AP&T Alimentary Pharmacology and Therapeutics

Randomised clinical trial: the effects of perioperative probiotic treatment on barrier function and post-operative infectious complications in colorectal cancer surgery – a double-blind study

Z. Liu, H. Qin, Z. Yang, Y. Xia, W. Liu, J. Yang, Y. Jiang, H. Zhang, Z. Yang, Y. Wang & Q. Zheng

Department of Surgery, Affiliated Sixth People's Hospital, Shanghai Jiao Tong University, Shanghai, China.

Correspondence to:
Prof. H. Qin, Department of Surgery, Affiliated Sixth People's Hospital, Shanghai Jiao Tong University, Shanghai 200233, China.
E-mail: hlqin@live.cn

Publication data
Submitted 23 August 2010
First decision 11 September 2010
Resubmitted 29 September 2010
Accepted 1 October 2010
EV Pub Online 25 October 2010

SUMMARY

Background
Infection following abdominal operation remains a major factor affecting the morbidity of patients after surgery.

Aim
To determine the effects of perioperative administration of probiotics on the gut barrier function and the surgical outcome in patients undergoing elective colorectal surgery.

Methods
One hundred patients with colorectal carcinoma were randomly divided into the control group ($n = 50$) and the probiotics group ($n = 50$). The probiotics were given orally for 6 days preoperatively and 10 days post-operatively. Outcomes were measured by bacterial translocation, gut permeability, the effect on the faecal microbiota, and the clinical outcomes such as infectious-related complications and gut defecation function.

Results
Compared with the control group, probiotics group had increased transepithelial resistance ($P < 0.05$), reduced transmucosal permeation of horseradish peroxidase and lactulose/mannitol ratio, reduced bacterial translocation ($P < 0.05$), decreased ileal-bile acid binding protein ($P < 0.05$) and positive rate of blood bacterial DNA ($P < 0.05$) and an enhanced mucosal tight junction protein expression. They had decreased blood enteropathogenic bacteria and increased faecal bacterial variety. The post-operative recovery of peristalsis, incidence of diarrhoea, and infectious-related complications were also improved.

Conclusion
Probiotics can improve the integrity of gut mucosal barrier by benefiting the faecal microbiota, and decreasing infectious complications in patients with colorectal cancer undergoing colorectomy.

Aliment Pharmacol Ther 2011; **33:** 50–63

doi:10.1111/j.1365-2036.2010.04492.x

INTRODUCTION

Advanced surgical techniques and improved perioperative care have considerably lowered post-operative morbidity and mortality in patients undergoing gastrointestinal surgery. However, infection following abdominal operation remains a major factor affecting the morbidity of the patients. Urinary tract infections, pneumonia, wound infection, intra-abdominal abscess and cholangitis are frequently observed among patients undergoing abdominal surgery for various medical conditions, such as biliary cancer surgery, pancreatico-duodenectomy and liver transplantation.[1, 2]

The exact pathophysiological mechanisms that predispose patients undergoing major abdominal surgery to infection are yet to be identified. However, bacterial translocation (BT) describes the passage of bacteria from the gastrointestinal tract to normally sterile tissues such as the mesenteric lymph nodes (MLNs) and other internal organs. BT occurred more frequently in patients who underwent emergency surgery and in those who received preoperative total parenteral nutrition (TPN). BT plays a significant role, but, in elective cases, this may be less significant and other pathways of microbial signalling that influence the host have recently been described.[3] MacFie *et al.*[3] demonstrated that BT occured in 927 surgical patients with an overall prevalence of about 14% and was associated with an increased incidence of post-operative septic morbidity over a 13-year period of study. Reddy *et al.*[4] reported that the cultured MLNs sampled after colonic mobilization were positive for bacteria in 79.6% of patients, compared with 11.4% in controls in whom MLNs were sampled prior to bowel mobilization. Physical injury of the intestinal mucosa that leads to the disruption of the gut barrier and increased intestinal permeability as well as gut microbial imbalance are among the main causes.[5, 6]

Probiotic therapy, which was first introduced by Lilly and Stillwell,[7] may improve clinical and laboratory outcome of patients undergoing gastrointestinal surgery. Probiotics are 'live microorganisms, which when administered in adequate amounts, confer a health benefit on the host'. Used in combination, probiotics and prebiotics are called synbiotics. Prebiotics are nondigestable food constituents that selectively alter growth or activity of one or a limited number of bacterial species in the colon in a manner that potentially improves the health of the host.[8] Recently, some probiotic strains have been rediscovered for prevention and therapy of several diseases, such as necrotizing enterocolitis,[9] antibiotics-induced and Clostridium difficile-associated diarrhoea,[10, 11] chronic inflammatory bowel disease,[12] acute pancreatitis (AP),[13] hepatic encephalopathy,[14] steatohepatitis[15] and atopic disease.[16] Probiotics therapy is able to modulate bacterial growth, vitamin B12 availability and weight loss in patients after Roux-en-Y gastric bypass surgery,[17] improving surgical outcome.[18–21] Thus far, several randomized controlled trials (RCTs) using probiotics/synbiotics preoperatively and/or post-operatively with a focus on the prevention of post-operative infections have been performed,[8, 22–26] demonstrating the clinical benefits among patients receiving viable probiotics. Therefore, we elected to use a probiotic in this intervention to study its clinical value in patients undergoing surgery for colorectal cancer.

However, the use of probiotics is not free of side effects or risk. Besselink *et al.*[27] report that patients with severe AP receiving probiotics have more infectious complications and an increased risk of mortality compared with the control patients receiving placebo. It is vital that clinical trials of probiotics adhere to the high standards required for RCTs to demonstrate clearly the preventive and therapeutic effectiveness as well as safety profiles. Therefore, we conducted a prospective, randomized clinical trial aimed at determining whether perioperative administration of probiotics could prevent post-operative alterations in intestinal permeability, integrity and microbiota, and improve the surgical outcome in patients undergoing elective colorectal surgery.

PATIENTS AND METHODS

Patients

One hundred and twenty patients with colorectal cancer who were scheduled to undergo radical colorectomy at Shanghai Sixth People's Hospital, Shanghai Jiao Tong University, between April 2007 and June 2009, were enrolled in this study. The inclusion criteria and exclusion criteria are listed in Table 1. The patients were randomized before surgery to either the placebo control group (the placebo group), receiving perioperative oral feeding with placebo, or the probiotics therapy group (the PRO group), receiving probiotics treatment preoperatively and post-operatively. The study design and protocols were reviewed and approved by the Human Research Review Committee of the Shanghai Sixth People's Hospital, and written informed consent for participation was obtained from each patient before enrolment into the study. In our study, 120 patients were assessed for eligibility, whereas only 114 patients were enrolled in this study because six patients were excluded from our

Table 1 | Inclusion and exclusion criteria of the patients in the study

Inclusion criteria
Age 25–75 years
The diagnosis was confirmed by biopsy and histological test
Undergone radical resection and no far away metastasis (including the liver etc.)
Exclusion criteria
Age more than 75 years
Pregnancy
Known lactose intolerance
Clinically significant immunodeficiency
Usage of antibiotics and additional gastrointestinal disorders (e.g. Crohn's disease or ulcerative colitis)
Received antibiotics for the last 10 days before surgery
Evidence of infection
Probiotics or prebiotics and excessive fibre intake within 2 weeks
Undergoing emergency operation
Bowel preparation for colonoscopy within 6 days prior to surgery
Undergoing proctectomy with low rectal anastomosis or surgery for polypoid lesion
Laparoscopic surgery
Patients received preoperative chemotherapy or radiation therapy

study either not meeting the inclusion criteria or refused by the participants. Therefore, of the original 120 patients, 114 were randomized to study medication, 100 of whom completed the entire study. Of the 114 patients, 56 were included in the control group, while 58 were in the PRO group. Only 100 subjects completed the trial (Figure 1), with 50 each in the control group and the PRO group. There were no significant differences in gender, age, BMI, cancer stage, and time between onset of symptoms and hospital admission between the two groups. No significant differences in the levels of albumin, Hb, creatinine, blood loss, surgical time and blood transfusion were found between the two groups (Table 2).

