Decrease in Frequency of Liquid Stool in Enterally Fed Critically Ill Patients Given the Multispecies Probiotic VSL#3: A Pilot Trial

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Background Diarrhea has adverse consequences for critically ill patients, health care staff, and health care costs.
Objective To evaluate the efficacy of the multispecies probiotic VSL#3 in reducing the mean number of episodes of liquid stool in enterally fed critically ill patients.
Methods A single-center, double-blind, randomized, placebo-controlled pilot study was done in a 6-bed intensive care unit in a 330-bed public hospital in Australia. A total of 45 adults (20 intervention, 25 control) who required enteral nutrition for more than 72 hours were given VSL#3 or a placebo twice daily. The frequency (mean number of episodes per patient per day) and weight (grams per day) were determined for both liquid stool and liquid and loose (unformed) stool.
Results The 2 groups of patients had no demographic or clinical differences. Patients received enteral nutrition for a mean of 8.5 days (SD, 5.4) and were studied for a mean of 11.9 days (SD, 5.6). Compared with the control group, the intervention group had a significant reduction in the frequency of liquid stools (incidence rate ratio, 0.50; 95% confidence interval, 0.27 to 0.93; $P = .03$). Smaller but still significant differences also occurred between the groups in both the frequency of episodes and the weight of liquid and loose (unformed) stool.
Conclusion VSL#3 was effective in reducing the frequency of liquid stool in critically ill patients receiving enteral nutrition. Probiotics possibly can minimize diarrhea in critically ill tube-fed patients, but more controlled clinical trials are needed. (*American Journal of Critical Care.* 2010;19:e1-e11)

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Diarrhea is relatively common in the intensive care unit (ICU), with a reported frequency of 15%[1] to 63%[2] in enterally fed critically ill patients. Diarrhea can prolong hospitalization and increase mortality.[1] Despite little evidence, some investigators suggest it may also increase nursing workload[3] and hospital costs,[4,5] and it can be upsetting for patients.[6]

Probiotics are therapeutic preparations of live microorganisms administered in sufficient dosage to be beneficial to health.[7] The therapeutic effects of these microorganisms appear to be strain specific.[8] Thus, research is needed to determine which probiotic strains are useful in the treatment of specific gastrointestinal health problems.

The mechanism of action of probiotics has not been fully elucidated. They may exert their effects through immunomodulation,[7,9] by suppressing the growth of enteropathogens by producing bacteriocins,[10] or by competition for intestinal wall adhesion sites and nutrients.[11] Although widely used by predominantly healthy persons, probiotics are increasingly being used to prevent and treat some diarrheal illnesses, including acute infectious diarrhea,[12-14] traveler's diarrhea,[15] diarrhea related to food allergies,[16] diarrhea associated with radiotherapy,[17] and some intestinal inflammatory diseases.[18-21]

Positive outcomes have also been reported for the probiotic treatment of diarrhea associated with use of antibiotics[22,23] and diarrhea caused by *Clostridium difficile*.[24,25] However, despite the potential of probiotics, some studies[26,27] have indicated that the agents are of little or no benefit in the treatment of myriad health conditions. Previous meta-analyses[22,28,29] indicated that evidence to determine the efficacy or safe use of probiotics to prevent diarrhea is incomplete. Variations in estimates of the efficacy of probiotics in the treatment and prevention of diarrhea may be due to the different probiotic preparations used[29] and to potential differences in the efficacy of probiotics in specific diseases and clinical circumstances. The number of cases in double-blind, randomized, controlled trials has been insufficient for estimates of the efficacy of specific probiotic strains and of the dosages in specific health disorders.

Diarrhea can prolong hospitalization, increase patient mortality, and increase nursing workload and hospital costs.

Regardless of its etiology, diarrhea is a common problem in ICU patients receiving enteral nutrition. Many causes, presumably most of which deplete the numbers of normal intestinal flora, have been implicated in the pathogenesis of diarrhea related to enteral feeding, including antibiotic therapy,[30] various alterations in intestinal microbiota due to intragastric feeding,[31] infection by enteropathogens,[32] colonization with *C difficile*,[33] contaminated enteral feeding preparations and delivery systems,[33,34] route of enteral feeding,[33] osmotically active medications,[35] low serum levels of albumin,[36] and impairment of colonic secretion of water and electrolytes.[37]

A recent study[32] indicated that great variability in intestinal flora occurs in patients who have diarrhea during enteral feeding. Compared with patients without diarrhea, those with diarrhea had higher counts of clostridia and lower counts of bifidobacteria. Whatever the cause of diarrhea, replacement of depleted intestinal microorganisms with viable probiotics may be beneficial in maintaining favorable microflora and a homeostatic environment in the gastrointestinal tract. Maintenance of resident flora in patients who are enterally fed might increase antimicrobial[32] and immunological activity,[8] suppress the colonization of enteropathogens,[38] and assist in the colonic reabsorption of water by stimulating the production of short-chain fatty acids.[39]

Probiotics are therapeutic preparations of live microorganisms that benefit intestinal health.

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Only a few studies have addressed the therapeutic use of probiotics to prevent diarrhea related to enteral feeding. Investigators in a multicentered, randomized, controlled trial (RCT)[30] found that the nonpathogenic yeast *Saccharomyces boulardii* reduced the number of days a patient had diarrhea by 25%. In a relatively recent RCT[40] involving 28 critically ill patients, the probiotic preparation VSL#3 modulated intestinal permeability and improved immune function but did not significantly alter the incidence of diarrhea between the patient groups. Similarly, in an earlier RCT[41] involving critically ill patients, *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* did not alter the risk of diarrhea.

Probiotics can enhance the immune system, improve the integrity of the mucosal barrier, actively colonize the intestinal tract, and inhibit the growth of enteropathogens.

