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Epidemiological and Microbiome Associations Between *Klebsiella pneumoniae* and Vancomycin-Resistant *Enterococcus* Colonization in Intensive Care Unit Patients

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Background. Prior colonization by *Klebsiella pneumoniae* and vancomycin-resistant *Enterococci* (VRE) is associated with subsequent infection, particularly in intensive care unit (ICU) populations. Screening for VRE colonization, but not *K. pneumoniae*, is routinely performed in some health care systems. Identification of patient factors associated with *K. pneumoniae* colonization could enable infection prevention.

Methods. ICU patients were screened for VRE and *K. pneumoniae* by rectal swab culture over 2 time periods: July–October 2014 (n = 1209) and January–May 2016 (n = 1243). Patient demographics, baseline laboratory data, comorbidities, and outcomes were analyzed. 16S rRNA gene-based analysis was performed on a subset of patients (n = 248) to identify microbiota characteristics associated with VRE and *K. pneumoniae* colonization.

Results. *K. pneumoniae* colonization (17.3% of patients in the 2014 cohort, 7.3% in 2016) was significantly associated with VRE colonization in multivariable analysis ($P = .03$ in 2016; $P = .08$ in 2014). VRE colonization was associated with poor underlying health, whereas *K. pneumoniae* colonization was associated with advanced age. The most prevalent operational taxonomic units were *Escherichia coli/Shigella* spp., *Klebsiella,* and *Enterococcus*, consistent with high rates of detectable *K. pneumoniae* and VRE by culture. Microbial community structure in noncolonized patients was significantly different from those with VRE, *K. pneumoniae,* or both, attributable to differences in the relative abundance of *Klebsiella* and *Enterococcus*.

Conclusions. *K. pneumoniae* co-colonizes with VRE and is a predominant taxon in ICU patients, but colonization was not associated with significant comorbidities. Screening for *K. pneumoniae* and VRE simultaneously could be an efficient approach for novel infection prevention strategies.

Keywords. *Klebsiella*; vancomycin-resistant *Enterococci*; colonization; infection; microbiome.

Klebsiella pneumoniae is a gram-negative bacillus that causes pneumonia, bloodstream infections, and urinary tract infections in hospitalized or immunocompromised patients [1]. It is a leading cause of health care–associated infections (HAIs), causing around 10% of HAIs annually [2]. Additionally, *K. pneumoniae* is becoming increasingly resistant to antibiotics, including carbapenems, complicating treatment of infections and leading the Centers for Disease Control and Prevention to label *Klebsiella* an urgent public health threat [3]. Prior colonization with *K. pneumoniae* is significantly associated with subsequent infection, and 80% of infections in colonized patients are caused by their colonizing strain [4, 5]. The acquisition of Carbapenem-resistant *K. pneumoniae* (CRKP) in the hospital is strongly associated with colonization pressure, and cohorting patients colonized with CRKP is more effective at preventing transmission than any other infection control measure alone [6–8]. Therefore, understanding risk factors for *K. pneumoniae* colonization could direct screening measures to vulnerable patient populations, preventing the spread of colonization throughout the hospital and identifying patients at risk for later infection.

Vancomycin-resistant *Enterococci* (VRE) is a gram-positive coccus that causes around 20 000 HAIs annually. Like *K. pneumoniae,* VRE colonization is a risk factor for later infection [9, 10], with a sensitivity of 94%, specificity of 78%, and relative risk of 24.15 for bacteremia [11]. To prevent patient-to-patient spread of VRE, many hospitals perform routine surveillance cultures of rectal swabs and place detectably colonized patients in contact precautions [12–14]. *K. pneumoniae* and VRE infect a similar patient population of hospitalized, immunocompromised patients, and previous antibiotic exposure is a risk factor for colonization with antibiotic-resistant strains of either organism [1, 13, 15]. We have previously shown high

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K. pneumoniae colonization rates in patients screened for VRE [4]. Despite colonizing similar patients, the biological and epidemiological links between VRE and *K. pneumoniae* are unclear.

Culturing VRE surveillance samples for *K. pneumoniae* and analyzing patient medical records could identify factors associated with colonization in patients susceptible to infection. Upon patient identification, 1 potential intervention strategy might involve manipulating the microbiota to better resist colonization by exogenous pathogens. Previous studies have begun to identify intestinal microbiota signatures associated with acquisition of VRE and other multidrug-resistant organisms [16–18]. For example, CRKP colonization has been associated with the presence of 2 specific operational taxonomic units (OTUs; *Desulfovibrio* and *Ruminococcaceae*) [19], but this finding may be specific to the specific *Klebsiella* clone. Identifying species found in the microbiota that are broadly associated with *K. pneumoniae* colonization could enable widely applicable approaches to infection prevention.

