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Stability of probiotics with antibiotics via gastric tube by simple suspension method: An *in vitro* study☆

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ABSTRACT

Data on the stability of probiotics with antibiotics delivered via gastric tube using the simple suspension method (SSM) are limited. Therefore, we investigated bacterial survivability in probiotics treated with antibiotics prepared by the SSM *in vitro*. Probiotics and antibiotics were suspended in 20 mL of sterilized hot water (55 °C) and then 1-mL of the suspensions were taken each at 10, 60, 120, 180 and 360 min. Thereafter, the samples were inoculated on 3 media and cultured at 37 °C for 24 h. Survival of probiotic strains was measured in colony-forming units. The growth of *Clostridium butyricum* did not change without antibiotics at all experimental times, but in the case of *Enterococcus faecium* tended to increase. On the other hand, the viable bacterial number of *C. butyricum* was decreased significantly by treatment with cefdinir, tosufloxacin, clarithromycin, or azithromycin, but was not altered by levofloxacin, minocycline, or vancomycin. The viable bacterial number of *E. faecium* was significantly decreased by treatment with tosufloxacin, levofloxacin, minocycline, vancomycin, or azithromycin, and was significantly increased by clarithromycin. In conclusion, our results suggest that the efficacy of probiotic therapies might be reduced by the SSM when specific antibiotics are used. Moreover, antibiotics might inhibit probiotic growth, although some probiotics are spore-forming and have high minimum inhibitory concentrations. Additionally, early administration of non-spore-forming bacteria might be desirable. Therefore, when patients are administered therapy combining probiotics and antibiotics by the SSM, we should consider the characteristics of the probiotics and the administration times.

Antibiotics are used for 30% of patients during hospitalization [1]. As a result, antibiotic-associated diarrhea (AAD) has become common and there are growing concerns about increasing durations of hospitalization and costs [2]. Hence, the prevention and treatment of AAD in clinical settings is important. However, antibiotics often cannot be stopped in immunocompromised patients such as those with leukemia. Therefore, probiotics are generally antibiotic-resistant bacterial strains that can be taken at the same time as antibiotics, and a meta-analysis found that they are effective for the prevention of AAD [2]. Also, critically ill patients are often administered these medicines via gastric tube [3].

The simple suspension method (SSM) was developed for gastric tube administration, which involves disintegrating tablets and capsules in 20 mL hot water (55 °C) for 5 min without grinding them. The medicines can then be administered via gastric tube [4,5]. However, when patients are administered probiotics and antibiotics concomitantly by the SSM, the probiotics are exposed to high concentrations of antibiotics because the solution only contains 20 mL water. Moreover, in Japanese hospitals, it can take 10–180 min to administer the drugs prepared via the SSM because the nurses are busy [6]. In these situations, probiotic strains might not be able to proliferate in the gastrointestinal tract. Hence, we hypothesize that therapy consisting of both probiotics and antibiotics prepared by the SSM might reduce the therapeutic effects against AAD. Hence, we performed an *in vitro* study of probiotics and antibiotics prepared by the SSM.

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Two probiotic medicines were used in this study, *Clostridium butyricum* (Miya-BM fine granules 1 g; Miyarisan Pharmaceutical Co., Ltd., Tokyo, Japan) and antibiotic-resistant *Enterococcus faecium* (BIOFERMIN-R powder 1 g; Biofermin Pharmaceutical Co., Ltd., Kobe, Japan). *C. butyricum* was cultured anaerobically in modified GAM agar medium (Nissui Pharmaceutical, Tokyo, Japan). *E. faecium* was cultured aerobically in EF agar medium (Nissui Pharmaceutical). Culture was performed at 37 °C. Antibiotics were used as follows in this study: 50 mg of cefdinir 10% granules (Astellas Pharmaceutical Co., Ltd., Tokyo, Japan), 150-mg tosufloxacin tablets (Taisho-Toyama Pharmaceutical Co., Ltd., Tokyo, Japan), 500-mg levofloxacin tablets (Daiichi-Sankyo Pharmaceutical Co., Ltd., Tokyo, Japan), 100-mg minocycline capsules (Pfizer Pharmaceutical Co., Ltd., Tokyo, Japan), 500-mg of vancomycin powder (Pfizer Pharmaceutical Co., Ltd.), 200-mg clarithromycin tablets (Mylan Pharmaceuticals Co., Ltd., Tokyo, Japan), and 250-mg azithromycin tablets (Pfizer Pharmaceutical Co., Ltd.).