Study design, probiotics treatment and patient care

A randomized, double-blind, placebo-controlled, prospective study design was employed. Equal randomization was accomplished using a computer-generated random allocation schedule. Envelopes numbered 1–120 contained the letter 'A' or 'B'. Placebo and probiotics were manufactured and labelled 'A' or 'B'. The capsule and its content looked identical in both groups. The smell and taste of the study substances were also identical. Only a nurse not directly involved in the trial was able to break the treatment codes in the event of an emergency.

Patients in the PRO group received daily encapsulated bacteria (Institute of Life Science of Only, Shanghai Jiao Tong University, Shanghai, China), containing *Lactobacillus plantarum* (CGMCC No. 1258, cell count $\geq 10^{11}$ CFU/g), *Lactobacillus acidophilus* (LA-11, cell count $\geq 7.0 \times 10^{10}$ CFU/g) and *Bifido-bacterium longum* (BL-88, cell count $\geq 5.0 \times 10^{10}$ CFU/g). An acid-resistant coating was used to prepare the capsules containing the PRO and placebo. Each patient in the PRO group received probiotics, 2 g/day, in a total daily dose of 2.6×10^{14} CFU. Patients in the placebo group received daily encapsulated maltodextrin and a 10-g sachet of maltodextrin. The intervention period lasted 16 days, i.e. 6 days preoperatively and 10 days post-operatively. All the subjects were interviewed by the study nurse, and reactions to the product, medications taken and any adverse events occurring in the 16-day period were recorded. The faecal samples were obtained at the first defecation after operation.

During the study period, no parenteral or enteral nutritional supplementation was given. All patients received a regular diet preoperatively, and a low-residue diet 1 day before surgery. For mechanical bowel preparation (MBP) 1 day before the surgery, all patients were given Soffodex, containing 2.4 g of monobasic sodium phosphate and 0.9 g of dibasic sodium phosphate. Parenteral hydration was given in the morning of the surgery supplied via a central venous catheter. A 12F catheter was placed through a jejunal limb during surgery for gastric aspiration to reduce colon anastomotic fluxion. For prophylaxis, 500 mg of metronidazole and 1 g of ceftriaxone were given 1 h before induction and continued for 48 h after the operation. Complications were registered daily post-operatively, and patients were re-examined at the outpatient clinic 1, 2 and 4 weeks after surgery. During the post-operative period, all patients received the regular parenteral hydration infusion.

MLN culture for BT

During the laparotomy, after mobilization of the bowel, MLN samples were harvested using a fresh surgical blade from the inferior mesenteric pedicle before its ligation. The MLN were divided equally into two parts, one for histopathology and another for bacterial culture.

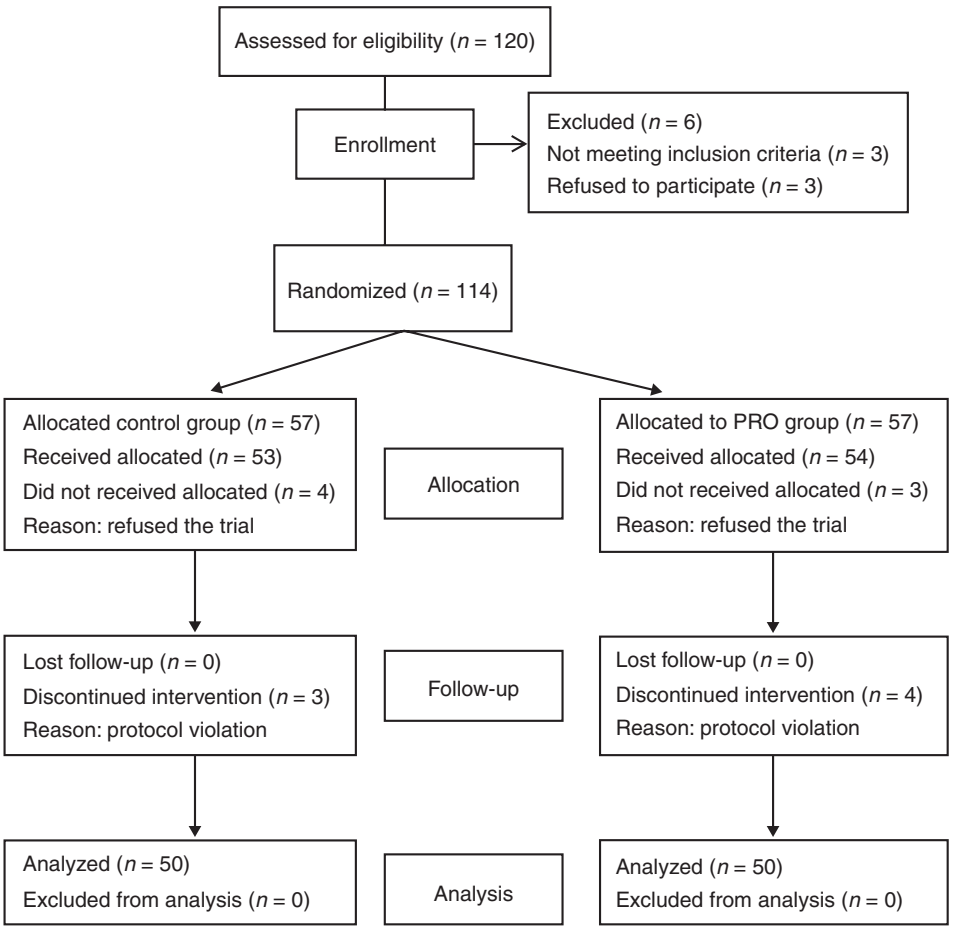

Figure 1 | Flowchart of the randomization procedure to enrol patients in the study.

Microbiological methods established by Reddy *et al.*[22] were followed for bacterial culture and identification.

Intestinal permeability assay

Intestinal permeability was assessed using the lactulose–mannitol (L/M) test on admission, on the third day and on the 10th day post-operatively. After an overnight fast, all subjects were given the oral test solution containing 10 g of lactulose (Sigma-Aldrich, Tokyo, Japan) and 5 g of mannitol (Sigma-Aldrich) in 60 mL of physiological saline. For the next 6 h, the subjects were at rest and no food or water was allowed. Complete 6-h urine was collected and mixed, and a 10-mL urine sample was taken and frozen at −20 °C until analysis. Urinary L/M concentrations were measured by gas–liquid chromatography.[13]

Measurement of intestinal fatty acid binding protein

The concentrations of intestinal fatty acid binding protein (I-FABP) in the plasma collected at 1 h preoperatively and 6, 12 and 24 h post-operatively were determined using an enzyme-linked immunosorbent assay that selectively detected human I-FABP (standard: 20–5000 pg/mL; Hycult Biotechnology, Uden, the Netherlands). The levels of ileal-bile acid binding protein (I-BABP; standard: 0.32–5 ng/mL) were also determined using the methods previously described.[28]

Measurements of colon mucosal short-circuit current (I_{SC}) for transepithelial resistance and horseradish peroxidase flux for transepithelial permeability

The samples of the normal colon segments were taken during surgery and immediately immersed in oxygenated Kreb's buffer; after being stripped of external muscle and myenteric plexus, the adjacent segments were mounted into Ussing chambers (World Precision Instruments; Narco Scientific, Mississauga, ON, Canada). Using the chamber, 0.6 cm^2 of tissue was exposed to 8 mL of circulating oxygenated Kreb's buffer containing (in mM) 115 NaCl, 1.25 $CaCl_2$, 1.2 $MgCl_2$, 2.0 KH_2PO_4 and 25 $NaHCO_3$ (pH 7.35). The chambers contained agar-salt

