Provisional chapter

Immune-Based Treatment Strategies for Patients with Recurrent Urinary Tract Infections – Where Are We?

Thomas Nelius, Christopher Winter,
Julia Willingham and Stephanie Filleur

Additional information is available at the end of the chapter

1. Introduction

Urinary tract infections (UTI) are among the most common bacterial infections in humans. Approximately 50%-60% of adult women experience a UTI during their lifetime. Furthermore, a significant number of patients can be characterized as having recurrent UTIs if they meet the criteria of ≥2 uncomplicated UTIs in 6 months or ≥3 positive cultures within the preceding 12 months. Applying this definition, it is estimated that recurrent UTIs affect 25% of women with a history of UTI.

E. coli represents the main causative pathogen in recurrent UTI and is responsible for approximately 80% of all episodes of infection. Further important pathogens include Proteus mirabilis, Staphylococcus saprophyticus, and Klebsiella pneumonia.

Symptomatic UTIs cause significant discomfort such as dysuria, polyuria, and suprapubic tenderness. If left untreated, a UTI can progress to acute pyelonephritis with the risk of permanent renal scarring and loss of renal function.

Patients with recurrent urinary tract infections undergo frequent antibiotic treatment and/or low-dose antibiotic prophylaxis. Additionally, a subset of patients is required to undergo a systematic radiological and endoscopic evaluation of the urinary tract in order to rule out any underlying structural abnormalities or urinary calculi. The immense use of antibiotics for the treatment of urinary tract infections has resulted in the development of considerable bacterial resistance and therefore, increasing difficulties in eradicating infections. Due to the development of bacterial resistance, UTIs are a substantial economic burden and a noteworthy public health issue. Therefore, new treatment strategies and preventive measures against UTIs such as immune-stimulation/modulation, vaccine development, the use of pro-

biotics, and the instillation of attenuated bacteria into the urinary bladder are currently being researched.

This chapter will review the most recent literature and provide up-to-date information on developments in immune-based treatment strategies for patients with recurrent UTIs from a pre-clinical and clinical point of view.

2. Material and methods

To identify all relevant materials, comprehensive literature searches were performed via the data sources: MEDLINE, EMBASE, CINAHL and OVID using the key words: urinary tract infection, urine culture, UTI, vaccines, adherence, fimbriae, biofilms, probiotics. Relevant articles and references between 1970 and 2012 were reviewed and analyzed. Reference lists from relevant review articles were also searched. Only articles published as formal papers in peer-reviewed journals were selected for inclusion if they reported findings of interest. The data base searches resulted in 710 articles, of which 75 of 710 pertained directly to immune-based treatment strategies. The entirety of these articles was reviewed, forming the basis for the current review.

3. Results

Plant-derived therapies have long been used in Ayurvedic and traditional Chinese medicine (Wollenweber, 1988). The interest in plant antimicrobials for treatment or prevention of UTIs has been driven both by the prevalence of antibiotic-resistant uropathogens and growing popularity of complementary and alternative medicine. Still, little evidence exists for the effectiveness of these treatments and therapeutic dose requirements. More than 5000 plant polyphenols have been identified so far. The spectrum of biological effects include anti-microbiol, anti-inflammatory and anti-carcinogenic activities (Beretz et al., 1978). Plant derived extracts contain different chemical compounds with multiple antimicrobial activities (Burt, 2004). The most studied species include cranberry, berberine, blueberry, bearberry, and certain herbs such as cinnamon. According to Ohno et al., the potential for bacteria to develop resistance to plant derived anti-microbials is relatively small (Ohno et al., 2003).

3.1. Cranberry

Cranberry, Vaccinium macrocapron, is the best known and most studied plant-derived therapy for UTIs (Seeram, 2008). Historically its antimicrobial effects were believed to be due increased excretion of hippuric acid and urinary acidification, although this was disproved in the 1950's (Bodel et al., 1959). More recent studies have proved Cranberry compounds fructose and proanthocyanidin to inhibit E. Coli adhesins *in vitro*. This finding has given rise to the currently held hypothesis that cranberry extracts prevent E. Coli adhesion to bladder mucosa, thus decreasing the incidence of UTIs (Howell et al., 2005, Lavigne et al., 2008, Liu

et al., 2006, Ofek et al., 1991, Ohnishi et al., 2006). More recently, other compounds such as flavanoids, anthocyanins, catechin, triterpenoids, organic acids and ascorbic acid were identified as constituents (Raz et al., 2004). A wide variety of cranberry products are employed as treatment, the most common are cranberry juice concentrate, cranberry juice cocktail, and capsules. In respect to prevention, randomised trials suggest that cranberry juice or cranberry-concentrate tablets reduce the risk of symptomatic recurrent infection by 12-20%, especially in pre-menopausal women (Avorn et al., 1994, Kontiokari et al., 2001, Stothers, 2002). The same results do not apply to men, elderly patients, or those requiring catheterization (Jepson &Craig, 2008). When compared to the current standard of antibiotic treatment, trimethoprim-sulfamethoxazole 480mg daily is more effective than cranberry capsules 500mg twice daily to prevent recurrent UTIs over 12 months (Beerepoot et al.). High withdrawal rates were common in these trials, as was the inability to confirm compliance with cranberry prophylaxis. While some randomized clinical trials could not demonstrate that cranberry is beneficial (Jepson et al., 2000), other clinical and epidemiological studies support the use of cranberry in maintaining a healthy urinary tract (Perez-Lopez et al., 2009). Another proposed mode of action is the non-enzymatic generation of nitric oxide under acidic conditions(MacMicking et al., 1997). Nitric oxide has significant anti-microbial activity. Up until now, no evidence exists that cranberry extracts are effective to treat UTIs, while some data support its use as prophylactic agent in the prevention of UTIs (Guay, 2009).

