

Lactobacillus and bifidobacteria combinations: A strategy to reduce hospital-acquired *Clostridium difficile* diarrhea incidence and mortality

Terry Graul^a, Alisha M. Cain^b, Kelly D. Karpa^{c,*}

^a American Board Certified Clin-Path Associates, PC, 1255 West Washington Street, Tempe, AZ 85281-1201, USA

^b Wilkes University School of Pharmacy, 84 West South Street, Wilkes-Barre, PA 18766, USA

^c An Apple A Day Health Solutions, LLC, 33 East Areba Ave, Hershey, PA 17033, USA

Summary

Incidence and virulence of *Clostridium difficile*-associated disease (CDAD) is increasing, particularly in institutional settings. Morbidity, mortality, and costs associated with this condition are high. Broad-spectrum antibiotics have long been recognized as the primary risk factor for CDAD due to disruption of protective normal gastrointestinal flora. We suggest that administration of appropriate lactobacilli and bifidobacteria probiotics could be employed as a strategy to protect hospitalized patients from CDAD by normalizing disrupted gastrointestinal flora, resulting in fewer cases per admission. Routine use of probiotics within institutional settings may substantially decrease healthcare costs. To date, relatively little is known about the role of probiotics as a means of preventing initial CDAD diagnosis. Although two reports suggestive of benefits have been published, these studies have been either too under-powered to draw definitive conclusions or have employed such restrictive inclusion criteria that results are not generalizable to most hospitalized adults. Since CDAD is an opportunistic infection associated with disrupted gut flora, it is logical to employ a strategy that modulates gut flora as a preventative approach. Herein, we report pilot data that is strongly suggestive that bifidobacteria and lactobacilli combinations may be effective in preventing this hospital-acquired infection and possibly reducing severity when diagnosed. These data are generalizable to all hospital patients since few exclusion criteria were employed. Limitations to these data are acknowledged since the pilot was not conducted in a placebo-controlled manner. Although generalizable, lack of a placebo precludes a definitive answer in terms of efficacy that lactobacilli and bifidobacteria may provide in CDAD prevention. Given substantial morbidity, mortality, and healthcare costs associated with CDAD, appropriately-designed clinical trials are warranted.

Introduction

Clostridium difficile is an opportunistic, gram-positive, anaerobic spore-forming bacillus that may cause unrelenting diarrheal disease, pseudomembranous colitis, and in severe cases, hemodynamic failure, colonic perforation, and peritonitis [1]. Symptomatically, diarrhea is the most common symptom of CDAD and may be accompanied by abdominal pain, nausea, anorexia, dehydration, malabsorption, malnutrition, fever, fecal leukocytosis, and toxic megacolon.

As an opportunistic pathogen, *C. difficile* may be carried by patients asymptotically, with the organism remaining dormant until conditions are acceptable for growth, proliferation, and infection. Frequently, that ideal time arises during administration of broad-spectrum antibiotics. Therefore, antimicrobial use is the main modifiable risk factor for CDAD. Antibiotics are believed to alter normal flora in the lower intestinal tract, perturbing the complex colonic ecology. Antibiotics are known to significantly decrease intestinal levels of anaerobic

*Corresponding author. Tel.: +1 717 533 3572; fax: +1 717 533 2006. E-mail address: kelly.karpa@verizon.net (K.D. Karpa).

bacteria. Within 3 days of initiation of antibiotic therapies, bifidobacteria populations decrease between 56% and 100% and lactobacilli decrease to 57% of baseline levels [2,3]. Broad-spectrum antibiotics have historically been cited as a key risk factor. Incidence of CDAD also correlates with duration of antimicrobial therapy [4–8]. Although nearly all antibacterial drugs have been reported to cause CDAD, agents such as clindamycin, cephalosporins, and fluoroquinolones are most often implicated [9–12].

Treatment of CDAD typically involves discontinuing the offending antibiotic and initiating treatment with metronidazole or oral vancomycin. Although these antibiotics suppress symptoms for most individuals, response rates as low as 78% for metronidazole are significantly lower than previously thought [13]. Furthermore, even among responders, an estimated 20–43% of individuals develop recurrences, typically within one to two months of the initial episode [14,15]. Patients that experience more than two episodes of CDAD have as high as a 50–65% chance of further recurrences [4]. An estimated 3 million individuals may live with chronic, persistent, relapsing CDAD and are plagued with diarrhea and related gastrointestinal symptoms [1].

