Mechanisms of action and applications of probiotics for the treatment of *Clostridium difficile* infection

Xinhua Chen[1], **Mingsheng Dong**[2] and **Xingmin Sun**[3*]

[1]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
[2]College of Food Science and Technology, Nanjing Agricultural University, Nanjing, P.R. China
[3] Department of Infectious Diseases and Global Health, Tufts University Cummings School of Veterinary Medicine, North Grafton, MA 01536, USA
*Corresponding author: X. Sun (xingmin.sun@tufts.edu)

Clostridium difficile is a spore-forming Gram-positive anaerobic bacillus, and is the leading cause of nosocomial diarrhea and colitis in the industrialized world. The incidence and mortality rates *of C. difficile* infection (CDI) have increased dramatically in the past decade. CDI is difficult to treat as antibiotic options are limited. Moreover, 15-35% of patients infected with *C. difficile* relapse following antibiotic treatment, which limits the ability of the colonic flora to inhibit *C. difficile* colonization. In an effort to improve outcomes and reduce recurrences of CDI, interest has been renewed in the development of nonantibiotic and adjunct approaches to therapy. Among these, probiotics have been investigated for primary and secondary prophylaxis against CDI, with varying success. This review discusses the mechanisms by which probiotics interact with *C. difficile* and its toxins, evaluates experimental models and clinical trials for probiotic use in the prevention or treatment of CDI and provides a framework for future research directions.

Keywords *Clostridium difficile* infection, probiotics, therapeutics

1. Introduction

C. difficile, a Gram-positive, spore-forming anaerobic bacillus, is the most common cause of nosocomial antibiotic-associated diarrhea and pseudomembranous colitis [1]. The incidence of CDI is rising steadily worldwide [2]. Antibiotic exposure is the most significant risk factor for the diseases [3]. Several hospital outbreaks of CDI with high morbidity and mortality which occurred in the last few years in North America have been attributed to the widespread and often inappropriate use of broad-spectrum antibiotics. The emergence of new and more virulent *C. difficile* strains also contributes to the increased burden of disease [4, 5]. Standard therapy depends on treatment with vancomycin, fidaxomicin or metronidazole, neither of which is fully effective [6]. Moreover, an estimated 15-35% of those infected with *C. difficile* relapse following treatment [7, 8]. In an effort to improve outcomes and reduce recurrences of CDI, interest has been renewed in the development of nonantibiotic and adjunct approaches to therapy. Given the inhibitory effects of the normal gastrointestinal flora against *C. difficile* growth and toxin release [9, 10], there has been considerable interest in the use of probiotics to prevent CDI.

2. *Clostridium difficile* infection

2.1. Virulence factors

CDI is primarily a toxin-mediated disease. Two exotoxins, toxin A (TcdA) and toxin B (TcdB), are the most extensively studied and thought to be major virulence factors of *C. difficile* [11-13]. Purified TcdA possesses potent enterotoxic and pro-inflammatory activities, as determined in ligated intestinal loop studies in animals [14]. TcdA is also cytotoxic to culture cells in nanogram quantities. TcdB has been previously reported to exhibit no enterotoxic activity in animals [15, 16], but recent studies have described enterotoxic and proinflammatory activities in human intestinal xenografts in immunodeficient (SCID) mice [17]. Furthermore, some strains of *C. difficile* do not produce TcdA yet can cause pseudomembranous colitis in some patients [18]. Recent genetic analyses of isogenic toxin mutants confirmed that TcdB is essential for virulence in a hamster model of infection [19]. The requirement for TcdA during infection, however, remains controversial. While $TcdA^+$ $TcdB^-$ mutants were less virulent in studies performed by Lyras et al.[20], the equivalent mutants in studies performed by Kuehne et al. [19] were as virulent as wild-type. A limited number of *C. difficile* isolates, including the epidemic NAP1/027 strain, produce a binary toxin that exhibits ADP-ribosyltransferase activity [21-23], but its role in the development of human disease is not well understood [24]. In addition to these toxins, several other factors may contribute to disease pathogenesis, such as fimbriae and other molecules facilitating adhesion, capsule production, and hydrolytic enzyme secretion [25-27]. Recent studies have shown that the surface layer proteins of *C. difficile* may contribute to bacterial colonization [28, 29].

 © FORMATEX 2013

2.2. Disease manifestation and therapeutic approaches

CDI is acquired by the ingestion of vegetative organisms or spores, most likely the latter [30, 31]. Spores survive exposure to gastric acidity and germinate in the colon. The clinical appearance of CDI is highly variable, from asymptomatic carriage, to mild self-limiting diarrhea, to more severe pseudomembranous colitis. The most common symptom is diarrhea. Other common clinical symptoms include abdominal pain and cramping, increased temperature and leucocytosis. In mild cases of CDI, oral rehydration plus withdrawal of antibiotics is often effective. When CDI cases are more severe, oral administration of metronidazole, vancomycin or fidaxomycin is recommended. This treatment however is associated with a relapse rate from 15 to 35% [7, 32]. After a first recurrence, additional recurrences are more likely, and some patients have experienced multiple recurrences over a period of one to two years. The primary treatment option for recurrent CDI is retreatment with antibiotics, a strategy which does not allow recovery of the normal colonic flora which is required for eventual recovery. Other options, such as anion-exchange resins, have limited efficacy and are potentially harmful [33]. Experimental treatments currently in clinical development include toxin-absorbing polymer, some antibiotics, and toxin-specific human monoclonal antibodies [34-37]. A toxoid vaccine inactivated by formaldehyde and administered intramuscularly is currently under clinical trial by Acambis [38, 39]. Several other protein or DNA vaccine candidates either targeting *C. difficile* toxins or other virulent factors such as surface-layer protein, pentasaccharide cell wall repeating unit, cysteine protease and flagellin have been under investigation in animal models. Probiotics including fecal transplantation have been investigated for primary and secondary prophylaxis against CDI, with varying success.

2.3. Antibiotic administration and the development of primary and recurrent CDI

The major risk factors for CDI are increasing age, prolonged hospital stay and antimicrobial use. The most important risk factor is alteration of bowel microflora and subsequent loss of colonization resistance associated with antimicrobial usage. There are particular classes of antimicrobials that are associated with the highest risk of *C. difficile* acquisition, including clindamycin, cephalosporins and β-lactam antimicrobials and more recently fluoroquinolones. Antibiotic-induced invasion of *C. difficile*, presumably due to a loss of colonization resistance, has been demonstrated in hamsters [40], mice [41, 42], and in human fecal bioreactors [43]. Reduced diversity of the intestinal community associated with *C. difficile* colonization in these models has been demonstrated, although the community members responsible for suppressing *C. difficile* have yet to be identified.