3.1.1. Berberine

Berberine sulfate is an alkaloid found in the Berberine arisata plant, as well as the roots of Oregon Grape (Mahonia aquifolium), Goldenseal (hydrastis canadensis), and Goldenthread (Coptis chinesis). It is present in the root, rhizome, and stem bark of the plants (Yarnell, 2002). Head et al. could show that berberine extracts are effective against a variety of organisms, including bacteria, viruses, fungi, and protozoans (Head, 2008). Growth inhibitory effects were described for several bacterial pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa,, E. coli, Bacillus subtilis and Chlamydia (Cernakova &Kostalova, 2002, Head, 2008, Scazzocchio et al., 2001). In one study berberine was found to decrease synthesis and expression of E. Coli Pap fimbriae, thus decreasing bacterial adhesion to epithelia *in vitro* (Sun et al., 1988). Similarly, berberine sulfate was found to prevent Streptococcus pyogenes to adhere to host cells (Sun et al., 1988). Another potentially important mode of action, is the alteration of the bacterial cell division through targeting the FtsZ protein (Domadia et al., 2008). However, the mechanisms behind the anti-microbial properties of berbine are not well studied. Although berberine has been included in Chinese urinary medications for centuries, no *in vivo* studies or clinical trials have been published to evaluate its effectiveness.

3.1.2. Blueberry

Blueberry, part of the Vaccinium genus, is closely related to cranberry. Studies suggest that high molecular weight pranthocyanidins found in wild blueberry inhibit E. Coli adhesion *in vitro* (Head, 2008, Ofek et al., 1996, Ofek et al., 1991). It was also shown, that the constituents

in blueberry extracts similarly to those in cranberry extracts inhibit uropathogenic E. coli (UPEC) from adhering to the uroepithelial cells using the mannose-resistant adhesin. This was measured by the ability of prepared blueberry fractions to suppress the agglutination of human red blood cells after incubation with UPEC strains (Schmidt et al., 2004). No relevant trials of blueberry alone for UTI prevention or treatment were found at this time.

3.1.3. Bearberry

Bearberry, Arcystaphylos uva-ursi, is a member of the Ericaceae family, as are cranberry and blueberry. The glycoside arbutin is the main constituent of bearberry leaves and dried preparation. Arbutin is metabolized into hydroquinone, which has a urinary availability of approximately 65% of the administered arbutin dose (Schindler et al., 2002). Hydroquinone is an antimicrobial according to *in vitro* studies, although there are no *in vivo* studies to prove its effectiveness (Frohne, 1970). Turi et al. showed that growth of clinical isolates of UPEC in the presence of Arcystaphylos uva-ursi extracts change microbial cell surface characteristics by increasing the microbial cell surface hydrophobicity, thereby decreasing their ability to adhere to host cells (Turi et al., 1997). Furthermore, Arcystaphylos uva-ursi was described as diuretic and anti-inflammatory agent, effects that are supportive in the treatment of UTIs (Beaux et al., 1999, Kubo et al., 1990).

Currently, bearberry is used in Germany to cure mild UTI, defined as asymptomatic or bacteriuria less than 10^5 colony-forming units/mL (Schindler et al., 2002). It is not, however, recommended for long-term prevention due to concern of hydroquinone carcinogenic effects (DeCaprio, 1999).

3.1.4. Trans-cinnamaldehyde

Trans-cinnamaldehyde is an extract of the bark of cinnamon (Adams et al., 2004). It has a wide margin of safety between conservative estimates of intake and no observed adverse effects (Adams et al., 2004). *In vitro* studies demonstrated antibacterial activity against Clostridium botulinum, S. aureus, E. coli O157:H7 and Salmonella Typhimurium. Recently, it was shown that trans-cinnamaldehyde has the ability to inhibit UPEC biofilm formation on urinary catheters and to inhibited the adhesion and invasion of uroepithelial cells by UPEC by down regulation of major virulence genes (Amalaradjou et al.). Other antimicrobial mechanisms include changes in the permeability of cell membranes, inhibitory effects on enzymes such as amino acid decarboxylases and inhibiting the production of virulence factors (Gill &Holley, 2006, Sikkema et al., 1994, Smith-Palmer et al., 2002).

These results justify further pre-clinical studies and make trans-cinnamaldehyde a potential candidate for use as an antimicrobial agent for controlling UTIs.