On the basis of the beneficial effects of probiotics in other populations of patients and in healthy individuals and the theorized mechanism of action via the restoration of favorable gut flora, we hypothesized that therapeutic administration of a probiotic preparation might also have benefits in critically ill patients. Importantly, the current uncertainty concerning probiotic effectiveness[30,40,41] made further investigation desirable.

The aim of the randomized, double-blind, placebo-controlled pilot study reported here was to estimate the prophylactic ability of the multispecies probiotic VSL#3 to decrease the frequency (mean number of episodes per patient per day) of liquid stool in critically ill ICU patients receiving enteral nutrition.

Few clinical studies have addressed the prophylactic use of probiotics in the treatment of diarrhea associated with enteral feeding.

Materials and Methods

This single-site, double-blind, randomized, placebo-controlled pilot study was conducted at Launceston General Hospital, a 330-bed, university-affiliated hospital in Tasmania, Australia. The hospital is a major gastrointestinal surgery referral center and accepts patients with major trauma, with the exception of patients with neurotrauma.

The study was done in the 6-bed general ICU between July 2005 and October 2006. The ICU admits approximately 500 patients per year, and about 60% to 75% receive mechanical ventilation. The unit accommodates both high-acuity patients treated with mechanical ventilation who require complex organ support and lower acuity high-dependency patients who require perioperative care. Patients with all forms of acute illnesses, except postoperative cardiothoracic and acute neurosurgical problems, are treated. The ICU is run as a "closed unit"; admission and treatment rights are limited to staff intensivists. Care was provided 24 h/d by an intensivist in conjunction with full-time junior medical staff (residents or registrars). This study was approved by the Human Research Ethics Committee (Tasmania) Network and the Griffith University Human Research Ethics Committee and was registered with the Australian Clinical Trials Registry. Written informed consent was obtained from patients or their next of kin.

The primary outcome was frequency of liquid stool (mean number of episodes per patient per day), because liquid stool is the type most closely associated with the term diarrhea. Secondary outcomes included the frequency (mean number of episodes per patient per day) of combined liquid and loose (unformed) stool and the weight (grams per day) of liquid stool and of liquid and loose (unformed) stool (per patient per day).

Study Sample

This study was intended as a pilot for a larger multicentered study. Calculation of the sample size indicated that 150 patients per group would be required to detect a 30% reduction in episodes of liquid stools and that 64 patients per group would be required to detect a 40% reduction with a 80% power if the episode rate is 1.01 per day (SD, 0.75) as observed in the first 15 patients in the pilot study.

All adult patients (18 years or older) who required enteral nutrition delivered through a feeding tube were considered for inclusion in the study if the attending intensivist expected that artificial nutritional support would be required for more than 72 hours and no contraindication to use of the gastrointestinal tract was present.

Exclusion criteria included expected death within 24 hours of admission, established treatment limitations, an expected interhospital transfer within 72 hours of admission, a specific surgical request not to use the gastrointestinal tract (eg, enteric anastomosis, imminent bowel resection or endoscopy), ischemic bowel, enteric fistula, bowel obstruction, and exacerbated inflammatory bowel disease. Additionally, patients were excluded if their treatment included established or recently commenced (<48 hours) parenteral nutrition and they had been started on enteral tube feeding before ICU admission. Because of the confounding effect on stool weight

and consistency, patients were also excluded if their initial illness was gastrointestinal hemorrhage or diarrhea or was complicated by these 2 entities. In patients with no contraindication, enteral nutrition was started within 24 hours of ICU admission.

For randomization of patients to the 2 groups, a hospital pharmacist not involved in recruitment, patient care, or data collection used Microsoft Excel's pseudorandom number generator. Assignments to the intervention or placebo groups were recorded in sequentially numbered, sealed opaque envelopes. All ICU staff, investigators, study patients, and the patients' families had no knowledge of group allocation.

Enteral Nutrition

The type of enteral nutrition administered was standardized; all patients received Isosource or Renal or Diabetic Resource (Novartis, Melbourne, Australia) formulas if clinically indicated. All enteral nutrition was administered via a naso-orogastric or nasojejunostomy tube according to the ICU "closed" enteral feeding protocol. Enteral feedings were started at 20 mL/h and were increased 20 mL/h every 4 hours until the target dosing rate was achieved. In instances in which gastric residual volumes exceeded 150 mL, intravenous prokinetic agents were introduced with feeding and were continued until individual target rates for enteral feeding were achieved. The target feeding rate was individualized for each patient and was based on weight and adjusted for metabolic demands; typical target rates were 25 to 35 cal/kg per day and 0.8 to 1.5 g protein per kilogram per day.

Both the control and the intervention groups received a nutritional supplement (Sustagen, Mead Johnson Co, Melbourne, Australia); the intervention group also received VSL#3. All enteral formulas and the nutritional supplement were free of fiber and prebiotic additives. The nutritional supplement was used to reconstitute the live freeze-dried VSL#3 product in a liquid form so that both preparations (placebo and intervention) appeared identical and had a similar viscosity. VSL#3 (VSL Pharmaceuticals, Gaithersburg, Maryland) contains live freeze-dried lactic acid bacteria, and each dose contains approximately 450 billion live lactic acid bacteria in defined ratios of lyophilized *Bifidobacterium breve, Bifidobacterium longum* ($>10 \times 10^9$/g), *Bifidobacterium infantis* ($>10 \times 10^9$/g), *L acidophilus, Lactobacillus plantarum, Lactobacillus casei, L bulgaricus, Streptococcus thermophilus* ($>100 \times 10^9$/g). The exact composition of the bacteria of the VSL#3 product is not specified by the distributor (Orphan Australia Pty Ltd, Dandenong, Victoria, Australia), and the bacterial count of the strains listed without numbers may vary between batches.

