

# Combination probiotics may prevent *Clostridium difficile* infection among elderly patients undergoing an orthopedic surgery

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The incidence of *Clostridium difficile* infection (CDI) is greater in elderly orthopedic patients. We conducted a retrospective case-control study by selecting elderly patients who underwent proximal femoral fracture surgery to investigate the effect of probiotics on CDI prevention. Cases were diagnosed with CDI by an enzyme-linked immunosorbent assay for *C. difficile* toxins using frozen stool specimens. The primary method of exposure was receipt of combination probiotics such as *Streptococcus faecalis*, *Bacillus mesentericus*, and *Clostridium butyricum*. The crude odds ratio between developing CDI and receiving combination probiotics was 0.074 (95% CI: 0.010–0.565;  $p=0.002$ ). Adjunctive combination probiotics among elderly patients who undergo proximal femoral fracture surgery likely reduces the probability of CDI.

**Key words:** *Clostridium difficile* infection, elderly patient, orthopedic surgery, combination probiotics

An improved understanding of prevention of *Clostridium difficile* infection (CDI) is important among elderly patients undergoing an orthopedic surgery, as CDI is associated with substantial morbidity [1]. We experienced CDI patients at Matsumoto Orthopedic Surgical Hospital with a rate of 4.9 cases per 10,000 patient bed-days in 2015, which is as many as in previous reports such as EUCLID (the European, multicentre, prospective biannual point prevalence study of *Clostridium difficile* Infection in hospitalized patients with Diarrhoea), which reported 7.3 cases per 10,000 patient bed-days in 2013 [2]. The incidence of CDI has been increasing markedly across Europe, North America, and Asia since 2000 [3]. There is moderate evidence concerning the effectiveness of probiotics to prevent CDI [4], but their effect in elderly orthopedic surgical patients has not been investigated.

We conducted a single-center retrospective case-control study by selecting elderly patients who underwent an orthopedic surgery between January 1, 2016, and December 31, 2017, to investigate the effect of combination probiotics on CDI prevention. Cases and controls were patients aged over 75 years who underwent an orthopedic surgery such as osteosynthesis or prosthesis replacement for proximal femoral fracture. Cases and controls required more than 3 days of antibiotic prescription for prophylaxis of surgical

site infection or infection of any causes. The cases had been diagnosed as having antibiotic-induced diarrhea by a physician, and the diagnosis was confirmed by an enzyme-linked immunosorbent assay (*C. DIFF* QUIK CHEK COMPLETE<sup>®</sup>) for glutamate dehydrogenase and *C. difficile* toxins A and B using frozen stool specimens. The primary method of exposure was receipt (vs. nonreceipt) of combination probiotics including *Streptococcus faecalis*  $2 \times 10^8$  CFU/day, *Bacillus mesentericus*  $1 \times 10^7$  CFU/day, and *Clostridium butyricum*  $5 \times 10^7$  CFU/day (Bio-Three tablets<sup>®</sup>, Toa Pharmaceutical Co., Ltd., Tokyo, Japan) for more than 14 days. Potential confounders included age, sex, choice of antibiotics, antibiotic polypharmacy, hypoalbuminemia, anemia, laxative use, and proton pump inhibitor (PPI) use. Crude odds ratios (ORs), numbers needed to treat (NNTs), and 95% confidence intervals (CIs) were calculated. Statistical analysis was performed by  $\chi^2$  test. The ethics committee of Matsumoto Orthopedic Surgical Hospital approved this study, and informed consent was obtained in the form of opt-out via an in-hospital bulletin board.

We enrolled 29 cases and 120 controls during the 24-month study period. There were no differences between cases and controls in age, female ratio, prescription pattern of antibiotics, antibiotic polypharmacy rate, hypoalbuminemia rate, anemia rate, and the rate of PPI use (Table 1). The crude OR between developing CDI and receiving combination probiotics was 0.074 (95% CI: 0.010–0.565;  $p=0.002$ ; Fig. 1). The NNT of combination probiotics for CDI prevention was calculated as 3 (95% CI: 8–3), which was considered as having a clinical benefit. The use of laxatives is thought to be a risk factor for CDI, as the crude OR between developing

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Table 1. Clinical characteristics of cases and controls

	Total (n=149)	Case (n=29)	Control (n=120)	p values
Age (years)	83.3	85.3	83.6	0.367
Female ratio (%)	72.5	65.5	74.2	0.349
Use of cephalosporin (%)	91.9	93.1	91.7	0.799
Antibiotic polypharmacy (%)	12.1	20.7	10.0	0.113
Serum albumin<3.0 g/dl (%)	28.9	31.0	28.3	0.773
Hb<10.0 g/dl (%)	32.9	44.8	30.0	0.127
Use of laxatives (%)	45.0	69.0	39.2	0.004
Use of proton pump inhibitors (%)	18.8	20.7	18.3	0.771
Use of combination probiotics (%)	26.8	3.5	32.5	0.002