3.1.5. Others

The existing literature lists many other herbs for the treatment of UTIs, while most of them lack a scientific basis for this purpose. They include Taraxacum officinalis leaf (dandelion), Juniperus communis (juniper), Hydrangea aborescens (hydrangea), Agrimonia eupatoria

(agrimony), Elymus repens (couchgrass), Althea officinalis (marshmallow), Zea mays (corn silk), Apium graveolens (celery seed), Ulmus fulva (slippery elm), Arctium lappa (burdock), and Mentha piperita (peppermint). For others some scientific background exists. Extracts from Salvia officinalis (Garden sage or common sage) has been shown to be active against Klebsiella, Enterobacter species, UPEC, Proteus mirabilis and Morganella morganii obtained from the urine samples from patients with UTIs (Pereira et al., 2004).

Another potentially useful herb is Barosma betulina (bachu). It has been used in the treatment of UTI and urethritis for a long time. It was found to have anti-microbial effects against uropathogens *in vitro* and diuretic properties (Simpson, 1998).

3.2. Vaginal probiotics/lactobacilli

Studies could demonstrate that Lactobacilllus species in the form of probiotics reduced the risk of UTIs and vaginal infections (Bruce &Reid, 2003, Reid et al.,1985). However, despite ongoing research the mechanisms are poorly understood (Spiegel et al., 1980). In the literature were different mode of action proposed including downregulation of pro-inflammatory cytokines (IL-6, IL-8, TNF-alpha) (Anukam et al., 2009), production of hydrogen peroxide, which protects against the UPEC (Czaja et al., 2007) production of a 29-kDa biosurfactant proteins which inhibits bacterial adhesion (Osset et al., 2001, Xia et al., 2006). Some of the commensal bacteria such as Lactobacillus species and Bifidobacteria species are known to produce immunoregulatory factors that modulate the immune response and may therefore decrease UTIs (Wilson et al., 1998). From a clinical standpoint, there is a close correlation between loss of the normal genital microbiota, particularly Lactobacillus species, and an increased incidence of genital and bladder infections. Vaginal colonization with Lactobacillus spp. was found to prevent recurrent UTIs (Bruce &Reid, 1988, Reid et al., 1992). A Phase I clinical trial assessed the safety of the use of vaginal Lactobacillus suppositories to prevent recurrent (Czaja et al., 2007). Reid et al. investigated in a randomized study the role of probiotic lactobacilli in controlling re-infection in women after treatment of an acute UTI (Reid et al., 1992). 235 patients were treated with antibiotics for three days. Recurrence occurred in 41% of the patients. Individuals with recurrence were randomly assigned to Lactobacillus suppositories treatment group or placebo suppository group. Treatment was given twice weekly for two weeks, then once a month for the next two months. The recurrence rate was 21% in the Lactobacillus group compared to 47% in the placebo group. Oral use of Lactobacilli was also investigated. Reid et al. demonstrated L. rhamonosus GR-1 and L. fermentum RC-14 taken orally lead to colonization of vaginal epithelium within one week. In pediatric patients the oral intake of L. rhamnosus GG resulted in a reduction in UTI incidence rate compared to the placebo group (Dani et al., 2002). These results are promising and warrant further pre-clinical and clinical studies for the use of probiotics in controlling UTIs.

3.3. Immuno-stimulation/vaccines

Currently, low-dose antibiotic treatment has been the most effective prophylaxis for the prevention of recurrent UTI. However, cessation of antibiotic treatment usually results in recurrence of infection in most patients (Ha &Cho, 2008). Furthermore, with the number of

antibiotic resistant strains of uropathogenic bacteria causing recurrent UTI on the rise, a substantial amount of research in alternative treatment modalities such as immuno-stimulation and attenuated vaccines is being performed. The target of such modalities consists of the Mucosa-Associated Lymphoid Tissue (MALT) lining much of the GI, respiratory, and genitourinary tract, the activation of specific receptors on dendritic cells within the genitourinary tract, and increasing the concentration bacteria-specific immunoglobulins (Ha &Cho, 2008). The objective of such therapies is to reduce the frequency and severity of UTI as well as reduce the consumption of antibiotics used to treat them.

Uro-Vaxom® (OM-89) is an extract of 18 strains of E. coli that is obtained by alkaline lysis, which destroys lipopolysaccharide (LPS) molecules produced by the gram negative bacteria and modifies other bacterial antigens, maintaining their antigenic potential (Ha &Cho, 2008). Taken orally, Uro-Vaxom® achieves immuno-stimulation by increasing the proliferation of Mucosal Associated Lymphoid Tissue (MALT) within the GI tract leading to elevated concentrations of bacteria-specific IgG, mucosal-derived IgA along with serum IgA. Furthermore, OM-89 increases the rate of dendritic cell maturation within the genitourinary tract, leading to increased numbers of circulating memory T-cells as well as an overall increase in inflammatory mediators such as TNF-alpha, IL-2, and IL-6 (Bessler et al., 2009). Treatment studies of sexually active females between the age of 20 and 50 shows that Uro-Vaxom® reduces the recurrence of UTI by 36% after 6 months of therapy compared to placebo (Bessler et al., 2009).

