare exposure, yet the validity of additional risk factors is debated within the literature.

The primary aim of this research was to evaluate the relationship between malnutrition and CDAD in a high-risk patient group, measure the relative contributions of various predictor variables, and assess patient outcomes. A secondary aim was to develop an efficient CDAD screening process that quickly and accurately identified susceptible patients and informed the preparation of a future interventional trial.

A one-month preliminary study was undertaken to assess CDAD risk factors among adult inpatients at Carle Foundation Hospital (CFH) in Urbana, IL; six risk factors were selected (advanced age, admission from another healthcare facility, recent hospitalization, recent antibiotic use, and a history of diarrhea and CDAD within the previous year), which were positively correlated with CDAD prevalence. The risk factors identified in the preliminary study then were used to stratify records from all adult inpatient admissions throughout 2014 for use in the primary study.

Records from patients with four or more risk factors were classified as high-risk, and were retained to investigate the relationship between malnutrition, CDAD, and outcomes in the primary study. Among high-risk patients, both malnutrition and malnutrition risk were individually associated with elevated CDAD (OR 2.62, 95% CI 1.78 to 3.87; P<0.0001 and OR 1.65, 95% CI 1.22 to 2.23; P=0.001, respectively). However, multiple regression identified only previous CDAD diagnosis, age ≥ 65, a nutrition consultation request, and Body Mass Index (BMI) as significant predictors of CDAD diagnosis. Neither malnutrition nor CDAD were individually associated with increased mortality, but among CDAD patients, those who were also malnourished experienced significantly higher mortality within 30 days of discharge than patients with CDAD alone (OR 5.49, 95% CI 2.50 to 12.04; P<0.0001).

INTRODUCTION

Within the last few decades, the developed world has experienced a profound increase in both the incidence and severity of *Clostridium difficile*-associated disease (CDAD), which accounts for nearly 500,000 infections and 15,000 deaths each year in the United States alone (Lessa et al., 2015). *C. difficile* has outpaced methicillin-resistant *Staphylococcus aureus* (MRSA) as the most common healthcare pathogen, and is currently the leading cause of gastroenteritis-related deaths (Miller et al., 2011). International concerns about antibiotic resistance and the emergence of hypervirulent *C. difficile* strains in the United States, Europe, and Canada, have warranted the reevaluation of CDAD treatment standards. Recent estimates indicate that 30-50% of antibiotics prescribed within hospitals and over 50% of those prescribed by outpatient facilities are either unnecessary or inappropriate. Paradoxically, antibiotic use, which enables opportunistic colonization by *C. difficile*, is the first line of treatment for CDAD. However, this problematic elimination of microbial diversity encourages future pathological

colonization (Pérez-Cobas et al., 2014), and even specific and narrow-spectrum antibiotics may not eradicate *C. difficile* spores. Some studies have suggested that antibiotic treatment is the main cause of CDAD recurrence, a phenomenon observed in approximately one in five successfully treated individuals, an estimated 83,000 first time recurrences each year (Shields et al., 2015). The waning effectiveness of current medications and high recurrence rates among previously treated patients highlight the need for non-antibiotic and prophylactic alternatives.

In recent years, microbiome-based research has provided considerable insight into the complex interactions involving nutrition, infection, and immunity; systematic manipulation of the microbiome through either pharmaceutical or dietary means could have vast implications for human health and represent a safe and invaluable alternative to conventional therapies for the treatment or prevention of CDAD. The connection between malnutrition and disease acquisition is not a new concept. The 1968 publication, *Interactions of Nutrition and Infection,* described numerous studies that demonstrated enhanced susceptibility to intestinal pathogens with nutritional deficiency and elevated morbidity and mortality among malnourished individuals (Scrimshaw, 1968). Additionally, malnutrition has been associated with excessive costs, comorbidities, complications, readmissions, and an extended length of stay (LOS) (Agarwal et al., 2013; Corkins et al., 2014; Correia and Waitzberg, 2003; Lim et al., 2012; Marco et al., 2011). Importantly, increased LOS and readmission among malnourished patients could prolong healthcare exposure and favor the development of healthcare-associated infections (HAI).

To date, the few publications that address nutritional status and CDAD are mostly correlative or preliminary in nature. In addition, the disease has predominantly been studied in medically unremarkable hospital-dwelling populations rather than the specific groups it is known to affect. The purpose of this retrospective review is to determine whether malnutrition affects

CDAD risk, validate well-established CDAD risk factors within our patient population (Cooper et al., 2013), assess patient outcomes, and begin to identify individuals that would most likely benefit from future clinical intervention.

METHODS

Study Design and Patient Sample

Research was conducted at Carle Foundation Hospital (CFH), a 393-bed regional care hospital, in collaboration with the University of Illinois. Prior to the primary study, a preliminary investigation was undertaken to validate risk factor selection among the hospital inpatient population. Upon request, the CFH Biomedical Research Center provided data from all adult inpatient admissions within the month of January, 2013. Each patient was assessed for the presence of six predetermined risk factors (advanced age, admission from another hospital unit or healthcare facility, hospitalization within 90 days of the current hospitalization, a history of diarrhea within one year prior to this hospitalization, and a documented CDAD diagnosis within one year prior to this hospitalization) upon admission and later CDAD acquisition.

The primary study utilized electronic data from all CFH adult inpatient admissions between January 2014 and December 2014. The decision to retrieve data based upon a set duration, rather than sample size, is consistent with medical chart review literature (Worster and Haines, 2004); the timeline was designed to account for the marked regional (Argamany et al., 2015) and seasonal variations (Brown et al., 2013; Gilca et al., 2012) in CDAD prevalence.

To validate the stratification of high-risk and low-risk groups, all inpatient data for individuals 18 years and older were evaluated by the number of risk factors each patient possessed and whether CDAD was acquired. When evaluating further aims, including those related to malnutrition and CDAD, patient outcomes, and CDAD severity, only adult inpatients

with four or more risk factors (patients designated as belonging to the high-risk group) were included. Due to the recurrent nature of CDAD and the potential for patients to incur multiple hospital visits throughout the year, all encounters with patients classified as high-risk during the study period were reviewed. Pediatric patients and outpatients were omitted from the study, but no further exclusions were made based upon primary diagnosis, comorbidity, or nutritional status.

Study variables

Patient files were searched for basic demographic information using the hospital's electronic medical record (EMR) system, as well as variables related to risk assessment, nutritional status, and CDAD acquisition within 30 days of hospital discharge. Malnutrition was identified by the presence of relevant ICD-9 codes, including those for unspecified protein-calorie malnutrition (263.9), cachexia (799.4), sarcopenia (728.2), and less common types of malnutrition (261, 262, 263, 263.1, and 263.8). In addition to malnutrition as a documented diagnosis, BMI, weight, requests for nutrition consultation, and information specific to the parameters set by AND/ASPEN (insufficient energy intake, weight loss, loss of muscle mass, loss of subcutaneous fat, edema, and decreased functional status) were requested (White et al., 2012). Further variables of interest included primary diagnoses, medication history, CDAD recurrence, and potential markers of CDAD severity (leukocyte counts, creatinine levels, the need for emergency GI surgery, death due to CDAD, and 30-day all-cause mortality).

Data retrieval and analysis

All patient data were obtained through RedCap, a secure web application used to build and manage databases and de-identify clinical data. Differences among groups were calculated using T-tests for continuous variables and chi-squared tests and odds ratios (OR) for categorical

variables. Multiple logistic regression was used to assess the relationship between predictor variables and the dichotomous dependent variable using the most parsimonious model to retain appropriate statistical power and minimize potential bias. The limit for statistical significance was set at $P<0.05$.

RESULTS

Preliminary 2013 Study

The preliminary study data provided by the Biomedical Research Center included 10,569 adult inpatient admissions throughout the month of January 2013. Patients were classified as high-risk if they possessed more than half of the selected risk factors (four or more). The high-risk group consisted of 193 people (1.8% of all adult admissions), but represented approximately half of all CDAD patients. The results are shown in **Table 4.1**. A diagnostic evaluation indicated that the proposed screening criteria for patients at high risk of acquiring CDAD exhibited low sensitivity (49.4%) and high specificity (98.9%). These data suggested that the population would be adequate for the proposed year-long primary study, and that the six designated risk factors were appropriate indicators of CDAD risk. A protocol for the primary study was submitted to the CFH Institutional Review Board, and accepted.

Primary 2014 Study

During the primary observation period, a total of 68,251 adult patients were admitted to CFH. As in the preliminary study, records were electronically retrieved and separated into low-risk (66,974) and high-risk (1,277) groups. Patients were ultimately classified by their highest risk factor value across encounters to maintain data uniformity and prevent investigator bias. The selection and continued use of the risk factors was validated among primary study patients, as the prevalence of CDAD diagnosis increased proportionally with CDAD risk (**Figure 4.1**); although

the proportion of high-risk patients to total inpatients remained similar to earlier results from the preliminary study (1.9%), the high-risk designation identified 78.3% of all CDAD patients within the 2014 study period and appeared to predict increased odds of CDAD diagnosis (OR 232, 95% CI 176 to 307; P<0.0001), as well as 30 day all-cause mortality (OR 11.3, 95% CI 9.5 to 13.3; P<0.0001). Results are represented in **Table 4.2.** The sensitivity and specificity of the risk factor screening were 78.3% and 98.5%, respectively.

The high-risk sample, which was utilized for the remaining study aims, contained 1,277 patients and represented 8,279 encounters. The most common risk factor was a history of recent hospitalization (98.9% of patients), followed by a history of recent antibiotic use, and age ≥65. The prevalence of the six selected risk factors within the high-risk study sample is summarized in **Table 4.3**.

Demographic information for patients at high-risk of acquiring CDAD is represented in **Table 4.4**. In general, the group contained more female than male records, but equal gender proportions between the CDAD-positive and CDAD-negative groups. Patients that developed CDAD during the observation period were generally younger (65.1 vs 74.7, P<0.0001), had a lower BMI (26.8 vs 28.1 kg/m^2, P=0.02), and possessed more risk factors than patients without the diagnosis (4.6 vs 4.1, P<0.0001). Similarly, CDAD patients accumulated more hospitalizations than controls (7.7 vs 6.1, P=0.0004).