The probiotic preparation was prepared in the pharmacy. One VSL#3 sachet was combined with 50 mL of vanilla-flavored nutritional supplement (Sustagen) and mixed into a suspension. The placebo suspension of the supplement was indistinguishable from the VSL#3 in terms of color, consistency, and taste. The mixture of VSL#3 and supplement was tested to ensure it maintained its viscosity, osmolality, and taste for up to 24 hours while refrigerated.

The intervention and placebo preparations were made up daily and used to fill 50-mL opaque syringes. Each day, 2 syringes identified by study number, date, and preparation time were delivered to the ICU and stored in the refrigerator. The probiotic preparation of VSL#3 or the placebo was administered to each study patient twice per day, approximately 12 hours apart, as a bolus. Administration was recorded in each patient's medication record by attending nurses. After each administration, the feeding tube was flushed with 30 mL of sterile water to maintain patency of the tube.

> A probiotic preparation of VSL#3 or a placebo was administered to each study patient twice per day, approximately 12 hours apart, as a bolus.

Data Collection

All fecal output was examined and was recorded by using the validated King's Stool Chart.[42] The chart uses a scoring system that involves characteristics of fecal frequency, weight, and consistency based on 3 categories of fecal weight and 4 categories of fecal consistency, with the aim of standardizing fecal output.[42] A comprehensive staff training program including the use of the stool chart was undertaken.

The study continued after the cessation of tube feeding; patients in whom tube feeding was stopped consumed the intervention or placebo preparation orally. Follow-up continued for 21 days after transfer from the ICU to the general unit or until discharge from the hospital.

Demographic and clinical data were also collected. All patients had daily biochemistry tests, including levels of serum albumin and total protein, and hematology profiles, including coagulation studies, as part of routine clinical management. Stool cultures and assays for *C difficile* toxins A and B were performed routinely for all patients who had diarrhea.

> Patients receiving VSL#3 had less frequent liquid stools and combined liquid and loose stools than did patients receiving placebo.

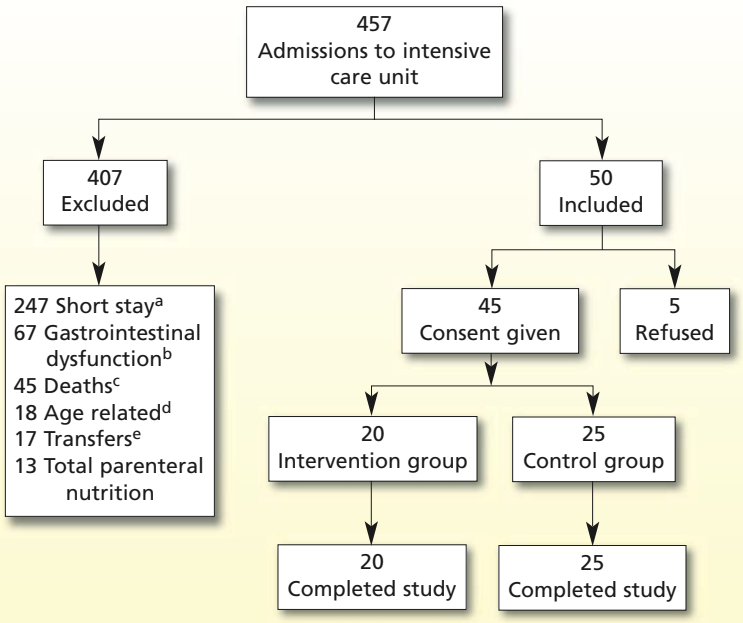

Figure Flow chart of sample recruitment.

[a] Patients admitted to intensive care unit for generally less than 72 hours.
[b] Not suitable for study inclusion because of suspected or known mechanical obstruction of the gastrointestinal tract.
[c] Death occurring within 24 hours of admission.
[d] Patients less than 18 years old.
[e] Patients transferred to other hospitals within 72 hours.

Statistical Analysis

Analysis was on an intention-to-treat basis. Demographic descriptors of the study population in the intervention and placebo groups were compared by using general linear modeling for continuous variables and logistic regression for categorical variables to determine randomization effectiveness for known or potential confounding variables. The absolute (per patient per day) and relative (incidence rate ratio [IRR]) incidence rates of episodes of liquid stools and of liquid or loose stools in each group were estimated by using repeated-measures Poisson regression. The daily absolute weight of stool and mean differences for liquid and liquid and loose (unformed) stools were estimated by using repeated-measures general linear modeling. Results are expressed as absolute and relative effect sizes with 95% confidence intervals (95% CI) of these estimates and *P* values for the comparisons. The analyses were also performed with adjustments for potential confounding variables; variables in the models were selected by using backward stepwise Poisson regression and general linear modeling, respectively, from a list of antibiotics (β-lactams, clindamycin, macrolides, ciprofloxacin, tetracyclines, cotrimoxazole, gentamicin, vancomycin, rifampicin, metronidazole, antifungals, and antivirals), age, sex, Acute Physiology and Chronic Health Evaluation II score and Simplified Acute Physiology Score II, and serum albumin levels, with forced inclusion of treatment group and variables with significance in models of other stool consistencies. Statistical analyses were performed by using STATA Statistics/Data Analysis, Version 10.1 (StataCorp, College Station, Texas).

Frequency of loose stool was positively associated with β-lactams, clindamycin, macrolides, and antifungals.

Results

Of 457 patients evaluated for potential enrollment, 407 did not fulfill the inclusion criteria, and 5 who did fulfill the criteria refused consent. A total of 45 patients were randomly assigned to the intervention (n = 20) or control (n = 25) group (see Figure). We had no reported or detected deviations from the study protocol, and all patients had follow-up. The pilot study was stopped after 15 months because of slower than expected recruitment and because a planned a priori interim analysis after the enrolment of 30 patients indicated a significant effect on the primary outcome (mean number of episodes of liquid and combined liquid and loose stool per patient per day).

