

## Relationship between the Severity of Diversion Colitis and the Composition of Colonic Bacteria: A Prospective Study

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**Background/Aims:** Diversion colitis is the inflammation of the excluded segment of the colon in patients undergoing ostomy. It has been suggested that a change in colonic flora may lead to colitis; however, direct evidence for this disease progression is lacking. The aim of this study was to evaluate the relationship between the severity of diversion colitis and the composition of colonic bacteria. **Methods:** We used culture methods and polymerase chain reaction to analyze the colonic microflora of patients who underwent rectal cancer resection with or without diversion ileostomy. In the diversion group, we also evaluated the severity of colonoscopic and pathologic colitis before reversal. **Results:** This study enrolled 48 patients: 26 in the diversion group and 22 in the control group. Significant differences were observed between the two groups in the levels of *Staphylococcus* ( $p=0.038$ ), *Enterococcus* ( $p<0.001$ ), *Klebsiella* ( $p<0.001$ ), *Pseudomonas* ( $p=0.015$ ), *Lactobacillus* ( $p=0.038$ ), presence of anaerobes ( $p=0.019$ ), and *Bifidobacterium* ( $p<0.001$ ). A significant correlation between the severity of colitis and bacterial composition was only observed for *Bifidobacterium* ( $p=0.005$ , correlation coefficient=-0.531). **Conclusions:** The colonic microflora differed significantly between the diversion and control groups. *Bifidobacterium* was negatively correlated with the severity of diversion colitis. (*Gut Liver* 2014;8:170-176)

**Key Words:** Diversion colitis; Colonic bacteria; Rectal neoplasms; Polymerase chain reaction

### INTRODUCTION

First described in 1981, diversion colitis refers to the inflammation of excluded segments of the colon in patients who have

undergone colostomy or ileostomy and have no history of inflammatory bowel disease.<sup>1</sup> Most patients who have had an ostomy display the endoscopic features of diversion colitis in the distal colon and rectum of the ostomy,<sup>2-5</sup> and they often complain of abdominal pain, tenesmus, mucus, and hemorrhagic discharge.<sup>6,7</sup> These symptoms are uncomfortable to patients and create anxiety, which can negatively affect quality of life.

Some studies have suggested that diversion colitis is caused by an alteration of colonic microflora at the colon-excluded fecal stream or changes in the metabolism of short chain fatty acids (SCFAs). Thus, SCFA enemas have been used to treat diversion colitis.<sup>8-11</sup> Other studies have suggested that oxidative DNA damage and a mutation of p53 can lead to diversion colitis.<sup>12-14</sup> Furthermore, the number of nitrate-reducing bacteria, which metabolize nitric oxide and are related to chronic inflammation of colon, are higher in patients with diversion colitis.<sup>15-17</sup> However, most of these results were derived from animal studies or reports after empirical treatment, and the exact cause of diversion colitis is still unknown.

We previously examined the frequency of diversion colitis among Koreans, and we assessed quality of life after ileostomy repair.<sup>18</sup> In that study, we found considerable variation in the severity of diversion colitis between patients, and we hypothesized that this variation might be related to differences in the cause of diversion colitis between patients. Therefore, the aim of the present study was to clarify the relationships between the severity of diversion colitis and the composition of colonic microflora. In addition, we compared the colonic microflora between patients with or without diversion ostomy to confirm that the bacteria related to the severity of diversion colitis were also related to the induction of diversion colitis.

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## MATERIALS AND METHODS

### 1. Patients

We prospectively enrolled a consecutive series of patients who underwent surgical resection for rectal cancer between September 2010 and June 2011 at Korea University Anam Hospital. All study protocols were approved by the Institutional Review Board at Korea University Anam Hospital. Patients in the diversion group had a temporary diverting ileostomy to protect the anastomosis, while patients in the control group did not have an ileostomy. Diverting ileostomy was performed protectively when the anastomosis was at high risk for leakage; for example, it was performed when there was severe colonic edema, an insufficient vascular supply to the colonic resection margin, a positive result on a leakage test after anastomosis, and preoperative history of radiotherapy. It was also performed therapeutically when an anastomotic leakage occurred. The factors responsible for the anastomotic leakage were assumed to have no effect on diversion colitis basically. Exclusion criteria for study participation were inflammatory bowel disease, such as Crohn disease or ulcerative colitis, or a medical history of antibiotic or probiotic use within the previous 6 weeks. The basic demographics of the patients were also recorded.

