CLINICAL TRIAL PROTOCOL

Prebiotic Inulin to Limit Antimicrobial-Resistant Infections During Critical Illness: A Phase 2 Clinical Trial

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Version 5

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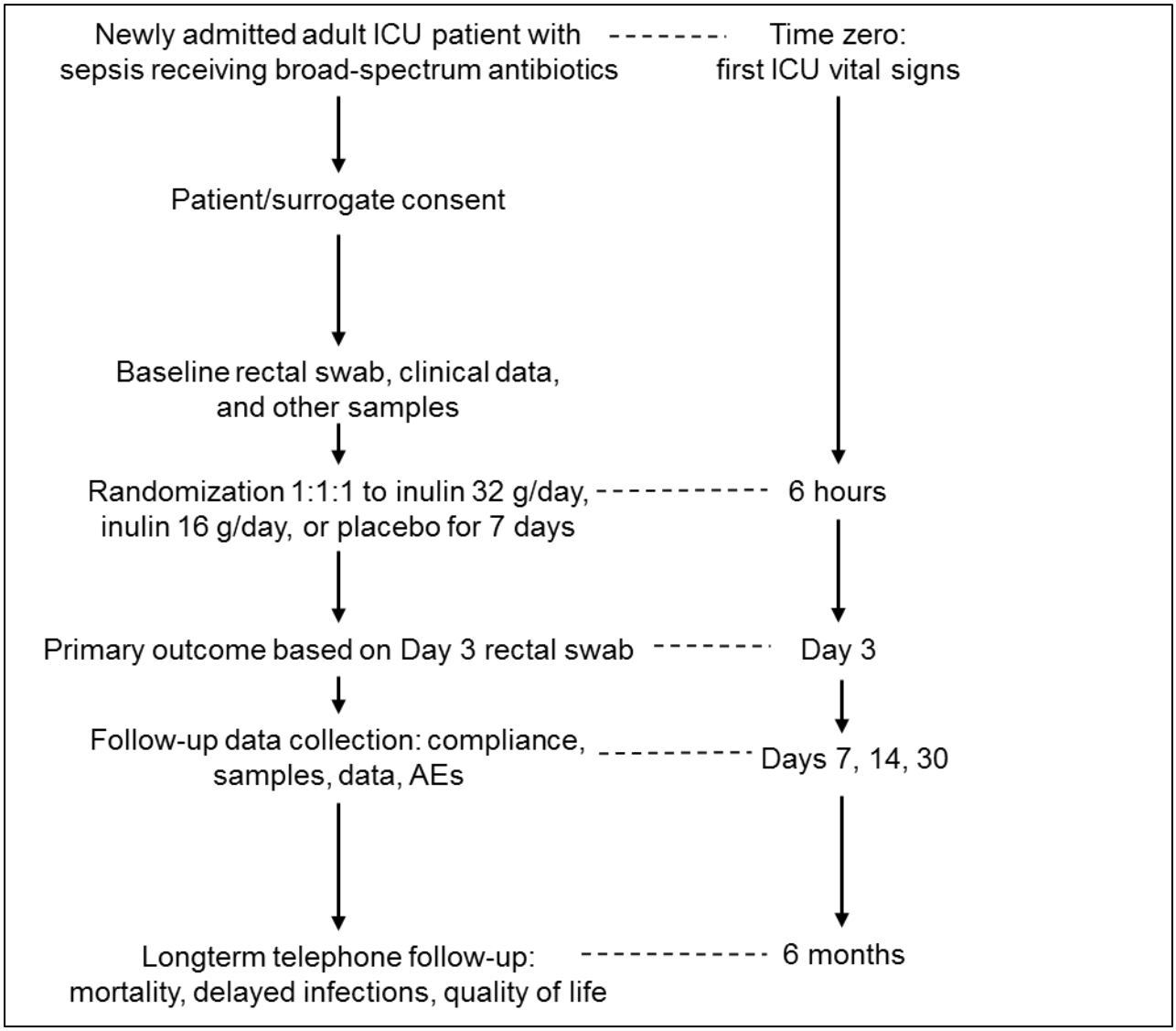
1. Table of Contents

2. Protocol synopsis

Title	Prebiotic Inulin to Limit Antimicrobial-Resistant Infections During Critical Illness: A Phase 2 Clinical Trial
Short title	Inulin in the ICU
Design	Randomized, double-blind, placebo-controlled trial in the ICU
Outcomes	*Primary outcome*: Change in relative abundance of SCFA producers through ICU Day 3 *Secondary outcome*: MDRO colonization status on ICU Day 3 *Additional outcomes*: 1. Feasibility 2. Rates of pre-specified AEs 3. Antibiotic resistance gene fraction on ICU Day 3 4. Culture-proven MDR infections through ICU Day 30 5. Fecal SCFA levels 6. Proportion of goal calories through ICU Days 3 and 7 7. Length of ICU stay 8. Length of hospital stay 9. Mortality through ICU Day 90 10. Self-assessed quality of life at 6 months 11. Times series data including ICU Day 7 outcomes
Intervention	1:1:1 allocation to inulin 32 g/day, inulin 16 g/day, or placebo dissolved in 250 cc sterile water and given in 2 daily doses for 7 days or until death/hospital discharge
Sample size	90 critically ill patients meeting Sepsis-3 criteria
Eligibility criteria	*Inclusion criteria*: 1. Medical ICU 2. Age ≥ 18 years old 3. Sepsis, defined according to the Sepsis-3 (2016) consensus as a known or suspected infection with a SOFA score of ≥2 points above baseline 4. Broad-spectrum antibiotics, received within the last 24 hours or ordered and pending administration 5. Able to complete enrollment within 24 hours of ICU admission for administration of the intervention within 48 hours of ICU admission *Exclusion criteria*: 1. Inability to receive oral or enteric fluids 2. Inulin allergy 3. Hyponatremia (serum sodium ≤128 mEq/L) 4. Immunosuppression, defined as CD4 count <200 or absolute neutrophil count <500 or reasonable expectation of either during the study intervention period 5. Surgery involving the intestinal lumen within 30 days or known intestinal strictures 6. Do Not Resuscitate (DNR) or Do Not Intubate (DNI) status, or “no escalation of care” orders 7. Lack capacity for consent and no appropriate Legally Authorized Representative (LAR).

2.1 Study diagram



3. Administrative information

Primary Investigator	Daniel E. Freedberg def2004@cumc.columbia.edu Columbia University Medical Center Division of Digestive and Liver Diseases 622 West 168th Street, P&S Building 3-401, New York, NY 10032 Research office: (212) 342-3302, Cell: (857) 998-9370
Co-Investigators	*All at Columbia University Medical Center* Julian Abrams: ja660@cumc.columbia.edu David Chong: dhc5@cumc.columbia.edu Zhezhen Jin: zj7@columbia.edu Anne-Catrin Uhlemann: au2110@@cumc.columbia.edu
Data and Safety Monitoring Officer	Daniel Brodie hdb5@cumc.columbia.edu Columbia University Medical Center Vivian and Seymour Milstein Family Heart Center 173 Fort Washington Avenue, 4-005A, New York, NY 10032 Phone: (212)305-9817
Other Key Personnel	Howard Andrews, Columbia University Data Coordinating Center, hfa1@columbia.edu Research Nurse to be hired

4. Funding

This project is funded by a Department of Defense Peer-Reviewed Medical Research Program Clinical Trial Award (PR181960) recommended for funding on February 20, 2019.

4.1 Role of funding bodies

The study will be designed and conducted, and the results analyzed, presented, and published by the investigators independent of the funding bodies.

5. Background and rationale for study

5.1 Background

Multidrug resistant (MDR) bacterial infections are a leading cause of morbidity, death, and healthcare-associated costs.[1-3] Nowhere is this more important than in the intensive care unit (ICU), where over half of all infections are MDR[4] and where sepsis from MDR bacteria confers 10-20% absolute increased mortality.[5-7]

This project hypothesizes that antibiotic resistance in the ICU is a problem of the human gastrointestinal microbiome, which is the critical reservoir for the bacteria and plasmids that encode antibiotic resistance genes.[8-10] The normal gut microbiota prevents colonization and subsequent infection with MDR organisms through competition for scarce resources and other mechanisms.[11] In the setting of critical illness, normal colonization resistance is lost and there are no currently available therapies to restore it.[12-15]

Specific gut bacteria contribute to colonization resistance. ICU patients have low levels of butyrate and other short chain fatty acids (SCFAs) which are derived from dietary fiber by anaerobic bacteria primarily within the Clostridial Clusters IV and XIVa (e.g., *Faecalibacterium prausnitzii*). SCFAs moderate colonic inflammation,[16] enlarge the pool of regulatory T cells,[17] and

contribute to the thickness of the colonic mucus layer and pathogen resistance.[18] During critical illness, levels of SCFA-producing bacteria and SCFAs are low, and these levels further decline with antibiotic treatment. Loss of SCFA-producing bacteria correlates with an increase in colonization with vancomycin-resistant *Enterococcus* and other antibiotic-resistant pathogens. We believe that preservation of SCFA-producing bacteria during critical illness is of major importance and can prevent the proliferation of colonizing organisms with antimicrobial resistance.

The prebiotic fiber **inulin** is a vegetable-derived polysaccharide that restores gastrointestinal colonization resistance against MDROs. SCFAs cannot be easily given to humans due to oral intolerability and rapid metabolism,[19,20] but inulin promotes growth of SCFA producers to raise SCFA levels. In animals, inulin-type fibers improve survival after pathogen challenge[21,22] even in the face of antibiotics.[18,23-25] In a randomized trial of 30 outpatients, inulin increased fecal levels of SCFA producers by 35-90%, with a 3-fold decrease of serum markers of bacterial translocation (e.g., lipopolysaccharide) relative to placebo.[26] Inulin is safe in ambulatory patients[27-35] with biologic effects seen at 16 g/day.[26] In the ICU, inulin-type fibers have been given up to 36 g/day without adverse events[36] and in our ICU cohort we have observed 30 g/day dietary fiber without intolerance. This trial will test whether inulin supplementation has similar effects in the ICU as it does in animal models and as it appears to in healthy volunteers: will it result in higher levels of SCFA-producing Clostridia, decreased gastrointestinal antibiotic resistance and lower risk for MDR infections?

5.2 Trial purpose and design

The purpose of this Phase 2 trial is to determine the feasibility and safety of inulin for the prevention of antibiotic resistant infections or colonization in critically ill patients. We will perform a double-blind, randomized controlled trial of inulin in the ICU with three arms (placebo, inulin 8 g twice daily, and inulin 16 g twice daily). Subjects will be treated with inulin or placebo for 7 days with bedside follow-up for 30 days or hospital discharge and with long-term telephone follow-up for 6 months.

6. Previous data

Our observational ICU data supports the importance of SCFA producers in the ICU. In animals, inulin protects against sepsis. Inulin increases SCFA producers in ambulatory studies and inulin-like fibers are safe in the ICU setting.

6.1 Evidence supporting the importance of SCFA producers in the ICU

We have assembled two large prospective cohorts now totaling over 500 ICU patients who have contributed rectal swabs and other data.[37-39] Results from these cohorts confirm the importance of SCFA producers in protecting against pathogen colonization and infection in the ICU.

- ***Cohort #1***.[40] A single rectal swab was performed on medical ICU admission for consecutive patients. Swabs were cultured for VRE and residual DNA was extracted for 16S rRNA gene sequencing; patients were then followed for up to 30 days for death or culture-proven infections. In 9 months, **301 patients** were enrolled of whom 91 (30%) were VRE colonized. Thirty-day mortality was 25% and an overlapping 21% patients developed culture-proven MDR infections.

Cohort #1 demonstrated that the bacteria causing infections in the ICU are present as gut colonizers at the time of admission. We tested whether colonizing MDROs predicted subsequent death or culture-proven infection. Presence of the colonizing organism at ICU admission was almost always associated with subsequent organism-specific infection, even after adjusting for

acute severity illness as captured by the Simplified Acute Physiology Score-3 (SAPS-3)[41,42] and other relevant clinical variables. This was true for VRE (aHR 5.85, 95% CI 2.25-15.2, 16 infections), *C. difficile* (aHR 19.0, 95% CI 4.40-81.8, 9 infections), *Pseudomonas* (aHR 2.37, 95% CI 1.04-5.43, 28 infections), and *Klebsiella spp*. (aHR 3.81, 95% CI 1.10-13.2, 28 infections). To interrogate the process by which MDRO colonization may lead to infection, we also examined MDRO domination defined as ≥30% 16S reads corresponding to a potential pathogen.[43,44] Because this cohort contained VRE culture data, we were able to test whether patients who had *Enterococcus* domination were in fact colonized by VRE. *Enterococcus* domination (15% of the cohort) and VRE colonization (30%) both predicted *all-cause* infection (**Figure 1**). When 30-day mortality was examined as a stand-alone outcome, VRE colonization/domination predicted 40% increased mortality, after adjusting for SAPS-3 score and other factors.