Culture-independent analysis of the microbiome has been used to monitor how various antimicrobials alter the gut microbiota and how certain changes are associated with lowered colonization resistance and risk of subsequent CDI. One *in vitro* study used a fermentation system to investigate the effect of broad- and narrow-spectrum antimicrobials directed against *C. difficile* and the gut microbiota [43]. Another group studied alteration in the gut microbiome associated with risk of developing nosocomial CDI [44]. Recurrent CDI is becoming an increasingly alarming and important problem. It has been proposed that patients with recurrent CDI have sufficiently altered indigenous microbiota such that colonization resistance is not restored after treatment directed against *C. difficile*. Support for reduced microbiota diversity in recurrent CDI was found in some patients recovering from CDI [45]. Recent studies show that fecal microbiota transplantation (FMT) is currently the most effective treatment for recurrent CDI [46]. Additionally, fecal transplantation was demonstrated to have a profound effect on the fecal microbiota of recipients [47]. Given the inhibitory effects of the normal gastrointestinal flora against *C. difficile* growth and toxin release [9, 10], there has been considerable interest in the use of probiotics to prevent CDI.

3. Probiotics

Probiotics are generally defined as live or live-attenuated microorganisms that confer a health benefit to the host such as improved intestinal function or recovery from certain gastrointestinal inflammation and infection [48]. Both bacterial and yeast have been used, including *Lactobacillus spp* and *Saccharomyces* species, to prevent or treat inflammatory or infectious gastrointestinal diseases [49, 50]. The best evidence for clinical efficicay comes from studies involving irratable bowel disease, acute pouchitis, and acute infectious diarrhea in infants and children [48, 51]. Since normal intestinal microbiota suppress C. *difficile* invasion of the intestinal tract, fecal transplantation is also considered as probiotic therapy in this review. Mechanisms of action of probiotics include colonization resistance to pathogens, modification of the intestinal microflora, enhancement of barrier function, immnomodultion of epithelical cells, dendritic cells, monocytes/macrophage, lymphocytes such as B lymphocytes, NK cells, and T cells[52-56].

© FORMATEX 2013

4. Potential mechanisms of probiotics against CDI

Both direct and indirect potential protective mechanisms have been proposed or demonstrated for probiotics used to treat or prevent CDI.

4.1. Direct effects on *C. difficile* or its toxins

4.1.1. Suppression of germination, colonization and adhesion of *C. difficile* spores or vegetative cells

Probiotics may suppress the ability of pathogens to colonize the lumen and subsequently adhere and invade the gastrointestinal mucosa. For example, *Lactobacilli* and *Bifidobacteria* inhibit *C. difficile* growth and adhesion to enterocytes [57]. This effect may be mediated by probiotic production of antimicrobials and acids produced by probiotics [58]. For example, lacticin 3147, a broad-spectrum bacteriocin produced by *Lactococcus lactis subsp*., inhibits a range of genetically distinct *C. difficile* isolates [59]. Several groups have mined the human intestinal microbiota for antibacterial activities produced by the microbiota that are specifically targeted to *C. difficile*. An *in vitro* screen had identified an intestinal *Bacillus thuringiensis* strain capable of producing a bacteriocin named Thuricin CD [60], which was shown to be as effective as metronidazole at inhibiting *C. difficile*.

Normal intestinal microbiota may suppress *C. difficile* invasion of the intestinal tract via the transformation of bile acids, which have profound effects on spore germination and vegetative growth of *C. difficile in vitro* [61]. Human synthesize two main primary bile acids, cholate and chenodeoxycholate (CDCA), which are conjugated to an amino acid (glycine or taurine) [62]. The microbiota plays two important roles in bile acid transformation. First, bile salt hydrolase enzymes secreted by bacteria deconjugate the bile acids from their amino acid in the intestinal lumen. Second, bacteria transform primary bile acids to secondary bile acids via the enzyme 7-dehydroxylase, converting cholate and CDCA into deoxycholate and lithocholate, respectively [63]. Cholate stimulates the germination of *C. difficile* spores, while CDCA has a strong inhibitory effect on spore germination. Antibiotic treatment might reduce the members of the microbiota that generate cholate or alter the ratio between cholate and CDCA. In support of this hypothesis, small intestine and cecal contents from mice that had been treated with antibiotics were able to support *C. difficile* spore germination at higher levels than control mice, and antibiotic treated animals and people have higher levels of cholate versus CDCA [64].

Normal intestinal microbiota may impact CDI by activating TLR5 or TLR4. In a mouse model of CDI, it was shown that TLR5 activation by flagellin (from *Salmonella*) was sufficient to prevent CDI after antibiotic treatment [65]. A 5 log reduction in *C. difficile* cell numbers was observed, indicating that TLR5 activation either prevents spore germination or elicits a bactericidal effect against vegetative *C. difficile*. TLR5 mutants were unable to prevent *C. difficile* colonization in response to flagellin activation. In contrast to TLR5, TLR4 appears to have a direct role in the recognition and response to CDI. When wild type, TLR4-deficient and TLR2-deficinet mice were treated with antibiotics and then subsequently challenged with *C. difficile*, TLR4-deficinet mice displayed more severe diseases than wild type and TLR2-deficient mice [66].

4.1.2. Inhibition of *C. difficile* toxin

Animal models indicate that pretreatment with S. *boulardii* can inhibit *C. difficile* toxins [67-70]. Pretreatment of rats with S. *boulardii* led to reduced binding of TcdA to the intestinal brush border, reduction in intestinal fluid secretion, and decreased intestinal permeability [70]. This effect was attributed to a protease secreted by *S. boulardii* that hydrolyzed TcdA and TcdB and inhibited their binding to their respective intestinal brush border receptors [67, 68].

Probiotics such as *lactobacilli*, and VSL#3 (a probiotic mixture) were shown *in vitro* to induce the expression of defensins and cryptidins [53, 71]. It has been reported that human alpha-defensins inhibited TcdB by interactions with high affinity [72]. Lactic acid produced by lactic acid bacteria such as *Streptococcus thermophilus* has potenital to decrease TcdA gene expression and TcdA toxin release into the extracellular milieu [73].

