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MAJOR ARTICLE

Epidemiology of Vancomycin-Resistant *Enterococcus faecium* and *Enterococcus faecalis* Colonization in Nursing Facilities

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Background. Vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* frequently colonize nursing facility (NF) residents, creating opportunities for vancomycin-resistant *Enterococcus* (VRE) transmission and dissemination of mobile genetic elements conferring antimicrobial resistance. Most VRE studies do not speciate; our study addresses this lack and compares the epidemiology of *E faecium* and *E faecalis*.

Methods. We enrolled 651 newly admitted patients from 6 different NFs and collected swabs from several body sites at enrollment, 14 days, 30 days, and monthly thereafter for up to 6 months. The VRE were speciated using a duplex polymerase chain reaction. We used multinomial logistic regression models to compare risk factors associated with colonization of *E faecium* and *E faecalis*.

Results. Overall, 40.7% were colonized with *E faecium*, *E faecalis*, or both. At enrollment, more participants were colonized with *E faecium* (17.8%) than *E faecalis* (8.4%); 3.2% carried both species. *Enterococcus faecium* was carried twice as long as *E faecalis* (69 days and 32 days, respectively), but incidence rates were similar (*E faecium*, 3.9/1000 person-days vs *E faecalis*, 4.1/1000 person-days). Length of stay did not differ by species among incident cases. Residents who used antibiotics within the past 30 days had a greater incidence of both *E faecium* (odds ratio [OR] = 2.89; 95% confidence interval [CI], 1.82–4.60) and *E faecalis* (OR = 1.80; 95% CI, 1.16–2.80); device use was most strongly associated with the incidence of *E faecium* colonization (OR = 2.01; 95% CI, 1.15–3.50).

Conclusions. Recent increases in vancomycin-resistant *E faecium* prevalence may reflect increased device use and longer duration of carriage.

Keywords. *Enterococcus faecalis*; *Enterococcus faecium*; nursing facilities; VRE.

On any given day, 1.7 million older Americans receive long-term or short-term postacute care in a nursing facility (NF) [1]. Infection is one of the top 5 leading causes of death among NF participants, but it is also one of the most preventable [2, 3]. Because of frequent hospitalization and antibiotic use, NF participants are at a particularly high risk for healthcare-associated infections due to multidrug-resistant organisms (MDROs) [4, 5].

One of the most serious threats to antibiotic resistance control efforts are bacteria of the genus *Enterococcus*, which are intrinsically resistant to many antibiotics and frequently resistant to vancomycin. In addition to causing an estimated 54 500 hospitalizations and 5400 deaths per year in the United States leading to $539 million in healthcare costs [6], vancomycin-resistant *Enterococcus* (VRE) is a reservoir of vancomycin resistance for other pathogenic bacteria such as methicillin-resistant *Staphylococcus aureus*. Thus, an effective way to prevent emergence of additional MDROs is to prevent VRE colonization.

The 2 most prevalent and clinically relevant *Enterococcus* species are vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* [7]. In previous years, most VRE spp infections were caused by *E faecalis* [8]. However, since 2002, an increase in the prevalence of vancomycin-resistant *E faecium* has been observed, with reports of vancomycin-resistant *E faecium* colonization being as common as those caused by vancomycin-resistant *E faecalis* in 2006 [9–11]. This could be due to *E faecium*'s intrinsic and acquired resistance to many classes of antibiotics [12], making it better adapted to the hospital and NF environment where antibiotic use is common. Although *E faecalis* also exhibits intrinsic and acquired resistance to a

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variety of antibiotic classes, the presence and level of resistance can differ between species [13, 14]. Colonization is the first step towards infection [15], with the caveat that *E faecium* and *E faecalis* strains vary in their propensity to cause disease [15, 16]. Most studies of VRE colonization and/or infection do not separate by species in their analysis.

In hospitalized patients, risk factors for VRE colonization (species unspecified) include recent intensive care unit admission, prolonged hospitalization, comorbidities, and invasive procedures [17–19]. One of the few studies comparing risk factors by enterococcal species focused on bloodstream infections. In this Canadian population-based surveillance study, the source of bacteremia was more likely urinary for *E faecalis* and gastrointestinal for *E faecium*. In this study, increased mortality and antibiotic resistance was largely associated with *E faecium* infection [20]. By contrast, risk factors for VRE in NFs are not well characterized, although the prevalence of all VRE in US NFs ranges between 5% and 18%, with one report as high as 50% [2]. Once colonized with VRE, the bacteria can be carried for several weeks. A South Korean study estimated the duration of carriage of vancomycin-resistant *E faecium* in participants discharged from hospitals to be 5.67–8.9 weeks [21]. Extended duration of carriage complicates VRE control efforts. Few estimates of incidence of VRE colonization exist in any setting due to the difficulty of obtaining longitudinal data.