Probiotics and antibiotics were placed together in 20 mL of sterilized hot water (55 °C) and suspended. Thereafter, 1-mL samples of the solutions were obtained at 10, 60, 120, 180, and 360 min. Sampling times were set in reference to administration times by nurses [6]. To remove the antibiotics, 1-mL samples were added to 4 mL of sterilized water, centrifuged at 15000 rpm for 1 min, and the supernatants were removed. To reduce the concentrations of the antibiotics after centrifugation, each 0.05-mL sample was diluted 100-fold by adding 4.95 mL of sterilized water and 0.1-mL

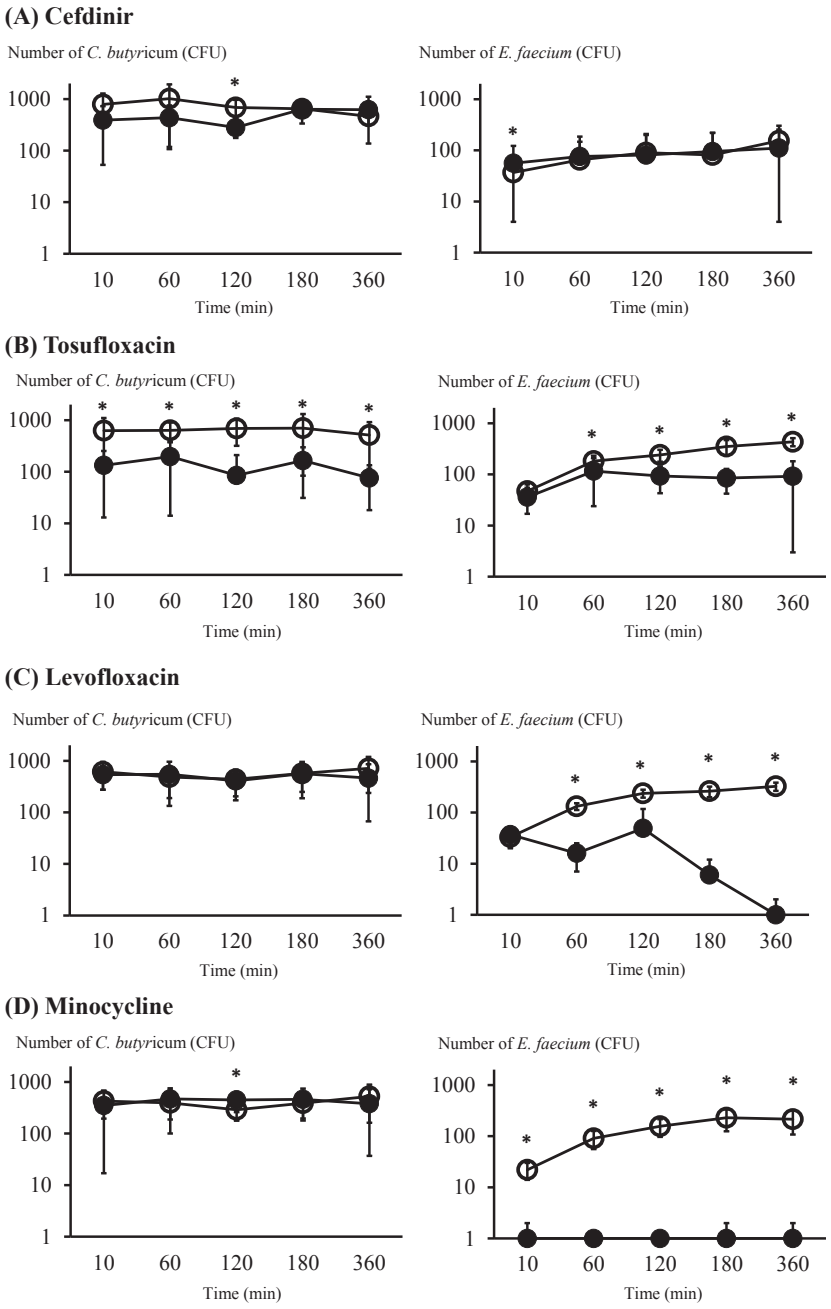

Fig. 1. Changing the viable bacterial number of *Clostridium butyricum* and *Enterococcus faecium*. Data are shown as the mean ± standard deviation ($N = 3$). Closed circles, antibiotic; open circles, control. *Welch's t-test ($P < 0.05$), control versus treatment with antibiotics.

