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Research Paper

Effect of probiotic supplementation on Clostridioides difficile stool concentration in hospitalized patients in coma: A preliminary study

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Abstract

Introduction: Clostridioides difficile (CD) is a major healthcare-associated pathogen responsible for antibiotic-associated diarrhea and severe colitis. Probiotics have been proposed as a preventive strategy. However, data on critically ill patients remain limited.

Aim: To evaluate the effect of probiotic supplementation on CD stool concentration in patients hospitalized in coma.

Material and methods: This prospective, randomized preliminary study was conducted from May to August 2023 at the Department of Medical Rehabilitation, Coma Ward (pol. *Budzik dla Dorosłych*). Fourteen patients in coma were randomized to receive either a new probiotic mixture (Labifid®, containing *Bifidobacterium animalis* AMT30 and *Bifidobacterium breve* AMT32) or standard hospital probiotics (*Lactobacillus rhamnosus*, *Saccharomyces boulardii*). Stool samples were collected at baseline, 1, 2, and 4 months afterwards.

Results and discussion: Baseline CD levels were comparable between groups (2.1×10^5 vs. 2.2×10^6 CFU/g; $p = 0.607$). At 1, 2, and 4 months, patients receiving the new probiotics demonstrated significantly lower CD concentrations than controls ($p = 0.032$, $p = 0.017$, and $p = 0.029$, respectively). After 4 months, CD levels in the new-probiotic group fell below the detection limit. No serious adverse events were observed.

Conclusions: Supplementation with *Bifidobacterium animalis* and *Bifidobacterium breve* was associated with a significant reduction in CD stool concentration in comatose patients. However, due to the small sample size, it should be interpreted with caution.

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1. INTRODUCTION

Inflammatory bowel diseases can arise from various causes, including infectious inflammation by bacteria, viruses, fungi, and protozoa. Among bacterial agents, Clostridioides difficile (CD) is a Gram-positive, anaerobic bacterium, widely recognized as the most common cause of antibiotic-associated diarrhea and a key healthcare-associated pathogen.[1] Its virulence is largely attributed to spore formation, which grants resistance to harsh environmental conditions, complicates decontamination in healthcare settings, and permits long-distance transmission.[1]

The principal virulence factors are toxins A and B. Toxin B plays a more crucial role in disrupting tight junctions of the intestinal epithelium, triggering inflammation and cytotoxicity.[1,2] They can cause inflammation and mucosal injury, ultimately leading to symptoms that range from mild diarrhea to fulminant colitis, bowel perforation, or septic shock.[3]

Disruption of the gut microbiota, most commonly via antibiotic use, predisposes to CD infection.[4,5] Additional risk factors include advanced age, comorbidities, immunosuppression, hospitalization (especially in intensive care units), residency in long-term care, intestinal stasis, presence of catheters, enteral or parenteral nutrition, prior chemotherapy or radiotherapy, and abdominal surgery.[6]

CD infection is a clinically significant pathogen due to its strong association with healthcare settings, potential for recurrence, and increasing frequency of severe, treatment-resistant disease, all of which heighten patient suffering, increase healthcare costs, strain healthcare systems, and elevate patient mortality.[1,7] However, there is a clear deficiency in the existing literature concerning the use of probiotics in comatose or critically ill populations. Data on strain-specific effects, optimal dosing, and clinical outcomes in this vulnerable group remain scarce, underscoring the need for further research.

2. AIM

The aim of this study was to assess the effect of probiotic administration on the concentration of CD in the stool of patients hospitalized due to coma. Specifically, the study compared changes in CD levels between a probiotic-treated group and a control group over a four-month observation period.

3. MATERIAL AND METHODS

This study is an initial, prospective, randomized investigation assessing the effect of probiotics on the concentration of CD in stool. The study was conducted from May to August 2023 at the Department of Medical Rehabilitation Coma Ward (pol. *Budzik dla Dorosłych*). Inclusion criteria were patients treated at the Coma Ward during the study period, regardless of the cause of admission.

Exclusion criteria included symptomatic CD infection at the time of enrollment and lack of consent from the legal guardian for participation in the study.

