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Full Paper

Clostridium butyricum therapy for mild-moderate *Clostridioides difficile* infection and the impact of diabetes mellitus

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The therapeutic effect of *Clostridium butyricum* for adults with *Clostridioides difficile* infection (CDI) was investigated. A retrospective study was conducted in medical wards of Tainan Hospital, Ministry of Health and Welfare, between January 2013 and April 2020. The disease severity of CDI was scored based on the Clinical Practice Guidelines of the IDSA/SHEA. Treatment success was defined as the resolution of diarrhea within six days of a therapeutic intervention without the need to modify the therapeutic regimen. In total, 241 patients developed CDI during hospitalization in the study period. The treatment success rates for the 99 patients with mild-moderate CDI among them were as follows: metronidazole, 69.4%; *C. butyricum*, 68.2%; metronidazole plus *C. butyricum*, 66.7%; and oral vancomycin, 66.7% (p=1.00). Patients with treatment success were less likely to have diabetes mellitus than those with treatment failure (38.2% vs. 61.3%, p=0.05). Patients treated with *C. butyricum* alone or in combination with metronidazole had shorter durations of diarrhea than those treated with metronidazole alone (3.1 ± 2.0 days or 3.5 ± 2.4 days vs. 4.2 ± 3.5 days; p=0.43 or 0.71), although the differences were not statistically significant. In conclusion, the treatment success rate of *C. butyricum* alone or in combination with metronidazole for patients with CDI was non inferior to that of metronidazole alone. The presence of diabetes mellitus in affected individuals is a risk factor for treatment failure.

Key words: *Clostridioides difficile* infection, *Clostridium butyricum*, diabetes mellitus, probiotics, metronidazole, mild-moderate

INTRODUCTION

Clostridioides difficile is the major cause of community- and healthcare-associated infections, ranging from mild diarrhea to pseudomembranous colitis or toxic megacolon, with a mortality rate of up to 25% to 40% [1–9]. Among patients with mild *C. difficile* infection (CDI), both metronidazole (MNZ) and vancomycin (VCM) therapy have been shown to result in substantial clinical cure rates; in contrast, for those with severe CDI, the clinical cure rate of MNZ therapy has been shown to be

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lower than that of VCM therapy [10]. So, MNZ and VCM were once regarded as the therapeutic options for mild-moderate and severe CDI, respectively, depending on the severity of disease.

However, the role of MNZ as the therapy of choice for mild-moderate CDI has been challenged in many studies [11, 12]. In a meta-analysis of MNZ and VCM treatment for CDI, MNZ was inferior to VCM in terms of both initial clinical cure rate and sustained cure rate [11]. Another pooled analysis of two multinational trials showed that MNZ was inferior to VCM in terms of clinical success rate [12]. Thus, the latest clinical guidelines updated by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) prefer oral VCM for either mild-moderate or severe CDI [13]. Nevertheless, oral VCM markedly disrupts the intestinal microbiota and leads to prolonged loss of colonization resistance to CDI and dense colonization by VCM-resistant *Enterococcus*, *Klebsiella pneumoniae*, and *Escherichia coli* [14]. Moreover, both MNZ and VCM therapy have been associated with substantial treatment failure and recurrence rates [15].

Another potential therapeutic option for mild-moderate CDI is the introduction of competing nonpathogenic organisms, *i.e.,* probiotics, into intestinal tract to restore microbial balance. Certain probiotic agents, such as *Lactobacillus* GG or *Saccharomyces boulardii*, have been studied for the prophylaxis or treatment of CDI [16, 17]. However, in a recent systemic review, there was insufficient evidence to suggest routine clinical use of probiotic therapy for the prevention or treatment of CDI [18]. The role of probiotics for the treatment of CDI is still controversial.