Within the year, 238 (18.6%) patients within the sample acquired CDAD, and among these cases, 139 (10.9%) experienced CDAD recurrence. As expected, CDAD risk was associated with CDAD incidence and CDAD recurrence (P<0.00001**; Table 4.5**).

Malnutrition, identified by ICD-9 code, was only documented for 133 (10.4%) patients. However, the odds of acquiring CDAD was 2.6 times higher for patients diagnosed with

malnutrition than for those without a malnutrition diagnosis (**Table 4.6**, 95% CI 1.8 to 3.9; P<0.0001). Similarly, according to nutrition consultation requests, CDAD was more prevalent among patients with high perceived-nutrition risk than patients with no perceived risk (OR 1.7, 95% CI 1.2 to 2.2; P=0.001). Malnutrition was also associated with CDAD recurrence, as patients without a malnutrition diagnosis were more likely to be CDAD negative (83.2% vs 65.4%) and less likely to experience recurrence (9.3% vs 24.8%) than their malnourished counterparts (P<0.00001).

Disparities in CDAD acquisition differed across BMI groups with decreased odds among overweight and obese patients (**Table 4.7**). No significant difference was observed between the underweight and normal patient groups. Interestingly, malnourished patients were represented within all BMI groups (24 patients with an underweight BMI, 52 with a normal BMI, 21 with an overweight BMI, and 34 with an obese BMI). Height and weight data were unavailable for the remaining two malnourished patients.

Lastly, to better assess the contributions of each predictor variable, multiple logistic regression analysis was performed. Among high-risk patients, the only independent variables from the univariate analysis that remained significant included a previous CDAD diagnosis, age ≥65, nutrition consultation requests, and BMI (**Table 4.8**). Neither malnutrition nor CDAD were individually associated with increased mortality, but taken together, malnourished CDAD patients had significantly higher mortality within 30 days of discharge (**Table 4.9**).

DISCUSSION

While the results of this study differ significantly from the available literature due to initial stratification by patient risk, both risk factor and prevalence data remained consistent among high-risk patients between the preliminary and primary study. Expectedly, CDAD was far

more prevalent in our high-risk sample (18.6%) than in recent approximations of the national average of 1.4-3.4% for all inpatients (Steiner et al., 2014; Zacharioudakis et al., 2015), but the low cumulative prevalence of CDAD (including patients from both the high and low risk groups) at CFH in 2014 (0.44%) is remarkable, and more difficult to explain. It is possible that CDAD went undiagnosed among low-risk patients, as people who are younger, or become infected without predisposing history, often experience less aggressive symptoms that may not merit hospitalization, let alone physician follow-up. The low prevalence observed in this study may also be a result of tracking individual high-risk patients, rather than reporting separate encounters, which was implemented to prevent overestimating incidence and prevalence due to inevitable treatment failure and recurrence. Furthermore, long-term (or at least repeated) patient assessment was necessary for detecting recurrent cases, and is more consistent with the format of the anticipated clinical intervention this review was meant to inform.

Alternatively, the low detection rate of iatrogenic malnutrition in this patient sample likely reflects a systemic and well-documented problem among healthcare institutions, more than atypical study design. Underdiagnosis of malnutrition has repeatedly been described by others (Barker et al., 2011; Gout et al., 2009) and represents an inability to identify malnourished patients, exacerbated by a lack of communication among healthcare providers and inconsistent reporting (Silver et al., 2018). Malnutrition is estimated to affect between 30-60% of hospitalized adults and elderly (Holst et al., 2013), which greatly exceeds the 10.4% of high-risk CFH patients diagnosed by the appropriate ICD-9 code. Further investigation into comparable data sets from recent years would be necessary to identify possible causes of error or variation.

As a retrospective study, we were unable to account for CDAD-positive patients who did not return to CFH for diagnosis. CDAD is regularly treated in long-term care facilities and

outpatient settings, and for many people, severe GI symptoms may not prompt or necessitate hospitalization. Patients that may have sought care elsewhere were considered CDAD-negative for the purpose of this data set. In addition, the timing of this project coincided with the nationwide transfer to an EMR system in 2014 and could contribute to the high frequency of missing data within this set. Variables that were not addressed in hospital records included insufficient energy intake, handgrip strength as a measure of functional status, and differentiation between muscle and fat mass.

In this study, electronic diagnosis of malnutrition was primarily determined by the presence of ICD-9 code 263.9. This particular code represents unspecified protein-calorie malnutrition, which is the most commonly assigned malnutrition code. Additional codes for cachexia (799.4), sarcopenia (728.2), and other types of malnutrition (261, 262, 263, 263.1, 263.8) were included in the query, but did not identify any additional subjects. Importantly, ICD-9 codes are obtained by physician assessment, and do not include input from designated nutrition professionals, regardless of malnutrition screening results, perceived risk, or whether a nutrition consultation was ordered. ICD-9 codes are known to severely underestimate malnutrition prevalence. A review of 227 cancer patients revealed that malnutrition prevalence estimates varied among sources and assessors. Estimates within the sample ranged from 8.8% based upon BMI and 9.3% based upon physician-assigned ICD-9 codes, to 26% based upon dietitian assessments (Platek et al., 2011). The kappa coefficient revealed a weak agreement between dietician diagnosis and physician ICD-9 codes (kappa=0.28), and fair strength of agreement between BMI and ICD-9 codes (kappa= 0.38). These data suggest that the identification of malnutrition by ICD-9 code is only marginally more reliable than diagnosis by BMI alone.

Obesity has previously been associated with decreased GI microbiota diversity (Kapur and Nagaraja, 2017), which is associated with predisposition to disease. Most research indicates that obesity causes low-level inflammation and metabolic dysfunction (Gregor and Hotamisligil, 2011), but there is some evidence to suggest that obesity could be protective due to the secretion of anti-inflammatory interleukin-1 receptor antagonist (IL-1RA) by leptin (Gabay et al., 2001). In contrast to the negative correlation between BMI and CDAD detected by this study, others have either failed to find an association between obesity and CDAD risk (Ciricillo et al., 2016; Punni et al., 2015), or have reported increased risk (Bishara et al., 2013; Leung et al., 2013; Mulki et al., 2017). Methodological differences regarding case and control matching, regression, inclusion criteria, and disease classification (community-associated CDAD vs healthcare-associated CDAD) likely affected reported outcomes.

In order to address the disparity between observed and expected malnutrition prevalence in the current study, perceived malnutrition risk was inferred by the presence or absence of a nutrition consultation request within each patient file. As nutritional consultations are initiated only by the presence of tube feeding, an affirmative response to patient questions involving recent and significant unintentional weight loss at admission, or by physician request, the high perceived-risk category likely identified most malnourished patients, but also contained a substantial portion of false positives. In a multi-site study of 1,330 participants, the malnutrition screening tool (MST) accurately identified 71% of malnutrition cases while the other 29% were found to be adequately nourished (Phillips and Zechariah, 2016).

The intent to retrospectively evaluate malnutrition using ASPEN/AND criteria was precluded by inconsistencies in electronic data reporting, incomplete patient charts, and otherwise unmeasured or unavailable parameters. Edema and weight loss were the only two

ASPEN/AND criteria reported, and missing weight data prevented the necessary calculation for loss over time. Furthermore, the presence of either edema or weight loss can obscure the detection of the other, especially without clinical context and being able to physically observe the patient.

The findings from both the regression analysis and mortality analysis were unanticipated, as they failed to approximate previous work by other investigators that showed increased LOS and mortality among malnourished patients (Wong et al., 2009), and higher malnutrition and mortality rates, more extensive antibiotic use, and a longer LOS among CDAD patients (Fry et al., 2010; Monge et al., 2013). Although dissimilarities can be attributed to the stratification of the sample and low prevalence of malnutrition diagnoses, the possibility that malnutrition contributes to CDAD risk, but closely models another significant factor or group of significant risk factors cannot be eliminated. The selected model, however, was well-fitted, as indicated by the insignificant Hosmer-Lemeshow statistic, high coefficient of determination (R^2), and low Variance Inflation Factors for all included variables, which suggested that observed proportions did not differ from expected proportions, 70% of the variation in the dependent variable was accounted for by the predictors, and multicollinearity was not a critical detraction from the regression results.

CONCLUSION

Several publications have listed malnutrition as a risk factor for CDAD, yet support for this claim within the primary literature is limited. In this stratified sample of high risk hospitalized adults, there was no significant association between CDAD and malnutrition following regression analysis. Although immediate nutritional interventions for CDAD

prevention may not yet be advisable at this time, further research regarding malnutrition identification and diagnosis, as well as its potential implications for human health, are warranted.