From the late 1990s to early 2000s, hospital discharge codes reported a doubling of CDAD incidence [4]. According to US vital statistics data, deaths related to *C. difficile* enterocolitis increased 203% between 1999 and 2003 [16]. Furthermore, patients with a *C. difficile* diagnosis were found to have four times higher mortality rates, were hospitalized 2.5 times longer, and hospital costs were more than twice as much as patients without CDAD [17]. More than \$1.1 billion US dollars are spent annually treating CDAD. Re-hospitalizations due to recurrences of hospital-acquired CDAD are estimated to cost each affected institution more than \$85,000 annually [18–20]. Citing the Association for Professionals in Infection Control and Epidemiology, a recent Chicago newspaper indicated that more than 300 individuals are dying each day in the United States due to CDAD [21]. This has become a bacterial infection of endemic concern; its spread has out-paced that of methicillin-resistant *Staphylococcus aureus*, leading this organism to be cited as one of the most ecologically-relevant microbes and deadliest bacterial threats of the present day [18,22].

Presumably underlying the increase in CDAD incidence and mortality rates is a strain of *C. difficile* that appears to be more virulent than historical isolates. In the 1970s and early 1980s, two *C. difficile* toxins—toxin A (an enterotoxin) and toxin B (a cytotoxin)—were identified and implicated in the pathogenesis of CDAD. These toxins act synergistically, resulting in damage to colonic epithelium [23,24]. Following recent outbreaks of *C. difficile* diarrhea in both Canada and the United States, a strain of *C. difficile*, termed BI/NAP1/027, has emerged as a leading cause of illness. This more virulent *C. difficile* strain [9,10,18,25–27] has been associated with higher risk of serious complications, urgent need for intensive care, and increased rate of recurrence following initial infection [11,27]. All of these issues may stem from this strain's ability to produce 16 to 23 times more toxins A and B than were produced by historical isolates [11,27]. The ability to produce more toxin is believed to be caused by a deletion in *tcdC*—a mutation that is postulated to result in loss of negative regulatory function for toxin production [18,28,29]. In addition, BI/NAP1/027 appears to be less responsive to standard antimicrobial therapies historically used to combat this infection. This strain also produces a third binary toxin, whose functional significance is not yet clear [10,11,25,26]. Increased virulence of this organism has been simultaneously accompanied by increased incidence of community-acquired CDAD in populations previously thought to be at low risk, namely children and healthy young women [9,11].

Considering substantial morbidity, mortality, and costs associated with CDAD, efforts from healthcare institutions must focus on effective prevention strategies. Currently, within institutional settings, preventative strategies for CDAD primarily focus on good hand-hygiene for health-care workers. However, *C. difficile* spores may persist in the environment for months. Standard cleaning agents do not eradicate the organisms. Thus, *C. difficile* may be spread in hospitals through inanimate objects such as glucometers, stethoscopes, bedpans, and bed rails. As an opportunistic pathogen, *C. difficile* is most detrimental when host defenses are weakened by antimicrobial agents—due to disruption of normal gastrointestinal flora. Therefore, replacement of normal colonic flora may provide a mechanism for prevention of nosocomially-acquired *C. difficile* infections.

Hypothesis

Probiotics, defined as live microorganisms that confer host benefits when administered in adequate amounts, may offer a viable mechanism for normalizing disrupted endogenous gastrointestinal flora. Based upon various putative mechanisms, we propose that routine use of probiotics in hospitalized patients taking antibiotics may prevent CDAD. Furthermore, probiotics may reduce severity of infections when diagnosed and result in substantial cost savings to the healthcare system.