Table 2 | Characteristics of the patients with colorectal cancer undergoing colorectomy in the study

Index	Placebo group (n = 50)	PRO group (n = 50)	P-value
Gender (male/female)	31/19	28/22	>0.05
Age (year)	65.7 ± 9.9	65.3 ± 11.0	>0.05
BMI (kg/m^2)	22.6 ± 2.0	22.8 ± 1.8	>0.05
Stage (A/B/C)	12/29/9	11/30/9	>0.05
Location of tumour			
Transverse colon	8	7	>0.05
Descending colon	10	5	>0.05
Sigmoid colon	21	25	>0.05
Rectum	11	13	>0.05
Preoperative albumin (g/dL)	37.5 ± 3.2	39.1 ± 3.5	>0.05
Preoperative Hb (g/L)	123.2 ± 19.6	125.3 ± 17.7	>0.05
Creatinine (mg/dL)	1.1 ± 0.16	1.2 ± 0.14	>0.05
Operative time (min)	130.8 ± 46.2	125.3 ± 18.3	>0.05
Intra-operative blood loss (mL)	410 ± 152	390 ± 174	>0.05
Transfusion during operation (mL)	450 ± 144	420 ± 142	>0.05
Usage of supplemental albumin post-operation (g)	90 ± 14	95 ± 17	>0.05
Preparation time preoperation (days)	7.3 ± 1.1	6.7 ± 0.87	>0.05
Cumulative length of antibiotics therapy (days)	8.7 ± 1.0	9.5 ± 0.98	>0.05
Metronidazole (n)	50	50	>0.05
Penicillin (n)	26	24	>0.05
Ceftriaxone (n)	24	26	>0.05

bridges to monitor the potential difference across the tissue and to inject the required short-circuit current (I_{SC}) to maintain a zero potential difference, as recorded via an automated voltage clamp (World Precision Instruments, Sarasota, FL, USA). Tissue conductance, representing passive permeability to ions, was calculated by Ohm's law. Mucosal to serosal transport of macromolecules was assessed by measuring transepithelial flux of horseradish peroxidase (HRP) as a model protein antigen. HRP activity was determined using a modified Worthington method[29] and the mucosal to serosal flux of HRP was expressed as pmol/h/cm^2.

Microbiological investigations and PCR assay for bacterial DNA fragment

Clinical samples comprised blood (40 mL), central lines (tips), urine (20 mL) and sputum (at 06:00 hours). Faecal samples were collected in a test tube, which was maintained anaerobically in an atmosphere of 7% H_2 and 5% CO_2 in N_2. Each clinical sample was taken from patients 72 h after the operation and sent immediately to the microbiological laboratory in the Department of Medical Microbiology. The specimens were cultured aerobically as well as under microaerophilic conditions and anaerobically at 35–37 °C for 24–48 hours. Anaerobic cultivation was performed in anaerobic chambers. Fungal cultures were made with the use of Sabouraud's medium (bioMérieux, France). The biochemical characteristics of the cultured strains were investigated using the API and/or ID tests (bioMérieux, Paris, France) according to the manufacturer's instructions. The remaining 20 mL of blood was collected in a sterile container containing EDTA for molecular detection of bacterial DNA. To determine the sensitivity of the PCR detection, serial dilutions of the spiked blood were tested until a negative result was found. The sensitivity of the test was 10 organisms/mL. Data analysis was accomplished as previously reported by Shang.[30]

Faecal bacterial anaerobic culture[31]

One day before the pre-treatment, 1 day before the operation, and 3 and 10 days after the operation, faecal samples were collected in a test tube, which was maintained anaerobically in an atmosphere of 7% H_2 and 5% CO_2 in N_2. The faecal sample (0.1 g) was placed into 9 mL of

Ringer's dilution solution used for standard bacterial culture under aerobic and anaerobic traditional conditions. To analyse the total *Lactobacillus* population, 10 colonies were picked randomly from a dilution agar plate containing about 100 colonies. The bacterial colonies were counted and identified using Microscan Autoscan-4 Machine (Dade Behringcom, Sacramento, CA, USA). All bacterial counts were transformed to logarithms (log 10 CFU) for statistical analysis. The low limit of bacterial detection with this procedure was 1000 CFU/g of faeces for the obligate anaerobes, and *Bacteroidaceae*, *Bifidobacterium* and 100 CFU/g of faeces for other bacteria.

Faecal bacterial DNA fingerprint profiling

During the post-operative 72 h, the faecal samples were examined by PCR-denaturing gradient gel electrophoresis (DGGE) profiles.[32] To extract bacterial DNA, 1 mL of faecal homogenate (0.2 mL of faecal homogenate were added to 500.0 μL, pH 7.0 PBS) was centrifuged at 14 600 *g* for 5 min at 5 °C. DNA was extracted from the resulting pellet with a Fast DNA kit (BIO 101, Vista, CA, USA). The V2-V3 region of the 16S rDNA gene (positions 339–539 in the *Escherichia coli* gene) of the bacteria in the faecal samples was amplified by using primers, bacterial ITS PS2 (5′-TG(C/T) ACA-CACCGCCCGT-3′) and PL2 (5′-GGG T(G/C/T) CCCCATTC(A/G)G-3′). The PCR was performed as previously reported.[13, 31] Electrophoresis was performed at 130 V (constant voltage) and 60 V for about 4.5 h. The gels were stained with an ethidium bromide solution (5 μg/mL) for 20 min, washed with deionized water and reviewed by UV transillumination.

Estimation of bacteria richness and diversity

The richness and diversity of faecal microbiota were estimated from the number of PCR-DGGE bands present in the NA fingerprint profiling.[32] The band numbers and frequencies were compared among different groups. 'Species' used in the indices referred to individual bands on the PCR-DGGE gels. These indices measured ecological diversity using various parameters, including species richness (the number of different species) and evenness (the distribution of individual species in the ecosystem). Band number corresponded to the number of individual bands in a single lane. Band frequency was calculated by measuring the percentage of all samples at a given time point containing a specific band. Mean percentage similarities (C_s values) were determined by the following equation:

$$C_s = \left[\frac{2j}{a+b}\right] \times 100\%$$

where a is the number of PCR-DGGE bands in lane 1, b is the number of PCR-DGGE bands in lane 2 and j is the number of common PCR-DGGE bands.

Claudin-1, JAM-1 and Occludin proteins expression by immunohistochemistry and fluorescence staining under a confocal laser scanning microscope

For immunohistochemistry (IHC) assays, paraffin sections were dewaxed in xylene and rehydrated in graded ethanol to distilled water.[33] Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide in methanol for 12 min. Primary antibodies were diluted 1:20 to 1:100 (rabbit monoclonal anti-human Claudin-1, JAM-1, Occludin; Zymed, California, USA) in 2% bovine serum albumin-PBS. Secondary antibodies were goat anti-rabbit immunoglobulin G from Immunotech (Luminy, France) and were diluted 1:20 in PBS containing 2% bovine serum albumin. The data were analysed using HPIAS1000 high definition colour imaging system (QianPing Image Co., Wuhan, China). Under the magnification of 400, the density was determined per field of vision by light-densitometer (five fields per slide). For fluorescence staining, the slides were incubated with fluorescein isothiocyanate-conjugated specific secondary antibody (Sigma) at room temperature in the dark. The density of the staining was detected by confocal laser scanning microscope (CLSM) (Bio-Rad MRC 1024, Bio-Rad, Richmond, CA, USA). Two groups were done in the same experimental session. Staining was absent from the negative control inserts in which the primary antibodies were omitted.