This study fills a gap in the literature by describing the epidemiology of *E faecium and E faecalis* using data among 6 NFs located in Southeastern Michigan, obtained during a 3-year span. We estimate and compare the prevalence, incidence rate, duration of carriage, and associations of known risk factors for vancomycin-resistant *E faecium* and *E faecalis* colonization.

METHODS

Study Design

We identified and characterized bacterial isolates, and we analyzed patient characteristics pertaining to overall health and medical care collected during a previously described prospective cohort study of recently admitted NF participants [22]. In brief, participants from 6 NFs in Southeastern Michigan were enrolled within 14 days of NF admission and followed for up to 6 months. Enrollment took place between November 2013 and May 2016. Prevalence of MDRO colonization was evaluated upon enrollment and throughout patient stay. Any NF patient recently admitted to the NF who (or his/her proxy) provided consent to collect surveillance samples and patient specific data was enrolled in the study. The only exclusion criterion was receiving end of life care. The Institutional Review Board at the University of Michigan approved the study protocol.

Sample Collection

Once participants were enrolled, trained research personnel reviewed each individual's medical records for age, sex, functional status, prior hospitalization length of stay, device use (defined as the presence of an indwelling urinary catheter or feeding tube), antibiotic use, wounds, and a physical self-maintenance score ranging from 6 (independent) to 30 (dependent) in 6 categories of self-maintenance (bathing, dressing, feeding, ambulation, grooming, and toileting) [23]. Microbiological samples were collected from participants' hands, nares, oropharynx, enteral feeding tube insertion site, urinary catheter insertion site, groin, perianal area, and wounds to assess MDRO colonization on the day of enrollment, day 14, and day 30 and then monthly for up to 6 months, enabling estimates of incidence.

Table 1. Population Demographics for Prevalent and Incident Cases of VRE Colonization From 6 Nursing Facilities in Southeastern Michigan

		Prevalent Cases			Person-Days		Incident per 1000 Person-Days			
Characteristic	Total (N = 651)	*E faecium* Only (N = 116)	*E faecalis* Only (N = 55)	Both Species (N = 21)	*E faecium*	*E faecalis*	*E faecium* (N = 55)	*E faecalis* (N = 62)	IRR	95% CI
Sex										
Male	275	52 (.19)	27 (.10)	9 (.03)	5381	5661	23 (4.27)	24 (4.24)	1.02	(0.57–1.80)
Female	376	64 (.17)	28 (.07)	12 (.03)	8889	9481	32 (3.60)	38 (4.01)	0.90	(0.56–1.44)
Race										
White	406	66 (.16)	33 (.08)	13 (.03)	8288	8683	33 (3.98)	38 (4.38)	0.91	(0.57–1.45)
Black	243	49 (.20)	22 (.09)	8 (.03)	5965	6417	22 (3.69)	24 (3.74)	0.99	(0.55–1.76)
Other	2	1 (.5)	0 (0)	0 (0)	17	42	0 (0)	0 (0)	-	-
Device use[b,c]	281	60 (0.21)	31 (0.11)	15 (0.05)	5079	4647	25 (4.92)	15 (3.23)	1.52	(0.81–2.96)
Antibiotic use[c]	392	95 (0.24)	40 (0.10)	18 (0.05)	6645	7185	33	26	1.37	(0.82–2.32)
Total		17.82%	8.44%	3.23%	14 270	15 142	3.86	4.09	0.94	(0.23–3.78)

Abbreviations: CI, confidence interval; *E*, *Enterococcus*; IRR, incidence rate ratio; VRE, vancomycin-resistant *Enterococcus*.

[a]There was no statistically significant difference in prevalence by sex or race.

[b]Device use was defined as the presence of an indwelling catheter or feeding tube.

[c]Past 30 days.