Solco Urovac, which is currently under phase 2 clinical trials, is a treatment modality that utilizes the vaginal mucosa via suppository to induce an immune response. The Solco Urovac vaccine consists of 6 E. coli strains along with several recombinant antigenic factors such as fimbriae proteins and 1 strain each of Proteus mirabilis, Morganella morganii, Klebsiella pneumonia, and Enterococcus faecalis (Hopkins et al., 2007). SolcoUrovac works in a similar manner to that of OM-89, however it achieves a higher overall mucosal concentration of IgA in the vagina and bladder. One characteristic of SolcoUrovac is that it must be administered on a booster schedule or else the maximum efficacy will not be achieved. The overall reduction of UTIs in female patients between the age of late teens to early 70s treated with SolcoUrovac plus multiple boosters was measured to be 46.0% (Hopkins et al., 2007).

Siderophores, such as the IroN-cell surface protein found on most strains of E. Coli, are potential targets for immune-therapy. A significant systemic-compartment IroN IgG-specific antibody response developed in serum. However, there was no IroN IgA-specific antibody response in either the systemic or the mucosal compartment. Subcutaneous immunization with denatured IroN resulted in a significant IroN immunoglobulin G (IgG)-specific response in serum but not a systemic or mucosal IroN-specific IgA response (Russo et al., 2003).

3.4. Inhibition of bacterial adhesion

UPEC strains express a number of virulence factors used for colonization of their host. One important virulence factor is located on type 1 pili, allowing UPEC to adhere and invade host cells within the urinary tract. FimH interactions with several host factors have been

documented. These include components of the glycocalyx that sparsely covers the bladder surface, carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family members, soluble Tamm-Horsfall protein, the glycosylphosphatidylinositol (GPI)-anchored protein CD48, the leukocyte adhesion molecules CD11 and CD18, and uroplakin 1a (Klein et al.). Adherence to mannose residues prevents the rapid clearance of E. coli from the urinary tract by the flow of urine and enables the invasion of the host cells (Wellens et al., 2008). The binding of type one pili to bladder epithelial cells activates the innate immune system via the Toll-like receptor 4 pathway which recruits neutrophils and other inflammatory mediators to the site of insult (Wellens et al., 2008). Next, the complement system opsonizes the UPEC and counter intuitively facilitates invasion of the bacteria into the epithelial cell.

Type 1 pili are the most prevalent fimbriae encoded by UPEC, consisting of the four subunits FimA, FimF, FimG, and FimH, the latter located at the tip of the pili (Wellens et al., 2008). The FimH moiety, which is the primarily subunit responsible for the initiation of bacterial adherence and invasion, is a prime target of immune-stimulation. Experiments done in mice have shown that both direct inoculation with immunogloblins against FimH, innoculation with synthetic peptides within the binding moiety for FimH, or gene knock-out of FimH could specifically block type 1 fimbriae-mediated bacterial adherence to bladder epithelial cells resulting in a dramatic loss of virulence(Thankavel et al., 1997). In experiments that utilized synthetic peptides to induce an immune response, dramatic increases in serum IgG and IgM were observed via ELISA (Thankavel et al., 1997). In addition, urine collections showed a marked increase in IgG and mucosal derived IgA along with elevated activity of mast cells within the bladder epithelium when faced with a bacterial challenge (Thankavel et al., 1997).

3.5. Inhibition of bacterial biofilms

Catheter–associated urinary tract infections are common and often related to the existence of bacterial bio-films. Progress in this area is limited. Preventive strategies include avoiding unnecessary catheterization, limit duration of catheterization, using closed drainage system, and appropriate hygiene, including frequent catheter changes and emptying of the drainage bags (Jacobsen et al., 2008, Newman, 1998). Many mechanisms of biofilm resistance against antibiotic treatment were reported over the years. The most important type of the biofilm resistance is the development of a diffusion barrier formed by the bio-film matrix (Ishida et al., 1998). The biofilm prevents access of antimicrobial agents and even of antibodies. Despite the antibiotic treatment, the infection often persists until the device is removed (Schierholz &Beuth, 2001). Over the last 2 decades different coatings were explored to reduce the risk of bacterial bio-film formation and bacterial colonization and subsequently infection. Coating substances included various antibiotics, silvercoating and others. Infections could not be prevented, however the “abacterial window” could be prolonged. Schaeffer et al. demonstrated that in patients with acute spinal cord injury, who received long-term urinary catheters, the silver-coated catheters delayed but did not prevent the onset of bacteriuria (Schaeffer et al., 1988). The same findings were reported for antibiotic impregnation of catheters (Darouiche et al., 1999, Guay, 2001, Johnson et al., 1999). These findings are important

for short-term use of urinary catheters but not for long-term use (Trautner &Darouiche, 2004). Several other substances were reported to inhibit development of bacterial bio-film formation including type A proanthocyanidins, hesperidin, apigenin,naringin and rhoifolina, and others. None of these substances made it into clinical trials so far.

