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# Pathogen colonization of the gastrointestinal microbiome at intensive care unit admission and risk for subsequent death or infection

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## Abstract

**Purpose:** Loss of colonization resistance within the gastrointestinal microbiome facilitates the expansion of pathogens and has been associated with death and infection in select populations. We tested whether gut microbiome features at the time of intensive care unit (ICU) admission predict death or infection.

**Methods:** This was a prospective cohort study of medical ICU adults. Rectal surveillance swabs were performed at admission, selectively cultured for vancomycin-resistant *Enterococcus* (VRE), and assessed using 16S rRNA gene sequencing. Patients were followed for 30 days for death or culture-proven bacterial infection.

**Results:** Of 301 patients, 123 (41%) developed culture-proven infections and 76 (25%) died. Fecal biodiversity (Shannon index) did not differ based on death or infection ( $p = 0.49$ ). The presence of specific pathogens at ICU admission was associated with subsequent infection with the same organism for *Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp., and *Clostridium difficile*, and VRE at admission was associated with subsequent *Enterococcus* infection. In a multi-variable model adjusting for severity of illness, VRE colonization and *Enterococcus* domination ( $\geq 30\%$  16S reads) were both associated with death or all-cause infection (aHR 1.46, 95% CI 1.06–2.00 and aHR 1.47, 95% CI 1.00–2.19, respectively); among patients without VRE colonization, *Enterococcus* domination was associated with excess risk of death or infection (aHR 2.13, 95% CI 1.06–4.29).

**Conclusions:** *Enterococcus* status at ICU admission was associated with risk for death or all-cause infection, and rectal carriage of common ICU pathogens predicted specific infections. The gastrointestinal microbiome may have a role in risk stratification and early diagnosis of ICU infections.

**Keywords:** Critical care, Nosocomial infection, Vancomycin-resistant *Enterococcus*, Microbiome, Colonization resistance, Mortality

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## Introduction

Infection is the leading cause of death in medical intensive care units (ICUs) and accounts for 40% of all ICU expenditures [1]. ICU patients are more likely than ward patients to acquire infections while hospitalized with an attributable mortality up to 25% [2]. Antimicrobials, often given in a probabilistic fashion, are the mainstay of treatment for ICU infections [3]. But antimicrobial resistance is widespread, and there is a need for early diagnostics to facilitate targeted antimicrobial therapy [4].

The gastrointestinal tract is the primary reservoir for the bacterial pathogens that cause most healthcare-associated infections [5]. Yet the majority of patients who are colonized with pathogens never develop infections [6]. It is unknown whether there are features within the gastrointestinal microbiome at the time of ICU admission that predict subsequent infection and how such features may interact with established predictors of ICU morbidity and mortality.

Commensal colonic anaerobes check the expansion of pathogens through a process known as colonization resistance [7]. Fecal biodiversity has been evaluated as a surrogate for colonization resistance and thus as a proxy for the overall health of the gastrointestinal microbiome [8, 9]. In previous studies, low fecal biodiversity has been associated with increased risk for death or infection [10, 11] and domination of the gastrointestinal microbiota by a single organism, often *Enterococcus*, has been identified as an independent risk factor for adverse outcomes [12–14]. It is uncertain, however, whether measures such as diversity and domination are appropriate predictors for risk for infection in a heterogeneous medical ICU population.

This prospective study sought to evaluate clinically relevant risk factors within the gastrointestinal microbiome by collecting rectal swabs from medical ICU patients at admission and following patients for 30 days for death or culture-proven infection.

## Methods

### Population

Unique adult patients were considered for the study if they were admitted to the medical ICU of Columbia University Medical Center (CUMC) between April 1, 2016 and August 31, 2017. Patients were eligible if they were either directly admitted to the ICU (i.e., through the emergency room) or if they were transferred to the ICU from a hospital ward. The study was approved by the institutional review board of CUMC with a waiver of informed consent.

