LACTIN-V: a probiotic choice in the prevention of UTIs in women

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DEDICATION

I dedicate this project to my family and friends who have always been supportive of my education and especially to my grandmother for giving me reason to research this topic.
I would like to thank Dr. Brian Harrington for his guidance throughout this project. He provided his extensive knowledge of microbiology and scientific writing, both important components of medicine. His support and patience were integral in the completion of this project and I am ever grateful for his assistance.
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INTRODUCTION

Infectious processes, whether caused by bacteria, fungi, parasites, viruses, or prions, are linked to a very large number of acute and chronic illnesses, such as cystitis, pyelonephritis, upper respiratory tract infections, lower respiratory tract infections, genital tract infections, gastrointestinal ailments, hepatitis, heart disease and even some cancers (Tierney, McPhee, & Papadakis, 2006). Great advances have been and are being made, especially using molecular (genetic) methods and immunotechniques, in determining the cause, or showing an association with infectious agents, of many of the most detrimental conditions. The importance of showing an association between an infectious agent and conditions such as atherosclerosis, some malignancies, Crohn’s disease, and diabetes, is the opening up of possible new treatment options directed against the infecting agent(s).

Antibiotics play a major role in treating many acute infective processes and may reduce the morbidities associated with infections; for example, by treating the acute infection (streptococcal pharyngitis) before the sequelae (rheumatic fever) can develop. Although the benefits of antibiotics are considerable, it is important to understand their limits. Numerous studies have shown that antibiotics are over used and misused (Carey & Cryan, 2003; Kardas, Devine, Golembesky, & Roberts, 2005), leading to a greater incidence in antibiotic resistant bacteria (ARB), such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus species (VRE). There are many ways in which this problem can be addressed. First, diagnostic and treatment criteria should be followed when initiating antibiotic therapy for infections. Second, each geographical area, health care site, and housing facility should track what types of ARB are present in the area to better screen and treat for them. Lastly,
we should develop means to prevent commonly-occurring infections therefore lessening the use of antibiotics and, in turn, giving bacteria fewer opportunities to develop antibiotic resistance.

There are many common infections that are treated with antimicrobial therapy. Many of which involve the respiratory tract and the genitourinary (GU) tract (Tierney, McPhee, & Papadakis, 2006). This review specifically focuses on the GU tract and urinary tract infections (UTIs) specifically. UTIs have a great impact on individual health and health care in America. UTIs affect both genders and all ages, although there are some trends in gender and age, therefore this paper will focus on the female population. There is a great cost and burden on health care involving the diagnosis and treatment of UTIs and untreated UTIs can have traumatic results to the individual, therefore possible ways to prevent UTIs are of great value. (Foxman, Barlow, D'Arcy, Gillespie, & Sobel, 2000; Gupta, Hooton, & Stamm, 2001; Gupta, Scholes, & Stamm, 1999; Stamm & Raz, 1999).
URINARY TRACT INFECTIONS

“Urinary tract infection is a general term that describes infection anywhere within the urinary system: urethra, bladder, ureters, or kidneys. Although a UTI can be caused by any infectious agent that successfully colonizes the urinary tract (including viruses and fungi), most are caused by bacteria” (Foxman & Brown, 2003). UTIs are among the most common community-acquired infections and have a large economic burden on health care, as well as consume a large amount of health care resources (Foxman, Barlow, D'Arcy, Gillespie, & Sobel, 2000). In 2000, a total of cost of $3.4 billion was attributed to the care of individuals with UTIs including 8.2 million office visits, 1.8 million emergency room visits, and 350,000 hospitalizations (Litwin et al., 2005). Antibiotic therapy is the primary treatment of UTIs, but as the occurrence of UTIs increases, the available optimal antibiotic choices may be decreasing due to the growing number of UTIs caused by antibiotic resistant bacteria (Gupta, Scholes, & Stamm, 1999). We may be faced with UTIs that are more difficult to treat and that may be unresponsive to current treatment. This would have a great effect on health care, as costs increase and more serious complications such as pyelonephritis, urosepsis, and perinephric and intrarenal abscesses can develop from mistreated UTIs (Stamm & Raz, 1999). Health care professionals must address this issue and find ways to prevent UTIs from occurring, therefore decreasing cost, antibiotic use, opportunity of ARB development, and morbidity and mortalities associated with UTIs.

The most common pathogen in uncomplicated UTIs, e.g. cystitis and in pyelonephritis, is *Escherichia coli* regardless of patient age, although the percentage of UTIs caused by *E. coli* decreases with age, being institutionalized, and being catheterized, especially with indwelling catheters. In these situations, other pathogens, some of which are intrinsically more resistant
than *E. coli* become more prevalent (Arslan, Azap, Ergonul, & Timurkaynak, 2005; Nicolle, 2001, 2002). Risks associated with an increased susceptibility to infection include: presence of a urinary catheter, incontinence, engaging in vaginal intercourse, spermicide use, female gender, pregnancy, diabetes, prostatic enlargement, and previous UTIs or antibiotic use (Foxman & Brown, 2003; Stamm & Raz, 1999). These risk factors and others vary with the age of the individual as demonstrated in Table 1.

