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Probiotic Use in Trauma Patients: A Pathway to Determining the Standard of Care to Improve Outcomes

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PROBIOTIC USE IN TRAUMA PATIENTS: A PATHWAY TO DETERMINING THE STANDARD OF CARE TO IMPROVE OUTCOMES

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By

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ABSTRACT

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Dissertation supervised by L. Kathleen Sekula

The gut microbiome consists of normally non-pathogenic bacteria, viruses, and fungi. These friendly microbes serve to maintain GI barrier function and integrity, play a role in host nutrient and drug metabolism, immunomodulation, and prevent pathogenic bacteria from colonizing or causing disease. The importance of a healthy microbiome for the overall health of the host is just recently being appreciated within the medical and science communities. Disruption of the microbiome places one at greater risk for illness and infection. Patients who suffer a traumatic injury are among those at highest risk for complications associated with microbial imbalance, or dysbiosis. In addition to the mechanisms of traumatic injury that can impair one's immune function, these patients are subjected to a variety of treatments and therapies that account for a tendency toward dysbiosis which can lead to healthcare-acquired infections. These include treatment with

antibiotics, proton pump inhibitors, and opioids as well as therapies that require invasive procedures and monitoring. One suggested means to restore immune function and for the prevention of HAIs is to supplement patients with probiotics to restore or replenish host microbiota. However, despite promising findings regarding the efficacy of probiotics for prevention and amelioration of certain HAIs, supplementation with probiotics is not without risks.

DEDICATION

I would like to dedicate this work to my family. Without their endless love, support and understanding, none of this could ever have been possible.

To my wonderful husband, Chris – Thank you for: being my biggest cheerleader, your endless encouragement, always listening to my frustrations, and helping me to manage my stress. Without you assuming all the household duties so that I could study and write, I never could have gotten through this. You were my research assistant, proof-reader, and study-buddy. I feel as though your name should be on the diploma next to mine.

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To my mom, Eileen Clites, I know you and dad are smiling down from heaven. I know how much you wanted to see me graduate. I always smiled when I heard you tell your friends that soon I would be "Doctor Vitko…….. although the Clites name should be on her diploma since I'm the one who raised her…." Even though God saw fit to take you before I could finish, I know how proud you are of me.

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Chapter 1

INTRODUCTION

Healthcare-associated infections (HAI) are a costly complication of hospitalization. These infections place a significant burden on the individual and healthcare system in terms of morbidity, mortality, and excess costs. Considered a marker of quality care, HAIs are one of the most common causes of preventable harm in healthcare. According to a study by the Centers for Disease Control (CDC), more than one million HAIs occur each year in the United States healthcare system. Many common HAIs can be prevented through certain measures, and hospitals and clinicians are implementing prevention strategies with some success. However, new and novel approaches to the prevention of HAIs must be explored.

Hospitalized trauma patients are at even greater risk for the development of a HAIs due to the nature of their injury. Trauma sets off a massive inflammatory response that can weaken the patient's immune system and can destroy gastrointestinal commensal bacteria, making them more susceptible to infection. Traditionally, infections or suspected infections are treated with antibiotics, a practice that has contributed to the development of multi-drug resistant organisms. In addition to killing pathogenic bacteria, they also disrupt the gastrointestinal (GI) microbiome, our "normal flora", as well. This added destruction of our gastrointestinal host defenses can contribute to the development of infections.

Commercially available probiotic supplements are living organisms which contain a variety of bacterial and fungal species normally found in a healthy gut. Consumption of these probiotic supplements can replenish depleted or destroyed GI host flora, thus

providing a barrier or restoring the normal immune defense to the host. Although there is sufficient evidence that probiotics are effective for a variety of health ailments, it is a largely understudied topic with respect to consistency in strains and methods and is generally a new area of research in the trauma population.

Since sepsis is a leading cause of late mortality in trauma patients, the ultimate goal is to prevent infections in this high-risk population. Treatment with antibiotics once an infection has developed is associated with many dangers including increased cost, treatment failure, *Clostridium* infections, and the development and contribution of multi-drug resistant organisms. This dissertation research aimed to address the research gap by specifically looking at the impact the administration of probiotic supplements to hospitalized trauma patients has on a variety of outcomes, primarily the development of HAIs.

This document is divided into the following sections:

1. Chapter 2 includes a published manuscript that provides an overview and summary of the current literature and evidence that relates to outcome measures associated with probiotic administration to hospitalized trauma patients.
2. Chapter 3 contains: the dissertation proposal that describes the research project's specific aims, research questions, background and significance, the theoretical framework, significance to nursing, research design and methods, plans for data analysis and interpretation, study limitations and challenges, and strategies to address threats to study validity.
3. Chapter 4 includes 3 manuscripts. First, a manuscript submitted to the American Journal of Nursing which is under revision is included. Next, an article

that has been accepted for publication in the Journal of Emergency and Critical Care Medicine is included. This will publish early 2019. Lastly, a manuscript written for the American Journal of Critical Care that describes the dissertation research study, statistical analyses, and results with discussion is included. This will be submitted upon completion of the doctoral defense.

Chapter 2

During the process of my literature review and search for a guiding theoretical framework, I realized the ethics of using probiotic supplements for trauma patients could be questioned. Despite the lack of clear clinical practice guidelines back by a robust body of literature, research that has been conducted in this area is very promising. However, case reports of adverse events still warrant caution be taken. For that reason, I decided to write a manuscript that discusses not only the research that has been done with probiotic supplementation for trauma patients, but potential ethical dilemmas their use may cause. This manuscript was accepted for publication by the American Journal of Trauma Nursing and appeared in the January, 2017 issue. Copyright permission has been received from the Journal of Trauma Nursing. The article follows.

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Probiotics for Trauma Patients: Should We Be Taking a Precautionary Approach?

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Abstract

The use of probiotics in the hospital setting is largely understudied and highly controversial. Probiotics are living organisms that, when taken internally, can produce an immunomodulating effect and improve the gastrointestinal (GI) mucosal barrier. Although used for centuries by healthy individuals for GI health, their use in the hospital setting is now gaining wide attention for the prevention of infectious complications such as antibiotic-associated diarrhea, *Clostridium difficile* infections, multiple-organ dysfunction syndrome, and ventilator-associated pneumonia. However, current understanding of the efficacy of probiotics in the acute care setting is confounded by the inconsistencies in the literature with regard to the strain of probiotic being studied, optimal dosage, and timing and duration of dosing, which make the formulation of clinical practice guidelines difficult. Although the safety of probiotics has been confirmed when used for the prevention and treatment of certain diseases, practitioners remain hesitant to administer them to their patients, citing the lack of high-quality studies clearly demonstrating efficacy and safety. Infection is a cause of late death in trauma patients, but only recently has research been conducted on the use of probiotics specifically for the

prevention of hospital-acquired infections in trauma patients. In the face of such limited but promising research, is it reasonable to use probiotics for the prevention of infection in hospitalized trauma patients and improve outcomes? Use of the "precautionary principle" may be useful in this instance.

Keywords: Precautionary principle, Probiotics, Trauma patients

The purpose of this research review was to examine the potential benefits of probiotic supplementation for hospitalized trauma patients versus the theoretical harm that can be caused by their use. The broad definition, mechanism of action, potential benefits, and regulation of probiotics are discussed first. Safety such as theoretical adverse effects, case reports of infection linked to probiotic use, and possible high-risk populations are then discussed. Finally, ethical considerations with using probiotic supplements with hospitalized trauma patients are presented.

History of Probiotics Use

Probiotics have been used for centuries, mostly consumed in the form of fermented foods, by healthy people for the prevention and treatment of a variety of ailments. Probiotics are living organisms, the "good bacteria" that normally resides in our gastrointestinal system (GI) ("microbiota") that help maintain gut barrier function and serve as part of our innate and adaptive immune systems. The ingestion of probiotics is thought to restore and replenish host microbiota that may have been depleted from insults such as injury, certain medications, and diet. When there is a depletion of our microbiota, pathogenic bacteria can grow and flourish and cause disease. Probiotic organisms are capable of modifying the intestinal microbiota, thereby influencing physiological and pathological processes of those who ingest them. It was not until the

late 1900s that researchers suggested that "bad" bacteria could be replaced with "good" bacteria in the gut and hence the concept of probiotics was formed (Food and Agricultural Organization of the United Nations [FAO]/World Health Organization [WHO], 2001; Schrezenmeir & de Vrese, 2001). Although the WHO has defined probiotics as living substances that, when ingested in adequate amounts, can provide certain health benefits to the host (FAO/WHO, 2001), there exists no legal definition of just what constitutes a probiotic. The Food and Drug Administration (FDA) classifies them as "live biotherapeutics" (Vaillancourt, 2006), and they are now commercially available to the general public in a variety of forms.

"Probiotics" is a very general term used to describe many different species and strains of healthy microbes. Naturally found in fermented foods such as yogurts, the probiotic species *Lactobacillus* and *Bifidobacterium* are the most commonly found organisms. These, along with other species such as the fungus *Saccharomyces*, are being commercially marketed for consumer use for the prevention of certain illnesses. Within each microbial species, a number of strains also exist. It is thought that certain species and strains have different host benefits, so the benefits of probiotics may be species-, strain-, dose-, and possibly time-specific. Although benefit may be demonstrated with one strain for a particular reason, it does not mean that this same strain can be used for a different ailment. In addition, the beneficial effects of any probiotic may only be realized if it is consumed at regular intervals, in sufficient quantities to deliver live bacteria to the gut, and over a period of time to repopulate the gut (Ross, Desmond, Fitzgerald, & Stanton, 2005).

Regulation of Probiotics

Although many health care clinicians believe that probiotics have certain benefits for their patients, many are reluctant to use them for patients or in clinical trials (Williams, Ha, & Ciorba, 2010). This is not surprising, considering that the current regulatory framework under which probiotic supplements fall may discourage researchers from conducting trials on their therapeutic benefits. The FDA does not provide strict oversight over the use of probiotics, as they usually are considered a supplement. The only FDA oversight for any supplement is that safe manufacturing practices are to be maintained to ensure products are free from contaminants. Manufacturers do not have to prove therapeutic dosage, nor efficacy claims (Vaillancourt, 2006). Probiotics are considered by the FDA to be "generally regarded as safe," although there are case reports in the literature where the development of infection in certain high-risk people has been linked to the use and misuse of probiotic supplements (Hempel et al., 2011; Salminen et al., 2004; Thygesen, Glerup, & Tarp, 2012) .

However, in the strictest sense, besides supplements, probiotics may fall under other FDA categories including foods, biologics, or drugs—depending on their intended use (Vaillancourt, 2006). There is some question in the health care community as to whether probiotic supplements should actually be regulated by another agency, such as the Center for Food Safety and Applied Nutrition (Hoffmann et al., 2013) , or if Investigational New Drug (IND) applications should be submitted to the FDA prior to clinical research with them. In the strictest sense, probiotics are dietary supplements, naturally occurring in food, and were marketed as such prior to 1994 when IND requirements were first instituted. Therefore, some argue that FDA-assigned IND

numbers should not be required (Hoffmann et al., 2013), even though research with probiotics may technically classify them as a drug. However, the FDA defines a drug as "a substance intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease or a substance (other than food) intended to affect the structure or any function of the body" ("Drugs@ FDA Glossary of Terms," 2012) . In this sense, probiotics are a "food," although they are commercially prepared, and would not be classified as a drug, although they are being used for prevention and treatment of illness.

Although most research with probiotics demonstrates effectiveness and safety, there is not an absolute consensus of such in the literature and results may differ even when the same probiotic species and strain have been used. One possible explanation for the varying results of clinical studies regarding the efficacy of probiotic supplements may again lie with the lack of regulatory oversight. Because probiotics are living organisms and not chemical substances, they are dynamic and are vulnerable to problems with viability and degradation. Not only these largely are dependent upon manufacturing, handling, and storage practices but these can also be influenced by the human host (H ill et al., 2014; Ross et al., 2005; Sarkar, 2016). Without strict regulatory oversight, such as is provided with FDA-approved drugs, many products may not possess the properties that were intended when manufactured. Currently, manufacturers are to declare the genre and species of probiotics in their product but not the viable levels of the populations (Sarkar, 2016).

Current Research with Probiotics

Much of the research on probiotic use has been conducted on otherwise healthy individuals. Although much of this research is not scientifically robust, the use of these

"good bacteria" has been linked to improvement in many conditions ranging from GI irregularity and certain environmental allergies to prevention of secondary *Candida* infections. With the growing interest in probiotic supplementation for the benefit of strengthening and altering host immunity, recent research has been conducted in the inpatient setting on acutely ill patients with the goal of preventing infections.

The majority of hospital research that has been conducted with probiotics is with their use for the prevention of antibiotic-associated diarrhea and *Clostridium difficile* (*C. diff*) infections. Much of this research has shown benefit for children and is now gaining momentum in the adult population. The most widely used species were *Lactobacillus* and *Saccharomyces boulardii* (a yeast), although many other species and strains were also used. A 2013 Cochrane Review that examined 23 randomized controlled trials (RCTs) conducted in children and adults found that probiotics are both safe and effective for the prevention of *C. diff* diarrhea (Goldenberg et al., 2013). More recent meta-analyses conducted in 2015 (McFarland, 2015b) and 2016 (Lau & Chamberlain, 2016) again concluded that the administration of probiotics was effective for preventing primary *C. diff* infections associated with antibiotic use. In 2016, Shen et al. also performed a meta-analysis of 18 RCTs that involved 6,129 adult hospitalized participants. There is a clear lack of homogeneity between these studies; 9 different species and strains were used in the various studies. Despite that, however, the authors concluded that not only were the various probiotics effective for the prevention of *C. diff* infections but also there were no reports of probiotic-related sepsis in any of the studies (Shen et al., 2016).

Another area of inpatient probiotics research is for the prevention of ventilator-associated pneumonia (VAP). Most commonly, strains of the *Lactobacillus* species have

been studied in mechanically ventilated patients who are at risk for the development of VAP. A meta-analysis published in 2010 (Siempos, Ntaidou, & Falagas, 2010) found a 39% reduction in the VAP rate with the administration of probiotics. Two other RCTs conducted since that publication (Barraud et al., 2010; Morrow, Kollef, & Casale, 2010) had conflicting results. Although Morrow et al. (2010) found that patients who were treated with probiotics had a lower incidence of VAP, Barraud et al (2010) found no difference between the probiotics and placebo groups. Again, however, results are difficult to combine and generalize due to significant heterogeneity in these studies.

Table 1 ***Synbiotic Forte (Medipharm, Kagerod, Sweden, and Des Moines, IA) Contains Lactobacilli + Bioactive Fibers: *Pediococcus pentosaceus* , *Leuconostoc mesenteroides* , *Lactobacillus paracasei* , and *Lactobacillus plantarum***

Author (Year)	Patient Population	Species	Outcomes Measures	Findings
Falcao de Arruda & de AguilarNascimento (2004)	TBI patients with enteral feedings	*Lactobacillus johnsonii*	Incidence of infection, ICU LOS, days of mechanical ventilation	Decreased infection rate and ICU LOS
Kotzampassi et al. (2006)	Multiple trauma, mechanically ventilated/ ventilator support	Synbiotic 2000 Forte	Infection rate, SIRS, sepsis, mortality, ICU LOS, days of mechanical ventilation	Decreased infection rate, SIRS, sepsis, and mortality
Spindler-Vessel et al. (2007)	Multiple trauma patients	Synbiotic 2000 Forte*	Changes in intestinal permeability, infection rate, mortality, ICU LOS, mechanical ventilation days, occurrence of multiple-organ failure	Fewer infections, lower intestinal permeability
Tan et al. (2011)	TBI patients with enteral feedings	*Bifidobacterium longum* + *Lactobacillus bulgaricus* + *Streptococcus thermophilus*	Infection rate, use of antibiotics, ICU LOS, 28-day mortality rate	Decreased incidence of nosocomial infections and ICU LOS

Note: ICU = intensive care unit; LOS = length of stay; SIRS = systemic inflammatory response syndrome; TBI = traumatic brain injury.

Research with trauma patients is fairly new, so there are very few published studies. Outcome assessment for these studies not only generally focused on overall hospital-acquired infections but also examined secondary outcomes such as VAP, length of stay (both intensive care unit [ICU] and hospital), and mortality rates. Results of these RCTs are summarized in Table 1. Overall, the administration of probiotics to trauma

patients has demonstrated positive outcomes for these patients, although there also is a lack of homogeneity in these studies. It may be reasonable to utilize outcomes of research with acutely and critically ill patients and generalize these findings to trauma patients; however, generalizability is not easily done. Different probiotic species and strains were used; different strengths, timing of administration, onset of first does, and duration of use vary widely among the studies. In addition, in many of the studies, the authors did not provide clear definitions, nor diagnostic criteria for what constitutes a hospital-acquired infection or VAP. Research with probiotics specifically in the trauma patient is certainly warranted. However, risks versus potential benefits must be considered.

Potential Risks Associated with Probiotics Use

Because probiotics have been primarily used by healthy people to improve GI function, their use in acutely ill patients does not come without controversy. The most commonly reported adverse effect associated with their consumption is mild GI effects such as abdominal cramps, flatulence, and nausea. However, more concerning adverse effects also have been sporadically reported. Because probiotics are living organisms, typically composed of bacteria and fungi, they theoretically could stimulate an excessive immune response in certain susceptible individuals or could cause an infectious process if they somehow establish outside of the GI system. These, along with some published case reports of probiotic-associated bacteremia, have led to safety concerns among some health care practitioners. Although the overwhelming majority of studies demonstrate that probiotic supplements are well tolerated and safe for use even in acutely and

critically ill patients, there are case reports that describe systemic infections thought to be linked with probiotic use.

There are more than 30 published case reports of *Saccharomyces* -positive blood cultures in patients receiving this probiotic species (Didari, Solki, Mozaffari, Nikfar, & Abdollahi, 2014; Doron & Snydman, 2015) and at least 8 cases of *Lactobacilli* bacteremia associated with its consumption (Doron & Snydman, 2015). In some of these cases, the patient also had a central venous access device, prompting experts to question if the method of entry into the bloodstream was the hands of the health care worker who was handling the probiotic. Therefore, strict adherence to hand washing and gloving when handling probiotic supplements to prevent this contamination should be strictly observed.

In 2011, Hempel et al. conducted an exhaustive review of the published probiotics literature. Sponsored by the National Institutes of Health and the FDA, this review included 622 studies of six probiotic species (*Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Enterococcus*, *Bacillus*, and *Streptococcus*), which concluded there is no evidence of increased risk to safety with the use of probiotics. However, the authors also stated that published studies do not consistently assess and report on safety of their studies, so researchers cannot assume absolute safety with the use of probiotics in intervention studies (Hempel et al., 2011).