### Rectal swabs

In the participating ICUs, a rectal surveillance swab is routinely performed on all patients at the time of

ICU admission to evaluate for VRE colonization status. Flocked rectal swabs (Copan) [15] were collected by nurses with patients turned onto the left lateral decubitus position immediately after transfer into the ICU bed and the swab inserted deeply into the rectal canal and rotated five times. Swabs were transported in liquid Amies media for VRE culture and then flash-frozen at  $-80^{\circ}\text{C}$ . Additional surveillance swabs were not collected routinely and were not available for the study.

### VRE culture

Swabs were streaked onto plates of chromogenic media impregnated with 6  $\mu\text{g}/\text{ml}$  of vancomycin (Remel). Plates were incubated at 33–37  $^{\circ}\text{C}$  under aerobic conditions for 24 h and interpreted categorically according to the manufacturer's instructions (VRE present vs VRE absent).

### 16S rRNA gene sequencing

Swabs were removed from media, cultured for VRE, and sequenced for the V3/V4 hypervariable regions of the 16S ribosomal RNA gene using the Illumina MiSeq platform with negative controls [16]. The QIIME pipeline [17] was used to merge paired-end reads with  $\geq$  Q20 quality Phred score and minimum overlap of 25%. Rarefaction was at 6000 reads based on curve plateaus for alpha diversity. Operational taxonomic units (OTUs) were clustered at 97% sequence similarity and aligned against the Greengenes database with noise filtering [18]. Sequencing data is available in the short read archive section of the National Center for Biotechnology Information (accession number SRP130887).

### Analysis of 16S data

Two approaches were used to identify differentially abundant taxa. First, a targeted approach was performed with the most common pathogens classified as present if one or more read was present after rarefaction ( $\geq 0.017\%$  relative abundance) and otherwise as absent. In this approach, OTU identifiers were assigned to specific pathogens at the lowest possible hierarchical level (species level for *E. coli*, *C. difficile*, and *S. aureus*, and genus level for *Pseudomonas*, *Klebsiella*, and *Enterococcus*). Taxa with median relative abundance  $\geq 1\%$  were further classified based on whether they showed domination using the cut-off of defined by Taur et al. of  $\geq 30\%$  16S reads [12]. Second, an untargeted approach was performed using LEfSe [19] to compare OTU differences between groups after excluding taxa present in  $< 10\%$  of samples; a least discriminant analysis cut-off of  $\geq 2.0$  applied. The Shannon index was selected a priori as a measure of within-sample fecal microbial diversity and the Chao index as a measure of fecal microbial richness.

### Death and infection

The primary outcome of the study was a composite of death or culture-proven infection based on cultures obtained during the 30 days after ICU admission. Vital status data were retrieved from the hospital EMR which was cross-referenced with the national social security death index. Culture-proven infection was independently ascertained with blinding to pathogen status by evaluating complete clinical data and using criteria adapted from sepsis-3 (Supplementary Table 1 for complete criteria) [20]. Culture-proven infections were further classified according to the infecting organism and the site of infection.

### Statistical approach

Clinical and laboratory-based co-variables from ICU admission were derived from the Simplified Acute Physiology Score-3 (SAPS3) and gathered from the electronic medical record using automated queries [21]. A modified SAPS3 score, calculated excluding the Glasgow Coma Scale and surgical site information, was examined both as a linear variable and as an ordinal variable classified into tertiles. For hematocrit and albumin, which have been shown to predict ICU outcomes but are not included in SAPS3 [22], standard laboratory cut-offs were used. Additional non-SAPS3 variables included recent prior hospitalization or surgery, an admission diagnosis of sepsis, immunosuppression, and treatments received at the time of ICU admission including antibiotics, which were further classified as narrow- or broad-spectrum (see Supplement). The final multivariable analysis was constructed using a Cox proportional hazards model with patients followed from the time of ICU admission until death, culture-proven infection, or for a maximum of 30 days. The proportional hazards assumption was verified by visual inspection of time-to-event data and by testing for a non-zero slope in the Schoenfeld residuals. Non-SAPS3 variables were assessed in the full model if they had evidence of a relationship with the outcome ( $p < .10$ ) and other variables as components of SAPS3. Variables were retained in the reduced model if they had a significant independent relationship with the composite outcome or if they altered the  $\beta$ -coefficient representing fecal microbial characteristics by at least 10%. All analyses were performed using Stata statistical software (version 14, StataCorp) and were conducted two-sided at the  $\alpha = 0.05$  level of significance.