Signs and symptoms usually associated with UTIs are increased urinary frequency and urgency, dysuria, fever, chills, cloudy, bloody, or malodorous urine, suprapubic, flank, or costovertebral pain, and increased incontinence (Midthun, 2004; Woods, 2005). The diagnosis of a UTI requires the presence of both signs and symptoms. Microbiological evidence is most often obtained by way of a urinalysis (UA) and/or culture on microbiological media that support growth of the bacteria and yeasts that commonly cause UTIs. In the UA, usually both the nitrite and leukocyte esterase levels are evaluated, as well as the presence of blood in the urine. An elevated leukocyte presence in the urine (pyuria), either determined enzymatically (the leukocyte esterase), microscopically (the visualization of these white blood cells), or by both techniques, suggests a bacterial infection. Situations that result in a false negative or a false positive result for either or both the nitrite test and the leukocyte esterase test can be attributed to many factors. Some of these factors are: ingestion of certain items such as large amounts of vitamin C, food dyes, synthetic additives and certain medications, difficulty in collecting a midstream urine sample, insufficient dietary nitrate consumption, the type of infecting organism(s), and less opportunity for a morning urine sample because of nocturia (Brown, 2002). Often overlooked is the fact that Gram-positive bacteria and yeasts, both of which are becoming increasingly found in UTIs, do not always give a positive nitrite test. Differential diagnoses of a UTI include:
bacterial vaginosis, sexually transmitted diseases, urethral syndrome, benign prostatic hypertrophy, and dysmenorrhea which are usually excluded by the results of the UA. The literature also stresses the importance of culturing the urine quantitatively, identifying infecting organisms and determining their quantitation, i.e. “colony count” (colony-forming units per milliliter of urine (cfu/mL), and testing significant isolates for their susceptibility or resistance to a panel of antibiotics (the “culture and sensitivity” or “C&S” test). Nicolle recommends doing this before instituting antimicrobial therapy (Nicolle, 2002) so that there is a greater chance of isolating and testing the pathogen(s), therefore ensuring that the pathogen is susceptible to the antibiotic (Nicolle, 2002). There is, however, inconsistencies in the literature as to what constitutes a “significant” colony count and this is especially true in the case of UTIs in women. Historically a colony count of more than or equal to $10^5$ CFU/mL of pathogenic bacteria is necessary to define a UTI. Less than this value may be a sufficient representation of a UTI especially with atypical microorganisms and in samples that are dilute or have spent less time in the bladder of the subject. For this reason, it is important to take other components into consideration, such as symptoms, risk factors, and medical history.

The recommended treatment of symptomatic UTI is oral antibiotic. *The Sanford Guide to Antimicrobial Therapy 2006* recommends the following as primary and treatment regimes: 3 days of trimethoprim/sulfamethoxazole-double strength dosed twice daily, 7 days of nitrofurantoin, or single dose of fosfomycin. As an alternative treatment, *The Sanford Guide* recommends: 3 days of ciprofloxacin (250 mg or extended release), gatifloxacin, or levofloxacin. (Gilbert, 2006). It is recommended that such antimicrobial therapy be initiated only when the patient is bacteriuric, defined as having more than or equal to $10^5$ CFU/mL of pathogenic bacteria in urine, and symptomatic (Nicolle, 2002). The appropriateness of antibiotic choice is a
current concern. Although TMP-SMX (a primary antimicrobial choice) and nitrofurantoin are the most optimal drugs in treatment of UTIs, one study found that these are only used in only 40.5% of cases (Kahan, Kahan, & Chinitz, 2003). Other antibiotics, such as fluoroquinolones and cephalosporins were used as alternatives (Kahan, Kahan, & Chinitz, 2003). Another study found the proportion of TMP-SMX use in UTIs has decreased over the past ten years, while the proportions of nitrofurantoin and fluoroquinolones have increased (Huang & Stafford, 2002). In 2007, the recommended treatment utilizing a TMP-SMX cost $2.34 compared to $26.99 and $22.73 of nitrofurantoin and ciprofloxacin respectively (Epocrates, 2007). One should question why there is a developing trend away from the defined standard of care and what effect this has on health care costs and resources associated with UTIs.

One reason for the increasing use of alternative antibiotic therapy may be a growing number of UTIs that are unresponsive to TMP-SMX. (Gupta, Hooton, & Stamm, 2001; Gupta, Scholes, & Stamm, 1999). UTIs are one of the most common community acquired infection (Foxman, Barlow, D'Arcy, Gillespie, & Sobel, 2000) and are often recurrent, therefore pathogenic bacteria have an opportunity to develop resistance to commonly used antibiotics, such as TMP-SMX. An infection by antibiotic resistant bacteria (ARB) presents a huge problem for health care. More research should be performed to determine the detriment of an infection by ARB and costs associated. Also, therapies other than antibiotics must be developed to use for prophylaxis of UTIs.
An option for the prevention of UTIs is probiotics. “Probiotics are defined as living microorganisms that, when administered in adequate amounts, confer a beneficial effect on the health of the host” (Senok, Ismael, & Botta, 2005). Currently probiotic use in UTI prevention is a developing area of attention and research. The history of probiotic discovery and the possible uses are important to understand before determining the suspected benefit on one’s health. Probiotics have been indicated for use in the oral cavity, the gastrointestinal system, and in the genitourinary system. Probiotic products are not regulated by the FDA, therefore there are minimal regulations and the mislabeling of products has been identified (Hamilton-Miller, Shah, & Winkler, 1999). Because of this problem, the Food and Agriculture Organization of the United Nations (FAO) along with the World Health Organization (WHO) have developed guidelines for the evaluation of probiotics in food (Joint FAO/WHO Working Group, 2002). Studies are necessary to identify which probiotics are useful, the most effective route of administration, and the proper formula.