FIGURE AND TABLES

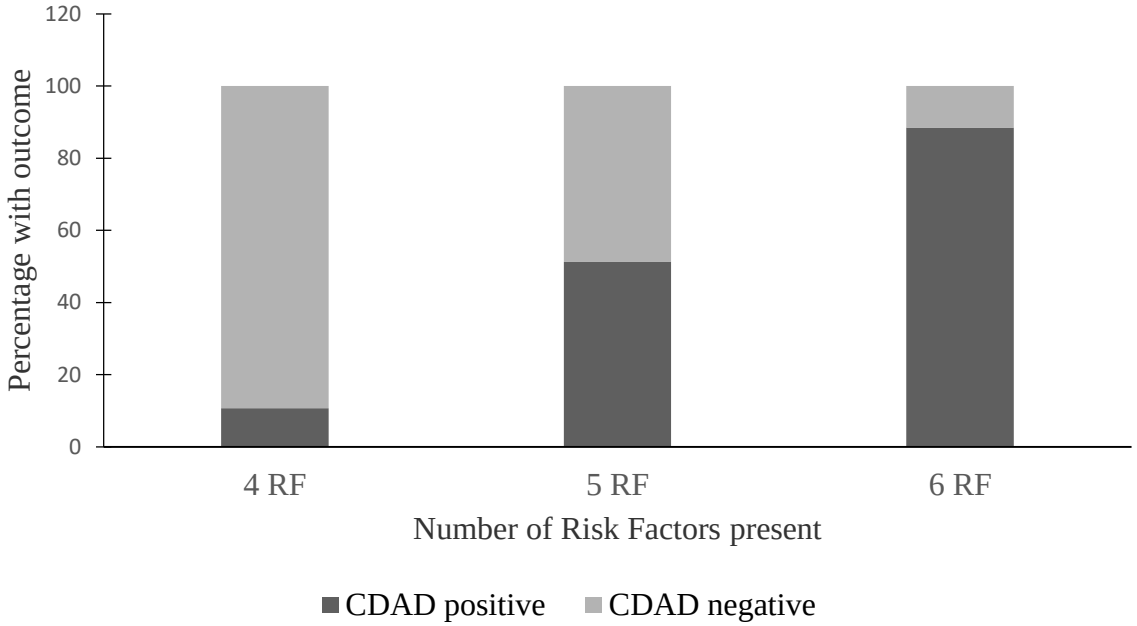

Figure 4.1. Predetermined Risk Factors (RF) predict *Clostridium difficile*-associated disease (CDAD) acquisition among high-risk patients during the 2014 primary study period. Columns represent the percentage of patients with a total of 4, 5, and 6 risk factors who were diagnosed with CDAD during the study period. The percentage of patients with a CDAD diagnosis increased proportionately with risk (x^2 266, $P < 0.00001$).

Table 4.1. Positive association between CDAD risk and CDAD acquisition during preliminary study

Risk Factors	N	CDAD prevalence[1]
0	4,696	0 (0)
1	3,015	8 (0.27)
2	2,039	24 (1.18)
3	626	48 (7.67)
4	156	44 (28.2)
5	32	29 (90.6)
6	5	5 (100.0)

Risk factor prevalence among patients diagnosed with *Clostridium difficile*-associated disease (CDAD) during the preliminary study period (January 2013). The first column represents the total number of risk factors possessed by study subjects. The second and third columns indicate the total number of patients represented by a given risk factor, and the percentage of patients diagnosed with CDAD for each risk factor, respectively. Values within the parenthesis represent the percentage of patients that developed CDAD per risk factor.

1. Prevalence expressed as percentage of patients with outcome.

Table 4.2. CDAD prevalence and patient death with risk factor stratification model.

Risk Stratification	N	CDAD[1]	Deaths[1]
Low-risk	66,974	66 (0.10)	1,024 (1.53)
High-risk	1,277	238 (18.6)	190 (14.9)

Risk stratification of all patients admitted throughout 2014 into low-risk (1-3 risk factors) and high-risk (4-6 risk factors) groups. Patients in the high-risk group had greater odds of being diagnosed with *Clostridium difficile*-associated disease (CDAD) (OR 232.2, 95% CI 175.6 to 307.1; P <0.0001) and death (OR 11.3, 95% CI 9.54 to 13.3; P <0.0001).

1. Prevalence expressed as percentage of patients with outcome.

Table 4.3. Prevalence[1] of *Clostridium difficile*-associated disease (CDAD) risk factors among the primary study sample.

Risk Factors	Total (percentage) of study sample with risk factor
Hospitalized within 90 days[2]	1,263 (98.9)
Antibiotics within 90 days[2]	1,251 (98.0)
Age ≥ 65 yr[†]	1,124 (88.0)
History of diarrhea within previous year[3]	892 (69.9)
Admit from another facility	616 (48.2)
Diagnosis of CDAD within the previous year[3]	298 (23.3)

All risk factors cumulative and specific to patient identifiers.

1. Prevalence expressed as percentage of patients with risk factor.

2. Risk factor was possessed within 90 days before patient entry into the hospital.

3. Risk factor was possessed within one year before patient entry into the hospital.

Table 4.4. Demographic information of primary study participants

Patient Characteristic	CDAD Cases (n=238) [‡]	Controls (n=1,039)
Age (SD)	65.1 (18.7)*	74.7 (11.2)
Sex (%)		
Male	94 (39.5%)	411 (39.6%)
Female	144 (60.5%)	628 (60.4%)
Number of Hospitalizations (SD)	7.74 (6.50)*	6.13 (5.41)
Risk Factor Total (SD)	4.62 (0.66)*	4.10 (0.31)
BMI	26.8 (7.89)*	28.1 (7.51)

*Compared with controls: P<0.05

Abbreviations: *Clostridium difficile*-associated disease (CDAD), Body Mass Index (BMI)

[†] Among the high-risk study sample, advanced age patients were evenly distributed by sex, and comprised 87.9% and 88.0% of the male and female patients, respectively.

[‡] Furthermore, no differences were observed when CDAD-positive patients were separated by age or gender (x^2 1.39, P=0.25).

Table 4.5. Association between *Clostridium difficile*-associated disease (CDAD) risk and CDAD development

Item	CDAD negative[1]	Incident CDAD[2]	Recurrent CDAD[3]	X^2	P value
4 Risk Factors n=1,052	939 (89.3)	60 (5.70)	53 (5.04)	290	<0.00001
5 Risk Factors n=199	97 (48.7)	29 (14.6)	73 (36.7)		
6 Risk Factors n=26	3 (11.5)	10 (38.5)	13 (50.0)		

Each risk factor is expressed by outcomes across each row.
Patients were assigned to a risk group based on their highest total represented throughout the study period.
[1.] CDAD negative: Represents patients who did not develop CDAD within the study period; all values within the column are presented as n (%).
[2.] Incident CDAD: Represents patients who developed a single case of CDAD within the study period; all values within the column are presented as n (%).
[3.] Recurrent CDAD: Represents patients who developed >1 separate case of CDAD within the study period; all values within the column are presented as n (%).

Table 4.6. Results of univariate analysis of potential risk factors.

Variable	Odds Ratio	95% CI	P value
Age ≥65 yr	0.08	0.06 to 0.12	<0.0001
History of diarrhea	3.60	2.40 to 5.39	<0.0001
Previous CDAD diagnosis	114	70.0 to 187	<0.0001
Malnutrition	2.62	1.78 to 3.87	<0.0001
Nutrition consultation request	1.71	1.27 to 2.30	0.0004
Admitted from healthcare facility	0.65	0.49 to 0.86	0.0029
Recent antibiotic use	0.54	0.23 to 1.24	0.14
Recent hospitalization	0.92	0.26 to 3.27	0.89

Among the high-risk patient sample, a history of diarrhea, previous *Clostridium difficile*-associated disease (CDAD) diagnosis, malnutrition, and request for nutrition consultation were associated with increased odds of obtaining a CDAD diagnosis within the current study, while advanced age (≥65 years) and admission from a healthcare facility were associated with decreased odds. Recent antibiotic use and hospitalization did not significantly affect the odds of CDAD diagnosis within the high-risk group.

Table 4.7. CDAD risk varies by BMI classification

Variable	Odds Ratio	95% CI	P value
Underweight vs normal	1.31	0.75 to 2.31	0.35
Overweight vs normal	0.68	0.47 to 0.98	0.04
Obese vs normal	0.65	0.45 to 0.93	0.02

All individual comparisons relative to normal Body Mass Index (BMI); underweight BMI<18.5 kg/m^2; normal BMI 18.5-24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI≥30 kg/m^2. Overweight and obese patients had lower odds of obtaining a *Clostridium difficile*-associated disease (CDAD) diagnosis than patients with a normal BMI; no significant difference between underweight and normal BMI patients was observed.

Table 4.8. Results of Multiple Logistic Regression Model

Variable	Coefficient	SE	OR Estimate	95% CI	Wald x^2	P value
Intercept	-2.22	0.57	-	-	-	-
Nutrition consultation request	0.53	0.26	1.70	1.20-2.83	4.14	0.04
Age ≥65 yr	-0.84	0.29	0.43	0.24-0.80	8.14	0.004
Previous CDAD diagnosis	4.71	0.28	111	64.7-192	289	<0.0001
BMI	-0.04	0.02	0.96	0.94-0.99	5.69	0.02

Significant predictor variables for *Clostridium difficile*-associated disease (CDAD) diagnosis in the current study included previous CDAD diagnosis, a nutrition consult request, advanced age (≥65 years), and Body Mass Index (BMI).

Table 4.9 Mortality within 30 days of patient discharge

Variable	Odds Ratio	95% CI	P value
CDAD vs control	0.90	0.60 to 1.36	0.63
Malnutrition vs control	1.53	0.97 to 2.41	0.07
Malnutrition and CDAD vs CDAD only	5.49	2.50 to 12.0	<0.0001

Neither *Clostridium difficile*-associated disease (CDAD) nor malnutrition were individually associated with mortality, but among patients with CDAD, malnutrition increased the odds of death within 30 days of patient discharge.

CHAPTER 5: ASSESSING RISK FACTORS AND MALNUTRITION IN PATIENTS AT RISK FOR *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DISEASE: 2016 FOLLOW-UP STUDY

ABSTRACT

Clostridium difficile-associated disease (CDAD) is a highly communicable and potentially lethal healthcare infection, and a significant burden on the U.S. healthcare system. A critical aspect of infectious disease management and prevention is the detection of patient risk factors, which can later be incorporated into medical interventions. In this chapter, we will retrospectively evaluate several previously-identified risk factors in a high-risk inpatient sample over the course of a year. As a follow-up to a 2014 CDAD surveillance study, results will be validated using a comparable data set from 2016, and will be assessed for changes over time.