The demographic and clinical characteristics of the 2 groups did not differ significantly (Table 1). Patients were in the study for mean of 11.9 days (SD, 5.6) and received enteral nutrition for a mean of 8.5 days (SD, 5.4). The 2 groups did not differ significantly in type of feeding tubes; 2 of 20 (10%) in the intervention group and 4 of 25 (16%) in the control group had nasojejunal tubes; the remaining patients had nasogastric tubes.

Compared with the control group, the intervention group had a reduction in the frequency of liquid stools (IRR, 0.50; 95% CI, 0.27 to 0.93; $P = .03$) and of combined liquid and loose stools (unformed) (Table 2). The 2 groups did not differ significantly in stool weight (Table 2). When these analyses were adjusted for potentially confounding variables, the variance of the results was reduced, and the clinically significant differences in the effect of VSL#3 on measures of stool frequency and consistency were also statistically significant (Table 3).

All patients received intravenous antibiotics of some form during the study period. We found significant positive associations between frequency of loose stool and certain antibiotics: β-lactams (IRR, 2.04; $P = .001$), clindamycin (IRR, 3.41; $P < .001$), macrolides (erythromycin or azithromycin; IRR, 2.62; $P < .001$), and antifungals (IRR, 2.09; $P = .003$).

We found negative associations for serum level of albumin (IRR, 0.72; $P = .004$) and use of jejunal feeding tubes (IRR, 0.78; $P = .02$; Table 4). No association was found between metoclopramide use and the frequency of liquid stools (IRR, 1.06; 95% CI, 0.64 to 1.75; $P = .82$) or liquid stool weight (mean difference, 0 g; 95% CI, -45 to 42; $P = .96$).

A total of 8 of the 45 patients died: 5 in the intervention group and 3 in the placebo group. Survival analysis indicated no significant difference in those mortality rates (hazard ratio, 1.22 for VSL#3 vs placebo; 95% CI, 0.31 to 4.83; $P = .78$; adjusted for age, use of nasojejunal tube, serum albumin level less than 25 g/L, and alternative feeding type).

Discussion

In this small double-blind, randomized, placebo-controlled pilot study, use of the probiotic VSL#3 was associated with a 50% reduction in the frequency (rate per day) of liquid stool in ICU patients expected to receive enteral nutrition for at least 72 hours. This outcome is similar to the results of another study[30] on diarrhea associated with enteral feeding with a comparable sample and method. The outcome is also comparable with the findings of other RCT studies in different groups of patients.[23,40]

We intended to recruit 150 patients, but recruitment (n = 45) was much slower than expected; because of changes in surgical coverage, fewer surgical patients were available who required enteral feeding. On the basis of our results, a sample size of 45 patients had an 80% power to detect a 47% reduction in frequency of liquid stools, and 99 patients in each group would be required to detect a 30% reduction in the frequency of liquid stools.

Although we found that the frequency of liquid stool decreased significantly, the absolute volume of stool in both groups was not particularly large. Some clinicians may not classify the amounts as diarrhea, a situation that highlights difficulties with current definitions of diarrhea. For example, the World Health Organization[43] defines diarrhea as 3 or more loose or watery stools in a 24-hour period; no amount is specified. No single standard definition of the term diarrhea exists; Lebak et al[44] found more than 33 different definitions in the literature. Criteria included in definitions of diarrhea include characteristics (loose/watery, formed/semiformed/nonformed) and frequency (>3 stools/day, >4 stools/day of stools passed with in a specific time frame [<24 hours]).[2] Other definitions include the previously mentioned criteria plus the volume of stool passed per episode (>200 g/d of liquid stool) and combinations of any of the criteria described here. A strength of our study is that we used a validated tool to quantify the fecal output of all patients by measuring the 3 dimensions of stool output: frequency, consistency, and weight of stool.

All patients were routinely tested for *C difficile*. Although reported rates of infection caused by *C*

Table 1
Demographic and clinical characteristics of the study patients

Descriptor	Mean (SD) VSL#3 (n = 20)	Mean (SD) Placebo (n =25)	*P*
Before recruitment			
Age, y	60.8 (15.6)	65.5 (9.8)	.23[a]
APACHE II score	22.2 (8.9)	23.8 (10.2)	.56[a]
SAPS II	43.9 (15.0)	46.1 (19.4)	.66[a]
Albumin	26.7 (6.0)	24.3 (5.1)	.18[a]
Sex, male/female	13/7	17/8	.83[b]
Emergency admission (emergency/elective)	15/5	20/5	.69[b]
Surgical admission (operative/nonoperative)	7/13	9/16	.95[b]
After recruitment			
Days in study	12.2 (5.1)	11.6 (6.0)	.72[a]
Days in intensive care unit	7.3 (5.7)	8. 1 (4.0)	.58[a]
Days in general care unit	4.9 (4.8)	3.5 (2.8)	.23[a]
Days of enteral feeding	8.8 (5.0)	8.2 (5.7)	.72[a]
Position of feeding tube (nasogastric/nasojejunal)	18/2	21/4	.56[b]
Hours of mechanical ventilation	144 (125)	161 (126)	.65[a]
Days of parenteral opioids	4.5 (4.5)	5.1 (4.2)	.61[a]
Days of sedation	4.9 (3.9)	4.9 (4.2)	.99[a]
Days of antibiotic	11.1 (4.9)	9.4 (5.8)	.31[a]
Prokinetics			
Metoclopramide	10 of 20	12 of 25	.55[b]
Erythromycin	10 of 20	8 of 25	.52[b]
Nasogastric feeding			
Isosource	15 of 20	19 of 25	.33[b]
Diabetes research	3 of 20	4 of 25	.69[b]
Renal	2 of 20	2 of 25	.28[b]
Antibiotics			
ß-lactams	15 of 20	15 of 25	.79[b]
Penicillins	19 of 20	20 of 25	.82[b]
Cephalosporins	8 of 20	9 of 25	.39[b]
Clindamycin	2 of 20	1 of 25	.48[b]
Macrolides	12 of 20	10 of 25	.63[b]
Ciprofloxacin	4 of 20	4 of 25	.73[b]
Tetracyclines	1 of 20	0 of 25	
Cotrimoxazole	2 of 20	1 of 25	.40[b]
Gentamicin	6 of 20	7 of 25	.92[b]
Vancomycin	4 of 20	4 of 25	.66[b]
Rifampicin	0 of 20	1 of 25	
Metronidazole	5 of 20	8 of 25	.55[b]
Antifungals	8 of 20	8 of 25	.59[b]
Antivirals	1 of 20	1 of 25	.85[b]