3.6. Stimulation of cyclic adenosine/forskolin

One important survival mechanism of UPEC is the creation of an intracellular reservoir. Within the epithelial cell, the UPEC are able to resist antibiotic treatment by binding to Rab 27 b/CD 63 positive vesicles. Forskolin, the active component of the Coleus forskohlii, has been proven to increase the content of cyclical adenosine monophosphate (cAMP) in urothelial cells, leading uropathogenic bacteria to exit the urothelial cells. It was shown that cAMP levels regulate the exocytosis of these vesicles depending on the bladder distension. These findings could lead to new approaches for the treatment and prevention of recurrent urinary tract infections (Bishop et al., 2007, Gonzalez-Chamorro et al.).

3.7. Hormone therapy

Hormonal deficiency in postmenopausal women results in thinning of the vaginal and urethral mucosa and more importantly to disruption of the normal vaginal flora and therefore to an increased risk for UTIs (Head, 2008). Several studies could demonstrate, that replacing estrogene in this patient population can reduce the incidence of UTIs.

In a randomized study postmenopausal women received either intra-vaginally administered estradiol or placebo. At the end of the study a significant reduction in the incidence of UTIs in the treatment group compared to the placebo group was noted. Of interest is also the observation that Lactobacilli that were absent in the vaginal cultures of patients of the treatment group at the beginning of the trial reappeared in 61% (Raz &Stamm, 1993).

3.8. Instillation of attenuated bacteria into the urinary bladder

Darouiche et al. conducted a prospective, randomized, placebo-controlled, double-blind pilot trial to examine the efficacy of bacterial interference in preventing urinary tract infection (Darouiche et al., 2005). In this study 27 patients with spinal cord injury were included. Patients were randomly assigned either to have their bladders inoculated with either E. coli 83972 (experimental group) or sterile normal saline (control group). Patients whose bladders became colonized with E. coli 83972 were half as likely (P=.01) than non-colonized patients to develop UTI during the subsequent year.

Billips et al. demonstrated recently that deletion of the O antigen ligase gene, waaL, from the uropathogenic E. coli isolate NU14 results in a strain that stimulates enhanced urothelial cytokine secretion (Billips et al., 2009). They could show that NU14 DwaaL stimulated an enhanced interleukin-6 secretion by mouse macrophages, compared with secretion by the wild type. Of great importance is the fact that mice vaccinated via instillation into the bladder developed protective responses that prevented persistent colonization after bladder challenge with NU14, yet NU14 DwaaL failed to persistently colonize the mouse bladder. They could

also show that the mice were additionally protected against challenge with a broad range of clinical UPEC isolates and developed immunity that lasted more than 8 weeks (Billips et al., 2009). These findings open a new avenue for future treatment strategies of recurrent urinary tract infection by caused by UPEC.

4. Discussion

Immune-based treatment strategies for patients with recurrent UTIs are of special interest. Several promising new approaches including bladder colonization with attenuated bacteria and intravesical vaccination were published recently and discussed in this chapter. Furthermore, studies suggest that the use of inhibitors of bacterial adherence to urothelial cell and inhibitors of biofilm formation receptors hold great promise. Moreover, stimulators of cyclic AMP inside urothelial cells and the recent advancements in the development of vaccines are an interesting initiative in this field. For some of the plant –based prevention and treatment strategies only little scientific evidence for the prevention and treatment of urinary tract infection exist.

Acknowledgment

We would like to thank the library staff from Texas Tech University Health Sciences Center, Lubbock for their extraordinary support and assistance. On a special note, we would like to thank again Carrie Gassett who showed great interest and support in our project.

Author details

Thomas Nelius, Christopher Winter, Julia Willingham and Stephanie Filleur

Texas Tech University Health Sciences Center, Departments of Urology and Microbiology, Lubbock, USA

References

[1] Adams, T. B., et al. (2004). The FEMA GRAS assessment of cinnamyl derivatives used as flavor ingredients. Food Chem Toxicol, Feb 2004), Print), 42(2), 157-185.

[2] Amalaradjou, M. A., Narayanan, A. ., & Venkitanarayanan, K. Trans-cinnamaldehyde decreases attachment and invasion of uropathogenic Escherichia coli in urinary

tract epithelial cells by modulating virulence gene expression. J Urol, Apr Electronic), 185(4), 1526-1531.

[3] Anukam, K. C., et al. (2009). Probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 may help downregulate TNF-Alpha, IL-6, IL-8, IL-10 and IL-12 (p70) in the neurogenic bladder of spinal cord injured patient with urinary tract infections: a two-case study. Adv Urol, Vol. Print)(2009), 680363.

[4] Avorn, J., et al. (1994). Reduction of bacteriuria and pyuria after ingestion of cranberry juice. Jama, Mar 9 1994), Print), 271(10), 751-754.

[5] Beaux, D., Fleurentin, J. ., & Mortier, F. (1999). Effect of extracts of Orthosiphon stamineus Benth, Hieracium pilosella L., Sambucus nigra L. and Arctostaphylos uva-ursi (L.) Spreng. in rats. Phytother Res, May 1999), X (Print), 13(3), 222-225.