Preliminary data indicate that nutritional status and CDAD development are interrelated, and that supplementing malnourished patients may relieve symptoms and restore resiliency within the GI tract. To test this hypothesis, patient records were stratified into risk-based groups following assessment of the six selected risk factors from our prior study—advanced age, admission from a healthcare facility, recent hospitalization, antibiotic use, a history of diarrhea, and a previous CDAD diagnosis. Patients with four or more risk factors were classified as high-risk and their records were retained for further analysis. As in 2014, numerical patient risk factor totals were directly proportional to CDAD diagnosis in 2016 ($P<0.00001$). The use of the six risk factors to screen for CDAD yielded a sensitivity and specificity of 38.7% and 99.1%, respectively.

In 2016, 84,641 patients were admitted to CFH, which included 447 (0.53%) CDAD patients and 1,240 (1.47%) deaths within 30 days of hospital discharge. The high-risk criteria identified 973 patients, 173 CDAD patients, and 184 deaths within 30 days of hospital discharge. Malnutrition (10.4% vs 14.7%, P=0.002), mortality (14.9% vs 18.9%, P=0.01), and nutrition consultation requests (27.3% vs 47.3%, P<0.00001) increased between 2014 and 2016, but CDAD prevalence remained unchanged among high-risk patients (18.6% vs 17.8%, P=0.60).

The two data sets produced similar results for risk factor prevalence, all demographic variables except for BMI, and all potential predictor variables during univariate analysis, including malnutrition (OR 2.04, 95% CI 1.35 to 3.07; P=0.0007). After regression analysis, only nutrition consultation requests (OR 1.96, 95% CI 1.24 to 3.10; P=0.004) and previous CDAD diagnosis remained significant predictors of CDAD among high-risk patients in 2016 (OR 53.0, 95% CI 32.7 to 85.6; P<0.0001).

The current study validated most of the findings from 2014, but the data did not support the counterintuitively decreased CDAD odds among overweight and obese inpatients. As expected, the selected risk factors predicted outcomes, and could be easily integrated into clinical practice or used to inform future interventions. The regression model indicated that malnutrition was not a significant predictor of CDAD among high-risk patients after accounting for more influential independent variables, such as a previous CDAD diagnosis. Further work is needed to elucidate the nature of the indirect relationship between malnutrition and CDAD and gain greater insights into the determinants of CDAD acquisition.

INTRODUCTION

Within the last twenty years, escalations in morbidity and mortality have primarily been attributed to the development of antibiotic-resistant organisms and hypervirulent *C. difficile*

strains. As such, infection control measures, which include the implementation of national surveillance programs, novel disinfection techniques, and antibiotic stewardship, have primarily fixated on environmental and external contributors to disease.

More recently, the popularization of microbiome research has drawn the focus inward. The importance of host-pathogen interaction is exemplified by a series of unique risk factors that confer susceptibility to CDAD in a manner that is both disproportionate, yet predictable. Research has consistently shown age to be one of the most critical risk factors for CDAD development, as it relates to both bacterial acquisition and CDAD-related mortality (Moshkowitz et al., 2007). Among hospitalized patients aged 65 years and older, interactions between the pathogen and host are often mediated by a combination of polypharmacy, reduced gastric acidity, immune senescence, increased intestinal transit time, and homogeneity of the intestinal microbiota (Asempa and Nicolau, 2017). Collectively, these manifestations of normal ageing can have detrimental health outcomes.

The prevalence of CDAD, which is nearly nine times more common in patients over the age of 65, is expected to rise precipitously as the U.S. population ages (Lessa et al., 2015). By the year 2030, this age group will comprise over 20% of the population, a larger proportion than ever before (Ortman et al., 2014). Elderly patients are not only more susceptible to CDAD, but statistically less resilient. Patients aged 65 years and older account for over 90% of CDAD deaths, and one in nine cases proves fatal within 30 days of diagnosis (Kochanek, 2011).

In addition to CDAD, many other pathologies are known to systematically affect older populations. Malnutrition affects approximately 60% of hospitalized people over age 65 (Mudge et al., 2011), and clinical improvements following nutritional supplementation have been specifically reported in this group (Snider et al., 2015).

METHODS

Study Design and Patient Sample

Research was conducted at Carle Foundation Hospital (CFH), a 393-bed regional care hospital, in collaboration with the University of Illinois. In 2014, an investigation was undertaken to validate risk factor selection among the hospital inpatient population. For the current study, all CFH adult inpatient admissions between January 2016 and December 2016 were assessed for the presence of the six predetermined risk factors from 2014 (advanced age, admission from another hospital unit or healthcare facility, hospitalization within 90 days of the current hospitalization, a history of diarrhea within one year prior to this hospitalization, and a documented CDAD diagnosis within one year prior to this hospitalization) upon admission. The decision to retrieve data based upon a set duration is consistent with medical chart review literature (Worster and Haines, 2004) and the timeline was designed to account for the marked regional (Argamany et al., 2015) and seasonal variations (Brown et al., 2013; Gilca et al., 2012) in CDAD prevalence.

Electronic medical records were first evaluated by the number of risk factors each patient possessed, and were subsequently stratified into high-risk and low-risk groups. Adult inpatients with four or more risk factors were designated as high-risk patients, and all encounters with patients classified as high-risk during the study period were reviewed to account for potential recurrence. Pediatric patients and outpatients were omitted from the study, but no further exclusions were made based upon primary diagnosis, comorbidity, or nutritional status.

Study variables

Patient files were electronically searched for basic demographic information, as well as variables related to risk assessment, nutritional status, CDAD acquisition within 30 days of

hospital discharge, CDAD severity, and 30-day all-cause mortality. Malnutrition was identified by the presence of relevant ICD-10 codes, including those for Kwashiorkor (E40), nutritional Marasmus (E41), marasmic Kwashiorkor (E42), unspecified severe protein-calorie malnutrition (E43), protein-calorie malnutrition of moderate and mild degree (E44), and unspecified protein-calorie malnutrition (E46). In addition to malnutrition as a documented diagnosis, BMI, weight, requests for nutrition consultations, and information specific to the parameters set forth by the AND/ASPEN consensus statement (insufficient energy intake, weight loss, loss of muscle mass, loss of subcutaneous fat, edema, and decreased functional status) were requested (White et al., 2012). Primary diagnoses, medication history, CDAD recurrence, and potential markers of CDAD severity (leukocyte counts, creatinine levels, the need for emergency GI surgery, death due to CDAD, and 30-day all-cause mortality) were collected to evaluate patient outcomes.

Data retrieval and analysis

Data were obtained through RedCap, a secure web application used to build and manage databases and de-identify clinical data. Differences between groups were calculated using T-tests for continuous variables and chi-squared tests and OR for categorical variables. Multiple logistic regression was used to assess the relationship between predictor variables and the dichotomous dependent variable using the most parsimonious model to retain appropriate statistical power and minimize potential bias. The limit for statistical significance was set at $P<0.05$.

RESULTS

2016 Data

CFH admitted 84,641 patients in 2016, but only 973 were at high-risk for acquiring CDAD. Among the high-risk patients, 173 developed CDAD, 143 were diagnosed with malnutrition, and 184 died within 30 days of hospital discharge. In contrast, a lower proportion

of both CDAD prevalence and deaths were observed among the 83,668 low-risk patients admitted within the same year **(Table 5.1)**. Although the high-risk designation represented only 1.2% of all inpatients, it captured nearly 40% of all CDAD patients. The sensitivity and specificity of the risk factor screening were 38.7% and 99.1%, respectively.

The prevalence of the six risk factors used to categorize high-risk patients is summarized in **Table 5.2**. As in 2014, an increasing number of CDAD risk factors was positively correlated with CDAD development in 2016 (P<0.00001). Patients who possessed only four risk factors were predominantly CDAD negative (88.8%), whereas patients who possessed six risk factors were far more likely to obtain a CDAD diagnosis (76.2%) (**Figure 5.1)**.

As in 2014, 2016 assessment of the high-risk demographics revealed that patients who developed CDAD throughout the study period were younger (68.2 vs 75.0 years, P<0.0001), required more hospitalizations (3.92 vs 2.44, P<0.0001), and possessed a higher number of CDAD risk factors (4.57 vs 4.11, P<0.0001) than their CDAD-negative counterparts (**Table 5.3**). However, there was no significant difference in gender (58.4% vs 55.3% female, P=0.41) or BMI between cases and controls (27.9 vs 28.4 kg/m^2, P=0.46).

Univariate analyses of several published risk factors were performed to help elucidate possible determinants of CDAD development in high-risk patients (**Table 5.4**). Previous CDAD diagnosis (OR 52.3, 95% CI 32.5 to 84.0; P<0.0001), a history of diarrhea (OR 2.78, 95% CI 1.91 to 4.03; P<0.0001), a request for nutrition consultation (OR 1.94, 95% CI 1.38 to 2.71; P<0.0001), and malnutrition (OR 2.04, 95% CI 1.35 to 3.07; P=0.0007) increased the odds of CDAD diagnosis, while decreased odds were calculated for advanced age (OR 0.18, 95% CI 0.12 to 0.27; P<0.0001) and admission from a healthcare facility (OR 0.42, 95% CI 0.30 to 0.58; P<0.0001). Neither recent antibiotic use (OR 2.36, 95% CI 0.71 to 7.80; P=0.16) nor recent

hospitalization (OR 1.30, 95% CI 0.29 to 5.87; P=0.73) affected CDAD odds. Following univariate analysis, multiple logistic regression analysis was conducted to explore the contributions of each individual predictor variable; only requests for nutrition consultations and previous CDAD diagnosis produced significant contributions to the model (**Table 5.5**). No interaction between malnutrition and BMI was observed.

2014 and 2016 comparison

A similar proportion of CDAD cases in high-risk patients were documented in 2016 and 2014 (17.8% vs 18.6%, P=0.60), although the 2016 data set contained fewer high-risk patients overall (973 vs 1,277). Nutrition consultations were conducted more frequently (47.3% vs 27.3%, P<0.00001) and a higher proportion of malnourished patients were detected (14.7% vs 10.4%, P=0.002) in 2016 than in 2014. **Figure 5.2** depicts comparisons for CDAD and malnutrition prevalence, as well as mortality, between the two data sets. There was no difference between the two years in the proportion of CDAD-positive patients provided with nutrition consultations (P=0.49) or diagnosed with malnutrition (P=0.24).

