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

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Summary: Colonization of vancomycin-resistant enterococci within nursing facilities can contribute to the increase of multi-drug resistant organisms. Studies often combine E. faecium and E. faecalis for analysis. Our findings show differences in risk factors and prevalence that suggest they should be examined separately.

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Abstract

**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. VRE were speciated using a duplex PCR. 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. *E. 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* (OR=2.89; 95% 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.
Introduction

On any given day, 1.7 million older Americans receive long-term or short-term post-acute care in a nursing facility (NF)[1]. Infection is one of the top five leading causes of death among NF participants, but 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 (HAIs) due to multidrug resistant organisms (MDRO)[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 5,400 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 two 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 VR E. faecium has been observed, with reports of VR E. faecium being as common as VE 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 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 \textit{E. faecalis} and gastrointestinal for \textit{E. faecium}. In this study, increased mortality and antibiotic resistance was largely associated with \textit{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 5-18%, 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 VR \textit{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 \textit{E. faecium} and \textit{E. faecalis} using data among six NFs located in Southeastern Michigan, obtained during a three-year span. We estimate and compare the prevalence, incidence rate, duration of carriage, and associations of known risk factors for VR \textit{E. faecium} and \textit{E. faecalis} colonization.

\textbf{Methods}

\textbf{Study Design}: We identified and characterized bacterial isolates, and analyzed patient characteristics pertaining to overall health and medical care collected during a previously described prospective cohort study of recently admitted NF participants [22]. Briefly, participants from six NFs in Southeastern Michigan were enrolled within 14 days of NF admission and followed for up to six 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 (PSMS) 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, day 30 and then monthly for up to six months, enabling estimates of incidence.

Samples were collected using sterile swabs (Bactiswab, Remel, Lenexa, KS), 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]. Further, 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 of VRE on BEV6 was confirmed by pyrrolidonyl arylamidase testing (PYR) (DrySlide, BD, Franklin Lakes, NJ).

Enterococcus Species Typing: This study included a duplex Polymerase Chain Reaction amplification for species confirmation. Isolate DNA was obtained by adding a single colony of VRE, identified by selective media, to 50 µL of sterile water. Amplification was
performed using primers targeting the *ddl* gene as described and validated by Tan TY *et al.* in 2015 with modifications [25]. The full PCR protocol used in this study is found in the appendix.

**Estimation of Prevalence, Incidence, and Duration of Carriage:** We used the observed prevalence of VR *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 VR *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]. Since participants were enrolled very close to the time of admission to a NF, the prevalence reflects the incidence at the facility from which they were admitted, thus the duration may be under or over-estimated 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 Chi-squared test, 2-tailed (table 1). We then compared the cumulative incidence of colonization for each species. Lastly, 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 two visits and those at risk for at least one 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 nursing facility (n=6) and individuals (n=441). We imputed values for the two 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 <0.05) were included in the full model. Model fit was assessed by comparing the mean 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]. Briefly, participants averaged 74.7 years of age (SD: 12.2); 42.2% were male, 62.4% were white, and 37.3% were African American. 33.2% of participants in the pilot study tested positive for VRE at enrollment. Here we describe the prevalence of VR *E. faecium* and/or VR *E. faecalis* only which gives slightly different numbers than published previously: 192 (29.5%) were positive for one or both species at enrollment. Specifically, 116 (17.8%) participants were colonized with VRE *faecium*, 55 (8.4%) with VRE *faecalis*, and 21 (3.2%) with both at enrollment (Figure 1). VR *E. faecium* and/or VR *E. faecalis* were isolated at least once throughout the study period from 265 (40.7%) of the 651 participants. At enrollment, prevalence of VRE *faecium* was higher than VRE *faecalis* in both sexes and races (Table 1). The incidence rates for the 55 participants who became colonized with VR *E. faecium* or both species during the course of the study were similar to the 62 participants who
became colonized with VRE. *faecalis* or both overall, and when stratified by sex and race (Table 1).

Over the course of the study there were 780 VR *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* vs *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 two 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, a quarter of those without *E. faecalis* at enrollment acquired *E. faecalis*, and by day 60 following enrollment, a 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*, and *E. faecium* and *E. faecalis*. The model included variables such as age, sex, race, and underlying medical conditions. The results showed that *E. faecium* colonization was associated with higher odds of colonization at the hands, nares, oropharynx, and enteral feeding tube insertion site, while *E. faecalis* colonization was associated with higher odds at the perianal area and wounds. The duration of carriage for *E. faecium* was longer than for *E. faecalis*, and the rate of new cases acquired was higher for *E. faecium*.
*faecalis,* or both at a particular visit for all individuals at risk who also had one 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 (OR=2.90; 95% CI: 1.70, 4.93) and antibiotic use (OR = 3.53; 95% CI: 2.26, 5.51) within the last 30 days, while 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). Additionally, 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 factors 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 (VR *E. faecium*: OR = 2.89; 95% CI: 1.82, 4.60; VR *E. faecalis*: OR= 1.80; 95% CI: 1.16, 2.80). Those who were colonized with both species had a significantly increased odds of testing positive for VR *E. faecalis* or both species at the next visit (VR *E. faecalis*: OR = 6.38; 95% CI: 1.25, 32.54; Both: OR=41.40; 95% CI: 3.74, 457.78). Increased length of stay at or beyond 30 days, compared to 14 days, did not increase odds of any colonization type when adjusting for the other risk factors.

