ORIGINAL ARTICLE

Defining the Role of the Environment in the Emergence and Persistence of *vanA* Vancomycin-Resistant Enterococcus (VRE) in an Intensive Care Unit: A Molecular Epidemiological Study

Andie S. Lee, MB BS, MSc;[1,2] Elizabeth White, BN;[3] Leigh G. Monahan, PhD;[4] Slade O. Jensen, PhD;[5,6] Raymond Chan, MB BS, PhD;[1,2] Sebastiaan J. van Hal, MBChB, PhD[1,5,6]

OBJECTIVE. To describe the transmission dynamics of the emergence and persistence of *vanA* vancomycin-resistant enterococcus (VRE) in an intensive care unit (ICU) using whole-genome sequencing of patient and environmental isolates.

DESIGN. Retrospective cohort study.

SETTING. ICU in a tertiary referral center.

PARTICIPANTS. Patients admitted to the ICU over an 11-month period.

METHODS. *VanA* VRE isolated from patients (n = 31) were sequenced using the Illumina MiSeq platform. Environmental samples from bed spaces, equipment, and waste rooms were collected. All *vanA* VRE-positive environmental samples (n = 14) were also sequenced. Data were collected regarding patient ward and bed movements.

RESULTS. The 31 patient *vanA* VRE isolates were from screening (n = 19), urine (n = 4), bloodstream (n = 3), skin/wound (n = 3), and intra-abdominal (n = 2) sources. The phylogeny from sequencing data confirmed several VRE clusters, with 1 group accounting for 38 of 45 isolates (84%). Within this cluster, cross-transmission was extensive and complex across the ICU. Directionality indicated that colonized patients contaminated environmental sites. Similarly, environmental sources not only led to patient colonization but also to infection. Notably, shared equipment acted as a conduit for transmission between different ICU areas. Infected patients, however, were not linked to further VRE transmission.

CONCLUSIONS. Genomic sequencing confirmed a predominantly clonal outbreak of VRE with complex transmission dynamics. The environmental reservoir, particularly from shared equipment, played a key role in ongoing VRE spread. This study provides evidence to support the use of multifaceted strategies, with an emphasis on measures to reduce bacterial burden in the environment, for successful VRE control.

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Vancomycin-resistant enterococcus (VRE) is endemic in many healthcare facilities, accounting for ~60% of bacteremias[1] and >80% of healthcare-associated infections due to *Enterococcus faecium* in some settings.[2] Furthermore, VRE infections are associated with significant mortality and morbidity,[3] in part due to limited antimicrobial treatment options.[4] Given the clinical impact of this pathogen, efforts to reduce cross-transmission have been implemented in many hospitals. However, the optimal approach to VRE control remains controversial.[5,6]

In late 2013, a shift from *vanB* to *vanA* VRE occurred across Australia.[7,8] Unlike the *vanB* gene, which usually integrates into the *E. faecium* chromosome, the *vanA* gene is often located on a plasmid.[9,10] The ease with which horizontal transfer of plasmids occurs suggests that the emergence of *vanA* VRE will likely lead to an overall larger burden of VRE. Indeed, there was a dramatic increase in *vanA* VRE incidence in our institution between 2013 and 2014, despite improvement in methicillin-resistant *Staphylococcus aureus* acquisition rates during this period (from 5.7 per 10,000 patient days to 3.4 per 10,000 patient days).

Affiliations: 1. Departments of Infectious Diseases and Microbiology, Royal Prince Alfred Hospital, Sydney, New South Wales, Australia; 2. Faculty of Medicine, University of Sydney, Sydney, New South Wales, Australia; 3. Infection Control Department, Royal Prince Alfred Hospital, Sydney, New South Wales, Australia; 4. The ithree institute, University of Technology Sydney, Sydney, New South Wales, Australia; 5. Antibiotic Resistance and Mobile Elements Group, Ingham Institute, Sydney, New South Wales, Australia; 6. Medical Sciences Research Group, School of Medicine, Western Sydney University, Sydney, New South Wales, Australia.

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We therefore undertook this molecular epidemiological study to better characterize the emergence of *vanA* VRE in our intensive care unit (ICU) using whole-genome sequencing of patient and environmental isolates to delineate transmission chains. We hypothesized that the development of a substantial environmental reservoir played a key role in the emergence and sustained transmission of *vanA* VRE in the unit.