Several mechanisms may account for probiotic efficacy in preventing CDAD. Some probiotic bacteria produce substances, termed bacteriocins, which are toxic to *C. difficile* spores. Generation of hydrogen peroxide and short-chain fatty acids, which lower pH in localized areas of the colon, may be used by probiotics as natural defense mechanisms to inhibit *C. difficile* spore germination [18,30]. These mechanisms are significant since none of the currently- available antibiotics are capable of eradicating the spore form of the pathogen. In addition, probiotic microorganisms have potential to increase the number of antibody-secreting cells in the intestinal mucosa, and augment immune responses against *C. difficile* and/or its toxins [31]. Moreover, probiotics, via competitive exclusion for available space and/or nutrients within the intestine, may compete with clostridia and prevent these opportunistic organisms from becoming pathogenic, a phenomenon termed “colonization resistance” [32].

Using polymerase chain reaction (PCR) to detect *C. difficile*, the organism has been found to endogenously colonize 53% of healthy, community-based adults. This suggests that low-level endemic carriage of *C. difficile* is far more common than previously recognized [33]. Furthermore, most *C. difficile* recurrences (90%) appear to be attributed to endogenous *C. difficile* [34], suggesting that it is not the presence of the organism that is problematic per se. Rather, it is the lack of other constituents within the normal gastrointestinal flora which predisposes individuals to become infected with CDAD. This realization underscores the importance of protecting vulnerable individuals from CDAD caused by disruption of their own normal gut flora. Administration of anaerobic probiotics appears to be a means to accomplish colonization resistance and protect patients from disrupted gut flora, thus preventing CDAD. Lack of adequate clinical trials in this area leaves only speculation as to the true benefits that may be realized with probiotics if these agents were routinely used in hospitals.

Evolution of the Hypothesis

To date, relatively little is known about the role of probiotics as a means of preventing initial CDAD, but it does appear that bacterial species selection may play an important determinant in success. Specifically, single strain *Lactobacillus* species may not be adequate. Two trials using *Lactobacillus rhamnosus* GG as a CDAD preventative failed to demonstrate that this supplement could reduce incidence of CDAD [35,36].

Two additional studies have been undertaken where two or more bacterial species are used in combination. The first, a double-blind, placebo-controlled trial was a pilot study performed by Plummer et al. in 2004. However, the study was under-powered [37]. It was initially planned that 400 hospitalized patients taking antibiotics would be recruited and a 50% difference in CDAD between those receiving placebo versus probiotic would be realized. However, as a consequence of low study recruitment (only 138 patients), analysis of the ability of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* to prevent CDAD was only able to discuss differences between proportions of patients that developed *C. difficile*-toxin positive stool cultures. When analyzing outcomes associated with the probiotic in patients that developed diarrhea, incidence of stool specimens testing positive for *C. difficile*-associated toxins was 2.9% for those who received probiotic compared with 7.25% in those who received placebo. While these results suggest a protective benefit of the lactobacilli/ bifidobacterial combination, low recruitment prevents a firm conclusion.

Another double-blind, placebo-controlled trial was conducted by Hickson et al., where incidence of CDAD following antibiotics and probiotics (*Lactobacillus casei*, *S. thermophilus*, and *Lactobacillus bulgaricus*) was

investigated [38]. Researchers noted an absolute risk reduction for CDAD of 17% ($p = 0.001$; 95% CI, 7-27%) [38]. While these results are encouraging, this study has received wide criticism for study design [39-41]. It has been suggested that the exclusion criteria utilized by Hickson et al. were so restrictive that their results cannot be generalized to the majority of hospitalized adults. Specifically, only 7% of individuals that were prescribed antibiotics while hospitalized were enrolled into this study. Thus, many clinicians have not willingly accepted these results.

Observational Study

Prior to the studies by Plummer et al. and Hickson et al., observational data collected at Valley Lutheran Medical Center suggested the potential for probiotics to prevent CDAD when physicians at one hospital were encouraged to regularly prescribe probiotics to their inpatients receiving antibiotics. A portion of these data were presented previously in poster format; however, these data were not published [42].