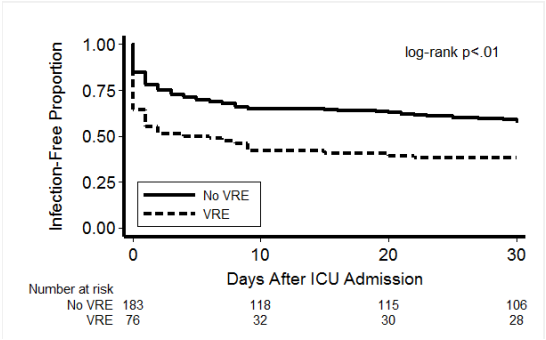


Figure 1. VRE colonization/domination was associated with death or culture-proven infection in the ICU. VRE colonization or domination was assessed at the time of ICU admission and patients were followed for all-cause culture-proven infection.

Next, we sought to determine the relationship between SCFA producers and VRE based on culture at the time of ICU admission. Only VRE was cultured within this cohort, so we did not have the ability to assess colonization with other MDROs using culture (e.g., Gram negative bacteria). Based on the 16S sequencing results, SCFA producers were classified as absent (<.01% relative abundance), low (.01% to 1%), or high (>1%) after Haak *et al*.[45] High levels of SCFA producers were associated with lower prevalence of VRE colonization based on culture: 40% when SCFA producers were absent, 27% when they were low, and 22% when they were high (p<.01). Results were similar for *Enterococcus* domination.

- ***Cohort #2***.[37,39] This cohort was designed to examine *dynamic* changes in the ICU microbiome, focusing on SCFA producers and MDROs. Swabs were performed in duplicate at ICU admission and 72 hours later (±4 hours). Swabs were then (1) cultured for MRSA, VRE, and MDR Gram negatives and (2) 16S sequenced. In 3 years, **230 patients** out of a target of 300 have been enrolled with 126 sequenced to date.

Cohort #2 demonstrated that SCFA-producing bacteria associate with preserved colonization resistance. Within cohort #2, we first performed an unbiased analysis using LEfSe[46] to ask which bacterial taxa changed most within individuals, comparing their admission and Day 3 samples. 14 taxa were identified as having significantly changed at a false-discovery adjusted p<.05. Ten taxa decreased over these first 72 hours of which 6 were SCFA producers. These changes were substantial in magnitude (e.g., 8-fold for *F. prausnitzii*).

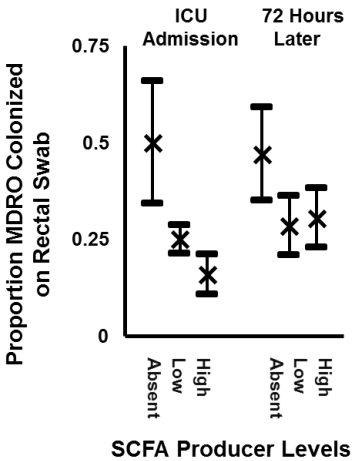


Figure 2. Higher levels of SCFA-producing bacteria associated with lower colonizing MDROs, both at the time of ICU admission and 72 hours later. Lines are 95% confidence estimates.

We wanted to know how SCFA producers associated with MDRO colonization. SCFA producers were again classified as absent, low, or high based on sequencing. MDROs were classified as present vs absent based on culture results. At both ICU admission and 72 hours later, there was a stepwise relationship

where higher SCFA producers was associated with decreased MDRO colonization (**Figure 2**, previous page). Comparing high to absent SCFA levels, the difference in rates of MDRO colonization was over 25%.

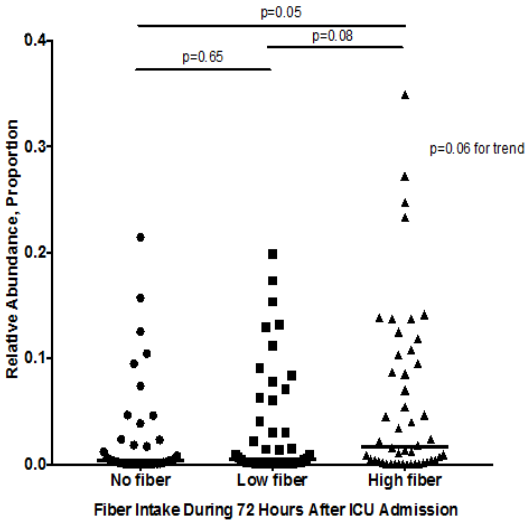


Figure 3. Higher fiber intake associated with higher levels of SCFA producers in the ICU. Fiber intake was classified into tertiles, with relative abundance of SCFA producers classified based on 16S sequencing.

Last, we sought to retrospectively assess the impact of dietary fiber on SCFA producers and MDROs. Observed fiber intake was low overall with a mean of 13.4 g over 72 hours, but some patients did receive large amounts of fiber (maximum 30 g/24 hours). There was no intolerance of fiber based on documentation of abdominal distension, diarrhea, or nausea/vomiting. Fiber intake was classified into tertiles and SCFA producers were examined as a continuous measure based on 16S sequencing. At ICU admission, baseline relative abundance of SCFA producers was similar across fiber intake groups. After 72 hours, higher fiber intake was associated with higher levels of SCFA producers (**Figure 3**). Higher fiber was also associated with less *Enterococcus* (median abundance .08% high fiber vs .35% no fiber, p=.03). This data is observational so there were baseline patient differences between those who did and did not receive high dietary fiber. To account for this, we built a multivariable model adjusted for acute severity of illness, receipt of antibiotics, and total caloric intake. In this model, there was a 0.30% median increase in SCFA producers after 72 hours per additional 10g of fiber intake (p<0.01). This suggests that dietary fiber has the potential to impact levels of SCFA producers, even during critical illness and with receipt of broad-spectrum antibiotics (78% of the cohort).

6.2 Inulin in animal models

Animal models show protective effects of inulin or similar compounds, which appear to be mediated by an increase in SCFA producers.[47,48] Importantly, these studies demonstrate that inulin is likely to be effective even when given concurrently with antibiotics. Animal models include cecal ligation and puncture (CLP),[21] lipopolysaccharide (LPS) injection,[22] challenge with *Citrobacter rodentium*[18] or pathogenic *Escherichia coli* strains,[47] and *Salmonella typhimurium* challenge with[23] or without antibiotics.[24,25] All models have their strengths and weaknesses, so it is remarkable that results align well across them. Inulin and similar fructo-oligosaccharides (FOS) seem to confer protective effects through multiple mechanisms all of which likely depend on SCFA fermenters. First, they maintain the protective colonic mucus layer. Inulin fermenters activate carbohydrate metabolism pathways and switch fuel utilization from dietary fiber to colonic mucus when inulin-like fibers are unavailable, causing thinning of the colonic mucus layer.[18,23] Second, the bacteria nourished by inulin have direct antagonistic effects on pathogens[25] by making protonated SCFAs which diffuse across Gram negative cell walls to delay pathogen growth.[24] Luminal acidification by SCFAs also drives broad microbiome shifts towards relatively pH-tolerant, Gram positive obligate anaerobes.[49,50] Third, SCFA producers induce host immunity. When SCFA-producing anaerobe levels increase during sepsis, there is induction of T regulatory cells (Tregs), reduction in activated dendritic cells, and downstream decreased DNA binding of NF-kB for an overall reduction in pro-inflammatory cytokines and reduced T cell anergy.[22] In these animal studies, the protective effect is large, with dramatic differences in outcomes including weight loss, colitis severity, and mortality.

6.3. Inulin in the ambulatory setting

Inulin has been studied in over 400 ambulatory patients where it has shown excellent safety and tolerability.[27-35] No serious adverse events have been reported in trials using doses up to 34 g/day inulin[51] or 55 g/day mixed fiber,[16] with self-resolving bloating the main side effect. Three trials specifically examined outcomes related to the ability of inulin to prevent colonization or infection. First, Dewulf *et al*. randomized 30 patients to inulin 16 g/day vs placebo; the inulin group had a doubling of *F. prausnitzii* and other SCFA-producers and concurrent decreases in serum CRP and LPS concentration.[26] Second, Lecerf *et al*. randomized 60 volunteers 1:1:1 to placebo, a combination xylo-oligosaccharide (XOS), or inulin-XOS.[52] The inulin group had the highest post-treatment SCFA levels and butyrate levels, the lowest circulating LPS, and decreased gene expression for the pro-inflammatory cytokines IL-1β, TNFα, and IL-12. Third, Miguez *et al*. found that an inulin-like fructo-oligosaccharides (FOS) preserved gut microbiome diversity after broad-spectrum antibiotics, mitigated loss of SCFA producers, and increased butyrate levels 3-fold.[53] **Table 1** lists selected studies of inulin in the ambulatory setting, focusing on those with results most relevant to this project.

Table 1. Selected ambulatory trials testing inulin alone or in combination

Author	Subjects	Intervention	Effect	AEs
Kruse[51]	8 healthy volunteers	Inulin 34 g/day	Increased *Bifidobacteria*, unchanged SCFA levels	Self-resolving bloating and flatulence.
Dewulf[26]	30 obese women	inulin 16 g/day (50% FOS enriched) x 3 months	Increased *Bifidobacteria* and *F. prausnitzii* and decreased LPS.	Self-resolving bloating and flatulence.
Lecerf[52]	60 healthy volunteers	inulin 3g/day plus 1g/day XOS	Increased fecal SCFAs, decreased LPS, attenuated LPS-induced serum changes in IL-1b and IL-13.	Self-resolving bloating and flatulence, small decrease in general well-being.
Azpiroz[27]	36 with IBS	inulin 8g/day x 4 weeks	Increased *Bifidobacteria*.	None reported.
Canfora[29]	44 obese pre-diabetic adults	inulin 15g/day x 12 weeks	Increased *Bifidobacteria*.	None reported.
Garcia-Peris[30]	31 adult women with gynecological cancer	inulin 12g/day x 4 weeks (50% FOS enriched)	Increased in *Lactobacillus* and *Bifidobacteria*.	Self-resolving bloating.
Holscher[31]	29 healthy adults	inulin 7.5g/day x 21 days (agave-derived)	Increased *Bifidobacteria*.	None reported.
Petry[32]	32 obese female adults	inulin 20 g/day x 4 weeks	Increased *Bifidobacteria*.	None reported.
Rahat-Rozenbloom[54]	25 healthy obese or lean adults	inulin 24 g/day x 3 days	Increased serum SCFAs.	None reported.
Ramirez-Farias[33]	12 healthy adults	inulin 10g/day x 16 days	Increased *Bifidobacteria* and *F. prausnitzii*, no change in fecal SCFAs.	None reported.
Vandeputte[35]	42 healthy adults	inulin 12g/day x 4 weeks	Increased *Bifidobacteria*.	None reported.

6.4 Inulin-like fibers in the ICU

Inulin has not been studied in the ICU but data can be extrapolated from ICU trials of similar fructo-oligosaccharides (FOS). To date, there are no reports of serious adverse effects or major intolerance with over 1,000 patients studied.[55] Doses have been used up to 36 g/day for 5 weeks.[36] This emerging safety data is robust enough that consideration of supplemental fiber has

been incorporated into relevant ICU nutrition guidelines.[56] Data on the effects of inulin-like FOS on infections in the ICU is scarce but promising. Three double-blind, randomized ICU trials tested a commercial synbiotic containing *Lactobacillus spp*. and mixed soluble prebiotic fiber including some inulin (up to 20 g/day total).[57-59] In these studies, the overall rates of ICU-acquired infections were 16-27% lower in the group receiving prebiotic fiber, although MDR infections were not separately examined. In an open-label trial of mixed fiber up to 24 g/day in 30 critically ill adults, the median hospital length of stay was 5 days shorter in the fiber group and the overall rate of complications, including feeding-related complications was lower.[60] This provides important reassurance given the concerns occasionally raised regarding prebiotics in patients with altered small bowel or colonic motility.[61,62] **Table 2** lists selected studies of fiber supplementation in the ICU, focusing on those with results most relevant to this project

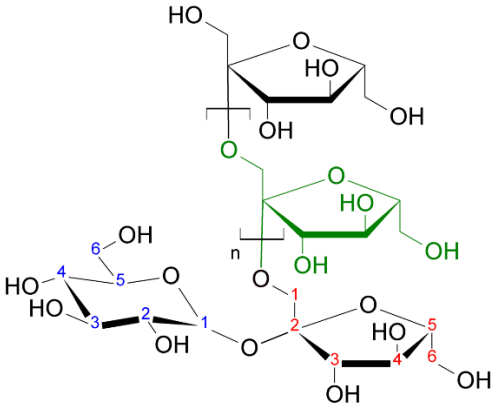


Table 2. Selected ICU-based trials testing inulin or similar fibers, ordered by decreasing amounts of fiber.

Author	Subjects	Intervention	Effect	AEs
O'Keefe[36]	13 ICU patients (most with pancreatitis)	32 g/day wheat dextrin up to 5 weeks	Higher fecal SCFA producers and SCFAs.	None reported.
Karakan[60]	30 ICU patients with pancreatitis	24 g/day mixed soluble and insoluble fiber (open label)	Shorter hospital stays and lower complications.	Self-resolving bloating.
Knight[58]	259 ICU patients on ventilation	20 g/day mixed fiber synbiotic including 5 g/day inulin	Trend towards lower rates of VAP.	None reported.
Kotzampassi[57]	65 ICU patients with SIRS	20 g/day mixed fiber synbiotic including 5 g/day inulin	Fewer infections and less diarrhea.	None reported.
Majid[63]	22 adults in the ICU	inulin 7g/day	No difference in *Bifidobacteria* and lower *F. prausnitzii*.	None reported.
Spapen[64]	25 ICU patients with septic shock	22 g/L guar fiber enteral feeding, dose individualized	Less diarrhea.	None reported.
Spindler-Vesel[59]	113 ICU trauma patients	17 g/day average mixed soluble fiber-synbiotic	Fewer infections.	Higher gastric residuals.