4.2. Indirect effects by modulating the host response

4.2.1. Stimulation of immune function

Probiotics stimulate immune function in a number of ways. Cellular and animal models have demonstrated that probiotics can have a profoundly anti-inflammatory effect via stimulation of the innate immune response [74-79]. The host immune response to CDI is crucial in influencing disease outcomes [80, 81]. For example, *S. boulardii* can up-regulate total and specific anti-TcdA secretory IgA expression in animal models of CDI [82, 83]. Serum antibodies against both TcdA and TcdB are crucial in recovery from acute CDI and in protection against recurrence [80, 84, 85].

 © FORMATEX 2013

4.2.2. Enhancement of Barrier function

Intestinal barrier function is maintained by several interrelated systems including mucus secretion, chloride and water secretion, and tight junction proteins. Disruption of epithelial barrier functions is seen in several conditions including CDI, inflammatory bowel disease, and enteric infections.

C. difficile toxins disrupt the cytoskeleton and tight junctions [86] of intestinal epithelial cells. It has been reported that TcdB induces redistribution of NHE3, the apical Na^+/H^+ exchanger [87]. In animal model, TcdA increases intestinal permeability and induces Cl^- secretion [88]. Enhancement of barrier function by probiotics has been observed both *in vitro* and *in vivo* [52]. The probiotic mixture VSL#3 normalized barrier integrity as assessed by short circuit current, transepithelial potential difference, and mannitol flux in mouse intestine [89]. The mechanisms by which probiotics enhance gut mucosal function may relate to alterations in tight junction protein expression by epithelial cells or chloride secretion via the apical Na^+/H^+ exchanger. Some probiotics limit chloride and water secretion. For example, *S. thermophilus* and *Lactobacillus acidophilus* reversed the increase in enteroinvasive *E. coli*-induced chloride secretion by epithelial cell lines [90]. Bifidobacteria dose dependently inhibited chloride secretion by human intestinal epithelial cells [91]. Butyrate produced by probiotics increased NaCl absorption by NHE_3 stimulation and transcription [92]. *L. acidophilus* increased surface expression of DRA (down regulated in adenoma), which is $Cl^-/HCO3^-$ exchangers, giving increased chloride absorption [93, 94]. Probiotic bacteria can also prevent tight junction protein redistribution. For example, *S. thermophilus* and *L. acidophilus* maintain or enhance cytoskeletal and tight junctional protein structures in epithelial cell lines [90]. *L. plantarum* upregulates gene coding for *de novo* synthesis of claudin and occludin [95, 96].

Some probiotics modify mucus secretion. For example, *L. rhamnosus* GG has been shown to increase gut mucin production, which improves the barrier defenses of the epithelium, and increases colonic water absorption, resulting in reduced diarrhea [97].

4.2.3. Inhibition of epithelial cell apoptosis

Once the protective epithelial barrier has been breached, *C. difficile* toxins and other proteins come into contact with submucosal macrophages, monocytes and dendritic cells and trigger the release of proinflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α [98-100].

Both *C. difficile* toxins and TNF-α induce apoptosis of intestinal epithelial cells and other cells [101]. Two soluble proteins from *L. rhamnosus* GG promote intestinal epithelial cell survival and growth. These proteins inhibit TNF-α-mediated apoptosis by activation of the anti-apoptotic factor Akt and protein kinase B. Furthermore, they inactivate the pro-apoptotic p38 mitogen-activating protein kinase signaling pathway in epithelial cells [102].

4.2.4. Inhibition of *C. difficile* toxin-induced inflammatory signal cascades

The probiotic yeast *S. boulardii* shows effective protection against TcdA in the murine ileal loop model and in cell culture assays. This protection results from interference of *S. boulardii* with the TcdA-induced inflammatory signal cascade activating Erk1/2 and JNK/SAPK pathways [103, 104]. *S. boulardii* can also produce a soluble anti-inflammatory factor that inhibits NF-kB-mediated IL-8 gene expression [105].

5. Efficacy of Probiotics in Animal Studies

5.1. Probiotics in hamster CDI model

Non-toxigenic *C. difficile* strains occur naturally and, when given to hamsters during or after antibiotic treatment, are able to harmlessly colonize the gut and prevent subsequent infection challenge with toxigenic strains of *C. difficile* [106]. *Bifidobacterium* strains were somewhat protective against CDI in the hamster model [107]. Moreover, oral treatment with a mixture of lactic acid bacteria and yeasts (*Lactobacillus plantarum*, *Lactobacillus kefir, Lactococcus lactis, Kluyveromyces marxianus* and *Saccharomyces cerevisiae*) from kefir significantly protected hamster from diarrhea and enterocolitis triggered by *C. difficile* [108]. These observations in hamsters support the use of a probiotic strategy for CDI prevention in humans receiving antibiotics, and provided pre-clinical validation for prophylaxia in human infection discussed below.

5.2. Probiotics in mouse CDI model

Oral administration of *Saccharomyces boulardii (Sb)* in gnotobiotic mice significantly decreased mortality following CDI. A single dose of Sb protected 16% of mice, whereas 56% were protected when Sb was given continuously in the drinking water. Direct inhibition of *C. difficile* numbers was not detected, but reduced toxin production was demonstrated [109].

Mice with active CDI given the probiotic *S. thermophilus* exhibited 46% less weight loss compared with untreated controls; moreover, less tissue damage, diarrhea, and lower toxin levels in cecal contents were evident in *S. thermophillus* treated mice. A significant, inverse correlation between the levels of luminal lactate and abundance of *C.*

difficile were noted suggesting that lactate produced by *S. thermophilus* is a factor impacting the progression of *C. difficile* infection in the murine system [110]. In addition, an exploratory method using a combination of *L. acidophilus* and epidermal growth factor (EGF) reduced the severity of *C. difficile* infection in mice [111].

Lawley *et al* recently identified a mixture of six phylogenetically diverse intestinal bacteria, which allowed normalization of a healthy colonic microbiota and clearance of chronic *C. difficile* 027/BI infection from mice. This demonstrates a rational approach to harness the therapeutic potential of health-associated microbial communities to treat *C. difficile* disease [112].

6. Clinical applications of probiotics

Since CDI is associated with disrupted gut flora and loss of normal barrier function, it is logical to employ strategies that modulate gut flora as prophylaxis or treatment for this infection [113]. Many probiotics are effective for acute gastroenteritis, persistent diarrhea, and diarrhea prevention [114]. Below is a brief summary of the clinical usage of probiotics in CDI.