Comparison of the mean QIC values revealed 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 (VR *E. faecium*: OR = 3.04; 95% CI: 1.02, 9.08; VR *E. faecalis*: OR = 4.18; 95% CI: 1.55, 11.29; Both: OR = 5.50; 95% CI: 1.50, 20.10). Additionally, usage of other classes was positively associated with the presence of VR *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 VR *E. faecium* (OR = 2.33; 95% CI: 1.34, 4.03) (Supplemental Table 1).

**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 VR *E. faecium* in the current study is consistent with previous studies in acute healthcare settings where prevalence was 19% [30,31], and among three Southern California NFs, where the overall VRE prevalence was 16% (prevalence varied from 7-19% depending on the NF) [32]. A Jerusalem study of 1,215 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 a NF from an acute-care facility [34]; this may explain the higher prevalence observed in our study as over 96% of participants were transferred to the NF from an acute-care facility, and were enrolled shortly after NF admission.

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The observation of a longer duration of *E. faecium* carriage, 69 days, compared to 32 for *E. faecalis*, is novel. We found only one 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 suggests *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 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 three 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 three 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. Further, although each of the colony morphotypes was sub-cultured 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 sub-cultured for testing. Thus, it is likely that we underestimated co-colonization. We observed 2 phenotypic colonies 5% of the time, of which 42% were different species. However, if co-colonization 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. Additionally, 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 one species, we could not detect it. Previous studies using stool samples have shown 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. Similarly 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.

In conclusion, we observed a higher prevalence of VR *E. faecium* compared to VR *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. Notably, device use was more strongly associated with increased incidence of VR *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 VR *E. faecium* colonization, and that of other pathogenic organisms [41,42].
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Potential Conflicts of Interest

All authors report no conflicts of interest.
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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 |
|--------------------------|-----------------|-------------|--------------------------------|
|                          | Total (N=651)   | E. faecium  | E. faecalis                     | E. faecium (N=55) | E. faecalis (N=62) | IRR** | 95% CI     |
|                          | Only (N=116)    | Only (N=55) | Only (N=21)                     |                  |                      |       |            |
| **Sex**                  | Male            | 275         | 52 (.19)                        | 27 (.10)         | 9 (.03)             | 5381   | 5661        | 23       | 24       | 1.02 (0.57, 1.80) |
|                          | Female          | 376         | 64 (.17)                        | 28 (.07)         | 12 (.03)            | 8889   | 9481        | 32       | 38       | 0.90 (0.56, 1.44) |
| **Race**                 | White           | 406         | 66 (.16)                        | 33 (.08)         | 13 (.03)            | 8288   | 8683        | 33       | 38       | 0.91 (0.57, 1.45) |
|                          | Black           | 243         | 49 (.20)                        | 22 (.09)         | 8 (.03)             | 5965   | 6417        | 22       | 24       | 0.99 (0.55, 1.76) |
|                          | Other           | 2           | 1 (.5)                          | 0 (0)            | 0 (0)               | 17     | 42          | 0 (0)    | 0 (0)    | -                  |
| **Device use***          | ++              | 281         | 60 (0.21)                       | 31 (0.11)        | 15 (0.05)           | 5079   | 4647        | 25       | 15       | 1.52 (0.81, 2.96) |
|                          | ++              | 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)       |

*There was no statistically significant difference in prevalence by sex or race  ** Incidence Rate Ratio
Table 2. Prevalence, incidence, and duration of carriage of vancomycin resistant *Enterococci* by species within 6 nursing facilities in Southeastern Michigan

|                        | Vancomycin resistant *E. faecium* | Vancomycin resistant *E. 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                              |

+ Device use was defined as the presence of an indwelling catheter or feeding tube
++ Past 30 days
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 one visit.

|                        | E. faecium only | E. faecalis only | Both Species |
|------------------------|-----------------|-----------------|--------------|
|                        | OR   | 95% CI | p-value | OR   | 95% CI | p-value | OR   | 95% CI | p-value |
| Non-White Race         | 0.99 | (0.62, 1.59) | 0.97    | 0.69 | (0.45, 1.07) | 0.10    | 1.06 | (0.40, 2.80) | 0.90    |
| Male                   | 1.08 | (0.67, 1.73) | 0.76    | 0.89 | (0.58, 1.38) | 0.61    | 1.56 | (0.58, 4.20) | 0.38    |
| Device Use*,**         | 2.90 | (1.70, 4.93) | <.0001  | 1.52 | (0.86, 2.24) | 0.15    | 3.81 | (1.45, 10.03) | 0.01    |
| Open Wound*            | 1.66 | (0.93, 2.97) | 0.09    | 1.34 | (0.80, 2.24) | 0.26    | 2.71 | (1.00, 7.33) | 0.05    |
| Antibiotic*            | 3.53 | (2.26, 5.51) | <.0001  | 1.86 | (1.22, 2.84) | 0.004   | 2.49 | (1.03, 7.03) | 0.04    |
| Physical Self-Maintenance Score*** | 1.04 | (0.99, 1.10) | 0.13    | 1.04 | (0.99, 2.27) | 0.13    | 1.01 | (0.90, 1.14) | 0.82    |

* Within the past 30 days.