6.5 Summary of previous experience with inulin

Our prospective ICU cohort data provides real-world data supporting the hypothesis that inulin, by nourishing SCFA producers, will lead to decreased pathogen colonization and infection in the ICU. Animal studies show that inulin confers protection across various pathogen challenge models and sepsis models including LPS injection and cecal ligation-and-puncture. Ambulatory studies of inulin demonstrate safety and generally show increases in SCFA producers related to inulin. Few ICU studies have tested inulin, but there is ample ICU experience with inulin-like fibers that provides reassurance that high doses of fiber are safe even in the context of sepsis and hypotension in the ICU.

7. Study objectives

The short-term goal of the study is to test inulin for the prevention of MDRO colonization and infection in the intensive care unit. The long-term goal of this research is to develop antibiotic-free strategies that prevent or ameliorate ICU-acquired infections.

8. Study Design

This will be a double-blind, randomized, placebo-controlled trial with two doses.

9. Intervention

Block randomization (blocks of six) will be performed with 1:1:1 random computer allocation of subjects into the three study arms. In the two inulin arms, chicory root-derived inulin (Cargill Inc., Wayzata, MN) will be diluted in 250 ml of sterile water and given at 8 or 16 g twice daily (**Figure 4**). The placebo arm will receive the same volume of sterile water twice daily, with the intervention given for 7 days or until death or hospital discharge. Prior studies suggest that these doses are likely to be safe and sufficient to confer benefit,[26,36] even in the setting of critical illness.[57,58,63,65] Subjects, investigators, and the treating ICU team will be blinded to the intervention arm, with blinding managed by the CUMC Research Pharmacy which will provide labeled, de-identified, semi-opaque fluid bags containing inulin/placebo in accordance with the U.S. Code of Federal Regulations governing labelling of new investigational drugs (21CFR 312.6). The intervention will be taken orally or given with enteric feeding by nursing staff overseen by the study team, with the first dose administered within 6 hours of ICU admission. The time course for the intervention and remaining study assessments is shown below in **Table 3**.

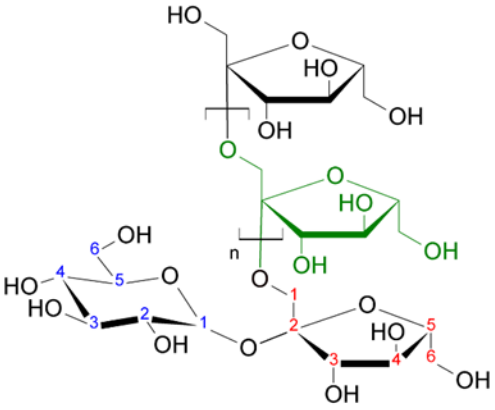


Figure 4. Chemical structure of inulin. Inulin is a naturally occurring polysaccharide derived from chicory root, agave, garlic, and other vegetable sources; it is a long chain carbohydrate of fructose polymers with β(1,2) glycosidic linkages which are indigestible by human enzymes but fermented by colonic anaerobic bacteria into short-chain fatty acids (chemical formula: $C_{6n}H_{10n+2}O_{5n+1}$).

Table 3. Time course of intervention and main assessments during the study.

	Inulin---------->			Monitoring---------->			
Type of Assessment	**Day 0**	**Day 3**	**Day 7**	**Day 14**	**Day 30**	**3 months**	**6 months**
Rectal swab	**X**	**X**	**X**	**X**	**X**		
Whole stool	**X**	**X**	**X**	**X**	**X**		
Clinical data	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Telephone follow-up						**X**	**X**

9.1. Storage, accountability, and dispensing

The study medication will be delivered to the CUMC Research Pharmacy as a powder via Swanson/Cargill protected from moisture and light and accessible only to authorized individuals. The medication will be stored at 20-25ºC in powder form and reconstituted before dispensation. Medication labels will be blinded. The Research Pharmacy will maintain a record of the inventory and disposition of all medication and placebo. This receipt record will include from whom the medication was received, to whom it was distributed, the date, quantity, and lot number. The study medication has the following characteristics (**Table 4**).

Table 4: Study medication characteristics.	
Product name:	Inulin powder
Manufacturer:	Cargill Inc., 15407 McGinty Rd W, Wayzata MN 55391-2365
Distributor:	Swanson Health, 4075 40th Ave SW, Fargo, ND 58104-3912
Appearance:	White powder
Flavor:	Neutral to sweet
Bulk density:	0.6 g/mL
Solubility:	>100 g/L
Moisture:	3.41%
Dry stability:	4 years

9.2. Rationale for the selection of duration and dose.

9.2.1. Rationale for duration. Inulin will be given for 7 days with the primary outcomes adjudicated after 3 days. The Day 7 data will be used to test whether an effect can be extended or whether a delayed effect is present. In our cohort data, 84/126 (67%) new infections occurred within 3 days and 97/126 (77%) within 7 days.[38] This shows that any ICU-based intervention seeking to prevent infections must work quickly if it is to be effective. Meanwhile, our preliminary cohort data suggests that fiber can have an impact even within this short timeframe (Figure 3). A final assessment will be made on the day of hospital discharge in subjects who are to be discharged prior to Day 14.

9.2.2. Rationale for dose. In the ambulatory setting, inulin impacts SCFA producer levels at doses of 12 to 16 g/day, with biologic effects relevant for ICU patients (e.g., decreases in serum LPS and CRP).[26,66] Approximately 16 g/day therefore represents the minimum dose at which an effect *might* be seen; this was selected for the low dose arm of the study. However, patients in these ambulatory studies were not critically ill and did not receive antibiotics. It is possible that inulin will have lesser effects in the ICU compared to the ambulatory setting, and logical to test higher doses. To select a maximum study dose, we referenced our own experience with dietary fiber in the prospective cohort and published tolerability data from ICU studies of inulin-like prebiotic fiber. In our prospective ICU cohort, 30 g/day was the maximum observed dose of dietary fiber, and was not associated with any intolerance. In published literature, O'Keefe *et al*. reported tolerance up to a maximum of 36 g/day wheat dextran in the ICU.[36] We therefore selected 32 g/day for the high dose study arm because it lies between these values and is exactly double a dose with proven biologic activity.

9.3. Regulatory

No subjects will be enrolled until after review and approval by the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO). The PI holds FDA IND [redacted] (FDA date: September 4, 2018) for inulin for the prevention of MDRO colonization/infection in the ICU or other at-risk settings. Safety and adverse effects will be overseen by the PI in conjunction with Daniel Brodie, the Director of Medical Intensive Care Units at our institution, who will serve as the Data Safety Monitoring Officer (DSMO) and will convene an appropriate Data Safety Monitoring Board (DSMB) for the study. Before initiating research, the study will be registered on clinicaltrials.gov.

9.4. Documentation of administration of the intervention

The study team will work with the treating ICU team to ensure that the study drug is delivered in a timely manner. The time and dose of each medication administration will be documented at each study assessment by referring to the documentation of medication administration within the

electronic medical record (EMR). Patients will be enrolled with a goal of administering the intervention within 24 hours of ICU admission and it will be considered a protocol deviation if the intervention is administered more than 48 hours from ICU admission.

10. Outcomes

Primary and secondary outcomes focus on the comparison of baseline to Day 3 samples. This project will also gather samples at ICU Days 7, 14, and 30. These additional samples will be used to construct longitudinal time series data and test for differences between groups that may be slower to emerge (e.g., baseline vs Day 7 comparisons).

10.1. Primary outcome: *Relative abundance of SCFA producers through ICU Day 3*. The within-individual change in the relative abundance of SCFA producers will be determined by 16S rRNA gene sequencing of rectal swabs and compared between intervention groups. From extracted rectal swab DNA, polymerase chain reaction (PCR) will be performed on a Bio-Rad T100 Thermal Cycler (Bio-Rad, Hercules, CA) to amplify the V4 hypervariable region of the 16S ribosomal RNA gene with primers derived from Klindworth *et al*.[67] appended with overhang sequences for compatibility with Illumina index and sequencing adapters (Illumina, San Diego, CA). The relative abundance of SCFA producers levels will be determined as the sum total across the bacterial species known to ferment the SCFA butyrate and subtracted within each individual to obtain the change (Day 3 minus baseline).[39,45,68-70]

10.2. Secondary outcome: *MDRO colonization status on ICU Day 3*. MDRO colonization status will be based on Day 3 rectal swab cultures, classified categorically and compared between intervention groups. MDROs will be defined as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and MDR Gram negative bacteria[71] (defined as Gram negatives with *in vitro* non-susceptibility for third-generation cephalosporins).[72] Based on culture results, Day 3 MDRO colonization status will be classified categorically and compared between groups.

10.3. Additional outcomes:

10.3.1. *Feasibility*. Descriptive demographic and clinical data will be compared across study groups to assess adequacy of randomization. Summary statistics (count data) will be recorded for patients across all 3 intervention arms to assess feasibility as (1) the enrollment rate (enrolled)/(enrolled+refusals) over each 12 month period; (2) protocol adherence (proportion of randomized subjects who receive 1 or more doses of the allocated intervention); and (3) fidelity of the intervention (defined as the proportion of high/low inulin allocated vs received and also categorically as receipt of 90% or more allocated doses of inulin across groups). Feasibility goals are as stated in Aim 1.

10.3.2. *Rates of pre-specified AEs*. There will be active ascertainment of AEs in the following categories, which will be compared between intervention groups. This will be done through the end of the intervention period (ICU Day 7) and also through Day 30/discharge to assess for delayed AEs.

- Stool frequency, measured by subject or nursing report by the number of spontaneous stools over the 24 hours preceding each assessment;

- Stool consistency, measured by subject or nursing report as an ordinal variable (0-4) over the 24 hours preceding each assessment;

- Abdominal bloating, measured by subject or nursing report as an ordinal variable (0-3) over the 24 hours preceding each assessment.

- Electrolyte abnormalities, measured by examining for differences in median values, minimum/maximum values, and proportions of values outside the normal range. This will be done for glucose, calcium, magnesium, phosphate, potassium, and sodium.

- Nausea, vomiting, obstruction, and abdominal pain. These will be recorded as present or absent based on bedside assessment of the subject or based on the nursing impression.

- Culture-proven infections, which will be considered both an AE and an outcome (see 10.3.4).

10.3.3. *Antibiotic resistance gene fraction on ICU Day 3*. DNA will be extracted from rectal swabs (MoBio PowerFecal, Carlsbad, CA) and assessed using multiplex quantitative PCR (Qiagen Cat. No. 330261, Valencia, CA) for 87 common antibiotic resistance genes.[73] Within-individual differences in multiplex qPCR results will be compared from baseline to Day 3 both as summed changes and within relevant gene categories (e.g., β-lactamase resistance genes) and compared between intervention groups.

10.3.4. *Culture-proven MDR infections through ICU Day 30*. Antibiotic-resistant infections will be defined as those showing all of the following: (1) site-specific symptoms or signs of infection, (2) initiation of targeted antimicrobials by the treating ICU team, and (3) molecular diagnostics or cultures showing an MDRO as adapted from the CDC and NHSN[74] and previously used by our group (**Table 4**).[40] Rates of infections will be compared between intervention groups as a sum total, and separately for 3 distinct periods: the intervention period of Day 0 to 7, the Day 7 to 30 period, and the Day 30 to 6 month period.

Table 4. Criteria used to adjudicate culture-proven MDR infections for. Criteria based on CDC/NHSN guidelines.

Criteria	Operationalization	Example
1. Site-specific symptoms or signs of infection.	• Pulmonary: documentation of new or worsening cough or imaging consistent with an infection • Urinary: fever, dysuria, increased urinary frequency, flank pain, mental status changes, or hypotension and clinical exclusion of other causes for infection. • Bloodstream: clinical evidence of a bloodstream infection (fever, chills, or hypotension) without alternative sources for infection • Stool: documentation of new loose stools or frequent stools ≥3 per 24 hours • Wound: appropriate surgical site incision or wound with discharge, warmth, or erythema.	Cough and a chest X-ray showing new infiltrate.
2. Positive cultures or other diagnostics with an MDRO.	• Pulmonary: quantitative or semi-quantitative sputum or bronchoscopic cultures • Urinary: ≥10^5 CFU/mL growth. • Bloodstream: growth of a recognized bloodstream pathogen from blood but not another site or growth of a NHSN commensal from blood but not another site.	Sputum culture growing >10^5 CFU/ml of *K. pneumoniae* resistant to ceftriaxone.

	• Stool: positive PCR for the *C. difficile* toxin B gene (Cepheid or BioFire) or positive toxin B EIA • Wound: growth of aseptically obtained organisms.	
3. Initiation of targeted antibiotics within 24 hours.	Antibiotics showing non-susceptibility using standard CLSI breakpoints or appropriate empiric antibiotics initiated at the time of clinical suspicion/testing and continued for ≥3 days or initiated within 24 hours following the availability of diagnostic testing showing a culture-proven infection.	Initiation of ceftriaxone when the X-ray was ordered; later changed to meropenem.

