Department of Pharmacy[1], Gifu Municipal Hospital; Laboratory of Clinical Pharmacy[2], Gifu Pharmaceutical University, Gifu, Japan

Risk factors for *Clostridium difficile*-associated diarrhea and the effectiveness of prophylactic probiotic therapy

T. Mizui[1], H. Teramachi[2], T. Tachi[2], K. Tamura[2], H. Shiga[2], N. Komada[2], M. Umeda[1], A. Koda[1], S. Aoyama[1], C. Goto[1], T. Tsuchiya[2]

Received February 04, 2013, accepted March 01, 2013

Hitomi Teramachi, Laboratory of Clinical Pharmacy, Gifu Pharmaceutical University, 1-25-4 Daigaku-nishi, Gifu 501-1196, Japan
teramachih@gifu-pu.ac.jp

Pharmazie 68: 706–710 (2013) *doi: 10.1691/ph.2013.3552*

Measures for prevention of *Clostridium difficile*-associated diarrhea, a common nosocomial infection, in hospital settings are urgently needed. This study was conducted to identify the risk factors contributing to *C. difficile*-associated diarrhea and to evaluate the clinical benefit of probiotics in its prevention. The study included 2716 patients at least 20 years old who received an injected antibiotic at any time between February 2010 and February 2011; a total of 2687 patients (98.9%) were assigned to the non-*C. difficile*-associated diarrhea group, and 29 patients (1.1%) were assigned to the *C. difficile*-associated diarrhea group. Univariate analysis revealed a significant difference between the two groups for the following factors: antibiotic therapy for $\geq$8 days; enteral nutrition; intravenous hyperalimentation; fasting; proton pump inhibitor use; H_2 blocker use; and serum albumin $\leq$2.9 g/dL ($p < 0.05$). Multivariate logistic regression analysis revealed a significant difference between the two groups for several factors. Antibiotic therapy for $\geq$8 days, intravenous hyperalimentation, proton pump inhibitor use, and H_2 blocker use were therefore shown to be risk factors for *C. difficile*-associated diarrhea. Prophylactic probiotic therapy was not shown to suppress the occurrence of *C. difficile*-associated diarrhea.

1. Introduction

Clostridium difficile is a Gram-positive anaerobic bacterium present in the intestines of some healthy persons. The use of antibiotic therapy in a *C. difficile* carrier disrupts the colonic microbiota, resulting in *C. difficile* growth and the consequent release of toxins A and B, which cause pseudomembranous colitis with fever and diarrhea. This form of diarrhea is called *C. difficile*-associated diarrhea (CDAD) (Bartlett et al. 1977).

C. difficile occurs as toxigenic strains that produce toxins A and B and nontoxigenic strains that produce no toxins. Toxigenic strains cause CDAD. Toxin A, an enterotoxin, exhibits intestinal loop activity, produces symptoms of bleeding and water retention, and causes diarrhea. Toxin B, a cytotoxin, has potent cytotoxicity (Geric et al. 2004).

Pseudomembranous colitis is a clinical manifestation of *C. difficile* infection. This is likely the most common nosocomial infection, accounting for a high percentage of infections in hospital settings. *C. difficile* produces spores, which persist in the hospital environment after an infection being able to infect other hospitalized patients (Hurley and Nguyen 2002).

Known risk factors for CDAD include advanced age, antibiotic use, proton pump inhibitor use, H_2 blocker use, and undernutrition, and gastric acid suppression multiplies the risk (Hookman and Barkin 2009; Dial *et al*. 2005). Prophylactic probiotic therapy has been postulated as a factor lowering the risk of CDAD. Probiotics maintain the colonic microbiota, thereby preventing the growth of pathogenic microorganisms. Probiotics have been cited in the prevention of CDAD and other diarrhea caused by pseudomembranous colitis (Hickson 2011). However, despite a large body of research (Dendukuri et al. 2005; Song et al. 2010; Avadhani and Miley 2011), the clinical benefit of probiotics has not been demonstrated.