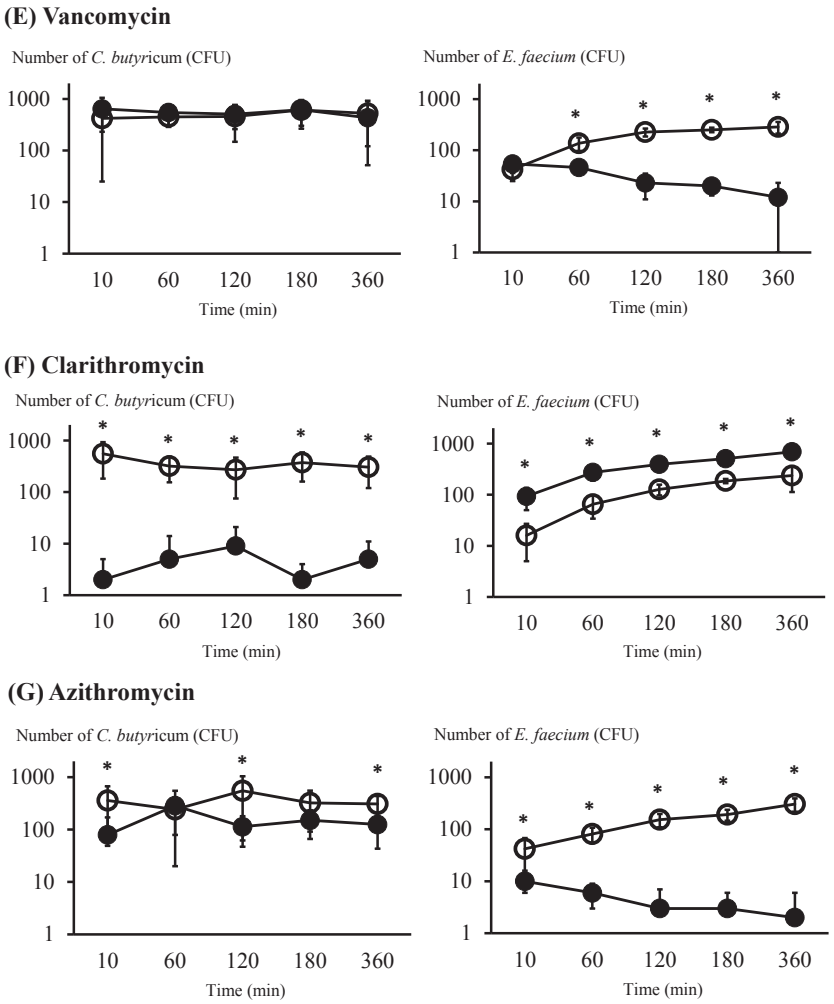

Fig. 1. (*continued*).

samples were each seeded in 3 media. After 24 h of culture, the probiotic strains were measured using the colony-forming unit (CFU) method from the mean of the 3 media. For each experiment, three replicates were performed, each on a different day, and the mean CFU and standard deviation of the three replicates were calculated for each experiment. For controls, we conducted the same experiment without antibiotics. Variables are reported as the mean and standard deviation. Statistical analysis was performed by Welch's *t*-test using Microsoft Excel 2007 compared differences between control and treatment with antibiotics at each time point. $P < 0.05$ was considered statistically significant.

Changes found among the viable bacterial number of the probiotics are shown in Fig. 1. The viable bacterial number of the *C. butyricum* controls did not change at any time, but those of *E. faecium* tended to increase over time. On the other hand, the viable bacterial number of *C. butyricum* was significantly decreased by mixing with cefdinir, tosufloxacin, clarithromycin, or azithromycin and remained unchanged by levofloxacin, minocycline, or vancomycin. The viable bacterial number of *E. faecium* was significantly decreased by mixing with tosufloxacin, levofloxacin, minocycline, vancomycin, or azithromycin, and was significantly increased by clarithromycin. At 120 min, the viable bacterial number of *C. butyricum* mixed with minocycline had increased significantly compared with the control (CFUs: 449 ± 189 vs 289 ± 112), while at 10 min, the viable bacterial number of *E. faecium* mixed with cefdinir had increased significantly compared with the control (CFUs: 66 ± 39 vs 33 ± 6). At 10 min, the viable bacterial number of *E. faecium* among the controls and with each individual antibiotic were: 33 ± 6 versus 66 ± 39 (cefdinir); 47 ± 17 versus 36 ± 19 (tosufloxacin); 33 ± 13 versus 37 ± 15 (levofloxacin); 22 ± 8 versus 0 ± 1 (minocycline); 42 ± 17 versus 54 ± 18 (vancomycin); 16 ± 11 versus 93 ± 43 (clarithromycin); and 42 ± 26 versus 10 ± 4 CFU (azithromycin).