On the basis of case reports, there is a certain population that may be considered "at risk" for safety issues with the administration of probiotic supplements. A systematic review on the safety of probiotics conducted in 2014 (Didari et al., 2014) examined published literature from 1984 through May 2013. It found overwhelming evidence that

suggests probiotics are safe, but certain high-risk populations may incur complications such as systemic infection and even death. These populations include immunocompromised patients and those who are critically ill. Populations that have been suggested by the FDA to be at risk also include those with structural heart disease/mechanical valves and patients with the potential for the translocation of the probiotic across the bowel wall such as the presence of an active bowel leak (Doron & Snydman, 2015). Positive effects, however, have also been found in these high-risk populations that were given probiotic supplementation, so a risk–benefit ratio must be considered before electing to use them in any high risk group.

Ethical Issues/Controversy

In the pursuit of evidence-based practice, there is a certain element of risk that must be assumed when researching new ideas, technologies, and drugs. This risk, however, must be minimalized and every effort should be made to weigh the risk–benefit ratio of pursuing new treatments. “First, do not harm” is an axiom at the root of medical and pharmacological education and is used to serve as a reminder to clinicians that every medical decision carries with it an element of harm (Smith, 2005). The goal in medical research is to use available knowledge to formulate hypotheses that can be used to potentially prevent, treat, or ameliorate illness and disease. Oftentimes, as with any new ideas, benefits must outweigh risks in the absence of true clinical certainness. But does not having enough evidence to support the use of probiotics justify not using them at all? Especially, when the majority of published studies show benefit with minimal risk of harm? It has been questioned whether cost–benefit analysis is appropriate for health regulation and policy making, especially when the assessed value is associated with

improved health and outcomes (Arrow, Cropper, Eads, & Hahn, 1996). Although the "precautionary principle" was developed and is usually applied in the context of environmental protection and global policy making, it may have applicability in health care decision making.

In 1998, the Wingspread Conference was convened to discuss and reach an agreement on the precautionary principle and its application in public health and environmental decision making ("Wingspread Conference on the Precautionary Principle," 1998). Participants in this conference came from a wide variety of disciplines and interest groups including environmental activists, scientists, and scholars from the United States, Europe, and Canada. Prior to this meeting, policies were aimed at allowing new technologies to be implemented on the basis of risk assessments, but many times these technologies or actions were later proven to be harmful. The precautionary principle aimed to put the burden of proof upon the scientists and companies that any activities they pursue were reasonably considered to be "harmless" and that these scientists are to be held accountable if any harm should occur. The principle was intended for the protection of the public and the environment when technologies were implemented or actions were taken that could negatively affect the public. Therefore, the basic element is that in the absence of scientific certainty, the onus of responsibility that chances of harm are minimized falls on the researcher. In other words, when an action can potentially pose a threat to human health, precautionary measures should be undertaken even if cause and effect relationships have not been fully established or are not clearly understood. Therefore, scientists would adopt a precautionary approach when implementing any medical treatment that lacks scientific certainty (Adler, 2002).

Without scientific inquiry, new evidence and new prevention and treatment strategies can never be realized, but proposed treatment should be based on sound hypothetical reasoning. Although absolute scientific certainty rarely can be found, it is clear that more research into the use of probiotics for trauma patients must be done. Therefore, in one interpretation and application of the precautionary principle, the use of probiotics being administered to patients in the acute care setting may fall under these auspices.

Clinical trials that have been conducted to date have overwhelmingly shown reduction in the incidence of hospital-acquired infections among certain patient groups that received probiotics during hospitalization (Barraud et al., 2010; Curtis & Epstein, 2014; Enomoto, Larson, & Martindale, 2013; Gu, Deng, Gong, Jing, & Liu, 2013) . Although several studies over the past decade have mostly demonstrated both efficacy and safety of probiotic supplementation, there is a clear lack of homogeneity in the research and many studies are based on small sample sizes. Therefore, any results whether favorable or unfavorable must be interpreted cautiously. Although it cannot be said that there is an absolute absence of risk associated with the administration of probiotic supplements to acutely ill patients, what medical therapy exists that carries an absolute absence of risk associated with its use? Potential for harm must be recognized and every effort must be made to prevent risk. When administering probiotics to acutely ill trauma patients, doing so should encompass careful consideration as in all high-risk populations. Monitoring parameters for nurses and physicians regarding potential untoward effects associated with probiotic administration, when to halt the use of the probiotics, and when to initiate laboratory testing is warranted.

Caution must be used when making decisions regarding the administration of probiotics to acutely ill patients, especially high-risk populations such as those patients who are critically ill, are severely immunocompromised, have acute pancreatitis, and live with mechanical heart valves or structural heart defects and those with major bowel surgery (Doron & Snydman, 2015; Hempel et al., 2011) . Therefore, although an absolute causal relationship has not been established between the administration of probiotic supplements and adverse effects, it is reasonable to be cautious and consider the utilization of any probiotic through a heuristic process until further evidence is published that would provide better guidance. Reasonable measures such as exclusionary criteria for high-risk populations and strict monitoring parameters and interventions must be undertaken to avoid a serious threat to human health.

Adopting the precautionary principle in its strictest form can stifle discovery. At its extreme, the principle calls for avoiding risk and the elimination of the use of substances that have not been proven to be safe, even if a causal link between the substance and harm has not been proven or lacks sufficient evidence (COMEST, 2005). If scientists focus solely on the risk associated with the administration of probiotics to acutely ill patients, then infection rates could continue to climb. Therefore, the risks that may be associated with the administration of probiotics to hospitalized patients should be weighed against the benefit that they may prevent deadly and costly hospital acquired infections. Currently, there is little evidence to support that probiotics cause harm (Hempel et al, 2011). Infection is a leading cause of late death after trauma; therefore, measures aimed at preventing infection are important to undertake. Hospitalized patients with traumatic injuries are at increased risk for infection, not only due to the nature of

their injuries that cause interruption of skin integrity and impaired host defense mechanisms but also from tissue hypoperfusion as a result of hemorrhage and multiple invasive procedures. In addition to the increased morbidity and mortality associated with infection, there is considerable financial burden to the health care system.

In a study based on the Healthcare Cost and Utilization Project Nationwide Inpatient Sample, Glance, Stone, Mukamel, and Dick (2011) reviewed the records of 155,910 trauma patients who incurred a hospital-acquired infection and found that they experienced longer lengths of hospital stays, increased mortality rates, and increased costs associated with their care. According to various online sources and in-store costs, the daily cost of probiotic supplementation averages approximately $2.00 per patient, whereas the cost to treat a hospital-acquired infection is $9.8 billion each year, averaging $11,000–$45,000 per patient depending on the infection site (Zimlichman et al., 2013).

Summary

There is growing interest in repopulating the human GI tract with "good bacteria" for immune system health. Prevention of infection in hospitalized trauma patients is imperative to improve outcomes, and there is a modest body of evidence to support that probiotic supplementation in this patient population is beneficial to achieve this goal. Because the research into probiotic use is relatively new, and there is a clear lack of homogeneity in published trials, one must cautiously interpret results from pooled estimates of risk where different types of probiotic strains, dosages, durations, etc., have been used to derive findings (McFarland, 2015a). Because researchers cannot clearly conclude that probiotic supplementation in the trauma population should be used to improve outcomes and carry relatively low risk for untoward adverse effects, efforts

should be made to aggressively monitor for and identify risk to human health that could have a causal relationship to probiotic administration. Recognizing the potential benefits of probiotic supplementation to hospitalized trauma patients while protecting those who could potentially be harmed by their administration is in line with the guidelines of the precautionary principle.

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Chapter 3

The Research Proposal

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Probiotic use in trauma patients: A pathway to determining the standard of care to improve outcomes

Introduction

Healthcare-associated infections (HAI) are a costly consequence of hospitalization, with increased financial costs as well as increased morbidity and mortality for the patient. Traditionally, infections or suspected infections are treated with antibiotics, a practice that has contributed to the development of multiple-drug resistant organisms. Antibiotics kill not only pathogenic bacteria but our host microbiome, or normal flora, as well. This destruction of our gastrointestinal (GI) host defenses can contribute to the development of infections.

Even in the absence of antibiotic use, certain patient populations are at risk for the development of HAIs due to the nature of their injury. Trauma patients are one such population. Traumatic injury causes a massive inflammatory response that destroys GI commensal bacteria and weakens host defense mechanisms.

Probiotics are living organisms which are commercially available that contain a variety of bacterial and fungal species normally found in a healthy gut. The on-going theory is that ingestion of probiotics assists in immune function by restoring depleted normal flora. Although there is sufficient evidence that probiotics are effective for a variety of health ailments, it is a largely understudied topic with any homogeneity and is a very new area for research in the trauma population.

Since infection is a leading cause of late mortality in trauma patients, the ultimate goal is to prevent infections in this high-risk population. Treatment with antibiotics once an infection is present is associated with many dangers including increased cost, treatment failure, *Clostridium difficile* (*C. diff*) infections, and the development and contribution of multiple-drug resistant organisms. For these reasons, a "Probiotics

Protocol" was designed and implemented at a 500 bed Level 1 trauma center in south western Pennsylvania whereas all trauma patients meeting inclusion criteria would be given two different probiotic supplements (See Appendix A). All the attending trauma surgeons agreed with ordering probiotics for their patients that met inclusion criteria. The aim was to decrease overall infection rates in the trauma patients in this hospital. The protocol must be ordered by the physician, physician resident, or trauma Nurse Practitioner as it was not included in the trauma electronic order set. At the time of the initiation of the protocol, the hospital was in the midst of changing electronic medical record keeping software, and no new order sets were being incorporated into the old software/system. As a result, clinicians had to complete a paper order sheet which was then faxed to pharmacy and transcribed into the computer.

At this hospital, residents rotate through services and typically are on the trauma service for only one month at a time. As residents rotated through the trauma service, the group exiting was responsible for communicating the use of the protocol and the necessary steps for implementation to the incoming group of residents. Additionally, the trauma attending physicians also were to instruct the incoming residents on this protocol. For various reasons, including those discussed previously, the protocol had not always been followed.

In order for the protocol to be implemented into the trauma admission order set, it needed to go through a series of committees for approval. Initially, the implementation of the protocol was met with considerable resistance from some members of the healthcare team, namely one clinical pharmacist and an infection control physician. Therefore, a meeting was held between those clinicians and members of the trauma team

(which was comprised of 3 trauma attending physicians, a clinical pharmacist, and this researcher) whereas literature was produced that demonstrated efficacy with minimal risk to harm to the patient population. After this input was provided into the probiotic administration protocol, the protocol was taken to the critical care/trauma committee and was approved for implementation. However, the protocol remained as a written protocol whereas the trauma admission orders were electronic.

Another hurdle with implementing the protocol with trauma patients involved computerized physician ordering software for the probiotic supplements. It was discovered after the initial implementation of the protocol date that the probiotics specific for the protocol (*Lactobacillus GG*, Culturelle® and *Saccharomyces boulardii*, Florastor®) were not easily found in the pharmacy database. This necessitated that prescribers search for the particular probiotic formulations in the database which often resulted in incorrect variations and frequency of probiotics being ordered.

It was not until January 2018 that the protocol was incorporated into the electronic trauma admission orders. Data collection for this research concluded in December 2017. After preliminary statistical analyses were run, it was apparent that due to the low numbers of patients who actually received probiotics, statistical significance could not be affirmed. Therefore, an extension of data collection through June 2018 was requested and granted by the IRB at both Duquesne University and Memorial Medical Center. Additionally, data extracted from the former medical record system/software (prior to 2017) was not suitable for statistical analyses due to a multitude of factors surrounding incomplete data collection.

Specific Aims

The purpose of this research study was to identify the impact of probiotics on certain health-related outcomes for hospitalized trauma patients. Specifically, the outcomes of healthcare-associated infections such as the incidence of pneumonia and ventilator-associated pneumonia (as applicable), *C. diff* infections, urinary tract infections, and bloodstream infections/bacteremia, as well as hospital length of stay, mortality, and mechanical ventilation days (as applicable) was measured. Not all trauma patients require mechanical ventilation, however for those who do, preventing ventilator-associated pneumonia is paramount.

Outcome measures:

- Healthcare-associated infections (HAIs)
  - Pneumonia (PNA)
  - Ventilator-associated pneumonia (VAP)
  - *Clostridium difficile* (*C. diff*) infections
  - Urinary tract infections (UTIs)
  - Bloodstream infections/Bacteremia (BSI)
- Probiotic usage
- Hospital length of stay (LOS)
- Days of mechanical ventilation (MV days)
- Mortality
- Antibiotic requirements

Research Question: Does the administration of probiotic strains *Lactobacillus rhamnosus GG* (*LGG,* Culturelle®) and *Saccharomyces boulardii* (*S. boulardii*, Florastor®) to hospitalized trauma patients improve the incidence of infections and their outcomes?

The specific aims of this research are as follows:

1. To determine whether administering the dual probiotic strains *LGG* and *S. boulardii* to trauma patients would reduce the incidence of hospital-acquired infections. Specific infections include: pneumonia, ventilator-associated pneumonia (in mechanically ventilated patients), urinary tract infections, blood stream infections/bacteremia, and *Clostridium difficile* infections.
2. To measure compliance with ordering probiotics (probiotics usage)
3. To determine whether administering the dual probiotic strains *LGG* and *S. boulardii* to trauma patients will affect the hospital length of stay, hospital mortality, and MV days (as applicable).
4. To determine whether antibiotic usage is different between patients receiving probiotics or not.

Goals of this study include:

1. Decrease the incidence of healthcare-associated infections. (Assessed by tracking data retrospectively)
2. Decrease trauma patients' length of stay.
3. Gain a better understanding of the role of probiotics in the acute care setting to improve other outcomes for trauma patients.

Probiotics have been used in other acute care populations with mostly favorable results, therefore the research hypothesis is that administering probiotics to trauma

patients will improve health-related outcomes. The long-term goals of this research are to determine if the use of probiotics for trauma patients will reduce the incidence of infectious complications. Results, coupled with what is already known in the field of probiotic use in the acute care setting, may be used to develop the best evidence-based protocol for trauma patients with regard to the aforementioned outcomes.

Background and Significance

Probiotics are living microorganisms that, when taken internally, can produce an immunomodulating effect and improve gastrointestinal mucosal barrier function through replenishing lost flora. Their use in the hospital setting is gaining worldwide attention for preventing infectious complications and improving outcomes in patients. Infection is a leading cause of late death (death which occurs more than a week after the initial traumatic injury) in trauma patients (Pfeifer, Tarkin, Rocos, & Pape, 2009). In many cases, traumatic injury disrupts not only the mechanical barriers that prevent infection, but the biological defense mechanisms as well (Hietbrink, Koenderman, Rijkers, & Leenen, 2006; Rijkers, 2011), causing immunological dysfunction and hyper-inflammatory responses that can alter immune function. This places trauma patients at an even greater risk for the development of a healthcare-associated infection. Over the past 10 to 15 years, several published studies have focused on the administration of probiotics to hospitalized patients. However, these studies clearly lack homogeneity with respect to species and strain selection, timing of initiation and duration of probiotic administration, and probiotic dosages. Further, only recently has research regarding the use of probiotics specifically for trauma patients been conducted. This proposed research will contribute

to the knowledge that currently exists and focus on the question, "can probiotics prevent infection and improve outcomes in trauma patients?"

It is becoming increasingly clear that probiotics have a beneficial effect on immunity and their clinical use in the hospital setting is gaining worldwide attention. Probiotics are living organisms that, when ingested in certain amounts, can provide certain benefits to humans (FAO/WHO, 2001) such as modulation of immune function (Doron & Gorbach, 2006; Isolauri, Sütas, Kankaanpää, Arvilommi, & Salminen, 2001; Macintyre & Childscymet, 2005; Tan, Zhu, Du, Zhang, & Yin, 2011). This change in immune function produces an immuno-protective effect that can aid in the prevention of infection. Clinical trials that have been conducted to date have overwhelmingly shown reductions in the incidence of hospital-acquired infections among certain patient groups that received probiotics during hospitalization (Barraud, Bollaert, & Gibot, 2013; Curtis & Epstein, 2014; Enomoto, Larson, & Martindale, 2013; W. J. Gu, T. Deng, Y. Z. Gong, R. Jing, & J. C. Liu, 2013), yet some health care providers remain reluctant to use them for their acutely ill patients (Williams, Ha, & Ciorba, 2010), citing the scientific uncertainty that surrounds the use of probiotics due to the lack of sufficient high-quality studies. Cause and effect relationships with probiotics use in the acute care setting have not been fully established and are not completely understood. However, as with any new ideas, benefits must outweigh risks in the absence of true clinical certainness; especially in the case where not having evidence does not justify inaction. The use of probiotics in the hospital setting remains a largely under-studied and highly controversial topic.

Probiotics have been utilized for centuries and have been shown to have a good safety profile, demonstrating effectiveness for a wide variety of ailments. A study

commissioned by the Agency for Health Care Research and Quality (AHRQ) confirms the safety of probiotics and efficacy for prevention and treatment of certain diseases, although the researchers acknowledge that published studies have limited safety reporting (S. Hempel, Newberry, S., Ruelaz, A., Wang, Z., Miles, J.N.V., Suttorp, M.J., Johnsen, B., Shanaman, R., Slusser, W., Fu, N., Smith, A., Roth, E., Polak, J., Motala, A., Perry, T., and Shekelle, P.G., 2011). Probiotics are not considered to be a pharmaceutical formula, but rather "dietary supplements" or "medical food", depending upon their use. Because they not considered to be medications, they are not stringently monitored and controlled by the Federal Drug Administration (FDA). The only FDA oversight for any dietary supplement or medical food calls for safe manufacturing practices and quality control measures to assure these products are made using good manufacturing practices with regards to sanitation, and the identity, purity, quality, strength, and composition are as labeled (Venugopalan, Shriner, & Wong-Beringer, 2010). Manufacturers may make claims on the label such as "supports digestive health", however they do not have to prove therapeutic dosage nor efficacy claims (Vaillancourt, 2006). This is an important consideration when using probiotics; without strict regulatory FDA oversight, labeling and content may differ with considerable variation. This included viability of the supposed living microbes in the container. Shelf life and strain survival are dependent upon many factors. Manufacturing, storage, and handling practices can impact stated dosage and strain viability as listed on the manufacturers' packaging (Huys et al., 2006; Theunissen, Britz, Torriani, & Witthuhn, 2005) which may lead to inaccurate and inconsistent research results. Further, packages may contain unlabeled microbes,

different amounts of live microbes, or may contain pathogenic micro-organisms if proper sanitation is not maintained during the manufacturing process.