In 2001, due to increasing use of broad-spectrum antibiotics, a multi-disciplinary team at Valley Lutheran Medical Center opted to institute a protocol, approved by both the Pharmacy and Therapeutics Committee and the Medical Executive Committee, to be followed for 3 months. Physicians were encouraged to write orders for a multi-species probiotic to be administered three times daily to nearly all inpatients prescribed antibiotics. Only patients undergoing active chemotherapeutic treatments were excluded. Each capsule of probiotic contained at least 5 billion cfu *Lactobacillus acidophilus*, 4 billion cfu *Bifidobacterium bifidum Bb12*, and 1 billion cfu *Bifidobacterium longum* per capsule (Florajen3, American Lifeline, Baraboo, WI, www.florajen.com) according to manufacturer's label. This product was selected because it contains no additives, preservatives, or allergens and because it contains a high number of viable organisms (CFU) which survive gastric acidity, adhere to intestinal epithelial cells, and colonize the digestive tract [43].

Diagnosis of CDAD was determined via enzyme linked immunosorbent assay (ELISA) for *C. difficile* toxins A and B (Premier Toxin A and B Kit, Meridian Bioscience, Cincinnati, OH) by the clinical microbiology laboratory at Valley Lutheran Medical Center. As part of a clinical nurse specialist's (T.G.) quality of care assessment of institutional problems and potential solutions, a retrospective chart review was conducted to see if regular probiotic prescribing during February through April of 2001 impacted the incidence or severity of antibiotic-associated diarrhea, specifically that caused by *C. difficile*. Data generated during this observational period was compared to that obtained from chart reviews of patients hospitalized and receiving antibiotics during the two preceding years; these patients served as a historical control population.

In addition, a reference group of patients diagnosed with CDAD were identified during the same February through April timeframes of 1999, 2000, and 2001 at Mesa Lutheran Hospital, a sister hospital located in the same town—about 12 miles from Valley Lutheran Medical Center. Both hospitals were part of the same healthcare system and staffed by many of the same healthcare professionals. Importantly, this facility also used the clinical microbiology laboratory at Valley Lutheran Medical Center for *C. difficile* diagnosis. Two other probiotic products, Bacid and Lactinex, were on formulary at Mesa Lutheran Hospital during all years of analysis and could be prescribed as deemed appropriate by attending providers. Florajen3 was not available at this institution. Raw data generated by chart reviews is presented in Table 1 as the number of individuals diagnosed with *C. difficile* per number of patients discharged during the years under investigation.

Given the substantial increase in morbidity and mortality attributed to *C. difficile* in hospitalized patients since 2001, data from the 2001 poster was recently re-examined using a log-linear model for binomial data. Our analysis of data was deemed to be exempt from investigational review board (IRB) oversight as determined by the IRB at Penn State Hershey College of Medicine Milton S. Hershey Medical Center (where one of us—KDK—is on faculty). Reasons for exemption include: statistical analysis of existing data that is publicly available and has been previously presented, individual subjects were not identifiable from the data that was analyzed, and the present data analysis does not involve use of protected health information as outlined by federal regulations.

A total of 4846 patients were discharged from Valley Lutheran Medical Center during the 2001 observational period. Of these patients, 6.03% (292 of 4846) were prescribed antibiotics and 175 of these individuals received antibiotics plus probiotics. Physicians failed to write probiotic orders (reasons not documented) for 117 eligible patients. Therefore, 59.9% of eligible patients received probiotics concomitantly when prescribed antibiotics.

Based upon 1999-2000 rates of CDAD at Valley Lutheran Medical Center and adjusted for incidence at Mesa Lutheran Hospital in 1999, 2000 and 2001, it is estimated that the percent reduction in incidence of CDAD that resulted from use of the Lactobacillus/Bifidobacteria combination was 66% (0.99-0.33%; p = 0.0027).

Similarly, assuming that the rate of antibiotic use per discharge was equal across years and at both hospitals, we can estimate the number of patients that were prescribed antibiotics based on the rate of antibiotic use per discharge at Valley Lutheran Medical Center during 2001 (292/4846 = 6.03%) (Table 1). This assumption appears to be reasonable since it has previously been demonstrated that although use of individual classes of antibiotics changes with time, total number of prescribed antibiotic units tends to remain relatively stable in hospitals around the country over extended time periods [44]. Again, using this assumption to adjust for 1999 and 2000 rates of CDAD per antibiotic use at Valley Lutheran Medical Center and Mesa Lutheran Hospital, incidence of CDAD was significantly reduced per antibiotic use during the months that the probiotic protocol was followed. Probiotic intervention is estimated to have reduced the incidence of CDAD by 11% (p = 0.0016; NNT = 9) in hospitalized patients, with 9 being the number needed to treat to prevent one case of CDAD.