Clostridium butyricum, one of the most commonly used probiotics, has been proven to be effective in many kinds of gastrointestinal disease, such as ulcerative colitis [19] and enterohemorrhagic *E. coli* (EHEC) colitis [20]. *C. butyricum* has been proven to have an inhibitory effect on *C. difficile ex vivo* and *in vivo* [21]. In a rat model of *C. difficile* infection, the cytotoxin titer in feces of rats treated with *C. butyricum* was lower than that of the control group [21]. The utility of *C. butyricum* in treating patients with mild-moderate CDI was investigated in the present study.

MATERIALS AND METHODS

A retrospective study was conducted in medical wards of Tainan Hospital, Ministry of Health and Welfare, in southern Taiwan. The study was approved by the institutional review board of National Cheng Kung University Hospital, Taiwan (approval number: B-ER-107-362). Adults with mild-moderate CDI between January 2013 and April 2020 were included.

Severity score assessment

The severity score of CDI was graded according to the Clinical Practice Guidelines updated by the IDSA and SHEA in 2017, and patients with a white blood cell (WBC) count of ≤15,000 cells/mL and serum creatinine level of <1.5 mg/dL were regarded as having mild-moderate CDI [13].

Clinical information evaluation

Clinical data, including age, nasogastric tube use, and underlying diseases, were recorded. Patients with an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2 for at least three months were regarded as having chronic kidney disease (CKD) [22]. The degree of glycemic control in diabetic adults was assessed based on the serum level of glycated hemoglobin A1c (HbA1c). Glycemic control was classified as being poor if the serum HbA1c level was >7% [23].

Medications, including systemic antibiotics (cephalosporins such as cefazolin, cefuroxime, cefmetazole, ceftriaxone, ceftazidime, and cefepime; penicillins such as penicillin derivatives like penicillin, oxacillin, and piperacillin and beta-lactam/beta-lactamase inhibitors like amoxicillin/clavulanic acid, ampicillin/sulbactam, and piperacillin/tazobactam; carbapenems such as ertapenem, imipenem, and meropenem; and glycopeptides such as VCM and teicoplanin), proton pump inhibitors, histamine H2-receptor antagonists, systemic steroids, and probiotics (*C. butyricum* MIYAIRI 588, Miyarisan®, Miyarisan Pharmaceutical, Japan), prescribed during hospitalization were recorded. *C. butyricum* MIYAIRI 588, a commonly used probiotic in Asia, contains 3.8–5.0 × 10^8 viable spores of the MIYAIRI 588 strain per package [24]. Most patients took one package of *C. butyricum* MIYAIRI 588 three times daily, as suggested by the manufacturer.

Definitions

Diarrhea was defined as at least three unformed bowel movements per day for at least two days. CDI cases referred to patients suffering from diarrhea without alternative explanations but with *C. difficile* toxin or *tcdB*-carrying isolates detected in fecal samples [25]. The duration of hospitalization preceding CDI was the period from admission to CDI onset, and only the first episode of CDI was included. Treatment success was defined as the resolution of diarrhea within six days, without the need to change the therapeutic regimen [10]; all other cases were considered to be treatment failure. Recurrence was defined as the recurrence of diarrhea with *C. difficile* toxin or *tcdB*-carrying isolates detected in fecal samples by day 21 after initial treatment success [10].

Laboratory diagnostic methods

Initially, the presence of toxigenic *C. difficile* isolates in unformed stool samples was determined by simultaneous measurements of *C. difficile* glutamate dehydrogenase (GDH) and toxin A/B using an enzyme immunoassay (EIA; Abbott, Santa Clara, CA, USA). In cases with discordant EIA results (GDH+/toxin-), stool samples were subjected to anaerobic culture on cycloserine-cefoxitin-fructose agar (CCFA; Creative Media Plate®, Creative Life Science Co, Ltd., New Taipei City, Taiwan) with incubation for 24 to 48 hours to isolate *C. difficile*. After harvesting *C. difficile* isolates, genomic DNA was extracted with a genomic DNA Mini Kit (Geneaid Biotech Ltd., New Taipei City, Taiwan). A multiplex polymerase chain reaction (PCR) was used to detect *tcdA*, *tcdB*, *cdtA*, *cdtB*, and *tcdC* deletion, as described previously [26]. The diarrheal patients were diagnosed as having CDI if *tcdB* was detected in the *C. difficile* isolates by multiplex PCR.