Therefore, the present study was conducted to identify the risk factors contributing to CDAD and evaluate the clinical benefit of probiotics in the prevention of CDAD.

2. Investigations and results

A total of 3212 patients received an injectable antibiotic while admitted. The non-CDAD group included 3183 patients (99.1%), and the CDAD group included 29 patients (0.9%) (Fig. 1). A total of 309 patients in the non-CDAD group with diarrhea for 2 or more consecutive days at any time from the start of antibiotic use to 1 month after the end of antibiotic use were excluded, and another 187 patients not taking a probiotic as prophylaxis were also excluded. No patient in the CDAD group satisfied any exclusion criterion. Following exclusion, 2687 of the 2716 patients (98.9%) were placed in the non-CDAD group, and 29 patients (1.1%) were placed in the CDAD group.

The demographics, number of days of antibiotic treatment, and statistical information on laboratory values of the 2716 study patients are shown in Table 1 with the results of univariate analysis in the non-CDAD and CDAD groups. The mean age was 66.7 years, and the age range was 20 to 99 years. Serum albumin, total cholesterol, and lymphocyte count data were available for 2376 patients (87.5%), 424 patients (15.6%), and 1075 patients

Table 1: Demographics and laboratory values of the CDAD and non-CDAD groups

	Total (n=2716)		Non-CDAD (n=2687)		CDAD (n=29)		p value
	n	Mean (median)	n	Mean (median)	n	Mean (median)	
Gender (Male/Female)	1527/1189		1506/1181		21/8		0.055
Age (years)	2716	66.7 (70.0)	2687	66.6 (70.0)	29	71.7 (73.0)	0.129
Period of antibiotic therapy (days)	2716	10.0 (6.0)	2687	9.9 (6.0)	29	19.2 (19.0)	0.000
Serum albumin (g/dL)	2376	3.3 (3.3)	2348	3.3 (3.3)	28	2.7 (2.6)	0.000
Total cholesterol (mg/dL)	424	164 (158)	423	164 (160)	1	N.D.	0.111
Total lymphocyte count (total/uL)	1075	1169 (1060)	1058	1172 (1062)	17	1026 (561)	0.137

(39.6%), respectively. The standard deviation was not determined for total cholesterol alone because only 1 patient in the CDAD group had an available total cholesterol value. The mean and median numbers of days of antibiotic therapy were 9.9 and 6.0 days, respectively, in the non-CDAD group and 19.2 and 19.0 days, respectively, in the CDAD group. These figures were significantly different ($p < 0.001$). The mean and median serum albumin levels were 3.3 and 3.3 g/dL, respectively, in the non-CDAD group and 2.7 and 2.6 g/dL, respectively, in the CDAD group. These figures were also significantly different ($p < 0.001$). The numbers of non-CDAD and CDAD group patients considered in each analysis and the results of univariate analysis are presented in Table 2. Significant differences between the non-CDAD and CDAD groups were detected for the following items: antibiotic therapy for ≥8 days; enteral nutrition; intravenous hyperalimentation; fasting; proton pump inhibitor use; H_2 blocker use; and serum albumin ≤2.9 g/dL. No significant difference was identified for prophylactic probiotic therapy. Similarly, no significant difference was detected for any specific probiotic formulation (Biofermin®: *Lactomin*, Biofermin-R®: *Resistant lactic acid bacterium*, Miya-BM®: *Clostridium butyricum*, Enteronon®-R: *Resistant lactic acid bacterium, faecalis BIO-4R*).

The numbers of non-CDAD and CDAD group patients taking each type of antibiotic and the results of univariate analysis are presented in Table 3. Second-generation cephem antibiotics were the most frequently used type (31.0%), followed by carbapenem antibiotics (28.0%) and third-generation cephem antibiotics (27.0%). Tetracyclines were the least commonly used type (2.1%). A significant difference was detected between the non-CDAD and CDAD groups in second-generation cephem antibiotics, third-generation cephem antibiotics, carbapenem antibiotics, and methicillin-Resistant *Staphylococcus aureus* (MRSA) agents. The use of third-generation cephem antibiotics, carbapenem antibiotics, and anti-MRSA agents was associated with an increase in CDAD occurrence, while the use of second-generation cephem antibiotics was associated with a decrease in CDAD occurrence.