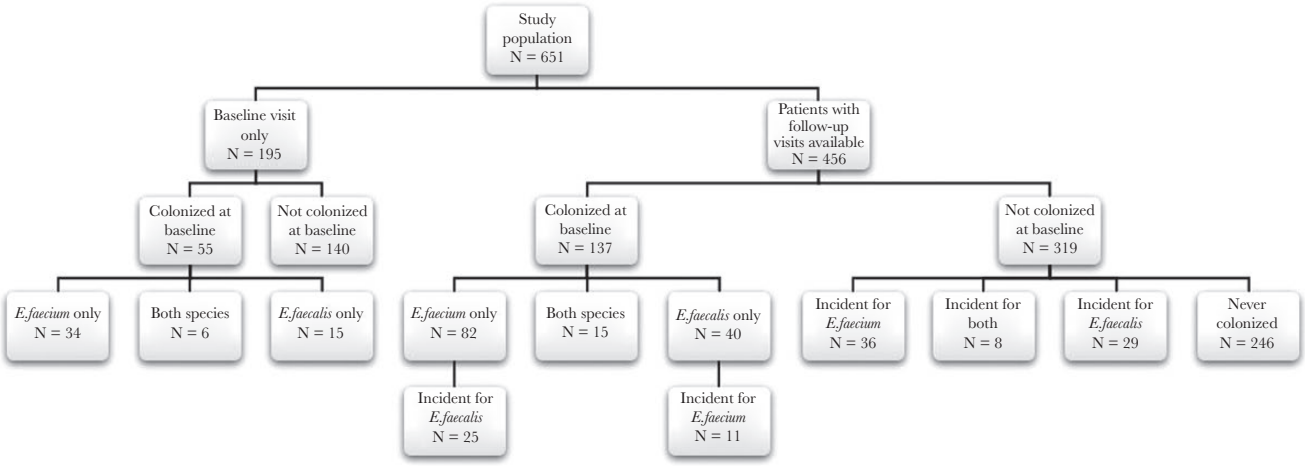


Figure 1. Study flow diagram showing colonization with vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* from 6 nursing facilities throughout Southeastern Michigan.

Samples were collected using sterile swabs (Bactiswab; Remel, Lenexa, KS) and then placed in transport media and cultured on bile-esculin plates with 6 mg/L vancomycin (BEV6). Growth sensitivity on the selected plates is similar between species [24]. Furthermore, because we isolated VRE directly, the risk of "crowding out" of VRE by sensitive *Enterococcus* is limited. Hand swabs were enriched overnight in brain-heart infusion broth at 36°C before culturing. Growth suggestive

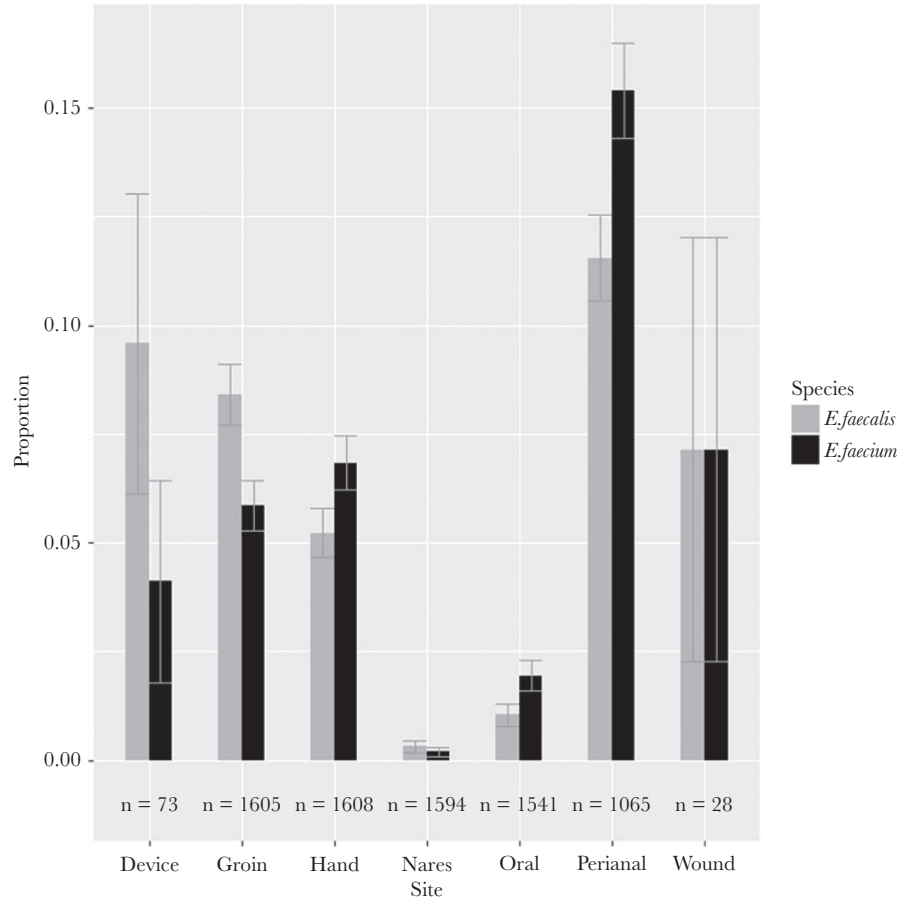


Figure 2. Proportion of swabs testing positive for *Enterococcus faecalis* or *Enterococcus faecium* at any visit by body site.