Statistical analysis was performed with statistical software (IBM SPSS Statistics, version 22.0). Continuous data were expressed as means ± standard deviations. The χ^2 test or Fisher's test was used for categorical variables, and Student t-test was used for continuous variables. A two-tailed p-value of less than 0.05 was considered to be statistically significant. Bonferroni correction was applied for multiple comparisons, and the results were considered to be statistically significant at a p-value of less than 0.05.

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RESULTS

Therapeutic success rates stratified by the different therapies

A total of 241 patients developed CDI during hospitalization between January 2013 and April 2020. Of them, 160 (66.4%) receiving anti-*C. difficile* antibiotic therapy or *C. butyricum* were included in the present study (Fig. 1). Their average age was 76.4 ± 12.9 years, and 72 (45.0%) of them were male (Table 1). Of the 160 patients with CDI, 102 (63.8) were diagnosed based on GDH+/toxin A/B+ results in stool samples, and 58 (36.3%) were diagnosed based on the detection of *tcdB*-carrying *C. difficile* isolates in stool. Overall, the growth of toxigenic *C. difficile*, which harbored at least *tcdB,* was detected in stool samples of 72 (45%) of the 160 patients with CDI. Furthermore, both *tcdC* deletion and binary toxin were detected in 9 (12.5%) of the 72 patients.

The included patients were stratified by the IDSA/SHEA severity score for CDI (Fig. 1), and 99 (61.9%) of them had mild-moderate CDI. The treatment success rates for the different therapeutic interventions in the 99 patients with mild-moderate CDI were as follows: MNZ, 69.4% (43/62); *C. butyricum,* 68.2% (15/22); MNZ plus *C. butyricum,* 66.7% (8/12); and oral VCM, 66.7% (2/3; p=1.00).

Factors associated with treatment success

The 99 patients receiving therapy for mild-moderate CDI were further stratified according to therapy outcome, *i.e.,* treatment success or failure (Table 1). Fewer patients with treatment success had diabetes mellitus than those with treatment failure (38.2% vs. 61.3%; p=0.05). However, among diabetic patients, fasting serum glucose or HbA1C levels were similar in the treatment success and failure groups (158.7 ± 88.9 mg/dL vs. 197.7 ± 114.6 mg/dL; p=0.20). There were no differences in terms of other comorbidities, concomitant use of non-anti-*C. difficile* antibiotics or gastric acid-lowering agents, and *tcdC* deletion in fecal *C. difficile* isolates between the treatment success and failure groups.

There was no correlation between treatment outcomes and WBC counts in the patients with mild-moderate CDI. Moreover, the treatment regimens containing MNZ, *C. butyricum*, or VCM were not associated with the treatment outcome. Patients with treatment success had shorter hospitalization durations than those with treatment failure (31.0 ± 25.7 days vs. 57.3 ± 50.0 days; p=0.03), but no difference in CDI recurrent rate or in-hospital mortality rate was noted between the treatment success and failure groups (Table 2).

Characters of patients treated with the different regimens

Compared with the 62 patients treated with metronidazole and 22 patients treated with *C. butyricum*, the 12 patients treated with the combination therapy (MNZ plus *C. butyricum*) were less likely to be male (16.7% vs. 50.0% and 27.3%; p=0.03). Patients treated with the combination therapy were more likely to have had prior carbapenem therapy than those treated with MNZ or *C. butyricum* alone, although the differences were not statistically significant (41.7% vs. 29.0% and 13.6%; p=0.18), as noted in Table 3.