### 2. Methods

#### 1) Sample collection and faecal bacteriological examination

Faecal or mucosal materials were sampled with a cotton rectal swab through the anus. In the diversion group, the sampling was performed before an endoscopic test, when patients were admitted for ileostomy repair, in order to ensure the results were not affected by endoscopy. Typically, ileostomy repair was performed within 2 or 3 months of the primary surgery. In the control group, the sampling was also performed at about 2 to 3 months after the primary surgery. The collected samples were immediately inoculated into liquid anaerobic transport media (thioglycolate broth) at the patients' bedside to ensure the survival of strict anaerobes. The samples were then transported to a laboratory as soon as possible. All manipulations were performed in an anaerobic chamber (atmosphere: N<sub>2</sub> 75%, H<sub>2</sub> 10%, CO<sub>2</sub> 15%). Transported samples were inoculated again on two blood agar plates (BAP), Mac, and *Brucella* medium. The series of plates with the BAP or Mac medium were then incubated for 24 hours at 35°C under anaerobic conditions in a 5% CO<sub>2</sub> incubator. The series of plates with the *Brucella* medium were incubated for 48 hours in an anaerobic jar. The incubated bacterial species were identified qualitatively.

#### 2) Polymerase chain reaction

Residual samples that remained after inoculation were diluted with 20% skim milk and stored in a refrigerator at -70°C. We then used a portion of these samples to perform DNA extraction

using a DNA extraction kit (Intron Biotechnology, Seoul, Korea). We selected 11 bacterial genera for polymerase chain reaction (PCR) analysis based on the other previous studies<sup>17,19,20</sup> and our preliminary culture experiments as follows: *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Enterobacteriaceae*, *Klebsiella*, *Pseudomonas*, *Lactobacillus*, *Bacteroides*, *Clostridium*, *Eubacterium*, and *Bifidobacterium*. Primer sets used in this study are summarized in Table 1. The value of T<sub>m</sub> (melting temperature) was set at more than 65°C to ensure maximum suppression of nonspecific reactions, and the length of the primer was 30 to 35 mer to improve specificity as much as possible. PCR amplification was performed with a Takara PCR machine (Takara Shuzo Co., Shiga, Japan). Amplification was identified with HotStart-Taq DNA polymerase (Qiagen Inc., Mississauga, ON, Canada), and the results were interpreted with 2.5% agarose gel electrophoresis. The detailed PCR analysis method described by Koyama *et al.*<sup>21</sup> was used, with modification. The bacterial species were identified qualitatively.

#### 3) Endoscopic and pathologic findings

Patients in the diversion group had a colonoscopy before ileostomy reversal and at approximately 2 to 3 months after the primary operation. The presence or absence of diversion colitis was assessed during the colonoscopy. Mechanical bowel preparation (MBP) was omitted before colonoscopy to reduce bias. The endoscopic severity of colitis at the proximal colon from the splenic flexure, the distal colon, and rectum were assessed by a single endoscopic specialist. The variables used to assess endoscopic and pathologic colitis were modified slightly from those used in previous studies.<sup>22</sup> Table 2 depicts the endoscopic and pathologic severity scoring used in this study. The factors for easy-touch bleeding, edema and mucosal hemorrhage were each scored on a scale from 0 to 1 or from 0 to 3, and these values were summed to obtain the severity score, which ranged from 0 to 7. Endoscopic severity was defined as the mean of the scores from each location. An endoscopic biopsy was also performed, and the pathologic severity of colitis was assessed by a single pathologist. The assessment criteria included acute inflammation, chronic inflammation, eosinophil count, crypt architecture distortion, follicular lymphoid hyperplasia, and crypt abscess. Each factor was scored from on a scale from 0 to 1 or from 0 to 3, and the values were summed to obtain the severity score, which ranged from 0 to 14. Pathologic severity was defined as the mean of the scores from each point. The final severity score was calculated by adding the endoscopic and pathologic severity scores, and it ranged from 0 to 21.