A leading development in prophylaxis of UTIs is CTV-05, a strain of Lactobacillus crispatus. CTV-05 is found in LACTIN-V, a vaginal insert undergoing phase II clinical trial. This review highlights important discoveries and progress in the development of this therapy. Of interest is the proposed mechanism of action, the indications, and the expectations of LACTIN-V. This therapy could be a break through in the prevention of UTIs and relieve some of the suffering, costs, and health resources associated with them, as well as change much of the thinking of probiotics and the possible uses and benefits.

The field of probiotics has been developing for more than 100 years. The research of probiotics dates back to the early 1900s when Elias E. Metchinkoff studied the role of lactic acid
produced by normal flora in the gut and the field has been developing ever since. Research involving types of probiotics and proposed uses for them is plentiful and growing. A review of the literature will highlight the possible role of probiotics in the treatment of many conditions, including UTIs. The literature reveals: 1) the possible mechanisms of protection by probiotics, 2) the importance of identifying the strains of probiotics, 3) the most common probiotic strains used, and 4) the current problems in the marketing of probiotic supplements. With the current expansion and interest in probiotics, much is being discovered and exploited. Taking a close look at current probiotic products and FAO/WHO recommendations, as well as literature will help differentiate the facts from fiction and reveal possible health benefits of probiotics.

Probiotics, as reviewed by many (de Vrese & Schrezenmeir, 2002; Gupta et al., 1998; Kopp-Hoolihan, 2001; Reid, Jass, Sebulsky, & McCormick, 2003; Senok, Ismaeel, & Botta, 2005), are proposed in the treatment of: gastroenteritis, diarrhea, *Helicobacter pylori* infections, oral ailments such as dental caries, respiratory tract infections, common viral infections, inflammatory bowel syndrome and diseases, cancer, allergies, and genitourinary tract infection, including UTIs. Such claims are often not supported by clinical research, therefore there is a new movement to establish scientific research behind such products, to have a standard evaluation, and to manufacture therapies to be approved and regulated (Joint FAO/WHO Working Group, 2002). Specifically, there may be a role for probiotic use in the treatment of UTIs in the female population. A UTI is most often caused by pathogenic bacteria ascending the urethra (Tierney, McPhee, & Papadakis, 2006). Pathogenic flora that colonize the GU tract causing a UTI are mainly uropathogens such as *Escherichia coli* and other *Enterobacteriaceae* (Falagas, Betsi, Tokas, & Athanasiou, 2006). “Indeed, the presence of pathogens dominating the vagina increases several fold the likelihood that a women will develop a symptomatic infection” (Reid,
Jass, Sebulsky, & McCormick, 2003). The theory behind probiotic use in UTI prophylaxis is to colonize the GU tract with probiotic strains of normal flora and inhibit pathogenic bacteria from colonizing and ascending the GU tract.

The ways in which probiotic strains protect the body are unknown although several mechanisms are theorized. Proposed protective mechanisms of probiotics are: 1) through the production of antimicrobial substances such as, hydrogen peroxides and acids, 2) through competition for binding sites, 3) through competition of nutrients, 4) through blocking pathogen adhesion, and 5) through their stimulation of modulators of the immune system (de Vrese & Schrezenmeir, 2002; Falagas, Betsi, Tokas, & Athanasiou, 2006; Hoesl & Altwein, 2005; Kopp-Hoolihan, 2001). At first glance probiotics seem like the miracle cure to common infections, but it is important to review the data behind these claims.

Many strains of probiotics are commercially available, as shown in Table 2. Strain identification is the initial step in the possible use of probiotics. Studies have found two important things: first, probiotic strains of the same species do not necessarily have the same properties or benefits (Ibnou-Zekri, Blum, Schiffrin, & von der Weid, 2003) and second, the strain of probiotics in available products is often misrepresented by their label (Hamilton-Miller, Shah, & Winkler, 1999). Therefore, much effort has been spent on properly identifying strains of normal flora and of that flora, which strains have the perceived beneficial properties that could act as probiotics. Probiotics are identified and chosen based on desirable properties such as: 1) the ability to survive transit through the digestive tract, 2) adherence to epithelial cells, 3) colonization potential in the respective target organ, 4) production of antimicrobial substances towards the pathogens (Hoesl & Altwein, 2005). Because each strain has specific properties, it is vital that identification is the initial step in the evaluation of a probiotic.
As mentioned above, the probiotic strain selected must be safe for human use. Because probiotics are viable organisms, there are a number of possible adverse reactions, such as: systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals, and gene transfer (Hoesl & Altwein, 2005; Joint FAO/WHO Working Group, 2002; Reid, Jass, Sebulsky, & McCormick, 2003). To assess for safety, the Working Group of the FAO/WHO set forth safety parameters to be met in their reported guidelines (Hoesl & Altwein, 2005; Senok, Ismaeel, & Botta, 2005). Probiotics, especially those used in dairy fermentation, have a long history of minimal adverse reactions but as new strains and uses for probiotics emerge, each safety profile must be examined and evaluated in all populations.

The FAO and the WHO have drafted *Guidelines for the Evaluation of Probiotics in Food* because of the recent boom in probiotics (Joint FAO/WHO Working Group, 2002). “A Working Group was convened by FAO/WHO to generate guidelines and recommend criteria and methodology for the evaluation of probiotics, and to identify and define what data need to be available to accurately substantiate health claims” (Joint FAO/WHO Working Group, 2002). Figure 1 is an outline of the guidelines from the report issued by the Working Group of the FAO/WHO in 2002.