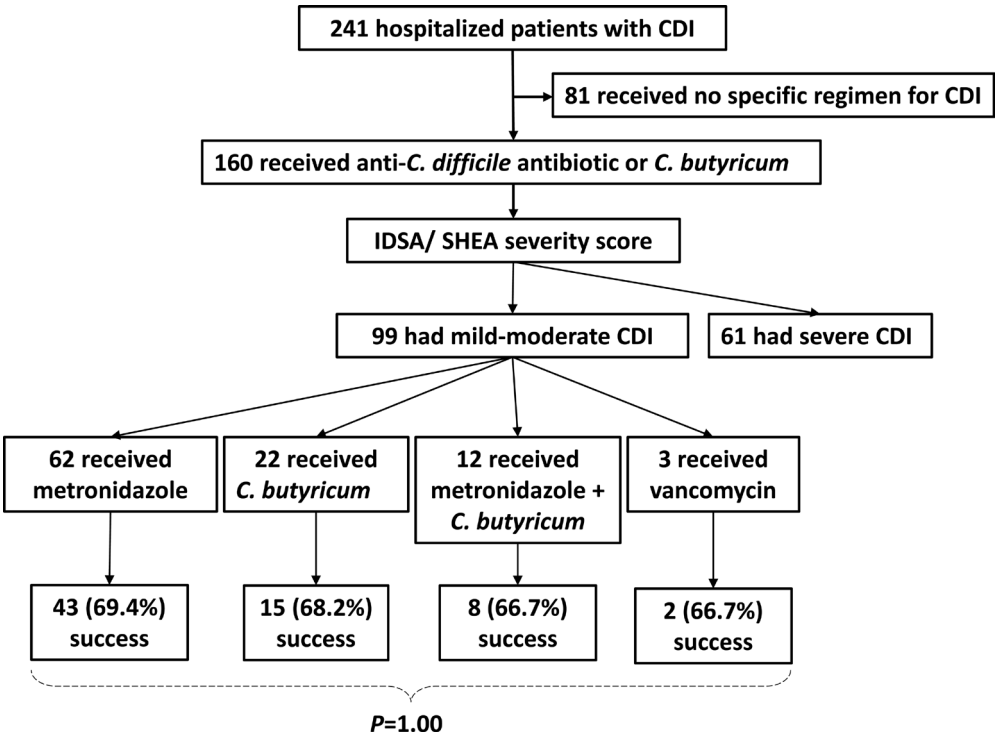

Fig. 1. Flowchart for hospitalized patients with mild-moderate *Clostridioides difficile* infection (CDI) and subsequent success rates of the different therapeutic regimens (metronidazole, *Clostridium butyricum*, metronidazole plus *C. butyricum*, and vancomycin).

*Mild-moderate CDI in the Infectious Diseases Society of America (IDSA) /Society for Healthcare Epidemiology of America (SHEA) severity criteria: leukocytosis with a white blood cell count of ≤15,000 cells/mL and a serum creatinine level <1.5 × baseline mg/dL.

*Success in therapy: resolution of diarrhea within six days of indicated therapy without change in therapeutic regimen.

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Table 1. Comparisons of the clinical characters and prior medications of the 99 adults with mild-moderate *Clostridioides difficile* infection (CDI), stratified by treatment success or failure of anti-CDI therapy