Post-operative clinical observations

Detailed daily records of post-operative courses were kept and infectious complications were recorded for up to 30 days after surgery. The diagnosis of bacterial infection was based on a previous reference.[23] Several types of complications were observed after surgery, including bacteriaemia, intra-abdominal abscess, incision infection and pneumonia. The SIRS incidence, the intra-abdominal drainage time, systemic circulation infectious rate and infections-related complication (including incision, central lines and pneumonia) were recorded. The first defecation, diarrhoea incidence (≥3 loose stools per day), the total time of continuous diarrhoea, duration of post-operative pyrexia (>38.5 °C), abdominal cramping and distension, intake time of fluid diet and solid diet, the post-operative hospital stay, the hypoalbuminaemia, and

the cumulative duration of antibiotic therapy were recorded. The patients underwent abdominal examination by one of two examiners (Zhen Yang and Yang Xia) to evaluate bowel sounds, abdominal cramping and distension on the post-operative days. Data were categorized as minimal change (0–1 for distension and cramping and 2–3 for bowel sounds) or significant change (2–4 for cramping and distention and 0–1 for bowel sounds) from preoperative activity. All patients' blood, central lines, urine and sputum were sampled for cultures during colorectomy post-operative once at 72 h, irrespective of the presence or absence of other infectious sources. For each set of blood cultures, 10 mL of blood was drawn under sterile conditions and then immediately inoculated into separate culture bottles (Organon Teknika, Durham, NC, USA) for aerobic and anaerobic cultures. Blood sample was incubated until bacterial growth was detected, or for 7 days.

Statistical analysis

A sample size calculation based on the published prevalence of BT indicated that approximately 44 patients would be required in each group to demonstrate a reduction in BT from 15% to 0% at $P = 0.05$ significance level with a power of 80%.[3] Results were analysed using SPSS 11.5 version for Windows (SPSS, Chicago, IL, USA). Densitometry was performed on immunoblots using a computer-assisted image analysis system (Quantity One, version 4.2.0; Bio-Rad, Hercules, CA, USA), and the results were presented as the fold increase in densitometry values. Quantitative data are expressed as mean ± standard deviation. Comparison of categorical data between groups was made using Pearson χ^2 test or, where indicated, Fisher's exact test. ANOVA or Kruskal–Wallis test was used for continuous variables, as appropriate. $P < 0.05$ was considered statistically significant.

RESULTS

The PRO treatment reduces infection risk and improves gut barrier function

BT in MLN. The PRO group had a significantly lower post-operative incidence of BT compared with the placebo group [18.0% (9/50) vs. 28.0% (14/50), $P < 0{\cdot}01$].

Intestinal permeability. On day three after the operation, the patients had elevated L/M ratios, indicative of increased permeability in both the placebo group (0.23 ± 0.06) and the PRO group (0.23 ± 0.08) compared with their preoperative L/M ratios (0.19 ± 0.05; 0.17 ± 0.04; $P < 0.05$) respectively. By day 10, the mean L/M ratio in the PRO group was significantly lower than that in the placebo group (0.18 ± 0.03 vs. 0.22 ± 0.04; $P = 0.04$).

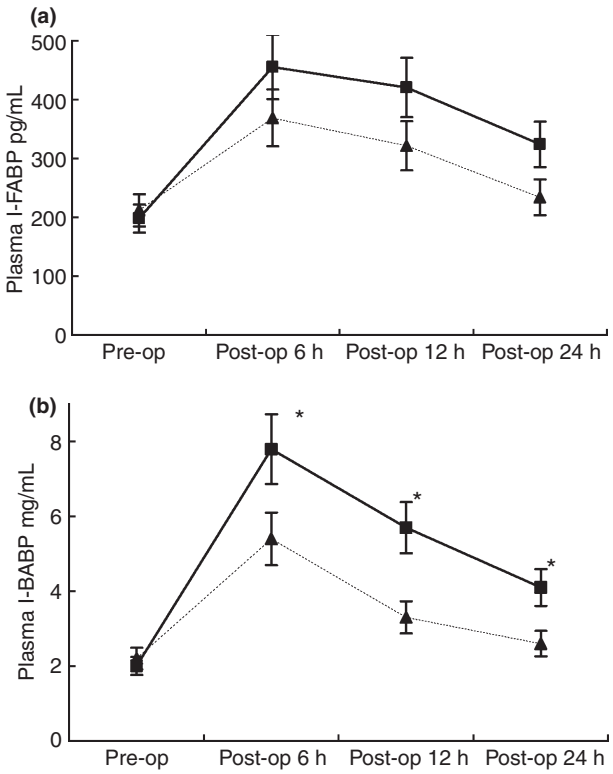

Figure 2 | The plasma levels of I-FABP and I-BABP after surgery. (a) The values of plasma levels of I-FABP post-operative 6, 12 and 24 h. (b) The values of plasma levels of I-BABP post-operative 6, 12 and 24 h. ■, the placebo group; ▲, the PRO group. * vs. PRO group, $P < 0.05$.

Plasma I-FABP and I-BABP. The plasma concentration of I-FABP increased shortly after surgery from a mean baseline value of 212 ± 30 pg/mL to a peaked value at 6 h of 456 ± 69 pg/mL. The mean levels of I-FABP in the PRO group were significantly lower than that in placebo group at the post-operative 6, 12 and 24 h (all $P < 0.01$; Figure 2a). Similarly, the mean I-BABP plasma concentrations also increased significantly at 6, 12 and 24 h after surgery compared with baseline values (Figure 2b). Considering that I-FABP is excreted by the kidneys and the high plasma values of I-FABP could be caused by impaired renal function in patients, diuresis during and after surgery was used and the plasma creatinine values (average values, 1.2 ± 0.22 mg/dL) were not

elevated, indicating that the elevation of plasma I-FABP was caused by enterocyte cell death.

Transepithelial resistance and flux of HRP of colon mucosa. The mean colon mucosal transepithelial resistance (TER) in the placebo group was significantly lower than that in the PRO group ($13.7 \pm 4.2\ \Omega/cm^2$ vs. $18.4 \pm 5.1\ \Omega/cm^2$, $P < 0.05$). The cumulative transmucosal permeation of HRP of the colon mucosa for 120 min in the placebo group was higher than that in the PRO group ($1.13 \pm 0.27\%$ vs. $0.61 \pm 0.15\%$, $P < 0.05$).

Blood bacterial culture and microbial DNA positive rate. During the post-operative 72-h period, the total rate of positive bacterial cultures (including blood, central lines and sputum) in the placebo group (30.0%, 15/50) was significantly higher than that that in the PRO group (14.0%, 7/50), $P < 0.05$ (Table 3). None of the patients had intraoperative or post-operative signs of diffuse peritonitis or sepsis. Therefore, antibiotic prophylaxis was not continued, and these cases were not defined as peritonitis. The bacterial positive rate of the blood in the placebo group (5/50, 10.0%) was not significantly higher than that in the PRO group (3/50, 6.0%, $P > 0.05$). Microbial DNA was found in all patients whose blood cultures were positive. However, the blood bacterial DNA positive rate in the placebo group (26.0%) was significantly higher than in the PRO group (14.0%), $P < 0.05$; especially, the highest copy value (4.4×10^5/mL) was in the placebo group.