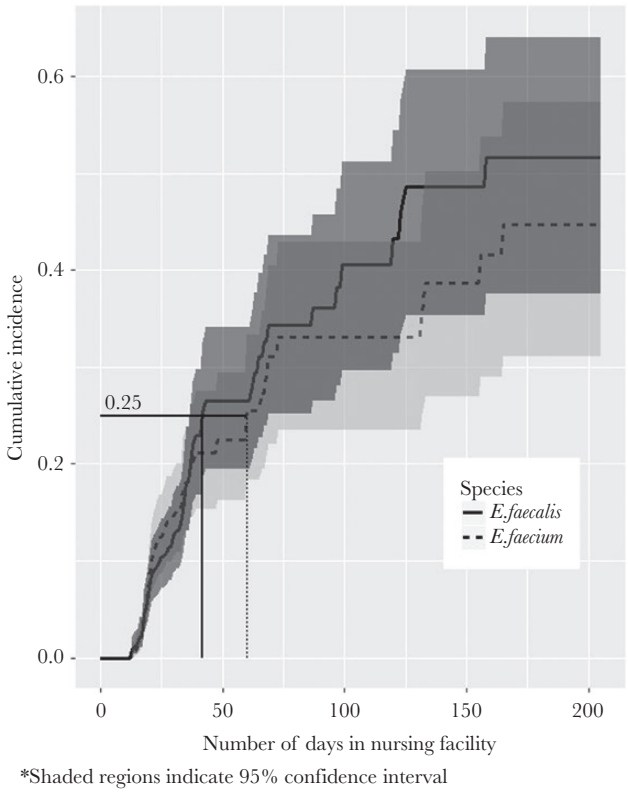
Table 2. Prevalence, Incidence, and Duration of Carriage of Vancomycin-Resistant *Enterococci* by Species Within 6 Nursing Facilities in Southeastern Michigan

Epidemiological Measure	Vancomycin-Resistant *Enterococcus faecium*	Vancomycin-Resistant *Enterococcus faecalis*
Prevalence	21.0%	11.7%
Prevalence odds	0.27	0.13
Incidence rate (per 1000 person-days)	3.9	4.1
Duration of carriage (days)	69	32

of VRE on BEV6 was confirmed by pyrrolidonyl arylamidase testing (DrySlide; BD, Franklin Lakes, NJ).

Enterococcus Species Typing

This study included a duplex polymerase chain reaction (PCR) amplification for species confirmation. Isolate deoxyribonucleic acid (DNA) was obtained by adding a single colony of VRE, identified by selective media, to 50 µL sterile water. Amplification was performed using primers targeting the *ddl* gene as described and validated by Tan et al with modifications [25]. The full PCR protocol used in this study is found in the Appendix.



*Shaded regions indicate 95% confidence interval

Figure 3. Cumulative incidence of vancomycin-resistant *Enterococcus* by species from participants in 6 nursing facilities throughout Southeastern Michigan

Estimation of Prevalence, Incidence, and Duration of Carriage

We used the observed prevalence of vancomycin-resistant *E faecalis* and *E faecium* at baseline under the assumption that the incidence rate and duration of carriage for each species did not change during the course of the study [4, 26]. Incidence rate was estimated using all those who were negative at baseline by species. Individuals who were colonized by one species contributed person-time at risk for the other species. Duration of carriage was estimated from the observed incidence and prevalence of vancomycin-resistant *E faecalis* and *E faecium*, by taking the quotient of the prevalence odds (P/(1-P)) and incidence rate observed, assuming the incidence and prevalence did not change over the study period [26, 27]. This assumption is consistent with an earlier report of this population [22]. Because participants were enrolled very close to the time of admission to an NF, the prevalence reflects the incidence at the facility from which they were admitted, thus the duration may be under- or overestimated if the incidence in the previous institutions is different than the current NFs.

Statistical Analysis

All statistical analyses were performed using SAS, version 9.4 (SAS Institute). The statistical significance of selected patient characteristics was assessed using χ^2 test, 2-tailed (Table 1). We then compared the cumulative incidence of colonization for each species. Finally, a time-varying multinomial logistic regression model with empirical covariance was used to estimate the odds of colonization with (1) *E faecium*, (2) *E faecalis*, or (3) both species. Only participants with at least 2 visits and those at risk for at least 1 species at baseline (so incidence could be estimated) were included in the regression analysis (n = 441).