[6] Beerepoot, M. A., et al. Cranberries vs antibiotics to prevent urinary tract infections: a randomized double-blind noninferiority trial in premenopausal women. Arch Intern Med, Jul 25 Electronic), 171(14), 1270-1278.

[7] Beretz, A., Anton, R. ., & Stoclet, J. C. (1978). Flavonoid compounds are potent inhibitors of cyclic AMP phosphodiesterase. ExperientiaAug 15 1978), Print), 34(8), 1054-1055.

[8] Bessler, W. G., et al. (2009). Immunomodulating effects of OM-89, a bacterial extract from Escherichia coli, in murine and human leukocytes. Arzneimittelforschung, Print), 59(11), 571-577.

[9] Billips, B. K., et al. (2009). A live-attenuated vaccine for the treatment of urinary tract infection by uropathogenic Escherichia coli. J Infect Dis, Jul 15 2009), Print), 200(2), 263-272.

[10] Bishop, B. L., et al. (2007). Cyclic AMP-regulated exocytosis of Escherichia coli from infected bladder epithelial cells. Nat Med, May 2007), Print), 13(5), 625-630.

[11] Bodel, P. T., Cotran, R. ., & Kass, E. H. (1959). Cranberry juice and the antibacterial action of hippuric acid. J Lab Clin Med, No. (Dec 1959), Print), 54, 881-888.

[12] Bruce, A. W. ., & Reid, G. (1988). Intravaginal instillation of lactobacilli for prevention of recurrent urinary tract infections. Can J Microbiol, Mar 1988), , 34(3), 339-343.

[13] Bruce, A. W. ., & Reid, G. (2003). Probiotics and the urologist. Can J Urol, Apr 2003), , 10(2), 1785-1789.

[14] Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods--a review. Int J Food Microbiol, Aug 1 2004), Print), 94(3), 223-253.

[15] Cernakova, M. ., & Kostalova, D. (2002). Antimicrobial activity of berberine--a constituent of Mahonia aquifolium. Folia Microbiol (Praha), Print), 47(4), 375-378.

[16] Czaja, C. A., et al. (2007). Phase I trial of a Lactobacillus crispatus vaginal suppository for prevention of recurrent urinary tract infection in women. Infect Dis Obstet Gynecol, , 2007(2007), 35387.

[17] Dani, C., et al. (2002). Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. Biol Neonate, Aug 2002), , 82(2), 103-108.

[18] Darouiche, R. O., et al. (1999). Efficacy of antimicrobial-impregnated bladder catheters in reducing catheter-associated bacteriuria: a prospective, randomized, multicenter clinical trial. UrologyDec 1999), Electronic), 54(6), 976-981.

[19] Darouiche, R. O., et al. (2005). Bacterial interference for prevention of urinary tract infection: a prospective, randomized, placebo-controlled, double-blind pilot trial. Clin Infect Dis, Nov 15 2005), Electronic), 41(10), 1531-1534.

[20] De Caprio, A. P. (1999). The toxicology of hydroquinone--relevance to occupational and environmental exposure. Crit Rev Toxicol, May 1999), Print), 29(3), 283-330.

[21] Domadia, P. N., et al. (2008). Berberine targets assembly of Escherichia coli cell division protein FtsZ. BiochemistryMar 11 2008), Print), 47(10), 3225-3234.

[22] Frohne, D. (1970). The urinary disinfectant effect of extract from leaves uva ursi]. Planta Med, Jan 1970), Print), 18(1), 1-25.

[23] Gill, A O &Holley, R A.(2006). Disruption of Escherichia coli, Listeria monocytogenes and Lactobacillus sakei cellular membranes by plant oil aromatics. Int J Food Microbiol, Apr 15 2006), Print), 108(1), 1-9.

[24] Gonzalez-Chamorro, F., & et, al. [. Urinary tract infections and their prevention]. Actas Urol Esp, Jan Electronic), 36(1), 48-53.

[25] Guay, D. R. (2009). Cranberry and urinary tract infections. Drugs, Print), 69(7), 775-807.

[26] Guay, D. R. (2001). An update on the role of nitrofurans in the management of urinary tract infections. Drugs, Print), 61(3), 353-364.

[27] Ha, U S &Cho, Y H.(2008). Immunostimulation with Escherichia coli extract: prevention of recurrent urinary tract infections. Int J Antimicrob Agents, Suppl 1, No. (Feb 2008), Print), 31, S63-S67.

[28] Head, K. A. (2008). Natural approaches to prevention and treatment of infections of the lower urinary tract. Altern Med Rev, Sep 2008), Print), 13(3), 227-244.

[29] Hopkins, W. J., et al. (2007). Vaginal mucosal vaccine for recurrent urinary tract infections in women: results of a phase 2 clinical trial. J Urol, Apr 2007), quiz 1591, 0022-5347 (Print), 177(4), 1349-1353.

[30] Howell, A. B., et al. (2005). A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. Phytochemistry, Sep 2005), Print), 66(18), 2281-2291.