The PRO treatment modulates microflora in patients

Faecal microflora and bacterial DNA fingerprint profile analysis. The numbers of bacteria, including *Bifidobacteria* and *Lactobacilli*, increased in the PRO group after surgery, whereas they decreased in the placebo group. In contrast, the numbers of microorganisms, including *Enterobacteriaceae, Pseudomonas* and *Candida*, were decreased in the PRO group and increased in the placebo group. The number of *Enterococci* increased after surgery in both groups (Table 4). The faecal bacterial DNA fingerprint profile by DGGE analysis represented the richness and diversity of the gut faecal bacterial community. Visual observation of profiles revealed that the faecal bacteria variety and the intensity in the placebo group were less than that in the PRO group (Figure 3). Further analysis of the bacterial richness and diversity showed that the richness of the healthy volunteers group, the control group and the PRO group were 5 ± 2.2, 2 ± 1.2 and 7 ± 2.8 respectively. The C_s values were 0.82 ± 0.36 between the healthy volunteers group and the control group, 0.46 ± 0.32 between the healthy volunteers and PRO group, and 0.22 ± 0.18 between the control and PRO group respectively. Statistic analysis indicated that the PRO group had an enhanced bacterial richness compared with both the healthy volunteers group and the control group ($P < 0.05$). The PRO group had a higher similarity to the healthy volunteers group compared with the control group ($P < 0.05$).

Claudin-1, JAM-1 and Occludin expression by IHC and Fluorescence staining. The tight junction (TJ) barrier function can also be affected by changes in the distribution of specific TJ proteins and/or their levels of expression. TJs associated proteins were continuously distributed with bright brown spots along membrane of the epithelial cells. Their borders of the Claudin-1, JAM-1 and Occludin were clear in the PRO group. In the

Table 3 | The results of bacterial culture of blood, incision, central lines and sputum

	Sample					
	Placebo group (n = 50)			PRO group (n = 50)		
Bacterium	Blood	Central lines	Sputum	Blood	Central lines	Sputum
Escherichia coli	3	3	3	3	0	1
Staphylococcus aureus	2	2	0	0	1	1
Klebsiella pneumoniae	0	0	0	0	0	0
Aeruginosin	0	2	0	0	0	1
Bacterial positive patient	5	7	3	3	1	3

Note: Total bacterial positive patients in the placebo group = (blood + venous tube + sputum) = (5 + 7 + 3) = 15 patients. Total bacterial positive patients in the PRO group = (blood + venous tube + sputum) = (3 + 1 + 3) = 7 patients. The total bacterial positive rate in the placebo group was 30.0% (15 in 50 patients); in the PRO group, 14.0% (7 in 50 patients), $P < 0.05$.

Table 4 | Changes in the faecal microflora perioperation (mean ± s.d., log 10 CFU/g of faeces)

Bacterial	Time							
	1 day before trial		1 day before operation		3 days after operation		10 days after operation	
	Control group	PRO group	Control group	PRO group	Control group	PRO group	Control group	PRO group
Total anaerobe counts	10.6 ± 0.5	10.7 ± 0.6	10.6 ± 0.6	10.8 ± 0.4	10.6 ± 0.6	10.8 ± 0.4	10.7 ± 0.9	11.0 ± 0.5
Bacteroidaceae	10.4 ± 0.7	9.8 ± 1.2	10.2 ± 0.8	10.2 ± 0.6	10.2 ± 0.8	10.2 ± 0.6	10.5 ± 0.5	10.5 ± 0.7
Bifidobacterium	9.7 ± 1.1	9.6 ± 1.2	8.3 ± 1.8	10.3 ± 0.9*†	8.3 ± 1.8	10.5 ± 0.9*†	8.8 ± 2.4	10.8 ± 0.4*†
Enterococcus	8.0 ± 1.3	7.9 ± 1.5	8.7 ± 1.1	8.6 ± 1.0	8.7 ± 1.1	8.6 ± 1.0	8.7 ± 1.1	8.9 ± 0.7
Lactobacillus	6.3 ± 1.8	5.6 ± 2.3	4.3 ± 1.9	7.2 ± 1.6	4.3 ± 1.9	7.2 ± 1.6	6.0 ± 1.7	7.4 ± 1.0
Staphylococcus	3.5 ± 1.3	3.8 ± 1.5	3.2 ± 1.0	3.6 ± 1.3	3.2 ± 1.0	3.6 ± 1.3	3.5 ± 1.2	3.6 ± 1.0
Enterobacteriaceae	7.5 ± 1.0	7.6 ± 1.1	7.7 ± 1.0	6.6 ± 1.6*†	7.7 ± 1.0	6.5 ± 1.6*†	8.3 ± 1.0	6.4 ± 1.2*†
Bacillus	2.7 ± 1.1	3.0 ± 1.9	2.1 ± 0.5	2.2 ± 0.9	2.1 ± 0.5	2.2 ± 0.9	2.8 ± 1.2	2.9 ± 1.3
Pseudomonas	2.5 ± 1.2	2.6 ± 1.5	3.5 ± 2.1	2.3 ± 1.2	3.5 ± 2.1	2.3 ± 1.2*	2.7 ± 1.3	2.1 ± 0.4
Candida‡	4.1 ± 1.4	3.7 ± 1.6	4.9 ± 1.7	3.1 ± 1.5*†	4.9 ± 1.7	3.1 ± 1.4*†	4.7 ± 1.7	3.1 ± 1.1*†

* $P < 0.05$ vs. control group.

† $P < 0.05$ vs. PRO group 1day before trial.

‡ As a yeast.

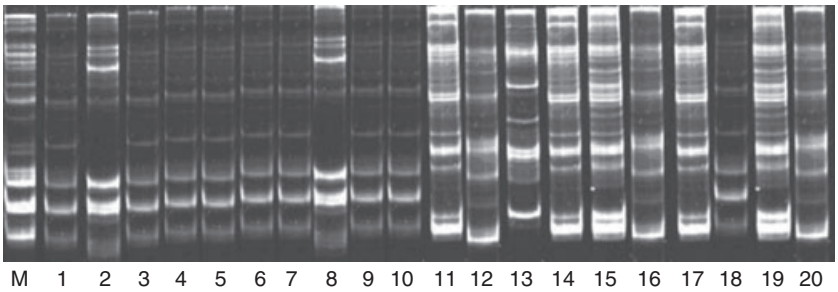

Figure 3 | Faecal bacterial DNA fingerprint profile. Lane M: 10 faecal samples/lane of the healthy volunteers; lanes 1–10: the control group (five patients' faecal/lane, mixture with 50 patient's faecal); lanes 11–20: the PRO group (five patients' faecal/lane, mixture with 50 patients' faecal).

placebo group, there was a substantial loss of claudin-1, JAM-1 and occludin from the TJs. The brown spot distributions were decreased and the degradation developed in the placebo group (Figures 4a,b). Confocal imaging was also performed to assess the distribution of the TJs. TJ-associated proteins were continuously distributed with bright red spots along the membrane of epithelial cells. The borders of Claudin-1, JAM-1 and Occludin were clear in the PRO group. In the placebo group, the fluorescence was dispersedly distributed and with some loss from the membrane as opposed to the uniform membrane staining. This loss was manifested by discontinuities in membrane staining, a reduction in staining intensity and, in some areas, a complete loss of staining (Figure 5).