Each visit was treated as an independent observation; we used generalized estimating equations to account for clustering within NF (n = 6) and individuals (n = 441). We imputed values for the 2 observations missing antibiotic use (0.2%) and the one missing device use (0.1%). The imputed dataset was created in SAS using the Multiple Imputation procedure with 100 imputations [28]. Open wound status, hospital stay, sex, minority status, Hispanic background, and number of days in the facility were used to impute the missing variables. All models were adjusted for visit and previous colonization status. Separate multinomial logistic regression models were performed for each confounder, and all significant variables ($P < .05$) were included in the full model. Model fit was assessed by comparing the mean Quasi-likelihood under the independence model criterion (QIC) value from the imputations of the model including only visit and previous colonization status to that of the full adjusted model [29].

RESULTS

The demographic data for the 651 participants from 6 NFs were described previously in the parent study [22]. In brief,

participants averaged 74.7 years of age (standard deviation, 12.2); 42.2% were male, 62.4% were white, and 37.3% were African American. A total of 33.2% of participants in the pilot study tested positive for VRE at enrollment. In this study, we describe the prevalence of vancomycin-resistant *E faecium* and/or vancomycin-resistant *E faecalis* only, which gives slightly different numbers than published previously: 192 (29.5%) were positive for 1 or both species at enrollment. Specifically, 116 (17.8%) participants were colonized with vancomycin-resistant *E faecium*, 55 (8.4%) with vancomycin-resistant *E faecalis*, and 21 (3.2%) with both at enrollment (Figure 1). Vancomycin-resistant *E faecium* and/or vancomycin-resistant *E faecalis* were isolated at least once throughout the study period from 265 (40.7%) of the 651 participants. At enrollment, prevalence of vancomycin-resistant *E faecium* was higher than vancomycin-resistant *E faecalis* in both sexes and races (Table 1). The incidence rates for the 55 participants who became colonized with vancomycin-resistant *E faecium* or both species during the course of the study were similar to the 62 participants who became colonized with vancomycin-resistant *E faecalis* or both overall and when stratified by sex and race (Table 1).

Over the course of the study, there were 780 vancomycin-resistant *E faecium* and *E faecalis* specimens obtained from the hands, nares, oropharynx, enteral feeding tube insertion site, catheter site, groin, perianal area, or wounds of the 265 colonized participants. Of the positive swabs, 52.2% were *E faecium* and 47.8% were *E faecalis* (individuals differed in the number of swabs collected, and because *E faecium* was carried longer, there were more positive swabs for *E faecium*). However, the average number of swabs per person did not differ between those who became positive for *E faecium* versus *E faecalis*. Although most (66.2%) positive swabs were collected from groin or perianal sites, given that a swab was taken, perianal swabs had the highest proportion testing positive for *E faecium* or *E faecalis* (Figure 2, Supplemental Figure 1). Prevalence of *E faecium* colonization (alone or with *E faecalis*) was 21.0%, and new cases were acquired at a rate of 3.9 cases per 1000 person-days, with an inferred duration of carriage of 69 days (Table 2). By contrast, *E faecalis* colonization prevalence was 11.7% and was acquired at a rate of 4.1 cases per 1000 person-days, with an inferred duration of carriage of 32 days.

During the study period, there were a total of 109 incident cases of VRE colonization: 55 cases of *E faecium* and 62 cases of *E faecalis*. Figure 3 shows similar cumulative incidences between enrollment and day 20 for the 2 species. A separation occurs after day 20, with *E faecium* having a slightly higher cumulative incidence, until day 38, after which *E faecalis* remains higher. By day 42, one quarter of those without *E faecalis* at enrollment acquired *E faecalis*, and by day 60 after enrollment, one quarter of those without *E faecium* at enrollment had acquired *E faecium*.

To further assess the risk of colonization, we used a time-varying multinomial logistic regression model to predict the odds of being colonized at any site with *E faecium*, *E faecalis*, or both at a particular visit for all individuals at risk who also had 1 or more follow-up visits. After adjusting for the number of days in the facility and previous colonization status at the most recent visit, the odds of *E faecium* colonization increased with device use (odds ratio [OR] = 2.90; 95% confidence interval [CI], 1.70–4.93) and antibiotic use (OR = 3.53; 95% CI, 2.26–5.51) within the last 30 days, whereas only antibiotic use (OR = 1.86; 95% CI, 1.22–2.84) within the last 30 days increased the odds of *E faecalis* colonization (Table 3). In addition, the odds of being colonized with both species at a given visit significantly increased with device use (OR = 3.81; 95% CI, 1.45–10.03) and antibiotic use (OR = 2.49, 95% CI, 1.03–7.03) in the past 30 days.