Patients were randomly assigned to two groups: those receiving the newly studied probiotics and those receiving the standard probiotics administered in the hospital according to regulations. Patients in the first group received Labifid® at a dose of two sachets twice daily, suspended and administered via PEG. Each sachet contained *Bifidobacterium animalis* AMT30 with 1.5×10^{10} Colony Forming Units per gram (CFU/g) and *Bifidobacterium breve* AMT32 with 5×10^{9} CFU/g). Patients in the standard group received Lakcid® Forte containing *Lactobacillus rhamnosus* and Enterol® with *Saccharomyces boulardii* CNCM I-745, Figure 1.

Stool samples were collected immediately before starting probiotics, and then at one, two, and four months after initiation. They were tested for the presence of CD using classical microbiological methods, applying serial dilution on

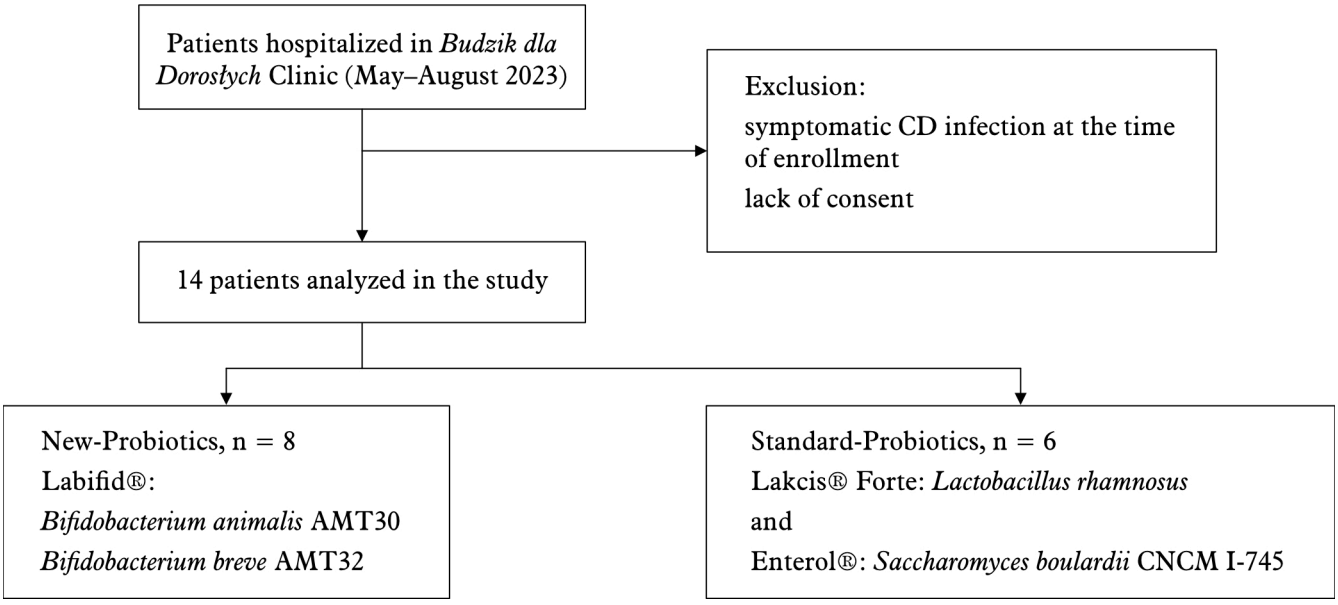

Figure 1. Flowchart of the study.

selective chromogenic media to determine the number of pathogens per 1 g of sample. Positive results were further analyzed molecularly using real-time PCR to confirm the presence of the pathogen and to detect toxins A and B. Genetic material was isolated with the Qiagen QIAamp Fast DNA Stool Mini Kit. Molecular analyses were performed using the VIASURE CD Real Time PCR Detection Kit and the VIASURE CD Toxins A/B Real Time PCR Detection Kit. Additionally, the patient database included information on age, cause of coma, and clinical outcomes.