The PRO treatment reduces post-operative infection complications

Among the 100 patients eligible for the analysis, no patient had problems related to leakage of the anastomosis, fistulas and abdominal haemorrhage. The data of per-protocol showed that the incidence of infection complications in the PRO group (14%) was less than that in the control group (46%) ($P < 0.05$). The

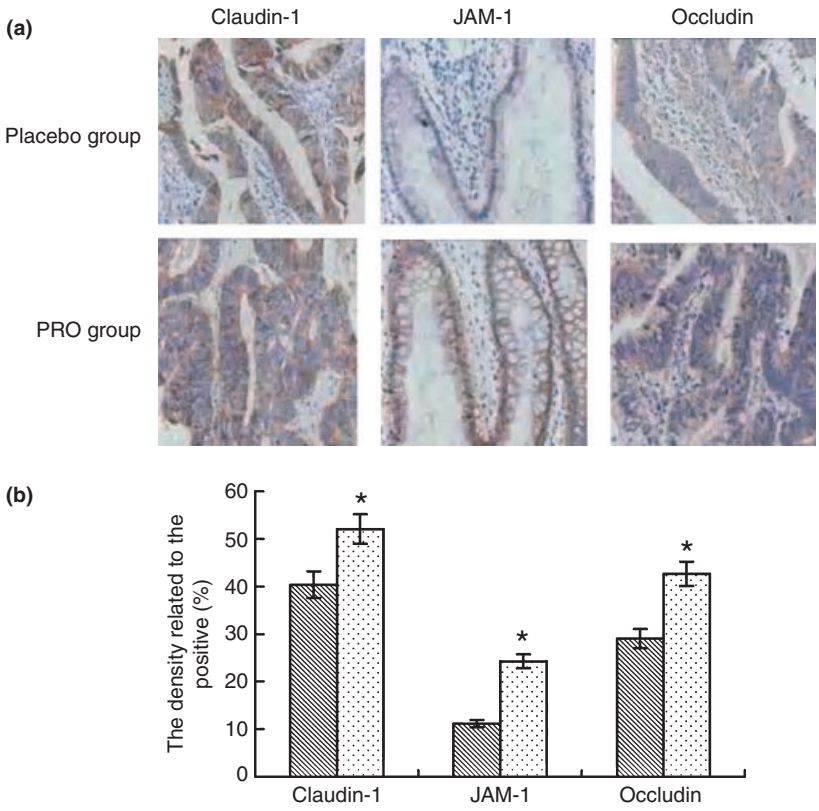

Figure 4 | Claudin-1, JAM-1 and Occludin expression assayed by immunohistochemistry: (a) the imaging of the TJ proteins expression; (b) the staining density. Under the magnification of 400×, the density was determined per field of vision by light densitometer (five fields selected per slide). ▧, the placebo group; ⊡, the PRO group. * vs. PRO group, $P < 0.05$.

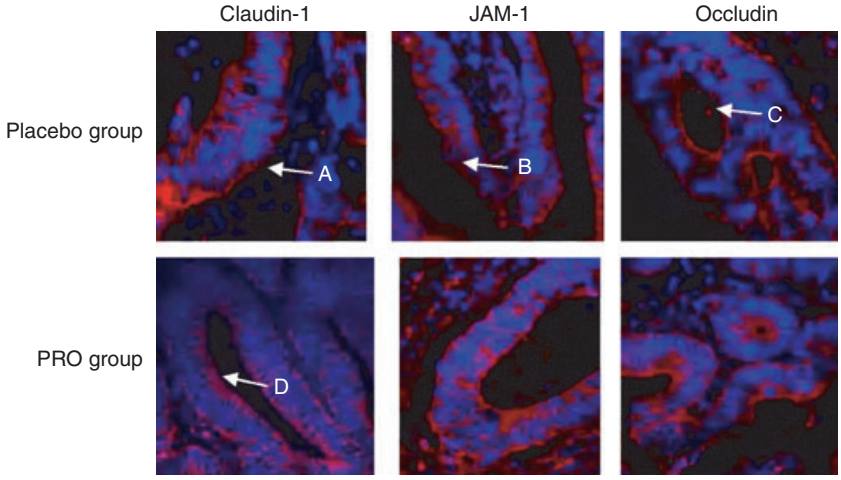

Figure 5 | Claudin-1, JAM-1 and Occludin expression by fluorescence: (A) discontinuities in membrane staining and a reduction in staining intensity; (B) a discontinuity; (C) some areas with a complete loss of staining; (D) a continuous and intense staining pattern. Magnifications: 400×.

detailed comparison between the two groups is shown in Table 5. There were no statistically significant differences in the SIRS incidence, intra-abdominal drainage time, urinary catheters time and intake time of fluid and solid diet between the two groups. No complication or side effects of probiotic use for patients were observed. Compared with the placebo group, the PRO group had a shorter time to have the first defecation (3.3 days vs. 4.2 days, $P < 0.05$), a lower diarrhoea incidence 10% vs. 30%, $P < 0.05$), lower incidences of abdominal cramping 26% vs. 38%, $P < 0.05$) and distension (22% vs. 36%, $P < 0.05$), and a shorter duration of pyrexia (>38.5 °C) (5.9 days vs. 7.2 days, $P < 0.05$). There were no statistically significant differences in the hypo-albuminaemia and cumulative length of antibiotic therapy and duration of post-operative hospital stay (12.7 days vs. 12.9 days, $P > 0.05$) between the two groups. No other post-operative complications occurred. Similar results were found in the data of intention-to-treat.

Table 5 | Comparison of post-operative outcomes between two groups on the day of discharge

	Per-protocol			Intention-to-treat		
Outcomes	Control (n = 50)	PRO (n = 50)	P-value	Control (n = 56)	PRO (n = 58)	P-value
SIRS incidence	84% (42/50)	80% (40/50)	>0.05	82% (46/56)	81% (47/58)	>0.05
Intra-abdominal drainage time (days)	4.7 ± 1.4	4.3 ± 1.4	>0.05	4.6 ± 1.4	4.4 ± 1.6	>0.05
Incision infection	10% (5/50)	6% (3/50)	>0.05	11% (6/56)	7% (4/58)	>0.05
Central lines infection	14% (7/50)	2% (1/50)	<0.05	14% (8/56)	3% (2/58)	<0.05
Pneumonia infection	10% (5/50)	4% (2/50)	<0.05	14% (8/56)	5% (3/58)	<0.05
Urinary infection	12% (6/50)	2% (1/50)	<0.05	13% (7/56)	5% (3/58)	<0.05
First defecation time (days)	4.2 ± 1.3	2.3 ± 1.2	<0.05	4.2 ± 1.2	2.6 ± 1.6	<0.05
Diarrhoea incidence	34% (17/50)	18% (9/50)	<0.05	34% (19/56)	17% (10/58)	<0.05
Urinary catheters time (days)	7.2 ± 2.2	6.8 ± 2.6	>0.05	7.0 ± 2.6	6.8 ± 2.2	>0.05
Abdominal cramping	38% (19/50)	26% (13/50)	<0.05	39% (22/56)	26% (15/58)	<0.05
Abdominal distension	36% (18/50)	22% (11/50)	<0.05	36% (20/56)	21% (12/58)	<0.05
Intake time of fluid diet (days)	3.6 ± 0.6	3.3 ± 0.5	>0.05	3.4 ± 0.5	3.5 ± 0.6	>0.05
Intake time of solid diet (days)	4.7 ± 0.7	4.5 ± 0.6	>0.05	4.9 ± 0.6	4.8 ± 0.8	>0.05
Side effects of probiotic use	N/A	N/A	N/A	N/A	N/A	N/A
Duration of post-operative pyrexia (>38.5 °C) (days)	7.2 ± 2.3	5.9 ± 1.0	<0.05	7.2 ± 2.1	6.0 ± 1.9	<0.05
Hypoalbuminaemia	18% (9/50)	12% (6/50)	>0.05	21% (12/56)	14% (8/58)	>0.05
Cumulative duration of antibiotic therapy	7.0 ± 2.4	5.9 ± 1.6	<0.05	7.2 ± 2.1	5.3 ± 1.7	<0.05
Post-operative hospital stay	12.9 ± 3.3	12.7 ± 2.2	>0.05	12.6 ± 3.3	12.3 ± 2.3	>0.05
Death case	0	0	>0.05	0	0	>0.05