METHODS

Study Design, Setting, and Participants

This retrospective cohort study was conducted at a 911-bed tertiary-care referral hospital in Sydney, Australia. The hospital provides solid-organ transplantation, hematopoietic stem-cell transplantation and pelvic exenteration services. The 2 general ICU wards (ICU-1 and ICU-2) care for both medical and surgical patients. The ICUs are close to each other, with potential movement of patients, staff, and equipment between units. After the emergence of *vanA* VRE was noted in 2013, VRE isolates from patients admitted to the ICUs from January to November 2014 were systematically stored and included in this study.

VRE Screening and Infection Control Precautions

Patients in the ICU undergo routine screening for VRE with rectal swabs collected on admission, weekly, and on discharge from the unit. Individuals colonized or infected with VRE are placed on contact precautions (using gowns and gloves) and are isolated in single rooms when available. ICU-1 has 13 beds with 3 (23%) single rooms, while ICU-2 has 17 beds including 7 (41%) single rooms. Bed spaces are terminally cleaned with a hypochlorite disinfectant when VRE-colonized or -infected patients are discharged from the ICU.

Environmental Sampling

Environmental sampling was performed in the ICUs in September 2014 to determine whether there was a reservoir to explain the increasing *vanA* VRE incidence. Samples were collected by premoistening swabs with normal saline then swabbing an area ≥5 cm in diameter. High-touch areas from bed spaces (ie, bed rails, bedside tables, infusion pumps, drawer handles, counters, patient stethoscopes, monitors, and computers), waste rooms (ie, door handle, pan sanitizer and taps), bathrooms (ie, light switch, shower taps, rails, call button, and sink taps) and shared equipment (ie, blood gas analyzer, point-of-care coagulation timer, patient slide, patient lifter, air-assisted patient transfer system (Hovermatt, HoverTech International, Allentown, PA), chlorhexidine wipe warmer, ultrasound, intravenous poles, electrocardiogram (ECG) machine, and ECG leads) were sampled. The bed spaces were randomly selected within each of the following categories in each ICU: current occupant VRE positive, previous occupant VRE positive, current occupant colonized with a multiresistant organism other than VRE (eg, methicillin-resistant *S. aureus*), and current occupant not colonized with a multiresistant organism.

Microbiology Methods

Screening and environmental samples were inoculated directly onto selective chromogenic agar (chromID VRE Agar, bioMérieux, Marcy-l'Étoile, France), incubated at 37°C, and read at 24 and 48 hours. Characteristically colored colonies were identified as *E. faecium* by the MALDI-TOF biotyper (Bruker Daltonics, Bremen, Germany). Presence of *vanA* or *vanB* genes was confirmed by polymerase chain reaction (PCR).[11] The first available patient and all environmental *vanA* VRE isolates were included in the study.

Data Collection

Data regarding admissions, patient days, hand hygiene compliance, and newly identified VRE patients were prospectively collected. Hand hygiene compliance was calculated as the number of compliant moments divided by total moments directly observed by trained auditors according to the National Hand Hygiene Initiative,[12] based on the World Health Organization's "Five Moments for Hand Hygiene."[13] For VRE patients, admission date, admitting specialty, ward and bed movements, and single-room isolation were also recorded. VRE acquisition was defined as isolation of VRE with no prior history of VRE colonization or infection, and VRE infection was defined as isolation of VRE from a sterile site or other specimen accompanied by signs of infection. ICU-acquired VRE was defined as new detection of VRE >48 hours after admission to the unit.

Statistical Analysis

Descriptive statistics included calculation of means for normally distributed variables and medians for nonparametric variables. For differences in proportions, the χ^2 test was used. Poisson regression was used to calculate differences in rates using 1,000 patient days as the exposure, VRE acquisition count as the dependent variable, and time period as the independent variable. All P values were 2-tailed, and $P < .05$ was considered statistically significant. Data were analyzed using Stata version 11.0 software (StataCorp, College Station, TX).