Another difficulty lies with the likelihood that certain diseases and illnesses may respond to specific strains of probiotics. Much of the research being conducted uses a variety of species and strains. The diversity of commercially available probiotic products is expanding, as is the variety of strains which are being studied. Lack of homogeneity in species and strains in current research makes it nearly impossible to claim efficacy based on pooled estimates. That is to say, not all strains are effective for every ailment. Further, it appears that some specific benefits of probiotic strains are also species-dependent (S. J. Salminen, Gueimonde, & Isolauri, 2005). There is strong evidence that the efficacy of probiotics is both strain-specific and disease-specific therefore any one probiotic may not fill all needs (L. V. McFarland, Evans, C. T., & Goldstein, E. J. , 2018).

Research suggests that certain probiotics offer promise for prevention of antibiotic-associated diarrhea, *C. diff* infections, multiple organ dysfunction syndrome, and ventilator-associated pneumonia (Alexandre et al., 2014; Bonten, 2010; Friedman, 2012; Johnston BC, 2012; Katz, 2006; Kogan; L. V. McFarland et al., 1994). Current understanding of the efficacy of probiotics in the acute care setting, however, is confounded by the inconsistencies in research with regard to the strain of probiotic being studied, optimal dosage, initiation and duration of dosing. Further, few high-quality studies have been conducted; suboptimal study populations, short duration of studies, differences in timing of initiating probiotic administration, and questionable statistical analysis methods have all been questioned by the research community. To date, two

readily available species of probiotics that are also consumer-popular are most commonly used.

A review of the literature shows the majority of probiotics researchers focus on antibiotic-associated diarrhea (AAD) and *C. diff* prevention and treatment, with the most widely studied strains being *S. boulardii* and *LGG* (L. V. McFarland et al., 1994; Shan et al., 2013; Surawicz et al., 2000; Tung, Dolovich, & Lee, 2009). *S. boulardii* has mostly been studied in adults, whereas the large majority of published trials with *LGG* were conducted on children and infants. All of these studies have demonstrated a favorable safety profile, although there have been a few case reports of *LGG* bacteremia (Boyle, Robins-Browne, & Tang, 2006; Husni, Gordon, Washington, & Longworth, 1997), *LGG* mitral valve endocarditis (Mackay, Taylor, Kibbler, & Hamilton-Miller, 1999), *LGG* liver abscess (Rautio et al., 1999), and *S. boulardii* fungemia complications (Bassetti, Frei, & Zimmerli, 1998; Hennequin et al., 2000; Lherm et al., 2002). However, a decade-long investigation into the increased use of *LGG* in Finland demonstrated there was no change in the reported cases of *Lactobacillus* bacteremia despite the 6-fold increase in usage over that time period (M. K. Salminen et al., 2002). Similarly, a study in Sweden also found no increased incidence of *Lactobacillus* bacteremia over a 6-year period despite the increased usage of a 3-strain Lactobacillus probiotic supplement (Sullivan & Erik Nord, 2006). Although nearly 100 cases of fungemia associated with *S. boulardii* use have been reported in the literature (Vandenplas, Brunser, & Szajewska, 2009), most of these cases have been attributed to GI translocation or through direct bloodstream contamination via a central venous catheter from the contaminated hands of healthcare workers who first handled the probiotic (Hennequin et al., 2000).

Adverse effects such as bacteremia are considered rare, with most cases occurring in immunocompromised patients. For this reason, it is recommended that probiotics not be administered to patients who are severely immunocompromised. It has also been proposed that minor risk factors for the development of systemic disease related to probiotic use include the presence of a central vein catheter, administration of the probiotic through a J tube, an impaired intestinal barrier, and administration in the presence of cardiac valvular disease (Boyle et al., 2006), therefore these factors should also be taken into consideration. The manufacturer of Florastor® in particular, warns that their product should not be administered to acutely ill patients and capsules should not be opened near patients that have a central line (Biocedex Pharma, 2018). Despite this warning, however, *S. boulardii* has been administered in the acute care setting with great frequency. Caution must be taken to avoid the contamination of the healthcare workers hands so as not to contaminate any central venous access device that could cause inadvertent administration of the microbe into a patient's bloodstream. The two probiotic strains to be used in this study are widely commercially available from reputable manufacturers and are stable at room temperature with a relatively long shelf-life.

A recent meta-analysis of randomized controlled trials published between 2004 and 2007 was conducted by Gu and colleagues (2013) to assess the role that probiotics have on the outcomes of trauma patients. Only five published randomized-controlled trials were identified, which furthers the need for more research to be done in this area. In this meta-analysis the primary outcome of interest, the incidence of hospital-acquired infections, was reduced in the probiotics group ($p=0.02$). Secondary outcomes such as length of stay were reduced in 2 trials ($p<0.001$) and the incidence of ventilator-

associated pneumonia was reduced in 3 of the trials (p=0.01). In the 5 trials that were included, only 2 used the same probiotic strain and one trial used a precursor to probiotics ("prebiotic"). Further, the definition of "nosocomial infection" was only made clear in 3 of the 5 studies, also true for the definition of "ventilator-associated pneumonia".

Given the available research findings in hospitalized patients that suggest improved outcomes with probiotic use, the researcher along with physicians in the Department of Trauma and Surgery at this 500 bed Level 1 trauma center in South Western Pennsylvania have developed and implemented a "Probiotics Protocol" for all hospitalized trauma patients meeting inclusion criteria. This protocol was created based on the most current scientific research that has been conducted in trauma and acutely ill patient populations. Consideration was used when making the exclusion criteria for the administration of probiotics; high-risk populations that have been identified in case reports are excluded from this protocol. Inclusion and Exclusion criteria are outlined in the table below.

Inclusion Criteria (ANY of the following):	Exclusion criteria (do NOT start probiotics if ANY criteria met):
○ Antibiotic administration ○ Mechanical ventilation ○ Anticipated LOS > 48 hrs	○ Age <18 ○ Current/recent use Immunosuppressive agents: ○ TNF blockers ○ MABs ○ Chemotherapy ○ Chronic corticosteroids ○ Anti-rheumatic drugs ○ Anti-rejection drugs ○ Cardiac valve surgery ○ HIV/AIDS ○ Active malignancy ○ Pregnancy

Probiotics Protocol: Inclusion and Exclusion Criteria

All patients meeting inclusion criteria were to be given a dual strain of probiotics (*S. boulardii* and *LGG*) starting within 24 hours of admission and continuing until discharge. Both probiotic preparations are available from the pharmacy in capsule form which can be opened and mixed with water for administration via feeding tubes. Compliance with this administration was validated through examination of the electronic medical record system.

Theoretical Framework

Traditional nursing theoretical frameworks cannot fully describe nor encompass the goals of this study. Although the ultimate goal of this research is to prevent infection in hospitalized trauma patients, it involves the administration of a non-FDA-approved supplement that must be ordered by a healthcare provider. In this sense, this study may lend itself to more of a "medical" study rather than a nursing one, even though outside of the hospital setting, probiotic supplements do not require a prescription. Prevention of infection in the hospital setting requires compliance with established protocols by all members of the healthcare team, although these measures often rely heavily on nurses. In this sense, although the probiotic supplement requires a prescriptive order, the goal is to reduce infections which certainly impacts nursing. However, this particular measure does not have a robust body of literature that clearly demonstrates the effectiveness and absence of harm. Considering there are no established clinical practice guidelines for the administration of probiotics to hospitalized, acutely ill patients, an ethical framework surrounding offering "good" and avoiding "harm" seemed most appropriate.

In the pursuit of evidence-based practice, there is a certain element of risk that must be undertaken when researching new ideas, technologies, and drugs. This risk,

however, must be minimized and every effort should be made to weigh the cost/benefit ratio of pursuing new treatments. "First, do no harm" is an axiom at the root of medical and pharmacological education and is used to serve as a reminder to clinicians that every medical decision has an element of harm (Smith, 2005). The goal in medical research is to use available knowledge to formulate hypotheses that can be used to potentially prevent, treat, or ameliorate illness and disease. However, as with any new idea, benefits must outweigh risks in the absence of true clinical certainness; especially in the case where not having enough evidence does not justify inaction.

When considering different ethical theories, the Precautionary Principle was first considered. This principle places the burden of proof upon the healthcare provider that any action taken or activities pursued were reasonably considered to be harmless and would most likely provide benefit in the absence of scientific proof. However, adopting the precautionary principle in its strictest form can stifle discovery. At its extreme, the principle calls for avoiding risk and the elimination of the use of substances that have not been proven to be safe, even if a causal link between the substance and harm has not been proven or lacks sufficient evidence (COMEST, 2005). The first manuscript for this dissertation was written based on that principle. After additional thought and research, a different guiding framework was ultimately chosen. The theory of expected utility is a normative theory of rational choice which can be applicable in medical decision making.

The Expected Utility Theory (EUT) is a theory of how people *should* make decisions and can be useful for medical decision-making when there is a lack of clear evidence of efficacy and risk. Here, the utility of each possible outcome of the action is weighted according to the probability that the action will lead to the intended outcome.

Based on the calculations of EUT, one can choose the action that will yield the highest expected utility. In other words, the probability of how great the benefit and how bad the risk of an action could be is used to guide decision making. In the case of administering probiotics in order to reduce HAIs, the EUT can be used.

Infection is a leading cause of late death in trauma patients, so prevention of infection in trauma patients is paramount. Potential harms (with every effort made to avoid harm) and costs associated with preventative measures clearly are much less than those associated with HAIs themselves. It is only recently that research has been conducted on the use of probiotics specifically for the prevention of hospital-acquired-infections in trauma patients but results are considerably positive. In the face of such limited but promising research, is it reasonable and ethical to use probiotics for the prevention of infection in hospitalized trauma patients to improve outcomes?

Certainly, there is substantial literature to support the overwhelming safety of probiotic supplementation but there is a paucity of case studies that suggest a cause-and-effect relationship between probiotics and septicemia. However, there is growing evidence that probiotics administered to acutely ill patients lead to favorable outcomes. Current literature supports that administering probiotics to acutely ill patients is ethically justified since the probability of the desired outcome (decrease in infection rates) would likely be realized for the greatest number of people.

Although risks associated with the use of probiotics in hospitalized patients has been documented in the literature in the form of case reports, the potential for causing harm (namely, the risk of actually causing an infection) has been minimized by excluding certain high-risk groups from receiving probiotics. However, since this risk still exists,

expected utility theory will also be used to calculate the probability of this potential outcome. Therefore, researchers can weigh the utility of each possible outcome (HAI, infection, antibiotic usage, LOS, mortality, etc.) according to the probability that administering probiotics will lead to each outcome.

Research Design and Methods

Research Design

This study was conducted using a cross sectional design through retrospective medical record review. The medical records of trauma patients admitted since the inception of the probiotics protocol in July 2015 who received the probiotics will constitute the intervention group. The medical records of trauma patients from the year prior to implementation of the probiotic protocol (July 2014 through June 2015) as well as patient who did not receive probiotics while the protocol was in place will comprise the control group. All data will be reviewed retrospectively extracting variables of interest and analyzed using SPSS software (version 25).

Setting, Population, Sampling Procedures, and Variables

The researcher, in conjunction with a group of trauma surgeons at the study hospital are presently implementing a "probiotic protocol": all adult (age >18) trauma patients admitted to the hospital and meeting certain inclusion criteria are administered two strains of probiotics, one containing *Lactobacillus rhamnosus GG* and the other *S. boulardii*. The protocol calls for the same strain, dosage, and frequency to be administered to each patient, allowing for homogeneity of the treatment. The probiotics protocol began in July 2015 and will continue indefinitely. Initially, the study design was to be a cohort one. It was anticipated that trauma patients who received probiotics

under the probiotics protocol will comprise the intervention cohort and trauma patients in the year prior to implementation of the probiotics protocol will serve as the control cohort (anticipated to be July 2014 through June 2015). There will be a group of patients who did not receive the probiotics protocol since the protocol was implemented. Reasons for this were discussed earlier. This group of patients who do not receive the probiotics will be evaluated as a third group for statistical purposes. If this group is not statistically different from patients prior to the inception of the protocol and they also did not receive the probiotics, then this third group will be combined with the control group for further statistical analyses. Due to difficulty obtaining complete data prior to 2017, the study design was changed to a cross-sectional one. Data from 2017 through June 2018 was examined and variables were used for examining associations between probiotic use and the outcomes of interest.

Outcome measures will be compared between the intervention and control groups: frequency of infectious complications (pneumonia, BSI/bacteremia, *C. diff* infection, VAP, and urinary tract infection), hospital length of stay, mortality, and duration of mechanical ventilation (as applicable, since not all patients will receive mechanical ventilation). In an effort to minimize confounding variables, demographics (age, gender, etc.) and Trauma Severity Scores (ISS/AIS) will be reported. Data on co-morbidities that may influence the development of infection will also be collected. This includes:

Asthma or Chronic obstructive pulmonary disease (COPD)

Heart failure (HF)

Alcohol consumption

Drug use

Diabetes Mellitus (DM)

Gastro-esophageal reflux disease (GERD)

High blood pressure (HBP)

Proton pump inhibitor (PPI) or H2 receptor blocker use (H2RB)

Hepatic failure

Renal failure

History of malignancy

Operative procedures performed

Procedures for Data Collection

After appropriate IRB approval, electronic medical records will be accessed, examined, and the demographics and data of interest will be collected. Additionally, descriptive statistics will be run in SPSS looking for outliers. Data will be stored on an encrypted flash drive and kept in a locked, secure location in the researcher's office. It is anticipated that not all patients who met criteria for receiving probiotics under the probiotics protocol actually did. This is due to many factors including resident turnover on the trauma service with lack of communication regarding the institution of the protocol and the hospital migrating to a new computer medical record/charting system. Due to the latter, the probiotics protocol was not uploaded into the "old" system which required that the physicians/resident physicians remember to order the probiotics on the paper order form. Therefore, the researcher anticipates that entire rows of data will be missing completely at random. In this instance, missing data may be handled through Regression Substitution whereas a multiple regression analysis will be used to estimate any missing values based on existing values. Another possibility is with the utilization

of dummy coding. This strategy will use all available information about the missing observations (received probiotics or not).

Plans for Data Analysis

Dependent Variables:

Categorical, dichotomous: HAIs (yes or no including infection site and causative organism), and mortality

Categorical, interval: hospital LOS, days of mechanical ventilation

Independent Variables:

Categorical, dichotomous: Probiotics (yes or no including which probiotics were received and duration of supplementation), gender, antibiotics received (including class, duration, and number of different antibiotics received)

Categorical, ordinal: Age, Injury Severity Score (ISS/AIS)

Data will be stored in Excel and downloaded into SPSS for Windows (version 25) for statistical analyses. Descriptive statistics will be calculated for the entire sample and for individual groups. Group differences will be tested with chi-square, and Fisher's exact test for numeric and categorical variables, respectively. Pearson's correlation test will be used to determine associations among variables. Student's t test will be used to compare the continuous variables between the groups as to whether or not they are homogenous (as assessed by the Levene test). Normality will be assessed using the Kolmogorov-Smirnov test and parametric data will be expressed as means, $\pm$ standard deviations, or medians according to the homogeneity of the samples. $P<0.05$ will be

established as the significance level (type I error). Incidence rates of infection and ventilator-associated pneumonia will be compared using risk ratios (RR) with 95% confidence intervals (CI). Two-tailed power analysis will be done on all end-point variables (infection rate, number of infections per patient, hospital length of stay, and days spent on mechanical ventilation) with the goal being to achieve at least 80% power (type II error). A priori two-tailed power analysis using an effect size of 0.5 and 0.80 power shows that approximately 50-60 patients per group (2 groups) will be needed. When an effect size of 0.3 is used, sample sizes increased to between 140-180, which is not considered feasible given the potential of participants in this hospital setting. It is impossible for the researcher to anticipate the number of patients that will be in each group prior to data collection.

Study Limitations, Potential Problems, Potential Strategies to Address Weaknesses

Retrospective studies may not detect rare instances of a disease or phenomenon. Therefore, the researcher will need to assess the incidence rates of the outcomes being mentioned and calculate a number needed to treat in order to assess the minimum number of patients in the study cohort to detect any changes in incidence levels in infections.

A second disadvantage is with the quality of the data that is obtained. The researcher has no control over deviations from the probiotics protocol. There may be instances such as patients not receiving probiotics at prescribed times, or initiation of administration not occurring within 24 hours over which the research has no control after the fact which can affect the outcomes. There is a great deal of reliance on the record keeping of others. This may have been ameliorated through education of nurses who will be caring for these trauma patients and physicians, residents, and nurse practitioners who

will be ordering the probiotics protocol. Attempts to educate resident physicians and nurse practitioners (who are responsible for most of the orders for trauma patients) were not always successful.

Since the outcomes (hospital-acquired infection, days spent on mechanical ventilation, LOS and mortality) will have already occurred in the study group, selection bias can occur in retrospective studies. This will be controlled for by including all trauma patients. Until the hospital database is accessed, the researcher cannot determine how many trauma patients this will include.

Comparability between exposed (probiotics/study group) and non-exposed (no probiotics/control group) may be difficult to achieve. Every effort will be made by the researcher to control for confounding variables and assessing homogeneity of the subjects through grouping demographics and trauma severity scores within groups.

Another issue that may affect data collection is that the researcher will need to obtain data from two different medical record systems. Migration to the new computer system occurred in October 2016. Some patient data from the previous system has been carried over to the new system, but at this point in time, the researcher does not know how far back this goes.

Strengths

This retrospective study will allow for the calculation of the incidence of multiple outcomes in the study (probiotics) group. Not only will the researcher be able to calculate absolute risk (incidence), for relative risk, risk difference, and attributable proportion (attributable risk %) of hospital-acquired infection, mechanical ventilation days, and length of stay can be calculated as well.

One major strength of this study design is that it is good for studying multiple outcomes. The outcome of hospital-acquired infections will have 4 subgroups (ventilator-associated pneumonia for mechanically ventilated patients, central line associated infections, urinary tract infections, and *Clostridium difficile* infections) as well as the outcome of length of stay and days of mechanical ventilation (as applicable).

Retrospective studies also are relatively inexpensive as the outcome and exposure have already occurred. They are also rather quick to perform, the only time needed is for data collection on existing data sets and time for statistical analyses and interpretation.

Plan for Dissemination of Results

The researcher intends to disseminate findings through peer-reviewed trauma journals and at educational venues and conferences. This study also will be presented at the Keystone Trauma and Research symposium and will be submitted to the Pennsylvania Trauma Society in partial fulfillment of accreditation standards for Level I trauma status for Conemaugh Memorial Medical Center in Johnstown, Pennsylvania.