Table 1

Incidence of CDAD diagnosis, total hospital discharges and estimated antibiotic use during the months of February through April in 1999, 2000, and 2001 at Valley Lutheran Medical Center and Mesa Lutheran Hospital.

Valley Lutheran Medical Center			Mesa Lutheran Hospital			
Year	Number of <i>C. dif-</i> <i>ficile</i> diagnoses	Discharges	Estimated rate of <i>C. dif-</i> <i>ficile</i> /antibiotic use (%)	Number of <i>C. dif-</i> <i>ficile</i> Diagnoses	Discharges	Estimated rate of <i>C. dif-</i> <i>ficile</i> /antibiotic use (%)
1999	40	3825	17.4 (40/230)*	24	3010	13.3 (24/181)*
2000	41	4050	16.8 (41/244)*	23	3124	12.2 (23/188)*
2001	16	4846	5.5 (16/292)**	25	3428	12.1 (25/207)*

* This estimate assumes a consistent rate of antibiotic use per discharge of 6.03%.

** p = 0.0016

Table 2

Average length of stay (LOS) for patients diagnosed with *C. difficile* during February through April in 1999, 2000, and 2001.

	Valley Lutheran Medical Center Average LOS (range)	Mesa Lutheran Hospital Average LOS (range)
1999	9.75 (2–42)	10.9 (3–34)
2000	9.53 (1–43)	8.8 (2–33)
2001	7.43 (2–31)	14.1 (2–63)

Although scarcity of data precludes definitive conclusions, there is a hint from collected data suggesting that even among those affected by CDAD at Valley Lutheran Medical Center in February through April of 2001, disease severity was lessened due to use of the probiotic protocol. Patients affected by CDAD were discharged to home faster (Table 2). Recent trends show patients affected by CDAD require 2.5 times longer length of hospital stays than non-CDAD-affected patients. If the present data is borne out in additional well-controlled studies, economic cost savings to the healthcare system could be substantial if probiotics were routinely prescribed to hospitalized patients.

Compared to the data of Hickson et al., the observational study conducted in Mesa, AZ, has the benefit of being widely generalizable since only patients receiving chemotherapy were categorically excluded from receiv-

ing the intervention. Nonetheless, positive trends observed are curtailed by other limitations, namely, lack of randomization, lack of placebo-control, use of historical controls, and need to rely upon assumptions during statistical analysis due to the methods utilized for data collection. Thus, definitive answers to the question of benefits from probiotics in preventing CDAD hospital-acquired infections have still not been reached. Despite limitations, this observational study is of utmost importance due to scarcity of data in this area. Routine use of combination probiotics in hospitalized patients prescribed antibiotics may have a substantial benefit on patient well-being, discharge outcomes, and healthcare costs.

Points to Consider in Future Research

Since CDAD is a condition associated with disrupted gut flora, it is logical to employ strategies that modulate gut flora as preventative approaches. However, as additional research is planned, investigators must consider the factors that have complicated our ability to understand how probiotics may fit into CDAD prevention protocols by designing trials that overcome obstacles encountered by others.

In the future, patient randomization to active and placebo groups, as well as treatment in a double-masked fashion, will be necessary to confirm the results obtained by the Mesa observational data. Prospectively-designed studies must assure that adequate statistical power can be attained. Knowing that adequate patient enrollment may be difficult with exclusion criteria inherent to these studies (recruitment as low as 7% among hospitalized patients taking antibiotics) [38], future investigators must adequately budget necessary staff, time, and financial resources in anticipation of slow recruitment. In addition, investigators must critically consider incidence rates of *C. difficile* in institutions where investigations are set to occur. Although some institutions may actually have CDAD incidence rates of 10% (as postulated in one study that was unable to demonstrate statistically significant results due to low enrollment) [37], most institutions probably do not have rates that high. Therefore, actual CDAD incidence rates must be known and accounted for when statistical analysis is computed to ensure that an adequate number of patients can be reasonably anticipated to be enrolled for study outcomes to yield meaningful results.