The objective of this study was to identify patient factors associated with colonization with *K. pneumoniae* in ICU patients. We screened samples from 2 patient cohorts, 1 from 2014 and 1 from 2016, for *K. pneumoniae* colonization and analyzed the associations between colonization and patient demographics, baseline laboratory data, and comorbidities. In addition, 16S rRNA sequencing of the microbiome was performed to determine if there was a microbiota signature associated with *K. pneumoniae* colonization. Understanding risk factors for *K. pneumoniae* colonization might inform surveillance practices for *K. pneumoniae* colonization in the hospital setting.

METHODS

Sample Collection

This study was conducted at Michigan Medicine, a tertiary care center with 1000 beds, and approved by the Institutional Review Board. Rectal swabs were obtained through Michigan Medicine's VRE surveillance program, wherein adult patients admitted to the intensive care unit (ICU) or hematology/oncology wards have a rectal swab taken upon admission and then weekly until a positive VRE culture is obtained or the patient is discharged. We included swabs collected from adults during 2 time periods: July 31, 2014, to October 31, 2014 (n = 1800), and January 10, 2016, to May 25, 2016 (n = 1824).

Bacterial Identification and Patient Population

Rectal swabs (ESwab Collection and Transport System, Becton Dickinson, Franklin Lakes, NJ, USA) were sent to Michigan Medicine's Clinical Microbiology laboratory and cultured to identify VRE using VRE*Select* chromogenic media (Bio-Rad, Hercules, CA, USA). Swabs were stored in glycerol at −80°C. To identify *Klebsiella pneumoniae* in the samples, swabs were either cultured on MacConkey agar plates before freezing (2014 cohort) or after thawing (2016 cohort), and 10 µL of sample was streaked. Three mucoid lactose-fermenting colonies were isolated and subcultured onto MacConkey agar plates. If <3 mucoid lactose-fermenting colonies were present from the sample, then all colonies were subcultured. Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) analysis was used for bacterial identification from the subcultures. Only patients with rectal swabs screened for both *K. pneumoniae* and VRE that were collected during patients' first encounter with Michigan Medicine during the study periods were included: 1209 for the 2014 cohort and 1243 for the 2016 cohort.

Demographic Definitions

Patient electronic medical records (EMRs) were reviewed for patient variables associated with colonization. Comorbidity data were collected from the patient's first encounter during the study. Comorbidities were defined using the Elixhauser Comorbidity Index [20]. The Elixhauser Comorbidity Score was calculated as previously described [21, 22]. Antibiotic usage was defined as antibiotic administration that occurred within 30 days preceding rectal swab collection and that started at least 48 hours before rectal swab collection. Patients were considered to have a *K. pneumoniae* clinical culture if their EMRs indicated a positive clinical blood, urine, or respiratory culture for *K. pneumoniae* within 90 days after their first rectal swab. Patients were considered to have a *K. pneumoniae* infection if the clinical culture met case definitions for a bloodstream infection, pneumonia, or a urinary tract infection within 90 days after their first rectal swab. Bloodstream infection was defined as any blood culture positive for *K. pneumoniae*. Pneumonia was defined based on new or progressive radiographic infiltrate plus at least 2 of 3 clinical features (fever >38°C, leukocytosis or leukopenia, and purulent secretions) [23]. Urinary tract infection was defined based on National Healthcare Safety Network (NHSN) case definitions [24].

Antibiotic susceptibility results (Sensititre, ThermoFisher Scientific) were extracted for *Klebsiella*-colonized patients with subsequent positive clinical cultures and reviewed for interpretations of extended-spectrum beta-lactamase and carbapenem resistance in the specimen report.

DNA Purification and Microbiota Analysis

The MagAttract PowerMicrobiome DNA/RNA Kit (Qiagen, Inc., Hilden, Germany) optimized for the EpMotion 5075 (Eppendorf, Hamburg, Germany) was used to isolate DNA from swabs. The V4 region of the bacterial 16S rRNA genes was polymerase chain reaction–amplified [25] from 1 µL of the sample DNA, as previously described [26]. The 500 cycle MiSeq Reagent Kit v.2 (Illumina, catalog No. MS-102-2003) was used to prepare amplicons for sequencing on a MiSeq (Illumina, San Diego, CA, USA) by the University of Michigan Microbial Systems Molecular Biology Laboratory using the manufacturer's protocols [26].

Sequences were quality-trimmed and processed using mothur v.1.39.5 [27] as described previously [19]; chimeras were removed using UCHIME [28] and reads aligned to the SILVA 16S rRNA sequence database [29]. Samples with <2500 sequences were excluded from further analysis. Using mothur's opticlust algorithm [30], sequences were clustered into OTUs at 97% similarity. The Ribosomal Database Project was used to classify OTUs [31]. Inverse Simpson's index, shared OTUs, and the Yue and Clayton dissimilarity index were calculated from unfiltered OTU data. Principal components analysis and analysis for molecular variance (AMOVA) were calculated in mothur. Basic R packages were used to visualize data. We created Dirichlet multinomial mixture models using R v.3.3.2 and used the DirichletMultinomial v.1.6.0 package [32] to assign all samples to community types, as proposed by Holmes et al. [33]. We determined the number of community types by comparing the Laplace approximation with the negative log posterior likelihood and identifying the point at which an increase in Dirichlet components results in minor improvements in model fit.