Guidelines to substantiate claims of probiotics are as follows: 1) identify strain by genus and species, 2) in vitro and animal studies to detect mechanism of protection and safety profile of strain, 3) double blind, randomizes, placebo-controlled human trial, preferable with an independent follow up study, 4) trial comparing probiotic with standard treatment, and 5) evaluate labeling integrity, including content identification, number of bacteria in contents, shelf life, storage conditions, and contact information for consumers. By setting forth such recommendations it is the desire that manufacturers of probiotics adopt such principles when
marketing products for the safety of the users and the legitimacy of probiotics (Hoesl & Altwein, 2005).
LACTOBACILLI

Lactobacilli are bacteria that may have a probiotic role in UTI prevention and of interest in this study. Lactobacilli are the most common component of the normal flora found in the healthy female vagina and decreased amounts have been associated with an increase in genitourinary (GU) infections, including UTIs (Burton, Cadieux, & Reid, 2003; Eschenbach et al., 1989; Gupta et al., 1998; Mijac, Dukic, Opavski, Dukic, & Ranin, 2006). Much research has been dedicated to identifying the most common and protective strains and distinguishing the mechanisms that lactobacilli use in protecting the GU tract. A literature review of in vitro studies, in vivo studies of animals and humans, and phase II clinical trial is included here to summarize the current knowledge and benefit of lactobacilli in preventing UTIs.

A focus of this paper is probiotics that may have the potential to be utilized in the treatment and prophylaxis of UTIs, specifically in the female population as UTIs affect females more often than males. One area of current research is dedicated to discovering and synthesizing strains of bacteria that mimic the normal flora of the female GU tract to be used as probiotics. As mentioned previously lactobacilli are the most common bacteria found in the healthy female GU tract. It is thought that this normal flora plays a role in protecting the vaginal mucosa from pathogenic bacteria by competition and through production of bactericidal substances (Hillier, Krohn, Klebanoff, & Eschenbach, 1992).

The determination and the identification of normal vaginal flora is the first step in developing potential probiotics. Determining factors that contribute to a change in the normal flora is important in understanding risk factors of developing UTIs. Also of interest is investigating whether restoration of the vaginal flora by the administration of probiotics strains reduces the occurrence of UTIs. Research of the most common species of lactobacilli found
within GU tract and the mechanisms of protection is the next step in the development of probiotics for UTI prophylaxis. Lastly, determining ability of the strain to adhere to vaginal mucosa, the mechanisms of lactobacilli administration, and the pharmacodynamics and pharmacokinetics will help determine whether there is possibility that the product can be beneficial or useful.

Properties of specific strains of lactobacilli have been shown to have protective mechanisms against pathogenic bacteria in vivo and in vitro studies (Boris, Suarez, Vazquez, & Barbes, 1998; Chan, Reid, Irvin, Bruce, & Costerton, 1985; Eschenbach et al., 1989; Ibnou-Zekri, Blum, Schiffrin, & von der Weid, 2003; Osset, Bartolome, Garcia, & Andreu, 2001; Vallor, Antonio, Hawes, & Hillier, 2001; Velraeds, van de Belt-Gritter, Busscher, Reid, & van der Mei, 2000). Evaluating these properties is an integral part in determining ability of a probiotic strain to be effective against pathogenic bacteria. As with any therapy there are adverse effects and disadvantages and because the use of lactobacilli is emerging as a probiotic agent, most of these are yet to be evaluated although past research shows that lactobacilli can cause endocarditis and disseminated infections (Apostolou et al., 2001).

Determining these and comparing them to the advantages and effectiveness will help discover if probiotic therapy is an effective choice for the treatment of UTIs. Most of the research has used healthy premenopausal women as subjects; it is necessary to determine the side effects and the safety profile of lactobacilli strains in other populations. Lastly, a review of studies of lactobacilli will shed light on the possible clinical role of lactobacilli for UTI prophylaxis.
Lactobacilli are gram positive anaerobes (some are facultatively aerobic) that colonize the moist surface of the vaginal epithelium, intestinal tract, and the oral cavity (Redondo-Lopez, Cook, & Sobel, 1990). The most common species include *acidophilus, fermentum, plantaum, brevis, crispatus, jensenii, casei, celllobiosus, leichmanii, and delbrueckii* (Antonio, Hawes, & Hillier, 1999). Colonization with lactobacilli is linked with decreased GU infections (Eschenbach et al., 1989; Gupta et al., 1998). To find reason for this, research has been directed to determining the beneficial effect that these bacilli have on the GU tract. Studies have found that the different species of lactobacilli have unique properties, some of which are considered protective, and that each species interacts with an environment differently. (Pelletier et al., 1997) Thus, the importance of identifying the exact species of lactobacilli is important. Within the past twenty years, the accuracy of species identification has become more advanced due to DNA fingerprinting and it has been discovered that many species of lactobacilli have been misidentified in past literature. Antonio, Hawes, and Hiller (1999) describe that the previous use of *L. acidophilus*, due to the misidentification that this species was the most dominant vaginal flora, “has been more a historic than a scientific designation” (Antonio, Hawes, & Hillier, 1999).