Future studies using probiotics may also seek to characterize both the genotype and toxinotype of *C. difficile* strains for which probiotics are efficacious and identify *C. difficile* strains that are non-responsive to probiotics. It is uncertain the extent to which Lactobacillus/Bifidobacteria combinations may impact the incidence of CDAD caused by the BI/NAP1/027 strain. No probiotic studies have attempted to genotype organisms recovered from affected patients. Future studies may focus on the degree to which probiotics can specifically prevent BI/NAP1/027 infection.

As additional studies are developed, adequate attention must be given to the strain(s) of bacteria tested. Previous work has shown that even similar species of probiotics may elicit vastly different biological responses. It cannot be assumed that all strains of probiotic bacteria produce the same beneficial outcomes as the strains studied herein, especially since numerous products marketed as “probiotics” are unlikely to meet all aspects of the definition. Additionally, it appears that combinations of different bacterial genera may be necessary to maximize therapeutic benefits [45]. Rationale for strain selection should also consider known characteristics of microorganisms such as resistance to gastric acid, bile sensitivity, ability to adhere to intestinal mucosa, and production of shortchain fatty acids, bacteriocins, and/or hydrogen peroxide metabolic byproducts. Evidence suggests that efficacy of bacterial probiotics against *C. difficile* may rely upon competitive exclusion mechanisms; thus, it is important that the majority of administered probiotic microorganisms remain viable until they reach the colon [32].

Similar to the necessity of using a rationally selected probiotic product, adequate consideration of placebo is also necessary. In order to demonstrate differences between treatment groups, the control group must be administered a placebo that contains only inert ingredients. Administration of placebo capsules that contain starches known to specifically stimulate growth and activities of intestinal flora (e.g. prebiotics) cannot be con-

sidered inactive and make poor placebos for probiotic efficacy studies since they can mask benefits that might be attained with probiotics.

Despite limitations, the Mesa, AZ data [42] lends support to our hypothesis that probiotics can prevent CDAD in hospitalized patients taking antibiotics. Furthermore, routine use of appropriate bacteriotherapeis could lead to substantial cost savings for the healthcare system. For less than \$1 per day, CDAD was prevented in many hospitalized patients taking antibiotics. In stark contrast, once the CDAD diagnosis is made, discharge may be delayed, hospitalization costs are doubled [17,20] and caregivers are exposed to a contagious, spore-forming infection [46].

Conclusions

In conclusion, lactobacilli/bifidobacteria probiotics may provide a safe and efficacious means for reducing incidence of CDAD in many patients. Given the substantial morbidity, mortality, and costs associated with CDAD, additional research into efficacy and putative mechanisms that underlie the protective effects of probiotic supplements is warranted. Numerous patients would benefit if this hypothesis is proven true, and in turn, morbidity, mortality, and economic burden would be diminished.

Acknowledgements

The authors would like to acknowledge American Lifeline, Inc. for their generous donation of Florajen probiotics (www.florajen.com) during the observational study in 2001.