Statistical Analysis

We compared the distributions of continuous variables among *K. pneumoniae*–colonized patients with those of noncolonized patients using the Student *t* test; for categorical variables, we used the chi-square or Fisher exact test. Variables significantly associated with *K. pneumoniae* colonization in either cohort at $\alpha = 0.05$ were entered into a multivariate logistic model predicting *K. pneumoniae* colonization. Univariate analysis comparing co-colonized, *K. pneumoniae*–colonized, VRE-colonized, and noncolonized patients was conducted using 1-way analysis of variance for continuous variables and the chi-square or Fisher exact test for categorical variables. Additional analyses comparing the colonized groups with the noncolonized group were performed using Tukey's multiple comparisons test for continuous variables and the chi-square or Fisher exact test for categorical variables with the hybrid Wilson/Brown method of estimating confidence intervals (Prism 7, Graph Pad). AMOVA was used to distinguish the microbiota structure between patient groups. The nonparametric Kruskal-Wallis test was used for multigroup microbiota comparisons.

RESULTS

VRE Colonization Is Associated With *K. pneumoniae* Colonization

Of the 1209 rectal swabs collected from eligible patients between July 31 to October 31, 2014 (2014 cohort), 209 (17.3%) were colonized with *K. pneumoniae.* In the 2016 cohort, collected from 1243 eligible patients between January 10 and May 25, 2016, 91 (7.3%) were colonized with *K. pneumoniae* (Supplementary Figure 1). In addition to increased prevalence of *K. pneumoniae* colonization in 2014 ($P < .0001$), women represented a higher percentage and white patients represented a smaller percentage of patients. Conversely, length of stay was significantly longer in the 2016 cohort (Table 1). Based on significant differences between the cohorts, the results were stratified based on cohort year, with the goal of finding consistent associations across 2 cohorts that differ by date and *K. pneumoniae* colonization rates.

To identify variables consistently associated with *K. pneumoniae* rectal colonization, univariate analysis was performed on demographic, comorbidity, and baseline laboratory data from each cohort (Table 2). The Elixhauser comorbidity score was not significantly associated with colonization in either cohort (2014: 7.57 ± 8.47 vs 7.14 ± 8.80; $P = .52$; 2016: 7.55 ± 6.37 vs 6.87 ± 7.57; $P = .40$). A positive clinical culture for *K. pneumoniae* was associated with rectal colonization in both the 2014 (odds ratio [OR], 5.0; 95% confidence interval [CI], 2.82–9.2; $P < .0001$) and 2016 (OR, 3.5; 95% CI, 1.38–9.07; $P = .0263$) cohorts. The sensitivity of detectable colonization for a subsequent positive culture was 48.9% (95% CI, 35.3%–62.8%) and 20.8% (95% CI, 9.2%–40.5%), and the specificity was 83.9% (95% CI, 81.8%–86.0%) and 92.9% (95% CI, 91.4%–94.3%) in the 2014 and 2016 cohorts, respectively. The negative predictive value for subsequent infection was consistently high in both cohorts (2014: 97.6%; 95% CI, 96.5%–98.4%; 2016: 98.4%; 95% CI, 97.4%–98.9%). We reviewed antibiotic

Table 1. Demographic Information and Colonization Rates for the 2014 and 2016 Cohort

Patient Variable, No. (%)	Year		*P* Value
	2014 (n = 1209)	2016 (n = 1243)	
Sex–female	591 (48.9)	548 (44.1)	.017
Age, mean ± SD, y	57.9 ± 16.4	58.9 ± 16.4	.119
Race–white	990 (81.9)	1059 (85.2)	.028
Black	128 (10.6)	121 (9.7)	
Other	91 (7.5)	63 (5.1)	
Admits from nursing home	3 (0.2)	2 (0.2)	.683
Length of stay, mean ± SD, d	11.5 ± 17.0	13.2 ± 17.4	.017
Death within 30 d	110 (9.1)	120 (9.7)	.637
Elixhauser comorbidity score, mean ± SD	7.22 ± 8.7	6.92 + 7.5	.363
Klebsiella pneumoniae–colonized	209 (17.3)	91 (7.3)	<.0001
VRE-colonized	118 (9.8)	96 (7.7)	.074
Primary service–surgery	216 (17.9)	224 (18.0)	.094
GynOnc	117 (9.7)	87 (7.0)	
Cardiac	119 (9.8)	100 (8.1)	
HemOnc	181 (15.0)	194 (15.6)	
Medical critical care	78 (6.5)	69 (5.6)	
Medicine	229 (19.0)	249 (20.0)	
Burn	17 (1.4)	13 (1.0)	
Cardiothoracic surgery	184 (15.2)	222 (17.9)	
Other	68 (5.6)	85 (6.8)	

Differences between the cohorts were calculated using the Student *t* test for continuous variables and the chi-square or Fisher exact test for categorical variables.