10.3.5. *Fecal SCFA levels*. The SCFAs butyrate, acetate, and propionate will be measured from whole stools on a 6490 triple quadrupole mass spectrometer and compared between intervention groups using the first stool sample produced after the Day 3 assessment. Patients who fail to produce a stool from Day 3 to 7 will be excluded from this analysis.

10.3.6. *Proportion of goal calories through ICU Days 3 and 7*. Proportion of goal calories consumed will be defined by manually extracting data from electronic nursing flow sheets. Intake during the intervention be calculated by multiplying enteral or oral feeding caloric content by the flow rate or percentage of meal consumption. A patient-specific calorie target will be calculated by a registered clinical dietician using the Mifflin-St. Jeor equation and the Penn State 2003b and 2010 equations, taking into consideration age, body mass index, ventilation status, and comorbidities.[75] The proportion of goal calories consumed will then be calculated as observed nutritional intake divided by calorie target. Day 3 and Day 7 proportions will be compared between intervention groups.

10.3.7. *Length of ICU stay*. Length of ICU stay through Day 30 will be compared between treatment groups, with Fine and Grey methods used to adjust for death as a competing risk. Length of ICU stay will be measured from the first to the last set of recorded ICU vital signs.

10.3.8. *Length of hospital stay*. Similarly, length of hospital stay through Day 30 will be compared between treatment groups, again adjusting for death as a competing risk. Length of hospital stay will be measured from the first set of recorded ICU vital signs to the last set of hospital recorded vital signs.

10.3.9. *Mortality through ICU Day 90*. Death data will be extracted from the hospital EMR which immediately captures in-hospital death and receives monthly mortality uodates from the National social security death index. Kaplan-Meier methods will be used to compared 30- and 90-day mortality between treatment groups.

10.3.10. *Self-assessed quality of life at 6 months*. Subjects or their caregivers will complete the EQ-5D-5L[76-78] and Katz ADL questionnaires[79,80] to test their quality of life at 3- and 6 months post ICU admission. Composite data will be compared between intervention groups.

10.3.11. *Time series data including Day 7 outcomes*. Time series data will be built and visualized for all relevant outcomes. We will explore the possibility of a delayed effect by testing for differences between groups from baseline to Day 7. If there is an observed effect, the Day 14 and Day 30 data will allow us to assess its durability.

11. Study population

11.1. Study setting

Patients will be enrolled from medical ICUs affiliated with Columbia University Medical Center. These ICUs include but are not limited to the following locations, all of which are in the general vicinity of Columbia University's medical campus:

ICU short designation	Full name	Hospital building, floor location	Street address
MICU-A	Medical ICU-A	Milstein, 4th floor	177 Fort Washington Ave, NY, NY
MICU-B	Medical ICU-B	Milstein, 4th floor	177 Fort Washington Ave, NY, NY
NICU	Neurological ICU	Milstein, 8th floor	177 Fort Washington Ave, NY, NY
AICU	Allen ICU	Allen Pavilion, 2nd floor	41 Broadway, NY, NY

The study will enroll 90 consecutive adult ICU patients who meet criteria for sepsis, are receiving appropriate antibiotics, and can complete a bedside assessment within 4 hours of ICU admission and receive the trial intervention within 6 hours of ICU admission. A replacement strategy will be used. If subjects are randomized but fail to receive the intervention or complete the Day 3 assessment, an additional subject will be enrolled to take their place with a final target enrollment of 30:30:30 patients who complete a minimum of 3 days of assessments. Specific inclusion and exclusion criteria are as follows:

11.2. Inclusion criteria

- Medical ICU. Subjects will be newly admitted to one of two CUMC medical ICUs during the study enrollment period;

- Age. Subjects will be eighteen or more years old at the time of ICU admission;

- Sepsis. Subjects will meet criteria for sepsis with life-threatening organ dysfunction caused by infection (Sepsis-3 third international consensus definition, 2016).[81] According to this definition, life-threatening organ dysfunction is operationalized as an increase in the Sequential [Sepsis-Related] Organ Failure Assessment (SOFA)[82] score of ≥2 points above baseline. Sepsis-3 criteria also require patients to have a known or suspected infection, defined as sampling of blood or any other body fluid for infection with concurrent administration of appropriate antibiotics (see Table 4);[81]

- Appropriate antibiotics. Subjects will receive appropriate antibiotics, which will be operationalized as (1) among subjects with positive culture data, the antibiotics within classes where the organism is susceptible based on standard Clinical and Laboratory Standards Institute (CLSI) cut-offs[72]; *or* (2) among subjects receiving empiric antibiotics (i.e., with negative cultures), antibiotics within classes with significant anaerobic activity. This will include β-lactam/β-lactamase inhibitor combination antibiotics, cephalosporins (generation 2 or greater), fluoroquinolones, lincosamides (clindamycin), metronidazole, and monobactams (a.g., meropenem). Vancomycin monotherapy is intentionally excluded. Antibiotics must be received within 24 hours before ICU admission or be actively ordered at the time of bedside screening;

- Prompt intervention. Subjects will complete study enrollment within 4 hours of ICU admission so that the intervention can be administered within 6 hours of ICU admission. The first set of recorded ICU vital signs will define the time of ICU admission.

11.3. Exclusion criteria

- Inability to receive oral or enteric fluids. Patients will be excluded if they are *nil per os* or are unable to take fluid by mouth or delivered via enteric nasal or oral tube because they would be unable to receive the study intervention;

- Inulin allergy. Patients will be excluded for safety reasons if they have a known chicory allergy. Serious chicory allergies are exceedingly rare although mild respiratory symptoms and IgE-mediated food allergies have been reported;[83]

- Hyponatremia. Patients will be excluded for hyponatremia with serum sodium ≤128 mEq/L because the intervention will be delivered in 500 ml of free water daily. If subjects develop hyponatremia (serum sodium serum sodium 125-128 mEq/L) after enrollment and randomization which persists at the time the subject is due to receive the study intervention, the study team will confer with the treating ICU team regarding whether the intervention should be deferred or further concentrated in order to minimize free water. If subjects develop severe hyponatremia (serum sodium serum sodium ≤124 mEq/L) after enrollment and randomization, the intervention will be withheld until 2 consecutive serum sodium values are within normal limits;

- Immunosuppression. Patients will be excluded if they have immunosuppression, as CD4 count <200 or absolute neutrophil count <500 or reasonable expectation of either during the study intervention period. We believe that the inulin works by modifying the gut bacteria. Excluding patients who lack the ability to mount an immune response improves trial homogeneity;

- Surgery involving the intestinal lumen. Patients will be excluded for surgeries involving the intestinal lumen within 30 days. The most common side effects from inulin are subjective bloating and abdominal distension.[26,28,30,52] Although this bloating appears to be mild and self-limited, patients with recent bowel surgeries may be at greatest risk for such symptoms and will therefore be excluded;

- DNR or DNI status. Such patients have limited treatment goals and will be excluded to minimize heterogeneity;

- No LAR. Patients will be excluded if they lack capacity for consent and have no appropriate Legally Authorized Representative (LAR) to act as surrogate decision-maker.

12. Enrollment strategy

Patients will be identified at the time of ICU bed allocation and screened for inclusion/exclusion criteria. After consultation with the treating ICU team, subjects who meet screening criteria will have the eligibility evaluation completed at the ICU bedside. This will be done after first obtaining agreement from the medical ICU attending of record that the patient/surrogate can be approached for the study. We anticipate that >50% of patients will lack decision-making capacity and will accept consent from a surrogate in these situations, as previously described by our group.[37] Issues related to altered capacity, privacy and time for decision-making, consent of LARs, and ongoing consent are discussed in detail in the **Human Subjects and Recruitment and Safety**

Procedures section. We expect that patients will usually be initially enrolled with surrogate consent. Patients who are on study will then be reassessed for capacity at each interaction, with the final determination of capacity made by the treating ICU team. When subjects regain capacity, they will make their own determination of whether or not to continue in the study. If subjects do not regain capacity by the time of hospital discharge, surrogate consent will be accepted as final. This approach, which we have used before,[37-39] preserves complete patient autonomy while recognizing that the target population for the study is unlikely to have decision-making capacity at the time of ICU admission.

Compliance statement regarding consent
This research will meet the requirements of **10 U.S. Code 980** for experimental human subjects research that "(1) the informed consent of the subject is obtained in advance; or (2) in the case of research intended to be beneficial to the subject, the informed consent may be obtained from a legal representative of the subject."

Retention
Potential loss of patients after randomization will be from death/discharge prior to ICU Day 3, or withdrawal of consent (e.g., after initial surrogate consent). In our ongoing ICU cohort studies, there has been 11.3% mortality within 3 days and a 0% Day 3 discharge rate. Additional patients will be enrolled for patients who do not complete the Day 3 assessment due to death or discharge. Thus, we expect to enroll 102 patients to obtain 90 who are able to donate a Day 3 sample and therefore can included in the intent-to-treat analysis.

12.1. Randomization and blinding
Randomization and data entry will take place through a secure, web-based system managed by the CUMC Data Coordinating Center (DCC) headed by Howard Andrews. This password-protected system is based on the Research Electronic Data Capture (REDCap) platform. The Research Nurse will use paper clinical research forms (CRFs) to document the initial screening process. When this information is entered into REDCap, the system will automatically generate a subject identifier and randomization code using 1:1:1 randomization in randomly permuted blocks of 6. This randomization code will be electronically communicated to the research pharmacy along with a separate key which will allow them to identify the random assignment. The Columbia Research Pharmacy will prepare the study intervention in indistinguishable semi-opaque fluid bags. Although inulin is readily soluble at the study doses, the opacity of these bags will eliminate any possibility that a small amount of precipitant might reveal the study assignment to the ICU nurses who deliver the intervention. Five drops of non-caloric flavoring syrup (McKesson Supply, Yonkers, NY, Product #570727) will be placed into the reconstituted inulin/placebo to prevent any possibility of accidental unblinding based on taste in patients who are alert. The patient, treating ICU team including the ICU nurse, and study team will remain blinded to each assignment until the completion of the trial, with the DCC preparing unblinded safety reports for the DSMO at pre-specified intervals.

12.1.1. Clinical need for unblinding
It seems unlikely that clinical need for unblinding will arise. If this occurs, the PI will coordinate with the DCC to facilitate unblinding for the treating ICU team. In any case of unblinding, data collection and follow-up will be maintained and the patient analyzed per-protocol.

12.2. Delivery of the intervention
The treating medical team will order the study intervention as a non-formulary medication to be delivered by ICU nurses, as they would any other medication, and the study team will work to ensure prompt delivery of the intervention with a goal of having it delivered within 24 hours from

the first set of ICU vital signs. Delivery of the intervention will be managed by ICU nurses who are not part of the study team by encouraging the subject to drink the liquid formulation or, in subjects who are not taking oral medications, by attaching the intervention to the patient's oral or nasogastric tube. The time of dosing will be recorded electronically within the patient EMR and documented by study staff on the CRFs and within REDCap.

12.3. Premature cessation of the intervention

Efforts will be made to ensure that all subjects who are randomized receive the study intervention. The initial medication order will specify that the study intervention is to be given q12h for a total of 14 doses. The study intervention will be stopped under the following conditions:

- Death;
- Discharge from the hospital;
- Request to withdraw from the study by the patient or surrogate. This request can be made at any time, without providing a reason. Consent to continue data collection and retain samples already collected will be sought in these situations;
- Definite clinical indication or contraindication becomes apparent. If the treating ICU team feels at any time that the patient needs or should not have fiber supplementation, the intervention will be immediately halted. The patients will remain in the study for follow-up and the intervention will be held as long as the indication/contraindication remains. In general, if the indication/contraindication resolves within the 7 day timeframe of the intervention, the intervention will be resumed. If the intervention is withheld for hyponatremia, the intervention will be resumed only once 2 consecutive serum sodium values are within normal limits;
- Adverse or serious adverse event related to the study intervention. Such events will constitute unanticipated problems. The patient will remain in the study for follow-up, but no further interventions will be administered.

13. Study assessments

The total study duration will be no more than 30 days of bedside assessments and no more than 6 months of telephone follow-up, both measured from the first recorded set of ICU vital signs. Samples will be gathered and bedside assessments made at the following intervals after ICU admission or until hospital discharge/death: baseline, 3 days, 7 days, 14 days, and 30 days. During study assessments, data will be combined from the following sources: (1) samples collected by the study team, (2) bedside assessments, and (3) data from the EMR.

13.1 Sample collection and storage

Sample collection is focused around **rectal swabs** because the timing of swabs (as opposed to whole stools) can be precisely dictated and because rectal colonization with MDR bacteria best reflects overall MDR colonization status[84-88] and risk for subsequent infection.[89] Samples are described below.

- Rectal swabs. At each study assessment, deep flocked nylon swabs of the rectum will be performed in duplicate to allow independent processing of swabs for 16S rRNA gene sequencing/multiplex qPCR and for bacterial culture of MDROs. Swabs will be inserted 5 cm rectally with patients in the left lateral decubitus position and rotated 5 times, with adequate

sampling verified by looking for fecal staining. Swabs to be sequenced will be flash-frozen at -80ºC for long-term cold storage[90] and batched extraction;[91] swabs to be cultured will be transported on soy broth with 20% glycerol (Becton Dickinson, Franklin Lakes, NJ) for culture or, when culture cannot be performed immediately, for storage overnight at 4º C and culture the following morning.[92]

- Whole stools. Nursing staff will collect the first spontaneous whole stool subsequent to each study assessment. 4 x 8 ml aliquots of stool will be flash-frozen and stored at -80 º C.