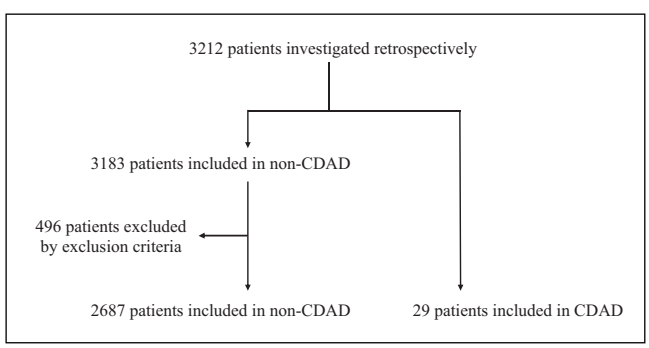

Fig. 1: Population analysis.

To investigate the relationship between prophylactic probiotic therapy and CDAD occurrence, multivariate logistic regression analysis was conducted using CDAD occurrence as the response variable and prophylactic probiotic therapy, age ≥65 years, antibiotic therapy ≥8 days, enteral nutrition, intravenous hyperalimentation, proton pump inhibitor use, H_2 blocker use, and serum albumin ≤2.9 g/dL as explanatory variables. To avoid issues such as multicollinearity and missing values, the other items were not included as explanatory variables. The results are shown in the forest plot in Fig. 2. The odds ratios (ORs) and 95% confidence intervals (95% CIs) are shown on the right of the Figure. There was a significant difference for the following factors: antibiotic therapy for ≥8 days (OR: 4.071, 95% CI: 1.333–12.430, $p = 0.014$), intravenous hyperalimentation (OR: 3.414, 95% CI: 1.469–7.934, $p = 0.004$), proton pump inhibitor use (OR: 3.224, 95% CI: 1.421–7.315, $p = 0.005$), and H_2 blocker use (OR: 2.376, 95% CI: 1.047–5.391, $p = 0.039$). However, no significant difference was found for prophylactic probiotic therapy, age ≥65 years, enteral nutrition, and serum albumin ≤2.9 g/dL.

3. Discussion

CDAD is not limited to sporadic occurrences and is known to affect multiple patients in the same hospital. Severe CDAD can cause extreme diarrhea, dehydration, melena, toxic megacolon, intestinal perforation, and even death (Hookman and Barkin 2009). CDAD can also prolong hospital stays and require readmission, increasing medical costs. The costs of handling *C. difficile* infections are astounding, leading some to find it worthwhile investing in preventive measures (Ghantoji *et al*. 2010). This study was conducted to collect further evidence on the risk factors for CDAD and investigate the clinical benefit of prophylactic probiotic therapy used to suppress CDAD.

The study produced valuable findings on CDAD risk factors but failed to show that prophylactic probiotic therapy suppressed CDAD occurrence. No significant difference was detected between the non-CDAD and CDAD groups even when prophylactic therapy with the probiotic formulations Biofermin®, Biofermin-R®, Miya-BM®, and Enteronon®-R was considered individually. It has been concluded elsewhere, however, that prophylactic therapy with probiotics containing a variety of microorganisms prevents antibiotic-associated diarrhea and *C. difficile* infections (Hickson 2011). Another study found that treatment with Miya-BM with an antibiotic lowered the detection rate of *C. difficile* (Kuroiwa et al. 1990). Further investigation of the relationship of probiotic types and CDAD occurrence with a larger sample size will be necessary.