Abbreviation: VRE, vancomycin-resistant *Enterococci.*

resistance among clinical cultures from *Klebsiella*-colonized patients. Consistent with the low institutional resistance rate for *K. pneumoniae*, 1 extended-spectrum beta-lactamase isolate was found in a 2016 clinical culture, and no CRKP were detected. Clinical infection was significantly associated with *K. pneumoniae* colonization in the 2014 cohort (OR, 5.5; 95% CI, 2.51–12.20; $P < .0001$). Infections were higher among patients who were colonized with *K. pneumoniae* in the 2016 cohort as well (OR, 2.85; 95% CI, 0.61–11.0); however, this did not reach statistical significance. In the cohort from 2014, patients colonized with *K. pneumoniae* were more likely to be female, have hypertension, have lower baseline albumin levels, and be older than noncolonized patients. In the 2016 cohort, *K. pneumoniae*–colonized patients were more likely to die within 30 days, have a neurologic disorder, and have underlying pulmonary circulation comorbidities. VRE colonization (2014: OR, 1.57; 95% CI, 0.95–2.57; 2016: OR, 2.20; 95% CI, 1.258–3.877) and low albumin baseline (2014: 3.44 ± 0.62 colonized vs 3.56 ± 0.65 not colonized; $P = .027$; 2016: 3.34 ± 0.58 vs 3.47 ± 0.62; $P = .072$) were potential shared risk factors for *K. pneumoniae* colonization between the cohorts.

In the final multivariable models, VRE colonization was associated with *K. pneumoniae* colonization with similar point estimates in both cohorts (2014: OR, 1.58; 95% CI, 0.92–2.62; 2016: OR, 2.07; 95% CI, 1.06–3.83), as was age >70 (OR, 1.73; 95% CI, 1.09–2.78; OR, 1.8; 95% CI, 0.96–3.47) (Table 3). Black race (OR, 1.38; 95% CI, 0.85–2.2; OR, 1.91; 95% CI, 0.94–3.63) had similar point estimates in both but did not reach statistical significance in either cohort. In the 2014 cohort, male gender was inversely associated with *K. pneumoniae* colonization (OR, 0.7; 95% CI, 0.51–0.96), but this was not observed in the 2016 cohort (OR, 1.14; 95% CI, 0.71–1.85). Similarly, hypertension was associated with colonization in the 2016 cohort (OR, 0.49; 95% CI, 0.22–0.97), but the point estimate was reversed in the 2014 cohort (OR, 1.33; 95% CI, 0.97–1.83).

VRE Colonization Is Associated With Comorbidities and Poor Outcomes

After establishing the association between *K. pneumoniae* and VRE colonization in ICU patients, we tested the hypothesis that co-colonized patients have more comorbidities and worse health outcomes. We grouped the cohorts into patients colonized with both *K. pneumoniae* and VRE, patients colonized with *K. pneumoniae* or VRE, and patients colonized with neither organism and performed univariate analysis with postanalysis, comparing each colonized group with the noncolonized group (Supplementary Table 1). Across both cohorts, the Elixhauser score was higher and albumin baseline was lower in the both-positive and VRE-positive colonization groups compared with the both-negative group. Also in both cohorts, death within 30 days, central lines, and urinary catheters were significantly associated with VRE colonization only, compared with the both-negative group. In the 2014 cohort but not the 2016 cohort, patients colonized with both organisms and only *K. pneumoniae* were more likely to have a later *K. pneumoniae* infection compared with patients colonized by neither. Overall, VRE and dual-colonized patients appeared to have poorer overall health compared with noncolonized patients, whereas *K. pneumoniae*–colonized patients did not.

Klebsiella and *Enterococcus* OTUs Dominate the Gut Microbiota in Colonized Patients

The positive association between VRE and *K. pneumoniae* colonization suggested that the microbiota may differ between colonized (either alone or with both) and noncolonized patients. To examine these differences and better understand potential mechanisms of colonization, we performed 16S rRNA gene-based analysis on a total of 248 available rectal swabs from the 2014 cohort. Of those swabs, 7 were from patients co-colonized with VRE and *K. pneumoniae*, 20 were from patients colonized with VRE, 31 were from patients colonized with *K. pneumoniae,* and 190 swabs were from patients not colonized with either organism at baseline.