As before, the most common risk factors among the high-risk group in 2016 included recent hospitalization, antibiotic use, and advanced age, whereas the rarest risk factor was again a previous CDAD diagnosis. Fewer CDAD patients were successfully identified by risk-factor evaluation in 2016, as only 38.7% of inpatients who developed CDAD were included in the high-risk group, compared to 78.3% in 2014. While the high-risk group contained a similar proportion of CDAD-positive cases between the years, the three-fold increase in CDAD cases among the low-risk group in 2016 severely depreciated the current sensitivity estimate. Importantly, in both years, the high-risk designation appeared to predict both CDAD development and mortality.

All demographic variables between cases and controls between 2014 and 2016 were consistent except for total hospitalizations and BMI. While patients with CDAD were hospitalized more frequently than their uninfected counterparts during both years, fewer hospitalizations were reported in 2016 overall. Additionally, in the 2016 data set, BMI did not differ between patients with CDAD and their uninfected counterparts, which is a critical departure from the lower BMI observed in conjunction with CDAD in 2014 (26.8 CDAD vs 28.1 control; P=0.02). BMI values rose insignificantly for both groups between 2014 and 2016 (27.8 in 2014 vs 28.3 in 2016, P=0.14), and more specifically from 26.8 to 27.9 for CDAD patients (P=0.16) and from 28.1 to 28.4 for controls (P=0.36). Furthermore, the decreased odds of CDAD measured for overweight and obese patients in 2014 were no longer seen in the current study (**Table 5.6**).

The same potential risk factors showed significance in univariate analyses between 2014 and 2016 and produced similar (though not identical) OR values. Regression analysis identified previous CDAD diagnosis and a request for nutrition consultation as significant predictors for both data sets, yet neither BMI nor advanced age were retained in 2016.

In regard to mortality, 2014 and 2016 data varied considerably. All-cause 30-day mortality increased between 2014 and 2016 for high-risk patients (14.9% vs 18.9%, P=0.01), and was associated with malnutrition (OR 1.78, 95% CI 1.18 to 2.67; P=0.006), but not CDAD (OR 0.80, 95% CI 0.51 to 1.24; P=0.3) in 2016. Conversely, neither malnutrition nor CDAD were individually associated with mortality in 2014, yet malnutrition was associated with elevated mortality specifically among CDAD patients; this relationship was absent in the 2016 data set (OR 1.74, 95% CI 0.72 to 4.29; P=0.2).

DISCUSSION

The 2016 follow-up study was designed, in part, due to updates in electronic medical record reporting requirements through the American Recovery and Reinvestment Act, as well as inconsistencies between our 2014 primary study results, preliminary research, and the existing literature. However, the results from the 2014 and 2016 CFH data sets were predominantly uniform. The current data set contained fewer high risk patients than the comparable data set from 2014, despite having more inpatient admissions overall. Risk factor selection was designed to identify patients who would most benefit from future study interventions, and was based on the need to rapidly and reliably identify patients at-risk for CDAD before bacterial exposure could occur. Although the same six predetermined risk factors were used to identify potential CDAD patients, sensitivity of the screening fell from 78.2% in 2014 to 38.7% in 2016.

Interestingly, CDAD prevalence increased among low-risk patients, from 0.10% in 2014 to 0.33% in 2016, but not among patients in the high-risk group. Nevertheless, the total 2016 CDAD prevalence for CFH, including patients from both risk groups, remained low (0.53%) in comparison with the national average of 1.4-3.4% (Steiner et al., 2014; Zacharioudakis et al., 2015).

Although low-risk patients exhibited a higher proportion of CDAD in 2016 than in 2014, fewer deaths occurred than were expected based on the earlier data set. It is possible that increased disease prevalence and slightly decreased mortality reflect a change in CDAD transmission, infectivity, and virulence patterns. Two of the selected factors used to stratify high-risk patients included recent hospitalization and admission from another facility. In the last few years, many studies have reported on the presence of CDAD beyond its canonical hospital (or even long-term care facility) setting as the appearance of CDAD cases in the community have become more widespread. While “healthcare-associated infection” is now generally preferred to

"hospital-associated infection", it should be noted that these terms are not interchangeable, and that many infections classified as community-associated are, in fact, still associated with healthcare exposure. In a 2013 study of community-associated CDAD, 82% of respondents experienced at least one visit to an outpatient healthcare setting such as a doctor's or dentist's office or dialysis clinic within 12 weeks before diagnosis (Chitnis et al., 2013).

In addition to their named transmission environments, differences in community-associated and healthcare-associated CDAD include infectivity, toxigenicity, patient selection, and outcomes. Community-associated CDAD often affects people who are younger and have no recent antibiotic exposure—people who were previously thought to be at low risk for infection. A 2015 surveillance article contrasted the 13.5% first-recurrence rate and 1.3% death rate of community-associated CDAD with the 20.9% first-recurrence rate and 9.3% death rate of healthcare-associated cases, and concluded that community-associated cases are less likely to cause recurrence or death; these outcomes were not entirely mediated by host selection either, as healthcare-associated isolates more commonly produced binary toxin and represented hypervirulent strains (Lessa et al., 2015). One possibility for the results of our 2016 study involves the pervasiveness of community-associated vs healthcare-associated CDAD by risk group. The low mortality despite elevated CDAD prevalence exhibited by patients in the low-risk group may represent less lethal community-associated cases, whereas the high-risk patients would more likely be afflicted with healthcare-associated CDAD due to admission from other facilities and recent hospitalization. This hypothesis is supported by the aforementioned risk factor prevalence and increase in mortality exclusively observed in the high-risk group. Although CDAD was not individually associated with death, the possibility that it is interacting with some other variable (or set of variables) cannot be eliminated.

Another uncertain aspect of our 2016 study is whether the values obtained for nutrition consultation requests and malnutrition diagnoses reflect an actual increase in prevalence, increased malnutrition awareness among staff (which thereby initiated more requests for consultations), or improved compliance and documentation. Records from 2016 proved to be more thorough for certain parameters, and contained fewer missing values than in 2014. For instance, patients for whom no height and weight information were available decreased from nearly 5% of patients in 2014 to approximately 0.5% of patients just two years later. However, as in 2014, it seems unlikely that all malnourished patients within the sample were appropriately diagnosed due to the uncharacteristically low prevalence of malnutrition within the data set. Nutrition literature estimates that one in three patients is malnourished upon hospital admission (Barker et al., 2011), and that nutritional status tends to decline throughout the hospital stay (Somanchi et al., 2011). Statistics for elderly patients are particularly disconcerting. A Canadian tertiary care center reported that 69% of their study sample (with a mean age of 66 years) was malnourished, with 30% classified as "severely malnourished" (Singh et al., 2006). Similarly, a Welsh study from 2013 indicated that 44% of hospitalized patients over the age of 60 years were malnourished, and that nutritional status was associated with mortality, LOS, and discharge destination (Rasheed and Woods, 2013).

Furthermore, significant discrepancies between malnutrition identification and documentation have been reported. Weight loss, appetite, oral intake, and functional status were documented for less than one-third of the malnourished patients in the Canadian tertiary care study (Singh et al., 2006), while earlier research reported that only 96 of 200 (48%) malnourished hospitalized patients had any record that contained nutritional information (McWhirter and Pennington, 1994). There is evidence of improved malnutrition documentation

within the last few years, as well as potential for future progress. A preliminary intervention confirmed that specific malnutrition education for hospital healthcare providers effectively enhanced EMR reporting of malnourished/high-risk patients from 26.7% to 38.3% within three months (Silver et al., 2018). Another collaborative project of 105 institutions showed a significant increase in malnutrition diagnosis from 4.0%-4.9% between 2014 and 2015 (Tobert et al., 2018). These recent statistics are improvements upon 2010 HCUP data, which reported diagnosis among only 3.2% of patients (Corkins et al., 2014), and are consistent with the increased prevalence of malnutrition diagnosis observed between the 2014 and 2016 CFH reviews.

Although the prevalence of malnutrition diagnosis among all CFH inpatients cannot be inferred with current data, 14.7% of the high-risk patient sample was assigned a malnutrition-related ICD-10 code within the EMR. It seems appropriate that the high-risk group contained a greater proportion of diagnoses than estimates from general inpatients, yet the available literature indicates that even this result is likely an underestimate. The extreme disparity between the prevalence of malnutrition among hospitalized patients and prevalence of identified and documented malnutrition diagnoses is a systemic issue, and requires further investigation.

Unlike earlier results, in 2016 BMI was non-significant between cases and controls. BMI increased for both groups between 2014 and 2016, but was 3.4-fold higher for CDAD patients. The proportion of malnourished patients in each BMI group was similar across the two data sets. For both years, overweight and obese patients made up slightly over 60% of the high-risk group, while underweight patients accounted for only approximately 6% of the total. Other studies have reported a range of values for overweight and obesity prevalence among hospitalized patients, from 45% of adult inpatients in a Norwegian study (Følling et al., 2014) to

74.5% in the American Health Cost and Utilization Project Nationwide Inpatient Sample (Akinyemiju et al., 2016). The CDC Behavioral Risk Factor Surveillance System described 26.3% and 27.5% of elderly Americans as obese in 2014 and 2016, respectively.

Increased odds of underweight patients developing CDAD did not reach statistical significance for either year, and the decreased odds of CDAD measured for overweight and obese patients in 2014 were not observed with the 2016 data; importantly, this retrospective study design cannot separate cause from consequence. Some studies have documented improved outcomes among overweight and obese hospital patients, such as decreased mortality and LOS, in conjunction with certain conditions in a phenomenon referred to as "the obesity paradox" (Yamauchi et al., 2014). The association is counterintuitive, as obesity also has been linked to many cardiovascular and metabolic disorders. Additionally, there is some evidence to suggest that obesity-related conditions may become more pronounced with age (Følling et al., 2014).