Antonia, Hawes, and Hiller (1999) isolated lactobacillus from 215 sexually active women by using whole-chromosomal DNA probes and comparing them to 20 American Type Culture Collection of lactobacillus strains. 71% of participants were colonized by lactobacilli. Of these, nearly one-third were colonized by *L. crispatus* and nearly one-fourth were colonized by *L. jensenii*. Nearly 100% of the species of *L. crispatus* and *L. jensenii* produced H₂O₂, thought to protect the vagina from pathogenic organisms. This study concluded that *L. crispatus* and *L. jensenii* are the most common vaginal flora of women of reproductive age (Antonio, Hawes, & Hillier, 1999). Another study involved the characterization of the microbial species found in the
vaginal tract of women of reproductive age using culture-independent methods (Zhou et al., 2004). Analyses of 16S rRNA sequences were compared to gene libraries. The results showed that samples were most commonly related to *L. crispatus* and *L. iners* (Zhou et al., 2004).

The inverse relationship between colonization of lactobacilli and genitourinary infections must be analyzed to demonstrate the effect normal flora has against opportunistic pathogens. One study found that H₂O₂-producing lactobacilli were found in 96% of women without a GU infection compared to 6% of women diagnosed with a bacterial vaginosis (BV), one of many GU infections (Eschenbach et al., 1989). The inverse relationship between BV and the presence of lactobacilli shows that lactobacilli may have a protective quality against uropathogens. Mijac et al. (2006) also found that the percentage of lactobacilli is lower in women with BV when compared to asymptomatic women although there was no statistical significance between asymptomatic women and those with vulvovaginal candidiasis or trichomoniasis. Therefore, it seems as though lactobacilli may only protect against specific pathogenic microorganisms.

The most common pathogen of uncomplicated UTIs is *E. coli*, although in young, sexually-active females *Staphylococcus saprophyticus* is an important uropathogen (Hovelius, Mardh, & Bygren, 1979; Hovelius & Mardh, 1984). Recent data from a university student health center found *Staphylococcus saprophyticus* represented 10% of all significant urine isolates (BGSU Student Health Service, unpublished data, 2003). It is of interest to research whether lactobacilli have protective properties against *E. coli*. Gupta et al. (1998) cultured vaginal samples of 140 women; 65 of whom had history recurrent urinary tract infections (case-patients) and 75 with no history of UTIs (control). In this study participants with recurrent UTIs are defined as having 3 or more UTIs in the past 12 months or having 2 or more UTIs in the past 6 months. None of the 140 women had symptoms of a UTI at the time of the study. Heavy
colonization of *E. coli* was found in 28% of the case-patients compared with 9% of the control group. The lactobacilli cultured from the vaginas were re-isolated and tested for production of H₂O₂. This study found that “women without H₂O₂-producing lactobacilli were more likely than women with H₂O₂-producing lactobacilli to have *E. coli* introital colonization (44% vs. 16%)” (Gupta et al., 1998). On a side note, this study also found that use of spermicide is associated with increased colonization of *E. coli* and decreased colonization of H₂O₂-producing lactobacilli, therefore showing that external factors can affect the normal flora. In conclusion this study supports the protective role of lactobacilli in the vagina and states “in women with altered vaginal flora, recurrent UTIs may be preventable by the repletion of H₂O₂-producing lactobacilli and inhibition of *E. coli* vaginal colonization through use of lactobacillus probiotic.”(Gupta et al., 1998). As mentioned previously, some pathogens other than *E. coli*, such as *S. saprophyticus*, seem to be more prevalent in certain populations. The impact and increased occurrence of UTIs caused by *S. saprophyticus*, as well as other atypical uropathogens, need to be evaluated as young sexually-active females is a group at increased risk for developing UTIs.

Lactobacilli have many unique properties as a genus and each species has its own specific properties. As the species identification is determined, the next step is determining the properties of the species and whether they have any protective value against pathogenic bacteria. One study found that the surface properties of lactobacilli vary by species (Pelletier et al., 1997). Many studies have evaluated the properties of lactobacilli that are thought to have a protective effect against pathogenic bacteria. Some of these properties are: the ability to adhere to vaginal epithelial cells (Chan, Reid, Irvin, Bruce, & Costerton, 1985), H₂O₂ production (Song et al., 1999; Vallor, Antonio, Hawes, & Hillier, 2001), biosurfactant production (Velraeds, van der Mei, Reid, & Busscher, 1996), ability to block uropathogen adhesion (Osset, Bartolome, Garcia,
& Andreu, 2001), and many others. The mentioned properties are thought to interact with uropathogens and make the environment one in which they cannot survive. (Boris, Suarez, Vazquez, & Barbes, 1998) The property of H₂O₂ production by lactobacilli strains is the most common discussed in current research, although there are many other mechanisms thought to be associated with the ability of lactobacilli inhibit the growth of pathogenic microorganisms.

The ability of certain species of lactobacilli to produce H₂O₂ has been shown by multiple studies (Song et al., 1999; Vallor, Antonio, Hawes, & Hillier, 2001). Other studies have evaluated whether these probiotics would adhere to vaginal epithelial cells and thereby have function within the vagina. Chan et al. (1985) incubated lactobacilli cells with uroepithelial cells and challenged the assay with radiolabeled uropathogens. The uropathogen adherence was then quantified. This study concluded two things: 1) lactobacilli readily adhere to uroepithelial cells and that the lopoteichoic acid component of the lactobacilli cell wall was responsible for this and 2) adherent lactobacillus cells greatly inhibited uropathogens from adhering and that the steric hindrance was the mechanisms causing this.