Genomic Analysis

Isolate sequencing was performed using a bench-top Illumina MiSeq sequencer and MiSeq V3 chemistry following library preparation (NextEra XT kit, Illumina, San Diego, CA) according to the manufacturer's instructions, generating 75 nucleotide paired-end reads. Single-nucleotide variants (SNVs) were determined from the pan-genome using kSNP3[14] with vancomycin resistance and multilocus sequence typing (MLST) obtained from *de novo* assemblies. A maximum-likelihood phylogeny was generated on the SNV matrix using

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RaxML version 8.2.9 (Exelixis, San Francisco, CA)[15] with hierarchical clustering.[16] Links between isolates were analyzed using the R package "outbreaker" software (R Foundation for Statistical Computing, Vienna, Austria).[12,17] This model determines the directionality of isolates based on genetic distance and sample isolation date assuming a single introduction event with no molecular clock rate. To minimize the impact of these assumptions, we limited this analysis to isolates from (1) the single dominant cluster (cluster 1) and (2) those obtained within a ±2 month window from the time of the environmental sampling, based on previous observations of VRE survival on surfaces for up to 2 months.[18]

RESULTS

In total, 1,729 patients were admitted to the 2 ICUs during the study period; 92 (5.3%) were VRE-positive on admission. Most patients colonized or infected on admission had *vanB* VRE (55 of 92; 60%), while 36 patients (39%) had *vanA* VRE and 1 patient was colonized with both *vanA* and *vanB* VRE. VRE acquisition rates in the ICUs rose from 3.1 per 1,000 patient days in 2013 to 7.0 per 1,000 patient days in 2014 (incidence rate ratio [IRR], 2.2; 95% CI, 1.4–3.5; $P < .001$), predominantly due to an increase in *vanA* VRE from 0.3 per 1,000 patient days to 3.9 per 1,000 patient days during this period (IRR, 11.2; 95% CI, 3.4–36.3; $P < .001$). Acquisition of *vanB* VRE remained relatively stable at 2.8 per 1,000 patient days in 2013 and 3.1 per 1,000 patient days in 2014 (IRR, 1.1; 95% CI, 0.6–1.9; $P = .69$).

Overall, 62 patients (3.6%) acquired VRE in the ICUs during the study period; 34 (55%) had *vanA* VRE and 28 (45%) had *vanB* VRE. Among the ICU-acquired *vanA* VRE, most (74%) were detected in ICU-1. For 31 patients with ICU-acquired *vanA* VRE, isolates had been stored and were therefore available for sequencing. Among these patients, 18 (58%) were male and the median age was 62 years (range, 26–87 years). Patients with

TABLE 1. Characteristics of ICU Patients With *vanA* VRE

Characteristic	ICU-1	ICU-2	Total
Total no. (%)	23 (74)	8 (26)	31 (100)
Male (%)	13 (57)	5 (63)	18 (58)
Age median y (range)	64 (26–87)	62 (34–68)	62 (26–87)
ICU length of stay, median d (range)	5 (2–48)	9 (3–61)	8 (2–61)
Admitting specialty (% of total in ward)			
Gastroenterology/hepatology	7 (30)	3 (38)	10 (32)
Gastrointestinal surgery	5 (22)	1 (13)	6 (19)
Hematology	2 (9)	2 (25)	4 (13)
Surgery (nongastrointestinal)	3 (13)	0 (0)	3 (10)
Respiratory medicine	2 (9)	1 (13)	3 (10)
Cardiology	1 (4)	1 (10)	2 (6)
Geriatric medicine	2 (9)	0 (0)	2 (6)
Renal medicine	1 (4)	0 (0)	1 (3)
Source of VRE isolate (% total in ward)			
Screening	14 (61)	5 (63)	19 (61)
Clinical culture			
Urine	2 (9)	2 (25)	4 (13)
Bloodstream	3 (13)	0	3 (10)
Skin/Wound	2 (9)	1 (13)	3 (10)
Intra-abdominal	2 (9)	0	2 (6)
VRE treatment while in ICU (%)			
VRE positive on screening	0/14 (0)	0/5 (0)	0/19 (0)
VRE positive on clinical cultures	4/9 (44)	0/3 (0)	4/12 (33)

NOTE. ICU, intensive care unit; VRE, vancomycin-resistant enterococcus.