Including all variables that had a significant association in the individual models in a single model showed similar results (Table 4). The magnitude and direction of the associations were similar for device use (OR = 2.01; 95% CI, 1.15–3.50) and antibiotic use (vancomycin-resistant *E faecium*, OR = 2.89 and 95% CI, 1.82–4.60; vancomycin-resistant *E faecalis*, OR = 1.80 and 95% CI, 1.16–2.80). Those who were colonized with both species had a significantly increased odds of testing positive for vancomycin-resistant *E faecalis* or both species at the next visit (vancomycin-resistant *E faecalis*, OR = 6.38 and 95% CI, 1.25–32.54; both, OR = 41.40 and 95% CI, 3.74–457.78). Increased length of stay at or beyond 30 days, compared with 14 days, did not increase odds of any colonization type when adjusting for the other risk factors.

Comparison of the mean QIC values revealed that the final model, including device use, antibiotic use, previous colonization history, and number of visits (mean QIC, 1275.46), was superior to a model containing previous colonization status and number visits alone (mean QIC, 1309.17).

To further analyze the effects of specific antibiotics, we assessed the usage of the top 3 antibiotic classes (cephalosporins, quinolones, and glycopeptides) and combined the remaining classes (aminoglycosides, carbapenems, lincosamides, lipopeptides, macrolides, nitrofurans, nitroimidazoles, oxazolidinones, penicillin, quinolone, sulfonamide, tetracycline, and triazole) into an "other" category for inclusion into the full model. Glycopeptide use had a positive association with all outcomes (vancomycin-resistant *E faecium*, OR = 3.04 and 95% CI, 1.02–9.08; vancomycin-resistant *E faecalis*, OR = 4.18 and 95% CI, 1.55–11.29; both, OR = 5.50; 95% CI and 1.50–20.10). In addition, usage of other classes was positively associated with the presence of vancomycin-resistant *E faecium* (OR = 2.21; 95% CI, 1.26–3.85). Addition of indicators for specific antibiotics did not change the association between device use and vancomycin-resistant *E faecium* (OR = 2.33; 95% CI, 1.34–4.03) (Supplemental Table 1).

Table 3. Separate Models Predicting VRE Colonization by Species, Adjusted for Number of Visit and Previous Colonization Status in 441 Nursing Facility Participants With More Than 1 Visit

	E faecium Only			*E faecalis* Only			Both Species		
Characteristic	OR	95% CI	*P* Value	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Non-white race	0.99	(0.62–1.59)	.97	0.69	(0.45–1.07)	.10	1.06	(0.40–2.80)	.90
Male	1.08	(0.67–1.73)	.76	0.89	(0.58–1.38)	.61	1.56	(0.58–4.20)	.38
Device use[a,b]	**2.90**	**(1.70–4.93)**	**<.0001**	1.52	(0.86–2.24)	.15	**3.81**	**(1.45–10.03)**	**.01**
Open wound[a]	1.66	(0.93–2.97)	.09	1.34	(0.80–2.24)	.26	2.71	(1.00–7.33)	.05
Antibiotic[a]	**3.53**	**(2.26–5.51)**	**<.0001**	**1.86**	**(1.22–2.84)**	**.004**	**2.49**	**(1.03–7.03)**	**.04**
Physical Self-Maintenance Score[c]	1.04	(0.99–1.10)	.13	1.04	(0.99–2.27)	.13	1.01	(0.90–1.14)	.82

Abbreviations: CI, confidence interval; *E*, *Enterococcus*; OR, odds ratio; VRE, vancomycin-resistant *Enterococcus*.

[a]Within the past 30 days.

[b]Defined as the presence of an indwelling catheter or feeding tube.

[c]Lower Physical Self-Maintenance Score indicates increased independence.

Bold text indicates values are statistically significant ($P < .05$).

DISCUSSION

Among the 441 participants with follow-up visits that were at risk for at least 1 species, 109 (24.7%) were newly colonized with VRE at some point during the 6 months of follow-up; half of these were colonized by *E faecium* (n = 55). Although the prevalence of colonization considering all body sites together was higher for *E faecium* than *E faecalis*, the difference in prevalence by species was almost entirely attributable to differences in inferred duration of carriage: the incidence of colonization was similar for *E faecium* and *E faecalis*, but *E faecium* was carried longer than *E faecalis*.