DISCUSSION

Several RCTs demonstrate that the use of probiotics/synbiotics in patients undergoing abdominal surgery is a promising approach to the prevention of post-operative infectious complications and is well tolerated by patients with minor side effects.[18] However, some investigators report[34, 35] that there is no evidence supporting any benefits from a preoperative use of pre- and probiotics (synbiotics) in patients with critical illnesses and undergoing elective abdominal surgery, and that in some cases, there is even an increased risk of mortality. Possible explanations for the lack of effectiveness in those studies include the relatively short post-operative period of administration (median time of 4 days), the oral (instead of jejunal) route of administration with unclear survival rate of the probiotics in the stomach due to low pH, and the high-risk operations, such as complicated colectomies, resulting in a high overall rate of BT and infections.[33]

In recent years, three important randomized studies on the effects of probiotics in surgical patients have reported [8, 23, 24] that the use of probiotics after surgery markedly improved intestinal microbial populations and significantly decreased the incidence of further infectious complications. Furthermore, the patients' quality of life was also improved, shortening the duration of post-operative hospital stay and the period needed for antibiotics administration. Our results were consistent with the previous three observations,[8, 23, 24] strongly suggesting the beneficial effects of probiotics in surgical patients undergoing colorectomy. Consistent with our previous studies,[13] our results showed that the infection-related complication and gut defecation function were improved in patients receiving perioperative oral probiotics treatment, suggesting that the use of probiotics could reduce the extent of damage to colon mucosa after surgery.

It is hypothesized that probiotics preserve epithelial barrier function. *In vitro* studies on epithelial monolayers showed that probiotics improved barrier function following *E. coli* infection or incubation with proinflammatory cytokines.[36, 37] In our previous study,[33] we documented that the transcutaneous electrical resistance stepped down and dextran integrated intensity stepped up with time after infection with EIEC, but after treating with *L. plantarum*, the changes in transcutaneous electrical resistance and dextran-integrated intensity were improved as compared with EIEC group. *Lactobacillus plantarum* prevented the damage of expression and rearrangement of Claudin-1, Occludin, JAM-1 and Zonula occludins-1 proteins induced by enteroinvasive *E. coli* (EIEC), and could ameliorate the injury of cytoskeleton protein F-actin infected with EIEC. Probiotics also preserve the intestinal epithelial barrier in several *in vivo* models, such as the IL-10 knockout colitis,[38] sepsis[31] and acute colitis.[39] Probiotics also protect against barrier dysfunction following psychological stress in rats.[40] Pathological BT to MLNs as a marker of impaired barrier function can also be effectively reduced by probiotic therapy.[31, 41] To the best of our knowledge, this is the first report to describe post-operative changes in colon mucosa integrity and permeability in surgical patients undergoing operation and receiving perioperative probiotics. We determined the L/M ratio, colon mucosa TER, HRP permeability and BT to assess intestinal integrity and permeability. This study demonstrated that surgical manipulation disrupted epithelial TJ structure, including claudin-1, JAM-1 and occludin distribution in colon mucosa, resulting in decreased TER and increased permeability to macromolecules. We also demonstrated, for the first time, using confocal laser scanning microscopy, that probiotics treatment stabilized cellular claudin-1, JAM-1 and occludin structure, thereby preventing surgical manipulation-induced damage of the integral TJ proteins in colon mucosa epithelium. Therefore, lower BT incidence, blood bacterial positive rate and microbial DNA positive rate were observed in the PRO group. We concluded that probiotics could alleviate intestinal villous cell injury and decrease the plasma levels of I-FABP induced by surgical procedures.

Gastrointestinal microbiota may be modulated by prebiotics and probiotics.[42] The preparation used in this study was selected based on previous works showing a reduction in the prevalence of *Enterobacteriaceae* in gastric aspirates of critically ill patients.[13, 43] Nevertheless, because a systematic stool collection is often difficult in surgical patients, sampling of faeces is a more practicable approach to providing important information concerning the intestinal microbiota. One of the important findings of this study was that the use of probiotics notably changed the faecal microbiota of surgical patients. In the PRO group, post-operative faecal *Bifidobacteria* and *Lactobacilli* increased, while *Enterobacteriaceae* and *Pseudomonas* decreased, as compared with placebo group. Of course, this was the predominant probiotic bacteria chosen. Additionally, in this study, we applied DGGE analysis of 16S rRNA gene fragments PCR amplified with general primers to follow the changes in the diversity of the gut bacterium populations. Although this molecular technique has a sensitivity of 90–99 per cent, bacteria at a concentration of $<10^9$ organisms per gram of faeces may not have been represented. Nevertheless, DGGE analysis has been very

useful in this and previous studies for following changes in the diversity of complex bacterial communities.[44] The results showed that there were much more bacterial groups and diversity of the bacterial community in the PRO group compared with the placebo group. Langlands *et al.*[45] found that prebiotics can change the composition of the mucosa-associated flora significantly in 29 subjects undergoing colonoscopy. Our results strongly suggested that the use of probiotics improved the capacity of the gut ecosystem to survive surgically induced injury, leading to fewer post-operative infections. Thus, we concluded that maintaining gut microbiota balance and diversity is important for enhancing host defences, especially during recovery from major surgery.

One limitation of our study may involve the disturbing effects of probiotics on microbiota. Our present study indicated that the faecal bacterial variety and the intensity in the placebo group were significantly less than that in the PRO group (received 500 mg of metronidazole and 1 g of ceftriaxone 1 h before induction and continued for 48 h after the operation). However, the number or bands of the DGGE analysis in the PRO group were also increased compared with healthy volunteers' stool as shown in Figure 3. Therefore, although it is quite possible that the intake of probiotics to some degree prevented antibiotics-induced disturbance in the microbiota,[4] the reduced disturbance of microbiota could also be a result of the surgical procedures because the colonic pathology after surgery may affect the resting gut microbiota, and these patients may have an altered gut microbiota compared with the control ('healthy') group. It is therefore still not confirmative that the improvement of gut microbiota could be just ascribed to probiotics. Both probiotics and surgical procedures could contribute to the amelioration of disturbance in the microbiota.

CONCLUSION

In summary, probiotics can improve the integrity of the gut mucosal barrier and balance of the gut microbiota, and they play a role in decreasing the infectious rate. We recommend preoperative oral intake of probiotics combined with post-operative treatment in patients who need gastrointestinal surgery.

ACKNOWLEDGEMENTS

Declaration of personal interests: None. *Declaration of funding interests*: This study was funded in full by the Ministry of Science and Technology of People's Republic of China (grant no. 2008CB517403). The clinical trial registration number is ChiCTR-TRC-00000423.