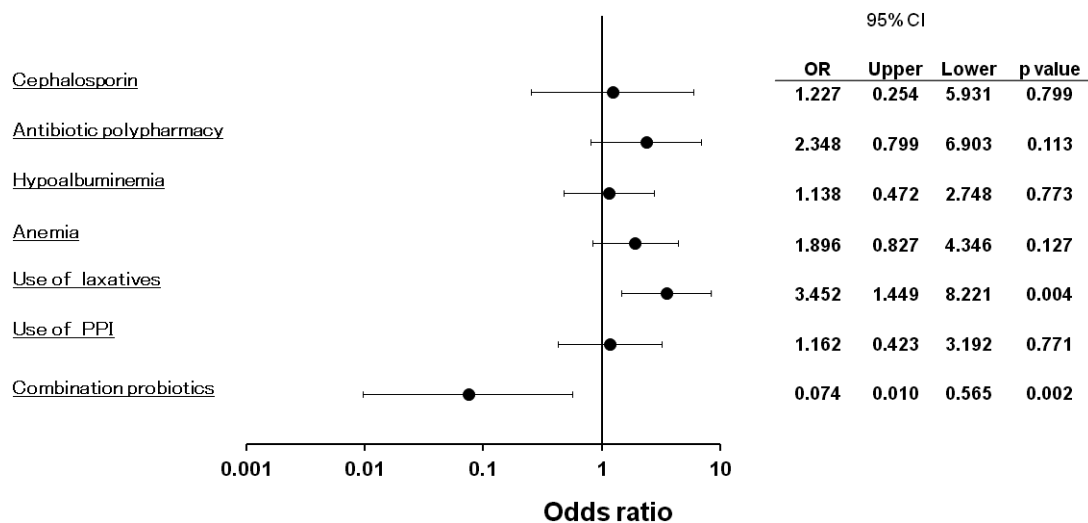


Fig. 1. Odds ratios and 95% confidence intervals for each item.

CDI and receiving laxatives was 3.452 (95% CI: 1.449–8.221;  $p=0.004$ ; Fig. 1). Laxatives used included magnesium oxide, sennoside, and lubiprostone, and there was no difference in prescription patterns between cases and controls.

Antibiotic prophylaxis with cephalosporins can reduce the incidence of deep wound infection in proximal femoral fracture surgery but may increase the risk of CDI [5]. Antibiotic treatment, even in prophylactic uses for surgical site infection, may disturb the resistance of gastrointestinal flora to colonization. *C. difficile* is an anaerobic, gram-positive, spore-forming bacillus that colonizes the intestinal tract after alteration of the normal gastrointestinal flora, usually by antibiotic therapy [6]. CDI is associated with increased cost, morbidity, and mortality in postoperative patients [1]. CDI prevention among orthopedic surgical patients is of extreme importance because prophylactic antibiotic treatment is usually provided after an operation. One potential strategy to prevent this side effect is the concurrent use of probiotic bacteria or yeast [7]. A growing body of evidence has

suggested that probiotics have a role in primary prevention of CDI to restore the balance in the intestinal microbiota [8]. Probiotics are live, nonpathogenic bacteria capable of colonizing the colonic mucosa. All strains used in this study (*Streptococcus faecalis*, *Clostridium butyricum*, and *Bacillus mesentericus*) showed high survivability in the presence of antibiotic agents under an *in vitro* intestinal model [9]. The possible mechanisms to prevent antibiotic-induced diarrhea or CDI include the production of bacteriocins/defensins, competitive inhibition with pathogenic bacteria, inhibition of bacterial adherence or translocation, reduction of luminal pH, and improvement of intestinal barrier function by increasing mucus production [10]. Combination probiotics affect intestinal bacterial flora by increasing anaerobic bacteria and decreasing the population of potentially pathogenic microorganisms, and they strongly inhibit the growth of harmful pathogenic bacteria such as toxigenic *Escherichia coli* [11]. In an *in vitro* study, combination probiotics appeared to stimulate the Th1 immune response, downregulate pro-

inflammatory cytokines such as tumor necrosis factor alpha and upregulate anti-inflammatory cytokine interleukin-10, resulting in improvement of immune balance [12]. Therefore, combination probiotics may have the potential to reduce the risk of CDI by improving intestinal flora and host immune function.

Non-antibiotic prescription drugs also have a notable impact on the overall architecture of the intestinal microbiome. For example, the use of PPI is linked to a decrease in *Clostridiales* and increase in *Actinomycetales*, *Micrococcaceae*, and *Streptococcaceae*, which are increased susceptibility to CDI [13]. However, PPI use was not associated with CDI in this study. On the contrary, the use of laxatives increased the risk of CDI. Laxatives themselves or chronic constipation appeared to be associated with striking changes in the colonic flora, intestinal permeability, and the systemic immune response [14]. Disturbances in bowel function in patients treated with laxatives might lead to disordered immunity and to decreased resistance to pathogenic flora.

In conclusion, treatment of elderly patients with combination antibiotics after they undergo orthopedic surgery likely reduces the probability of CDI; however, the current evidence remains limited due to variable outcome measures. Systematic reviews have provided evidence for the efficacy of probiotics in preventing CDI [15, 16], but current guidelines do not recommend probiotic use for prevention of CDI [17]. One possible reason for the lack of recommendation is that the optimal probiotic dose, species, and formulation remain unknown [17]. However, combination probiotics may mitigate the CDI burden. Additional high-quality studies are needed to establish appropriate recommendations.

#### Disclosure Statement

Drs Nagamine, Matsumoto, and Nakamura contributed to the concept/design. Drs Nagamine and Matsumoto contributed to the data acquisition and the analysis/data interpretation. Drs Nagamine and Nakamura drafted this article. No funding was received for the preparation of this article. The authors have no conflicts of interest relevant to the content of the article. This content has not been published or submitted for publication elsewhere.

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