#### 3. Interpretation of results and statistical analysis

We decided to positive for certain bacteria when it was detected by cultural method, PCR method, or both. Categorical data were analyzed with the chi-square test or Fisher exact test, and numerical data were analyzed with the Student t-test. A

**Table 1.** Primer Sets Used in This Study

Target organism	Primer set	Sequence (5' to 3')
<i>Staphylococcus</i>	GAP	CTCACAGAAAAGGTGACAAACGTCGTGCTCG GTCGCCAACTGACATTACACGAGTTTGTGTAGCG
<i>Streptococcus</i>	TUF	CGTGACCTTCTTTCAGAATACGACTTCCCAGG GAACACCACGAAGAAGAACACCAACGTTGTCC
<i>Enterococcus</i>	DDL	GCCTTATGTAGGCGCGGGTGTCTTAGC CTGGAACATGCGCTGGGATTTGCATTTCAATCG
<i>Enterobacteriaceae</i>	wecE	CATGCTCAGATTGAACGCTGGCGG CTCCAGATCTCTACGCATTTACCCTG
<i>Klebsiella</i>	LAMB	TACTCTGCGCAAATTCCTCTGGCGG GAGAGAGCTTACCGAAGCCAAGGTCAACG
<i>Pseudomonas</i>	OPRL	CGTCGAGCTGAAGAAGTAAGAAGTCGTTATGCCAAG CTGCTGGAGCTGCATGAACAGTTCGC
<i>Lactobacillus</i>	Aminopeptidase (pepN)	CAAGACCGTGATGCATAGCCGACCTGAG CCTTTGAGTTCAACCTTGGCGTCTACTCC
<i>Bacteroides</i>	Enterotoxin	GATAACCGAATGTCTTGGTACGTGGTCCAAAG ATACAATCGCCGTGCCAGTGGTTC G
<i>Clostridium</i>	TIF-2	GACCTAAAACCTCAACTGGAATTGATGGACCAGC CTGAGCATGGATTAGTTGTTGAAGACTGGGGTGG
<i>Eubacterium</i>	27 kDa-2 protein	TATCGACTGGGACGCGAAGGACG CGATGGACAGGTGCACGCAACG
<i>Bifidobacterium</i>	16S ribosomal RNA	CGTCCGGTGTGAAAGTCCATCGCTTAACG TGACGACGACCATGCACCACCTGTG

**Table 2.** Endoscopic and Pathological Severity Scoring

Variable	Findings	Severity score	Total severity score
Endoscopic	Easily touch bleeding	Absence (0), presence (1)	0-7
	Edema	None (0), mild (1), moderate (2), severe (3)	
	Mucosal hemorrhage	None (0), mild (1), moderate (2), severe (3)	
Pathologic	Acute inflammation	None (0), mild (1), moderate (2), severe (3)	0-14
	Chronic inflammation	None (0), mild (1), moderate (2), severe (3)	
	Eosinophil count	None (0), mild (1), moderate (2), severe (3)	
	Crypt architecture distortion	None (0), mild (1), moderate (2), severe (3)	
	Follicular lymphoid hyperplasia	Absence (0), presence (1)	
	Crypt abscess	Absence (0), presence (1)	

p-value less than 0.05 was considered statistically significant. Cutoff values for severity were determined as the one- and two-thirds points (0.33 and 0.67) in the frequency analysis in order to divide patients into mild, moderate, and severe categories. Bivariate correlations were performed to identify the relationships between the severity of colitis and the composition of colonic microflora in the diversion group, and the degree of correlation was expressed with the Spearman rho correlation coefficient. All analyses were conducted with SPSS version 12.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### 1. Demographic and clinical characteristics

A total of 50 patients were recruited, but two patients were declined to participate; one received antibiotics within 6 weeks, and one did not undergo colonoscopy before ileostomy reversal due to anastomotic stricture. Thus, the diversion group consisted of 26 patients, and the control group, 22 patients. The two groups did not differ in demographic and clinical characteristics (Table 3). There were no significant differences between groups in sex, age, body mass index, presence of diabetes, or history of radiation or chemotherapy.