Overall diversity of the microbiota communities (inverse Simpson's diversity) did not significantly differ across the 4 groups (Figure 1A). However, beta-diversity indices, reflective of microbial community similarity between the groups, demonstrated that co-colonized individuals exhibited a more similar microbiota to *K. pneumoniae*–colonized patients than to VRE-colonized or noncolonized patients (Figure 1B). Further, co-colonized patients and *K. pneumoniae* patients shared more operational taxonomic units (OTUs) than co-colonized patients and VRE-colonized patients (Supplementary Figure 2). AMOVA based on the Yue and Clayton theta dissimilarity index [34] indicated significant differences between groups overall ($P < .001$) (Supplementary Table 2) and between the noncolonized group compared with the co-colonized group ($P = .048$), the VRE-positive group ($P < .001$), or the *K. pneumoniae*–positive group ($P < .001$). The community composition also differed significantly between the *K. pneumoniae*– and VRE-colonized groups ($P = .003$) (Figure 1C). Community modeling by Dirichlet multinomial mixtures (DMMs) separated the samples into 4 community state types (CSTs) (Supplementary Figure 3) [32, 33]. Although no single CST was correlated with colonization, patients colonized with any combination of *K. pneumoniae* and VRE were significantly more likely to have CST1 or 2 compared with CST 3 and 4 combined ($P = .05$). Principal coordinate analysis with overlayed biplots of OTUs indicated that 2 OTUs of *Enterobacteriaceae* (OTU1 and OTU2) and 1 *Enterococcus* OTU (OTU3) were strong components in driving these influences (Figure 1C). Further comparisons of the sequences clustered into each of the OTUs revealed that OTU1 contained sequences classified as *Escherichia coli/Shigella*, whereas OTU2 contained *K. pneumoniae, K. variicola,* and *Enterobacter* species. OTU3 contained sequences classifying as *Enterococcus*

Table 2. Clinical and Demographic Characteristics of Patients With and Without *Klebsiella pneumoniae* Colonization

	2014 Cohort			2016 Cohort		
Variable	No.	Kp-Colonized	*P* Value	No.	Kp-Colonized	*P* Value
Female	591	116 (19.63)	.035	548	37 (6.75)	.494
Male	618	93 (15.05)		695	54 (7.77)	
Age			.024			.254
<50 y	347	42 (12.10)		344	21 (6.10)	
51–60 y	275	51 (18.55)		249	14 (5.62)	
61–70 y	306	59 (19.28)		333	26 (7.81)	
71+ y	281	57 (20.28)		317	30 (9.46)	
Race			.386			.119
White	990	169 (17.1)		1059	71 (6.7)	
Black	128	27 (21.1)		121	14 (11.5)	
Other	91	13 (14.2)		63	6 (9.5)	
Length of stay			.954			.877
<4 d	357	63 (17.65)		333	22 (6.61)	
4–7 d	267	48 (17.98)		277	20 (7.22)	
7–14 d	302	52 (17.22)		267	19 (7.12)	
14+ d	283	46 (16.25)		366	30 (8.20)	
Death in 30 d	110	23 (20.91)	.292	120	15 (12.50)	.022
No death in 30 d	1099	186 (16.92)		1123	76 (6.77)	
Later *K. pneumoniae* clinical culture	47	23 (48.9)	<.0001	24	5 (20.8)	.0263
No later *K. pneumoniae* clinical culture	1162	186 (16.0)		1219	86 (7.1)	
Later *K. pneumoniae* infection	25	13 (52.0)	<.0001	11	2 (18.1)	.1897
No later *K. pneumoniae* infection	1184	196 (16.6)		1232	89 (7.2)	
VRE-colonized	96	23 (23.96)	.072	118	16 (13.56)	.006
Not VRE-colonized	1113	186 (16.71)		1125	75 (6.67)	
Neurologic disorder	95	16 (16.84)	>.999	65	11 (16.92)	.005
No neurologic disorder	1114	193 (17.32)		1178	80 (6.79)	
Congestive heart failure	127	25 (19.69)	.457	123	14 (11.38)	.097
No congestive heart failure	1082	184 (17.01)		1120	77 (6.88)	
Pulmonary circulation disorder	72	13 (18.06)	.872	67	10 (14.93)	.026
No pulmonary circulation disorder	1137	196 (17.24)		1176	81 (6.89)	
Peripheral vascular disease	90	20 (22.22)	.194	99	7 (7.07)	>.999
No peripheral vascular disease	1119	189 (16.89)		1144	84 (7.34)	
Hypertension	449	92 (20.49)	.027	198	10 (5.05)	.233
No hypertension	760	117 (15.40)		1045	81 (7.75)	
Admitted overnight 180 d prior	701	124 (17.69)	.664	676	55 (8.14)	.907
Not admitted overnight 180 d prior	508	85 (16.73)		567	36 (6.35)	
Antibiotic exposure	110	12 (10.91)	.064	170	10 (5.88)	.438
No antibiotic exposure	1099	197 (17.93)		1073	81 (7.55)	
Central line	92	12 (13.04)	.263	147	7 (4.76)	.205
No central line	1117	197 (17.93)		1096	84 (7.66)	
Urinary catheter	87	17 (19.54)	.564	136	5 (3.68)	.084
No urinary catheter	1122	192 (17.11)		1107	86 (7.77)	
Hemoglobin baseline			.204			.554
<9.9	313	58 (18.53)		319	29 (9.09)	
10–11.7	303	58 (19.14)		316	22 (6.96)	
11.8–13.4	303	55 (18.15)		303	19 (6.27)	
13.5+	288	38 (13.19)		303	21 (6.93)	

Differences between colonized and not-colonized patients were calculated using the Student *t* test for continuous variables and the chi-square or Fisher exact test for categorical variables.
Abbreviations: Kp, *Klebsiella pneumoniae*; VRE, vancomycin-resistant *Enterococci*.

species. Consistent with numbering convention, these were the most predominant OTUs in these samples.