### Results

A total of 301 patients were included in the study. There were 123 patients (41%) who developed 211 distinct culture-proven infections and 76 patients (25%) who died within the 30 days following ICU admission. Table 1

describes the characteristics of patients at ICU admission stratified by death or culture-proven infection. The SAPS3 score was well calibrated to the outcome (Supplementary Table 2). The most common culture-proven infections were from *Staphylococcus aureus* (28 patients), *Escherichia coli* (26), *Pseudomonas aeruginosa* (23), *Enterococcus* spp. (22, including 16 VRE infections), *Klebsiella pneumoniae* (21), and *Clostridium difficile* (9). The most common infection sites were respiratory (58 patients), blood (27), and urine (19). Supplementary Table 3 describes infections according to organism and infection site.

### Microbiome features at ICU admission

The presence of potential pathogens at ICU admission was common based on sequencing data: *Enterococcus* spp. (88%), *Klebsiella* spp. (61%), *S. aureus* (29%), *Pseudomonas* spp. (22%), *C. difficile* (6%), and *E. coli* (4%). Among these organisms, only *Enterococcus* had  $>1\%$  relative abundance (Supplementary Table 4). Domination by *Enterococcus* ( $\geq 30\%$  reads) was present in 44 patients (15%) where it was also the most abundant organism; VRE was cultured in 91 patients (30%). *Enterococcus* domination was strongly associated with culture showing VRE (77% positive VRE culture in dominated patients vs 22% in other patients,  $p < .01$ ).

### Patient factors associated with the microbiome

Fecal microbial diversity and richness were decreased in patients who were transferred to the ICU from a hospital ward compared to those admitted directly to the ICU (both  $p < .01$ ). Diversity and richness were inversely correlated with the duration of pre-ICU hospitalization (Spearman  $p < .01$  for both), and were also inversely correlated with VRE colonization and *Enterococcus* domination (Supplementary Table 5). *Enterococcus* domination was more common in patients transferred to the ICU (20 vs 8%,  $p < .01$ ) and also more common with a longer duration of pre-ICU hospitalization (median difference +11 days,  $p < .01$ ); both of these results were similar when VRE status was assessed by culture. Pathogen status at admission was not clearly associated with transfer to the ICU from a hospital ward for any of the other common pathogens, and no other baseline patient factors were significantly associated with admission microbiome features.

### Microbiome features associated with death or infection

Neither fecal biodiversity (Shannon index) nor richness (Chao) differed between those who died or developed infections compared to those who did not ( $p = .49$  and  $p = .46$ , respectively). Principal components analysis did not separate samples based on death or infection (Supplementary Fig. 1).

**Table 1 Demographic, clinical, and laboratory characteristics at the time of ICU admission, stratified by death or infection status during the next 30 days**