6.1. *S. boulardii* in CDI

Saccharomyces boulardii (*Sb*) is a non-pathogenic yeast that has been used for many years as a probiotic agent to prevent or treat a wide variety of human gastrointestinal disorders of diverse etiologies [104, 115]. Preclinical and experimental studies of *Sb* have demonstrated anti-inflammatory, antimicrobial, enzymatic, metabolic and anti-toxin activity [103, 104]. In a double-blind, randomized, placebo-controlled study in patients with recurrent CDI, Sb was used in combination with metronidazole or vancomycin. A majority of the control subjects (antibiotics alone) experienced recurrence (65%) compared to only 35% of those receiving *Sb* plus antibiotics (P=0.04) [116]. A subsequent trial by the same investigators showed that in subjects with a history of recurrent CDI, a significant decrease in further recurrences occurred only in a group treated with high-dose vancomycin (2g per day) and *Sb* (16.7% versus 50% in control; P=0.05) [117]. In patients with recurrent CDI, *Sb* 500 mg twice daily for 28 days is safe and may be beneficial [118]. However, a recent clinical trial suggested that in elderly hospitalized patients, *S. boulardii* was not effective in preventing the development of AAD including CDI [119]. *S. cerevesiae* preparations or baker's yeast are also effective in recurrent CDI but no controlled study data are available [120, 121].

6.2. *Lactobacillus*

Lactobacillus organisms are part of the normal flora residing in the gastrointestinal and female genital tracts [122]. *L. rhamnosus* GG (LGG), an *L. casei* species variant [123], is most commonly used. LGG has shown positive results for recurrent CDI [124, 125]. In the only randomized pilot study conducted, patients (average age 78 y; median of 1 previous CDAD episode) were treated with LGG or placebo and were followed for 39 days after treatment was completed. The recurrence rate was not significantly different between the groups (8 pts. [37.5%] with LGG vs. 6 pts. [14.3%] with placebo) [126].

6.3. Non-pathogenic *C. difficile* strains

Asymptomatic colonization of patients with *C. difficile* (toxigenic or nontoxigenic strains) is associated with decreased risk of CDI [127]. The use of nontoxigenic *C. difficile* to prevent primary or recurrent CDI is an attractive strategy because the spores can be administered orally [128]. However, the mechanism by which nontoxigenic *C. difficile* prevents colonization by toxigenic strains has not been elucidated [128]. Human safety trials of nontoxigenic *C. difficile* were completed in 2010, and phase II trials involving patients are currently ongoing. The efficacy of this approach remains to be known.

6.4. Multistrain probiotic therapy

Ecologic®AAD/OMNiBiOTiC® 10 (Winclove Bio Industries BV, Amsterdam, the Netherlands) consists of equal ratios of 10 bacterial probiotic strains: On the basis of a retrospective chart review of a series of only ten CDI patients where recurrence was expected, all patients on adjunctive probiotic therapy with this multistrain cocktail showed complete clinical resolution [129]. Another multi-center randomized controlled trial is currently underway to evaluate combinational usage of two strains of lactobacilli and two strains of bifidobacteria in the prevention of antibiotic-associated diarrhea in older people admitted to hospital [130].

6.5. Fecal microbiota transplatation (FMT)

FMT involves the "infusion of a fecal suspension from a healthy individual into the gastrointestinal tract of an individual with colonic disease."[131]. With cure rates between 90-95% reported by numerous investigators, FMT is

 © FORMATEX 2013

emerging as a powerful clinical therapy for recurrent CDI [131, 132]. In a randomized, controlled trial by van Nood et al [133], FMT was effective in 81% of patients with recurrent CDI, consistent with a systematic review of uncontrolled case series in which FMT through the stomach or small intestine showed an overall response rate of 80% vs. control recovery of the anecdotally reported overall response rate for FMT through colonoscopy or enema is 92% [134].

7. Future Research Directions

Although the multistrain probiotic mixtures and *S. boulardii* may be effective in the prevention of CDI, these findings are largely based on small case studies. Randomized, controlled and double-blinded, multi-centered studies are needed to confirm these preliminary positive findings and to establish the efficacy and safety of these probiotics in CDI prevention or treatment [135].

Despite the fact that probiotics such as *lactobacilli* and *bifidobacteria* have a long record of safety, experience with other forms of probiotic is more limited [136]. There is still risk particularly in the context of certain forms of host susceptibility and uncertainty regarding the potential for transfer of antibiotic resistance with probiotics. It was recommended that the safety of probiotics should be considered on a strain-by-strain basis [136]. Special caution should be given to debilitated and immunosuppressed patients, for whom the risks may outweigh benefits [122].

Along the same line, despite the reported high cure rates, FMT has several limitations: Due to safety issues, screening of all FMT donors is recommended including a careful review of their health history and blood and stool test to detect any previous exposure to transmissible agents [132]. Furthermore, because it is aesthetically unappealing and logistically challenging, the use of feces may eventually be replaced by defined mixtures of cultured bacteria that confer colonization resistance against *C. difficile* [134]. Therefore, defining specific commensal bacterial species to target *C. difficile* is an attractive non-antibiotic based strategy. The mouse model study with a simple, defined bacteriotherapy resolves relapsing CDI and human FMT efficacy in CDI are supportive for the rational design of multistrain probiotics for CDI, utilizing microbiome analysis in the clinical settings.

Acknowledgements This work was funded through NIH award K01DK092352 (X. Sun), Tufts Collaborates 2013 (X. Sun) and Crohn's & Colitis Foundation of America Career Development Award (X. Chen). The authors thank Dr. J Thomas Lamont for his critical review of the manuscript.