The use of second-generation cephem antibiotics, third-generation cephem antibiotics, carbapenem antibiotics, and anti-MRSA agents resulted in a difference in CDAD occurrence, and the occurrence of CDAD increased in association with the use of third-generation cephem antibiotics, carbapenem

Table 2: Univariate analysis of therapy-related items and CDAD onset

	Total (n = 2716)	Non-CDAD (n = 2687)	CDAD (n = 29)	p value
Male	1527 (56.2%)	1506 (56.0%)	21 (72.4%)	0.055
Age≥65 years	1753 (64.5%)	1731 (64.4%)	22 (75.9%)	0.138
Period of antibiotic therapy≥8 days	1109 (40.8%)	1084 (40.3%)	25 (86.2%)	0.000
Enteral nutrition	170 (6.3%)	165 (6.1%)	5 (17.2%)	0.032
Intravenous hyperalimentation	266 (9.8%)	252 (9.3%)	14 (48.2%)	< 0.001
Fasting	853 (31.4%)	833 (31.0%)	20 (69.0%)	< 0.001
Proton pump inhibitor	732 (27.0%)	714 (26.6%)	18 (62.0%)	< 0.001
H_2 blocker	524 (19.3%)	509 (18.9%)	15 (51.7%)	< 0.001
Serum albumin ≤2.9 g/dL	785 (n = 2376, 33.0%)	767 (n = 2348, 32.7%)	18 (n = 28, 64.3%)	0.001
Total cholesterol ≤139 mg/dL	131 (n = 424, 30.9%)	130 (n = 423, 30.7%)	1 (n = 1, 100.0%)	N.D.
Total lymphocyte count ≤1199 total/mL	623 (n = 1075, 58.0%)	612 (n = 1058, 57.8%)	11 (n = 17, 64.7%)	0.379
Preventive probiotic therapy	102 (3.8%)	100 (37.2%)	2 (6.9%)	0.298
-Biofermin®	64 (2.4%)	63 (2.3%)	1 (3.4%)	0.501
-Biofermin-R®	20 (0.7%)	19 (0.7%)	1 (3.4%)	0.194
-Miya-BM®	22 (0.8%)	21 (0.8%)	1 (3.4%)	0.211
-Enteronon®-R	2 (0.1%)	2 (0.1%)	0 (0.0%)	0.979

Table 3: Univariate analysis of antibiotic types and CDAD onset

	Total (n = 2716)	Non-CDAD (n = 2687)	CDAD (n = 29)	p value
Penicillin	561 (20.7%)	553 (20.6%)	8 (27.6%)	0.236
First generation cephem	370 (13.6%)	363 (13.5%)	7 (24.1%)	0.089
Second generation cephem	842 (31.0%)	839 (31.2%)	3 (10.3%)	0.009
Third generation cephem	732 (27.0%)	718 (26.7%)	14 (48.3%)	0.011
Carbapenem	760 (28.0%)	747 (27.8%)	13 (44.8%)	0.038
Aminoglycoside	222 (8.2%)	222 (8.3%)	0 (0.0%)	0.083
New quinolone	112 (4.1%)	109 (4.1%)	3 (10.3%)	0.115
Lincomycin	70 (2.6%)	70 (2.6%)	0 (0.0%)	0.467
Tetracycline	56 (2.1%)	54 (2.0%)	2 (6.9%)	0.119
Fosmycin	83 (3.1%)	83 (3.1%)	0 (0.0%)	0.405
Anti-MRSA agent	94 (3.5%)	87 (3.2%)	7 (24.1%)	0.000

antibiotics, and anti-MRSA agents. Although, fundamentally, any antibiotic should be able to cause CDAD (Hookman and Barkin 2009), further investigation of the relationship between antibiotic type and CDAD occurrence will be necessary with a larger sample size.