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Appendix A

Probiotic Administration Protocol

For all at-risk adult trauma patients
(unless any 1 or more exclusion criteria exists)

Inclusion Criteria (ANY of the following):

- Antibiotic administration
- Mechanical ventilation
- Anticipated LOS > 48 hrs

Exclusion criteria (do NOT start probiotics if ANY criteria met):

- Age <18
- Current/recent use Immunosuppressive agents:
  - TNF blockers
  - MABs
  - Chemotherapy
  - Chronic corticosteroids
  - Anti-rheumatic drugs
  - Anti-rejection drugs
- Cardiac valve surgery
- HIV/AIDS
- Active malignancy
- Pregnancy

Probiotics should be started as soon as inclusion criteria met and continued throughout hospitalization:

___ Florastor 250mg po/NGT/CP/PEG/J tube BID

AND

___ Culturelle 1 tablet po/NGT/CP/PEG/J tube once daily

If infection suspected, discontinue both probiotics and order:

Routine sputum culture and Fungal sputum culture
Blood cultures X 2 set
Urine culture

Physician signature: _____
Date/Time:_____

Chapter 4

This chapter contains 3 manuscripts:

1. A manuscript that is currently under revision
2. A manuscript that has been accepted and will publish early in 2019
3. A final manuscript describing the research study, findings, and conclusions.

Chapter 4
Part 1

September 2018

Probiotics: Help or Hype? Can probiotics prevent infection in trauma patients?

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Abstract

There is increasing interest in the use of probiotics in the acute care setting for prevention and treatment of infection. Although several studies with probiotics have been conducted over the past decade that show clinical efficacy and safety, there is a clear lack of homogeneity in these studies with respect to species and strain selection, timing of initiation and duration of probiotic administration, and dosages. Outcomes generally studied include prevention and treatment of *Clostridium difficile* infections, prevention of ventilator-associated pneumonia, and other healthcare-acquired infections. Research in the use of probiotics in the trauma population to prevent infection in increasing. Although the role of probiotic administration in trauma patients remains unclear, published research is quite promising. However, the results of these studies must be interpreted cautiously due to several limitations.

Key words: probiotics, trauma, health-care associated infection

Introduction

As the use of probiotics increases throughout the healthcare system for the prevention of infection, it is of importance that all healthcare clinicians understand the benefits and the risks of using probiotics in acutely ill patients. The purpose of this article is to provide the current state of the research regarding use of probiotics in the acute care setting, particularly in the trauma population, to prevent infection and improve outcomes. Benefits of the use of probiotic supplementation as well limitations to current research will be presented so that healthcare providers can better interpret the evidence that exists both for and against probiotic use in the hospital setting.

The Human Microbiome

What we once referred to as "normal flora" is now being termed the "human microbiome," the intactness or breakdown of which influences our physiology and our susceptibility to disease and illness (Jacobs, Haak, Hugenholtz, & Wiersinga, 2017; Stavrou & Kotzampassi, 2017; Young, 2017). The resident organisms that comprise the microbiome serve as ecological, mechanical, and immune barriers (Fasano & Shea-Donohue, 2005; Fawzy, Genena, & Sewify, 2017). The intestines and the resident microbiota, which are comprised of trillions of living species of bacteria, archea, fungi, and viruses are important parts of the human immune system. The resident microbiota are responsible for maintaining barrier function which serves to keep the non-pathogenic "friendly" bacteria in and diminishes the possibility for pathogenic bacteria to translocate and cause disease (Preidis & Versalovic, 2009). The microbiome also serves as a part of our innate and adaptive immunity. Although the microbiome is present at birth, it is constantly developing and changing with the introduction of things we ingest such as

foods and medications (G. D. Wu et al., 2011; H. Wu, Tremaroli, & Bäckhed, 2015). An individual's microbiota are constantly being shaped by the hosts' genetics, environmental exposures (Jonkers, 2016), diet, host factors, and events or substances, all of which may disrupt this complex host-microbe interaction (Fishman & Thomson, 2015). Disruption of the microbiome of an individual can decrease one's host defenses and cause increased susceptibility to infection and disease.

Trauma and the Microbiome

Traumatic injury is one of those events that can alter our microbiome in many ways. Infection and sepsis is a leading cause of late death in trauma patients (Lenz, Franklin, & Cheadle, 2007; Lord et al., 2014; Morgan, 1992), therefore prevention of infection is paramount in this population. Mechanisms of traumatic injury cause immunological dysfunction and hyper-inflammatory responses that can alter immune function and significantly affect morbidity and mortality (Akrami & Sweeney, 2018; Howard et al., 2017).

The host response to traumatic injury and related tissue damage involves a cascade of inflammatory mediator responses that places these patients at risk for secondary infections and complications of the inflammatory response (Stoecklein, Osuka, & Lederer, 2012). This immune dysfunction can lead to a concept known as "immune paralysis" which increases the risk for the development of sepsis and multiple organ dysfunction which may lead to death (Hietbrink et al., 2006; Lenz et al., 2007). Further, a study performed by Menges and colleagues (1999) found that patients with severe trauma exhibit a marked depression in immunity characterized by an increase in cytotoxic T cells and a decline in helper lymphocytes. This multi-organ dysfunction, a syndrome

known as “MODS”, involves multiple mediators involved with the inflammatory response – most notably pro-inflammatory cytokines (Desborough, 2000; Lenz et al., 2007; Oberbeck, 2006). This non-specific inflammatory and immune response causes the release of mediators that can also disturb normal gastrointestinal flora and impair barrier function, contributing to translocation of intestinal microbiota. The result of this dysfunction is that normally non-pathogenic intestinal GI flora can migrate and become pathogenic in other areas of the body.

The procedures and medications that are utilized in caring for the trauma patient can add further insult to the injurious events caused by trauma. For mechanically ventilated patients and those in the Intensive Care Unit, these further insults can be numerous.

Adding Insult to Injury

The cascade of inflammatory reactions that is set off by a traumatic injury is intended to boost the immune defenses of the host; the greater the extent of the trauma, the greater the degree of inflammation. This inflammatory process, however, can lead to immune dysfunction which serves to actually place the patient at greater risk for the development of secondary infection and complications of the inflammatory process (Lord et al., 2014; Stoecklein et al., 2012). Additionally, treatments that are common practice for traumatically-injured patients can further compromise a patient’s immune defenses.

Hospital-associated infections are leading causes of mortality, morbidity, and increased financial burden (Glance, Stone, Mukamel, & Dick, 2011). Things that we do to our patients in the hospital, from invasive procedures to treatment modalities and even medications, can disrupt not only the mechanical barriers that prevent infection but the

biological defense mechanisms as well (Rijkers, 2011). Patients who survive an initial injury or disease process still may die as a result of the subsequent immune reactions that are induced by the event itself due to immunological dysfunction and hyper-inflammatory responses that can alter immune function.

Invasive Procedures

Invasive procedures can also disturb a person's natural barriers to infection. Central venous access lines are often necessary, as are indwelling foley catheters. These can serve as an entry point for pathogenic bacteria. Additionally, endotracheal intubation can lead to ventilator-associated pneumonia. Trauma patients often undergo surgical procedures, sometimes more than just once.

Stress Ulcer Prophylaxis

Ventilator bundle of care, considered best practice for patients receiving mechanical ventilation, includes the use of peptic ulcer disease prophylaxis which can be in the form of H2 receptor blockers (H2RBs) or proton pump inhibitors (PPIs) (Neuville, Mourvillier, Bouadma, & Timsit, 2017). According to a recent observational multi-center study, PPIs are the most widely used off-label medication in the ICU, with "stress ulcer prophylaxis" being cited as the most common indication for use (Barletta et al., 2015). The composition of human intestinal flora is influenced by gastric acid; the acidic environment of the stomach normally functions as a barrier to prevent GI flora from translocating and causing infection (Beasley, Koltz, Lambert, Fierer, & Dunn, 2015). Additionally, since the administration of PPIs and H2RBs raise the gastric pH, certain enteric pathogens can thrive in this less acidic environment, most notably some strains of *Escherichia coli* and *Clostridium difficile* (Bavishi & Dupont, 2011). Infections such as

pneumonia, enteric infections, and bacterial gastroenteritis have also been linked with increasing intestinal pH through the use of PPIs (Kanno et al., 2009; Yang & Metz, 2010). A study by Thorens et al. (1996) found that 53% of patients treated with omeprazole developed bacterial overgrowth explained by pronounced inhibition of gastric secretion. Histamine also plays many roles in the human body including the regulation of several components of the immune response (Jutel, Akdis, & Akdis, 2009). Thus, H2 receptor blockade can negatively affect immune response.

Catecholamines and Vasoactives

Catecholamines are released as part of the stress response. The immunomodulatory effect that catecholamines have in the body were first documented over 100 years ago; recent studies demonstrate the effects that these catecholamines have on a person's cellular immune function and peripheral immune system. Catecholamines lead to the release of pro-inflammatory cytokines (Desborough, 2000; Lenz et al., 2007; Oberbeck, 2006). Although the release of these chemical mediators occurs naturally, secondary to a traumatic event, synthetic catecholamines are often administered to these patients as well. Epinephrine, norepinephrine and dopamine, commonly referred to as vasopressors when utilized in the clinical setting, are used in the trauma population to support blood pressure during times of hypotension or other reasons where a higher mean arterial pressure is clinically warranted.

The Effects of Antibiotics on the Microbiome

Antibiotics, although life-saving, are a major cause of disruption of the human microbiome which can lead to antibiotic-associated diarrhea and *Clostridium difficile* (*C.diff*) infection, both of which are leading causes of morbidity and mortality in

critically ill patients (McDonald et al., 2018). More than half of all hospitalized patients receive at least one dose of antibiotics (Baggs, Fridkin, Pollack, Srinivasan, & Jernigan, 2016). Even a single dose of an antibiotic can alter the microbiota enough to allow *C. diff* to cause infection (Buffie et al., 2012), and even short term antibiotic usage can alter the intestinal microbiome for up to 24 months (Stavrou & Kotzampassi, 2017). Although the purpose of antibiotic administration is to treat infection by eradicating pathogenic bacteria, they kill the "good" bacteria as well, affecting our GI immune defenses. An overgrowth of pathogenic bacteria is then able to grow and flourish.

Endogenous and Exogenous Opioids

Morphine is endogenously-produced in people during the inflammatory response. It, along with other opioids, is also administered exogenously for the purpose of analgesia and sedation in critical care settings, those with traumatic injuries, and those undergoing surgical procedures (Mora et al., 2012). Morphine is a powerful immunosuppressant that also slows down gut motility, delaying transport time which potentially increases the risk for translocation of bacteria across the intestinal wall (Balzan, de Almeida Quadros, De Cleva, Zilberstein, & Cecconello, 2007). Researchers have also determined that chronic morphine use significantly alters the composition of the gut microbiota allowing rapid growth of gram positive pathogens (Banerjee et al., 2016).

The Importance of Prevention

In a study based on the Healthcare Cost and Utilization Project Nationwide Inpatient Sample, researchers found that trauma patients (n=155,91 records reviewed) who incurred a healthcare-associated infection (HAI) such as sepsis, pneumonia, infection with staphylococcus or *Clostridium difficile*-associated disease, experienced

greater hospital length of stays, increased mortality and costs associated with care (Glance et al., 2011). Specifically, trauma patients who developed sepsis had a 6-fold higher odds of dying than those who did not become septic ($p<.001$). Trauma patients with other HAIs not characterized as sepsis still had a 1.5-1.9 fold higher odds of mortality than those who did not develop an HAI ($p<.005$). For the non-HAI group, costs were significantly lower ($p<.001$) as was length of stay ($p<.001$). This study further supports the need to prevent HAIs in the trauma population.

Restoring the Microbiome with Probiotics

Probiotics are non-pathogenic "friendly" bacteria. Mostly used by healthy people to restore or maintain GI health, recent studies have provided some insight into just how probiotics can affect our microbiota and regulate colonization of pathogens in the gut (Kitazawa et al., 2015; Williams et al., 2010). The use of probiotics has been used for more than a century, usually in the form of fermented foods, in fact even Hippocrates acknowledged the importance of our GI tract in preventing disease; and how important it is to maintaining a healthy gut. "Death sits in the bowels, a bad digestion is the root of all evil" (Hippocrates, ca. 400 BC). However, it was not until the late 1900's that researchers suggested that "bad" bacteria could be replaced with "good" bacteria in the gut and, hence, the concept of probiotics was formed (FAO/WHO, 2001; Schrezenmeir & de Vrese, 2001).

Use of probiotic supplements in the acute care setting is gaining worldwide attention, but their clinical effectiveness is not entirely clear. The administration of these living organisms is used to re-establish normal non-pathogenic human microflora and prevent sequelae involving the disruption of our human gut microbiome. Probiotics

restore the balance of normal gut microbiota, which inhibits bacterial translocation and decreases pathogenic bacteria that emit inflammatory toxins, which then in turn restores proper immune function. There is considerable evidence that certain strains of probiotics, when ingested in adequate amounts, demonstrate both anti-inflammatory and anti-pathogenic effects for the host offering promise for the use to both prevent and treat certain diseases (Isolauri et al., 2001).

The use of probiotics in the acute care setting has steadily increased over the past 10 years. A descriptive study examined the discharge records of nearly 2 million patients in 2012 in a sample of 145 US hospitals (Sarah, Jernigan, & McDonald, 2016). During that year, more than 50,000 (2.6%) patients in 96% of those hospitals received probiotics. Although this may not seem like a large number, this study revealed that probiotic usage in hospitalized patients tripled between 2006 and 2012. The question that remain, however, is what strain of probiotic is best for which condition and at what dosage and duration?

Current Research with Probiotics

Clostridium difficile-Associated Diarrhea (CDAD)

Perhaps the strongest evidence supporting the use of probiotics is for the prevention and treatment of antibiotic associated diarrhea and *Clostridium difficile* infections. A recent meta-analysis examined 26 RCTs involving 7,957 patients (Chamberlain & Lau, 2016). Probiotic use reduced the risk of the development of CDAD by 60.5%, especially among hospitalized patients; adults and children alike. *Lactobacillus*, *Saccharomyces* (a yeast) and a mixture of probiotics were all beneficial in reducing risk (63.7%, 58.5%, and 58.2% respectively).

Even more recent, a retrospective review of patients admitted with a traumatic injury between 2008 and 2014 who received antibiotics (N=4632) found that the implementation of a *C. diff* bundle, which included the administration of probiotics, significantly reduced the incidence of CDI infection (Bommiasamy et al., 2018). The form of probiotics used in this hospital included Nancy's yogurt ™ (Springfield Creamery, Eugene, Oregon) and kefir, which contain a combination of 4 strains of *Lactobacillus*, *Bifidobacterium lactis*, and *Streptococcus thermophilus*. Although components of this bundle were initiated in a step-wise fashion, CDI rates among patients started to decline once probiotic administration was implemented and prior to every component of the bundle was enacted (Bommiasamy et al., 2018). This study also reported that no adverse effects, including clinical infection, occurred as a result of the use of probiotics.

Ventilator-Associated Pneumonia (VAP)

A recent meta-analysis that included 13 random control trials (RCTs) and 1,969 patients found that the administration of probiotics was associated with a lower incidence of VAP (p=0.002) (Weng et al., 2017). The authors concluded that it is likely that probiotics offer some benefits for mechanically ventilated patients.

Hospital-Acquired Infections

Gu and colleagues (W.-J. Gu, T. Deng, Y.-Z. Gong, R. Jing, & J.-C. Liu, 2013) conducted a meta-analysis of RCTs published between 2004 and 2007 and concluded that the incidence of HAIs were reduced in trauma patients who received probiotics as part of early enteral nutrition (p=0.02). Again in 2016, a meta-analysis of 33 RCTs which included 2,972 patients showed that the administration of probiotics to critically ill

trauma patients resulted in a significant reduction in overall infections (Kotzampassi, Giamarellos-Bourboulis, Voudouris, Kazamias, & Eleftheriadis, 2006).

Regulation of Probiotics

Commercially prepared probiotics are considered a "supplement" or "medical food" and therefore not regulated by the FDA. This means that once the probiotic preparation leaves the manufacturer, there is no control over quality, viability, or content. Independent studies have been conducted that show the composition of microbes in several commercially prepared probiotic supplements are not what is written on the label (Huys et al., 2006). This can contribute greatly to inconsistencies in outcomes and further lead to a lack of homogeneity in studies; even when the same brand of probiotic is used.

Safety of Probiotic Supplements

While the majority of existing evidence suggests that probiotics are safe, serious adverse events associated with probiotics, although rare, have been reported (Doron & Snydman, 2015). At risk populations include immunocompromised patients, patients with pancreatitis, and patients with mechanical heart valves although other patient populations have been reported to experience adverse events. Risk-benefit ratios should be weighed when considering use in at-risk patients. (Didari, Solki, Mozaffari, Nikfar, & Abdollahi, 2014). While there are many studies that examine the efficacy of probiotics for the prevention and treatment of a variety of conditions, there are few studies that have specifically examined the safety of these supplements when used in the acute care setting.

Although probiotic organisms are naturally-occurring commensal bacteria present in the human microbiome, the safety of administration of manufactured probiotic organisms to acutely ill hospitalized patients has been questioned. There are published case reports of probiotic supplementation potentially being associated with fungemia (Thygesen, Glerup, & Tarp, 2012), bacteremia (M. K. Salminen et al., 2004), and sepsis but these are relatively few (S. Hempel et al., 2011). Mostly these case reports are anecdotal and considered by some to be negligible in numbers when compared with the large number of patients who are given probiotic supplements each day. Others may argue that adverse events related to probiotic supplementation may not be recognized or reported in the literature therefore infection caused by probiotics may be grossly under-acknowledged. Hempel et al (2011) evaluated 622 intervention studies that addressed the safety of probiotic supplements since August 2010. They concluded that while the intervention studies do not indicate an increased risk of adverse events, they found that there is not a structured system for assessing and reporting untoward events which could contribute to the rarity of adverse events in the literature. Only 387 of these studies specifically reported the presence or absence of adverse events, but these events were poorly documented. Therefore, in certain patient populations, a precautionary approach must be taken (Vitko, Sekula, & Schreiber, 2017).

No Clear Clinical Practice Guidelines

Although research supports benefits to the administration of probiotics in trauma patients, many limitations to that research exist. Differences in probiotic species, dosing, timing, and duration cause significant heterogeneity in these studies. Systematic reviews of the published literature, therefore, cannot yield a true meta-analysis due to the lack of

homogeneity in studies. Of the meta-analyses presented here, few studies used the same strain, dosage, and duration.

Although varying results in outcomes with the use of probiotics may be due to these differences, another aspect that must be considered is the viability of the probiotic preparation itself. Probiotics are living organisms that generally are sensitive to heat and pH extremes. In studies where a probiotic supplement has not been shown to be efficacious, one must consider not only the aforementioned limitation, but consider the viability of the probiotic organisms themselves as they reach the intestines. This is not only a consideration once the probiotic is ingested, but proper handling and storage on the shelf should be considered as well.