Most of the potential risk factors identified by univariate analysis were not significant predictors in the logistic regression model. While malnutrition and CDAD were correlated in both the 2014 and 2016 data sets, it was not determined to be an independent predictor of CDAD in either study, and may have been overwhelmed by more influential predictors. Variance Inflation Factors for the regression model were below two for every predictor variable, which indicates that collinearity was within an acceptable range, and did not play a pivotal role in our study. Additional model fit statistics, such as the residual chi-square test and the Hosmer and Lemeshow Test also yielded insignificant values, and suggests that the regression model was appropriate.

As in 2014, there were several limitations to our study, including patients who did not return to CFH for diagnosis or treatment and were, therefore, presumed to be CDAD-negative,

inconsistent and incomplete data reporting, and an inability to determine disease causation through this type of retrospective analysis. The main differences between the 2014 and 2016 data sets were the transition from ICD-9 to ICD-10 coding and inconsistencies regarding the criteria that constitute an individual hospitalization, which dictated the removal of patient hospitalization totals as a parameter from the regression model to avoid undue influence.

Results from both studies indicate that overall CDAD prevalence at CFH was low in comparison with the national average, and that mortality rates, even among high-risk CDAD patients, which approximated 14% in 2014 and 16% in 2016, were well within other hospital estimates of 9%-37% (Walters et al., 2014; Hota et al., 2012). The vast majority of deaths within the high-risk group occurred among elderly patients; patients over 65 years accounted for 91% and 94% of the total high-risk deaths in 2014 and 2016, respectively, which is consistent with existing studies (Kochanek et al., 2011). Although the high-risk group contained a greater proportion of malnutrition diagnoses than studies assessing diagnosis among all hospitalized patients, the percentage of patients identified as malnourished by ICD-10 code in the 2016 study likely represents only half of the actual malnourished patient population (Barker et al., 2011). Underdiagnosis of malnutrition is hardly unique to CFH, but increased identification or documentation of both nutrition consultations and malnutrition diagnoses between 2014 and 2016 are indicative of significant improvement.

CONCLUSION

The selected CDAD risk factors first identified in the preliminary 2013 data and validated by the primary 2014 and 2016 studies could be easily integrated into the EMR to automatically alert hospital staff of high-risk patients before bacterial exposure occurs and effectively help redirect resources toward these patients. Similar patient screening procedures have been

successfully implemented for other lethal and expensive diseases, such as sepsis, and have decreased disease-related fatalities, conserved costs, and improved patient outcomes (Jones et al., 2015). However, due to the uniquely infectious and transmissible nature of *C. difficile*, isolating high-risk patients, enacting contact precautions, and providing careful monitoring would not only protect the selected patients, but the entire hospitalized population.

Although local infectious disease surveillance is vital and can help direct opportunities for future intervention, it cannot delineate cause and effect. Interventional studies designed to ameliorate malnutrition are not immediately warranted for CDAD prevention or treatment, yet are unlikely to cause significant harm. Further research is needed to better understand the nature of the association between malnutrition and CDAD, which appear to be indirectly related among high-risk inpatients.

FIGURES AND TABLES

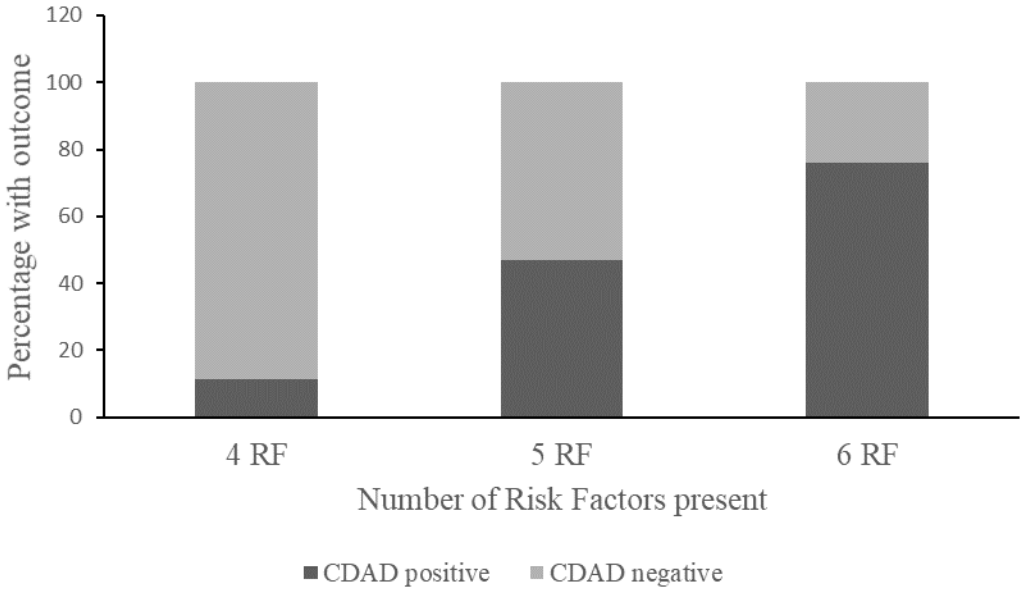

Figure 5.1. Predetermined Risk Factors (RF) predict *Clostridium difficile*-associated disease (CDAD) acquisition among high-risk patients during the 2016 primary study period. Columns represent the percentage of patients with a total of 4, 5, and 6 risk factors who were diagnosed with CDAD during the study period. The percentage of patients with a CDAD diagnosis increased proportionately with risk (x^2=154, p<0.00001).

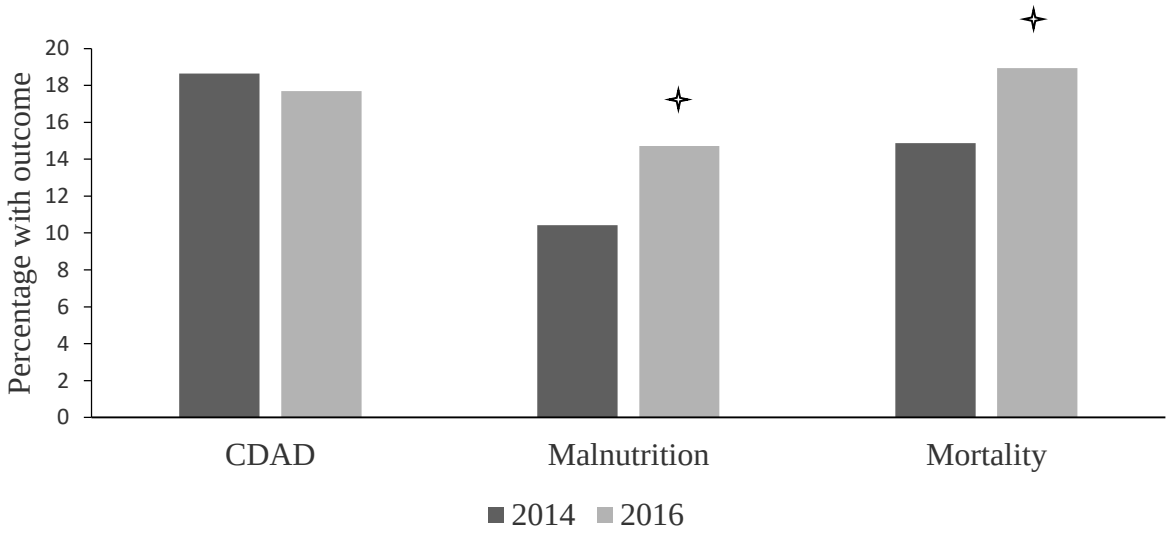

Figure 5.2. No difference was observed in *Clostridium difficile*-associated disease (CDAD) prevalence in high risk patients between 2014 and 2016 (p=0.57), but malnutrition (p=0.002) and death (p=0.01) increased in 2016.

Table 5.1. CDAD prevalence and patient death with risk factor stratification model.

Risk Stratification	N	CDAD[1]	Deaths[1]
Low-risk	83,668	274 (0.33)	1056 (1.26)
High-risk	973	173 (17.8)	184 (18.9)

Risk stratification of all patients into low risk (1-3 risk factors) and high risk (4-6 risk factors) groups. Patients in the high-risk group had greater odds of being diagnosed with *Clostridium difficile*-associated disease (CDAD) (OR 65.8, 95% CI 53.7 to 80.6; P<0.0001) or death (OR 18.2, 95% CI 15.4 to 21.7; P<0.0001).

1. Prevalence expressed as percentage of patients with outcome.

Table 5.2. Prevalence[1] of *Clostridium difficile*-associated disease (CDAD) risk factors among high-risk patients

Risk Factors	Total (percentage) of study sample with risk factor
Hospitalized within 90 days[2]	959 (98.6)
Antibiotics within 90 days[2]	938 (96.4)
Age ≥ 65 yr[§]	851 (87.5)
Admit from another facility	613 (63.0)
History of diarrhea within previous year[3]	547 (56.2)
Diagnosis of CDAD within the previous year[3]	221 (22.7)

All risk factors cumulative and specific to patient identifiers.

[1.] Prevalence expressed as percentage of patients with risk factor.

[2.] Risk factor was possessed within 90 days before patient entry into the hospital.

[3.] Risk factor was possessed within one year before patient entry into the hospital.

Table 5.3. Demographic information of high-risk patients

Patient Characteristic	CDAD Cases (n=173)[**]	Controls (n=800)
Age (SD)	68.2 (18.1)*	75.0 (12.1)
Sex (%)		
Male	72 (41.6%)	358 (44.8%)
Female	101 (58.4%)	442 (55.3%)
Number of Hospitalizations (SD)	3.92 (3.21)*	2.44 (1.68)
Risk Factor Total (SD)	4.57 (0.66)*	4.11 (0.33)
BMI	27.9 (8.03)	28.4 (7.84)

*Compared with controls: P<0.05

Abbreviations: *Clostridium difficile*-associated disease (CDAD), Body Mass Index (BMI)

[§] Among the high-risk study sample, advanced age patients were evenly distributed by sex, and comprised 89.8% and 85.6% of the male and female patients, respectively (P=0.53).