- Oral swabs. Two cotton-tipped oral swabs will be used to gather a composite of the oral flora by brushing the following consecutive locations x 10 each: left cheek, right cheek, hard palate, tongue, floor of the mouth, and maxillary gingivae (same as the Human Microbiome Project).[93] Swabs will be flash frozen at -80 º C.

- Urine. ICU nurses will withdraw 2 x 8 ml of urine using standard technique from the urinary catheter sampling port. In patients who lack a urinary catheter, clean catch urine samples will be accepted and aliquoted as above.

- Blood. Residual blood from clinical samples drawn immediately prior to each study assessment will be retrieved by the study team. For each subject, one 6 mL serum-separator and one 8 mL EDTA tube will be retrieved. Buffy coat will be separated by centrifuge and with 1-2 mL aliquots generated for plasma/buffy coat and stored at -80 º C. This strategy spares subjects the need for an additional blood draw.

Oral swabs, urine, and blood will be stored for future testing. The rationale for collecting these additional samples is that they are non-invasive, can be easily collected, and will provide opportunities for future studies seeking to explore additional markers or mechanisms (e.g., urinary 3-indoxyl sulfate for gut microbiome disruption[94] or micro-RNAs in sepsis).[95] All sample storage will be in the PI's -80ºC freezer unit (P&S-8) except for the fresh rectal swab used for culture which will be stored within the Uhlemann lab (P&S-9). Assays performed from rectal swabs and whole stools (SCFAs) are described below.

13.2. Sample assays

The assays described below will be performed from rectal swabs or whole stools (for fecal SCFA levels). Residual sample material will be retained in -80ºC storage for the duration of the study.

- *16S rRNA gene sequencing*. 16S rRNA gene sequencing will be performed to determine the **primary outcome**. From extracted rectal swab DNA, polymerase chain reaction (PCR) will be performed on a Bio-Rad T100 Thermal Cycler (Bio-Rad, Hercules, CA) to amplify the V4 hypervariable region of the 16S ribosomal RNA gene with primers derived from Klindworth *et al*.[67] appended with overhang sequences for compatibility with Illumina index and sequencing adapters (Illumina, San Diego, CA). Samples will be purified with Agencourt AMPure XP beads (Beckman Coulter, Jersey City, NY) and quantified using the Quant-iT broad range dsDNA Assay Kit (Thermo Fisher Scientific, Fair Lawn, NJ). Libraries will be normalized, pooled, and denatured in preparation for cluster generation and sequencing, which will be performed on the Illumina MiSeq 300PE.

- *Bacterial culture*. Aerobic culture will be performed to determine the **secondary outcome**. Culture has been selected for the primary outcome because culture identifies viable bacteria, remains the most common clinical method for testing for MDRO colonization in

the ICU, and best predicts longterm risk for organism-specific infection.[96-99] Additional analyses will utilize alternative methods for classifying MDROs. Cultures will identify MRSA, VRE, and MDR Gram negatives, which cause 94% of all MDR bacterial infections in the ICU.[6] Rectal swabs will be gently vortexed to dissociate DNA from the swabs into the broth media. Media will then be split into equal aliquots and plated under a laminar flow hood for MRSA (Spectra-MRSA, Remel), VRE (Spectra-VRE, Remel), and ESBL or *Klebsiella pneumonia* carbapenemase (KPC)-producing bacteria (Uhlemann lab). In addition, we will plate cultures on Gram-negative selective agar (MacConkey II agar, Becton Dickinson) directly and at three serial dilutions for semi-quantitation (10^{-2}, 10^{-4}, and 10^{-6}). While the main analysis will be based on a categorical classification of MDROs (present vs absent), an exploratory analysis will be conducted using semi-quantitative culture data. Preliminary data show that these dilutions will yield a countable number of colonies. After 18-24 hours of aerobic growth, selective plates will be classified as positive vs negative for growth according to the media characteristics. The colony-forming units (CFUs) on the non-selective plates for Gram positives/negatives will be counted to provide relative quantification of each MDRO based on the total CFU counts from the non-selective plates. If multiple non-selective plates show growth, the plate with 10-100 colonies will be used for CFU counting with results normalized to the 10^{-2} plate. From these plates, organisms will be identified using the VITEK 2 system (bioMerieux) and AST-N010/020 cards with confirmatory testing as needed. Resistant isolates will be stored for whole genome sequencing analyses.

- Quantitative PCR. Quantitative PCR for resistance genes will be performed as an additional outcome. DNA will be extracted from rectal swabs (MoBio PowerFecal, Carlsbad, CA) and assessed using multiplex quantitative PCR (Qiagen Cat. No. 330261, Valencia, CA) for 87 common antibiotic resistance genes.[73] In this assay, 250 ng of template genomic DNA will be added to each reaction on a 96-well plate which will be run on a StepOnePLus RT-PCR machine using the following cycling conditions: initial denaturation for 10 min at 95º C, cycling 45 x 15 sec at 95º C denaturing followed by 2 min at 60º C annealing and extension. Controls will detect the presence of bacterial DNA, PCR inhibitors and background (no template control (NTC)). PCR cycle thresholds (C_T) of <35 will be considered positive for the presence of any given gene. Adequate reactions will be determined by C_T values of <29 for pan-bacterial reference genes (e.g., *16S rRNA*, *gyrA*, *recA*, *rpoB*)[100] and positive PCR controls (PPC) C_T values of <24. Linearity and sensitivity for this PCR have been determined using synthetic templates over a 6-log serial dilution ranging from 1 to 1 million copies, with results verified across multiple antibiotic resistance genes using pyrosequencing.[73]

- *Fecal SCFA levels*. Fecal SCFAs levels will be assayed as an additional outcome. Whole stools will be aliquoted and diluted with 80% ethanol to be agitated at 4°C. After centrifugation, 20 µl of supernatant from the homogenized samples will be mixed with 20 µl of 200 mM N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (1-EDC HCl) in 5% pyridine and 40 uL of 100 mM 2-Nitrophenylhydrazine (2-NPH) in 80% acetonitrile (ACN) with 50 mM HCl. After a brief incubation, ACN will be added to the solution which will be injected onto a 6490 triple quadrupole mass spectrometer (Agilent, Santa Clara, CA) and tested for concentrations of the SCFAs butyrate, acetate, and propionate.

13.3. Bedside assessments including data gathered from the EMR

During each study assessment, a study-specific Case Report Form (CRF) will be used to record the following data: origin for admission (classified as from the hospital floor, operating room, emergency room, or outside ICU transfer based on the most recent location prior to CUMC ICU

admission); the Glasgow Coma Scale (GCS);[101] the use of life support (classified as present vs absent) including the use of mechanical ventilation, hemodialysis, or additional life-support devices such as extra-corporeal membrane oxygen (ECMO) or an intra-atrial balloon pump (IABP); and the presence or absence of intravenous lines or catheters including a central venous catheter, urinary catheter, arterial catheter, nasal or oral gastric tube, or rectal tube. Using duplicate data entry, anonymized CRF data will be transferred into the secure REDCap database. Additional clinical variables will be entered directly into REDCap from the EMR. These variables will include laboratory analyses, all of the component variables of the Sequential Organ Failure Assessment (SOFA) score,[82] measures related to nutritional intake, and ICU interventions including administration of vasopressors, proton pump inhibitors, and antibiotics (with the name of the antibiotic and route of administration recorded).

13.3.1. Tolerability

Patients who are alert will be asked regarding abdominal bloating which will be graded on a 4-point Likert scale (0=none, 1=a little, 2=a moderate amount, 3=a lot/severe). When patients are not alert, this will be graded as "unable." ICU nurses will be asked to grade the average consistency of stools within each preceding 24 hours on a 5-point scale (0=like water, 1=like porridge, 2=loose but with chunks, 3=formed, soft, 4=formed, hard). This information will also be recorded from the EMR into REDCap as a separate entry. When subjects have no stools within the preceding 24 hours, this will be recorded as "no stools." The frequency of stools within the preceding 24 hours will be recorded as an integer at each study assessment. Again, this will be done based both on the nurse's in-person report and separately based on EMR documentation.

ICU nurses will be additionally asked regarding the presence or absence of the following symptoms during the 24 hour period prior to each study assessment: nausea, vomiting, bowel obstruction, and abdominal pain. This data will be recorded categorically on the CRFs/REDCap and will appear on DSMO reports.

13.3.2. Nutritional intake

Information related to nutritional intake will be gathered from the EMR including goal calories, actual calories consumed, and the amount of dietary fiber consumed in grams. All ICU patients at our institution receive a complete clinical nutritional assessment with calculation of goal calories based on the Penn State Equation[102] with the modified equation used for individuals >60 years old or those with BMI >30 as described by Frankenfeld *et al*. in 2009.[103,104] Actual calories and grams of fiber consumed will be based on the specific components of the tube feeds received or, among patients on an *ad lib* diet, the food consumed. The nutritional components of all CUMC diets are completely specified in terms of calories, fat, protein, carbohydrate, and fiber content. To calculate calories and fiber consumed, the study team will refer to the proportion of each meal consumed as recorded by ICU nurses and to the volume of tube feeds delivered which is captured hourly in the EMR. In those receiving tube feedings, the interval number of tube feeding interruptions will be recorded at each assessment or recorded as 0 in those receiving *ad lib* diets.

13.3.3. Long-term telephone follow-up

Telephone interviews will be conducted with subjects or their surrogates 3 months after ICU admission and again 6 months after ICU admission. In the event that subjects are still hospitalized, these interviews will be conducted at the bedside. Vital status will be ascertained and, to minimize loss of follow-up information, we will interview subjects or surrogates to determine (1) discharge location type (classified as home, short-term rehabilitation, or long-term rehabilitation including skilled care facilities), (2) hospital admission to non-CUMC facilities after ICU discharge, (3) physician office visits after ICU discharge, and (4) infections or other adverse events after ICU discharge. If there are non-CUMC hospital admissions or other healthcare

interactions, permission will be requested to obtain relevant records to determine the nature of infections and to assess for potential medium- to long-term AEs related to the study intervention. Among surviving subjects, the EQ-5D-5L (EuroQol 2009) will be used to determine health status across the domains of mobility, self-care, activity levels, pain, and anxiety/depression.[76,77] Use of this highly validated instrument will facilitate standardization and generalizability of results and will provide preliminary data for post-ICU discharge monitoring for later phase trials. This instrument can be applied via telephone in 5 minutes and was recommended in 2017 for post-ICU assessment of overall well-being after a three-round modified Delphi process involving clinical researchers from over 16 countries, patients/caregivers, clinicians, and research funders.[78] EQ-5D-5L responses have the potential to vary depending on whether answers are provided on patients versus surrogates,[105] and therefore we will additionally assess post-ICU disability using Katz' Index of Independence in ADLs (Activities of Daily Living).[79] Although focused on functional status rather than well-being, Katz' ADLs has been validated as an important patient-centered outcome across dozens of populations and unlike the EQ-5D-5L has high agreement between patients and caregivers.[80]

14. Data collection and management

The Columbia DCC will oversee use of a custom-designed, REDCap-based clinical trial data management system. REDCap is a widely used web-based relational data system developed at Vanderbilt University with NIH support to collect research information in 21CRFPart11, FISMA and HIPAA-compliant environments. REDCap functionality that will be used in this trial include randomization, subject scheduling, *ad hoc* reporting, data verification and audit trails. Paper CRFs will be used to collect bedside data, while readily available selected electronic data (e.g., laboratory values) will either be directly "pulled" from Columbia clinical servers and merged into the REDCap database or manually entered into REDCap. REDCap 'Events' will be specified corresponding to the study assessment timepoints: baseline, Days 3, 7, 14, 30, 3 months, and 6 months. Data of each type will be associated in the system with the appropriate time period. REDCap "Anytime" functionality will be used to capture adverse events and other incidents that can occur at any time, i.e. events that are not associated with fixed time periods. The REDCap system has built-in data verification aspects that will be utilized for the trial including minimum and maximum ranges and other logical checks, and automatic checks for completion of individual items and forms.

14.1. Data security within REDCap

The servers on which DCC REDCap project data are stored are located in a physically and electronically secure environment maintained by institutional IT staff on the CUMC campus. The DCC confirms project data access privileges on a monthly basis, and REDCap requires strong passwords, which must be changed periodically. In this trial, to ensure data security, explicit identifiers needed for recruitment and scheduling (names, addresses, phone numbers, email addresses, medical record numbers) will be maintained separately and will *not* be stored in the REDCap database.

14.2. Record retention

All electronic and paper records will be retained for a minimum of 6 years from the trial end date in accordance with New York State law and meeting or exceeding Federal minimums. Consent forms, CRFs, and other relevant paper documents will be moved to secure longterm paper storage once the trial has been closed and the data locked. Record retention will be prolonged beyond 6 years as necessary if required by the funding agency.

15. Safety and adverse events (AEs)

Adverse health events, laboratory irregularities, and even death are expected in ICU patients with sepsis and it would be difficult or impossible to accurately document and label all adverse health events. This is a common problem faced by ICU trials. The solution recommended by academic critical care research groups (e.g., Cook *et al*.) is to pre-specify AEs of interest for careful monitoring. In keeping with this approach, this study will produce detailed reports of these AEs for the DSMC at standard intervals while only unanticipated problems that also meet criteria for serious adverse events (SAEs) will be reported to the DSMC in real time.[106] AEs will be reported for all individuals who have received one or more doses of the intervention. This study has pre-specified AEs in the following categories.