Other limitations to published studies include the number of participants that is necessary in order to achieve statistical significance and meet intent-to-treat numbers. Most studies have small sample sizes which render them underpowered. Further, few studies included what clinical definition was being used for ventilator-associated pneumonia, since at the time of the studies there was not a standardized and universal clinical diagnostic criterion set for the diagnosis. Next, HAIs were often grouped together, with few studies actually reporting just what infection was present in the patient. Severity of traumatic injury certainly has an effect on outcomes as well; there lacked consistency on how severity was measured which can affect outcome measurement. More severely injured or multi-injury patients are generally at greater risk for immune dysfunction and increased infection rates.

Conclusions and Implications for Future Research

The use of probiotics in the acute care setting is increasing, yet there remain relatively few high-quality RCTs that have specifically examined probiotic role in the trauma population. Further, meta-analyses of existing RCTs that pooled results from trials using probiotics to prevent different diseases are flawed. Since the research into probiotic use is relatively new and there is a clear lack of homogeneity in published trials, one must cautiously interpret results from pooled estimates of risk where different types of probiotic strains, dosages, durations, etc. have been used to derive findings (L. V. McFarland, 2015). Further, effects of probiotics may be strain and species specific, meaning that findings of one study demonstrating effectiveness of a particular species may not be applicable to other conditions. Caution must be taken with attempts to generalize findings, even within similar strains. For example, there are 11 Lactobacillus species and 7 Bifidobacterium species currently known (Senok, Ismaeel, & Botta, 2005). In order for clinical practice guidelines to be developed, there must be greater homogeneity in studies with larger multi-center randomized-controlled trials being conducted with the trauma population. Additionally, definitions of infections/clinical diagnostic criteria should be standardized as well as utilization of the same trauma severity scoring systems in order to better generalize findings.

Implications of Key Findings

Although one cannot clearly conclude that probiotic supplementation in the trauma population should be used to improve outcomes such as decreased infection rate, ventilator-associated pneumonias, mechanical ventilator days, decreased antibiotic usage, decreased hospital and ICU length of stay, morbidity, and mortality, the current evidence

strongly suggests that probiotics positively contribute to these outcomes with relatively low risk in most patients. Until more rigorous research is performed using the same strains, dosages, and timing of probiotic administration, it may be reasonable to use current research for the use of probiotics in this population as benefit seems to be likely.

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Chapter 4
Part 2

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Probiotics in the critical care unit: fad, fact, or fiction?

Abstract: The gut microbiome consists of normally non-pathogenic bacteria, viruses, and fungi. These friendly microbes serve to maintain gastrointestinal (GI) barrier function and integrity, play a role in host nutrient and drug metabolism, immunomodulation, and prevent pathogenic bacteria from colonizing or causing disease. Healthy people have used probiotics for centuries to promote and restore GI health by restoring the normal flora. Probiotics continue to captivate consumers in the current health conscious society for their provocative health claims. The importance of a healthy microbiome for the overall health of the host is just recently being appreciated within the medical and science communities, however. Disruption of the microbiome places one at greater risk for illness and infection. Critically ill patients are among those at highest risk for complications associated with microbial imbalance, or dysbiosis. Patients being cared for in intensive care units are subjected to a variety of treatments and therapies that account for a tendency toward dysbiosis. These include treatment with antibiotics, proton pump inhibitors, and opioids as well as therapies that require invasive procedures and monitoring. With the consequential compromise to one's immunity, critically ill patients are at the greatest risk for healthcare-associated infections (HAIs). One suggested means to restore immune function and for the prevention of HAIs is to supplement patients with probiotics. Probiotics may serve to prevent and ameliorate the effects of a number of HAIs, including: ventilator associated pneumonia (VAP), Clostridium difficile infection (CDI), catheter-associated urinary tract infection (CAUTI), and surgical-site infection (SSI). Despite promising findings regarding the efficacy of probiotics for a number of

conditions, supplementation with probiotics is not without risks. Consumers and prescribers must be educated on what probiotics can and cannot do and should understand that not all probiotics are the same.

Keywords: Probiotics; critical illness; cross infection; microbiota

Introduction

Probiotics, mostly in their "natural" form, have been used by otherwise healthy people to promote and restore gastrointestinal (GI) health. The term "probiotics" is not new; in fact, probiotics have been around for centuries, mostly consumed in the form of fermented food for the associated lenitive effects. Probiotics are the normally non-pathogenic bacteria, viruses, and fungi that reside in one's GI tract. These friendly microbes make up the human GI microbiome, or flora, and serve to maintain GI barrier function and integrity, play a role in host nutrient and drug metabolism, immunomodulation, and prevent pathogenic bacteria from colonizing or causing disease. The importance of a healthy microbiome for the overall health of the host is just recently being appreciated within the medical and science communities.

It is widely known that healthy GI flora is important for overall health of the host, and the disruption of this leaves one with increased susceptibility to illness and disease. Here we will provide an overview of the current understanding of the human microbiome, alterations caused to it by critical illness and associated procedures/therapies, and the use of probiotics to restore GI flora for the prevention and amelioration of infection.

Function of the microbiome

Long before existence of microorganisms was known, fermented products were used therapeutically to treat a number of ailments, including fevers, the common cold, and GI distress (nausea and diarrhea). It is now understood that this "normal flora" is part of one's natural defense against pathogenic invaders. The entire human microbiome

consists of viruses, bacteria, fungi, archaea, and single-celled eukaryotes (1). In fact, it is estimated that there are more than 100 trillion bacterial cells comprised of more than 35,000 species that constitute our microbiome (2). Most of these organisms are naturally found in the GI tract, yet others exist as part of the respiratory or genitourinary tract as well as upon our integument (3). Although the healthy gut microbiota consists of varying species and numbers throughout the entire GI tract, nearly 75% of the microbes exist within the large intestine alone (4). When discussing illness or disease related to disruption of the GI flora, it is generally referring to colonic bacteria, and these bacteria usually are what are trying to be restored.

Even ancestral scholars acknowledged the importance of maintaining a healthy GI tract in preventing disease. As early as 400 BC, Hippocrates asserted "death sits in the bowels… a bad digestion is the root of all evil". The mechanism through which a healthy gut augments one's immunity is complex and multifaceted, but it can be understood through three barriers of immunity.

The first barrier is the ecological barrier, which is the inhabitant flora of our intestines. The second mechanical barrier resides at the cellular level; it is the intact mucosal epithelia that form a direct, physical barrier against pathogenic organism invasion or translocation of otherwise harmless resident bacteria. The third barrier is an immune barrier, comprised of a slew of host immune cells, including intraepithelial lymphocytes, macrophages, neutrophils, natural killer (NK) cells, aggregated mesenteric lymph nodes (Peyer's patches) and immunoglobulin A. Disruption of any of these barriers will often have nocuous consequences (5).

As aforementioned, the ecological barrier consists of the trillions of "good" viruses, bacteria, and fungi residing in the human body. Some of the most prevalent and commonly supplemented organisms harbored in the GI tract are of the Bifidobacterium and Lactobacillus species. More specifically, these include strands *Bifidobacterium animalis*, *Bifidobacterium infantis*, *Bifidobacterium lactis*, *Lactobacillus casei*, *Lactobacillus rhamnosus GG*, *Lactobacillus reuteri*, and the yeast *Saccharomyces boulardii* (6). While each strand

of species confers upon the host its own immunologic and nonimmunologic benefits, these organisms collectively modulate a healthy gut and immune system through a variety of similar means. Most importantly, these resident organisms compete against pathogens for binding sites and nutrients utilized as growth substrates (7). Additionally, these friendly organisms produce vitamins (B, K) that can be used for growth of other non-pathogenic organisms, exert anti-inflammatory effects through increasing or decreasing certain cytokine and interleukin activity (8), and stimulating an innate immune response through activation of helper-T cells, macrophages, NK cells, and immunoglobulins (4).

The normal flora also influences the maintenance of mechanical barrier function—the second barrier. When this mechanical barrier of epithelial cells is disrupted, pathogens as well as bacteria which exert no harmful effect inside the GI tract can translocate and cause disease elsewhere. The breakdown of the GI barrier has been linked to several diseases, including: inflammatory bowel disease, chronic kidney disease, necrotizing pancreatitis, celiac disease, food allergy, *Clostridium difficile* infection (CDI), and sepsis (3,9).

Breakdown and disruption of the GI flora derives from a number of processes, including dehydration and malnutrition, but also may be inadvertently incurred through the administration of antibiotics and other pharmacological therapies (10). The resulting microbial imbalance, or dysbiosis, in turn, affects the third barrier of defense by altering levels of host immune mediators while inducing both chronic inflammation and metabolic dysfunction. What's more, the composition of the gut microbiome is influenced by various environmental factors, such as lifestyle, diet and hygiene preferences as well as the physiological effects of traumatic injury and critical illness (4,11). Additionally, the procedures and medical therapies that patients are subjected to during hospitalization can further disrupt the GI flora, making a patient even more susceptible to infection. Each of these influences will be discussed in more detail later.

The effect of enteral nutrition on the microbiome

It is understood that a variety of inherent host factors influence the composition and integrity of the microbiome. Perhaps the most important of these is the host's enteral nutrition, or diet; for, dietary and bacterial metabolites influence immune responses and gut microbiome physiology (12). Therefore, nutritional strategies directed at restoring the natural flora may have particular utility in the critically ill, given that these individuals are most susceptible to alterations of the microbiome. Most of the research has focused on the role fats, carbohydrates, and protein have on gut microbial composition (13). However, greater intake of fiber is thought to strengthen the intestinal barrier, increase peristalsis, and reduce gut inflammation (14).

Fiber, once consumed, is fermented to short chain fatty acids, which include acetate and butyrate. These fatty acids bind G-protein-coupled receptors (GPR43, GPR41), which foster homeostasis and the regulation of inflammatory responses in the gut. More specifically, the G-proteincoupled receptors bound to metabolites augment epithelial integrity and IgA antibody responses (12). Conversely, enteral antibiotic intake may disrupt gut and immune homeostasis by altering the encompassed short-chain fatty acid metabolites, consequently promoting the inflammatory status of the intestinal mucosa (15).

Adapting diets specific to patient needs, including supplementation with probiotics, prebiotics and synbiotics, are possible nutritional strategies for improving gut and microbiome homeostasis. Prebiotics, which are often found as complex carbohydrates in fruits, vegetables and grains, are undigested and unabsorbed until reaching the large intestine where selective fermentation occurs. This promotes the growth and metabolic activity of host flora, which further promotes gut-barrier homeostasis. Probiotics, as previously discussed, may inhibit the growth of enteric pathogens through completive exclusion. They also interact with resident microbiota to modulate host immune function (14).

Critical illness and infection prevention

Increased risk

Patients in critical care units constitute a small percentage of total hospital admissions, yet they account for approximately 25% of all healthcare-associated infections (HAIs) (16).

Infection as a complication of critical illness contributes to increased ICU length of stay (LOS), costs associated with admission, resistance to antimicrobials, and morbidity and mortality (17). HAIs are those infections not present and without evidence of incubation at time of admission but develop or become clinically evident after 48 hours of admission. According to the CDC and the results of the 2014 HAI Prevalence Survey, a total of 722,000 HAIs were documented in U.S. acute care hospitals in 2011. What's more, an astonishing 75,000 patients died as a result of these infections (18). The five most prevalent HAIs were: surgical-site infection (SSI), ventilator-associated pneumonia (VAP), CDI, central-line-associated bloodstream infection (CLABSI), and catheter-associated urinary tract infection (CAUTI) (19). The role that probiotics may play in post-surgery infections, VAP, and CDI will be discussed here. The limited information on CAUTI prevention will also be discussed. The reasons that patients in critical care units incur more infections are many, but the pure nature of critical illness as well as the multitude of treatment modalities necessary for care are major factors.

Disruption of our innate barrier defense mechanisms

The physical barriers afforded by our innate immune response such as skin and the mucosal lining of our respiratory, GI, and urinary tract, can be disrupted either by injury or from procedures common in the critical care unit. Skin barriers are breached by intravenous lines and surgical procedures. Respiratory barriers are compromised by endotracheal intubation. Gastric tubes, inserted nasally or orally, as well as indwelling urinary catheters can disrupt mucosal barriers within our GI/GU systems.

Endogenous insult also occurs, perpetrated by certain medications and procedures. All of these serve as means to treat illness, injury, and disease, yet at the same time have infelicitous and puissant consequences.

Common pharmaceutical treatments that alter the microbiota

Antibiotics

It is widely understood and accepted that antibiotics have bactericidal and bacteriostatic effects against both pathogenic and non-pathogenic "good" bacteria. These effects include major changes in the gut microbiota taxonomic diversity which accounts for decreased ability for competitive exclusion. That is, antibiotics destroy multitudes of good bacteria that allow pathogenic bacteria to survive due to less competition for binding sites and growth substrates. However, of principal concern regarding the use of broad-spectrum antibiotics is the opportunity for resistant strains to emerge and be promulgated through horizontal gene transfer among surviving organisms. This accounts for a two-fold insult to human hosts; for, not only are hosts experiencing an alteration of the normal gut microbial diversity, but also pathogenic microbes are being adapted to survive against the current best means for eradication—antimicrobial therapy (4).

It is estimated that more than half of all hospitalized patients received at least one antibiotic during their stay (20). Research demonstrates how GI flora destroyed by just one dose of an antibiotic often takes months to years to recover and host flora may never return to a pre-antibiotic state (21). Further, one-third of antibiotics prescribed in U.S. hospitals involve prescribing problems including prescribing

antibiotics for a patient who is not clinically indicated (22). With an understanding of antibiotic-induced dysbiosis, it becomes important for clinicians to focus their efforts on preventing infection and treating HAIs with non-antibiotic strategies whenever possible. Probiotic administration is just one of the suggested strategies for accomplishing this.

H2 receptor blockers/proton pump inhibitors

Prevention of stress-induced ulceration of the GI mucosa is quite common in intensive care units. Usually this is achieved through pharmacological measures such as the administration of H2 receptor blockers and proton-pump inhibitors. Although they offer GI protective effects, the acid secretion suppression and neutralization of GI acidity can be hospitable for a number of pathogenic organisms to flourish, namely *Escherichia coli* and *Clostridium difficile* (CD) (23). Other infections associated with an increase in gastric pH and subsequent bacterial overgrowth include pneumonia and bacterial gastroenteritis (24).

Catecholamines

In times of critical illness and stress, the adrenal glands secrete glucocorticoids from the cortex and catecholamines from the medulla in order to activate the sympathetic nervous system (SNS). This “fight or flight” response allows for the body to act upon the perceived threat at hand. In addition to these hormones being secreted endogenously, these substances are administered as exogenous pharmacologic medications commonly meant to support a falling blood pressure along with treatment for a multitude of other conditions. It is known that elevated levels of

cortisol and epinephrine contribute to impairment of the immune system, placing the patient at a heightened risk for infection (25).

Opioids

Critical illness is often accompanied by pain, either as a result of an injury or a disease process. Opioid analgesics are used frequently to treat this pain, but also may be used for sedative properties. Regardless of the intended use, opioids possess powerful immunosuppressive properties. Another well known side effect of opioids is slowing of GI motility. Delayed peristalsis incurred with opioid administration can increase the risk of translocation of bacteria out of the GI system where they can become pathogenic to the host (26). Additionally, treatment with opioids is known to place the patient at an increased risk for CDI due to the associated alterations in the GI microbiome and immune function (27,28).

Probiotics: What are they, how they vary, and how they can restore the microbiome

Probiotics can be simply defined as microbial cells that confer beneficial effects on the health of a human host and are naturally found in many foods (5). Some of the most common probiotic-rich foods include yogurt, cultured vegetables (sauerkraut and kimchi), kefir, and kombucha (see *Table 1*). However, recent interest has emerged in consuming probiotic supplements in pill, powder, or capsule form to achieve these same benefits. There has been an incredible increase in the number of manufacturers producing and marketing various species and strains of these beneficial microbes to assist in not only digestive health, but also for immune support and as an

adjunct to a healthy lifestyle. Under the Dietary Supplement Health and Education Act (DSHEA), a manufacturer of a supplement cannot claim its substance has the capacity to diagnose or cure any particular disease. However, making broad claims such as "improves overall health and well-being" has proven to be quite a draw for the current health-conscious society. Since probiotics mostly are used as a dietary supplement, they do not face stringent regulation by the Food and Drug Administration (FDA).

Food	Probiotic strain/species	CFUs
Activia® by Dannon	*Lactococcus lactis* *Lactobacillus bulgaricus & lactis* *Streptococcus thermophiles*	5-10 billion CFU/4 oz.
Plain Greek yogurt	*Bifidus* *Lactobacillus acidophilus* *Lactobacillus bulgaricus & casei* *Streptococcus thermophiles*	6 million – 1 billion CFU per 4 oz.
Plain yogurt	*Bifidus* *Bifidobacterium lactis* *Lactobacillus acidophilus, bulgaris, & lactis* *Streptococcus thermophilus*	>100 million per 4 oz.
Cultured Vegetables (saurerkraut, kimchi)	*Lactobacillus acidophilus, brevis, fermentum, mesenteroides, pentosus, plantarum, and fallux*	Varies 4-6 million CFU/g
Kefir	*Bifidobacterium breve, lactis, & longum* *Lactobacillus acidophilus, casei, lactis, plantarum, reuteri, & rhamnosus* *Leuconostoc cremoris* *Sacharomyces florentinus* *Streptococus diacetylactis*	40 billion CFU/4 oz.
Kombucha	*Bacillus coagulans lactospore*	Varies Generally 10 million CFUs per 8 oz.

Table 1 Probiotic-containing foods
*CFU's, colony-forming units

Brand	Probiotic Species	CFU's
Align® Proctor and Gamble, Ohio	*Bifidobacterium infantis 5624*	1 billion per capsule
Culturelle® iHealth, Inc., Connecticut	*Lactobacillus rhamnosus GG*	10 billion per capsule
Florastor® Biocodex, Inc., California	*Saccharomyces boulardii lyo CNCM-I745*	250 mg per capsule
Ultimate Flora Extra Care® ReNew Life, Inc., Florida	*Lactobacillus acidophilus, bulgaricus, casei, paracasei, plantarum, rhamnosus, & Salivarius*	50 billion per capsule
Synbiotic 2000 Forte Medifarm, Sweden	*Lactobacillus paracasei, Pediococcus pentosaceus, Lactobacillus plantarum Leuconostoc mesenteroides*	40 billion per sachet containing powdered probiotic mixture
Floratrex® Global Healing Center, Texas	23 strains including: *Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus, Bacillus, Enterococcus, and Pediococcus species*	25 billion per capsule
Raw Probiotics Women® Chrysalis Nutrition and Health, LLC, Colorado	32 strains of bacteria and yeast including: Lactobacillus, Bifidobacterium, Streptococcus, Leuconostoc, *Kluyveromyces, Brettanomyces, Debaryomyces, Saccharomyces, & Torulaspora*	28 billion CFU per capsule

Table 2 Common probiotic supplements

CFU's, colony-forming units

The fad

The draw of being "healthy" and "boosting immunity" is captivating the interest of both the researcher and the consumer, and manufacturers and marketing companies are capitalizing on this. In fact, the probiotic movement has spread such that you can find probiotics in foods like granola bars, bottled water and juices, and even chocolates. With the growing interest and application of probiotics for specific conditions and overall health claims, these "healthy microbes" will continue to find their way from the shelves to our bellies. Some of the more common and popular brands include: Align®, Culturelle®, Florastor®, Ultimate Flora Extra Care®, Synbiotic 2000 Forte®, Floratrex®, and Raw Probiotics Women® (see *Table 2*).