[**] Furthermore, no differences were observed when CDAD-positive patients were separated by age or gender (x^2 2.40, P=0.12).

Table 5.4. Results of univariate analysis of potential risk factors.

Variable	Odds Ratio	95% CI	P value
Age ≥65 yr	0.18	0.12 to 0.27	<0.0001
Admitted from healthcare facility	0.42	0.30 to 0.58	<0.0001
Previous CDAD diagnosis	52.3	32.5 to 84.0	<0.0001
History of diarrhea	2.78	1.91 to 4.03	<0.0001
Nutrition consultation request	1.94	1.38 to 2.71	<0.0001
Malnutrition	2.04	1.35 to 3.07	0.0007
Recent antibiotic use	2.36	0.71 to 7.80	0.16
Recent hospitalization	1.30	0.29 to 5.87	0.73

Among the high-risk patient sample, a history of diarrhea, previous *Clostridium difficile*-associated disease (CDAD) diagnosis, malnutrition, and request for nutrition consultation were associated with increased odds of obtaining a CDAD diagnosis within the current study, while advanced age (≥65 years) and admission from a healthcare facility were associated with decreased odds. Recent antibiotic use and hospitalization did not significantly affect the odds of CDAD diagnosis within the high-risk group.

Table 5.5. Results of Multiple Logistic Regression Model

Variable	Coefficient	SE	OR Estimate	95% CI	Wald x^2	P value
Intercept	-3.64	0.24	-	-	-	-
Nutrition consultation request	0.67	0.23	1.96	1.24-3.10	8.19	0.004
Previous CDAD diagnosis	3.97	0.25	53.0	32.7-85.6	262	<0.0001

Significant predictor variables for *Clostridium difficile*-associated disease (CDAD) diagnosis in the current study included previous CDAD diagnosis and a nutrition consult request.

Table 5.6. BMI classification does not affect *Clostridium difficile*-associated disease (CDAD) risk

Variable	Odds Ratio	95% CI	P value
Underweight vs normal	1.78	0.90 to 3.50	0.10
Overweight vs normal	1.38	0.91 to 2.10	0.13
Obese vs normal	0.96	0.63 to 1.47	0.85

All individual comparisons relative to normal Body Mass Index (BMI); underweight BMI<18.5 kg/m^2; normal BMI 18.5-24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI≥30 kg/m^2. All results were non-significant.

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

The interactions between diet, energy metabolism, and the microbiome are extremely complex, and the means by which GI epithelium, pathogens, and the microbiota mediate immunity is not entirely known. Acknowledging the critical nature of the gastrointestinal microbiota, taken together with the compromised nutritional status often observed during disease development and progression, makes the provision of adequate nutritional care in healthcare settings seem not only intuitive, but necessary.

A 2017 article, aptly titled "Kick the Bucket" reads "…the burden of *Clostridium difficile* outpaces goals. Unless innovative approaches are tried, we risk culling elderly, immunosuppressed, and otherwise debilitated populations" (Delaney, 2017). Although this claim may initially seem hyperbolic, it makes an impassioned and convincing argument in favor of readdressing outdated treatment standards, and represents a reality that is bleak, but ultimately valid. The convergence of an ageing U.S. population large enough to precipitate an epidemic, increasing institutional accountability for infections (especially those viewed as preventable), and a lack of progress on national CDAD reduction goals set forth by both HHS (HHS, 2013) and the independent non-profit health-accreditation organization known as The Joint Commission (2018), indicates that CDAD is unlikely to improve without directed change, and that novel prophylactic and treatment strategies are desperately needed.

This dissertation has reviewed the risks and costs associated with standard CDAD treatment, emphasized the importance of prevention due to poorer patient outcomes and recurrent disease, detailed a systematic review of an understudied but likely beneficial prophylactic solution, analyzed CDAD risk factors with a focus on malnutrition, and assessed

CDAD infection patterns and outcomes over time for high-risk hospital patients with the intent to inform future clinical interventions. This research was conducted in four parts. First, an initial review of the clinical and scientific literature was conducted to identify therapies that had shown promise in CDAD patients. From this investigation, a hypothesis about the role of prebiotics was constructed based on a small and heterogenous collection of clinical research regarding prebiotics and CDAD, but more importantly, the efficacy of prebiotics for other digestive diseases, and their history in animal and in vitro research.

To determine whether a clinical study could responsibly be undertaken, the existing clinical research was assembled into a systematic review that centered around the PICO question: "Can prebiotic consumption prevent or reduce the prevalence of CDAD in adults receiving antibiotic treatment?" Since very few articles had evaluated prebiotic administration for CDAD, studies involving CDAD and synbiotics were also included, with the rationale that they contained prebiotics. Ultimately, it was concluded that most of the studies suggested prebiotic safety and efficacy, but current research could not provide the necessary evidence to make a valid recommendation. Although conducting a meta-analysis on three studies seemed like a scientific rarity, it revealed valid and critical information. The fact that only three of the five available studies included the desired outcome variable highlighted all of the heterogeneity and design flaws within the research itself. However, due to heterogeneity, a random effects model was deemed to be appropriate, as it was unreasonable to expect that each of these studies produced a uniform effect. The results of the random effects model included an OR of 0.43, a broad 95% confidence interval of 0.15 to 1.01, and the borderline P value of 0.05. Essentially, this finding was non-significant and inconclusive. Future clinical prospective trials in this area are necessary, as they would undoubtedly provide invaluable data on the usefulness of prebiotic

supplementation. Importantly, it would be necessary to intensively evaluate the study design, including sample size, inclusion criteria, selected outcomes, and the handling of missing data prior to the initiation of the trial. The existing literature would be greatly improved by current, well-conducted randomized controlled trials with large sample sizes and a reasonable, rather than excessive, number of outcome measures. In summary, although four studies claimed a prebiotic or synbiotic benefit, only two were statistically verified. The only conclusion that could fairly be asserted was that prebiotic therapy may reduce CDAD, but insufficient and low-quality evidence precludes the objective assessment of clinical benefit.

While designing the 2014 retrospective review, we were working under the hypothesis that the disproportionate distribution of CDAD risk and prevalence would be mirrored by an uneven benefit derived from potential interventions. The chart reviews were designed to help prepare for an interventional study that would exclusively target patients who were likely to acquire CDAD. Prior to conducting the 2014 review, a literature search was performed and a one-month preliminary study was initiated to test the validity of well-known CDAD risk factors at CFH; due to the ubiquitousness of *C. difficile* spores in healthcare settings and because CDAD can be acquired at any time (although illness may not manifest for up to two days), it was imperative that study participants be recruited and begin the intervention within 48 hours of admission. Risk factors were selected partially due to their ability to be provided at the time of admission since *C. difficile* could be acquired in the time it would take to complete a thorough patient examination or return lab analyses. Results from the preliminary study showed that the selection of six risk factors, including advanced age, admission from a healthcare facility, recent hospitalization, antibiotic use, a history of diarrhea, and a previous CDAD diagnosis, seemed not

only adequate to detect and predict CDAD risk, but would avoid the unnecessary recruitment and treatment of excess patients.

For the 2014 primary study, a full year of records was requested for all patients possessing four or more risk factors to prevent interference by variations in seasonal risk. CDAD diagnoses, primary diagnoses, variables that related to malnutrition and weight, medication history, and likely markers of CDAD severity were delivered through RedCap, and were then analyzed using unpaired t-tests, chi-squared tests, OR with 95% confidence intervals, and multiple logistic regression. The high-risk group consisted of 1,277 inpatients, and included 238 CDAD positive patients, and 133 patients with diagnosed malnutrition. A total count of all low risk patients, including deaths and those with CDAD, was compared with the high-risk group to ensure that risk stratification was managed appropriately. The relative frequency of risk factors among high-risk patients varied from 23.4% to 98.9%. Demographics differed between cases and controls in all measured fields except gender.

An odds ratio was calculated for every potential predictor variable, and all significant terms were considered for inclusion in the final regression model. Among high-risk patients, previous diarrhea, previous CDAD, malnutrition, and requests for nutrition consultation were associated with significantly increased odds of CDAD diagnosis, whereas advanced age and admission from a healthcare facility were associated with decreased odds. Regression analysis identified requests for nutrition consultations, advanced age, previous CDAD, and BMI as significant predictors of CDAD diagnosis. Neither CDAD nor malnutrition were associated with elevated mortality.

As expected, CDAD prevalence was considerably higher in our high-risk patient sample than the national average for all patients, and as others have described, malnutrition was likely

underreported in our study. We were unable to control for patients who did not return to CFH, and inconsistencies in data reporting undermined our ability to detect malnutrition using AND/ASPEN criteria or assess severity beyond 30-day all-cause mortality. We specifically hoped to infer malnutrition diagnoses by calculating specified weight changes over time in conjunction with edema, but were not able to do so due to restrictions in the data set.

This study was unique in that it only employed a high-risk group that consisted of unmatched CDAD patients and controls. Many retrospective studies are performed using large database sourcing, and may not include control patients, or match cases and controls to reduce confounding variables. Another difference between our work and the work of others was an attempt to control for reinfection, relapse, and treatment failure by tracking data for individual patients rather than for individual encounters. We did not gain strong support for our hypothesis that malnutrition is associated with CDAD diagnosis and, notably, our study design was unable to detect causation.

The novel study design, while planned and necessary, became an unintended obstacle during the analysis and interpretation of the 2014 project, as it became increasingly apparent that the newly acquired results had no basis for comparison in the current literature. These uncertainties were only compounded by the possibility that missing observations from the 2014 data set were an arbitrary consequence of recent electronic reporting mandates for acute care facilities, which were widely regarded as vague, unnecessary, and unfavorable at the time. Essentially, the preliminary and primary studies had provided data that were being interpreted in a vacuum; this played a role in the decision to undertake a second retrospective review using a comparable data set, but from a more recent year.