3.1. Statistical analysis

All analyses were performed using Statistica version 13.PL (StatSoft Inc.). Descriptive statistics were used to summarize the data. The Shapiro-Wilk test was employed to assess the normality of continuous variables. Since most data did not follow a normal distribution, results for continuous variables are expressed as medians with interquartile ranges (IQR). The Mann–Whitney U test was used to compare differences between two independent groups. For categorical variables, comparisons were made using the chi-square test or Fisher's exact test, depending on the data distribution. A p-value of ≤0.05 was considered indicative of statistical significance.

3.2. Ethical considerations

The data were anonymized. The study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments. The study was approved by The Bioethics Committee of the University of Warmia and Mazury in Olsztyn (24/2022).

4. RESULTS

A total of 14 patients were included in the study: 8 in the new-probiotic group and 6 in the standard-probiotic group. The median age was comparable between the groups (26.0 vs. 29.0 respectively, $p = 0.897$), Table 2. The most common cause of admission in the probiotic group was traumatic brain injury (62.5%) and cardiac arrest (50.0%) in the standard-probiotic group ($p = 0.297$). No statistically significant differences were found between groups regarding the discharge ($p = 0.062$).

Analysis of CD concentration in stool showed no significant difference between groups at baseline (2.1×10^5 vs. 2.2×10^5, respectively, $p = 0.607$), Table 2. After one, two, and four months, the CD concentration was significantly lower in the new-probiotic group compared to the standard-probiotic group ($p = 0.032$, $p = 0.017$, $p = 0.029$), Figure 2.

5. DISCUSSION

In our study administration of probiotics was associated with a significant reduction in CD concentration in stool compared to the control group. The difference between groups was statistically significant. In the new-probiotic group after four months the concentration of CD was below the limit of detection. These results suggest a potential early suppressive effect of probiotics on CD colonization or proliferation, which may be beneficial in the management of patients with CD infections, including those in critical condition requiring continuous care.

Table 1. Characteristics of patients.

	New-probiotics, n = 8	Standard-probiotics, n = 6	P-value
Age	26.0 (22.0–39.5)	29.0 (25.0–38.0)	0.897
The reason of admission			
Traumatic brain injury	5 (62.5%)	2 (33.3%)	0.297
Cardiac arrest	3 (37.5%)	3 (50.0%)	
Status epilepticus	0	1 (16.6%)	
Discharge status			
Discharged home in a coma	7 (87.5%)	2 (33.3%)	0.062
Emerged from coma	1 (22.5%)	1 (16.6%)	
Death	0	3 (50.0%)	

Table 2. The quantity of CD in stool.

Variable, median (IQR)	New-probiotics, n = 8	Standard-probiotics, n = 6	P-value
Before	2.1×10^5 (1.0×10^2–9.6×10^5)	2.2×10^6 (1.0–8.6×10^6)	0.607
After 1 month	7×10^4 (1.0×10^4–3.9×10^5)	5.8×10^6 (1.2×10^6–7.4×10^6)	0.032
After 2 months	2.7×10^5 (1.0–9.4×10^5)	9.0×10^7 (3.5×10^6–8.6×10^7)	0.017
After 4 months	1.0 (1.0–1.0)	2.0×10^6 (6.0×10^4–2.2×10^6)	0.029