	No death or infection <i>N</i> = 133	Death or infection <i>N</i> = 168	<i>p</i> value
SAPS3 characteristics at ICU admission			
Age (year)			.11
< 40	27 (20%)	18 (11%)	
40–59	40 (30%)	43 (26%)	
60–69	27 (20%)	48 (29%)	
70–74	10 (8%)	14 (8%)	
75–79	13 (10%)	15 (9%)	
≥ 80	16 (12%)	30 (18%)	
Hospital ward stay prior to admission			.68
≥ 28 days	11 (8%)	15 (9%)	
15–27 days	12 (9%)	14 (8%)	
1–14 days	47 (35%)	70 (42%)	
None (admission through ER)	63 (47%)	69 (41%)	
SAPS3 reason for admission			.54
Rhythm disturbances	1 (1%)	4 (2%)	
Hypovolemic shock	1 (1%)	4 (2%)	
Coma/obtunded	5 (4%)	8 (5%)	
Septic shock	5 (4%)	10 (6%)	
Liver failure	3 (2%)	6 (4%)	
Focal neurological deficit	0 (0%)	1 (1%)	
Severe pancreatitis	0 (0%)	1 (1%)	
Other	118 (89%)	134 (80%)	
SAPS3 preexisting comorbidities			.06
Heart failure	3 (2%)	13 (8%)	
Cirrhosis or AIDS	5 (4%)	9 (5%)	
Cancer	7 (5%)	16 (10%)	
None of the above	118 (89%)	130 (77%)	
Use of vasopressors	64 (38%)	104 (62%)	<.01
Temperature > 35 °C	3 (2%)	8 (5%)	.25
Heart rate > 120 bpm	7 (5%)	20 (12%)	.05
Systolic blood pressure (mmHg)			.58
≥ 120	79 (59%)	90 (54%)	
70–119	53 (40%)	76 (45%)	
< 70	1 (1%)	2 (1%)	
PaO <sub>2</sub> /FIO <sub>2</sub> (on mechanical ventilation) (mmHg)			.14
< 100	7 (5%)	9 (5%)	
≥ 100	38 (29%)	66 (39%)	
PaO <sub>2</sub> < 60 mmHg (not on mechanical ventilation)	5 (4%)	8 (5%)	.67
White blood cell count ≥ 15 × 10 <sup>9</sup> /L	45 (34%)	62 (37%)	.58
Platelet count (× 10 <sup>3</sup> /ml)			.03
> 100	113 (85%)	131 (78%)	
50–99	14 (11%)	19 (11%)	
20–49	3 (2%)	17 (10%)	
< 20	3 (2%)	1 (1%)	
pH ≤ 7.25	14 (11%)	21 (13%)	.60
Creatinine (μmol/L)			.24
> 309.4	25 (19%)	43 (26%)	
176.8–309.3	22 (17%)	36 (21%)	

Table 1 continued

	No death or infection N = 133	Death or infection N = 168	p value
106.1–176.7	33 (25%)	35 (21%)	
< 106.1	53 (40%)	54 (32%)	
Bilirubin (μmol/L)			.06
> 102.6	14 (11%)	28 (17%)	
34.2–102.6	11 (8%)	24 (14%)	
< 34.2	108 (81%)	116 (69%)	
Additional clinical characteristics at ICU admission			
Previous hospital admission within 90 days	28 (21%)	59 (35%)	<.01
Major surgery within 30 days	11 (8%)	8 (5%)	.21
Sepsis in admitting diagnosis	22 (17%)	58 (35%)	<.01
Immunosuppression	39 (29%)	43 (26%)	.47
Myeloablative chemotherapy	7 (5%)	15 (9%)	.23
Hematocrit < 35%	86 (65%)	123 (73%)	.11
Albumin < 221 μmol/L	14 (11%)	44 (26%)	.09
Treatments received at admission			
Mechanical ventilation	45 (34%)	75 (45%)	.06
Hemodialysis	22 (17%)	32 (19%)	.57
Proton pump inhibitors	39 (29%)	60 (36%)	.24
Antibiotics			<.01
None	40 (30%)	24 (14%)	
Narrow-spectrum only	8 (6%)	13 (8%)	
Broad spectrum with or without narrow-spectrum	85 (64%)	131 (78%)	

SAPS3 simplified acute physiology score-3, ICU intensive care unit, ER emergency room, AIDS acquired immunodeficiency syndrome