The 2016 chart review was a validation study that examined the same parameters among high-risk patients stratified by the six risk factors derived from the 2014 preliminary study; it also compared study variables between the two years. Identical methods were employed to maintain consistency, although a few notable differences between the data represented in the two sets could not be addressed. Improved data reporting between 2014 and 2016 was expected, although it remains unclear whether this result was intentional, or driven by contemporaneous EMR updates, as we had initially hypothesized.

The 2016 high-risk group contained 973 patients, including 173 CDAD patients, 143 patients with a malnutrition diagnosis (which is definitively different from malnourished patients, as previously mentioned), and 184 deaths. Between 2014 and 2016, results were mostly similar, yielding increased confidence in the results obtained from our previous study. Although CDAD prevalence did not change, we observed increased malnutrition diagnoses, requests for nutrition consultations, and mortality among the high-risk patients. Conversely, low-risk patients exhibited slightly fewer deaths than would be expected based upon 2014 data, yet CDAD was simultaneously raised by three-fold in this group; owing to the increased CDAD prevalence and decreased mortality in the low-risk group, coupled with a contradictory elevation in mortality and equal CDAD prevalence among high-risk patients, it seemed plausible that the low-risk patients may have been afflicted by community-associated CDAD, which exhibits a different infection pattern than healthcare-associated CDAD. The only other notable differences between the two years were a significant difference in BMI between cases and controls, decreased odds of CDAD diagnosis in overweight and obese patients, and inclusion of advanced age and BMI as significant predictors in the regression model in 2014.

One of the most interesting aspects of these studies was the distribution of malnourished patients across all four BMI categories, which directly contradicts the popular belief that malnutrition only afflicts underweight individuals (or that healthcare professionals should be reluctant to diagnose overweight or obese individuals with malnutrition). Weight and BMI cannot serve as adequate indicators of nutritional status or bodily composition on their own; just as underweight individuals can be adequately nourished, overweight and obese individuals can suffer from malnutrition.

The limitations of the validation study include inconsistencies between the methods used to classify "total hospitalizations", which can be visualized by extreme differences between 2014 and 2016 data for this particular variable. Documentation of hospitalization was noted not only upon admission, but also during transfer to other hospital units, and occasionally, upon the initiation of various procedures. It seems that multiple hospitalizations were attributed to what were likely single hospitalizations in 2014, and created artificially inflated totals. While the difference between cases and controls within the same year are likely to represent a true difference, the inexplicable decrease in hospitalizations between 2014 and 2016 is suspect. Another limitation related to the existing EMR system was the inability to access detailed prescribing history (e.g. which conditions the listed medications were prescribed for) and information regarding the resolution of prior illnesses, as these parameters were necessary for accurate CDAD recurrence and comorbidity assessment.

This research has important implications for several audiences, primarily healthcare institutions, medical researchers and educators, and hospitalized patients, long-term care facility residents, and the people who care for them. The results of these studies, taken together with information drawn from other clinical and academic resources, indicate that environmental

surveillance, common-sense risk reduction, and nutrition education may all improve patient outcomes. The main ways in which CDAD is propagated throughout a facility are through environmental contact with spores, contact with infected, but not necessarily symptomatic individuals, and the sharing of contaminated spaces. Since the six risk factors identified by our chart reviews captured a high proportion of subsequent CDAD cases among less than two percent of all patients admitted to the hospital within each year, the introduction of an automated risk factor-based screening process, administered upon admission, could be used to prospectively recognize patients at-risk for CDAD. Positive EMR screening results could be applied pragmatically to redirect resources toward high-risk patients to encourage ancillary hygiene, antibiotic restriction, vigilant oversight, contact precautions, and patient isolation. The allocation of private rooms, appropriate signage, auxiliary nursing and janitorial staff, and attentive care to a marginal number of high-risk inpatients could have an inordinately beneficial impact on morbidity, mortality, operating costs, Medicare reimbursements, and facility infection rates.

In 2008, a pilot study was initiated at Houston Methodist Hospital in response to rampant sepsis-induced mortality and ineffective management (Jones et al., 2015). The program was developed, implemented, and assessed over four years before being expanded by a federal grant in 2012. As screening compliance increased, fewer sepsis-related deaths occurred. The risk-based patient screening procedure for sepsis reduced costs and mortality, and provides a clear precedent for early intervention within the hospital setting.

In addition to the timely detection of high-risk CDAD patients, the current research illustrates the need for systematic and standardized malnutrition screening, assessment, and reporting. Although minor improvements in the prevalence of diagnosis have been noted over time (which were supported by our 2014 and 2016 retrospective study results), increased

identification and documentation will not remedy systemic malnutrition without purposeful education and organized treatment. In the U.S. and Canada, accredited physicians, physician assistants, and nurses are required to complete Continuing Medical Education (CME) or a field-specific equivalent to maintain competency and ensure they remain updated within their field. Nutrition-based CME credits are available online at no cost, and a 2017 study showed that these courses may help account for a lack of nutritional training in medical school, particularly with regard to lifestyle diseases (Hicks and Murano, 2017). A single one-hour online lesson was sufficient to increase physician knowledge of type 2 diabetes, which was a critical improvement, as 50% of the surveyed physicians were unable to define the purpose of Medical Nutrition Therapy and 85% were unfamiliar with the prevalence of diabetes in the U.S. prior to the course. In another study, over 95% of clinicians reported an intention to change their practice by asking more questions, listening more, assessing patients' willingness to change, and adjusting counseling based on a patients' willingness to change after a two-hour workshop (Sargeant et al., 2008). However, time constraints, complacency with current skills, a lack of self-efficacy, and the fear of missing influential opportunities with patients impaired their ability to adopt and continue newly acquired practices. It remains essential that physicians are educated (and, perhaps, continually reeducated) on the recognition of malnutrition and evidence-based nutrition interventions (Tappenden et al., 2013).

Physicians are first confronted with inadequate nutrition education in the early phases of their post-baccalaureate career. In an invited commentary from *JAMA Internal Medicine,* a third-year Harvard medical student compared the 60 hours of cardiology delivered during a single year to the nine hours of nutrition education offered throughout all four years of medical school (Morris, 2014), and claimed that "…a poorly trained physician workforce should be viewed, in

and of itself, as a structural contributor to diet-related disease". The lack of funding and trained and interested professors, as well as the misguided perception that nutrition is insufficiently science-based, contribute to curriculum decisions. Furthermore, archaic and discredited information, such as the use of albumin and prealbumin as the foremost indicators of nutritional status, is still disseminated in nursing and medical schools (Singh et al., 2006; Smith, 2017).

Nutritional knowledge is lacking at both the highest and lowest positions on the healthcare spectrum, and is aggravated by the overwhelming plurality of nutrition screening methods, competing professional organizations and associations, unclear regulatory requirements, and incomplete clinical documentation. The Joint Commission began mandating universal malnutrition-risk screening in 1995 for all patients in acute care facilities within 24 hours of admission (Patel et al., 2014). However, hospitals that do not seek Joint Commission accreditation (including CFH) are not subject to screening compliance, and may not even utilize a standardized and validated screening tool in favor of more subjective methods. At a basic level, the tool selected for screening should be appropriate for the individual in question, and may involve the screening setting (e.g. community, subacute, or acute care) anthropometry, age, functional status, and the presence of comorbidities and psychological diagnoses. Regardless of which tool is ultimately used or whether a facility is accredited, nutrition education is important for all healthcare providers, particularly front line staff and nurses responsible for screenings.

A comprehensive nutrition care model should be adopted and sustained by all healthcare providers to focus on malnutrition identification, diagnosis, and documentation, as well as the implementation of appropriate nutritional interventions, monitoring, and communication with the patient and other providers up to, and following, discharge (Tappenden et al., 2013). Communication among providers includes both initial and intermittent nutrition screenings,

followed by the forwarding of positive results to a dietician, who then performs the nutritional assessment, appropriately transcribes any nutrition-based diagnoses into the EMR and, if applicable, directs nutritional intervention. Implementing a standardized workflow can provide more synchronous, coordinated, and holistic patient care, and in many facilities, advancements in technology have automated this process to enhance efficiency and reliability.

Since it is unreasonable to dismiss a modifiable risk factor that accurately predicts both outcomes and costs, the overall purpose of this work was to build upon previous reports and examine whether nutritional intervention might be warranted for patients with an illness that is historically resistant to treatment. Improving options for CDAD prevention and treatment may ultimately require collaboration by experts from multiple disciplines, such as bioinformatics, bioengineering, surgery, general medicine, gastroenterology, gerontology, nursing, dietetics, social work, environmental sanitation, immunology, and microbiology. Until a solution is discovered, hospitals will remain a potential access point for people who are unable to obtain or wait for primary care; it is in the best interest of healthcare institutions to encourage productive partnerships between physicians and basic scientists, to exercise judicious antibiotic stewardship techniques, prescribe narrow-spectrum antibiotics whenever possible, and exercise extreme caution with patients who require these antibiotics, are elderly, immunosuppressed, or otherwise experience a disruption in their gut microbiota that endangers digestive and immune equilibrium. While some healthcare facilities and employer-sponsors of health insurance have cited expense as a reason for resisting the implementation of nutrition-based wellness programs, they are failing to recognize the tradeoff between the relatively minor cost of improved nutritional education and care, and potentially devastating costs due to unnecessary resource utilization, prolonged illness, and uncontrolled comorbidities (Sacks et al., 2009). Advances in technology

have helped facilitate the dissemination of nutrition education, and have literally paid off for many employers who offer employee health promotion programs.

However, as the avenues for CDAD transmission continually increase beyond hospitals and other healthcare settings, preventive strategies need to evolve in order to provide adequate protection for both canonical and atypical CDAD patients. Approximately 345,000 CDAD cases occur outside of the hospital each year, which underscores the demand for affordable, accessible, and widespread prophylaxis (Lessa et al., 2015). The impact of nutritional status on immunity and gut microbial maintenance suggests that supplementation, particularly with prebiotics or probiotics, may help ensure protection against CDAD, as it has with other enteric pathogens.

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