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; SAPS, Simplified Acute Physiology Score.
[a] Difference between the means of the 2 groups for continuous variables estimated by using general linear modeling.
[b] Difference between the distributions of categorical variables in the 2 groups estimated by using ordinal logistic regression, with duration of treatment taken into account.

Table 2
Effect of probiotic VSL#3 on diarrhea

Stool episodes	Episodes per patient per day, mean (SD)		VSL#3 vs placebo		
	VSL#3 (n = 20)	Placebo (n = 25)	Unadjusted IRR[a]	95% Confidence interval	*P*
Liquid[b]	0.53 (0.54)	1.05 (1.08)	0.50	0.27 to 0.93	.03
Liquid and loose[b,c]	0.96 (0.79)	1.48 (1.23)	0.65	0.40 to 1.05	.08
All stools	1.30 (0.73)	1.68 (1.19)	0.77	0.55 to 1.09	.14
Stool weight[d]	**Mean, g/day**		**Mean Δ**	**95% Confidence interval**	***P***
Liquid stools	87 (102)	159 (192)	-72	-159 to 15	.10
Liquid and loose[c]	140 (163)	217 (143)	-77	-167 to 12	.09
All stools	182 (116)	254 (169)	-73	-156 to 11	.09

[a] Incidence rate ratio (IRR) estimated by using repeated-measures Poisson regression.
[b] Mean number of episodes of diarrhea per patient per day.
[c] Liquid and loose stool category is a combined category of liquid stools (shown separately) and loose stools (not shown).
[d] Stool weight (g/d) means and mean difference (Δ) estimated by using repeated-measures general linear modeling.

Table 3
Effect of probiotic VSL#3 on diarrhea adjusted for antibiotic use, nasojejunal tube use, albumin <25 g/L, and alternative feeding type

Stool episodes	Episodes per patient per day, mean (SD)		VSL#3 vs placebo		
	VSL#3 (n = 20)	Placebo (n = 25)	Adjusted IRR[a]	95% Confidence interval	*P*
Liquid[b]	0.48 (0.29)	0.93 (0.70)	0.59	0.40 to 0.85	.005
Liquid and loose[b,c]	0.94 (0.61)	1.33 (0.79)	0.76	0.57 to 1.01	.06
All stools	1.29 (0.54)	1.55 (0.80)	0.84	0.64 to 1.09	.18
Stool weight[d]	**Mean, g/day**		**Mean Δ**	**95% Confidence interval**	***P***
Liquid stools	97 (54)	151 (125)	-54	-113 to 5	.07
Liquid and loose[c]	154 (87)	205 (126)	-51	-116 to 14	.13
All stools	196 (80)	242 (132)	-46	-109 to 17	.16

[a] Incidence rate ratio (IRR) estimated by using repeated-measures Poisson regression.
[b] Mean number of episodes of diarrhea per patient per day.
[c] Liquid and loose stool category is a combined category of liquid stools (shown separately) and loose stools (not shown).
[d] Stool weight (g/d) means and mean difference (Δ) estimated by using repeated-measures general linear modeling.

difficile are as high as 20% in enterally fed patients,[33] no episodes of *C difficile* infection occurred in either group of patients during the study. However, because *C difficile* infection can develop up to 4 weeks after the cessation of antimicrobial therapy, some undetected positive associations between *C difficile* and diarrhea may have occurred. The apparent absence of *C difficile* infection in this study is comparable to the finding in a recent RCT[23] in which a probiotic was administered to reduce the incidence of diarrhea associated with use of antibiotics and infection by *C difficile.* In that study,[23] the intervention group had no episodes of *C difficile* infection, whereas 17% of the placebo group did.

During the study, prokinetics were prescribed for patients who were experiencing high gastric residual volumes of enteral feeding. Use of metoclopramide was not associated with the frequency and weight of liquid stools. Erythromycin was associated with increased frequency and weight of liquid

Table 4
Effect of probiotic VSL#3 on stool episode frequency adjusted for antibiotic use (including details of all associations in the model)

Type of episode by group	Episodes per patient per day, mean (SD)		VSL#3 vs placebo		
	VSL#3 (n = 20)	Placebo (n = 25)	Unadjusted IRR[a]	95% Confidence interval	*P*
Liquid[b]					
Treatment group	0.48 (0.29)	0.93 (0.70)	0.59	0.40 to 0.85	.005
ß-lactams			2.17	1.41 to 3.34	<.001
Clindamycin			2.52	1.77 to 3.60	<.001
Macrolides			2.59	1.50 to 4.46	.001
Antifungals			1.70	1.04 to 2.79	.04
Alternative nasogastric feeding[c]			1.33	0.88 to 2.02	.17
Nasojejunal tube			0.77	0.64 to 0.92	.005
Albumin <25 g/L			2.32	1.39 to 3.88	.001
Liquid and loose[b,d]					
Treatment group	0.94 (0.61)	1.33 (0.79)	0.76	0.57 to 1.01	.06
ß-lactams			1.54	1.24 to 1.92	<.001
Clindamycin			1.90	1.33 to 2.70	<.001
Macrolides			1.91	1.43 to 2.56	<.001
Antifungals			1.34	1.06 to 1.70	.02
Alternative nasogastric feeding[c]			1.45	1.13 to 1.86	.003
Nasojejunal tube			0.93	0.80 to 1.08	.32
Albumin <25 g/L			1.71	1.24 to 2.36	.001
All stools					
Treatment group	1.29 (0.54)	1.55 (0.80)	0.84	0.64 to 1.09	.18
ß-lactams			1.32	1.02 to 1.69	.03
Clindamycin			1.75	1.31 to 2.34	<.001
Macrolides			1.83	1.14 to 2.92	.01
Antifungals			1.24	0.89 to 1.73	.20
Alternative nasogastric feeding[c]			1.35	1.02 to 1.78	.04
Nasojejunal tube			0.93	0.81 to 1.07	.33
Albumin <25 g/L			1.38	1.03 to 1.86	.03