The facts

Both foods and supplements have been developed and marketed with the intention to enhance wellness in healthy individuals as well as for the dietary management of various diseases. In the U.S., probiotics are regulated as dietary supplements unless a particular product is marketed for having a role in treating or preventing a particular disease. Rather than focusing on quality, safety, and efficacy, regulational oversight focuses on the legitimacy of any claims made by the manufacturer (29). Depending on the intended use, regulatory oversight and requirements for probiotics differ greatly (30). "Nutritional supplements" are considered a food and therefore regulated by the FDA's Center for Food Safety and Applied Nutrition. However, if the intent is to use a substance for a cure, mitigation, treatment, or prevention of a disease, then it can be classified as a drug

by the FDA (31). Manufacturers explicitly state on labeling that the product "is not intended to diagnose, treat, or mitigate disease" in order to be in compliance with the FDA. However, clinicians are often using probiotics for those purposes.

Manufacturing practices, conditions, and ingredients play a major role in the determination of product characteristics and properties. Again, current law may allow for a variety of formulations to be sold under the same brand which may account for a product different from the original. Such regulatory deficits may have dire consequences on consumers as well as for prescribers using these preparations as part of clinical guideline-recommended management of various problems or diseases. A prescriber can be liable for prescribing a formulation of a product not properly tested for safety or efficacy. For these reasons, current regulations are not sufficient to protect consumers or providers. Further regulatory oversight is warranted (29).

As aforementioned, when used for nutritive value, probiotics are considered a supplement and therefore not subject to the scrutiny of the FDA. In light of the recent research examining their use for preventing and treating certain diseases, the FDA responded by defining probiotics as a biotherapeutic product and mandating that any clinical research done with probiotics requires an Investigational New Drug (IND) application when conducting research that is beyond using them as nutritive supplements. This holds probiotics to increased scrutiny and places barriers to advancing research in this area. Manufacturers must provide required information to the FDA. Exclusion criteria set forth by the FDA includes pregnancy, immunosuppression, structural heart disease, or a leaky bowel wall.

The fiction

It is important to dispel a common myth regarding probiotics—all probiotics are not the same. In fact, a particular supplement itself may vary bottle to bottle. Several commercially available products vary between the actual microbial composition (at either the species or genus level) and what is labeled on the container (32). Further, the colony count of species may be far different from labeling, due, in part, to mishandling the product once it leaves the manufacturing facility. Accounting for this variability is the lack of regulation and oversight over the probiotic market. Probiotics are live organisms and are extremely sensitive to their environment. The manufacturing process and handling of the products themselves are not always the same. In addition, many of the properties of probiotics are not only species but strain specific (33). This means that safety and efficacy of findings should not be generalized to similar products (29).

Use of probiotics/evidence-based use of probiotics

VAP

VAP, a type of hospital acquired pneumonia, is the second most common nosocomial infection in the U.S. and the most frequent HAI in intensive care units (34). In fact, it is estimated that as many as 30% of mechanically ventilated patients develop VAP (35). Similar to other HAIs, VAP is associated with poor clinical outcomes and high financial burden, with estimates close to an additional $40,000 of hospital costs per patient (34). VAP can be diagnosed in any critical care patient who has been mechanically ventilated for at least 48 hours and demonstrates clinical symptoms of pneumonia along with displaying relevant radiographic criteria (36). VAP has long been an outcome indicator of quality of care and infection prevention strategies among critical care units. More

recently, the concept of infectionrelated ventilator complications (IVAC) and ventilator associated complications (VAC) have been proposed by the CDC to expand upon VAP as more objective measures of quality of care, since the diagnostic criteria of VAP may be interpreted differently by clinicians (37). A further discussion on the nosology and etiology of VAP, IVAC, and VAC are beyond the scope of this discussion.

Bundles of care specific to ventilated patients have been proposed and implemented across the world as effective healthcare-associated pneumonia prevention strategies (37). It is predicted that incidence of VAP can be decreased by 50–60% if evidence-based care bundles are instituted and properly followed by all medical staff a part of the care of mechanically ventilated patients (38). Essential components of these bundles, as explicated by the Institute for Healthcare Improvement, include: daily interruption of sedation with wakening and weaning trials, elevation of the head of the bed to at least 45 degrees at all times, deep vein thrombosis prevention with pharmaceuticals and sequential compression devices, pharmacologic prevention of intestinal bleeding and gastric ulcers, and frequent oral care, perhaps with chlorhexidine (39). In light of current research regarding the efficacy of probiotics on decreasing incidence of VAP, it may be worth considering probiotic supplements as part of ventilator care bundles.

With an understanding of how probiotics positively influence host gut-barrier health, it should come as no surprise that there is a growing body of evidence demonstrating the positive effects probiotics have on incidence of hospital-acquired infections, including VAP. Probiotic therapy may prevent and treat VAP by restoring non-pathogenic bacteria that compete with pathogens for binding sites and growth substrates, modulating host immune response, and augmenting gut mucosal barrier function. One particular species

of probiotics substantiated by the literature regarding safety and efficacy is *Lactobacillus rhamnosus GG* (LGG). In a study by Morrow *et al.*, administration of LGG was associated with a significant reduction in the incidence of VAP with microbiological confirmation on invasive lower respiratory tract samples (40). However, when examining the total body of evidence pertaining to the therapeutic use and safety of probiotics, there remains inconsistency of results (34). Further research is warranted to better understand what exact species and dosing as well as what time is ideal for introduction to the host to prevent and combat VAP.

CDI

CD and CDI is a frequent cause of hospital acquired infection and significantly increases a patient's morbidity and mortality. In 2008, 66 out of 100,000 patients were infected with CD; a rate which doubled from that just eight years prior. Risk factors for CDI are advanced age (age greater than age 65), prolonged hospital stay, female sex, immunocompromised, and recent antibiotic administration, among others (41). In fact, just one dose of an antibiotic can severely alter a host's microbiome to the point where opportunistic pathogens like CD can proliferate and cause illness for an extended period of time (42). Current recommended treatment approaches include metronidazole for mild to moderate cases of CDI and vancomycin for severe cases. These antibiotics often result in recurrent CDI, however, due to their broad-spectrum coverage that destroys not only CD but also host microbiota. Recent studies aspire to find a new, narrow-spectrum antibiotic, such as thuricin CD, with specific anti-CD coverage to reduce the collateral destruction on host microbiota (42).

In addition to the effect a more narrow-spectrum antibiotic may have on mitigating the destruction of host flora during treatment of CD, concurrent supplement with probiotics may prove beneficial. A recent meta-analysis by Johnson *et al.* found moderate-quality evidence suggesting that supplemental therapy with probiotics resulted in a significant reduction in incidence of CD-associated diarrhea without any association of increased adverse events. In fact, when examining 20 trials that included over 3,800 patients, Johnson *et al.* saw a 66% reduction of CDassociated diarrhea when patients were supplemented with species of *Bifidobacterium*, *Lactobacillus*, *Saccharomyces* and/or *Streptococcus* in patients receiving antibiotics. Trials that used multiple species showed greater effects than those using a single species. Their findings offer reason to encourage the use of probiotics in patients receiving antibiotics who are at risk for CDI and CD-associated diarrhea (43).

Furthermore, Lau and Chamberlain also conducted a meta-analysis to ascertain the efficacy of probiotics on reducing the incidence of CDI. They examined 26 randomized controlled trials that included a total of 7,957 patients. Lau and Chamberlain found that probiotics had an effect of 60.5% reduction on the incidence of CD-associated diarrhea. Specifically, treatment with Lactobacillus, Saccharomyces, or a mixture of probiotic species reduced CD-associated diarrhea by 63.7%, 58.5%, and 58.2%, respectively. Again, the variation and heterogeneity of trials accounts for a major limitation of findings; however, probiotics should still be considered a valuable adjunct in the therapeutic regimen of patients receiving antibiotics unless otherwise contraindicated (44). Finally, Goldenberg and colleagues conducted a Cochrane review that found probiotics decreased the risk of CD-associated diarrhea by 60% in patients who were not

immunocompromised or in a severely debilitated state. Based on data from this meta-analysis of 31 randomized controlled trials including over 8,670 patients, researchers concluded that moderate certainty evidence suggests that concurrent probiotic use along with antibiotics is a safe and effective strategy for preventing CD-associated diarrhea (45).

CAUTI

A urinary tract infection (UTI) occurs when the urethra is colonized with uropathogens or fecal flora. These organisms include *E. coli* (50%), *Proteus* (15%), *Enterobacter* (15%), and *Klebsiella* (15%), among others with less significant rates. Uropathogens can further spread down the urethra to the bladder and possibly the kidneys via the ureters. Such UTIs account for many cases of cystitis and pyelonephritis (46). What's more, significant comorbidities resulting from UTIs include urethral stricture, abscess or fistula formation, bacteremia, and sepsis. An astonishing 25% of all sepsis cases emanate from UTIs (47).

Among all cases of healthcare-associated UTI, approximately 75% are associated with a urinary catheter. It is estimated that between 15–25% of hospitalized patients receive urinary catheters at some point in their stay (48). Indwelling urinary catheters are often necessary for critically ill patients in order to monitor intake and output and to alleviate urinary outflow obstruction, for example, but they are a major source of preventable infection. Improper handling and poor hygiene can result in contamination of the catheter which allows for pathogenic organisms to invade the urinary tract. Additionally, urinary catheter drainage bags serve as potent bacterial reservoirs.

The risk of contracting a CAUTI increases with prolonged use of the catheter. Therefore, urinary catheters should be removed as soon as clinically warranted. Unfortunately, the research regarding the efficacy of probiotics on the prevention of CAUTI is limited. At this time, there is not enough evidence to assert that probiotics may be an effective choice for CAUTI prophylaxis. However, in light of recent research regarding the efficacy of probiotics for preventing UTIs in general, studies with the intent to ascertain the efficacy of probiotics for prevention of CAUTI specifically is warranted.

Recently, researchers sought to review the safety and efficacy of probiotics in the prevention of UTI, given that probiotics have other potential uses as prophylactic therapies. Schwenger *et al.* reviewed the effect probiotics had on morbidity and mortality compared to placebo or no therapy in patients susceptible to UTI. They included nine studies that involved over 730 patients. The focus of these studies was to quantify differences in incidence of recurrent UTI. Researchers found that no significant benefit with probiotics therapy compared with placebo or no treatment; however, a benefit cannot be ruled out for several crucial reasons. These include a data set that was too limited and use of small studies with poor methodological reporting (47).

SSI

Roughly 1.5 million of the 80 million surgeries performed in the U.S. each year is complicated by a SSI. Post-SSIs are the most common of all HAIs, comprising approximately 30% of the total number of these infections. SSIs can add 7 to 11 additional postoperative days to a patient's stay and increase mortality by up to 11 times. Among perioperative patients, 77% of deaths are directly attributed to SSIs (49). Septic morbidity and mortality associated with surgical and medical treatments is high and

rising all over the world, with estimates around 200,000 annual deaths in the U.S. attributed to sepsis (50). Researchers and clinicians are continuously exploring ways to reduce the incidence of infective complications and other surgical adverse events in perioperative patients. Evidence suggests that surgical trauma disrupts the gut microbiome and allows for translocation of normal gut flora. The gut itself is closely linked to the initiation of systemic inflammatory processes that are, in part, responsible for the development of sepsis following surgery.

Recently, perioperative nutrition modulation of gut flora is increasingly being used as an outcome improvement strategy. Probiotic, prebiotic, and synbiotic therapies are being substantiated by the literature as effective means to mitigate the disruptive effect surgical trauma has on gut function. For reference, a prebiotic is a food substance that positively influences growth and survival of host microbiota and a synbiotic is a product that contains both prebiotics and probiotics (51).

A meta-analysis performed by Kinross *et al.* explored the effect probiotic and synbiotic therapy had on postoperative sepsis rates (52). Over 960 elective surgery patients from 13 randomized controlled trials were sampled with the primary outcome measure being postoperative sepsis rate. Of the 962 patients, 304 received synbiotics and 182 received probiotics either postoperatively or pre- and postoperatively. Probiotics used were of the *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Bifidobacterium* species. Researchers found that the rate of postoperative sepsis was reduced in both the probiotic and synbiotic groups compared to the control group.

Prevention above all else

Although decreasing costs associated with the development of infections is important, the main reason to prevent HAIs is to improve outcomes and save lives. Deaths attributed to critical illness/sepsis have been increasing more quickly than any other cause of mortality in this population, making infection prevention strategies even more critical (34).

Safety and risks associated with probiotics

The rapidly expanding consumer probiotic market is reaching into the acute care setting. While research with probiotics for the prevention and treatment of certain ailments in otherwise healthy people is not new, the theoretical benefit of restoring a healthy microbiome for a person that is acutely ill and at increased risk for infection is being explored with great interest. Their use in the acute care setting has been quite promising, but the use of probiotics in the critical care setting has caused significant controversy. Research with probiotics demonstrates their efficacy in preventing and treating various conditions, particularly those involving the GI tract, but can they be harmful? There are certain patient populations in whom risks versus benefits must be carefully considered. Although the overwhelming evidence supports that probiotics are safe, there are case reports of risks associated with probiotic use as well as some theoretical risks that have been posed in certain patient populations.

The most widely cited safety concern surrounding probiotics is the possibility for bacteremia and fungemia associated with their use in certain high risk groups. Adverse effects of probiotics are not widely reported in studies, yet case reports of infection possibly related to concomitant probiotic use have been reported. Most of these

infections occurred in “vulnerable” groups (53). Even though their overall safety has been confirmed in literature reviews, caution still needs to be taken in certain situations. A systematic review published in 2014 concluded that probiotics are safe, yet caution should be taken when using probiotics in populations such as those that are critically ill in the recent postoperative period and immunocompromised (54). Case reports of infection also have been reported in patients with short bowel syndrome, central venous catheters, and patients with cardiac valve disease or mechanical heart valves (53,55,56). The risk of infection may also be related to improper handling of the probiotics themselves.

Systemic infections have been cited; cases of Lactobacillus bacteremia and fungemia associated with the administration of *Lactobacillus* probiotics and *Saccharomyces* species in patients who also had central lines have been reported (30,55,57,58). These preparation (usually capsules or sachets) often need opened for administration through a feeding tube which is a practice that can potentially spread the microbes into the air and cause them to contaminate the hands of healthcare workers (59). Improper hand washing, then, can cause a translocation to a central line catheter where the microbes have direct entry into systemic circulation. Although the manufacturers of some of these products list on the label that they are not to be administered to patients with central lines, this practice still does occur. Precautions must be taken to avoid the accidental contamination of the central line of the patient receiving the probiotic or those with central venous catheters in the close proximity to the patient receiving the probiotic (60).

Other safety concerns have been cited by researchers and healthcare practitioners, including the possibility of gene transfer from the microbe itself, toxins being produced,

and effects on a person's immunological system. It is theoretically possible for certain microbes to transfer gene resistance to the host. Certain Lactic acid bacteria have genes resistant to common antibiotics such as macrolides and chloramphenicol (55). Although this does remain a possibility, no literature to date supports this theoretical risk to humans.

Commercially available probiotics may contain a single or multiple strain of a particular microbial species. This could either be multiple strains of the same species, or strains from more than one genus (29). Different products contain varying amounts of bacteria or fungi, so safety related to the quantity of living microbes being ingested is a concern. Additionally, with each species or strain added to a preparation, concern regarding the adverse effect profile, or safety, of each is necessary.

It is known that probiotics affect both our innate and adaptive immune systems, so concern has been raised over the possibility of over-stimulating immune function in certain individuals. Theoretically, this could lead to an "awakening" of an autoimmune disease in the host. However, this too has not been evident in the literature thus far (8). There is a scarcity of studies that specifically examine the safety profile of probiotics when administered to the critically ill. Considering the long history of safe use of probiotics coupled with the actual and potential risks associated with improper administration or certain vulnerable groups, it is best for clinicians and researchers to take precautions. This includes conducting a careful risk/benefit assessment for certain patient groups. When using probiotics, active surveillance for cases of infection associated with probiotic use along with laboratory confirmation of causative organisms

and vigilant reporting of same is important for establishing a safety profile for their use in the critically ill population.

Conclusions

Despite lack of clear, scientific evidence on efficacy, the appeal for consumption and sale of probiotic supplements and probiotic foods continues to grow. Beyond the role they play in promoting GI health, probiotics have been studied and shown to facilitate restoration of the microbiome. Despite lack of homogeneity and the number of high quality studies, published research shows much promise for the use of probiotics to restore altered microbiota and therefore confer substantial benefits to the critically ill; namely the prevention and, in some cases, amelioration of infection. Therefore, an ethical conundrum exists for healthcare providers and a risk-benefit ratio must be explored. Does the administration of probiotics to critically ill patients, despite lack of clear clinical guidelines, offer significant benefit?

Certainly, a precautionary approach is warranted (61). It is important for clinicians to distinguish between the reality of what is presented in the marketing of probiotics, what is known from research, and purely theoretical benefits and potential harms associated with their use. In essence, current evidence supports a role for probiotics in the critical care setting. However, results, in their current form, must be interpreted cautiously in order to ascertain what this legitimate role may be and which species and strains will provide the most benefit.

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Chapter 4
Part 3

The final manuscript for the research project is included. A discussion of the data analysis and significance of the findings is included.

Using probiotics to decrease the incidence of infection in hospitalized trauma patients.

Running Title: Probiotics for trauma patients

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Key Words: Probiotics, Healthcare-associated infection, Trauma

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Abstract

Background: Infection is a leading cause of death in patients who survive trauma. Traumatic injury/critical illness cause dysbiosis; disruption in intestinal flora that normally affords immune protection. Probiotic usage in trauma patients for infection prevention is being studied.

Objectives: To investigate the influence of probiotic supplementation on infection rate in trauma patients. Variables: probiotic usage, mechanical ventilator days, hospital length of stay (LOS), mortality, and antibiotic usage.

Methods: Cross-sectional study in a Level 1 trauma center. Trauma admission medical records from January 2017 through June 2018 were reviewed for infection events and probiotic usage. Infection rates for patients receiving probiotics was compared with those who did not.