- Bloating: graded 0-3 based on patient report: patient mean and maximum values will be reported;

- Stool consistency and frequency: graded 0-4 for consistency and with an integer denoting frequency over 24 hours based on nursing report. For each patient, mean and maximum values will be reported;

- Electrolyte imbalances: For each patient, the minimum and maximum values for serum calcium, magnesium, phosphate, potassium, and sodium will be reported. This will be done for the 7 days of the intervention period and separately from Day 7 through Day 30/discharge;

- Gastrointestinal AEs: the gastrointestinal AEs of nausea, vomiting, bowel obstruction, and abdominal pain will be recorded as present or absent for each assessment and separated from other AEs to improve early pattern recognition by the DSMO;

- Infections: Infections are both an AE and an outcome and will be adjudicated as previously described. For each infection, the date of the infection, suspected site, organism, and antibiotics received will be recorded. Infections will be further classified as MDR vs non-MDR using the criteria previously described;

- Death: death will be gathered from the hospital EMR, which inerfaces with the social security death index.

15.1. Serious adverse events

Federal Regulation 62 Oct. 7, 1997 defines SAEs as those events occurring at any dose which:

- Result in death;

- Are life-threatening, meaning the patient was at risk for death at the time of the event;

- Require hospitalization or prolong hospitalization;

- Results in significant disability or incapacity;

- Is a congenital abnormality/birth defect;

- Or does not meet these criteria but is thought by the investigator to be unusual and potentially serious.

SAEs in this trial will be AEs meeting the above Fed. Reg. 62 definition.

15.2. Adjudicating and reporting adverse events as unanticipated problems

Unanticipated problems (UPs) will be any unforeseen medical event thought likely related to the study intervention. Such events will be monitored for in real time. UPs that do not meet criteria for seriousness (same as SAE criteria) will be reported to the DSMO and IRB at pre-specified intervals. UPs that meet criteria for seriousness will be reported within 24 hours from our notification of the event. This reporting will include:

- Contact of the DSMO;
- Contact of the Columbia IRB;
- Fax or email scan containing when the event occurred, the name of the reporter, callback information, the protocol number, and a description of the event;
- Availability of the PI to provide follow-up information regarding the event.

The proposed research is pending review by the Columbia University IRB (IRB #AAAS2576) and will be reviewed by the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO) prior to implementation. Prior to initiation of research, the study will be registered on clinicaltrials.gov.

Safety and adverse effects will be overseen by the PI in conjunction with the Data and Safety Monitoring Officer (DSMO). **Daniel Brodie**, the Director of the Medical Intensive Care Units at our institution will serve as the trial DSMO and will convene an appropriate Data and Safety Monitoring Committee (DSMC) to oversee interim study results. The primary responsibility of the DSMC will be to review interim data in terms of safety and make recommendations whether the study should be changed, halted, or terminated. Full details of the DSMC procedures and processes as well as additional details regarding AE and SAE reporting are in the DSMP.

15.2.1. Compliance statement regarding research monitoring

The DSMB under Dr. Brodie will function as a **Research Monitor** and is responsible to oversee the safety of the research and report observations/findings to the IRB or a designated institutional official. The Research Monitor will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. The Research Monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.

15.3. Unblinding for adverse events

All possible efforts will be made to retain blinding for the duration of the trial. However, if a treating clinician feels that blinding must be removed in order to assess a possible adverse reaction quickly and correctly, the PI will facilitate this process by working through the DCC.

16. Study termination and completion

The study may be terminated at any time for safety concerns by the PI, DSMC, IRB, or other appropriate regulatory bodies. Otherwise the study will continue until 90 patients are enrolled who complete the Day 3 assessment and will be considered complete when all study assessments and analyses have been concluded.

17. Statistical methods

17.1. Overall plan for statistical analysis

After enrollment is complete, the REDCap dataset will undergo DCC cross-checks for quality and accuracy. The PI will review blinded data to identify errors which, if they exist, will be rectified by reference to paper CRFs or to the EMR. After quality checks are complete, the REDCap dataset will be closed and locked. The biostatistician will then perform an intent-to-treat analysis to determine the primary and secondary outcomes. The DSMO will have complete access both to the raw data and to the analytic code used by the biostatistician and will nominate a qualified monitor who will review this work for rigor. Once this is complete, the primary and secondary outcomes will be finalized. At this point, the locked data will be made available to the PI and other investigators for additional analyses. For all analyses, alpha <0.05 will be considered statistically significant and two-sided testing will be performed. When death is a competing risk, Fine and Gray regression analyses will be performed.

17.2. Determination of the primary and secondary outcomes

Primary outcome: within-individual change in relative abundance of SCFA producers. 16S rRNA gene sequencing data will be processed using the QIIME platform with appropriate rarification and filtering.[107] The primary analysis will be intent-to-treat, restricted to the subjects who complete the Day 3 assessment and contribute data. Within-individual change in SCFA producers will be calculated as Day 3 minus baseline relative abundance and an ANOVA or Kruskal-Wallis (KW) test (if skewed data) will be used to assess for differences across the three groups. If there is no significant difference between the low- and high-dose inulin arms, these arms will be combined for comparison against placebo in the final analysis using a two-sample t-test or rank-sum test. Although the primary analysis will be with the Day 3 samples, unadjusted and adjusted longitudinal data analysis will be performed using the generalized estimating equation approach with working independence correlation structure.

Secondary outcome: MDRO colonization. Subjects who culture positive from rectal swabs from Day 3 for MRSA, VRE, or a Gram negative with non-susceptibility to a third generation cephalosporin will be classified as MDRO colonized and all other subjects as not MDRO colonized. The proportion of patients who are MDRO colonized on Day 3 will be compared between intervention groups using a chi-squared test or Fisher's test. For this analysis, patients in the inulin arms will be combined if testing shows no significant difference between these inulin arms. Because baseline colonization status is likely to be an important predictor of Day 3 colonization status, we will test whether baseline MDRO colonization rates differ between intervention groups. If such a difference is observed, we will reanalyze Day 3 MDRO colonization with stratification according to baseline MDRO colonization (baseline MDRO colonized vs not colonized). Exploratory analyses will examine Day 7 MDRO colonization status and semi-quantitative culture data using ANOVA or Kruskal-Wallis testing. The difference in rates of Day 3 MDRO colonization will be taken as the effect size estimate for future trials.

17.3. Power calculation and sample size

Primary outcome: within-individual change in relative abundance of SCFA producers. The study is powered to detect a difference in SCFA producer levels that is equal to or greater than the observed difference in SCFA producer levels from our prospective cohort (6.6% for high vs 3.7% low dietary fiber). The rationale for this is that inulin must be at least as impactful as mixed dietary fiber to be a viable therapeutic. The proposed sample size of 30:30:30 will yield 80% power to detect a minimum difference of 0.73 standard deviations (SD) comparing any one inulin arm to the placebo arm using a two-sided t-test with 0.05 significance level and assuming equal variance. If the inulin arms have similar changes in SCFA producer levels, they will be combined and the study would have 80% power to detect a minimum 0.63 SD for inulin compared to placebo at the .05 significance level. This sample size will also have 80% power to detect 0.33 SD differences among the 3 means versus the alternative of equal means using an F-test at the .05 significance level.

Secondary outcome: MDRO colonization. Power calculations were performed using the observed 42% rate of MDRO colonization in our prospective ICU cohort and 1:1:1 allocation to the intervention. Because this is an early phase study conducted with the expectation that promising results will be followed up by definitive larger studies, sample size calculations are based on one-sided testing with alpha .10 using a t-test based on the binomial distribution. Under these assumptions, a sample size of 90 patients will yield 80% power to detect a 20.4% or less rate of MDRO colonization on Day 7 with inulin, assuming that the two inulin arms are combined. If the inulin arms are not combined, there will be 80% power for a 17.3% or less rate of Day 7 MDRO colonization comparing any one inulin intervention arm vs placebo. The two-sided 95% confidence interval for the effect size estimate within the combined inulin arms would be 7.1 to 26.6% for an estimate centered on 15% and 10.8 to 32.3% for an estimate centered on 20%.

17.4. Interim analyses

Interim analyses will be performed for safety, and the DSMC will have the ability to halt the trial at any time. Full DSMP reports will be produced after 10, 20, 30, and 45 patients are randomized and at least semi-annually based on the date of enrollment of the first patient. Toxicity monitoring will be performed using a frequentist approach and targeting an excess rate of toxic death of no more than 5%. Toxicity will be monitored for each arm at the same 4 time points. Sequential boundaries will be calculated using the Pocock method. If the number of toxicities differs in any one study by 3, 4, 5, or 7 patients corresponding to the assessments above, enrollment will be halted and toxicities will be further evaluated to assess if it is appropriate to terminate the study. This stopping boundary yields a probability of early stopping of at most 0.05 when the dose-limiting toxicity is equal to 0.05. Toxic death will be adjudicated by the DSMC. There are no plans for futility monitoring because of the relatively small size of the proposed trial, non-invasiveness of the samples collected, and the potential for longitudinal study results to add significant mechanistic understanding to MDRO colonization in the ICU.

18. Publication policy

Study results will be disseminated as widely as possible by publication and presentation. The PI is ultimately responsible for publications arising from the study. Funding sources will be recognized.

Appendix A: References

1. Neidell MJ, Cohen B, Furuya Y, Hill J, Jeon CY, Glied S, Larson EL. Costs of healthcare- and community-associated infections with antimicrobial-resistant versus antimicrobial-susceptible organisms. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2012;55:807-815.
2. National Nosocomial Infections Surveillance S. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. American journal of infection control 2004;32:470-485.
3. Slayton RB, Toth D, Lee BY, Tanner W, Bartsch SM, Khader K, Wong K, Brown K, McKinnell JA, Ray W, Miller LG, Rubin M, Kim DS, Adler F, Cao C, Avery L, Stone NT, Kallen A, Samore M, Huang SS, Fridkin S, Jernigan JA. Vital Signs: Estimated Effects of a Coordinated Approach for Action to Reduce Antibiotic-Resistant Infections in Health Care Facilities - United States. MMWR. Morbidity and mortality weekly report 2015;64:826-831.
4. Garrouste-Orgeas M, Timsit JF, Tafflet M, Misset B, Zahar JR, Soufir L, Lazard T, Jamali S, Mourvillier B, Cohen Y, De Lassence A, Azoulay E, Cheval C, Descorps-Declere A, Adrie C, Costa de Beauregard MA, Carlet J, Group OS. Excess risk of death from intensive care unit-acquired nosocomial bloodstream infections: a reappraisal. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2006;42:1118-1126.
5. Blot S, Koulenti D, Dimopoulos G, Martin C, Komnos A, Krueger WA, Spina G, Armaganidis A, Rello J, Investigators E-VS. Prevalence, risk factors, and mortality for ventilator-associated pneumonia in middle-aged, old, and very old critically ill patients*. Crit Care Med 2014;42:601-609.
6. Tabah A, Koulenti D, Laupland K, Misset B, Valles J, Bruzzi de Carvalho F, Paiva JA, Cakar N, Ma X, Eggimann P, Antonelli M, Bonten MJ, Csomos A, Krueger WA, Mikstacki A, Lipman J, Depuydt P, Vesin A, Garrouste-Orgeas M, Zahar JR, Blot S, Carlet J, Brun-Buisson C, Martin C, Rello J, Dimopoulos G, Timsit JF. Characteristics and determinants of outcome of hospital-acquired bloodstream infections in intensive care units: the EUROBACT International Cohort Study. Intensive Care Med 2012;38:1930-1945.
7. Kollef KE, Schramm GE, Wills AR, Reichley RM, Micek ST, Kollef MH. Predictors of 30-day mortality and hospital costs in patients with ventilator-associated pneumonia attributed to potentially antibiotic-resistant gram-negative bacteria. Chest 2008;134:281-287.
8. Sommer MO, Dantas G, Church GM. Functional characterization of the antibiotic resistance reservoir in the human microflora. Science 2009;325:1128-1131.
9. Donskey CJ. Antibiotic regimens and intestinal colonization with antibiotic-resistant gram-negative bacilli. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2006;43 Suppl 2:S62-69.
10. Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MO, Dantas G. The shared antibiotic resistome of soil bacteria and human pathogens. Science 2012;337:1107-1111.
11. Britton RA, Young VB. Role of the intestinal microbiota in resistance to colonization by Clostridium difficile. Gastroenterology 2014;146:1547-1553.
12. Zaborin A, Smith D, Garfield K, Quensen J, Shakhsheer B, Kade M, Tirrell M, Tiedje J, Gilbert JA, Zaborina O, Alverdy JC. Membership and behavior of ultra-low-diversity pathogen communities present in the gut of humans during prolonged critical illness. MBio 2014;5:e01361-01314.
13. Shimizu K, Ogura H, Hamasaki T, Goto M, Tasaki O, Asahara T, Nomoto K, Morotomi M, Matsushima A, Kuwagata Y, Sugimoto H. Altered gut flora are associated with septic