[a] Incidence rate ratio (IRR) estimated by using repeated-measures Poisson regression, adjusted for daily use of listed antibiotics and albumin levels less than 25 g/L: variables in the models were selected by using backward stepwise Poisson regression from a list of antibiotics (ß-lactams, clindamycin, macrolides, ciprofloxacin, tetracyclines, cotrimoxazole, gentamicin, vancomycin, rifampicin, metronidazole, antifungals, and antivirals), prokinetics, age, sex, score on Acute Physiology and Chronic Health Evaluation II and Simplified Acute Physiology Score, use of alternative enteral feeding, nasojejunal tube, and serum albumin levels less than 25 g/L, with forced inclusion of treatment group and variables with significance in models of other stool consistencies.
[b] Mean number of episodes of diarrhea per patient per day.
[c] The 37 patients who received Isosource nasogastric feeding are compared with patients who received alternative nasogastric feedings (4 Diabetes Resource and 4 Renal feedings).
[d] Liquid and loose stool category is a combined category of liquid stools (shown separately) and loose stools (not shown).

stools, but we could not determine whether this finding was due to the prokinetic effect of the drug or to its antibiotic action. Nasojejunal placement of the feeding tube and serum level of albumin (<25 g/L) were associated with increased frequency and weight of liquid stool as expected,[33,36] but because of our small sample numbers, we could not analyze this interaction.

Any broad-spectrum antibiotic, such as cephalosporins, penicillin, and clindamycin,[45] can predispose patients to diarrhea. Most patients in our study received intravenous antibiotics. We found significant positive associations between certain antibiotics (β-lactams, clindamycin, erythromycin, and antifungals) and the frequency and weight of liquid and liquid and loose (unformed) stools. The multivariate analysis indicated that those antibiotics were associated with increased likelihood of episodes of liquid and loose stool and that the effect of VSL#3 in reducing that likelihood was independent of such confounding effects. Although most of our patients received intravenous antibiotics in the ICU, we did not collect data on use of oral antibiotics, a step that might have been useful. Most likely oral antibiotics produce higher local gastrointestinal concentrations of antibiotic than that achieved by intravenous administration. Thus, compared with intravenous antibiotics, oral antibiotics might attenuate the beneficial effects of probiotics.

Many probiotic preparations are available, with different single or multiple species. The potential of improved synergy or the ability of multistrain

probiotics to work together to enhance intestinal health may provide a rational basis for future research and potential clinical use,[46] although any therapeutic hypotheses would need to be tested in RCTs. The large numbers of potential single and multiple combinations of species and dosages create a daunting volume of empirical trial data that needs collecting.

The probiotic VSL#3 contains 8 different bacterial species that have been effective in managing serious gastrointestinal disorders.

The probiotic we used, VSL#3, is a commercially available, high-potency preparation containing 8 different bacterial species that have been effective in the management of serious intestinal disorders in patients with gastrointestinal disease.[17-21] VSL#3 survives gastric fluid, bile, and pancreatic secretions and can effectively colonize the gastrointestinal tract, regulate intestinal immunomodulary responses, and restore healthy levels of resident intestinal bacteria.[18,21,47] Although we found that VSL#3 reduced the number of episodes of liquid stools in ICU patients receiving enteral feeding, we cannot extrapolate and suggest which species in the preparation might be more efficacious than another species or whether the combined species might have worked synergistically to reduce the numbers of liquid stools. The reduction in the episodes of liquid stool in our study is similar to the results of a recent study[23] in which a multistrain probiotic drink was used to reduce the incidence of antibiotic-associated diarrhea.

More rigorous trials with larger sample sizes are required to test the clinical use of probiotics and to define the safety of probiotic use in critically ill patients.

Use of a high-potency multispecies probiotic in ICU patients receiving enteral feedings who have diarrhea is extremely limited. Thus, our findings are a proof of concept: VSL#3 can alter the risk of diarrhea in enterally fed ICU patients. The choice of probiotic preparation most likely is an important consideration for future research. For example, some preparations may actually contain inactive organisms, whereas others may have organisms that do not survive transit through the gut. Multiple RCTs with VSL#3 should be followed by similar studies on different preparations to determine the relative effectiveness of each preparation in specific health conditions.

Our study has limitations that may narrow the generalizability of the findings. The study was undertaken at a single site, with a limited case mix, and some important categories of critically ill patients were excluded from the sample, in particular neurosurgical patients. Only about 10% of ICU admissions were eligible, producing a small sample size. Despite the study limitations, particularly the high severity-of-illness scores of the patients in our sample, and given the potential beneficial effects of probiotics on diarrhea, further investigation is required. Although we attempted to classify our patient groups (eg, operative vs nonoperative), because of the small sample size, potential subgroups of patients who might benefit more than other patients could not be identified. Another limitation was use of a nutritional supplement as the placebo and as the preparation used to reconstitute the freeze-dried VSL#3 to a liquid form. Although the 2 main types of enteral nutrition used to feed patients (Diabetic Resource and Isosource) were relatively iso-osmolar (300-490 mOsm), retrospectively, we discovered that the supplement is hyperosmolar (671 mOsm) and therefore may be a confounder because of its high osmolarity and potential to cause diarrhea.