Results: After exclusion for infection-probiotic timing and stratification by patient acuity, 1,055 cases were analyzed. Adjusting for patient acuity, the high patient acuity group was too underpowered for interpretation. For both groups, results were impacted by a low probiotic usage rate; 85% of the patients eligible to receive probiotics did not. In the low patient acuity group, the infection rate for those receiving probiotics was 8.2% versus 1.8% in the group not receiving probiotics. The sample sizes varied (n=147 and n=856, respectively) which warrants caution with interpretation of results.

Conclusion: No benefit for the prevention of infection was found in this study. Several study limitations likely influenced results; low infection rates and low probiotics usage rates.

Background

Results of previous research are mixed and have shown both benefits and (potential) risks to probiotic use in trauma patients. For patients who survive the initial "first hits" of trauma, namely severe organ injury, hypoxia, and hypovolemia/hemorrhage, sepsis is a leading cause of death (Goris & Draaisma, 1982; Lenz et al., 2007; Lord et al., 2014). Trauma patients who experience a healthcare-associated infection (HAI) not only have increased mortality rates and longer lengths of stay, but also have increased costs associated with their care (Glance et al., 2011). The yearly costs associated with treating HAIs exceeds 9.8 billion dollars (Zimlichman et al., 2013). Patients who suffer a traumatic injury are at increased risk for developing HAIs, particularly with multidrug resistant organisms (Fawzy et al., 2017), due to a host of factors including the nature of the traumatic injury itself. Traumatic injury and the stress of a critical illness cause an intestinal dysbiosis; an overgrowth of pathogenic bacteria that replaces or overwhelms the gastrointestinal (GI) microbiome of commensal organisms (Manzanares, Langlois, & Wischmeyer, 2017; Lee E Morrow & Wischmeyer, 2017; Young, 2017).

The microbiome and resident microbiota, the collection of all genomes of all microbes in an ecosystem and the microbes that inhabit that ecosystem (Lynch, 2016), play a crucial role in homeostasis and immune response, and normally serve to protect us from infection and disease that is caused from potentially pathogenic microbes (Vieira, Teixeira, & Martins, 2013; Young, 2017). There is increased personal interest in the use of probiotics to aid in overall digestive health, but there is growing professional interest as well for a variety of uses. Research into restoring the human microbiome in critically

ill patients for the treatment and prevention of certain diseases has been increasing over the last decade. Considerable research has been conducted on the use of probiotics for prevention of ventilator-associated pneumonia (Bo et al., 2014; Cook et al., 2016; Zeng et al., 2016), *Clostridium difficile* (*C. diff*) infection (Barker et al., 2017; Evans & Johnson, 2015), antibiotic-associated diarrhea (Mantegazza et al., 2017; Vandenplas et al., 2009), *helicobacter pylori* eradication (Zhang, Qian, Qin, He, & Zhou, 2015) as well as many other disease processes. However, controversy still exists on their role and application to hospitalized patients; especially those that are critically ill or suffer a traumatic injury (Fawzy et al., 2017; L. E. Morrow, Gogineni, & Malesker, 2012). Beyond the question of efficacy, those in the medical and science community cite concerns over the safety of using a substance for patients that does not have strict Federal Drug Agency (FDA) regulatory oversight (Doron & Snydman, 2015; L. E. Morrow et al., 2012; Venugopalan et al., 2010; Vitko et al., 2017).

Background of Probiotics

Probiotics are living organisms that are found in many forms. They are naturally occurring in fermented foods, mostly dairy products, but are also commercially available and sold over-the-counter in pill, powder, and capsule form. They can be found added to a wide variety of foods and drinks that can be purchased in nearly all supermarkets. Probiotics, when used for the intent of supplementing the diet are considered "dietary supplements" as defined by the Dietary Supplement Health and Education Act. In this use, they are not regulated nor face stringent rules and regulations by the FDA. When a "supplement" is prescribed by a physician for the intent of dietary management of a

medical condition, it can be considered a “medical food”. The contents of a medical food must have scientific research to back any claims such as being “for digestive health”, but the FDA does not require proof of these studies. Further, information about any possible adverse effects is not required on the labeling.

The only oversight the FDA has with a dietary supplement or a medical food is that they are manufactured using Good Manufacturing Practice (de Simone, 2018) and the contents are reasonably expected to be safe for human consumption (Venugopalan et al., 2010). Manufacturers of supplements, however, do not necessarily comply with assuring proper sanitation and quality control within their manufacturing facilities. In fact, in fiscal year 2017 the FDA found that nearly one-half of the 656 facilities it inspected had violations in proper sanitation practices (Cohen, 2018).

Another area of concern surrounding the non-regulation by the FDA involves oversight once the supplement leaves the manufacturer. These substances are not subjected to any quality control once they leave the manufacturing plant (de Simone, 2018; Kolacek et al., 2017). Most probiotics that are marketed to consumers are prepared in pill, capsule, or powder form. Since probiotics are living organisms, viability from manufacturer to consumer can be a challenge especially when they are not “naturally” occurring such as in normally refrigerated foods. In pill or capsule form, they usually are lyophilized, affording them stability at room temperature and therefore a longer shelf life. However, these living organisms are easily killed by poor handling practices such as humidity and heat extremes (Kolacek et al., 2017). Therefore, any probiotic preparation must be viewed as “buyer beware” since the label may differ from the actual number of live colonies within the package(de Simone, 2018; Kolacek et al., 2017; Sanders et al.,

2010). Another issue of quality control is the labeling of the packaging. Labeling and actual content of the bottle can vary with regard to the species or strain of the probiotic (Huys et al., 2006; Long, 2018; Theunissen et al., 2005). This must also be taken into consideration when using probiotics in the clinical setting, especially when any type of research is being conducted regarding a certain strain or dosage/potency for efficacy.

The tide may be changing, however, with regards to the regulation of the growing probiotic market. In January 2017, scientifically-based best practice guidelines were released by the Council for Responsible Nutrition and the International Probiotics Association. These guidelines call for self-regulatory practices to include standards for labeling, storage, and stability testing of foods and supplements that contain probiotics(Wong, October, 2017). Perhaps as more stringent regulatory guidelines are put into place, assuming manufacturing companies follow these guidelines, consumers may begin to place more confidence and trust into these substances.

Inconsistency in the Research

The published research on the use of probiotics in the acute care setting has mostly shown favorable findings with respect to decreased infections and improved outcomes. However, the development of clinical practice guidelines on their use has been difficult since, as many systematic reviews and meta-analyses have shown, there is considerable heterogeneity with respect to the strain, dosage, and timing of probiotic administration(Manzanares, Lemieux, Langlois, & Wischmeyer, 2016; L. V. McFarland, 2015; Petrof et al., 2012). Further, many trials are of poor quality, single-center, or with an insufficient number of patients (Bo et al., 2014; Manzanares et al., 2016; Petrof et al., 2012; Siempos, Ntaidou, & Falagas, 2010; Zorzela, Ardestani, McFarland, & Vohra,

2017). And, as mentioned before, unless the probiotic is being tested within the acute care setting for species content and viability, efficacy based on these parameters may vary. Of note, however, there is research being conducted on "dead" or "modified" (inactivated by heat or sonication) probiotics examining whether killed or attenuated probiotic organisms may still confer some immunity or anti-inflammatory benefits (Theunissen et al., 2005; Zorzela et al., 2017), (Sarkar, 2018). Possible differences in safety risks associated with living versus non-living probiotic organisms is also being investigated.

Concerns over the safety of probiotics being administered to hospitalized patients, including those that are critically ill, was addressed by the Agency for Healthcare Research and Quality. They commissioned a comprehensive review based on 622 published studies and concluded that although the available evidence does not indicate an increased risk to safety with the administration of probiotics, they cannot confidently confirm their safety due to a lack of adverse events related to the use of probiotics being documented in the literature (S. Hempel et al., 2011). Other researchers examining the safety of probiotics in published studies also caution interpretation since many of the studies they reviewed were too small to detect events and others did not specifically report safety or adverse event data (Zorzela et al., 2017).

There have been case reports in the literature of fungemia or bacteremia associated with the administration of *Saccharomyes boulardii* (a yeast) and some strains of *Lactobacillus*, but these reports are rather rare considering the millions of doses of probiotics that have been administered to hospitalized patients (Doron & Snydman, 2015). So, although it appears that administering probiotics to even higher risk

populations is likely safe, there still exists some doubt and skepticism among healthcare providers. A conundrum exists in that replenishing depleted microbiota may offer immune protection to those at most risk, yet the supplement meant to protect may actually cause an infection.

Current research on not only the efficacy but the safety of living versus modified probiotics is relatively new but on-going. The principal theoretical risk associated with probiotic administration is the possibility that living microbials, although part of our normal commensal flora, can actually cause an infection if they somehow colonize outside of the GI tract (Boumis, Capone, Galati, Venditti, & Petrosillo, 2018; Doron & Snydman, 2015; S. Hempel et al., 2011; Perry & Doron, 2018). Additionally, ill-effects from potential contaminants to the probiotic substance from the manufacturing process or handling of the substance is possible. Although a review in 2010 found that heat-killed probiotics had fewer adverse effects when compared to living (Sanders et al., 2010), a more recent review found that the rate and type of adverse events caused by living and modified probiotics to be largely similar(Zorzela et al., 2017). Clearly additional research is warranted.

Probiotics in Patients with Alterations in Health

Probiotics have been used for centuries by healthy people. The recent interest in using them in people with altered health status warrants some caution. Vigilance should be used when considering the administration of probiotics to certain populations, such as those who are immunocompromised, those with mechanical heart valves, or those with the potential of translocation of the probiotic across the bowel wall (Didari et al., 2014;

Doron & Snydman, 2015). Other reports of infection, including systemic infection, likely caused by probiotics may have occurred due to contamination of the air and hands of health care workers. Probiotics are living microbials that are beneficial to humans when they inhabit the GI tract, but can become pathogenic outside of the GI system. This can happen when probiotics in powder form or capsules are opened for administration through an enteral tube and then unknowingly transmitted to a patient through a route other than for which they were intended. Case reports of systemic fungal infections related to *Saccharomyces boulardii* precipitated the manufacturer to label Florastor® with the warning "not (to) use Florastor® probiotics in the acute-care setting" and "not (to) use for any individual with a central line or port or in the surroundings of any patient with a central line or port" .

Despite the lack of clear clinical practice guidelines, inconsistencies in the literature regarding strains and efficacy, and caution statements regarding the potential for causing infection, current evidence strongly suggests that probiotics are largely safe and may be efficacious for certain outcomes. Prevention of infection and, ultimately, decreasing the need for antibiotics, is imperative for the vulnerable trauma population.

The goal in medical research is to use available knowledge to formulate hypotheses that can be used to potentially prevent, treat, or ameliorate illness and disease. However, as with any new ideas, benefits must outweigh risks in the absence of true clinical certainness; especially in the case where not having enough evidence does not justify inaction. The Expected Utility Theory (EUT) is a theory of how people *should* make decisions and can be useful for medical decision-making when there is a lack of clear evidence of efficacy and risk. Current literature supports that administering

probiotics to acutely ill patients is ethically justified since the probability of the desired outcome (decrease in infection rates) would likely be realized for the greatest number of people. In this study, we examined the effects of the administration of probiotics on incidence of infection in hospitalized trauma patients meeting inclusion criteria.

Objectives

Two of the most commonly studied and readily available probiotics are *Lactobacillus Rhamnosus GG* (LGG, Culturelle®) and the yeast *Saccharomyces Boulardii* (*S. boulardii*, Florastor®). These two probiotic types are widely available and have rather stable shelf lives. The objectives of this study were to examine through retrospective data collection whether there is a difference in the incidence of infection rates in trauma patients who have and have not received either *LGG* and/or *S. boulardii* probiotic supplements during hospitalization. Additionally, since the administration of probiotics to this population was new and somewhat inconsistent, we examined probiotics usage rates. Secondary outcomes also included: hospital length of stay, ventilator days (as applicable), mortality and antibiotic requirements.

Materials and Methods

Study Setting

A Level I trauma center and teaching hospital in western Pennsylvania that receives an average of 1200-1400 trauma patients per year was the setting for this study. The impetus for this study began in 2015 when some trauma physicians began prescribing probiotic supplements for their patients based on findings of recent studies that suggested improved outcomes such as reduction of *C. diff* and ventilator-associated pneumonia (VAP). At this time, there was a push to implement a probiotics

administration protocol whereby all adult trauma admissions that did not meet exclusion criteria would be administered two strains of probiotic supplementation; *LGG* and *S. boulardii*. The attending trauma surgeons agreed that probiotics should be ordered on admission, however since it was not possible at the time for this "probiotics protocol" to be included on the electronic admission order set, compliance with ordering was low. Toward the end of the study period, the use of probiotics did increase (10% ordering compliance in 2017 and 26% ordering compliance in 2018) and finally, near the conclusion of data collection, a formal probiotics administration protocol was approved and included in the electronic trauma admission order set (See Table 1).

Table 1: Patient Population Inclusion/Exclusion

All adult trauma admissions, 18 years of age and older, admitted between January 1, 2017 and June 30, 2018 adhering to the following inclusion/exclusion criteria:

Inclusion Criteria (ANY of the following):	Exclusion Criteria (do NOT start probiotics if ANY criteria are met):

○ Antibiotic administration
○ Mechanical ventilation
○ Anticipated LOS > 48 hrs
	○ Age <18
	○ Current/recent use Immunosuppressive agents:
	  ○ TNF blockers
	  ○ MABs
	  ○ Chemotherapy
	  ○ Chronic corticosteroids
	  ○ Anti-rheumatic drugs
	  ○ Anti-rejection drugs
	○ Cardiac valve surgery
	○ HIV/AIDS
	○ Active malignancy
	○ Pregnancy

Table 1: *All adult trauma patients meeting above criteria were to be prescribed LGG 1 capsule daily and S. boulardii 250mg twice daily*

Measurement of Outcomes

The primary outcome was the development of a HAI. Secondary outcomes were probiotic usage (compliance with ordering of probiotic supplements according to the protocol), hospital length of stay (LOS), mechanical ventilation days (MV, as applicable), mortality, and antibiotic requirements.

Methods

Upon receiving IRB approval from Duquesne University and Conemaugh Memorial Medical Center, medical records for trauma admissions between the specified dates (July 2014 through December 2017) were reviewed for incidences of infection. Additionally, medication administration records were reviewed for probiotic and antibiotic usage. Timing of initiation and duration of probiotic and/or antibiotic usage was extracted and then compared to those patients that developed an infection. At the time of IRB approval, the study hospital had recently transitioned electronic health record systems. Data prior to January 2017 was not suitable for this project due to the lack of detail necessary for analysis and was, therefore, not included. Statistical analysis was performed on data obtained from January 2017 through June 2018.

Data Collection and Statistical Analysis Plan

Data was extracted from multiple databases: trauma, infection control, and EPIC medical record system. The study population was determined from the hospital trauma

database filtered by the inclusion/exclusion criteria. The Infection Prevention Department provided an accounting of all healthcare-associated infections as identified per the current Centers for Disease Control (CDC-NHSN) regulatory and reporting guidelines; additional infection events were obtained from the trauma database. These infections were recorded in accordance with the quality control and constant outcome improvement processes designed and implemented by the Department of Trauma. Finally, the hospital EPIC report team created a detailed data extraction tailored for this project. The EPIC data extraction contained all needed details regarding medication taken and timing thereof. The information from these multiple extractions were coalesced and correlated into a single study data set for analysis. The EPIC data was aggregated by unique medical record number (MRN)-admit date pair; after which, the type, dose count taken, timing, and days taken for antibiotics and probiotics were calculated. MRN-admit pair was then used to combine all needed information from the three data extractions per each unique patient encounter. The final data set was imported into SPSS version 25 for analysis. An overall alpha equal to 0.05 was set.

Results

The unsuitability of data prior to January 2017, as described earlier, necessitated collection of additional data. Following IRB approval (amendment) allowing an increase of the study time period, the data collection process detailed previously was repeated for records from January 2017 through June 2018, yielding a data set of 1,059 records of admitted trauma patients. Infection events were obtained from two separate data bases; infection control department and the trauma database. We identified 4 infection events

from the infection control database that occurred either prior to the initiation of probiotics, or probiotics were started on the same day. These were excluded from analysis since there would be no influence on probiotics to infection in those cases. When examining the trauma database for infection events, we were unable to determine onset of date of infection related to probiotics administration so we could not determine how many, if any, of these infections occurred independent from probiotic usage. This is a major limitation to analysis and findings.

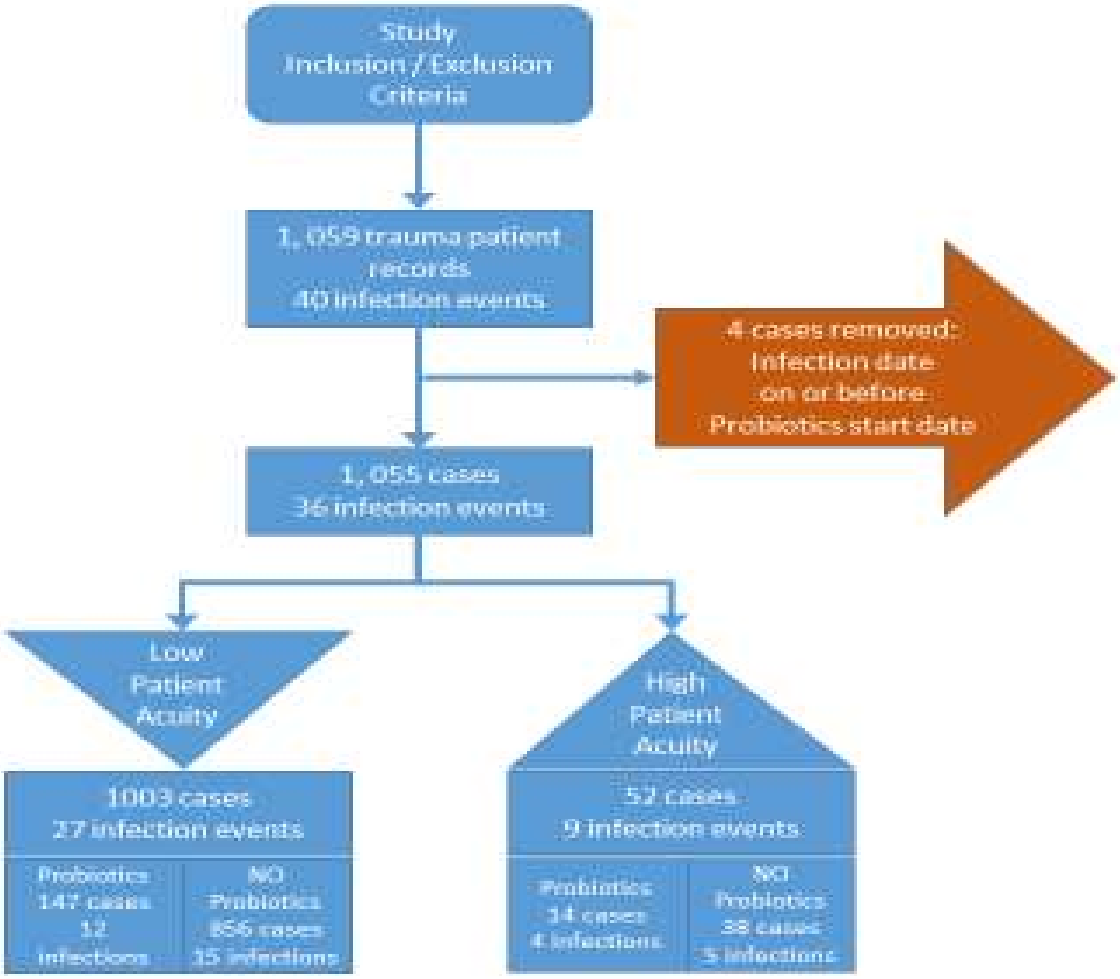

Figure 1: *Flow diagram of sample data set derivation*

Attempting to accommodate the potential confounding influence of patient acuity, the data was stratified by ISS, specifically, cases with ISS > 25 (n=52) were stratified into a high patient acuity group, while the remaining cases with ISS </=25 (n=1,003) were placed into the low patient acuity group (see Figure 1). The sample was analyzed as a whole and the two patient acuity groups were studied independently in attempt to quantify the potential confounding of high patient acuity (see Table 2).