complications and death in critically ill patients with systemic inflammatory response syndrome. Dig Dis Sci 2011;56:1171-1177.
14. Filius PM, Gyssens IC, Kershof IM, Roovers PJ, Ott A, Vulto AG, Verbrugh HA, Endtz HP. Colonization and resistance dynamics of gram-negative bacteria in patients during and after hospitalization. Antimicrob Agents Chemother 2005;49:2879-2886.
15. Lankelma JM, van Vught LA, Belzer C, Schultz MJ, van der Poll T, de Vos WM, Wiersinga WJ. Critically ill patients demonstrate large interpersonal variation in intestinal microbiota dysregulation: a pilot study. Intensive Care Med 2017;43:59-68.
16. O'Keefe SJ, Li JV, Lahti L, Ou J, Carbonero F, Mohammed K, Posma JM, Kinross J, Wahl E, Ruder E, Vipperla K, Naidoo V, Mtshali L, Tims S, Puylaert PG, DeLany J, Krasinskas A, Benefiel AC, Kaseb HO, Newton K, Nicholson JK, de Vos WM, Gaskins HR, Zoetendal EG. Fat, fibre and cancer risk in African Americans and rural Africans. Nat Commun 2015;6:6342.
17. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 2013;500:232-236.
18. Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, Pudlo NA, Kitamoto S, Terrapon N, Muller A, Young VB, Henrissat B, Wilmes P, Stappenbeck TS, Nunez G, Martens EC. A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. Cell 2016;167:1339-1353 e1321.
19. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev 2001;81:1031-1064.
20. Lewis SJ, Heaton KW. Increasing butyrate concentration in the distal colon by accelerating intestinal transit. Gut 1997;41:245-251.
21. Morowitz MJ, Di Caro V, Pang D, Cummings J, Firek B, Rogers MB, Ranganathan S, Clark RSB, Aneja RK. Dietary Supplementation With Nonfermentable Fiber Alters the Gut Microbiota and Confers Protection in Murine Models of Sepsis. Crit Care Med 2017;45:e516-e523.
22. Di Caro V, Cummings JL, Alcamo AM, Piganelli JD, Clark RSB, Morowitz MJ, Aneja RK. Dietary Cellulose Supplementation Modulates the Immune Response in a Murine Endotoxemia Model. Shock 2018;
23. Schroeder BO, Birchenough GMH, Stahlman M, Arike L, Johansson MEV, Hansson GC, Backhed F. Bifidobacteria or Fiber Protects against Diet-Induced Microbiota-Mediated Colonic Mucus Deterioration. Cell Host Microbe 2018;23:27-40 e27.
24. Jacobson A, Lam L, Rajendram M, Tamburini F, Honeycutt J, Pham T, Van Treuren W, Pruss K, Stabler SR, Lugo K, Bouley DM, Vilches-Moure JG, Smith M, Sonnenburg JL, Bhatt AS, Huang KC, Monack D. A Gut Commensal-Produced Metabolite Mediates Colonization Resistance to Salmonella Infection. Cell Host Microbe 2018;
25. Brugiroux S, Beutler M, Pfann C, Garzetti D, Ruscheweyh HJ, Ring D, Diehl M, Herp S, Lotscher Y, Hussain S, Bunk B, Pukall R, Huson DH, Munch PC, McHardy AC, McCoy KD, Macpherson AJ, Loy A, Clavel T, Berry D, Stecher B. Genome-guided design of a defined mouse microbiota that confers colonization resistance against Salmonella enterica serovar Typhimurium. Nat Microbiol 2016;2:16215.
26. Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PG, Neyrinck AM, Bindels LB, de Vos WM, Gibson GR, Thissen JP, Delzenne NM. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. Gut 2013;62:1112-1121.

27. Azpiroz F, Molne L, Mendez S, Nieto A, Manichanh C, Mego M, Accarino A, Santos J, Sailer M, Theis S, Guarner F. Effect of Chicory-derived Inulin on Abdominal Sensations and Bowel Motor Function. J Clin Gastroenterol 2017;51:619-625.
28. Bonnema AL, Kolberg LW, Thomas W, Slavin JL. Gastrointestinal tolerance of chicory inulin products. J Am Diet Assoc 2010;110:865-868.
29. Canfora EE, van der Beek CM, Hermes GDA, Goossens GH, Jocken JWE, Holst JJ, van Eijk HM, Venema K, Smidt H, Zoetendal EG, Dejong CHC, Lenaerts K, Blaak EE. Supplementation of Diet With Galacto-oligosaccharides Increases Bifidobacteria, but Not Insulin Sensitivity, in Obese Prediabetic Individuals. Gastroenterology 2017;153:87-97 e83.
30. Garcia-Peris P, Velasco C, Lozano MA, Moreno Y, Paron L, de la Cuerda C, Breton I, Camblor M, Garcia-Hernandez J, Guarner F, Hernandez M. Effect of a mixture of inulin and fructo-oligosaccharide on Lactobacillus and Bifidobacterium intestinal microbiota of patients receiving radiotherapy: a randomised, double-blind, placebo-controlled trial. Nutr Hosp 2012;27:1908-1915.
31. Holscher HD, Bauer LL, Gourineni V, Pelkman CL, Fahey GC, Jr., Swanson KS. Agave Inulin Supplementation Affects the Fecal Microbiota of Healthy Adults Participating in a Randomized, Double-Blind, Placebo-Controlled, Crossover Trial. J Nutr 2015;145:2025-2032.
32. Petry N, Egli I, Chassard C, Lacroix C, Hurrell R. Inulin modifies the bifidobacteria population, fecal lactate concentration, and fecal pH but does not influence iron absorption in women with low iron status. Am J Clin Nutr 2012;96:325-331.
33. Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P. Effect of inulin on the human gut microbiota: stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii. Br J Nutr 2009;101:541-550.
34. Tovar AR, Caamano Mdel C, Garcia-Padilla S, Garcia OP, Duarte MA, Rosado JL. The inclusion of a partial meal replacement with or without inulin to a calorie restricted diet contributes to reach recommended intakes of micronutrients and decrease plasma triglycerides: a randomized clinical trial in obese Mexican women. Nutr J 2012;11:44.
35. Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, Theis S, Verbeke K, Raes J. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. Gut 2017;
36. O'Keefe SJ, Ou J, Delany JP, Curry S, Zoetendal E, Gaskins HR, Gunn S. Effect of fiber supplementation on the microbiota in critically ill patients. World J Gastrointest Pathophysiol 2011;2:138-145.
37. Terry MA, Freedberg DE, Morris MC. An Alternative Consent Process for Minimal Risk Research in the ICU. Crit Care Med 2017;
38. Freedberg DE, Zhou MJ, Cohen ME, Annavajhala MK, Khan S, Moscoso DI, Brooks C, Whittier S, Chong DH, Uhlemann AC, Abrams JA. Pathogen colonization of the gastrointestinal microbiome at intensive care unit admission and risk for subsequent death or infection. Intensive Care Med 2018;
39. Livanos AE, Snider EJ, Whittier S, Chong DH, Wang TC, Abrams JA, Freedberg DE. Rapid gastrointestinal loss of Clostridial Clusters IV and XIVa in the ICU associates with an expansion of gut pathogens. PLoS One 2018;13:e0200322.
40. Freedberg DE, Zhou MJ, Cohen ME, Annavajhala M, Khan S, Moscoso DI, Brooks CA, Whittier S, Chong DH, Uhlemann AC, Abrams JA. Pathogen colonization of the gastrointestinal microbiome at intensive care unit admission and risk for subsequent death or infection. Intensive Care Medicine, Accepted for publication June 4, 2018
41. Metnitz PG, Moreno RP, Almeida E, Jordan B, Bauer P, Campos RA, Iapichino G, Edbrooke D, Capuzzo M, Le Gall JR, Investigators S. SAPS 3--From evaluation of the

patient to evaluation of the intensive care unit. Part 1: Objectives, methods and cohort description. Intensive Care Med 2005;31:1336-1344.
42. Moreno RP, Metnitz PG, Almeida E, Jordan B, Bauer P, Campos RA, Iapichino G, Edbrooke D, Capuzzo M, Le Gall JR, Investigators S. SAPS 3--From evaluation of the patient to evaluation of the intensive care unit. Part 2: Development of a prognostic model for hospital mortality at ICU admission. Intensive Care Med 2005;31:1345-1355.
43. Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, No D, Gobourne A, Viale A, Dahi PB, Ponce DM, Barker JN, Giralt S, van den Brink M, Pamer EG. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. Blood 2014;124:1174-1182.
44. Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, Lee YJ, Dubin KA, Socci ND, Viale A, Perales MA, Jenq RR, van den Brink MR, Pamer EG. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2012;55:905-914.
45. Haak BW, Littmann ER, Chaubard JL, Pickard AJ, Fontana E, Adhi F, Gyaltshen Y, Ling L, Morjaria SM, Peled JU, van den Brink MR, Geyer AI, Cross JR, Pamer EG, Taur Y. Impact of gut colonization with butyrate-producing microbiota on respiratory viral infection following allo-HCT. Blood 2018;131:2978-2986.
46. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker discovery and explanation. Genome Biol 2011;12:R60.
47. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, Tobe T, Clarke JM, Topping DL, Suzuki T, Taylor TD, Itoh K, Kikuchi J, Morita H, Hattori M, Ohno H. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature 2011;469:543-547.
48. Rivera-Chavez F, Zhang LF, Faber F, Lopez CA, Byndloss MX, Olsan EE, Xu G, Velazquez EM, Lebrilla CB, Winter SE, Baumler AJ. Depletion of Butyrate-Producing Clostridia from the Gut Microbiota Drives an Aerobic Luminal Expansion of Salmonella. Cell Host Microbe 2016;19:443-454.
49. Duncan SH, Louis P, Thomson JM, Flint HJ. The role of pH in determining the species composition of the human colonic microbiota. Environ Microbiol 2009;11:2112-2122.
50. Walker AW, Duncan SH, McWilliam Leitch EC, Child MW, Flint HJ. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. Appl Environ Microbiol 2005;71:3692-3700.
51. Kruse HP, Kleessen B, Blaut M. Effects of inulin on faecal bifidobacteria in human subjects. Br J Nutr 1999;82:375-382.
52. Lecerf JM, Depeint F, Clerc E, Dugenet Y, Niamba CN, Rhazi L, Cayzeele A, Abdelnour G, Jaruga A, Younes H, Jacobs H, Lambrey G, Abdelnour AM, Pouillart PR. Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. Br J Nutr 2012;108:1847-1858.
53. Miguez B, Gomez B, Parajo JC, Alonso JL. Potential of fructooligosaccharides and xylooligosaccharides as substrates to counteract the undesirable effects of several antibiotics on elder faecal microbiota: a first in vitro approach. J Agric Food Chem 2018;
54. Rahat-Rozenbloom S, Fernandes J, Cheng J, Gloor GB, Wolever TM. The acute effects of inulin and resistant starch on postprandial serum short-chain fatty acids and second-meal glycemic response in lean and overweight humans. Eur J Clin Nutr 2017;71:227-233.
55. Manzanares W, Lemieux M, Langlois PL, Wischmeyer PE. Probiotic and synbiotic therapy in critical illness: a systematic review and meta-analysis. Crit Care 2016;19:262.

56. McClave SA, Taylor BE, Martindale RG, Warren MM, Johnson DR, Braunschweig C, McCarthy MS, Davanos E, Rice TW, Cresci GA, Gervasio JM, Sacks GS, Roberts PR, Compher C, Society of Critical Care M, American Society for P, Enteral N. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). JPEN J Parenter Enteral Nutr 2016;40:159-211.
57. Kotzampassi K, Giamarellos-Bourboulis EJ, Voudouris A, Kazamias P, Eleftheriadis E. Benefits of a synbiotic formula (Synbiotic 2000Forte) in critically Ill trauma patients: early results of a randomized controlled trial. World J Surg 2006;30:1848-1855.
58. Knight DJ, Gardiner D, Banks A, Snape SE, Weston VC, Bengmark S, Girling KJ. Effect of synbiotic therapy on the incidence of ventilator associated pneumonia in critically ill patients: a randomised, double-blind, placebo-controlled trial. Intensive Care Med 2009;35:854-861.
59. Spindler-Vesel A, Bengmark S, Vovk I, Cerovic O, Kompan L. Synbiotics, prebiotics, glutamine, or peptide in early enteral nutrition: a randomized study in trauma patients. JPEN J Parenter Enteral Nutr 2007;31:119-126.
60. Karakan T, Ergun M, Dogan I, Cindoruk M, Unal S. Comparison of early enteral nutrition in severe acute pancreatitis with prebiotic fiber supplementation versus standard enteral solution: a prospective randomized double-blind study. World J Gastroenterol 2007;13:2733-2737.
61. Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, Nieuwenhuijs VB, Bollen TL, van Ramshorst B, Witteman BJ, Rosman C, Ploeg RJ, Brink MA, Schaapherder AF, Dejong CH, Wahab PJ, van Laarhoven CJ, van der Harst E, van Eijck CH, Cuesta MA, Akkermans LM, Gooszen HG, Dutch Acute Pancreatitis Study G. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008;371:651-659.
62. McIvor AC, Meguid MM, Curtas S, Warren J, Kaplan DS. Intestinal obstruction from cecal bezoar; a complication of fiber-containing tube feedings. Nutrition 1990;6:115-117.
63. Majid HA, Cole J, Emery PW, Whelan K. Additional oligofructose/inulin does not increase faecal bifidobacteria in critically ill patients receiving enteral nutrition: a randomised controlled trial. Clin Nutr 2014;33:966-972.
64. Spapen H, Diltoer M, Van Malderen C, Opdenacker G, Suys E, Huyghens L. Soluble fiber reduces the incidence of diarrhea in septic patients receiving total enteral nutrition: a prospective, double-blind, randomized, and controlled trial. Clin Nutr 2001;20:301-305.
65. Dilli D, Aydin B, Zenciroglu A, Ozyazici E, Beken S, Okumus N. Treatment outcomes of infants with cyanotic congenital heart disease treated with synbiotics. Pediatrics 2013;132:e932-938.
66. Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, Theis S, Verbeke K, Raes J. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. Gut 2017;66:1968-1974.
67. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glockner FO. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 2013;41:e1.
68. Van den Abbeele P, Belzer C, Goossens M, Kleerebezem M, De Vos WM, Thas O, De Weirdt R, Kerckhof FM, Van de Wiele T. Butyrate-producing Clostridium cluster XIVa species specifically colonize mucins in an in vitro gut model. ISME J 2013;7:949-961.
69. Vital M, Howe AC, Tiedje JM. Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. MBio 2014;5:e00889.
70. Tye H, Yu CH, Simms LA, de Zoete MR, Kim ML, Zakrzewski M, Penington JS, Harapas CR, Souza-Fonseca-Guimaraes F, Wockner LF, Preaudet A, Mielke LA, Wilcox SA, Ogura Y, Corr SC, Kanojia K, Kouremenos KA, De Souza DP, McConville MJ, Flavell