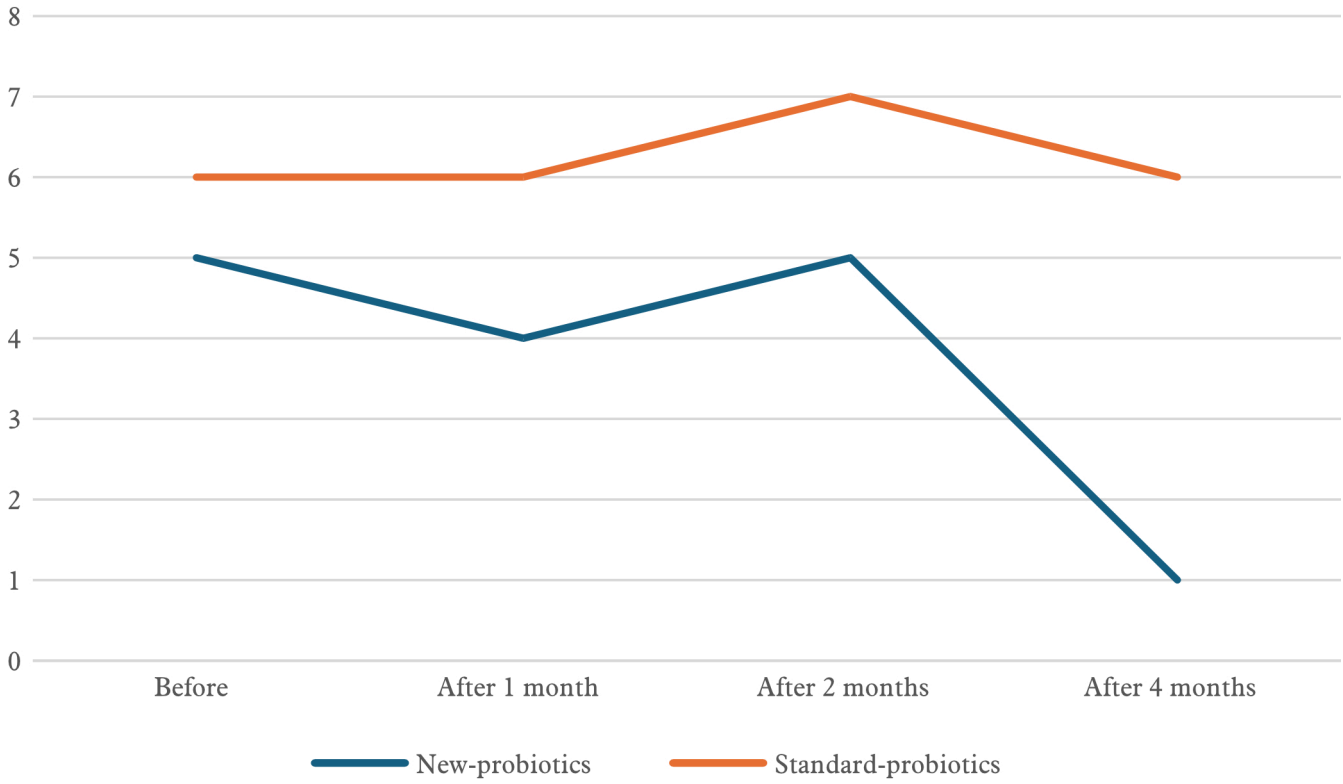

Figure 2. Median orders of magnitude of Clostridium difficile counts (CFU/g of stool) in new-probiotics and standard-probiotics group.

Our results are consistent with the in vitro study of Valdés-Varela et al.[8] They demonstrated that *Bifidobacterium breve* and *Bifidobacterium longum* significantly suppressed CD growth and toxin production, especially when co-cultured with short chain fructooligosaccharides.[8] In our study, a similar inhibitory effect was observed in vivo after administration of a probiotic mixture containing *Bifidobacterium breve* and *Bifidobacterium animalis*, despite the absence of added prebiotics. This suggests that either strain-specific properties or potential synergistic interactions may contribute to CD suppression in human patients. Animal models also confirm the potential of *Bifidobacterium* strains to reduce CD colonization and toxicity. Trejo et al. demonstrated that administration of *Bifidobacterium bifidum* CIDCA 5310 significantly decreased the incidence of enterocolitis and mortality in a hamster model of CD infection.[9]

A meta-analysis of 31 randomized controlled trials with 8,672 patients provides moderate-certainty evidence that probiotics effectively prevent CD-associated diarrhea.[10] Adverse events of CD infection were generally more frequent in control groups. Goldenberg et al. indicated that probiotics are safe when used short-term alongside antibiotics in non-immunocompromised patients.[10] It supports considering probiotics to prevent diarrhea and its complications in high-risk hospitalized patients. Similar conclusions can be found in another study.[11]