The observed 21% prevalence (95% CI, 18%–24%) of vancomycin-resistant *E faecium* in the current study is consistent with previous studies in (1) acute healthcare settings where prevalence was 19% [30, 31] and (2) among 3 Southern California NFs, where the overall VRE prevalence was 16% (prevalence varied from 7% to 19% depending on the NF) [32]. A Jerusalem study of 1215 participants from a single, long-term care facility estimated the prevalence of VRE at 9.6% [33]. Other studies have reported prevalence estimates as high as two thirds among those transferred to an NF from an acute-care facility [34]; this may explain the higher prevalence observed in our study because over 96% of participants were transferred to the NF from an acute-care facility, and they were enrolled shortly after NF admission.

The observation of a longer duration of *E faecium* carriage, 69 days, compared with 32 for *E faecalis*, is novel. We found only 1 study estimating duration of carriage, and that was limited to *E faecium*. In that study of 17 participants, the average duration of carriage was 54 days [35]. The shorter duration of carriage for *E faecalis* coupled with the similar incidence rates suggest that *E faecalis* may be more transmissible: new cases are being acquired at a similar rate but is cleared from the host more rapidly.

Risk factors associated with VRE colonization (all species combined) have been previously identified in the literature; however, we found very few studies comparing risk factors by species type. For example, a German study of patients in geriatric clinics, nursing homes, and ambulatory care identified a positive association between VRE colonization and the presence of wounds and with immobility [36]. An Australian point-prevalence survey in a tertiary hospital study reported

Table 4. Multivariate Model Adjusted for Number of Visit and Previous Colonization Status Predicting VRE Colonization in 441 Nursing Facility Participants With More Than 1 Visit

	E faecium			*E faecalis*			Both Species		
Characteristics	OR	95% CI	*P* Value	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Device use[a,b]	**2.01**	**(1.15–3.50)**	**.01**	1.24	(0.69–2.22)	.48	**3.12**	**(1.14–8.55)**	**.03**
Antibiotics[a]	**2.89**	**(1.82–4.60)**	**<.0001**	**1.80**	**(1.16–2.80)**	**.01**	1.79	(0.71–4.53)	.22
14 days[c]	ref	ref	ref	ref	ref	ref	ref	ref	ref
30 days[c]	0.62	(0.35–1.10)	.10	1.38	(0.80–2.38)	.24	1.21	(0.40–3.68)	.73
30+ days[c]	**0.47**	**(0.25–0.88)**	**.02**	0.98	(0.59–1.62)	.93	0.75	(0.27–2.11)	.59
Previous *E faecium*	**8.62**	**(5.09–14.60)**	**<.0001**	**3.02**	**(1.63–5.61)**	**.005**	**18.14**	**(4.49–73.29)**	**<.0001**
Previous *E faecalis*	1.60	(0.68–3.75)	.28	**10.78**	**(6.15–18.89)**	**<.0001**	**25.53**	**(6.61–98.66)**	**<.0001**
Previous both species	3.71	(0.38–36.57)	.26	**6.38**	**(1.25–32.54)**	**.03**	**41.40**	**(3.74–457.78)**	**.002**

Abbreviations: CI, confidence interval; *E*, *Enterococcus*; OR, odds ratio; ref, reference; VRE, vancomycin-resistant *Enterococcus*.

[a]In the past 30 days.

[b]Defined as the presence of an indwelling catheter or feeding tube.

[c]Number of days in nursing facility.

Bold text indicates values are statistically significant ($P < .05$).

a link between exposure to meropenem, increased length of stay, and age of 65 and older [37]. A study examining the risk factors for patients admitted to acute-care hospitals, intermediate-term care facilities, and long-term care facilities found positive associations between indwelling urinary catheters and prior VRE carriage, similar to results observed here. However, when the analysis was stratified by facility type, they did not find any significant risk factors when examining long-term care facilities only. The 3 point-prevalence estimates of VRE for long-term care facilities in their study (0.3%–1.1% over the course of 3 years) were much lower than our estimate of 29.5% (95% CI, 26%–32%) decreasing power to detect associations [38]. These 3 studies combined those positive with *E faecium* or *E faecalis* into a single VRE classification. The multinomial regression analysis in our study analyzed the species separately and uncovered distinct risk factors for *E faecium* and *E faecalis* colonization. This might suggest that one species is driving the associations reported when *E faecium* and *E faecalis* are analyzed together. As we continue to observe changes in the prevalence of *E faecium*, identification of risk factors at the species level will be of greater importance.