References

- [1] Karpa KD. A gut wrenching experience: probiotics and diarrhea. In: Bacteria for breakfast: probiotics for good health. Victoria, BC, Canada: Trafford; 2003. p. 104-43.
- [2] Seki H, Shiohara M, Matsumura R, et al. Prevention of antibiotic-associated diarrhea in children by *Clostridium butyricum* MIYARI. *Ped Internat* 2003;45:86-90.
- [3] Imase K, Takahashi M, Tanaka A, et al. Efficacy of *Clostridium butyricum* preparation concomitantly with Helicobacter pylori eradication therapy in relation to changes in the intestinal microbiota. *Microbiol Immunol* 2008;52:156-61.
- [4] Sunenshine RH, McDonald LC. *Clostridium difficile*-associated disease: new challenges from an established pathogen. *Cleve Clin J Med* 2006;73(2): 187-97.
- [5] Bergogne-Berezin E. Treatment and prevention of antibiotic associated diarrhea. *Int J Antimicrob Agents* 2000;16(4):521-6.
- [6] Pepin J, Saheb N, Coulombe M-A, et al. Emergence of fluoroquinolones as the predominant risk factor for *Clostridium difficile*-associated diarrhea: a cohort study during an epidemic in Quebec. *Clin Infect Dis* 2005;41(9):1254-60.
- [7] Gaynes R, Rimland D, Killum E, et al. Outbreak of *Clostridium difficile* infection in a long-term care facility: association with gatifloxacin use. *Clin Infect Dis* 2004;38(5):640-5.
- [8] Loo VG, Poirier L, Miller MA, 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):2442-9.
- [9] O'Marra NB. Severe infections caused by *Clostridium difficile*—a growing problem. *Pharmacist Lett* 2006;22:220108.
- [10] Bartlett JG. Narrative review: the new epidemic of *Clostridium difficile* associated enteric disease. *Ann Intern Med* 2006;145(10):758-64.
- [11] Warny M, Pepin J, Fang A, et al. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *Lancet* 2005;366(9491):1079-84.
- [12] Woelfel JA. Proton pump inhibitors associated with increased risk of *Clostridium difficile* diarrhea. *Pharmacist Lett* 2004;20:200804.
- [13] Musher DM, Aslam S, Logan N, et al. Relatively poor outcome after treatment of *Clostridium difficile* colitis with metronidazole. *Clin Infect Dis* 2005;40(11):1586-90.
- [14] McFarland LV, Surawicz CM, Rubin M, Elmer GW, Greenburg RN. Recurrent *Clostridium difficile* disease: epidemiology and clinical characteristics. *Infect Control Hosp Epidemiol* 1999;20(1):43-50.
- [15] Surawicz CM, McFarland LV, Greenberg RN, 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):1012-7.
- [16] Wysowski DK. Surveillance of prescription-related mortality using death certificate data. *Drug Saf* 2007;30(6):533-40.