Characters	All n=99	Treatment failure n=31	Treatment success n=68	p-value
Age, years	77.4 ± 12.6	77.3 ± 12.5	77.5 ± 12.7	0.94
tcdC deletion isolates (72)*	9/72 (12.5)	3/72 (4.2)	6/72 (8.3)	0.73
Male gender	41 (41.4)	14 (45.2)	27 (39.7)	0.66
Underlying disease				
Hypertension	63 (63.6)	20 (64.5)	43 (63.2)	1.00
Diabetes mellitus	45 (45.5)	19 (61.3)	26 (38.2)	0.05
Fasting serum glucose, mg/dL	170.6 ± 98.0	197.7 ± 114.6	158.7 ± 88.9	0.20
HbA1C (35)*, %	6.7 ± 1.9	6.8 ± 2.2	6.6 ± 1.7	0.70
HbA1C >7.0%, %	9/35 (25.7)	5/35 (14.3)	4/35 (11.4)	1.00
Chronic kidney disease	38 (38.4)	11 (35.5)	27 (39.7)	0.82
Old stroke	38 (38.4)	11 (35.5)	27 (39.7)	0.82
Dementia	31 (31.3)	12 (38.7)	19 (27.9)	0.35
Congestive heart failure	17 (17.2)	6 (19.4)	11 (16.2)	0.78
Malignancy	16 (16.2)	4 (12.9)	12 (17.6)	0.77
Parkinsonism	13 (13.1)	4 (12.9)	9 (13.2)	1.00
Coronary artery disease history	12 (12.1)	3 (9.7)	9 (13.2)	0.75
Chronic obstructive pulmonary disease	10 (10.1)	3 (9.7)	7 (10.3)	1.00
Liver cirrhosis	2 (2.0)	1 (3.2)	1 (1.5)	0.53
Concomitant medications				
Cephalosporins	43 (43.4)	15 (48.4)	28 (41.2)	0.52
Cefazolin, iv	3 (3.0)	1 (3.2)	2 (2.9)	1.00
Cefuroxime, iv/o	7 (7.1)	4 (12.9)	3 (4.4)	0.20
Ceftazidime or ceftriaxone, iv	24 (24.2)	7 (22.6)	17 (25.0)	1.00
Cefepime, iv	10 (10.1)	4 (12.9)	6 (8.8)	0.72
Fluoroquinolones, iv/o	6 (6.1)	3 (9.7)	3 (4.4)	0.37
Penicillins other than piperacillin-tazobactam, iv/o	5 (5.1)	2 (6.5)	3 (4.4)	0.65
Piperacillin-tazobactam, iv	3 (3.0)	0	3 (4.4)	0.55
Carbapenem, iv	27 (27.3)	10 (32.3)	17 (25.0)	0.47
Glycopeptide, iv	10 (10.1)	5 (16.1)	5 (7.4)	0.28
Proton pump inhibitors, iv/o	25 (25.3)	9 (29.0)	16 (23.5)	0.62
H2-receptor antagonists, iv/o	11 (11.1)	5 (16.1)	6 (8.8)	0.31
Steroid, iv/o	26 (26.3)	6 (19.4)	20 (29.4)	0.34
Anti-diarrheal agents	59 (59.6)	22 (71.0)	37 (54.4)	0.13

Continuous parameters are expressed as patient numbers (%) or means ± standard deviations.
iv: intravenous; o: oral.
*Available case or isolate number.

Table 2. Laboratory findings, therapeutic regimens, and clinical outcomes of the 99 adults with mild-moderate *Clostridioides difficile* infection (CDI), stratified by treatment success or failure of anti-CDI therapy

Clinical variables	All n=99	Treatment failure n=31	Treatment success n=68	p-value
White blood cell count, cells/mm^3	8,900 ± 3,000	8,400 ± 2,600	9,200 ± 3,200	0.20
Treatment regimens				
Metronidazole-containing	74 (74.7)	23 (74.2)	51 (75.0)	1.00
Clostridium butyricum-containing	34 (34.3)	11 (35.5)	23 (33.8)	1.00
Vancomycin-containing	3 (3.0)	1 (3.2)	2 (2.9)	1.00
Hospitalization duration, days	43.2 ± 40.7	57.3 ± 50.0	31.0 ± 25.7	0.03
In-hospital mortality	20 (20.2)	9 (29.0)	11 (16.2)	0.18
Recurrence	6 (6.1)	0	6 (8.8)	0.17

Clinical variables are expressed as patient numbers (%) or means ± standard deviations.