References

[1] Cloud, J. and C.P. Kelly, *Update on Clostridium difficile associated disease.* Curr Opin Gastroenterol, 2007. 23(1): p. 4-9.
[2] Archibald, L.K., S.N. Banerjee, and W.R. Jarvis, *Secular trends in hospital-acquired Clostridium difficile disease in the United States, 1987-2001.* J Infect Dis, 2004. 189(9): p. 1585-9.
[3] Bartlett, J.G., *Narrative review: the new epidemic of Clostridium difficile-associated enteric disease.* Ann Intern Med, 2006. 145(10): p. 758-64.
[4] Loo, V.G., et al., *A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality.* N Engl J Med, 2005. 353(23): p. 2442-9.
[5] McDonald, L.C., et al., *An epidemic, toxin gene-variant strain of Clostridium difficile.* N Engl J Med, 2005. 353(23): p. 2433-41.
[6] Zar, F., et al., *A Comparison of Vancomycin and Metronidazole for the Treatment of Clostridium difficileâ€"Associated Diarrhea, Stratified by Disease Severity.* Clinical Infectious Diseases, 2007. 45(3): p. 302-307.
[7] Barbut, F., et al., *Epidemiology of recurrences or reinfections of Clostridium difficile-associated diarrhea.* J Clin Microbiol, 2000. 38(6): p. 2386-8.
[8] Tonna, I. and P.D. Welsby, *Pathogenesis and treatment of Clostridium difficile infection.* Postgrad Med J, 2005. 81(956): p. 367-9.
[9] Borriello, S.P. and F.E. Barclay, *An in-vitro model of colonisation resistance to Clostridium difficile infection.* J Med Microbiol, 1986. 21(4): p. 299-309.
[10] Borriello, S.P., *The influence of the normal flora on Clostridium difficile colonisation of the gut.* Ann Med, 1990. 22(1): p. 61-7.
[11] Elliott, B., et al., *Clostridium difficile-associated diarrhoea.* Intern Med J, 2007. 37(8): p. 561-8.
[12] Kelly, C.P., C. Pothoulakis, and J.T. LaMont, *Clostridium difficile colitis.* N Engl J Med, 1994. 330(4): p. 257-62.
[13] Voth, D.E. and J.D. Ballard, *Clostridium difficile toxins: mechanism of action and role in disease.* Clin Microbiol Rev, 2005. 18(2): p. 247-63.
[14] .Kurtz, C.B., et al., *GT160-246, a toxin binding polymer for treatment of Clostridium difficile colitis.* Antimicrob Agents Chemother, 2001. 45(8): p. 2340-7.
[15] Lyerly, D.M., et al., *Biological activities of toxins A and B of Clostridium difficile.* Infect Immun, 1982. 35(3): p. 1147-50.
[16] Lyerly, D.M., et al., *Effects of Clostridium difficile toxins given intragastrically to animals.* Infect Immun, 1985. 47(2): p. 349-52.
[17] Savidge, T.C., et al., *Clostridium difficile toxin B is an inflammatory enterotoxin in human intestine.* Gastroenterology, 2003. 125(2): p. 413-20.
[18] Shin, B.M., et al., *Emerging toxin A-B+ variant strain of Clostridium difficile responsible for pseudomembranous colitis at a tertiary care hospital in Korea.* Diagn Microbiol Infect Dis, 2007.

[19] Kuehne, S.A., et al., *The role of toxin A and toxin B in Clostridium difficile infection.* Nature. 467(7316): p. 711-3.
[20] Lyras D, O.C.J., Howarth PM, Sambol SP, Carter GP, Phumoonna T, Poon R, Adams V, Vedantam G, Johnson S, Gerding DN, Rood JI, *Toxin B is essential for virulence of Clostridium difficile.* Nature, 2009.
[21] Carter, G.P., et al., *Binary toxin production in Clostridium difficile is regulated by CdtR, a LytTR family response regulator.* J Bacteriol, 2007. 189(20): p. 7290-301.
[22] McMaster-Baxter, N.L. and D.M. Musher, *Clostridium difficile: recent epidemiologic findings and advances in therapy.* Pharmacotherapy, 2007. 27(7): p. 1029-39.
[23] .Blossom, D.B. and L.C. McDonald, *The challenges posed by reemerging Clostridium difficile infection.* Clin Infect Dis, 2007. 45(2): p. 222-7.
[24] Stare, B.G., M. Delmee, and M. Rupnik, *Variant forms of the binary toxin CDT locus and tcdC gene in Clostridium difficile strains.* J Med Microbiol, 2007. 56(Pt 3): p. 329-35.
[25] Borriello, S.P., et al., *Virulence factors of Clostridium difficile.* Rev Infect Dis, 1990. 12 Suppl 2: p. S185-91.
[26] Seddon, S.V., I. Hemingway, and S.P. Borriello, *Hydrolytic enzyme production by Clostridium difficile and its relationship to toxin production and virulence in the hamster model.* J Med Microbiol, 1990. 31(3): p. 169-74.
[27] .Borriello, S.P., *Pathogenesis of Clostridium difficile infection.* J Antimicrob Chemother, 1998. 41 Suppl C: p. 13-9.
[28] Calabi, E., et al., *Binding of Clostridium difficile surface layer proteins to gastrointestinal tissues.* Infect Immun, 2002. 70(10): p. 5770-8.
[29] O'Brien, J.B., et al., *Passive immunisation of hamsters against Clostridium difficile infection using antibodies to surface layer proteins.* FEMS Microbiol Lett, 2005. 246(2): p. 199-205.