### Specific infections

*Enterococcus* domination as assessed by 16S sequencing was not associated with subsequent *Enterococcus* infection (9% domination vs 7% other patients, Fig. 1). However, culture for VRE at ICU admission was associated with subsequent *Enterococcus* infections (18 vs 3%, respectively,  $p < .01$ ). Patients with *E. coli* present at ICU admission were more likely to develop subsequent *E. coli* infection compared to patients without *E. coli* ( $p = .05$ ). This was also true for *C. difficile* ( $p < .01$ ), *P. aeruginosa* ( $p = .02$ ), and *K. pneumoniae* ( $p < .01$ ), but not for *S. aureus* (Fig. 1).

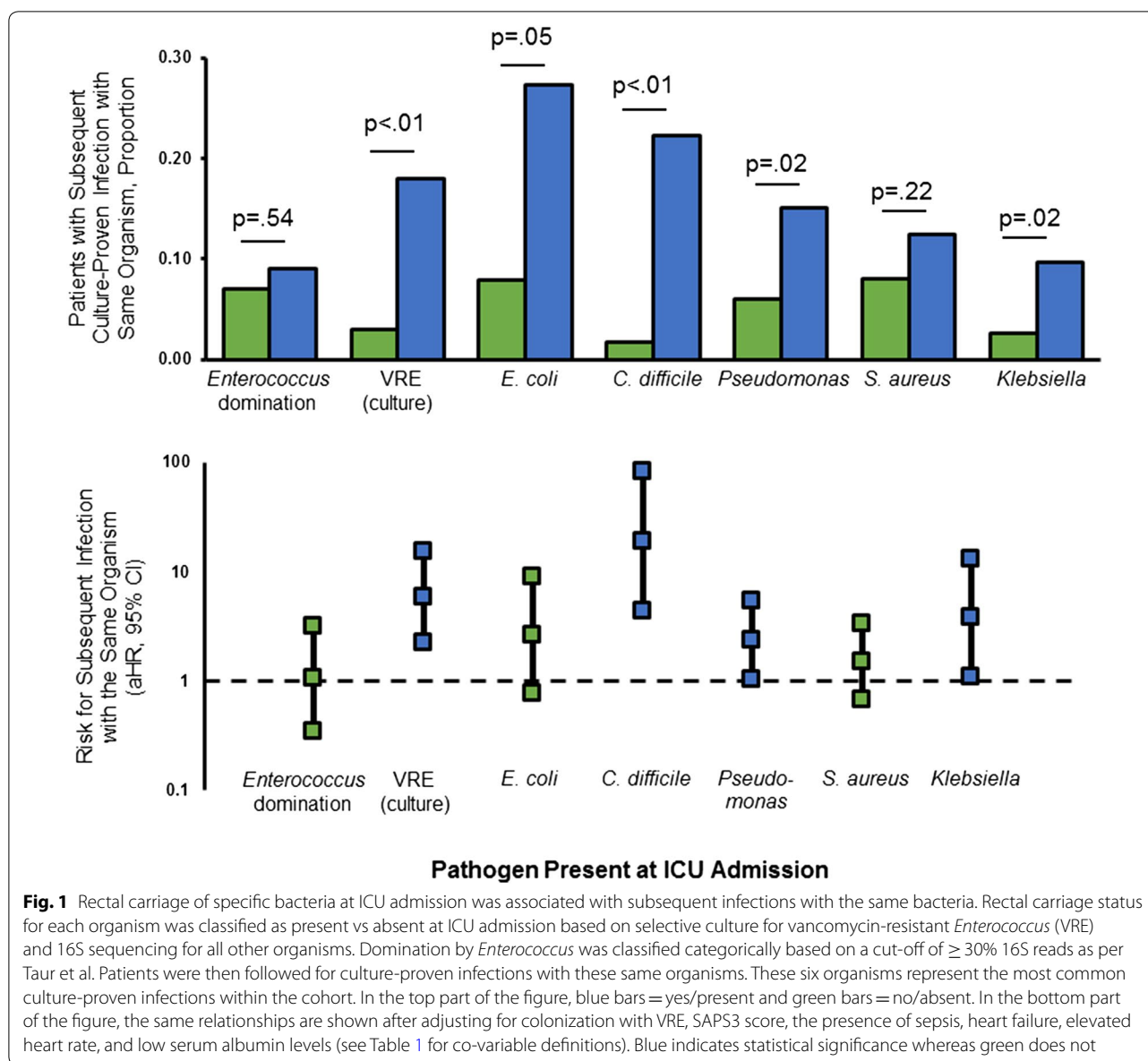
### Death or all-cause infection

Colonization with VRE or *Enterococcus* domination at ICU admission were associated with a 19 and 22% increased risk for death or infection, respectively ( $p < .01$  for both). When these outcomes were examined separately, VRE colonization was associated with 30-day mortality ( $p = .03$ , Supplementary Fig. 2) and, among those who survived, with all-cause infection ( $p < .01$ , Supplementary Fig. 3). Results were similar for *Enterococcus* domination. VRE colonization and *Enterococcus* domination were also associated with increased length of ICU stay (median differences +1 and +2 days,  $p = .04$  and

$p < .01$ , respectively). Patients who were discordant for *Enterococcus* domination and VRE had increased risk for death or infection regardless of which test was positive (Fig. 2). Presence of the other common pathogens at ICU admission did not associate with subsequent death or all-cause infection. Alternative cut-points for these organisms were explored but this did not alter the results (Supplementary Table 6). In an untargeted analysis, twenty-two OTUs differed in patients who died or developed infections compared to patients who did not (Supplementary Table 7). Among 14 OTUs that were increased in those who died or developed infections, seven were assigned to the Enterobacteriaceae or Pseudomonadaceae families.

### Multivariable model for death or all-cause infection

A Cox proportional hazards regression model was constructed to examine specific pathogens as risk factors for death or all-cause infection after ICU admission (Table 2). Colonization with VRE at ICU admission was a significant predictor of death or infection in the final model and results were similar when *Enterococcus* domination was entered in the model in lieu of VRE colonization. There was a trend towards increased 30-day mortality associated with VRE and with *Enterococcus*