REFERENCES

1. Tran KTC, Smeenk HG, van Eijck CHJ, *et al.* Pylorus preserving pancreaticoduodenectomy versus standard Whipple procedure – a prospective, randomized, multicenter analysis of 170 patients with pancreatic and periampullary tumors. *Ann Surg* 2004; **240**: 738–45.
2. Schroeder RA, Marroquin CE, Bute BP, Khuri S, Henderson WG, Kuo PC. Predictive indices of morbidity and mortality after liver resection. *Ann Surg* 2006; **243**: 373–9.
3. MacFie J, O'Boyle C, Mitchell CJ, Buckley PM, Johnstone D, Sudworth P. Gut origin of sepsis: a prospective study investigating associations between bacterial translocation, gastric microflora, and septic morbidity. *Gut* 1999; **45**: 223–8.
4. Reddy BS, Gatt M, Sowdi R, MacFie J. Surgical manipulation of the large intestine increases bacterial translocation in patients undergoing elective colorectal surgery. *Colorectal Dis* 2006; **8**: 596–600.
5. Saadia R, Schein M, MacFarlane C, Boffard KD. Gut barrier function and the surgeon. *Br J Surg* 1990; **77**: 487–92.
6. Welsh FKS, Ramsden CW, MacLennan K, *et al.* Increased intestinal permeability and altered mucosal immunity in cholestatic jaundice. *Ann Surg* 1998; **227**: 205–12.
7. Lilly DM, Stillwell RH. Probiotics: growth-promoting factors produced by microorganisms. *Science* 1965; **147**: 747–8.
8. Sugawara G, Nagino M, Nishio H, *et al.* Perioperative synbiotic treatment to prevent postoperative infectious complications in biliary cancer surgery: a randomized controlled trial. *Ann Surg* 2006; **244**: 706–14.
9. Lin HC, Su BH, Chen AC, *et al.* Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 2005; **115**: 1–4.
10. D'Souza AL, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. *BMJ* 2002; **324**: 1361.
11. Parkes GC, Sanderson JD, Whelan K. The mechanisms and efficacy of probiotics in the prevention of *Clostridium difficile*-associated diarrhoea. *Lancet Infect Dis* 2009; **9**: 237–44.
12. McCarthy J, O'Mahony L, Dunne C, *et al.* An open trial of a novel probiotic as an alternative to steroids in mild/moderately active Crohn's disease. *Gut* 2001; **49**: A2447.
13. Qin HL, Zheng JJ, Tong DN, *et al.* Effect of *Lactobacillus plantarum* enteral feeding on the gut permeability and septic complications in the patients with acute pancreatitis. *Eur J Clin Nutr* 2008; **62**: 923–30.
14. Liu Q, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology* 2004; **39**: 1441–9.
15. Li Z, Yang S, Lin H, *et al.* Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology* 2003; **37**: 343–50.
16. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic

disease: a randomised placebo-controlled trial. *Lancet* 2001; **357**: 1076–9.
17. Woodard GA, Encarnacion B, Downey JR, *et al.* Probiotics improve outcomes after Roux-en-Y gastric bypass surgery: a prospective randomized trial. *J Gastrointest Surg* 2009; **13**: 1198–204.
18. Pitsouni E, Alexiou V, Saridakis V, Peppas G, Falagas ME. Does the use of probiotics/synbiotics prevent postoperative infections in patients undergoing abdominal surgery? A meta-analysis of randomized controlled trials. *Eur J Clin Pharmacol* 2009; **65**: 561–70.
19. Correia MI, Nicoli JR. The role of probiotics in gastrointestinal surgery. *Curr Opin Clin Nutr Metab Care* 2006; **9**: 618–21.
20. van Santvoort HC, Besselink MG, Timmerman HM, van Minnen LP, Akkermans LM, Gooszen HG. Probiotics in surgery. *Surgery* 2008; **143**: 1–7.
21. Kinross J, von Roon AC, Penney N, *et al.* The gut microbiota as a target for improved surgical outcome and improved patient care. *Curr Pharm Des* 2009; **15**: 1537–45.
22. Reddy BS, Macfie J, Gatt M, Larsen CN, Jensen SS, Leser TD. Randomized clinical trial of effect of synbiotics, neomycin and mechanical bowel preparation on intestinal barrier function in patients undergoing colectomy. *Br J Surg* 2007; **94**: 546–54.
23. Rayes N, Seehofer D, Theruvath T, *et al.* Effect of enteral nutrition and synbiotics on bacterial infection rates after pylorus-preserving pancreatoduodenectomy: a randomized, double-blind trial. *Ann Surg* 2007; **246**: 36–41.
24. Kanazawa H, Nagino M, Kamiya S, *et al.* Synbiotics reduce postoperative infectious complications: a randomized controlled trial in biliary cancer patients undergoing hepatectomy. *Langenbecks Arch Surg* 2005; **390**: 104–13.
25. Rayes N, Seehofer D, Theruvath T, *et al.* Supply of pre- and probiotics reduces bacterial infection rates after liver transplantation – a randomized, double-blind trial. *Am J Transplant* 2005; **5**: 125–30.
26. Alberda C, Gramlich L, Meddings J, *et al.* Effects of probiotic therapy in critically ill patients: a randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr* 2007; **85**: 816–23.
27. Besselink MG, van Santvoort HC, Buskens E, *et al.* Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Lancet* 2008; **371**: 651–9.
28. Lieberman JM, Sacchettini J, Marks C, Marks WH. Human intestinal fatty acid binding protein: report of an assay with studies in normal volunteers and intestinal ischemia. *Surgery* 1997; **121**: 335–42.
29. Kiliaan AJ, Saunders PR, Bijlsma PB, *et al.* Stress stimulates transepithelial macromolecular uptake in rat jejunum. *Am J Physiol* 1998; **275**(5 Pt 1): G1037–44.
30. Shang S, Fu J, Dong G, Hong W, Du L, Yu X. Establishment and analysis of specific DNA patterns in 16S-23S rRNA gene spacer regions for differentiating different bacteria. *Chin Med J (Engl)* 2003; **116**: 129–33.
31. Qin HL, Shen TY, Gao ZG, *et al.* Effect of lactobacillus on the gut microflora and barrier function of the rats with abdominal infection. *World J Gastroenterol* 2005; **11**: 2591–6.
32. Schwiertz A, Gruhl B, Lobnitz M, Michel P, Radke M, Blaut M. Development of the intestinal bacterial composition in hospitalized preterm infants in comparison with breast-fed, full-term infants. *Pediatr Res* 2003; **54**: 393–9.
33. Qin H, Zhang Z, Hang X, Jiang YL. Plantarum prevents enteroinvasive *Escherichia coli*-induced tight junction proteins changes in intestinal epithelial cells. *BMC Microbiol* 2009; **9**: 63.
34. McNaught CE, Woodcock NP, MacFie J, Mitchell CJ. A prospective randomised study of the probiotic *Lactobacillus plantarum* 299V on indices of gut barrier function in elective surgical patients. *Gut* 2002; **51**: 827–31.
35. Anderson AD, McNaught CE, Jain PK, MacFie J. Randomised clinical trial of synbiotic therapy in elective surgical patients. *Gut* 2004; **53**: 241–5.
36. Resta-Lenert S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). *Gut* 2003; **52**: 988–97.
37. Resta-Lenert S, Barrett KE. Probiotics and commensals reverse TNF-alpha- and IFN-gamma-induced dysfunction in human intestinal epithelial cells. *Gastroenterology* 2006; **130**: 731–46.
38. Madsen K, Cornish A, Soper P, *et al.* Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology* 2001; **121**: 580–91.
39. Mennigen R, Nolte K, Rijcken E, *et al.* Probiotic mixture VSL#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G1140–9.
40. Zareie M, Johnson-Henry K, Jury J, *et al.* Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. *Gut* 2006; **55**: 1553–60.
41. Ewaschuk J, Endersby R, Thiel D, *et al.* Probiotic bacteria prevent hepatic damage and maintain colonic barrier function in a mouse model of sepsis. *Hepatology* 2007; **46**: 841–50.
42. Guarner F, Malagelada JR. Gut flora in health and disease. *Lancet* 2003; **361**: 512–9.
43. Jain PK, McNaught CE, Anderson AD, MacFie J, Mitchell CJ. Influence of synbiotic containing *Lactobacillus acidophilus* La5, *Bifidobacterium lactis* Bb 12, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and oligofructose on gut barrier function and sepsis in critically ill patients: a randomised controlled trial. *Clin Nutr* 2004; **23**: 467–75.
44. Konstantinov SR, Awati A, Smidt H, Williams BA, Akkermans AD, de Vos WM. Specific response of a novel and abundant *Lactobacillus amylovorus*-like phylotype to dietary prebiotics in the guts of weaning piglets. *Appl Environ Microbiol* 2004; **70**: 3821–30.
45. Langlands SJ, Hopkins MJ, Coleman N, Cummings JH. Prebiotic carbohydrates modify the mucosa associated microflora of the human large bowel. *Gut* 2004; **53**: 1610–6.