We compared the relative abundance of these predominant OTUs in each of the patient groups (Figure 2). The OTU2 containing *Klebsiella* was significantly more abundant in samples from patients colonized with *K. pneumoniae* alone ($P < .0001$) or co-colonized with VRE ($P = .013$) compared with patients colonized with neither organism. Similarly, *Enterococcus*

Table 3. Multivariable Logistic Regression Model for *Klebsiella pneumoniae* Colonization in 2014 and 2016

	2014 Cohort		2016 Cohort	
Variable	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value
VRE colonization	1.58 (0.92–2.62)	.08	2.07 (1.06–3.83)	.03
Male gender	0.7 (0.51–0.96)	.03	1.14 (0.71–1.85)	.6
Race (ref. white)				
Black	1.38 (0.85–2.2)	.18	1.91 (0.94–3.63)	.06
Other	0.71 (0.27–1.59)	.45	1.29 (0.3–3.88)	.68
Unknown	1.13 (0.44–2.55)	.78	2.94 (0.44–11.63)	.17
Days in hospital	0.99 (0.98–1)	.22	1 (0.98–1.01)	.6
Albumin ≤3	0.95 (0.69–1.31)	.77	0.87 (0.47–1.53)	.64
Age (ref. <50 y)				
50–60 y	1.66 (1.04–2.66)	.03	0.84 (0.37–1.84)	.66
60–70 y	1.72 (1.1–2.71)	.02	1.17 (0.6–2.31)	.65
Over 70 y	1.73 (1.09–2.78)	.02	1.8 (0.96–3.47)	.07
Death within 30 d	1.25 (0.74–2.05)	.39	1.68 (0.85–3.15)	.12
Neurologic disorder	1.07 (0.58–1.88)	.81	2.07 (0.9–4.4)	.07
Pulmonary circulation disorder	0.96 (0.47–1.82)	.91	2.18 (0.95–4.57)	.05
Hypertension	1.33 (0.97–1.83)	.08	0.49 (0.22–0.97)	.05

Abbreviations: CI, confidence interval; VRE, vancomycin-resistant *Enterococci*.

(OTU3) was higher in samples from patients colonized by VRE ($P = .0002$). OTU1 (containing *E.coli/Shigella* sequences) was less abundant in patients colonized with *K. pneumoniae* compared with no colonization but did not reach statistical significance ($P = .08$). To determine if these dominant OTUs were driving the overall differences observed in the microbiomes based on colonization state, we removed *Klebsiella* OTU2 and *Enterococcus* OTU3 and repeated our analyses. In this modified data set, AMOVA did not detect significant differences between the 4 colonization states ($P = .182$).

DISCUSSION

We and others have shown previously that *K. pneumoniae* colonization is associated with subsequent infection [4, 5]. This study sought to identify patient factors associated with *K. pneumoniae* colonization in ICU patients, including VRE colonization, which might be used to target screening programs to patients most likely to benefit from infection prevention interventions. In 2 separate cohorts of 1209 and 1243 patients, the only consistent patient factor associated with *K. pneumoniae* colonization was advanced age. By contrast, colonization with VRE alone or VRE plus *K. pneumoniae* was associated with worse baseline comorbidities than *K. pneumoniae* alone. However, there was an important microbiological factor: VRE colonization was associated with *K. pneumoniae* colonization. Importantly, the differences in the microbiome between groups seem to be driven by *Klebsiella* and *Enterococcus* themselves, with a higher abundance of a *Klebsiella* OTU in *Klebsiella* and co-colonized groups and a higher abundance of an *Enterococcus* OTU in VRE-colonized groups compared with the noncolonized group.

Outcomes of colonization include potential transmission to other patients and increased risk of infection in the colonized patient. This study was not designed to detect transmission, although there are reports of transmission from a colonizing strain from ICU settings across the globe [4, 5]. Further, *K. pneumoniae* infections occur frequently in colonized patients, as shown in a previous analysis of the 2014 cohort, where a significant association between infection and colonization was observed [4]. The current analysis using the first collected rectal swabs from each patient's first hospital encounter and their fecal microbiome demonstrated the same association with subsequent *Klebsiella* clinical cultures.

The consistent association between VRE and *K. pneumoniae* colonization has several implications. Because VRE status is routinely determined in units with an active surveillance program, detectable VRE colonization could be used to identify patients who are potentially colonized with *K. pneumoniae*. As a biological implication, this association suggests that VRE and *K. pneumoniae* interact in the fecal microbiome. Unlike infections caused by other health care–associated pathogens, such as *Clostridium* (*Clostridioides*) *difficile* [35], we did not observe differences in the overall diversity of the microbiota across the patient groups. This suggests that *K. pneumoniae* does not require a depauperate microbiome, lacking in number and variety of species, to persist and may be found in the range of intestinal microbial communities seen in this hospital. VRE has similar dynamics, although it tends to colonize patients with more comorbidities than *K. pneumoniae*.