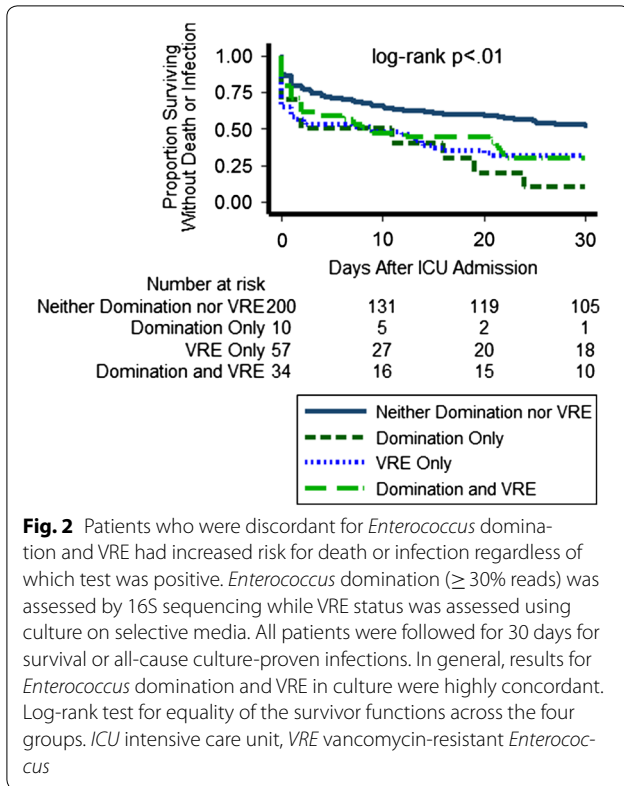


domination that was not statistically significant (respectively aHR 1.29, 95% CI 0.80–2.07 and aHR 1.24, 95% CI 0.68–2.27). Among those without VRE colonization, *Enterococcus* domination strongly associated with the combined outcome (aHR 2.13, 95% CI 1.05–4.29). There were no significant changes in these models after excluding 81 subjects who died or developed infections within 72 h of ICU admission, and also no changes when SAPS3 was classified as a linear variable. Presence of any of the other common bacterial pathogens did not significantly predict death or all-cause infection (Supplementary Table 8). However, the presence of taxa corresponding to *C. difficile*, *Pseudomonas*, and *Klebsiella* all predicted subsequent infections with the same organisms after

adjusting for the variables in the final model (Fig. 1). Neither fecal microbial diversity nor richness was significant in the final model (aHR 1.10, 95% CI 0.93–1.30 and aHR 1.00, 95% CI 0.99–1.01, respectively).

## Discussion

This prospective study found that the presence or absence of specific bacteria at ICU admission was associated with death or culture-proven infection during the next 30 days. Taxa corresponding to the common pathogens *Enterococcus*, *Pseudomonas*, *Klebsiella*, and *C. difficile* were each individually associated with subsequent infection with the same organisms. *Enterococcus* was unique: domination of the gastrointestinal microbiome



by *Enterococcus* or VRE colonization based on culture predicted death or all-cause infection; even among those who were not VRE colonized, *Enterococcus* domination remained a strong predictor of death or infection. Overall gastrointestinal diversity and richness, predictors of death or infection in other populations, were not associated with death or infection in this cohort.

Previous studies have demonstrated that pathogens can be cultured from stool or swabs that predict specific infections. Organism-specific colonization has been established as a risk factor for subsequent infection for VRE [13], MRSA [23], *C. difficile* [24], *Enterobacteriaceae* [25], and other bacteria. The role of quantitative culture in the ICU has also been investigated, especially for the diagnosis of ventilator-associated pneumonia [26]. This study focused on 16S sequencing rather than culture and found that the presence or absence of certain taxa predicted common infections. These findings highlight the potential for stool testing as a guide to early empiric therapy in the ICU. For *Enterococcus*, the pathogen that best predicted death or infection, sequencing results were closely comparable to results from VRE cultures. Gain and loss of resistance is common for hospital *E. faecalis* lineages and domination may be an important marker even in the absence of vancomycin resistance [27].

Microbiome-based interventions such as probiotics have been tested in the ICU to decrease infection, but no currently available therapies are proven to improve gut colonization resistance. Surveillance for gastrointestinal bacterial pathogen colonization usually focuses on multi-drug resistant organisms [28] and remains controversial [29]. Translating the identification of colonized patients into a subsequent reduction in risk for infection will only be possible if effective interventions are available [30] and if appropriately high-risk patients can be selected prior to implementing any intervention. This study's results suggest that *Enterococcus* burden may complement VRE status as a useful surrogate target for studies testing probiotics or conventional infection control-based strategies. Future studies may also wish to examine whether a tipping point can be identified for *Enterococcus* domination,

**Table 2** The full and reduced Cox proportional hazards models for death or infection

Risk factors at ICU admission	Full model hazard ratio, 95% CI	Reduced model hazard ratio, 95% CI
VRE colonization	1.43 (1.04–1.97)	1.46 (1.06–2.00)
SAPS3 tertile		
Tertile 1	Reference	Reference
Tertile 2	1.84 (1.22–2.79)	1.93 (1.29–2.88)
Tertile 3	1.87 (1.23–2.83)	2.01 (1.36–2.98)
Hospitalization within 90 days	1.43 (1.04–1.98)	1.42 (1.03–1.96)
Mechanical ventilation	1.19 (0.86–1.64)	–
Receipt of antibiotics		
None	Reference	–
Narrow-spectrum only	1.70 (0.86–3.37)	
Broad-spectrum	1.31 (0.83–2.08)	
Sepsis	1.69 (1.21–2.36)	1.72 (1.25–2.39)
Albumin < 221 $\mu\text{mol/L}$	1.60 (1.11–2.29)	1.60 (1.12–2.28)

VRE vancomycin-resistant *Enterococcus*, ICU intensive care unit, CI confidence interval

and whether specific interventions can affect the relative abundance of *Enterococcus* in ICU patients.