Although the study did not reveal any significant difference in CDAD prevention for prophylactic probiotic treatment, probiotic therapy, an issue considered in the study, was included in the multivariate analysis. Antibiotic therapy for ≥8 days, intravenous hyperalimentation, proton pump inhibitor use, and H_2 blocker use were shown to be risk factors for CDAD. Proton pump inhibitor use and H_2 blocker use suppress gastric acid secretion, elevating pH and allowing more *C. difficile* to survive past the stomach and grow in the small and large intestines to cause CDAD. Proton pump inhibitor use is reported to increase the risk of CDAD occurrence 2.03-fold (95% CI:

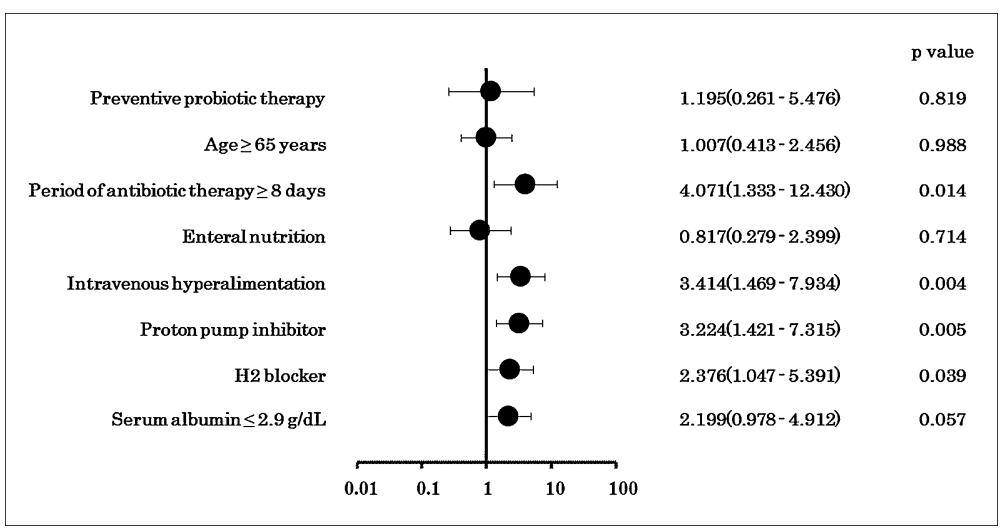

Fig. 2: Multivariate logistic regression analysis of items related to CDAD onset.

1.21–3.41), and antacid treatment increases the risk of CDAD occurrence 1.99-fold (95% CI: 1.19–3.31) (Kim et al. 2010; Yearsley et al. 2006). The present results are similar, indicating that proton pump inhibitor use increases the risk of CDAD occurrence 3.224-fold (95% CI: 1.421–7.315), and H_2 blocker use increases the risk 2.376-fold (95% CI: 1.047–5.391). Intravenous hyperalimentation was shown to increase the risk of CDAD occurrence 3.414-fold (95% CI: 1.469–7.934). Halim et al. (1997) found intravenous hyperalimentation to be a risk factor, but they did so in an analysis that included enteral nutrition. Others found that enteral nutrition users had a higher risk of CDAD recurrence (Chonan and Maeta 2010), and, in their research, all enteral nutrition formulations were administered as a bolus into the stomach. The amino acid arginine in enteral nutrition formulations was shown to promote *C. difficile* growth (Kato and Kato 2001). The findings of the present study, which considered intravenous hyperalimentation and enteral nutrition separately, suggest that enteral nutrition may not always be related to CDAD. The findings also suggest that CDAD can be prevented by switching from intravenous hyperalimentation to enteral nutrition as soon as possible. Undernutrition has also been reported as a CDAD risk factor (Takatsuka et al. 2010). Although there was no significant difference in CDAD occurrence in patients with a serum albumin level $\leq$2.9 g/dL, the P value of 0.057 approached significance. This is in line with the claim by Hookman et al. (2009) that hypoalbuminemia is a risk factor for CDAD.