Another study characterized, to the species level, lactobacilli from vaginal samples using a DNA-DNA hybridization method and investigated the species’ ability to produce H₂O₂. Results showed that *L. crispatus* is the most prevalent strain of vaginal normal flora and that 60% of the *L. crispatus* recovered were strongly positive for H₂O₂ production (Song et al., 1999). Another study hypothesized that “H₂O₂-producing lactobacilli are more likely to sustain long-term vaginal colonization than are lactobacilli that do not produce H₂O₂” (Vallor, Antonio, Hawes, & Hillier, 2001). This study showed that 70% of the lactobacilli colonized from 101 women over 303 visits produced H₂O₂. In favor of the hypothesis, women who were colonized by H₂O₂-producing lactobacilli at the first visit were significantly more likely to remain colonized for the following
visits at 4 months and 8 months (Vallor, Antonio, Hawes, & Hillier, 2001). These studies show that H₂O₂ may function as a bactericide as well as a survival mechanism of the lactobacilli.

The above studies show promise for the use of lactobacilli in GU health and prevention of GU infections, such as UTIs, by promoting normal vaginal flora and protecting against pathogenic microorganisms. Several strains of lactobacilli have been identified and studied. Strains are chosen for prophylactic use based on several factors; some of which are the ability to adhere to vaginal epithelial cells and the ability to produce H₂O₂. Studies have shown that *L. crispatus* has these factors.
LACTIN-V

The identification of normal vaginal flora and the discovery of their mechanisms of protection against UTIs are important findings which may have possible therapeutic value in the field of urology. Currently LACTIN-V, a probiotic lactobacilli vaginal insert, is undergoing phase II clinical trial for the prevention of recurrent UTIs. This trial is being sponsored by The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). LACTIN-V is composed of *Lactobacillus crispatus*, CTV-05. An analysis of the current clinical trial will define the use, application, and expected effect of LACTIN-V. Reasoning why this strain was selected will be further explained below. Also presented within this review is a brief synopsis of studies that support the role of LACTIN-V. As mentioned previously, *L. crispatus* CTV-05 is one of the most common strains colonized from healthy (free from symptoms and uropathogenic bacteria) female vaginas (Antonio, Hawes, & Hillier, 1999; Zhou et al., 2004). This strain has been identified by DNA fingerprinting and has demonstrated H₂O₂ production. (Antonio & Hillier, 2003). The selection of *L. crispatus* CTV-05 for LACTIN-V can be evaluated by referring to studies which involve this strain. *L. crispatus* has been shown to adhere to vaginal epithelial cells (Kwok et al., 2006), produce H₂O₂ (Mijac, Dukic, Opavski, Dukic, & Ranin, 2006; Osset, Bartolome, Garcia, & Andreu, 2001; Song et al., 1999; Vallor, Antonio, Hawes, & Hillier, 2001), and be inversely related to vaginal colonization of uropathogens (Boris, Suarez, Vazquez, & Barbes, 1998; Osset, Bartolome, Garcia, & Andreu, 2001). Therefore, evidence shows that CTV-05 has the ability to adhere and grow in the environment in which it is being administered. Also, with studies demonstrating the ability of CTV-05 to produce H₂O₂, there is evidence of a mechanism that is shown to be bactericidal.
The effects of *L. crispatus* have been evaluated in animal models. Although a perfect animal model does not exist, it gives information on how a therapeutic agent may respond once administered in humans. One study evaluated whether a vaginal capsule of CTV-05 would alter the vaginal microflora of the primate Macaca nemestrina commonly known as the pigtail macaque. This study found that colonization of CTV-05 was found in 30% of subjects and was tolerated well, as there were no colposcopic changes in the vagina or cervix of any of the subjects.

Another study evaluated the identification of CTV-05 after the insertion of gelatin capsules containing $10^8$ CFU of CTV-05 in females. The purpose of this study was to determine if repetitive element sequence-based PCR (rep-PCR), a DNA-fingerprinting technique, can distinguish the administered probiotic strain from other lactobacilli strains (Antonio & Hillier, 2003). After administration of the probiotic capsule, samples were taken from the vaginas of the participants and analyzed using DNA fingerprinting. Identification of the inserted CTV-05 strain from these samples provides evidence of successful colonization of the strain. This study resulted in accurate identification and successful colonization of CTV-05 after probiotic administration in 7 of the 9 women participants (Antonio & Hillier, 2003). This study provides background support of the use of CTV-05 as found in LACTIN-V as it successfully colonized subjects.

The results of in vitro and in vivo studies provide support for the possible use of LACTIN-V in humans for the prophylaxis of UTIs. The phase II clinical trial is only the beginning of a long and appropriate evaluation of LACTIN-V. Much is left to be discovered; mainly the effectiveness of LACTIN-V, how it compared to standard antibiotic treatment of UTIs, and the safety profile in humans have yet to be adequately studied. Previous work has
given a glimpse of what LACTIN-V may be able to accomplish and if successful, the field of probiotics will continue to grow and develop.