[30] .Roberts, K., et al., *Aerial dissemination of Clostridium difficile spores.* BMC Infect Dis, 2008. 8: p. 7.
[31] Dubberke, E.R., et al., *Prevalence of Clostridium difficile environmental contamination and strain variability in multiple health care facilities.* Am J Infect Control, 2007. 35(5): p. 315-8.
[32] Walters, B.A., et al., *Relapse of antibiotic associated colitis: endogenous persistence of Clostridium difficile during vancomycin therapy.* Gut, 1983. 24(3): p. 206-12.
[33] Gerding, D.N., C.A. Muto, and R.C. Owens, Jr., *Treatment of Clostridium difficile infection.* Clin Infect Dis, 2008. 46 Suppl 1: p. S32-42.
[34] McVay, C.S. and R.D. Rolfe, *In vitro and in vivo activities of nitazoxanide against Clostridium difficile.* Antimicrob Agents Chemother, 2000. 44(9): p. 2254-8.
[35] .Anton, P.M., et al., *Rifalazil treats and prevents relapse of clostridium difficile-associated diarrhea in hamsters.* Antimicrob Agents Chemother, 2004. 48(10): p. 3975-9.
[36] .Hinkson, P.L., et al., *Tolevamer, an anionic polymer, neutralizes toxins produced by the BI/027 strains of Clostridium difficile.* Antimicrob Agents Chemother, 2008. 52(6): p. 2190-5.
[37] Taylor, C.P., et al., *Open-label, dose escalation phase I study in healthy volunteers to evaluate the safety and pharmacokinetics of a human monoclonal antibody to Clostridium difficile toxin A.* Vaccine, 2008.
[38] Kotloff, K.L., et al., *Safety and immunogenicity of increasing doses of a Clostridium difficile toxoid vaccine administered to healthy adults.* Infect Immun, 2001. 69(2): p. 988-95.
[39] Sougioultzis, S., et al., *Clostridium difficile toxoid vaccine in recurrent C. difficile-associated diarrhea.* Gastroenterology, 2005. 128(3): p. 764-70.
[40] Razaq, N., et al., *Infection of hamsters with historical and epidemic BI types of Clostridium difficile.* J Infect Dis, 2007. 196(12): p. 1813-9.
[41] Chen, X., et al., *A mouse model of Clostridium difficile-associated disease.* Gastroenterology, 2008. 135(6): p. 1984-92.
[42] .Sun, X., et al., *Mouse relapse model of Clostridium difficile infection.* Infect Immun. 79(7): p. 2856-64.
[43] Rea, M.C., et al., *Effect of broad- and narrow-spectrum antimicrobials on Clostridium difficile and microbial diversity in a model of the distal colon.* Proc Natl Acad Sci U S A. 108 Suppl 1: p. 4639-44.
[44] Manges, A.R., et al., *Comparative metagenomic study of alterations to the intestinal microbiota and risk of nosocomial Clostridum difficile-associated disease.* J Infect Dis. 202(12): p. 1877-84.
[45] Chang, J.Y., et al., *Decreased diversity of the fecal Microbiome in recurrent Clostridium difficile-associated diarrhea.* J Infect Dis, 2008. 197(3): p. 435-8.
[46] Gough, E., H. Shaikh, and A.R. Manges, *Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection.* Clin Infect Dis. 53(10): p. 994-1002.
[47] Khoruts, A., et al., *Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent Clostridium difficile-associated diarrhea.* J Clin Gastroenterol. 44(5): p. 354-60.
[48] Suwantarat, N. and D.A. Bobak, *Current Status of Nonantibiotic and Adjunct Therapies for Clostridium difficile Infection.* Curr Infect Dis Rep. 13(1): p. 21-7.
[49] Pham, M., D.A. Lemberg, and A.S. Day, *Probiotics: sorting the evidence from the myths.* Med J Aust, 2008. 188(5): p. 304-8.
[50] Quigley, E.M., *Prebiotics and probiotics; modifying and mining the microbiota.* Pharmacol Res. 61(3): p. 213-8.
[51] Guandalini, S., *Probiotics for children with diarrhea: an update.* J Clin Gastroenterol, 2008. 42 Suppl 2: p. S53-7.
[52] Ng, S.C., et al., *Mechanisms of action of probiotics: recent advances.* Inflamm Bowel Dis, 2009. 15(2): p. 300-10.
[53] Oelschlaeger, T.A., *Mechanisms of probiotic actions - A review.* Int J Med Microbiol. 300(1): p. 57-62.
[54] Hickson, M., *Probiotics in the prevention of antibiotic-associated diarrhoea and Clostridium difficile infection.* Therap Adv Gastroenterol. 4(3): p. 185-97.
[55] Hell, M., et al., *Probiotics in Clostridium difficile infection: reviewing the need for a multistrain probiotic.* Benef Microbes. 4(1): p. 39-51.
[56] Parkes, G.C., J.D. Sanderson, and K. Whelan, *The mechanisms and efficacy of probiotics in the prevention of Clostridium difficile-associated diarrhoea.* Lancet Infect Dis, 2009. 9(4): p. 237-44.
[57] Trejo, F.M., et al., *Inhibition of Clostridium difficile growth and adhesion to enterocytes by Bifidobacterium supernatants.* Anaerobe, 2006. 12(4): p. 186-93.