RA, Gerlic M, Kile BT, Papenfuss AT, Putoczki TL, Radford-Smith GL, Masters SL. NLRP1 restricts butyrate producing commensals to exacerbate inflammatory bowel disease. Nat Commun 2018;9:3728.
71. Siegel JD, Rhinehart E, Jackson M, Chiarello L, Healthcare Infection Control Practices Advisory C. Management of multidrug-resistant organisms in health care settings, 2006. American journal of infection control 2007;35:S165-193.
72. (CLSI) CaLSI. Performance standards for antimicrobial susceptibility testing. CLSI Supplement M100. 2017(27th Ed.).
73. Fosbrink M, Wilt G, Chen L, Kreisworth B, Devgan V. Identification of antibiotic resistance genes in Klebsiella pneumoniae isolates and metagenomic samples using real-time PCR arrays. Sample to Insight, accessed on-line at https://www.slideshare.net/QIAGENscience/identification-of-antibiotic-resistance-genes-in-klebsiella-pneumoniae-isolates-and-metagenomic-samples-using-realtime-pcr-arrays. April 6, 2016.;
74. Magill SS, Hellinger W, Cohen J, Kay R, Bailey C, Boland B, Carey D, de Guzman J, Dominguez K, Edwards J, Goraczewski L, Horan T, Miller M, Phelps M, Saltford R, Seibert J, Smith B, Starling P, Viergutz B, Walsh K, Rathore M, Guzman N, Fridkin S. Prevalence of healthcare-associated infections in acute care hospitals in Jacksonville, Florida. Infection control and hospital epidemiology 2012;33:283-291.
75. Gottschlich MM, DeLegge MH, Guenter P, American Society for P, Enteral N. *The A.S.P.E.N. nutrition support core curriculum : a case-based approach : the adult patient.* Silver Spring, MD: American Society for Parenteral and Enteral Nutrition; 2007.
76. Janssen MF, Pickard AS, Golicki D, Gudex C, Niewada M, Scalone L, Swinburn P, Busschbach J. Measurement properties of the EQ-5D-5L compared to the EQ-5D-3L across eight patient groups: a multi-country study. Qual Life Res 2013;22:1717-1727.
77. Herdman M, Gudex C, Lloyd A, Janssen M, Kind P, Parkin D, Bonsel G, Badia X. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res 2011;20:1727-1736.
78. Needham DM, Sepulveda KA, Dinglas VD, Chessare CM, Aronson Friedman L, Bingham Iii CO, Turnbull AE. Core Outcome Measures for Clinical Research in Acute Respiratory Failure Survivors: An International Modified Delphi Consensus Study. Am J Respir Crit Care Med 2017;
79. Katz S, Downs TD, Cash HR, Grotz RC. Progress in development of the index of ADL. Gerontologist 1970;10:20-30.
80. Schandl A, Bottai M, Holdar U, Hellgren E, Sackey P. Early prediction of new-onset physical disability after intensive care unit stay: a preliminary instrument. Crit Care 2014;18:455.
81. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL, Angus DC. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315:801-810.
82. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, Reinhart CK, Suter PM, Thijs LG. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 1996;22:707-710.
83. Cadot P, Kochuyt AM, van Ree R, Ceuppens JL. Oral allergy syndrome to chicory associated with birch pollen allergy. Int Arch Allergy Immunol 2003;131:19-24.
84. Thurlow CJ, Prabaker K, Lin MY, Lolans K, Weinstein RA, Hayden MK, Centers for Disease C, Prevention Epicenters P. Anatomic sites of patient colonization and

environmental contamination with Klebsiella pneumoniae carbapenemase-producing Enterobacteriaceae at long-term acute care hospitals. Infection control and hospital epidemiology 2013;34:56-61.
85. Ray AJ, Pultz NJ, Bhalla A, Aron DC, Donskey CJ. Coexistence of vancomycin-resistant enterococci and Staphylococcus aureus in the intestinal tracts of hospitalized patients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2003;37:875-881.
86. Klotz M, Zimmermann S, Opper S, Heeg K, Mutters R. Possible risk for re-colonization with methicillin-resistant Staphylococcus aureus (MRSA) by faecal transmission. International journal of hygiene and environmental health 2005;208:401-405.
87. Acton DS, Plat-Sinnige MJ, van Wamel W, de Groot N, van Belkum A. Intestinal carriage of Staphylococcus aureus: how does its frequency compare with that of nasal carriage and what is its clinical impact? Eur J Clin Microbiol Infect Dis 2009;28:115-127.
88. Bhalla A, Aron DC, Donskey CJ. Staphylococcus aureus intestinal colonization is associated with increased frequency of S. aureus on skin of hospitalized patients. BMC Infect Dis 2007;7:105.
89. Dangerfield B, Chung A, Webb B, Seville MT. Predictive value of methicillin-resistant Staphylococcus aureus (MRSA) nasal swab PCR assay for MRSA pneumonia. Antimicrob Agents Chemother 2014;58:859-864.
90. Sinha R, Chen J, Amir A, Vogtmann E, Shi J, Inman KS, Flores-Munguia R, Sampson JN, Knight R, Chia N. Collecting Fecal Samples for Microbiome Analyses in Epidemiology Studies. Cancer Epidemiol Biomarkers Prev 2015;
91. Walker AW, Martin JC, Scott P, Parkhill J, Flint HJ, Scott KP. 16S rRNA gene-based profiling of the human infant gut microbiota is strongly influenced by sample processing and PCR primer choice. Microbiome 2015;3:26.
92. Bonten MJ, Nathan C, Weinstein RA. Recovery of nosocomial fecal flora from frozen stool specimens and rectal swabs: comparison of preservatives for epidemiological studies. Diagnostic microbiology and infectious disease 1997;27:103-106.
93. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature 2007;449:804-810.
94. Farowski F, Els G, Tsakmaklis A, Higgins PG, Kahlert CR, Stein-Thoeringer CK, Bobardt JS, Dettmer-Wilde K, Oefner PJ, Vehreschild JJ, Vehreschild M. Assessment of urinary 3-indoxyl sulfate as a marker for gut microbiota diversity and abundance of Clostridiales. Gut Microbes 2018;1-9.
95. Seeley JJ, Baker RG, Mohamed G, Bruns T, Hayden MS, Deshmukh SD, Freedberg DE, Ghosh S. Induction of innate immune memory via microRNA targeting of chromatin remodelling factors. Nature 2018;559:114-119.
96. Ziakas PD, Thapa R, Rice LB, Mylonakis E. Trends and significance of VRE colonization in the ICU: a meta-analysis of published studies. PLoS One 2013;8:e75658.
97. Garrouste-Orgeas M, Timsit JF, Kallel H, Ben Ali A, Dumay MF, Paoli B, Misset B, Carlet J. Colonization with methicillin-resistant Staphylococcus aureus in ICU patients: morbidity, mortality, and glycopeptide use. Infect Control Hosp Epidemiol 2001;22:687-692.
98. Loo VG, Bourgault AM, Poirier L, Lamothe F, Michaud S, Turgeon N, Toye B, Beaudoin A, Frost EH, Gilca R, Brassard P, Dendukuri N, Beliveau C, Oughton M, Brukner I, Dascal A. Host and pathogen factors for Clostridium difficile infection and colonization. N Engl J Med 2011;365:1693-1703.
99. Frencken JF, Wittekamp BHJ, Plantinga NL, Spitoni C, van de Groep K, Cremer OL, Bonten MJM. Associations Between Enteral Colonization With Gram-Negative Bacteria and Intensive Care Unit-Acquired Infections and Colonization of the Respiratory Tract. Clin Infect Dis 2017;

100. Rocha DJ, Santos CS, Pacheco LG. Bacterial reference genes for gene expression studies by RT-qPCR: survey and analysis. Antonie Van Leeuwenhoek 2015;108:685-693.
101. Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A practical scale. Lancet 1974;2:81-84.
102. Frankenfield DC, Rowe WA, Smith JS, Cooney RN. Validation of several established equations for resting metabolic rate in obese and nonobese people. J Am Diet Assoc 2003;103:1152-1159.
103. Frankenfield DC, Coleman A, Alam S, Cooney RN. Analysis of estimation methods for resting metabolic rate in critically ill adults. JPEN J Parenter Enteral Nutr 2009;33:27-36.
104. Frankenfield DC, Ashcraft CM, Galvan DA. Prediction of resting metabolic rate in critically ill patients at the extremes of body mass index. JPEN J Parenter Enteral Nutr 2013;37:361-367.
105. Dinglas VD, Chessare CM, Davis WE, Parker A, Friedman LA, Colantuoni E, Bingham CO, Turnbull AE, Needham DM. Perspectives of survivors, families and researchers on key outcomes for research in acute respiratory failure. Thorax 2017;
106. Cook D, Lauzier F, Rocha MG, Sayles MJ, Finfer S. Serious adverse events in academic critical care research. CMAJ 2008;178:1181-1184.
107. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, Fornelos N, Haiser HJ, Reinker S, Vatanen T, Hall AB, Mallick H, McIver LJ, Sauk JS, Wilson RG, Stevens BW, Scott JM, Pierce K, Deik AA, Bullock K, Imhann F, Porter JA, Zhernakova A, Fu J, Weersma RK, Wijmenga C, Clish CB, Vlamakis H, Huttenhower C, Xavier RJ. Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nat Microbiol 2018;

Appendix B: Abbreviations and terms

ADLs	activities of daily living
AE	adverse event
ANOVA	analysis of variance
APACHE	acute physiology and chronic health evaluation
ASPEN	American Society for Parenteral and Enteral Nutrition
BMI	body mass index
CDC	Centers for Disease Control and Prevention
CDI	*Clostridium difficile* infection
CFU	colony-forming units
CLP	cecal ligation and puncture
CRF	case report form
CUMC	Columbia University Medical Center
DNI	Do not intubate
DNR	Do not resuscitate
DCC	Data Coordinating Center
DSMC	data and safety monitoring committee
DSMO	data and safety monitoring officer
ECMO	extra-corporeal membrane oxygen
EDTA	ethylenediamineteatraacetic acid
EMR	electronic medical record
EQ-5D-5L	EuroQol 5-dimension 5-level questionnaire
ER	emergency room
ESBL	extended-spectrum beta-lactamase
FDA	Food and Drug Administration
GCS	Glasgow coma scale
GI	gastrointestinal
GMP	good manufacturing practices
GRAS	generally recognized as safe
HRPO	human research protection office
IABP	intra-atrial balloon pump
ICD	International classification of diseases
ICU	intensive care unit
IND	investigational new drug
IQR	interquartile range
IRB	institutional review board
KPC	*Klebsiella pneumonia* carbapenemase
LAR	legally authorized representative
LDA	least discriminant analysis
LEfSe	linear discriminant analysis effect size

LPS	lipo-polysaccharide
MDR	multidrug resistant
MDRO	multidrug resistant organism
MRSA	methicillin-resistant *Staphylococcus aureus*
NHSN	National Health and Safety Network
OR	operating room
ORP	Office of Research Protections
OTU	operational taxonomic unit
PCR	polymerase chain reaction
PI	primary investigator
PICRUSt	phylogenetic investigation of communities by reconstruction of unobserved states
PPIs	proton pump inhibitors
PRMRP	peer-reviewed military research projects
QIIME	Quantitative Insights Into Microbial Ecology
qPCR	quantitative polymerase chain reaction
REDCap	Research Electronic Data Capture
rRNA	ribosomal ribonucleic acid
RT-PCR	real-time polymerase chain reaction
SAE	serious adverse event
SCFA	short-chain fatty acids
SD	standard deviation
SAPS3	simplified acute physiology score-3
SIRS	systemic inflammatory response syndrome
SOFA	sequential organ failure assessment
TNF	tumor necrosis factor
USAMRMC	U.S. Army Medical Research and Materiel Command
VRE	vancomycin-resistant *Enterococcus*
WGS	whole genome sequencing