In the phase II clinical trial, 100 participants are enrolled; they are all premenopausal women with history of recurrent urinary tract infection. Half of the participants will receive the placebo and the other half will undergo treatment with LACTIN-V. This regime consists of vaginal suppositories of \textit{L. crispatus} CTV-05 with a dose of \(5 \times 10^8\) CFU/suppository. The administration schedule is as follows: daily suppository for days 1-5, followed by weekly suppositories for the next 10 weeks. Therefore the case patients will receive a total of 15 suppositories over 11 weeks. “The purpose of this study is to evaluate the safety of the vaginal suppository LACTIN-V (\textit{Lactobacillus crispatus} CTV-05) as well as the efficacy of LACTIN-V use in preventing recurrent urinary tract infections (RUTI) in women” (National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), 2007). Both the growth and presence of lactobacilli and uropathogens will be evaluated, as well as UTI symptoms and any adverse reactions to LACTIN-V. Participants will be evaluated 5 times during the study. Laboratory procedures at the visits will evaluate the effect of LACTIN-V on the subjects. The laboratory procedures are as follows:

1) microscopic examination of wet mount slides of vaginal fluid at all visits.

2) urine dipstick tests and urinalysis at all visits.

3) urine pregnancy testing at visits 1, 2, and 5.

4) pap smear at visit 1.

5) testing for gonorrhea and chlamydia at visit 1.

6) urine culture at all visits.

7) gram stain and culture of vaginal fluid specimens at all visits.
The hypothesis is that the vaginas will become colonized by *L. crispatus* and this strain will reduce the recurrence rate of UTIs.

(National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), 2007)
CONCLUSION/DISCUSSION

LACTIN-V and other similar products will continue to develop if the phase II clinical trial of LACTIN-V has promising results in the prophylaxis of UTIs. Many people are ailed by UTIs and health care resources and costs utilized reflect their common occurrence. Certain factors increase one’s risk of developing a UTI as mentioned previously. The population that LACTIN-V is being tested on is sexually active premenopausal females that have a history of recurrent UTIs. There are other groups that are at increased risk for UTIs, many of which are recurrent, that the use of probiotics has yet to be evaluated in. These groups include postmenopausal women, pregnant women, those that are incontinent, nursing home patients, and immunocompromised individuals. It is important to investigate whether a prophylactic therapy is beneficial for the majority of the individuals at increased risk and whether the safety profile of the therapy changes based on the characteristics of the user. For example, one review states that immune suppressed individuals and those with underlying conditions are more at risk for adverse reactions such as: endocarditis, meningitis, pneumonia and local suppurative infections (Apostolou et al., 2001). Therefore, probiotics may not be a safe option in many individuals. As more studies are completed, information regarding lactobacilli will emerge. Discovering the benefits remains as one of the most important findings of such studies but it is equally important to monitor the safety profile of lactobacilli in each study. Thus far, few serious adverse effects regarding the use of lactobacilli have been noted. This could be due to two reasons, among others: 1) few human studies have been performed and 2) the characteristics of the subjects lack diversity. It is important to evaluate the safety profile in all populations, especially in those that are at the most risk for UTIs.
The most common pathogen associated with UTIs is *E. coli*. For this reason, most research regarding lactobacilli has been dedicated to the characteristics of lactobacilli that protect the vaginal mucosa from *E. coli*. One reason that LACTIN-V is composed of CTV-05, a strain of *L. crispatus*, is because CTV-05 has shown protective properties against *E. coli* on vaginal epithelial cells. The evidence of lactobacilli’s effects against uropathogens other than *E. coli* is lacking. It is important to investigate whether properties of lactobacilli are effective against other organisms. If LACTIN-V proves to have a promising effect against UTIs caused by *E. coli*, we need to consider the possibility that UTIs caused by atypical organisms may increase. Therefore two areas are in need of more research: 1) the effect of lactobacilli on a wider spectrum of bacteria and 2) if preventing *E. coli* associated UTIs would cause other uropathogens to become more prevalent.

One reason for finding ways to prevent infections, such as UTIs, is to limit antibiotic use and thereby lessen the chance of bacteria to become resistant to commonly used antibiotics. There is data regarding the emergence and increased occurrence of infections caused by ARBs. Of interest to this review is the effect ARBs have on UTIs; whether they are becoming more common, if increased morbidity and mortality are associated, and the related cost. To date there is minimal data regarding the specific role of ARB in UTIs. This information would support the need for preventive medicine in the prophylaxis of UTIs.

The main role of probiotics, such as lactobacilli/LACTIN-V, is in preventative medicine. As discussed above, the use of probiotics has been proposed in the treatment for a number of ailments. The use of probiotics will gain more credibility if multiple studies demonstrate the benefits. LACTIN-V is in the process of being evaluated and compared to the current standard
treatment. By undergoing clinical trials, LACTIN-V is on course to be possibly reviewed by the FDA. It is important to keep in mind that few drugs/therapies that undergo clinical trials are actually proven as appropriate treatments and later approved by the FDA. Therefore, although LACTIN-V shows promise, there is a great chance that it may never be approved or utilized in the prophylaxis of UTIs.

In conclusion, due to prevalence of UTIs and the emergence of ARB there is a need to develop means to treat UTIs without using antibiotic therapy. Probiotic choices, such as LACTIN-V, show promise as preventative therapies by showing protective effects against uropathogens and ability to adhere to vaginal epithelial cells. Much needs to be further evaluated before determining whether LACTIN-V is beneficial as a therapy such as: its safety profile in a diverse population, its protective value against a spectrum of uropathogens, and if its use is practical and possible in the majority of at risk populations (e.g. nursing home patients). Current research of lactobacilli and LACTIN-V supports reason to further evaluate its use in preventive medicine and hopes are that it will provide an alternative treatment option to UTIs and lessen their occurrence and the minimize the development of ARB.
REFERENCES


Bowling Green State University Student Health Center Laboratory (2003) Bacterial Antibiogram, Fall Semester


Table 1. Estimated incidence, markers of exposure to potential uropathogens (risk factors), and host susceptibility for urinary tract infection for females by age.