The study did not show that prophylactic probiotic therapy suppresses the occurrence of CDAD. Since CDAD was diagnosed using only a toxin test with a detection accuracy of 60–80%, without combined use of a culture method, it is possible that false-negative patients who in fact should have tested positive were included in the non-CDAD group. However, given that patients who had diarrhea for $\geq$2 consecutive days during the period from the day antibiotic therapy was initiated, at which point CDAD was not diagnosed, to one month after completion of antibiotic therapy were excluded from the study on the basis that the possibility of CDAD could not be ruled out, false-negative patients were most likely excluded. In other words, some of the patients who would have been diagnosed with CDAD by the culture method were excluded from the present study, and possible effects on the present findings cannot be ruled out. In addition, because prophylactic probiotic therapy is not uniformly provided within the facility and is given according to the judgment of each physician, bias may exist among physicians. It appears that careful clinical monitoring of patients with any of the risk factors of antibiotic therapy for $\geq$8 days, intravenous hyperalimentation, proton pump inhibitor use, or H_2 blocker use is more beneficial than administering prophylactic probiotic therapy.

4. Experimental

4.1. Study population

The study included 3212 patients at least 20 years old who were admitted to Gifu Municipal Hospital and received an injected antibiotic at any time from February 2010 to February 2011. According to the Ethical Guidelines for Clinical Research, the study was conducted after undergoing a review by the Ethics Committee of Gifu Municipal Hospital. As mandated by the Gifu Municipal Hospital Ethics Committee, the data were analyzed only after being anonymized in a linkable fashion to protect patient privacy.

4.2. Information determined

The following information was retrospectively determined from electronic medical records:

1) Patient sex and age
2) CDAD following antibiotic use
3) Diarrhea persisting for 2 or more consecutive days at any time from the start of antibiotic use to 1 month after the end of antibiotic use
4) Antibiotic type and number of days used
5) Laboratory values indicative of nutritional status measured 2 or fewer days after the start of antibiotic use (serum albumin, total cholesterol, lymphocyte count)
6) Enteral nutrition, intravenous hyperalimentation, and fasting of 3 or more days during antibiotic use
7) Use of a proton pump inhibitor for 3 or more days during antibiotic use
8) Use of an H_2 blocker for 3 or more days during antibiotic use
9) Use of prophylactic probiotic therapy for 3 or more days during antibiotic use

CDAD was defined as a positive result on the toxin test. In line with the official Japanese definition of elderly persons, patients 65 years old or older were considered elderly.

CONUT was used to evaluate laboratory values indicative of undernutrition (de Ulíbarri and González-Madroño 2005). Laboratory values were classified as indicating satisfactory to mildly poor and moderately to severely poor nutrition. An assessment of moderately to severely poor nutrition constituted undernutrition. Specifically, serum albumin $\leq$2.9 g/dL constituted hypoalbuminemia, total cholesterol $\leq$139 mg/dL constituted hypocholesterolemia, and a lymphocyte count $\leq$1199/μL constituted hypolymphemia.

Prophylactic probiotic therapy was defined as the initiation of a probiotic on or before the first day of antibiotic treatment.

4.3. Exclusion criteria

All patients with diarrhea for 2 or more consecutive days at any time from the start of antibiotic use to 1 month after the end of antibiotic use without a diagnosis of CDAD were excluded from the study population because of an inability to exclude the possibility of CDAD.

Patients who used a probiotic for 3 or more days during antibiotic use but not for prophylaxis were also excluded from the study population because of the difficulty they would have presented in evaluating the relationship between CDAD onset and prophylactic probiotic therapy. This criterion was not applicable to patients who began using a probiotic after CDAD onset. However, patients who were given probiotics after the onset of CDAD were not excluded from the study.

4.4. Statistical analysis

Statistical analysis was performed with SPSS 18.0J. For univariate analysis, the Mann-Whitney U-test was used to test differences in age, numbers of days of antibiotic therapy, and laboratory values, and Fisher's exact probability test was used to test differences between the other items in the CDAD (patients who developed CDAD) and non-CDAD (patients who did not develop CDAD) groups. On multivariate analysis, multiple logistic regression analysis with the non-CDAD and CDAD groups as the response variables was used. P values less than 0.05 constituted a significant difference.

References

Avadhani A, Miley H. (2011) Probiotics for prevention of antibiotic-associated diarrhea and *Clostridium difficile*-associated disease in hospitalized adults - a meta-analysis. J Am Acad Nurse Pract 23: 269–274.