**Table 3.** Demographic and Clinical Characteristics of the Study Participants

Characteristic	Diversion (n=26)	Control (n=22)	p-value
Sex, male:female	15:11	10:12	0.409
Age, yr	64.4±10.9 (47–81)	59.8±14.7 (20–87)	0.223
Body mass index, kg/m <sup>2</sup>	23.2±3.4 (12.7–30.3)	23.6±3.2 (17.8–29.9)	0.660
Diabetes mellitus	4	5	0.526
Duration for diversion, mo	3.4±1.7 (1.0–8.5)	-	-
Chemotherapy			0.747
FOLFOX	5	1	
IV 5-fluorouracil	0	5	
Oral 5-fluorouracil	2	0	
Capecitabine	1	1	
Radiotherapy	0	2	0.121

Data are presented as mean±SD (range).

IV, intravenous; FOLFOX, oxaliplatin-based chemotherapy.

## 2. Comparison of bacterial presences in the diversion and control groups

Table 4 depicts the comparison of bacterial presences in both groups. The two groups differed significantly in *Staphylococcus* (0 patient vs 4 patients,  $p=0.038$ ), *Enterococcus* (11 patients vs 22 patients,  $p<0.001$ ), *Klebsiella* (9 patients vs 22 patients,  $p<0.001$ ), *Pseudomonas* (0 patient vs 5 patients,  $p=0.015$ ), *Lactobacillus* (21 patients vs 22 patients,  $p=0.038$ ), presence of anaerobes (20 patients vs 22 patients,  $p=0.019$ ), and *Bifidobacterium* (8 patients vs 20 patients,  $p<0.000$ ).

## 3. Correlation between the severity of diversion colitis and the composition of colonic bacteria

All the patients in the diversion group showed some degree of diversion colitis. The sum of the endoscopic and pathologic severity scores for diversion colitis were normally distributed (Fig. 1). The mean of the severity score was  $4.2\pm 1.5$  (range, 1.8–9), and the cutoff values were 3.482 and 4.727 when patients were classified as having mild, moderate, or severe diversion colitis. Eight patients belonged to the mild severity group, 10 patients belonged to the moderate group, and eight patients belonged to the severe group. The presence of bacteria in each group is shown in Table 5. Only *Bifidobacterium* was significantly associated with the severity of diversion colitis ( $p=0.005$ , correlation coefficient= $-0.531$ ). The inverse correlation between the presence of *Bifidobacterium* and the severity of diversion colitis is depicted in Fig. 2.

**Table 4.** Comparison of Bacterial Presences between the Diversion and Control Groups

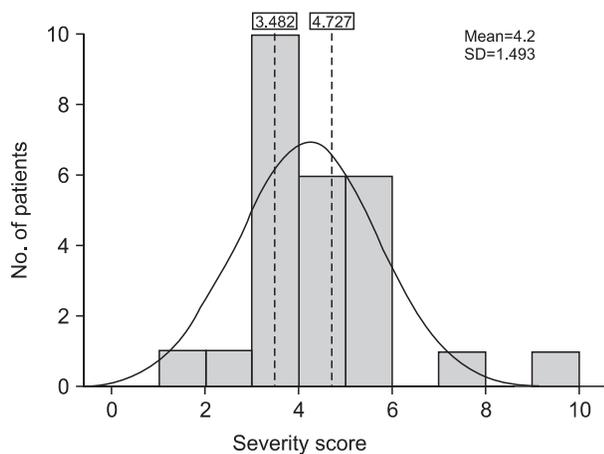
Variable	Diversion	Control	p-value
Aerobes	26 (100)	22 (100)	-
<i>Staphylococcus</i>	0	4 (18.2)	0.038
<i>Streptococcus</i>	6 (23.1)	6 (27.3)	0.745
<i>Enterococcus</i>	11 (42.3)	22 (100)	<0.001
<i>Lactococcus</i>	0	2 (9.1)	0.121
<i>Enterobacteriaceae</i>	26 (100)	22 (100)	-
<i>Klebsiella</i>	9 (34.6)	22 (100)	<0.001
<i>Pseudomonas</i>	0	5 (22.7)	0.015
<i>Lactobacillus</i>	21 (80.8)	22 (100)	0.038
Anaerobes	20 (76.9)	22 (100)	0.019
<i>Bacteroides</i>	3 (11.5)	7 (31.8)	0.088
<i>Fusobacterium</i>	2 (7.7)	1 (4.5)	0.662
<i>Prevotella</i>	0	1 (4.5)	0.282
<i>Peptostreptococcus</i>	0	1 (4.5)	0.282
<i>Clostridium</i>	6 (23.1)	6 (27.3)	0.745
<i>Propionibacterium/Actinomyces</i>	1 (3.8)	0	0.363
<i>Eubacterium</i>	12 (46.2)	7 (31.8)	0.322
<i>Bifidobacterium</i>	8 (30.8)	20 (90.9)	<0.001
Total bacteria	26 (100)	22 (100)	-

Data are presented as number (%). All numbers are the number of patients positive for the presence of bacteria.