Generalizing our results to other populations should be done with caution and considering the limitations in the study protocol. Not all body sites were swabbed from every individual at each visit, so our incidence is possibly underestimated. Furthermore, although each of the colony morphotypes was subcultured for testing, it is possible that multiple phenotypes might have been indistinguishable on the plate. In that case, the predominant isolate from each culture was most likely to be subcultured for testing. Thus, it is likely that we underestimated cocolonization. We observed 2 phenotypic colonies 5% of the time, 42% of which were different species. However, if cocolonization truly occurs as much as 10% of the time (but is not detectable phenotypically), we would have to test 28 colonies from each plate [39]. Therefore, our incidence estimates best represent that of the predominant colonizing organism. In addition, our use of enrollment samples to estimate the duration of carriage assumes incidence and prevalence of VRE at the patient's previous location was the same for everyone and remain constant over time. Although our previous study [22] found a constant prevalence within the 6 NFs, this might not have been true in the hospitals where participants stayed previously: participants were referred from multiple hospitals. Moreover, due to the large variety of antibiotic classes observed in our population, our analysis stratifying by class only highlighted the 3 most common antibiotic classes. If other antibiotic classes are more likely to select for 1 species, we could not detect it. Previous studies using stool samples have shown that VRE cultures may overpredict the absence of continued carriage [40], and the possibility of sudden reversion to a positive result soon after antibiotic administration means we cannot definitively say the apparent acquisition of VRE is not due to the unmasking of chronic VRE colonization. Likewise, changes in VRE concentrations to below detectable levels could explain loss of carriage. However, the isolation of VRE directly on agar plates containing vancomycin reduces the concern of vancomycin-susceptible enterococci dominating our cultures, which may be expected in patients not treated with glycopeptides. Nonetheless, our findings suggest that the transmission of VRE may vary by species and that increases in *E faecium* prevalence may reflect increased device use and longer duration of carriage.

CONCLUSIONS

In conclusion, we observed a higher prevalence of vancomycin-resistant *E faecium* compared with vancomycin-resistant *E faecalis* that was most likely attributable to its longer duration of carriage rather than some other factor. Whether other factors—such as increased virulence or exposure to specific antibiotic classes—also contribute should be considered in future studies. It is notable that device use was more strongly associated with increased incidence of vancomycin-resistant *E faecium* colonization (OR = 2.01; 95% CI, 1.15–3.50). Minimizing duration of device use and following good hygiene practices while inserting, maintaining, and removing devices would likely reduce vancomycin-resistant *E faecium* colonization and that of other pathogenic organisms [41, 42].

Supplementary Materials

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.

Supplemental Figure 1. Percentage of participants sampled at each site at each visit.

Supplemental Table 1. Multivariate Model Adjusted for Number of Visit and Previous Colonization Status Predicting VRE Colonization in 441 Nursing Facility Participants With More Than 1 Visit

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APPENDIX

Speciation of isolates: Identification of vancomycin-resistant *Enterococcus* (VRE) species was conducted using *Enterococcus faecium*-HRM-F (5'-TTTACAAGCTGCT GGTGTGC-3'), *E faecium*-HRM-R (5'-AACCCATATTCG CAGGTTTG-3'), *Enterococcus* faecalis-HRM-F (5'-GTGGC TTAAGTCGCTGTGAT-3'), and *E faecalis*-HRM-R (5'-AGGC ATGGTGTTCAATTCAT-3') primer pairs (Invitrogen) to amplify the 74-base-pair fragment of the *ddl E faecalis* gene and the 140-base-pair fragment of the *ddl E faecium* gene as described by Tan et al [25]. The reaction volume consisted of 12.5 µL GoTaq Green Master Mix (Promega), 2.5 µL *E faecalis* primer mix containing 10 µM forward and reverse primers, 2.5 µL *E faecium* primer mix containing 10 µM forward and reverse primers, 5 µL nuclease-free polymerase chain reaction (PCR) water, and 2.5 µL bacterial lysate to a total of 25 µL amplification reaction. Three controls were included in each PCR: nuclease-free PCR water as a negative control, known *E faecalis* positive lysate, and known *E faecium*-positive lysate. The PCR amplification was performed in an S1000 thermal cycler (Bio-Rad), with the following conditions: an initial denaturation step at 94°C for

5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 45 seconds, and a single final extension step at 72°C for 5 minutes with a 4°C hold step. Amplified DNA fragments were then separated by electrophoresis on 2% agarose gel. Vancomycin-resistant *E faecalis* and vancomycin-resistant *E faecium* samples were separated by species based upon the previous visualized banding patterns.