<table>
<thead>
<tr>
<th>Females (age)</th>
<th>Estimated % incidence per year</th>
<th>Markers of exposure to potential uropathogens</th>
<th>Host susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infancy (&lt;1y)</td>
<td>2.1</td>
<td>Catheterization Hospitalization Urethral-anal distance</td>
<td>Genetic predisposition Anatomic abnormalities</td>
</tr>
<tr>
<td>Childhood</td>
<td>3</td>
<td>Catheterization Hospitalization Urethral-anal distance</td>
<td>Genetic predisposition Anatomic abnormalities</td>
</tr>
<tr>
<td>Adulthood, pre menopausal (17 to 39) (40-59)</td>
<td>15.2 &amp; 11.4</td>
<td>Vaginal intercourse Other sexual activity Catheterization Hospitalization Urethral-anal distance</td>
<td>Genetic predisposition Anatomic abnormalities Trauma (vaginal intercourse, condom use) Obstruction (diaphragm use, pregnancy) Changes in vaginal flora (spermicide use, antibiotic use, menstrual cycle, diaphragm use, pregnancy) Diet Exposure to cold</td>
</tr>
<tr>
<td>Post menopausal (60-79)</td>
<td>9.7</td>
<td>Sexual activity Catheterization Hospitalization Urethral-anal distance</td>
<td>Genetic predisposition Anatomic abnormalities Trauma (sexual activity, condom use) Changes in vaginal flora (menopause, hormone-replacement therapy, antibiotic use) Anatomic changes with again or disease (cystocele, prolapse) Diet Exposure to cold</td>
</tr>
<tr>
<td>Senior (80 and older)</td>
<td>10.5</td>
<td>Sexual activity Catheterization Hospitalization Urethral-anal distance</td>
<td>Genetic predisposition Anatomic abnormalities Trauma (sexual activity, condom use) Changes in vaginal flora (hormone-replacement therapy, antibiotic use) Anatomic changes with again or disease (cystocele, prolapse) Diet</td>
</tr>
</tbody>
</table>

Table 2. Commercially Available Probiotics

<table>
<thead>
<tr>
<th>Lactobacillus species</th>
<th></th>
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<tbody>
<tr>
<td>L. acidophilus</td>
<td></td>
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<tr>
<td>L. casei</td>
<td></td>
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<tr>
<td>L. fermentum</td>
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<tr>
<td>L. gasseri</td>
<td></td>
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<tr>
<td>L. johnsonii</td>
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<tr>
<td>L. lactis</td>
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<tr>
<td>L. paracasei</td>
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<tr>
<td>L. plantarum</td>
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<tr>
<td>L. reuteri</td>
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<tr>
<td>L. rhamnosus</td>
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<tr>
<td>L. salivarius</td>
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<tr>
<td>Bifidobacterium species</td>
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<tr>
<td>B. bifidum</td>
<td></td>
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<tr>
<td>B. breve</td>
<td></td>
</tr>
<tr>
<td>B. lactis</td>
<td></td>
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<tr>
<td>B. longum</td>
<td></td>
</tr>
<tr>
<td>Streptococcus species</td>
<td></td>
</tr>
<tr>
<td>S. thermophilus</td>
<td></td>
</tr>
<tr>
<td>Yeasts &amp; Molds</td>
<td></td>
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<tr>
<td>Saccharomyces boulardii</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Guidelines for the Evaluation of Probiotics for Food Use

Strain identification by phenotypic and genotypic methods (Detailed in Section 3.1)
- Genus, species, strain
- Deposit strain in international culture collection

Functional characterization (Detailed in Section 3.2)
- \textit{In vitro} tests
- Animal studies

Safety assessment (Detailed in Section 3.3)
- \textit{In vitro} and/or animal
- Phase 1 human study

Double blind, randomized, placebo-controlled (DBPC) phase 2 human trial or other appropriate design with sample size and primary outcome appropriate to determine if strain/product is efficacious (Detailed in Section 3.4)

Preferably second independent DBPC study to confirm results

Phase 3, effectiveness trial is appropriate to compare probiotics with standard treatment of a specific condition

Labeling (Detailed in Section 3.5)
- Contents – genus, species, strain designation
- Minimum numbers of viable bacteria at end of shelf-life
- Proper storage conditions
- Corporate contact details for consumer information.

Probiotic Food

Abstract

Objective. LACTIN-V, a vaginal insert composed of Lactobacilli crispatus, is undergoing phase II clinical trial for evaluation in the treatment/prevention of recurrent UTI. Lactobacilli and UTIs were evaluated to assess the possible role of LACTIN-V in UTI treatment. Method. A review of literature on urinary tract infections, probiotics, lactobacilli, and LACTIN-V was utilized using MEDLINE, PUBMED, and OHIOLINK data bases. Results. L. crispatus, as in LACTIN-V, was chosen for use as a probiotic due to its protective properties and ability to adhere to vaginal epithelial cells. The presence of vaginal lactobacilli is inversely related to UTIs. Conclusion. L. crispatus has protective properties against E. coli. The administration of LACTIN-V to restore normal flora shows promise as a treatment option in UTIs. Needing more research is the safety profile of LACTIN-V in a more diverse population, the practicality of use, and its effect on a wider range of uropathogens.