Our results indicated that some mixtures of antibiotics and probiotics decreased the viable bacterial number. Specifically, *C. butyricum* did not tolerate being mixed with cefdinir, tosufloxacin, clarithromycin, or azithromycin. Moreover, *E. faecium* did not tolerate tosufloxacin, levofloxacin, minocycline, vancomycin, or azithromycin. These results suggest that the therapeutic effects against AAD may be reduced by combination therapies containing probiotics and antibiotics prepared by the SSM.

The viable bacterial number of the *C. butyricum* control did not change over time, while that of *E. faecium* tended to increase over time. *C. butyricum* is a spore-forming bacterium, and thus might not be able to germinate in water without nutrition. On the other hand, because *E. faecium* is a non-spore-forming bacterium, it might be able to germinate in water.

The minimum inhibitory concentrations (MICs) for *C. butyricum* are as follows: cefepime, 64 μg/mL; ceftazidime, 128 μg/mL; tosufloxacin, 0.25 μg/mL; levofloxacin, 1 μg/mL; vancomycin, 0.5 μg/mL;

and clarithromycin, <0.0625 μg/mL [7]. The probiotics and antibiotics are mixed in just 20 mL of water when preparing the SSM; thus, the probiotics are exposed to high concentrations of antibiotics at greater levels than the MICs. We predicted that the viable bacterial number of *C. butyricum* would not decrease in the presence of any of the antibiotics because *C. butyricum* is a spore-forming bacterium. However, in this study, only levofloxacin, minocycline, and vancomycin did not reduce the viable bacterial number of *C. butyricum*. Therefore, these antibiotics might be suitable for administration by gastric tube using the SSM. The reasons behind the inhibition of *C. butyricum* growth in the presence of some antibiotics remain unclear and could not be explained by antibiotic MICs, properties such as lipophilicity, or the pH of the solution. Germination might be inhibited by antibiotics adhering to the spores.

The MICs for *E. faecium* are as follows: cefepime and ceftazidime, >1024 μg/mL; tosufloxacin, 1 μg/mL; levofloxacin, 4 μg/mL; vancomycin, 0.5 μg/mL; and clarithromycin, >512 μg/mL [7]. In this study, the viable bacterial number of *E. faecium* decreased significantly when the bacteria were mixed with each of the antibiotics except cefdinir and clarithromycin. *E. faecium* is a non-spore-forming bacterium; thus, it might have had a greater effect than did *C. butyricum*. However, 10 min after the suspensions were prepared, the viable bacterial number were decreased in the presence of only minocycline and azithromycin. Hence, cefdinir, tosufloxacin, levofloxacin, vancomycin, and clarithromycin might be suitable for administration via gastric tube using the SSM if they are administered concomitantly with *E. faecium* within 10 min of the mixture being prepared. On the other hand, the viable bacterial number of *E. faecium* was greater than that of the control when the bacteria were mixed with clarithromycin. We suggest the following reasons: the MIC of clarithromycin for *E. faecium* may be high and the excipients in clarithromycin may have provided rich nutrition for *E. faecium*.

Our study has some limitations. First, it was an *in vitro* study. Probiotics might be able to grow in the bowel even if the viable bacterial number of probiotics is decreased by antibiotics before administration. Also, some antibiotics such as clarithromycin have reduced antibiotic activity due to changing pH levels [8]; however, since the effects of gastric acid were not considered in this study. Therefore, the clinical impact is unclear. Second, we attempted to reduce the effects of the antibiotics on the probiotics by centrifugation and dilution prior to culture; however, traces of the antibiotics might have remained in the solutions and inhibited the growth of the probiotics. Also, this method is not standardized. Nevertheless, we considered this method to be appropriate because of the difference in mass between the bacteria and antibiotics. In addition, it is unclear whether antibiotics inhibit the growth of spore-forming probiotics.

In conclusion, our results suggest that the efficacy of probiotic therapy might be reduced by using the SSM when specific antibiotics are used. Moreover, some antibiotics might inhibit the growth of probiotics, even if the probiotics are spore-forming and the MIC values are high. Additionally, early administration of live bacteria might be desirable. Therefore, for *C. butyricum*, levofloxacin, minocycline, and vancomycin might be suitable for administration by gastric tube using the SSM. For *E. faecium*, cefdinir and chlarithromycin might be suitable for administration by gastric tube using the SSM. Tosufloxacin, levofloxacin, and vancomycin might also be suitable for administration via gastric tube using the SSM if they are administered concomitantly with *E. faecium* within 10 min of the mixture being prepared. Finally, when patients are administered therapy combining probiotics and antibiotics prepared using an SSM, we should consider the characteristics of the probiotics and the administration time.

Conflict of interest

The authors declare that they have no competing interests.

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