 © FORMATEX 2013

[58] Corr, S.C., C.G. Gahan, and C. Hill, *Impact of selected Lactobacillus and Bifidobacterium species on Listeria monocytogenes infection and the mucosal immune response.* FEMS Immunol Med Microbiol, 2007. 50(3): p. 380-8.
[59] .Rea, M.C., et al., *Antimicrobial activity of lacticin 3,147 against clinical Clostridium difficile strains.* J Med Microbiol, 2007. 56(Pt 7): p. 940-6.
[60] .Rea, M.C., et al., *Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against Clostridium difficile.* Proc Natl Acad Sci U S A. 107(20): p. 9352-7.
[61] Britton, A.R., and Young, B. Vincent, *Interaction between the intestinal microbiota and host in Clostridium difficile colonization resistance.* Cell, 2012. 20(7): p. 313-319.
[62] Ridlon, J.M., D.J. Kang, and P.B. Hylemon, *Bile salt biotransformations by human intestinal bacteria.* J Lipid Res, 2006. 47(2): p. 241-59.
[63] Ridlon, J.M., D.J. Kang, and P.B. Hylemon, *Isolation and characterization of a bile acid inducible 7alpha-dehydroxylating operon in Clostridium hylemonae TN271.* Anaerobe. 16(2): p. 137-46.
[64] Giel, J.L., et al., *Metabolism of bile salts in mice influences spore germination in Clostridium difficile.* PLoS One. 5(1): p. e8740.
[65] Jarchum, I., et al., *Toll-like receptor 5 stimulation protects mice from acute Clostridium difficile colitis.* Infect Immun. 79(4): p. 1498-503.
[66] Ryan, A., et al., *A role for TLR4 in Clostridium difficile infection and the recognition of surface layer proteins.* PLoS Pathog. 7(6): p. e1002076.
[67] Castagliuolo, I., et al., *Saccharomyces boulardii protease inhibits Clostridium difficile toxin A effects in the rat ileum.* Infect Immun, 1996. 64(12): p. 5225-32.
[68] Castagliuolo, I., et al., *Saccharomyces boulardii protease inhibits the effects of Clostridium difficile toxins A and B in human colonic mucosa.* Infect Immun, 1999. 67(1): p. 302-7.
[69] Castex, F., et al., *Prevention of Clostridium difficile-induced experimental pseudomembranous colitis by Saccharomyces boulardii: a scanning electron microscopic and microbiological study.* J Gen Microbiol, 1990. 136(6): p. 1085-9.
[70] Pothoulakis, C., et al., *Saccharomyces boulardii inhibits Clostridium difficile toxin A binding and enterotoxicity in rat ileum.* Gastroenterology, 1993. 104(4): p. 1108-15.
[71] Schlee, M., et al., *Probiotic lactobacilli and VSL#3 induce enterocyte beta-defensin 2.* Clin Exp Immunol, 2008. 151(3): p. 528-35.
[72] .Giesemann, T., G. Guttenberg, and K. Aktories, *Human alpha-defensins inhibit Clostridium difficile toxin B.* Gastroenterology, 2008. 134(7): p. 2049-58.
[73] Kolling, G.L., et al., *Lactic acid production by Streptococcus thermophilus alters Clostridium difficile infection and in vitro Toxin A production.* Gut Microbes. 3(6): p. 523-9.
[74] Lammers, K.M., et al., *Effect of probiotic strains on interleukin 8 production by HT29/19A cells.* Am J Gastroenterol, 2002. 97(5): p. 1182-6.
[75] O'Hara, A.M., et al., *Functional modulation of human intestinal epithelial cell responses by Bifidobacterium infantis and Lactobacillus salivarius.* Immunology, 2006. 118(2): p. 202-15.
[76] Otte, J.M. and D.K. Podolsky, *Functional modulation of enterocytes by gram-positive and gram-negative microorganisms.* Am J Physiol Gastrointest Liver Physiol, 2004. 286(4): p. G613-26.
[77] Schlee, M., et al., *Induction of human beta-defensin 2 by the probiotic Escherichia coli Nissle 1917 is mediated through flagellin.* Infect Immun, 2007. 75(5): p. 2399-407.
[78] Hart, A.L., et al., *Modulation of human dendritic cell phenotype and function by probiotic bacteria.* Gut, 2004. 53(11): p. 1602-9.
[79] Drakes, M., T. Blanchard, and S. Czinn, *Bacterial probiotic modulation of dendritic cells.* Infect Immun, 2004. 72(6): p. 3299-309.
[80] Kelly, C.P., et al., *Human colonic aspirates containing immunoglobulin A antibody to Clostridium difficile toxin A inhibit toxin A-receptor binding.* Gastroenterology, 1992. 102(1): p. 35-40.
[81] Kyne, L., et al., *Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A.* N Engl J Med, 2000. 342(6): p. 390-7.
[82] Buts, J.P., N. De Keyser, and L. De Raedemaeker, *Saccharomyces boulardii enhances rat intestinal enzyme expression by endoluminal release of polyamines.* Pediatr Res, 1994. 36(4): p. 522-7.
[83] Qamar, A., et al., *Saccharomyces boulardii stimulates intestinal immunoglobulin A immune response to Clostridium difficile toxin A in mice.* Infect Immun, 2001. 69(4): p. 2762-5.
[84] Kyne, L.W.M., Qamar A, Kelly CP, *Association between antibody response to toxin A and protection against recurrent Clostridium difficile diarrhoea.* Lancet, 2001. 357: p. 189-193.
[85] Leav, B.A., et al., *Serum anti-toxin B antibody correlates with protection from recurrent Clostridium difficile infection (CDI).* Vaccine. 28(4): p. 965-9.
[86] Pothoulakis, C., *Effects of Clostridium difficile toxins on epithelial cell barrier.* Ann N Y Acad Sci, 2000. 915: p. 347-56.
[87] Hayashi, H., et al., *Inhibition and redistribution of NHE3, the apical Na+/H+ exchanger, by Clostridium difficile toxin B.* J Gen Physiol, 2004. 123(5): p. 491-504.
[88] Moore, R., et al., *C. difficile toxin A increases intestinal permeability and induces Cl- secretion.* Am J Physiol, 1990. 259(2 Pt 1): p. G165-72.
[89] Madsen, K., et al., *Probiotic bacteria enhance murine and human intestinal epithelial barrier function.* Gastroenterology, 2001. 121(3): p. 580-91.
[90] Resta-Lenert, S. and K.E. Barrett, *Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC).* Gut, 2003. 52(7): p. 988-97.
[91] Heuvelin, E., et al., *A Bifidobacterium probiotic strain and its soluble factors alleviate chloride secretion by human intestinal epithelial cells.* J Nutr. 140(1): p. 7-11.