Bartlett JG, Onderdonk AB, Cisneros RL, Kasper DL. (1977) Clindamycin-associated colitis due to a toxin-producing species of *Clostridium* in hamsters. J Infect Dis 136: 701–705.

Chonan K, Maeta K. (2010) Factors Inducing Recurrence of *Clostridium difficile*-related Diarrhea. J Jpn Soc Hosp Pharma. 46: 665–667.

Dial S, Delaney JA, Barkun AN, Suissa S. (2005) Use of gastric acid–suppressive agents and the risk of community-acquired *Clostridium difficile*-associated disease. JAMA 294: 2989–2995.

Dendukuri N, Costa V, McGregor M, Brophy JM. (2005) Probiotic therapy for the prevention and treatment of *Clostridium difficile*-associated diarrhea: a systematic review. CMAJ 173: 167–170.

Geric B, Rupnik M, Gerding DN, Grabnar M, Johnson S. (2004) Distribution of *Clostridium difficile* variant toxinotypes and strains with binary toxin genes among clinical isolates in an American hospital. J Med Microbiol 53: 887–894.

Ghantoji SS, Sali K, Lairson DR, DuPont HL, Garey KW. (2010) Economic healthcare costs of *Clostridium difficile* infection: a systematic review. J Hosp Infect 74: 309–318.

Halim HA, Peterson GM, Friesen WT, Ott AK. (1997) Case-controlled review of *Clostridium difficile*-associated diarrhoea in southern Tasmania. J Clin Pharm Ther 22: 391–397.

Hickson M. (2011) Probiotics in the prevention of antibiotic-associated diarrhoea and *Clostridium difficile* infection. Ther Adv Gastroenterol 4: 185–197.

Hookman P, Barkin JS. (2009) *Clostridium difficile* associated infection, diarrhea and colitis. World J Gastroenterol 15: 1554–1580.

Hurley BW, Nguyen CC. (2002) The spectrum of pseudomembranous enterocolitis and antibiotic-associated diarrhea. Arch Intern Med 162: 2177–2184.

Ignacio de Ulíbarri J, González-Madroño A. (2005) CONUT: A tool for Controlling Nutritional Status. First validation in a hospital population. Nutr Hosp 20: 38–45.

Kato H, Kato N. (2001) Analysis of *Clostridium difficile* isolates from nosocomial outbreaks at three hospitals in diverse a1reas of Japan. J Clin Microbiol 39: 1391–1395.

Kim JW, Lee KL, Jeong JB, Kim BG, Shin S, Kim JS, Jung HC, Song IS. (2010) Proton pump inhibitors as a risk factor for recurrence of C*lostridium-difficile*-associated diarrhea. World J Gastroenterol 16: 3573–3577.

Kuroiwa T, Iwanaga M, Kobari K, Higashionna A, Kinjyo F, Saito A. (1990) Preventive effect of Clostridium butyricu M588 against the proliferation *Clostridium difficile* during antimicrobial therapy. J Japan Assoc for Infect Dis 64: 1425–1432.

Song HJ, Kim JY, Jung SA, Kim SE, Park HS, Jeong Y, Hong SP, Cheon JH, Kim WH, Kim HJ, Ye BD, Yang SK, Kim SW, Shin SJ, Kim HS, Sung JK, Kim EY. (2010) Effect of probiotic Lactobacillus (Lacidofil® Cap) for the prevention of antibiotic-associated diarrhea: a prospective, randomized, double-blind, multicenter study. J Korean Med Sci 25: 1784–1791.

Takatsuka H, Sasayama T, Takai N. (2010) Poor nutrition status may predispose *Clostridium difficile*-associated diarrhea patients to higher incidence of recurrence. J Japan Soc Environm Infect 25: 272–276.

Yearsley KA, Gilby LJ, Ramadas AV, Kubiak EM, Fone DL, Allison MC. (2006) Proton pump inhibitor therapy is a risk factor for *Clostridium difficile*-associated diarrhea. Aliment Pharmacol Ther 24: 613–619.