In these data, the risk associated with gastrointestinal colonization depended on the organism. The most dramatic results were seen with *Enterococcus* whereas *C. difficile*, *Pseudomonas*, and *Klebsiella* were each individually associated with specific infections but not with overall infection. Enterococci clones such as *E. faecium* ST796 exhibit broad antibiotic resistance and starvation tolerance, characteristics that help them to thrive under unique ecological conditions including the selective pressure of vancomycin [31]. Selective killing of Gram-negative bacteria by antibiotics reduces RegIII- $\gamma$  levels and allows expansion of Gram-positives including *Enterococcus* [32]. As a result, when the right conditions exist, *Enterococcus* exhibits a densely dominating phenotype [33]. When other conditions exist, such as high levels of nitrate and colonic inflammation, a more diverse set of pathogens may appear including multiple Gammaproteobacteria species [34]. This may explain why *Enterococcus* associated with all-cause infection whereas the other organisms did not: the ecological features of *Enterococcus* mean that it uniquely reflects loss of gastrointestinal colonization resistance.

This study's results regarding fecal microbial diversity contrast with the results of Taur et al., who found that fecal microbial diversity prior to BMT was an important predictor of death and infection [12]. Compared to healthy volunteers, ICU patients have low fecal microbial diversity [35, 36], which further declines with prolonged admission [37], and are likely to show *Enterococcus* domination [38]. BMT patients also have low diversity compared to healthy subjects, but have profound immunological changes compared to ICU patients. In BMT patients, lack of transplant engraftment mediates the development of infection and high gastrointestinal biodiversity may reflect a Th1 and Th17 phenotype driven by obligate anaerobes that defends against infection [39]. In contrast for ICU patients, who do not have to completely reconstitute their immunity, diversity may be less important as a marker of loss of colonization resistance. Acuity of illness also may be a factor. ICU patients are heterogeneous and include patients who lack sufficient time to lose diversity between the onset of illness and ICU admission.

There are several strengths to this study. It was prospective, relatively large, incorporated both culture and sequencing data, and involved a standard medical ICU population that facilitates generalization of the results. The primary outcome—death or culture-proven infection—was important and rigorously adjudicated. The study also has limitations. There is no single gold standard for the ascertainment of infection in the ICU; the definition used here was designed to maximize specificity rather than sensitivity.

Sequencing results equate OTUs with specific bacteria but rarely distinguish bacteria at the species level (e.g., *E. faecium* vs *E. faecalis*) and may not correlate with culture-based tests. This study used rarefied data to focus on relatively abundant gut bacteria, and alternative methods would be necessary to examine very scarce taxa. Last, although the types of bacterial infections observed here parallel results from other ICUs in the developed world, results from this study may not apply to all institutions or ICUs [40].

In sum, the specific pathogens responsible for most medical ICU infections could be identified at the time of ICU admission. Colonization with VRE and domination by *Enterococcus* were important risk factors for all-cause infection and mortality, with domination a risk factor even in the absence of VRE. The presence of VRE or *Enterococcus* domination may be useful surrogate outcomes for studies seeking to intervene on the gastrointestinal microbiome to prevent infections in critically ill patients or similarly at-risk patient populations.

#### Electronic supplementary material

The online version of this article (<https://doi.org/10.1007/s00134-018-5268-8>) contains supplementary material, which is available to authorized users.

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#### Compliance with ethical standards

#### Conflicts of interest

None of the authors have conflicts of interest.

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