[92] Malakooti, J., et al., *Transcriptional regulation of the intestinal luminal Na(+) and Cl(-) transporters.* Biochem J. 435(2): p. 313-25.
[93] Borthakur, A., et al., *The probiotic Lactobacillus acidophilus stimulates chloride/hydroxyl exchange activity in human intestinal epithelial cells.* J Nutr, 2008. 138(7): p. 1355-9.
[94] Singla, A., et al., *LPA stimulates intestinal DRA gene transcription via LPA2 receptor, PI3K/AKT, and c-Fos-dependent pathway.* Am J Physiol Gastrointest Liver Physiol. 302(6): p. G618-27.
[95] Karczewski, J., et al., *Regulation of human epithelial tight junction proteins by Lactobacillus plantarum in vivo and protective effects on the epithelial barrier.* Am J Physiol Gastrointest Liver Physiol. 298(6): p. G851-9.
[96] Wells, J.M., *Immunomodulatory mechanisms of lactobacilli.* Microb Cell Fact. 10 Suppl 1: p. S17.
[97] Mack, D.R., et al., *Probiotics inhibit enteropathogenic E. coli adherence in vitro by inducing intestinal mucin gene expression.* Am J Physiol, 1999. 276(4 Pt 1): p. G941-50.
[98] Vohra, P. and I.R. Poxton, *Induction of cytokines in a macrophage cell line by proteins of Clostridium difficile.* FEMS Immunol Med Microbiol. 65(1): p. 96-104.
[99] 99.Sun, X., et al., *Essential role of the glucosyltransferase activity in Clostridium difficile toxin-induced secretion of TNF-alpha by macrophages.* Microb Pathog, 2009. 46(6): p. 298-305.
[100] Pechine, S., et al., *Immunological properties of surface proteins of Clostridium difficile.* J Med Microbiol, 2005. 54(Pt 2): p. 193-6.
[101] Solomon, K., et al., *Monocytes are highly sensitive to clostridium difficile toxin A-induced apoptotic and nonapoptotic cell death.* Infect Immun, 2005. 73(3): p. 1625-34.
[102] Yan, F., et al., *Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth.* Gastroenterology, 2007. 132(2): p. 562-75.
[103] Chen, X., et al., *Saccharomyces boulardii inhibits ERK1/2 mitogen-activated protein kinase activation both in vitro and in vivo and protects against Clostridium difficile toxin A-induced enteritis.* J Biol Chem, 2006. 281(34): p. 24449-54.
[104] Chen, X., et al., *Saccharomyces boulardii inhibits EGF receptor signaling and intestinal tumor growth in Apc(min) mice.* Gastroenterology, 2009. 137(3): p. 914-23.
[105] Sougioultzis, S., et al., *Saccharomyces boulardii produces a soluble anti-inflammatory factor that inhibits NF-kappaB-mediated IL-8 gene expression.* Biochem Biophys Res Commun, 2006. 343(1): p. 69-76.
[106] Sambol, S.P., et al., *Colonization for the prevention of Clostridium difficile disease in hamsters.* J Infect Dis, 2002. 186(12): p. 1781-9.
[107] Trejo, F.M., G.L. De Antoni, and P.F. Perez, *Protective effect of bifidobacteria in an experimental model of Clostridium difficile associated colitis.* J Dairy Res, 2013: p. 1-7.
[108] Bolla, P.A., et al., *Protective effect of a mixture of kefir-isolated lactic acid bacteria and yeasts in a hamster model of Clostridium difficile infection.* Anaerobe, 2013. 21: p. 28-33.
[109] Corthier, G., F. Dubos, and R. Ducluzeau, *Prevention of Clostridium difficile induced mortality in gnotobiotic mice by Saccharomyces boulardii.* Can J Microbiol, 1986. 32(11): p. 894-6.
[110] .Kolling, G.L., et al., *Lactic acid production by Streptococcus thermophilus alters Clostridium difficile infection and in vitro Toxin A production.* Gut Microbes, 2012. 3(6): p. 523-9.
[111] Kaur, S., et al., *Effect of Lactobacillus acidophilus & epidermal growth factor on experimentally induced Clostridium difficile infection.* Indian J Med Res, 2011. 133: p. 434-41.
[112] Lawley, T.D., et al., *Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing Clostridium difficile disease in mice.* PLoS Pathog, 2012. 8(10): p. e1002995.
[113] Graul, T., A.M. Cain, and K.D. Karpa, *Lactobacillus and bifidobacteria combinations: a strategy to reduce hospital-acquired Clostridium difficile diarrhea incidence and mortality.* Med Hypotheses, 2009. 73(2): p. 194-8.
[114] Preidis, G.A., et al., *Probiotics, enteric and diarrheal diseases, and global health.* Gastroenterology, 2011. 140(1): p. 8-14.
[115] Chen, X. and C.P. Kelly, *Saccharomyces species*, in *James Versalovic and Michael Wilson editors. Therapeutic Microbiology: Probiotics and Other Strategies*. 2008, American Society of Microbiology: Washington DC. p. 51-60.
[116] .McFarland, L.V., et al., *A randomized placebo-controlled trial of Saccharomyces boulardii in combination with standard antibiotics for Clostridium difficile disease.* Jama, 1994. 271(24): p. 1913-8.
[117] Surawicz, C.M., et al., *The search for a better treatment for recurrent Clostridium difficile disease: use of high-dose vancomycin combined with Saccharomyces boulardii.* Clin Infect Dis, 2000. 31(4): p. 1012-7.
[118] .McFarland, L.V., *Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease.* Am J Gastroenterol, 2006. 101(4): p. 812-22.
[119] .Pozzoni, P., et al., *Saccharomyces boulardii for the prevention of antibiotic-associated diarrhea in adult hospitalized patients: a single-center, randomized, double-blind, placebo-controlled trial.* Am J Gastroenterol, 2012. 107(6): p. 922-31.
[120] .Chia, J.K., S.M. Chan, and H. Goldstein, *Baker's yeast as adjunctive therapy for relapses of Clostridium difficile diarrhea.* Clin Infect Dis, 1995. 20(6): p. 1581.
[121] .Kovacs, D.J. and T. Berk, *Recurrent Clostridium difficile-associated diarrhea and colitis treated with Saccharomyces cerevisiae (Baker's yeast) in combination with antibiotic therapy: a case report.* J Am Board Fam Pract, 2000. 13(2): p. 138-40.
[122] Segarra-Newnham, M., *Probiotics for Clostridium difficile-associated diarrhea: focus on Lactobacillus rhamnosus GG and Saccharomyces boulardii.* Ann Pharmacother, 2007. 41(7): p. 1212-21.
[123] Alvarez-Olmos, M.I. and R.A. Oberhelman, *Probiotic agents and infectious diseases: a modern perspective on a traditional therapy.* Clin Infect Dis, 2001. 32(11): p. 1567-76.
[124] Biller, J.A., et al., *Treatment of recurrent Clostridium difficile colitis with Lactobacillus GG.* J Pediatr Gastroenterol Nutr, 1995. 21(2): p. 224-6.
[125] Gorbach, S.L., T.W. Chang, and B. Goldin, *Successful treatment of relapsing Clostridium difficile colitis with Lactobacillus GG.* Lancet, 1987. 2(8574): p. 1519.

 © FORMATEX 2013

[126] Lawrence, S.J., J.R. Korzenik, and L.M. Mundy, *Probiotics for recurrent Clostridium difficile disease.* J Med Microbiol, 2005. 54(Pt 9): p. 905-6.
[127] .Shim, J.K., et al., *Primary symptomless colonisation by Clostridium difficile and decreased risk of subsequent diarrhoea.* Lancet, 1998. 351(9103): p. 633-6.
[128] Gerding, D.N. and S. Johnson, *Management of Clostridium difficile infection: thinking inside and outside the box.* Clin Infect Dis, 2010. 51(11): p. 1306-13.
[129] Hell, M., et al., *Probiotics in Clostridium difficile infection: reviewing the need for a multistrain probiotic.* Benef Microbes, 2013. 4(1): p. 39-51.
[130] Allen, S.J., et al., *A multicentre randomised controlled trial evaluating lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea in older people admitted to hospital: the PLACIDE study protocol.* BMC Infect Dis, 2012. 12: p. 108.
[131] Borody, T.J. and J. Campbell, *Fecal microbiota transplantation: techniques, applications, and issues.* Gastroenterol Clin North Am, 2012. 41(4): p. 781-803.
[132] Senior, K., *Faecal transplantation for recurrent C difficile diarrhoea.* Lancet Infect Dis, 2013. 13(3): p. 200-1.
[133] .van Nood, E., et al., *Duodenal infusion of donor feces for recurrent Clostridium difficile.* N Engl J Med, 2013. 368(5): p. 407-15.
[134] Kelly, C.P., *Fecal microbiota transplantation--an old therapy comes of age.* N Engl J Med, 2013. 368(5): p. 474-5.
[135] Na, X. and C. Kelly, *Probiotics in clostridium difficile Infection.* J Clin Gastroenterol, 2011. 45 Suppl: p. S154-8.
[136] Shanahan, F., *A commentary on the safety of probiotics.* Gastroenterol Clin North Am, 2012. 41(4): p. 869-76.

© FORMATEX 2013