Table 3. Clinical characters and prior medications of the 96 adults with mild-moderate *Clostridioides difficile* infection treated with metronidazole alone, *Clostridium butyricum* alone, or both in combination

Characters	Metronidazole n=62	*C. butyricum* n=22	Combination n=12	p-value
Age, years	78.7 ± 11.1	75.4 ± 15.6	78.4 ± 10.6	0.54
Male gender	31 (50.0)	6 (27.3)	2 (16.7)	0.03
Underlying disease				
Hypertension	41 (66.1)	12 (54.5)	8 (66.7)	0.61
Diabetes mellitus	30 (48.4)	9 (40.9)	4 (33.3)	0.58
Chronic kidney disease	27 (43.5)	8 (36.4)	3 (25.0)	0.46
Old stroke	25 (40.3)	7 (31.8)	6 (50.0)	0.57
Dementia	17 (27.4)	9 (40.9)	5 (41.7)	0.39
Congestive heart failure	12 (19.4)	3 (13.6)	2 (16.7)	0.83
Malignancy	12 (19.4)	0	2 (16.7)	0.09
Chronic obstructive pulmonary disease	7 (11.3)	3 (13.6)	0	0.43
Coronary artery disease history	6 (9.7)	4 (18.2)	2 (16.7)	0.52
Parkinsonism	5 (8.1)	5 (22.7)	3 (25.0)	0.10
Liver cirrhosis	2 (3.2)	0	0	0.57
Concomitant medications				
Cephalosporins	26 (41.9)	12 (54.5)	4 (33.3)	0.44
Cefazolin, iv	3 (4.8)	0	0	0.43
Cefuroxime, iv/o	4 (6.5)	3 (13.6)	0	0.31
Ceftazidime or ceftriaxone, iv	14 (22.6)	7 (31.8)	3 (25.0)	0.69
Cefepime, iv	6 (9.7)	2 (9.1)	1 (8.3)	0.99
Fluoroquinolones, iv/o	5 (8.1)	1 (4.5)	0	0.53
Penicillins other than piperacillin-tazobactam, iv/o	2 (3.2)	3 (13.6)	0	0.12
Piperacillin-tazobactam, iv	2 (3.2)	0	1 (8.3)	0.41
Carbapenem, iv	18 (29.0)	3 (13.6)	5 (41.7)	0.18
Glycopeptide, iv	7 (11.3)	2 (9.1)	1 (8.3)	0.93
Proton pump inhibitors, iv/o	15 (24.2)	5 (22.7)	2 (16.7)	0.85
H2-receptor antagonists, iv/o	7 (11.3)	3 (13.6)	1 (8.3)	0.90
Steroid, iv/o	17 (27.4)	5 (22.7)	1 (8.3)	0.36

Clinical variables are expressed as patient numbers (%) or means ± standard deviations.
iv: intravenous; o: oral.

There were no differences in treatment success rate, recurrence rate, or in-hospital mortality rate among patients with mild-moderate CDI receiving the MNZ, *C. butyricum*, or combination therapies (MNZ plus *C. butyricum*). Patients treated with *C. butyricum* alone or *C. butyricum* in combination with MNZ had a shorter duration of diarrhea than those treated with MNZ alone (3.1 ± 2.0 days and 3.5 ± 2.4 days vs. 4.2 ± 3.5 days), although the differences were not statistically significant (Table 4).

Among the 22 patients treated with *C. butyricum*, those with treatment success tended to be less likely to have diabetes mellitus (26.7% vs. 71.4%; p=0.07) and to have lower fasting serum glucose (129.5 ± 55.5 mg/dL vs. 244.3 ± 243.2 mg/dL; p=0.50) and HbA1C (6.0 ± 1.0 mg/dL vs. 8.2 ± 3.9 mg/dL; p=0.42) levels than those with treatment failure, but the differences were not statistically significant (Table 5).

Of the three patients treated with oral VCM, all were male. Treatment success was noted in two (66.7%) of the patients, and their diarrheal durations were 3 and 4 days, respectively. Diarrhea did not resolve until 14 days of VCM therapy in one patient, who was categorized as having treatment failure.