- [17] Pennsylvania Health Care Cost Containment Council. *Clostridium difficile* infection in Pennsylvania hospitals. Issue 11; May 2007. Available from: www.phc4.org/reports/researchbriefs/051107/docs/researchbrief051107.pdf [accessed 12.02.09].
- [18] McFarland LV, Beneda HW, Clarride JE, Raugi GJ. Implications of the changing face of *Clostridium difficile* disease for health care practitioners. *Am J Infect Control* 2007;35(4):237-53.
- [19] Miller MA, Hyland M, Ofner-Agostini M, Gourdeau M, Ishak M. Canadian Hospital Epidemiology Committee. Canadian Nosocomial Infection Surveillance Program. Morbidity, mortality, and healthcare burden of nosocomial *Clostridium difficile*-associated diarrhea in Canadian hospitals. *Infect Control Hosp Epidemiol* 2002;23(3):137-40.
- [20] Kyne L, Hamel MB, Polavaram R, Kelly CP. Healthcare costs and mortality associated with nosocomial diarrhea due to *Clostridium difficile*. *Clin Infect Dis* 2002;34(3):346-53.
- [21] McCoppin R. Hospitals fight a drug-resistant bug that can kill. *Chicago Daily Herald*; 19th January 2009. Available from: <http://www.dailyherald.com/story/?id=265009&src=120>. [accessed 12.02.09].
- [22] Stirling B, Littlejohn P, Willbond ML. Nurses and the control of infectious disease: understanding epidemiology and disease transmission is vital to nursing care. *Can Nurse* 2004;100(9):16-20.
- [23] Bartlett JG, Onderdonk AB, Cisneros RL, et al. Clindamycin-associated colitis due to a toxin-producing species of *Clostridium* in hamsters. *J Infect Dis* 1977;136(5):701-5.
- [24] Taylor NS, Thorne GM, Bartlett JG. Comparison of two toxins produced by *Clostridium difficile*. *Infect Immun* 1981;34(3):1036-43.
- [25] Miller MA. Clinical management of *Clostridium difficile*-associated disease. *Clin Infect Dis* 2007;45(Suppl. 2):S122-8.
- [26] Ricciardi R, Rothenberger DA, Madoff RD, Baxter NN. Increasing prevalence and severity of *Clostridium difficile* colitis in hospitalized patients in the United States. *Arch Surg* 2007;142(7):624-31.
- [27] McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. *N Engl J Med* 2005;353(23):2433-41.
- [28] Eggertson L. *Clostridium difficile* may have killed 2000 in Quebec: study. *CMAJ* 2005;173(9):1020-1.
- [29] Johnson S, Gerding DN, Olson MM, et al. Prospective, controlled study of vinyl glove use to interrupt *Clostridium difficile* nosocomial transmission. *Am J Med* 1990;88(2):137-40.
- [30] Karpa KD. Probiotics for *Clostridium difficile* diarrhea: putting it into perspective. *Ann Pharmacother* 2007;41(7):1284-7.
- [31] Gorbach SL. Probiotics and gastrointestinal health. *Am J Gastroenterol* 2000;95(Suppl. 1):S2-3.
- [32] Seal D, Borriello SP, Barclay F, Welch A, Piper M, Bonnycastle M. Treatment of relapsing *Clostridium difficile* diarrhea by administration of a non-toxicogenic strain. *Eur J Clin Microbiol* 1987;6(1):51-3.
- [33] Iizuka M, Konno S, Itou H, et al. Novel evidence suggesting *Clostridium difficile* is present in human gut microbiota more frequently than previously suspected. *Microbiol Immunol* 2004;48(11):889-92.
- [34] Noren T, Akerlund E, Back E, et al. Molecular epidemiology of hospital-associated and community-acquired *Clostridium difficile* infection in a Swedish county. *J Clin Microbiol* 2004;42(8):3635-43.
- [35] Thomas MR, Litin SC, Osmon DR, Corr AP, Weaver AL, Lohse CM. Lack of effect of *Lactobacillus GG* on antibiotic-associated diarrhea: a randomized, placebo-controlled trial. *Mayo Clin Proc* 2001;76(9):883-9.
- [36] Arvola T, Laiho K, Torkkeli S, et al. Prophylactic *Lactobacillus GG* reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. *Pediatrics* 1999;104(5):e64.
- [37] Plummer S, Weaver MA, Harris JC, Dee P, Hunter J. *Clostridium difficile* pilot study: effects of probiotic supplementation on the incidence of *C. difficile* diarrhoea. *Int Microbiol* 2004;7(1):59-62.
- [38] Hickson M, Souza AL, Muthu N, et al. Use of probiotic *Lactobacillus* preparation to prevent diarrhea associated with antibiotics: randomized double blind placebo controlled trial. *BMJ* 2007;335(7610):80 [epub ahead of print, accessed 11.07.07].
- [39] Wilcox MH, Sandoe JA. Probiotics and diarrhoea: data are not widely applicable. *BMJ* 2007;335(7612):171.
- [40] Billyard T. Probiotics and diarrhea: no high risk antibiotics? *BMJ* 2007; 335(7612):171.
- [41] Miller M. Commentary. *Evid Based Med* 2008;13:2-46.
- [42] Graul T. Reduction of *Clostridium difficile* in acute care: *Lactobacillus acidophilus* and bifidobacterium as Florajen3 protocol. American Association of Nurse Practitioners; 2001.
- [43] Juntunen M, Kirjavainen PV, Ouwehand AC, Salminen SJ, Isolauri E. Adherence of probiotic bacteria to human intestinal mucus in healthy infants and during rotavirus infection. *Clin Diagn Lab Immunol* 2001;8(2):293-6.
- [44] McFarland LV, Surawicz CM, Greenberg RN, et al. Prevention of beta-lactam-associated diarrhea by *Saccharomyces boulardii* compared with placebo. *Am J Gastroenterol* 1995;90(3):439-48.
- [45] Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal Microbiome in recurrent *Clostridium difficile*-associated diarrhea. *J Infect Dis* 2008;197(3):435-8.
- [46] Austin N. Spores, babies, and alcohol. A nurse's battle with *C. difficile*. *RN* 2007;70(3):39-43.