McFarland et al. demonstrated the effectiveness of probiotics in the primary prevention of CD infections.[12] However, their impact may be limited in cases of recurrent infections. In recurrent infections, the mechanisms are more complex, often involving the persistence of bacterial spores, treatment resistance, and lasting disturbances of the gut microbiota that probiotics alone are unable to adequately restore. Saltzman et al. reached different conclusions in their retrospective analysis.[13] According to their study, patients who received probiotics within 24 hours of initiating antibiotic therapy had a twofold higher risk of developing CD infection. Despite attempts to adjust for confounding variables, the authors pointed out the possibility of indication bias, as sicker patients were more likely to be prescribed probiotics.[13] The authors also suggested that administration of inappropriate probiotic strains might further disrupt the gut microbiota, potentially contributing to increased susceptibility.

Allen et al., in a large, randomized placebo-controlled trial involving patients over 65 years of age, found no evidence that probiotic administration was effective in preventing antibiotic-associated diarrhea in the elderly, although there was a trend toward reduced CD diarrhea in the probiotic group.[14] Which is also consistent with the findings of our study.

The observed reduction in CD levels following administration of *Bifidobacterium animalis* and *Bifidobacterium breve* can be attributed to several interconnected mechanisms. Firstly, these probiotic strains are known to strengthen the gut barrier function by enhancing the integrity of the intestinal epithelium, thereby preventing pathogen translocation

and reducing inflammation.[15] Moreover, they modulate host immune responses by promoting anti-inflammatory cytokines and stimulating the production of immunoglobulin A.[16] It may contribute to mucosal immunity against pathogens like CD. Sanchez et al demonstrated that *Bifidobacterium* species produce short-chain fatty acids such as acetate and lactate, which lower the gut pH and create an inhospitable environment for CD growth and toxin production.[17] Moreover, some metabolites produced by *Bifidobacterium* strains can directly inhibit pathogen adherence and biofilm formation, further limiting CD colonization.[18] Collectively, these actions may contribute to the reduction in CD colonization and toxicity observed in probiotic-treated hosts. There is growing evidence that modulation of the gut microbiota may have systemic therapeutic effects or, conversely, contribute to disease development.[19,20] Therefore, studies on the microbiota have become an important part of modern biomedical research.

5.1. Strengths of the study

A major strength of this study is its integration with relevant prior literature and biological evidence. By linking the experimental data with clinical observations in a unique population of comatose patients, this study bridges the gap between preclinical and clinical research. Moreover, it provides one of the first prospective randomized insights into probiotic supplementation in critically ill or comatose individuals.

5.2. Limitations of the study and future directions

This study has several limitations. The small sample size limits the generalizability of the findings and may reduce statistical power. Moreover, we did not assess changes in the overall gut microbiota composition. In our study, no clinical signs of CD infection were monitored, so the clinical relevance of decreased CD levels remains uncertain. Despite these limitations, our preliminary findings support the potential role of specific probiotics in modulating CD levels in high-risk populations, such as patients in coma. Larger, multicenter randomized controlled trials are warranted to confirm these effects and explore clinical outcomes such as infection rates, symptom severity, and recurrence. The use of quantitative microbiological and molecular methods for pathogen assessment further strengthens the reliability of the results.

6. CONCLUSIONS

Bifidobacterium animalis and *Bifidobacterium breve* may significantly reduce CD concentration in the stool of patients in coma, particularly in the early phase of intervention. Although the clinical significance requires further investigation, these findings support the potential of targeted probiotics as a preventive strategy in high-risk hospitalized populations.

Ethics approval

The data were anonymized. The study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments. The study was approved by The Bioethics Committee of the University of Warmia and Mazury in Olsztyn (24/2022).

Conflict of interest

E.S.-T. was employed by Probios Ltd., which provided the probiotics used in the study.

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None declared.

Author Contributions

Study design: ES-T, MG, PS
Data collection: ES-T, MG, EZ, PS
Statistical analysis: ND-G
Data interpretation: ND-G
Manuscript preparation: ND-G, AB
Literature search: ND-G, AB
Funds collection: ES-T

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