**Defined as the presence of an indwelling catheter or feeding tube
*** Lower Physical Self-Maintenance Score indicates increased independence

Table 4: Multivariate model adjusted for number of visit and previous colonization status predicting VRE colonization in 441 nursing facility participants with more than one visit.

|                  | E. faecium | E. faecalis | Both Species |
|------------------|------------|-------------|--------------|
|                  | OR  | 95% CI   | p-value | OR  | 95% CI   | p-value | OR  | 95% CI   | p-value |
| **Device use***, **  |    |          |         |    |          |         |    |          |         |
| 2.01             | 1.15, 3.50 | 0.01       | 1.24   | 0.69, 2.22 | 0.48    | 3.12   | 1.14, 8.55 | 0.03    |
| **Antibiotics***  |    |          |         |    |          |         |    |          |         |
| 2.89             | 1.82, 4.60 | <0.0001   | 1.80   | 1.16, 2.80 | 0.01    | 1.79   | 0.71, 4.53 | 0.22    |
| **14 Days***     |    |          |         |    |          |         |    |          |         |
| ref              | ref    | ref       | ref    | ref | ref       | ref     | ref | ref       | ref     |
| 0.62             | 0.35, 1.10 | 0.10       | 1.38   | 0.80, 2.38 | 0.24    | 1.21   | 0.40, 3.68 | 0.73    |
| 0.47             | 0.25, 0.88 | 0.02       | 0.98   | 0.59, 1.62 | 0.93    | 0.75   | 0.27, 2.11 | 0.59    |
| **Previous E. faecium** |    |          |         |    |          |         |    |          |         |
| 8.62             | 5.09, 14.60 | <.0001    | 3.02   | 1.63, 5.61 | 0.005   | 18.14  | 4.49, 73.29 | <.0001  |
| **Previous E. faecalis** |    |          |         |    |          |         |    |          |         |
| 1.60             | 0.68, 3.75 | 0.28       | 10.78  | 6.15, 18.89 | <.0001  | 25.53  | 6.61, 98.66 | <.0001  |
| **Previous both species** |    |          |         |    |          |         |    |          |         |
| 3.71             | 0.38, 36.57 | 0.26       | 6.38   | 1.25, 32.54 | 0.03    | 41.40  | 3.74, 457.78 | .002    |

* In the past 30 days

** Defined as the presence of an indwelling catheter or feeding tube

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**Table 4:** Multivariate model adjusted for number of visit and previous colonization status predicting VRE colonization in 441 nursing facility participants with more than one visit.

In the past 30 days

Defined as the presence of an indwelling catheter or feeding tube
*** Number of days in NF
Figure Legends

Figure 1: Study flow diagram showing colonization with vancomycin resistant *E. faecalis* and *E. faecium* from 6 Nursing facilities throughout Southeastern Michigan

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

Figure 3. Cumulative incidence of VRE by species from participants in 6 nursing facilities throughout Southeastern Michigan
Appendix

Speciation of isolates: Identification of VRE species was conducted using *E. faecium*-HRM-F (5’- TTTACAAGCTGCTGGTGTGC-3’), *E. faecium*-HRM-R (5’- AACCCATATTTCAGGTTTG-3’), *E. faecalis*-HRM-F (5’- GTGGCTTAAGTCGCTGTGAT-3’), and *E. faecalis*-HRM-R (5’- AGGCATGGTGTTCAATTCAT-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 TY *et al* [25]. The reaction volume consisted of 12.5 µL GoTaq Green Master Mix (Promega), 2.5 µL of *E. faecalis* primer mix containing 10 µM forward and reverse primers, 2.5 µL of *E. faecium* primer mix containing 10 µM forward and reverse primers, 5 µL of nuclease-free PCR water, and 2.5 µL of 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 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, extension at 72°C for 45 sec, and a single final extension step at 72°C for 5 min with a 4°C hold step. Amplified DNA fragments were then separated by electrophoresis on 2% agarose gel. VRE. *faecalis* and VRE. *faecium* samples were separated by species based upon the previous visualized banding patterns.
The image is a flowchart titled "Figure 1". It illustrates the study population flow from baseline visit with colonization status and follow-up visits. The chart details the progression of patients through colonization status at baseline and follow-up visits.
Figure 3

Cumulative Incidence

Number of Days in Nursing Facility

Species
- E.faecalis
- E.faecium

*Shaded regions indicate 95% confidence interval