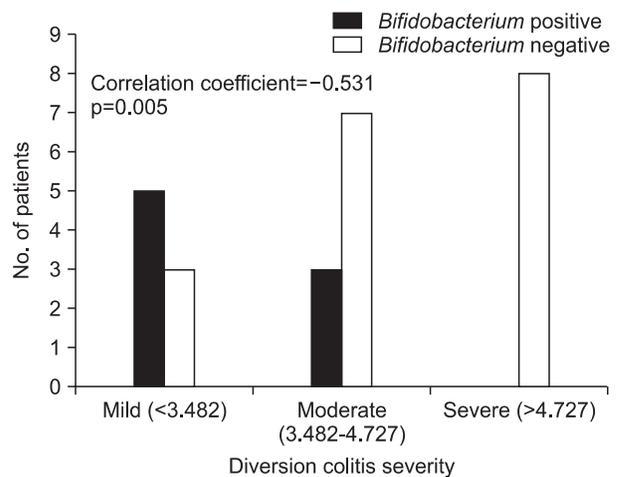
## DISCUSSION

Diversion colitis is reported in 70% to 100% of patients in Western countries who underwent diversion ostomy, and many of these patients experienced a variety of symptoms.<sup>3,8</sup> It is generally accepted that a change in the colonic mucosa, which uses metabolites from stool as an energy source due to the interruption of the faecal stream, may lead to colitis; however, direct evidence is still lacking. Well-designed studies for diversion colitis may be lacking because it is difficult to design controlled experiment and to interpret results given that the colonic microflora include innumerable bacteria. Furthermore, there are many factors that can potentially affect colonic microflora, such as age, obesity, metabolic disease, and a history of antibiotic or probiotic use, all of which are difficult to control. In addition, anaerobes are difficult to collect, transfer, and handle. To overcome this problem, we expended considerable effort to increase the detection of anaerobes. For example, we inoculated the specimens into anaerobic transport media immediately at the patient's bedside and promptly transferred the samples to the lab.

We only included rectal cancer patients in our study because these patients usually undergo temporary diversion ileostomy



**Fig. 1.** Distribution of the severity scores for diversion colitis (n=26). SD, standard deviation.



**Fig. 2.** Correlation between *Bifidobacterium* and the severity of diversion colitis.

**Table 5.** Comparison of Bacterial Presence in Relation to the Severity of Diversion Colitis

	Mild	Moderate	Severe	p-value	Correlation coefficient
Aerobes	8	10	8	-	-
<i>Streptococcus</i>	1	3	2	0.571	0.116
<i>Enterococcus</i>	2	5	4	0.381	0.179
<i>Enterobacteriaceae</i>	8	10	8	-	-
<i>Klebsiella</i>	5	1	3	0.312	-0.206
<i>Lactobacillus</i>	7	7	7	1.000	0.000
Anaerobes	7	6	7	1.000	0.000
<i>Bacteroides</i>	0	1	2	0.127	0.307
<i>Fusobacterium</i>	0	2	0	1.000	0.000
<i>Clostridium</i>	1	3	2	0.571	0.116
<i>Propionibacterium</i> / <i>Actinomyces</i>	0	0	1	0.209	0.255
<i>Eubacterium</i>	3	4	5	0.335	0.197
<i>Bifidobacterium</i>	5	3	0	0.005	-0.531
Total bacteria	8	10	8	-	-

All numbers are the number of patients positive for the presence of bacteria.

to protect against anastomotic leakage. Although the effects of rectal cancer on the distribution of colonic bacteria are unknown, we did not believe it was necessary in order to identify the effects by switching the faecal stream. However, the distinct characteristics of colonic microflora among patients who have undergone chemotherapy or radiation therapy should be considered in future studies. Few studies have examined the effects of chemotherapy or radiation therapy on colonic microflora. Because these therapies can damage the colonic mucosa directly, their effects should be distinguished from those of diversion colitis.<sup>23-26</sup>