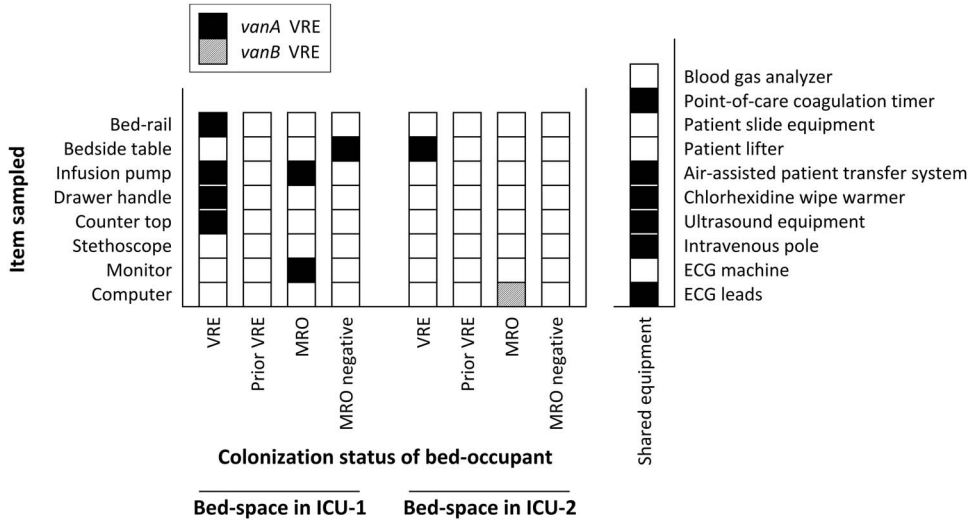

FIGURE 1. Isolation of vancomycin-resistant enterococcus (VRE) from environmental samples. Detection of VRE from environmental samples collected from ICU-1, ICU-2 and shared equipment. Results from sampling of bed spaces are labelled with the colonization status of the bed occupant at the time of sampling. NOTE. VRE, vancomycin-resistant enterococcus; MRO, multiresistant organism; ECG, electrocardiogram.

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vanA VRE were most frequently admitted under gastroenterology/hepatology (n = 10), gastrointestinal surgery (n = 6), or hematology (n = 4) specialties (Table 1). Overall, 19 screening isolates (61%) and 12 clinical isolates (39%) were identified (Table 1).

Of the 92 environmental samples, 14 (15%) were positive for *vanA* VRE, compared with only 1 (1%) positive for *vanB* VRE. ICU-1 had widespread environmental contamination, particularly surrounding the VRE-colonized patient (Figure 1). VRE was also detected, although at fewer sites, around other patients. Notably, however, VRE was not isolated from the bed space where the prior room occupant had been VRE colonized. In contrast, in ICU-2, *vanA* VRE was only recovered from 1 site. Importantly, more than half of the sampled equipment shared between the ICUs was also contaminated (Figure 1). The patient transfer system and ultrasound machine, items which come into direct patient contact, were particularly heavily colonized.

Genomic Analysis Results

The phylogeny (based on the pan-genome SNV matrix) revealed 4 distinct clusters. *In silico* MLST supported the clustering with identical sequence types within each cluster. A single cluster (Figure 2) predominated (84% of isolates), within which all isolates were not typeable as a result of deletion of the *pstS* allele.[19] Cross-transmission events were observed with identical isolates between patient and environmental genomes (median SNV between isolate pairs 13 SNVs; range, 5–55).

Genomic analyses of directionality (of the dominant cluster 1) confirmed the importance of the environment, including shared equipment, as the potential source of ongoing transmission (Figure 3). For example, an infusion pump (labeled "A" in Figure 3) was the source for several patient colonization and infection episodes, as well as further environmental contamination. Most transmission events from environmental sources were to patients close to the contaminated area (ie, within 1 bed space). In contrast, most transmission events that occurred at a distance (>1 bed space away) within the same ICU or between the 2 ICUs were related to patient sources, suggesting healthcare workers as potential conduits of transmission. Interestingly, isolates from VRE-infected patients were not linked with any additional isolates.

Enhanced Infection Control Interventions and Monitoring of VRE Rates

Review of the environmental data led to implementation of a number of interventions. These included enhanced monitoring and feedback of VRE acquisition, hand hygiene, and environmental contamination data. These measures were facilitated by meetings with key stakeholders including ICU (medical and nursing), executive, environmental services, infection control and infectious diseases staff (Figure 4 and Table S1 in Supplementary Appendix). Where widespread VRE contamination was documented, cleaning in the unit was intensified, with particular attention to ICU-1 and shared equipment.