DISCUSSION

C. butyricum was non inferior to MNZ in the treatment of mild-moderate CDI in our retrospective study. VCM has been suggested as a replacement for MNZ as the drug of choice for mild-moderate CDI, but its use is associated with profound microbiota disruption and colonization with antimicrobial agent-resistant pathogens [14]. Although some probiotic agents have been studied for the prophylaxis or treatment of CDI, there was still insufficient evidence for the routine clinical use of a probiotic therapy in the treatment of CDI [18]. *C. butyricum* has been shown to have *in vivo* [21] and clinical [27] anti-*C. difficile* effects in previous studies. Our study suggests that *C. butyricum* can be considered as another therapeutic choice for mild-moderate CDI.

The favorable effect of *C. butyricum* in the treatment of mild-moderate CDI might be due to its beneficial effect on the microbiome [27]. *C. butyricum* is one of the facultative and strictly anaerobic bacteria that colonize in the neonate intestine [28]. In a *in vivo* study of the gut microbiota of sows, the addition of 0.2% *C. butyricum* to commercial diet increased the relative abundance of *Bacteroidetes*, decreased the relative abundances of *Proteobacteria*, *Gemmatimonadetes*, and *Actinobacteria,* and decreased the *Firmicutes*/*Bacteroidetes* ratio [29]. In another

Table 4. Outcomes of the patients with mild-moderate *Clostridioides difficile* infections (CDIs) treated with metronidazole, *Clostridium butyricum*, or both in combination

Outcome	Metronidazole n=62	*C. butyricum* n=22	Combination n=12	p-value[a]	p-value[b]	p-value[c]
Duration of diarrhea, days	4.2 ± 3.5	3.1 ± 2.0	3.5 ± 2.4	0.43	0.71	0.78
Treatment success	43 (69.4)	15 (68.2)	8 (66.7)	1.00	1.00	1.00
Recurrence of CDI	3 (4.8)	2 (9.1)	1 (8.3)	0.60	0.52	1.00
In-hospital mortality	13 (21.0)	4 (18.2)	3 (25.0)	1.00	0.72	0.68

Clinical outcomes are expressed as patient numbers (%) or means ± standard deviations.
[a]Compare the metronidazole group with the *C. butyricum* group.
[b]Compare the metronidazole group with the combination group.
[c]Compare the *C. butyricum* group with the combination group.

Table 5. Clinical characters of the 22 adults with mild-moderate *Clostridioides difficile* infection (CDI) receiving *Clostridium butyricum*, stratified by treatment success or failure

Characters	Treatment failure n=7	Treatment success n=15	p-value
Age, years	75.9 ± 20.4	75.1 ± 13.6	0.92
Male gender	2 (28.6)	4 (26.7)	1.00
Underlying disease			
Diabetes mellitus	5 (71.4)	4 (26.7)	0.07
Fasting serum glucose, mg/dL	244.3 ± 243.2	129.5 ± 55.5	0.50
HbA1C (7)*, %	8.2 ± 3.9	6.0 ± 1.0	0.42
Hypertension	5 (71.4)	7 (46.7)	0.38
Chronic kidney disease	2 (28.6)	6 (40.0)	1.00
Old stroke	1 (14.3)	6 (40.0)	0.35
Dementia	2 (28.6)	7 (46.7)	0.65
Parkinsonism	2 (28.6)	3 (20.0)	1.00
Congestive heart failure	0	3 (20.0)	0.52
Coronary artery disease history	1 (14.3)	3 (20.0)	1.00
Chronic obstructive pulmonary disease	0	3 (20.0)	0.52
White blood cell count, cells/mm^3	7,900 ± 2,500	9,600 ± 2,900	0.16

Clinical variables are expressed as patient numbers (%) or means ± standard deviations.
*Available numbers of cases: 4 in the failure group and 3 in the success group.

study in piglets by the same study group, *C. butyricum*-based probiotics improved growth performance, enhanced intestinal morphology, changed hypothalamic neurotransmitters, and modulated colonic microflora [30]. Among pediatric patients with antibiotic-associated diarrhea receiving *C. butyricum*, the rate of diarrhea decreased from 59% to 5% after therapy, with an increase in the level of anaerobes and without an obvious decrease in the level of *Bifidobacterium* species [27].