Although our data suggest that VSL#3 reduced the frequency of liquid stool in critically ill patients, the study was not designed or large enough to assess the potential detrimental effects attributable to VSL#3 (eg, secondary sepsis or impaired function of the gastrointestinal tract). The heterogeneous underlying illnesses of the patients further limited our ability to assess mortality. Although we found no significant differences in mortality between the groups, the wide confidence intervals of the hazard ratio estimates preclude any firm conclusions about the safety of the use of the preparation. We did not monitor directly for adverse effects, because monitoring would have required vastly larger numbers of patients to exclude clinically relevant but uncommon adverse effects. The lack of monitoring limits the scope of our study. Potential risks include superinfection or breakthrough invasion into the blood with individual probiotic strains; these problems would be most likely in patients who are immunocompromised[48,49] or have severe acute pancreatitis.[50] In our study, all patients with liquid or soft stools had cultures of blood, urine, and sputum and assays for *C difficile* infection 3 times per week as routine monitoring for sources of infection. We found no cultures positive for individual probiotic strains.

Finally, measurement of the outcome relied on direct observation of stool by nursing staff. Although staff members were trained for this task, and periodic compliance checks were undertaken to ensure adherence, some episodes of diarrhea could have been missed or recorded incorrectly.

Conclusions

Patients with diarrhea who were given a probiotic preparation had a 50% reduction in the number of episodes of liquid stool per day and smaller, but still clinically significant, reductions in the number of episodes of liquid and loose (unformed) stool and in stool weight. Diarrhea remains an important problem for both patients and health care providers. Probiotics used in the correct context may be an appropriate response to the problem of diarrhea, and further investigation is warranted. The balance of beneficial and adverse effects in patients who might receive this treatment can be reliably estimated only by doing much larger scale RCTs and meta-analysis of multiple RCTs.

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REFERENCES

1. Montejo J. Enteral nutrition-related gastrointestinal complications in critically ill patients: a multicenter study. The Nutritional and Metabolic Working Group of the Spanish Society of Intensive Care Medicine and Coronary Units. *Crit Care Med.* 1999;27(8):1447-1453.
2. Smith C, Marien L, Brogdon C, et al. Diarrhea associated with tube feeding in mechanically ventilated critically ill patients. *Nurs Res.* 1990;39(3):148-152.
3. Bleichner G, Thomas O, Sollet J. Diarrhea in intensive care: diagnosis and treatment. *Int J Antimicrob Agents.* 1993;3(1): 33-48.
4. Dobb GJ. Diarrhea in the critically ill. *Intensive Care Med.* 1986;11:7-9.
5. Adam S, Batson S. A study of problems associated with the delivery of enteral feed in critically ill patients in five ICUs in the UK. *Intensive Care Med.* 1997;23:261-266.
6. Majid HA, Emery PW, Whelan K. Attitudes of patients and nurses towards diarrhoea during tube feeding [abstract]. *J Hum Nutr Diet.* 2008;21(4):395.
7. Isolauri E, Sütas Y, Kankaanpää P, Arvilommi H, Salminen S. Probiotics: effects on immunity. *Am J Clin Nutr.* 2001;73(2 suppl):444S-450S.
8. Pessi T, Sütas Y, Saxelin M, Kallioinen H, Isolauri E. Antiproliferative effects of homogenates derived from five strains of candidate probiotic bacteria. *Appl Environ Microbiol.* 1999;65(11):4725-4728.
9. Sheih YH, Chiang BL, Wang LH, Liao CK, Gill HS. Systemic immunity-enhancing effects in healthy subjects following dietary consumption of the lactic acid bacterium *Lactobacillus rhamnosus* HN001. *J Am Coll Nutr.* 2001;20(2 suppl):149-156.
10. Zamfir M, Callewaert R, Cornea PC, Savu L, Vatafu I, De Vuyst L. Purification and characterization of a bacteriocin produced by *Lactobacillus acidophilus* IBB 801. *J Appl Microbiol.* 1999;87(6):923-931.
11. Goldin B, Gorbach S, Saxelin M, Barakat S, Gualtieri L, Salminen S. Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. *Dig Dis Sci.* 1992;37(1):121-128.
12. Van Niel CW, Feudtner C, Garrison MM, Christakis DA. Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics.* 2002;109:678-684.
13. Bartlett JG. Antibiotic-associated diarrhea. *N Engl J Med.* 2002;346:334-339.
14. Szajewska H, Mrukowicz J. Probiotics in the treatment and prevention of acute infectious diarrhoea in infants and children; a systematic review of published randomized, double-blind, placebo-controlled trials. *J Pediatr Gastroenterol Nutr.* 2001;33(suppl 2):17-25.
15. McFarland LV. Meta-analysis of probiotics for the prevention of traveler's diarrhea. *Travel Med Infect Dis.* 2007;5(2):97-105.
16. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol.* 1997; 99:179-185.
17. Delia P, Sansotta G, Donato V, et al, Use of probiotics for prevention of radiation-induced diarrhea. *World J Gastroenterol.* 2007;13(6):912-915.
18. Gionchetti P, Rizzello F, Venturi A, et al. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology.* 2000;119(2):584-587.
19. Mimura T, Rizzello F, Helwig U, et al. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut.* 2004;53(1):108-114.
20. Fedorak RN, Gionchetti P, Campieri M, et al. VSL3 probiotic mixture induces remission in patients with active ulcerative colitis [abstract]. *Gastroenterology.* 2003;124(4 suppl 1):A377.
21. Bibiloni R, Fedorak RN, Tannock GW, et al. VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. *Am J Gastroenterol.* 2005;100:1539-1546.
22. D'Souza AL, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic-associated diarrhoea: meta-analysis. *BMJ.* 2002;324:1361-1367.
23. Hickson M, D'Souza A, Muthu N, et al. Use of probiotic lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. *BMJ.* 2007;335(7610):80.
24. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic-associated diarrhea and the treatment of *Clostridium difficile* disease. *Am J Gastroenterol.* 2006;101:812-822
25. Plummer S, Weaver MA, Harris JC, Dee P, Hunter J. *Clostridium difficile* pilot study: effects of probiotic supplementation on the incidence of *C. difficile* diarrhea. *Int Microbiol.* 2004;7(1):59-62.
26. Anderson AD, McNaught CE, Jain PK, MacFie J. Randomised clinical trial of symbiotic therapy in elective surgical patients. *Gut.* 2004;53(2):241-245.
27. McNaught CE, Woodcock NP, MacFie J, Mitchell CJ. A prospective randomised study of the probiotic *Lactobacillus plantarum* 299V on indices of gut barrier function in elective surgical patients. *Gut.* 2002;51(6):827-831.
28. Cremonini F, Di Caro S, Nista EC, et al. Meta-analysis: the effect of probiotic administration on antibiotic-associated diarrhoea. *Aliment Pharmacol Ther.* 2002;16(8):1461-1467.
29. Allen SJ, Okoko B, Martinez E, Gregorio G, Dans LF. Probiotics for treating infectious diarrhoea. *Cochrane Database Syst Rev.* 2004;(2):CD003048.
30. Bleichner G, Blehaut H, Mentec H, Moyse D. *Saccharomyces boulardii* prevents diarrhea in critically ill tube-fed patients: a multicenter, randomized, double-blind placebo-controlled trial. *Intensive Care Med.* 1997;23(5):517-523.
31. Whelan K. Enteral-tube feeding diarrhea: manipulating the