Our microbiota analysis also indicates that *K. pneumoniae* and *Enterococcus* are predominant taxa in the microbiome of ICU patients. Intestinal domination with *Enterococcus* and CRKP is

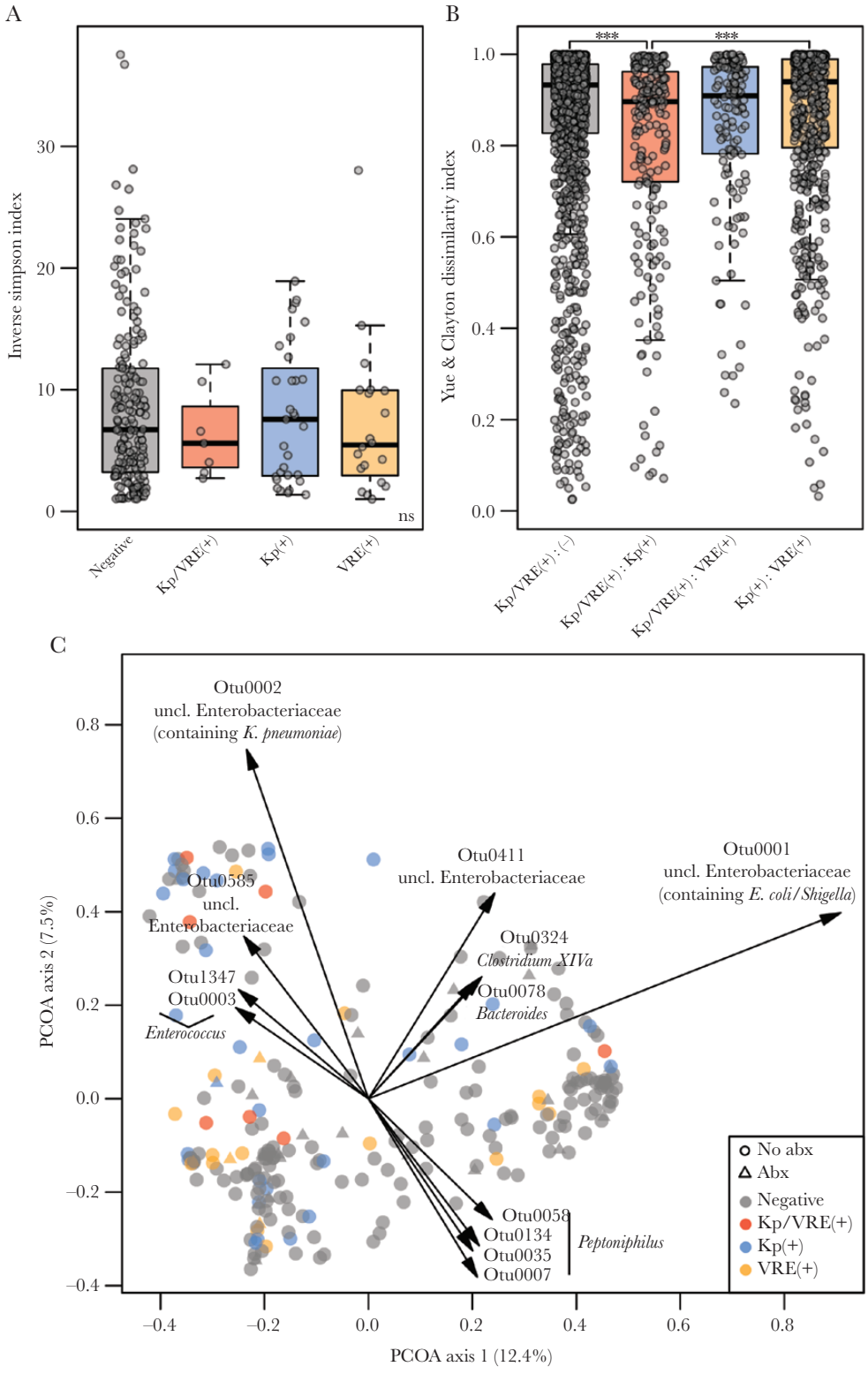

Figure 1. A, Alpha diversity (inverse Simpson index) and (B) beta diversity, or community structure similarity (Yue & Clayton dissimilarity) in the microbiota of noncolonized ("negative"), co-colonized ("Kp/VRE(+)"), *K. pneumoniae*–colonized ("Kp(+)"), and VRE-colonized ("VRE(+)") patients (Kruskal-Wallis adjusted with Benjamini-Hochberg method and Dunn's post hoc; $^{*}P < .01$; $^{**}P < .001$; $^{***}P < .0001$). C, Principal components analysis of the fecal microbiota community in all 4 patient groups, based on Yue & Clayton dissimilarity (AMOVA, $P < .05$). Abbreviations: AMOVA, analysis for molecular variance; Kp, *Klebsiella pneumoniae*; VRE, vancomycin-resistant *Enterococci*.