We sampled faecal and mucosal materials with a cotton

swab through the anus. Patients in the diversion group could not defecate because they had no stool in the distal colon of the diversion ileostomy. In addition, the data for the patients who underwent colonoscopy could have been biased towards anaerobes because the detection of anaerobic bacteria may be decreased due to the air used for bowel inflation if stool is collected using colonoscopy. Neut *et al.*<sup>19</sup> used a rectal swab to sample faecal material of 16 patients with diverting stoma and 16 healthy controls, and they found that the diversity of flora, especially strict anaerobes such as *Eubacterium* and *Bifidobacterium*, was significantly reduced. They also found that anaerobes were obtained less frequently from the stomal site than the rectal site, even among the patients with diverting ostomy, and they hypothesized that the lower anaerobic count in the stomal sample might be explained by greater oxygen exposure and lower humidity at that site. Thus, the results of the swab method may be affected by the sample site or the degree of sterilization.

To compensate for the limitations of culture method, we also used PCR to evaluate colonic flora. To our knowledge, this is the first study to use PCR to evaluate the relationships between colonic flora and diversion colitis, and this technique could improve the stability and sensitivity of the results. Ideally, we would have performed PCR analysis for all recognized bacteria, but this approach was not possible in practice because the number of bacteria is nearly infinite. Therefore, we relied on the findings from our culture experiments and previous studies to select the bacteria for PCR analysis.<sup>17,19,20</sup> Unfortunately, this strategy also had some limitations: the selection of a standard is ambiguous and dependent on existing information. Thus, the use of PCR alone is not ideal because it may cause researchers to overlook important bacteria, and the most reliable approach may be to use both a culture method and PCR. In addition, use of the common base sequencing of genus unit during PCR, which we used in our study, could be helpful. The merit of the combined methods that were used in this study is that the PCR

method has high sensitivity for the detection of essential bacteria while the culture method detects other bacteria not selected for the PCR method, without exception.

We found that *Bifidobacterium* was the only bacteria that displayed both a significant difference between the diversion and control groups, and a significant (and negative) association with the severity of diversion colitis. According these results, we believe that *Bifidobacterium* seems to be related to the pathophysiology of diversion colitis. This finding is in agreement with the results of Watanabe *et al.*<sup>20</sup> *Bifidobacterium* are usually regarded as beneficial 'probiotic' bacteria that confer various health benefits on the host, including up-regulation of the systemic immune response, stimulation of cellular immunity, and protection against infection.<sup>27,28</sup> Shimizu *et al.*<sup>29</sup> reported that patients with severe systemic inflammatory response syndrome had significantly lower levels of *Lactobacillus* and *Bifidobacterium*, and they suggested that abnormal gut flora may affect systemic inflammatory responses after severe insults. We found that the detection ratio of *Bifidobacterium* was inversely proportional to the severity of diversion colitis, which is in agreement with these previous studies. Future studies should focus specifically on *Bifidobacterium* to better understand its role in diversion colitis.

To improve our understanding of the significantly different bacterial composition between the diversion and control groups, it would be helpful to perform additional studies, such as analyses of faecal organic acid, which act between bacteria and the colonic mucosal layer. Watanabe *et al.*<sup>20</sup> found that the count of bacterial microflora, such as *Bifidobacterium* and total *Lactobacillus*, was significantly lower among patients who had MBP than patients who did not have MBP (the no-MBP group). In addition, the levels of faecal organic acids, such as acetic acid, propionic acid, and butyric acid, in intraoperative faecal material were significantly lower and the levels of lactic acid were significantly higher in the MBP group than the no-MBP group. The succinic acid level was significantly higher after surgery than before surgery in the MBP group. These results suggest that a change in the faecal stream can alter colonic bacteria and organic acids sequentially. Thus, future studies in this area should help determine the cause-and-effect relationship of diversion colitis. It would also be interesting to determine if colonic microflora recover after ileostomy reversal and how long recovery takes.

In conclusion, we found that the abundance of several types of bacteria differed significantly between the diversion and control groups. In addition, *Bifidobacterium* was inversely proportional to the severity of diversion colitis, so it might be related to the occurrence of this condition. Future studies are needed on the pathophysiology of diversion colitis and the changes in colonic microflora, which could lead to the development of novel treatments for diversion colitis.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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