The modifying effect of *C. butyricum* on the metabolome has also been shown to contribute to the therapeutic effect of *C. butyricum* on mild-moderate CDI [31]. When orally administered, *C. butyricum* spores germinate and grow in the intestinal tract, and they produce large amounts of SCFAs, such as butyrate and acetate [31]. It has also been reported that *C. butyricum* MIYAIRI 588 fermented metabolites, such as butyrate, by promoting the generation of regulatory T-cells in the intestine through the induction of transforming growth factor-β1 from lamina propria dendritic cells, which was mainly Toll-like receptor 2 dependent [32]. Butyrate is a kind of short-chain fatty acid (SCFA; chain length 1–6), and SCFAs are the main products of dietary fibers fermented by anaerobic gut bacteria and serve as substrates for energy metabolism [33].

Irrespective of MNZ, *C. butyricum*, or combination therapy, one factor associated with treatment failure in our study was the presence of diabetes mellitus. In previous studies, patients with diabetes mellitus were more likely to have *C. difficile* colonization [34], to develop severe CDI [35], or to develop recurrent CDI [36]. Moreover, diabetes mellitus has been linked to MNZ treatment failure in patients with CDI [37]. The possible mechanism involved in the development of type 2 diabetes in humans has been associated with decreased expression of vacuolar ATPase, which is involved in endocytosis and is critical in protective immunity [38]. A decrease in intestinal colonization of non-toxigenic strains of *C. difficile*, which are competitors of toxigenic strains of *C. difficile,* has also been noted in the guts of diabetic patients [39].

The treatment success rate of the combination regimen (MNZ plus *C. butyricum*) was not significantly higher than those of the MNZ and *C. butyricum* monotherapies in our study. Moreover, patients treated with the combination therapy more often had prior exposure to carbapenems, which may lead to dramatic alteration of the intestinal microbiota in cases of intestinal graft-versus-host disease [40, 41]. However, there was a limited case number of cases in the present study, and this may have resulted in the

absence of significant therapeutic superiority in the combination group.

The therapeutic effect of pharmacological interventions in the patients receiving an anti-diarrheal drug was difficult to assess, because patients with more severe diarrhea were more likely to be treated with anti-diarrheal drugs. Further clinical studies are warranted to determine the beneficial or detrimental effects of antidiarrheal drugs on mild-moderate CDI.

There were some limitations in this study. First, there was a limited number of cases, so it was difficult to find significant differences for certain study outcomes in comparisons with MNZ and *C. butyricum* alone. Second, this work was a retrospective study. Physicians might use MNZ or probiotics for CDI according to the clinical setting, and clinical settings were difficult to determine retrospectively in this study. Third, only one test result for the fasting serum glucose level was collected in our study, and not all of the diabetic patients had data for HbA1C. Therefore, the influence of long-term glycemic control on the clinical outcome for CDI could not be adequately evaluated.

In conclusion, for adults with mild-moderate CDI, the treatment success rate of *C. butyricum* alone or in combination with MNZ was non inferior to that of MNZ alone. However, underlying diabetes mellitus in affected individuals is a risk factor for treatment failure.

AUTHOR CONTRIBUTIONS

JCL and YPH initiated the study design. JCL, CWC, and LCC collected clinical data, and CCL and YPH analyzed the data. JCL and YPH wrote the manuscript, and IHH, PJT, and WCK revised and approved the manuscript.

CONFLICTS OF INTEREST

All authors report no conflicts of interest relevant to this article.

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