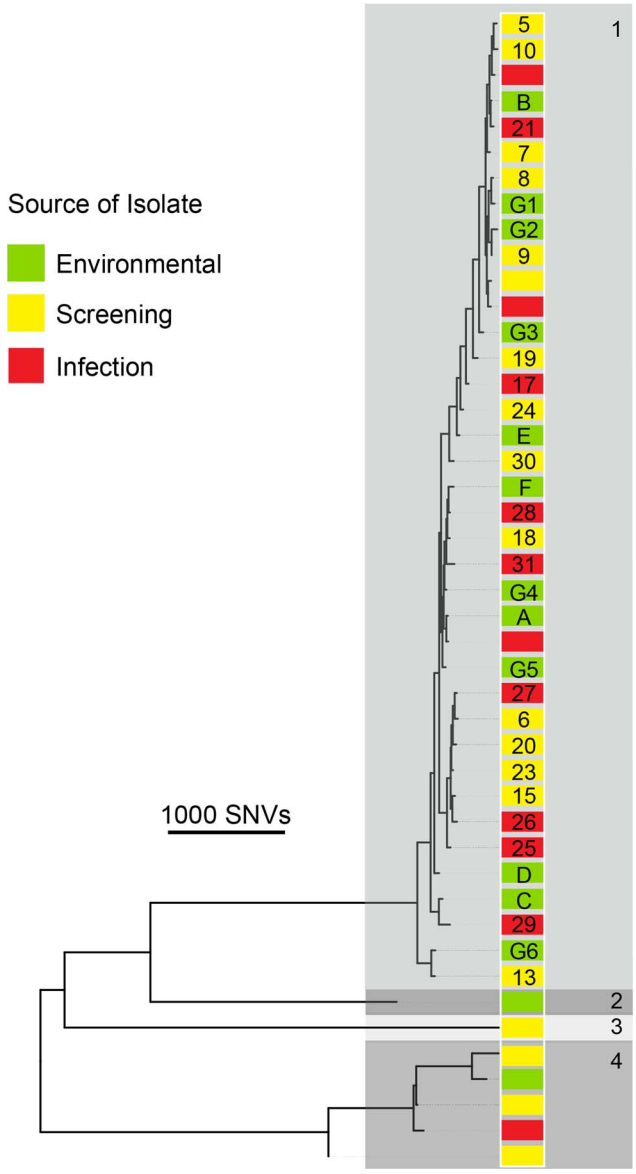

FIGURE 2. Maximum-likelihood phylogenetic tree. The phylogeny of all sequenced isolates (n = 45) with the isolate identifier and source of isolation depicted by the legend to the left of the tree. Four clusters were observed (see text for details). Members of the largest cluster (cluster 1 outlined by the top grey box) all classified as a single multilocus sequence type. Further analysis was directed at sequences within the predominant cluster that met inclusion criteria (ie, isolates with an identifier). Identifiers are shown to allow for cross-referencing between Figures 2 and 3. NOTE. SNV, single-nucleotide variant.

Hand hygiene compliance rates were lower in ICU-1 than in ICU-2 during the period of environmental sampling (46% and 75% respectively; $P < .001$), but it improved to 76% ($P < .001$) over the following 12 months (Figure 4). *VanA* VRE acquisition