[31] Ishida, H., et al. (1998). In vitro and in vivo activities of levofloxacin against biofilm-producing Pseudomonas aeruginosa. Antimicrob Agents Chemother, Jul 1998), Print), 42(7), 1641-1645.

[32] Jacobsen, S. M., et al. (2008). Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev, Jan 2008), Electronic), 21(1), 26-59.

[33] Jepson, R G &Craig, J C.(2008). Cranberries for preventing urinary tract infections. Cochrane Database Syst Rev, Vol. X (Electronic)(1), CD001321.

[34] Jepson, R. G., Mihaljevic, L. ., & Craig, J. (2000). Cranberries for treating urinary tract infections. Cochrane Database Syst Rev, Vol. X (Electronic)(2), CD001322.

[35] Johnson, J. R., Delavari, P. ., & Azar, M. (1999). Activities of a nitrofurazone-containing urinary catheter and a silver hydrogel catheter against multidrug-resistant bacteria characteristic of catheter-associated urinary tract infection. Antimicrob Agents Chemother, Dec 1999), Print), 43(12), 2990-2995.

[36] Klein, T et al. FimH antagonists for the oral treatment of urinary tract infections: from design and synthesis to in vitro and in vivo evaluation. J Med Chem, Vol. 53, No. 24, (Dec 23 pp. 8627-8641, 1520-4804 (Electronic)

[37] Kontiokari, T., et al. (2001). Randomised trial of cranberry-lingonberry juice and Lactobacillus GG drink for the prevention of urinary tract infections in women. Bmj, Jun 30 2001), Print), 322(7302), 1571.

[38] Kubo, M., et al. (1990). Pharmacological studies on leaf of Arctostaphylos uva-ursi (L.) Spreng. I. Combined effect of 50% methanolic extract from Arctostaphylos uva-ursi (L.) Spreng. (bearberry leaf) and prednisolone on immuno-inflammation]. Yakugaku Zasshi, Jan 1990), Print), 110(1), 59-67.

[39] Lavigne, J. P., et al. (2008). In-vitro and in-vivo evidence of dose-dependent decrease of uropathogenic Escherichia coli virulence after consumption of commercial Vaccinium macrocarpon (cranberry) capsules. Clin Microbiol Infect, Apr 2008), X (Print), 14(4), 350-355.

[40] Liu, Y., et al. (2006). Role of cranberry juice on molecular-scale surface characteristics and adhesion behavior of Escherichia coli. Biotechnol Bioeng, Feb 5 2006), Print), 93(2), 297-305.

[41] Mac, Micking. J., Xie, Q. W. ., & Nathan, C. (1997). Nitric oxide and macrophage function. Annu Rev Immunol, Print), 15(1997), 323-350.

[42] Newman, D. K. (1998). Managing indwelling urethral catheters. Ostomy Wound Manage, Dec 1998), passim, 0889-5899 (Print), 44(12), 26-28.

[43] Ofek, I., Goldhar, J. ., & Sharon, N. (1996). Anti-Escherichia coli adhesin activity of cranberry and blueberry juices. Adv Exp Med Biol, Print), 408(1996), 179-183.

[44] Ofek, I., et al. (1991). Anti-Escherichia coli adhesin activity of cranberry and blueberry juices. N Engl J Med, May 30 1991), Print), 324(22), 1599.

[45] Ohnishi, R., et al. (2006). Urinary excretion of anthocyanins in humans after cranberry juice ingestion. Biosci Biotechnol Biochem, Jul 2006), Print), 70(7), 1681-1687.

[46] Ohno, T., et al. (2003). Antimicrobial activity of essential oils against Helicobacter pylori. Helicobacter, Jun 2003), Print), 8(3), 207-215.

[47] Osset, J., et al. (2001). Assessment of the capacity of Lactobacillus to inhibit the growth of uropathogens and block their adhesion to vaginal epithelial cells. J Infect Dis, Feb 1 2001), , 183(3), 485-491.

[48] Pereira, R. S., et al. (2004). Antibacterial activity of essential oils on microorganisms isolated from urinary tract infection]. Rev Saude Publica, Apr 2004), , 38(2), 326-328.

[49] Perez-Lopez, F. R., Haya, J. ., & Chedraui, P. (2009). Vaccinium macrocarpon: an interesting option for women with recurrent urinary tract infections and other health benefits. J Obstet Gynaecol Res, Aug 2009), Print), 35(4), 630-639.

[50] Raz, R., Chazan, B. ., & Dan, M. (2004). Cranberry juice and urinary tract infection. Clin Infect Dis, May 15 2004), Electronic), 38(10), 1413-1419.

[51] Raz, R. ., & Stamm, W. E. (1993). A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. N Engl J Med, Sep 9 1993), Print), 329(11), 753-756.

[52] Reid, G., Bruce, A. W. ., & Taylor, M. (1992). Influence of three-day antimicrobial therapy and lactobacillus vaginal suppositories on recurrence of urinary tract infections. Clin Ther, Jan-Feb 1992), , 14(1), 11-16.

[53] Reid, G., et al. (1985). Prevention of urinary tract infection in rats with an indigenous Lactobacillus casei strain. Infect Immun, Aug 1985), , 49(2), 320-324.