colonic microbiota with probiotics and prebiotics. *Proc Nutr Soc.* 2007;66:299-306.
32. Whelan K, Judd P, Tuoy K, Gibson G, Preedy V, Taylor M. Fecal microbiota in patients receiving enteral feeding are highly variable and may be altered in those who develop diarrhea. *Am J Clin Nutr.* 2009;89:240-247.
33. Bliss DZ, Johnson S, Savik K, Clabots CR, Willard K, Gerding DN. Acquisition of *Clostridium difficile* and *Clostridium difficile*-associated diarrhea in hospitalized patients receiving tube feeding. *Ann Intern Med.* 1998;129(12):1012-1019.
34. Okuma T, Nakamura M, Totake H, Fukanaga, Y. Microbial contamination of enteral feeding formulas and diarrhea. *Nutrition.* 2000;16(9):719-722.
35. Seifert CF, Johnston BA. A nationwide survey of long-term care facilities to determine the characteristics of medication administration through enteral feeding catheters. *Nutr Clin Pract.* 2005;20:354-362.
36. Hwang TL, Lue MC, Nee YJ, Jan YY, Chen MF. The incidence of diarrhea in patients with hypoalbuminemia due to acute or chronic malnutrition during enteral feeding. *Am J Gastroenterol.* 1994;89(3):376-378.
37. Bowling TE, Raimundo AH, Gimble GK, Silk DB. Colonic secretory effect in response to enteral feeding in humans. *Gut.* 1994;35:1734-1741.
38. Resta-Lenert S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). *Gut.* 2003;52(7):988-997.
39. Bowling TE, Raimundo AH, Gimble GK, Silk DB. Reversal by short-chain fatty acids of colonic fluid secretion induced by enteral feeding. *Lancet.* 1993;342:1266-1268.
40. Alberda C, Gramlich L, Meddings J, et al. Effects of probiotic therapy in critically ill patients: a randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr.* 2007;85(3):816-823.
41. Heimburger D, Sockwell D, Geels W. Diarrhea with enteral feeding: prospective reappraisal of putative causes. *Nutrition.* 1994;10(5):392-396.
42. Whelan K, Judd PA, Taylor MA. Assessment of fecal output in patients receiving enteral tube feeding: validation of a novel chart. *Eur J Clin Nutr.* 2004;58(7):130-137.
43. World Health Organization. *The Treatment of Diarrhoea: A Manual for Physicians and Senior Health Workers.* 4th rev. Geneva, Switzerland: World Health Organization, Division of Diarrhoeal and Acute Respiratory Disease Control; 2005.
44. Lebak K, Bliss D, Savik K, Patten-Marsh K. What's new on defining diarrhea in tube-feeding studies? *Clin Nurs Res.* 2003;12(2):174-204.
45. Gupta V, Garg R. Probiotics. *Indian J Med Microbiol.* 2009; 27(3):202-209.
46. Timmerman H, Niers L, Ridwan B, et al. Design of multispecies probiotic mixture to prevent infectious complications in critically ill patients. *Clin Nutr.* 2007;26(4):450-459.
47. Venturi A, Gionchetti P, Rizzello F, et al. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Aliment Pharmacol Ther.* 1999;13(8): 1103-1108.
48. Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Shetty AK. Lactobacillus sepsis associated with probiotic therapy. *Pediatrics.* 2005;115(1):178-181.
49. Salminen MK, Rautelin H, Tynkkynen S, et al. Lactobacillus bacteremia, clinical significance, and patient outcome, with special focus on probiotic *L. rhamnosus* GG. *Clin Infect Dis.* 2004;38(1):62-69.
50. Besselink, M, van Santvoort H, Buskens E, et al; Dutch Acute Pancreatitis Study Group. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial [published correction appears in *Lancet.* 2008;371(9620):1246]. *Lancet.* 2008;371(9613):651-659.