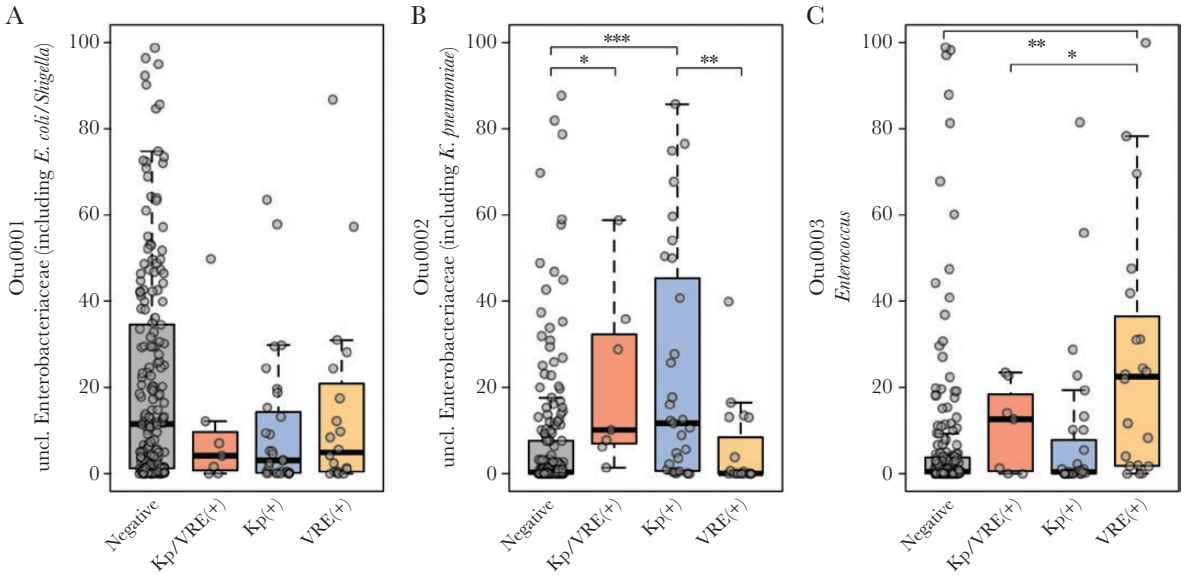

Figure 2. Relative abundance of OTU0001, OTU0002, and OTU0003 in noncolonized ("negative"), co-colonized ("Kp/VRE(+)"), *K. pneumoniae*–colonized ("Kp(+)"), and VRE-colonized patients ("VRE(+)"; Kruskal-Wallis adjusted with Benjamini-Hochberg method and Dunn's post hoc; $^{*}P < .01$; $^{**}P < .001$; $^{***}P < .0001$). Abbreviations: Kp, *Klebsiella pneumoniae*; VRE, vancomycin-resistant *Enterococci*.

associated with subsequent infections [36, 37]. Data from our study indicate that detectable colonization may represent dominance of that taxa in the microbiome, and not simply its presence, perhaps explaining why detectable colonization with VRE or *K. pneumoniae* is also associated with subsequent infection.

A previous study demonstrated that CRKP and VRE neither compete nor synergize in the murine model, and the 2 organisms reside within the same intestinal regions but occupy distinct metabolic niches [38]. Further, different indigenous OTUs classified as Enterobacteriaceae in mice impact susceptibility to another enteric pathogen, *Salmonella* [39]. It is possible that similar mechanisms in humans exist, where 1 endogenous, nonpathogenic organism can confer protection against other similar organisms.

A strength of this study was the use of 2 large cohorts of patients separated over time with extensive available chart data and known VRE and *K. pneumoniae* colonization status. VRE colonization was associated with *K. pneumoniae* colonization in each cohort despite significant differences in overall *K. pneumoniae* colonization rates between cohorts. An intriguing possibility is that these differences in colonization rates may be related to differences in ambient temperature between the summer of 2014 and winter of 2016 (Supplementary Figure 1). *K. pneumoniae* bacteremia rates are highly correlated with temperature [40]. Perhaps this fluctuation is due to changing colonization rates that in turn affect infection rates. The significant differences in demographics and overall comorbidities between cohorts were both a strength and a limitation, highlighting the robust association between *K. pneumoniae* and VRE but perhaps confounding identification of other variables associated with colonization.

In summary, this study demonstrates a reproducible association between detectable VRE and *K. pneumoniae* colonization and suggests that each can colonize varied intestinal microbial communities found in hospitalized patients.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Data availability. Raw sequences are available in SRA (BioProjectID: PRJNA556249, BioSampleIDs: SAMN12685404-SAMN12685651).

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