[54] Russo, T. A., et al. (2003). The Siderophore receptor IroN of extraintestinal pathogenic Escherichia coli is a potential vaccine candidate. Infect Immun, Dec 2003), Print), 71(12), 7164-7169.

[55] Scazzocchio, F., et al. (2001). Antibacterial activity of Hydrastis canadensis extract and its major isolated alkaloids. Planta Med, Aug 2001), Print), 67(6), 561-564.

[56] Schaeffer, A. J., Story, K. O. ., & Johnson, S. M. (1988). Effect of silver oxide/trichloroisocyanuric acid antimicrobial urinary drainage system on catheter-associated bacteriuria. J Urol, Jan 1988), Print), 139(1), 69-73.

[57] Schierholz, J. M. ., & Beuth, J. (2001). Implant infections: a haven for opportunistic bacteria. J Hosp Infect, Oct 2001), Print), 49(2), 87-93.

[58] Schindler, G., et al. (2002). Urinary excretion and metabolism of arbutin after oral administration of Arctostaphylos uvae ursi extract as film-coated tablets and aqueous solution in healthy humans. J Clin Pharmacol, Aug 2002), Print), 42(8), 920-927.

[59] Schmidt, B. M., et al. (2004). Effective separation of potent antiproliferation and antiadhesion components from wild blueberry (Vaccinium angustifolium Ait.) fruits. J Agric Food Chem, Oct 20 2004), Print), 52(21), 6433-6442.

[60] Seeram, N. P. (2008). Berry fruits for cancer prevention: current status and future prospects. J Agric Food Chem, Feb 13 2008), Print), 56(3), 630-635.

[61] Sikkema, J;de., Bont, J. A. ., & Poolman, B. (1994). Interactions of cyclic hydrocarbons with biological membranes. J Biol Chem, Mar 18 1994), Print), 269(11), 8022-8028.

[62] Simpson, D. (1998). Buchu--South Africa's amazing herbal remedy. Scott Med J, Dec 1998), , 43(6), 189-191.

[63] Smith-Palmer, A., Stewartt, J. ., & Fyfe, L. (2002). Inhibition of listeriolysin O and phosphatidylcholine-specific production in Listeria monocytogenes by subinhibitory concentrations of plant essential oils. J Med Microbiol, Jul 2002), , 51(7), 567-574.

[64] Spiegel, C. A., et al. (1980). Anaerobic bacteria in nonspecific vaginitis. N Engl J Med, Sep 11 1980), , 303(11), 601-607.

[65] Stothers, L. (2002). A randomized trial to evaluate effectiveness and cost effectiveness of naturopathic cranberry products as prophylaxis against urinary tract infection in women. Can J Urol, Jun 2002), Print), 9(3), 1558-1562.

[66] Sun, D., Abraham, S. N. ., & Beachey, E. H. (1988). Influence of berberine sulfate on synthesis and expression of Pap fimbrial adhesin in uropathogenic Escherichia coli. Antimicrob Agents Chemother, Aug 1988), Print), 32(8), 1274-1277.

[67] Sun, D., Courtney, H. S. ., & Beachey, E. H. (1988). Berberine sulfate blocks adherence of Streptococcus pyogenes to epithelial cells, fibronectin, and hexadecane. Antimicrob Agents Chemother, Sep 1988), Print), 32(9), 1370-1374.

[68] Thankavel, K., et al. (1997). Localization of a domain in the FimH adhesin of Escherichia coli type 1 fimbriae capable of receptor recognition and use of a domain-specific antibody to confer protection against experimental urinary tract infection. J Clin Invest, Sep 1 1997), Print), 100(5), 1123-1136.

[69] Trautner, B W &Darouiche, R O.(2004). Catheter-associated infections: pathogenesis affects prevention. Arch Intern Med, Apr 26 2004), Print), 164(8), 842-850.

[70] Turi, M., et al. (1997). Influence of aqueous extracts of medicinal plants on surface hydrophobicity of Escherichia coli strains of different origin. Apmis, Dec 1997), Print), 105(12), 956-962.

[71] Wellens, A., et al. (2008). Intervening with urinary tract infections using anti-adhesives based on the crystal structure of the FimH-oligomannose-3 complex. PLoS One, Electronic), 3(4), e2040.

[72] Wilson, M., Seymour, R. ., & Henderson, B. (1998). Bacterial perturbation of cytokine networks. Infect Immun, Jun 1998), , 66(6), 2401-2409.

[73] Wollenweber, E. (1988). Occurrence of flavonoid aglycones in medicinal plants. Prog Clin Biol Res, Print), 280(1988), 45-55.

[74] Xia, Y., Yamagata, K. ., & Krukoff, T. L. (2006). Differential expression of the CD14/TLR4 complex and inflammatory signaling molecules following i.c.v. administration of LPS. Brain Res, Jun 20 2006), , 1095(1), 85-95.

[75] Yarnell, E. (2002). Botanical medicines for the urinary tract. World J Urol, Nov 2002), Print), 20(5), 285-293.