Multiple binomial logistic regression (mBLR) was employed on the data set as a whole (N=1,055) to ascertain the effects of Any Antibiotics Taken (N,Y), Probiotics (N,Y), Sex (Female, Male), ICU days (N,Y), MV days (N,Y), ISS, ICU free days, LOS, and Age on the likelihood of developing an HAI. Linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box-Tidwell (1962) procedure. A Bonferroni correction was applied using all fourteen terms in the model resulting in statistical significance being accepted when P < .00357 (Tabachnick & Fidell, 2014). Based on this assessment, all continuous independent variables were found to be linearly related to the logit of the dependent variable. Next, multicollinearity was assessed using a criteria of Variance Inflation Factor (VIF) >/= 3, which resulted in the removal of LOS (VIF=5.099) and ICU free days (VIF = 3.849). Following removal, reassessment of multicollinearity showed VIF of all remaining variables to be < 1.800. Twenty-four standardized residuals had an absolute value > 2 standard deviations; all were kept in the analysis. With a cut-value of 3.5%, the mBLR model was statistically significant, $\chi^2(7) = 64.800$, $P < .0005$ (Hosmer-Lemeshow Test, $P = .617$). The model explained 23% (Nagelkerke R2) of the variance in acquisition of an HAI, with a 78.9% overall percentage correct. Sensitivity was 77.8%, specificity was 78.9, positive

predictive value was 11.5% and negative predictive value was 99.0%. Of the seven predictor variables, two were statistically significant, as shown in Table 2.

Two variables were revealed as statistically significant predictors of an HAI in this sample:

- ICU days (categorized as 0 days and > 0 days),
- Probiotics (No, Yes)

	B	S.E.	Wald	df	*P*	Odds Ratio, [Exp(B)]	95% C.I. for Odds Ratio	
							Lower	Upper
ICU days, cat(1)	1.866	0.472	15.626	1	***0.000***	***6.461***	2.562	16.293
Probiotics (N,Y)(1)	1.261	0.384	10.764	1	***0.001***	***3.528***	1.661	7.492
Any Antibiotics Taken(1)	0.788	0.427	3.402	1	*0.065*	2.199	0.952	5.082
ISS	0.033	0.021	2.579	1	0.108	1.034	0.993	1.077
Age	0.009	0.010	0.796	1	0.372	1.009	0.990	1.028
Vent(1)	0.285	0.493	0.335	1	0.563	1.330	0.506	3.494
Sex(1)	-0.155	0.405	0.146	1	0.702	0.857	0.388	1.893
Constant	-6.022	0.975	38.139	1	0.000	0.002		

Table 2. Results of the Multiple Binary Logistic Regression, N = 1,055

The interpretation does not change when the above analysis is repeated following stratification by ISS. (See Table 3)

ISS </= 25	B	S.E.	Wald	df	Sig.	Odds Ratio, Exp(B)	95% C.I. for Odds Ratio	
							Lower	Upper
ICU days, cat(1)	1.887	0.497	14.446	1	0.000	6.602	2.495	17.472
Probioitcs (N,Y)(1)	1.442	0.442	10.668	1	0.001	4.230	1.780	10.050
Any Antibiotics Taken(1)	0.845	0.488	3.004	1	*0.083*	2.328	0.895	6.054
Age	0.011	0.011	0.969	1	0.325	1.011	0.989	1.034
ISS	0.026	0.040	0.425	1	0.514	1.026	0.949	1.110
Sex(1)	0.301	0.462	0.424	1	0.515	1.351	0.546	3.341
Vent(1)	-0.126	0.579	0.047	1	0.828	0.882	0.283	2.744
Constant	-6.388	1.156	30.505	1	0.000	0.002		

Table 3: mBLR Output for Low Patient Acuity Group

(Note: Analysis of the ISS > 25 group is too underpowered for interpretation)

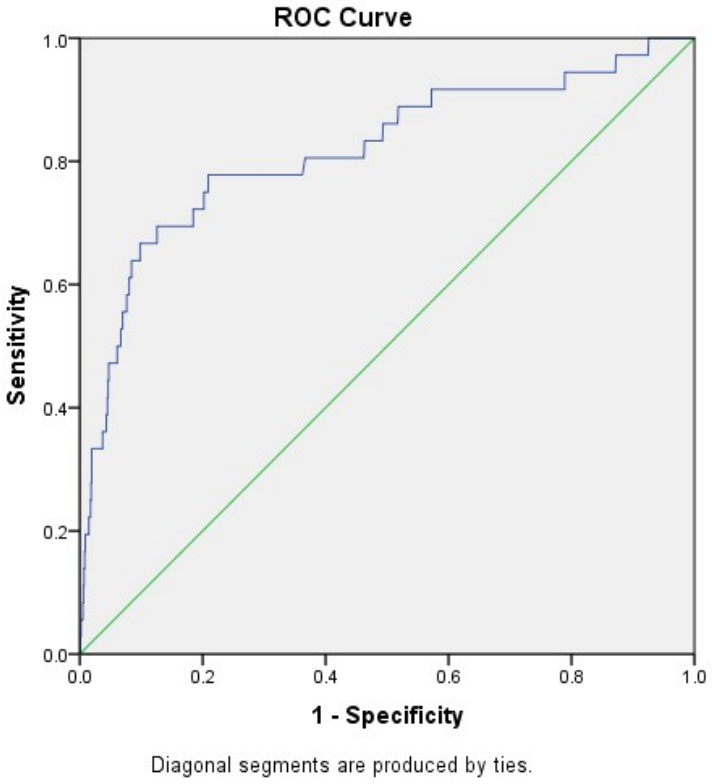

Figure 2: AUC for mBLR, ISS </= 25, Outcome of Infection

The area under the ROC curve was .817 (95% CI, .732 to .902), which is an excellent level of discrimination according to Hosmer et al. (2013).

Obviously, the two unexpected results are:

1) Taking an Antibiotic(s) is not a statistically significant variable and is associated with an increase in the odds of acquiring an HAI
2) Taking Probiotics is a highly statistically significant variable and is associated with an increase in the odds of acquiring an HAI

One reasonable and probable reason for this is demonstrated in Table 4.

	Frequency	Valid Percent	Cumulative Percent
No Abx & No Probiotics	428	40.6	40.6
Yes Abx & No Probiotics	466	44.2	**84.7**
Yes Abx & Yes Probiotics	111	10.5	95.3
No Abx & Yes Probiotics	50	4.7	100.0
Total	1055	100.0	

Table 4: Composite Variable: [Any Antibiotics Taken (N,Y) with Probiotics (N,Y)]

The probiotics protocol was *not* followed in 85% of the study sample.

Furthermore, the mBLR suffers from a low event condition given an overall 3.4% infection rate (36/1,055).

	Patient Acuity		
	Low (ISS </=25)	**High** (ISS > 25)	*P*-value
N =	1003	52	
Infection rate, % (count)	2.7 (27)	17 (9)	< .0001
LOS, days			
mean	5.66	9.9	< .0005
median	4	8	
mode	3	6	
Mortality, %			
lived	97	73	< .0005
died	3	27	
Sex, %			
female	60	46	0.046
male	40	54	
Age, years			
mean	70.91	60.54	0.014
median	76	62.50	
mode	86	78	
ISS			
mean	9.51	31.58	< .0005
median	9	29.50	
mode	9	26	
ICU free days			
mean	5.07	4.63	0.142
median	4	4	
mode	3	4	
ICU LOS, days			
mean	0.58	5.27	< .0005

median	0	3
mode	0	0

Table 2: *Comparison of patient acuity groups*

Probiotic usage

Clinician compliance with ordering probiotics remains a challenge to the investigation of the impact of probiotics on infection events. Prior to the probiotics protocol being incorporated into the (EPIC) electronic admission order set, only about 10% of eligible patients received probiotics. Although compliance increased afterwards, it remained suboptimal. Overall compliance/usage rates for the study period were 15% (161/1,055), with a statistically significantly different compliance rate between the low patient acuity at 15% (147/1003) and high patient acuity group at 27% (14/52), ($P <$ .0165, z-test for independent proportions.) The data also revealed that, in addition to patients with an ISS > 25, those admitted to a general trauma surgical unit were also more likely to have received probiotics.

Low Patient Acuity (ISS </=25)

	Probiotics		
	Yes	**No**	*P*-value
N =	147	856	
Infection rate, % (count)	8.2 (12)	1.8 (15)	< .0001
LOS, days			
mean	7.18	5.40	
median	5	4	< .0005
mode	3	3	
Mortality, %			
lived	97	97	.807
died	3	3	
Sex, %			

female	63	60	.399
male	37	40	
Age, years			
mean	65.04	71.92	.001
median	70	77	
mode	86	86	
ISS			
mean	10.39	9.36	.100
median	9	9	
mode	9	9	
ICU free days			
mean	5.74	4.95	.043
median	4	4	
mode	3	3	
ICU LOS, days			
mean	1.40	0.44	.026
median	0	0	
mode	0	0	

Table 6: *Comparison between those that did and did not receive probiotics in the low patient acuity group*

Infection

Of the 1,059 patients, 40 infections were recorded; 4 patients were excluded due to an infection event occurring prior to or on the same day as the start of probiotic usage. These 4 cases were excluded from final outcome analyses since probiotic administration could not have impacted the infection in these cases. The infection event date could not be determined for the 23 infection events recorded in the trauma database, this being a major limitation that likely affects our analysis. Of the remaining 36 infection events, 27 infections occurred in the low patient acuity group (in 15 of those cases, the patient did not receive probiotics) and 9 infection events in the high patient acuity group (in 5 of those cases, the patient did not take probiotics).

In the low patient acuity group, 8.2% of the patients who had probiotics also had had an infection (12/147), as compared with 1.8% infection rate for those who did not (15/186), In the high patient acuity group, 28.6% of patients taking probiotics had an infection (4/14), as compared to 13.2% infection rate for those who did not take probiotics (5/38). Although consistent with the mBLR analysis, these are unexpected results – that those patients taking probiotics had higher infection rates. Note that although not statistically significant predictors of HAI by the mBLR, both ICU LOS and hospital LOS in the low acuity group, were statistically significantly longer for those that took probiotics versus those that did not.

Mortality

Mortality rate in the patients not taking probiotics was 3.4% (29/856), as compared to 2.7% in those taking probiotics (4/147). Although there were differences between pre- and post- implementation of the addition of probiotics to the electronic order set, mortality was not statistically significantly different on probiotics, taken or not, (P = .807, Fisher's Exact Test used due to an expected cell count < 5).

MV days

A crosstab of MV days (N,Y) vs ISS category and MV days (N,Y) vs Probiotics (N,Y) for the low patient acuity group only showed a statistically significant association, Fisher's Exact Test, P < .0005 and P = 018, respectively.. Importantly, the observed count of patients with > 0 vent days that took probiotics was 1.7x (17/9.8) more than expected, whereas all other (observed-expected) differences were within +/- 7.2 cases.

Hospital LOS

The positively skewed distributions of hospital LOS were not statistically significantly different between the probiotics and no probiotics groups, mean days = 5.44 and 5.04, respectively. Given homoscedasticity (Levene statistic = 2.123, $P = .145$) and violation of normality (Shapiro-Wilk, $P < 0.0005$), the Mann-Whitney U Test was applied ($U = 59{,}025.500$, $z = 1.921$, $P < 0.055$).

Antibiotic requirements

Consistent with the mBLR, a Chi-square crosstab of Any Abx taken and Pt Acuity Group is not statistically significant ($P = .656$). However, a statistically significant association does exist between Any Antibiotic Taken and Probiotic Taken, ($P < .0005$), whereby a patient is about twice as likely to have both prescribed as opposed to either an antibiotic or probiotic alone. Furthermore, this finding is due to the low patient acuity group, $P < .0005$, versus $P = .125$ for high patient acuity (See Table 7).

The statistical analysis strongly indicates the presence of treatment bias (probiotics and antibiotics), unmitigated confounding, and likely the need for additional explanatory variables, e.g. measurement of the GI microbiome (pre, during, and post treatment), timing of probiotics relative to other treatment (antibiotics), and duration of antibiotic and probiotic regiments. A 2:1, nearest neighbor without replacement propensity score matching (PSM) utilizing logistic regression estimation with a matching order of largest, caliper setting of .2, and probiotics as the treatment was employed in an attempt to combat the bias and confounding. The covariates included were: dichotomized ISS (split at 25), Any antibiotics (Yes/No), Any H2 blocker (Yes/No), Any PPI (Yes/No),

ICU days (Yes/No), Mechanical ventilation (Yes/No), LOS, ICU free days, Age, and Sex. The PSM yielded a balanced (L1 from .859 to .707 and no unbalanced covariates) matched sample of: N = 446 with 16 infections (3.6%). The mBLR was rerun on this matched sample with no change in statistical interpretation from the original mBLR detailed previously.

% (count)	Low Patient Acuity Group	High Patient Acuity Group
N =	1003	52
% treated with antibiotics	55% (547/1003)	58% (30/52)
P - value	.653	
Of those: *% received probiotics*	18% (100/547)	21% (11/52)
P - value	.610	
Statistical Test	z-test for independent proportions	

Table 7: Antibiotic Treatment Pattern by Patient Acuity

Discussion

Two main issues prevent more definitive conclusions from the statistical analyses, namely, low infection rate (3.4%) and the compliance/usage rate of probiotics (15%). The utilization rate was likely negatively impacted by the conversion to EPIC and the process issues that followed during implementation. However, it should be noted that similar ordering compliance rates were found with a study performed by Berry and colleagues(Berry, 2017). They also found low utilization rates even after implementation of an electronic ordering system and formal recommendations of usage by the Medical

Executive Committee. They concluded that practicing physicians were reluctant to order probiotics even after formal recommendation was made by the Medical Executive Committee and education took place. The authors questioned whether ordering physicians had fear of active patient infection, lack of knowledge on probiotics, or whether other factors were influential in their decision. This is an area for further study in our institution.

Other than Injury Severity Score (ISS), an exhaustive investigation of patient acuity was not done due to difficulties with variables of interest not being available such as APACHE scores, co-morbidities; and surgical procedures performed - these are likely confounding variables. Also, the low patient acuity group was shown to have statistically significantly values of LOS and MV occurrence that would likely place those patients at a higher risk of infection.

Limitations

Our study had several limitations. First, as outlined above, it was conducted during a period of paper to electronic ordering modification which likely contributed to lower than expected probiotic utilization rates. Next, low infection rates relative to sample size, particularly after ISS stratification, produced a study that was nontrivially statistically underpowered. There also was a disparity in sample size between the 12 months prior to inclusion of the probiotics order set in electronic admission orders (n= 680) and the 6 months afterwards (n=379). The timing of the initiation of probiotics in relation to the onset of infection was impossible to determine in the trauma database. Although we did exclude 4 infection events based on the timing of initiation of probiotic

usage, there likely were more events that should have been excluded. Further, it is not known how long or how many doses of a probiotic one must take to replenish lost microbiota, so further investigation into this related to infection onset date should be more closely examined. Besides ISS scores, other potential confounding variables that may influence infection were not specifically tracked; namely other means of determining acuity (APACHE, etc.), co-morbid conditions, previous infections, or surgeries. We had no knowledge of the patients' GI microbiome prior to injury. Lastly, this was conducted at a single medical center.

Future Studies

First, investigation into reasoning for the low probiotic utilization rates should be performed and a plan should be developed to increase compliance with the probiotics protocol as indicated. Another area for investigation is to examine individual provider compliance with the protocol. Ordering compliance between attending physician, resident, or trauma nurse practitioners may be different. Once increased utilization is realized, strength of statistical analyses should improve to perhaps provide more solid answers to the research questions. Also, potential co-morbid factors as discussed earlier should be considered when examining the impact probiotics may or may not have on infection risk. This may be influential if a larger sample size is available.

Conclusions

The analyses reveal the importance of hospital processes, specifically regarding the ordering (yes/no) and timing of the probiotics protocol. Probiotics usage seemed to

trend higher for sicker patients and were ordered with increased frequency on certain hospital units. Further, being a relatively novel adjunct therapy that clinicians may not know about or fully trust, communication about the ordering of probiotics needs to be intense and frequent. In this hospital, residents rotate through the trauma service so communication regarding the expectation for ordering probiotics should be clear. This study reached no definitive conclusions regarding the use of probiotics in trauma patients. Some possible reasons for this lack of conclusions may by explained for reasons already stated. However, given the inconclusiveness of past research that has shown both benefit as well as the drawbacks of probiotic use speaks to the necessity of further research.

Summary of Key Points

Healthcare-associated infections are a costly complication of hospitalization. Hospitals are implementing prevention strategies with modest success. New and novel approaches for the prevention of HAIs must be explored.

- Although literature shows that benefits of probiotics likely outweigh any potential risks, some providers may still be hesitant to order them for acutely ill patients

- Trauma patients are at increased risk for the development of an HAI due to the nature of a traumatic injury, but as a result of a host of other factors as well

- Additional research is warranted in pursuit of ascertaining the appropriate use case(s), utility and interaction effects of probiotic usage in the trauma population

- No adverse events related to the administration of probiotics were identified in this study

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