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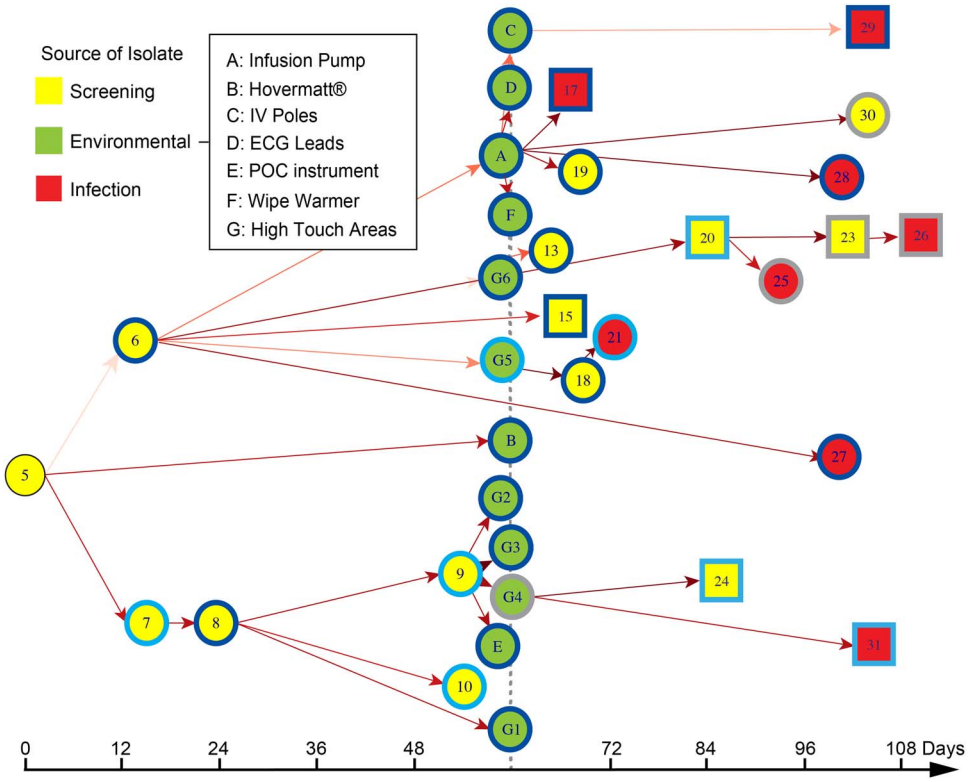

FIGURE 3. Inter- and intra-intensive care unit (ICU) transmission dynamics. Transmission chains and directionality of cluster 1 sequenced isolates within ±2 months of the date of environmental sampling. Arrows between samples indicate the likely ancestor or transmission chain of each isolate with darker arrow colors representing higher likelihoods of the parent isolate being the true ancestor. The time scale is provided on the x-axis with isolate source depicted using colors according to the legend at the top left of the figure. Environmental isolates are further categorized into shared equipment and high-touch areas in the legend. Circular shapes indicate a nonisolation area, and square shapes indicate that the patient was in a single room at the time the first positive VRE sample was collected. All shapes are highlighted with either dark blue or turquoise to reflect adjacent (within 1 bed space either side of the index isolate) and distant (>1 one bed space away) intra-ICU transmission respectively. Grey borders represent inter-ICU transmission events. For example, isolate 9 (a screening isolate on day 56) obtained from a nonisolated patient led to contamination of a high-touch area (G4, in the other ICU on day 60). This high-touch region was subsequently the source for a distant (>1 bed space apart) colonization (patient 24) and an infection event (patient 31) ~ 26 and 41 days later in the same ICU (intra-ICU events). Both patients were isolated at the time of first VRE detection. NOTE. IV, intravenous; ECG, electrocardiogram; POC, point-of-care.

rates continued to increase in the ICUs between 2014 and 2015 then remained stable in 2016 (Figure 4 and Table S2 in Supplementary Appendix). The shift from predominantly *vanB* to *vanA* VRE observed in 2014 persisted in subsequent years (Figure 4).

DISCUSSION

Increasing VRE incidence in the ICU was explained by multiple concurrent outbreaks of *vanA* VRE, with a single clone of a recently characterized lineage[19] emerging as the dominant circulating strain. The spread of VRE continued from patient to patient, with colonized patients acting as sources of transmission. In addition, patients transmitted VRE to the environment, including to fixed and shared equipment, which was then implicated as the source of further transmission events both within the same unit and across units.

The importance of the environment as a VRE reservoir has previously been documented.[20,21] However, our study provides an in-depth understanding of the role of the environment by detailed delineation of VRE transmission chains using discriminatory genomic data showing identical isolates on a pan-genome level. Notably, reusable medical equipment was demonstrated to be an important source for healthcare-associated infections. Cleaning and disinfection of these devices are frequently overlooked, often due to a lack of designated responsible personnel.[22,23] This situation is particularly concerning for VRE due to its ability to survive on dry surfaces for prolonged periods and to withstand attempts at disinfection.[23]

It is possible that increasing *vanA* VRE incidence may reflect the emergence of a strain with greater ability to persistent in the environment and/or enhanced transmissibility. Although most patients colonized on admission to the ICU harbored *vanB*

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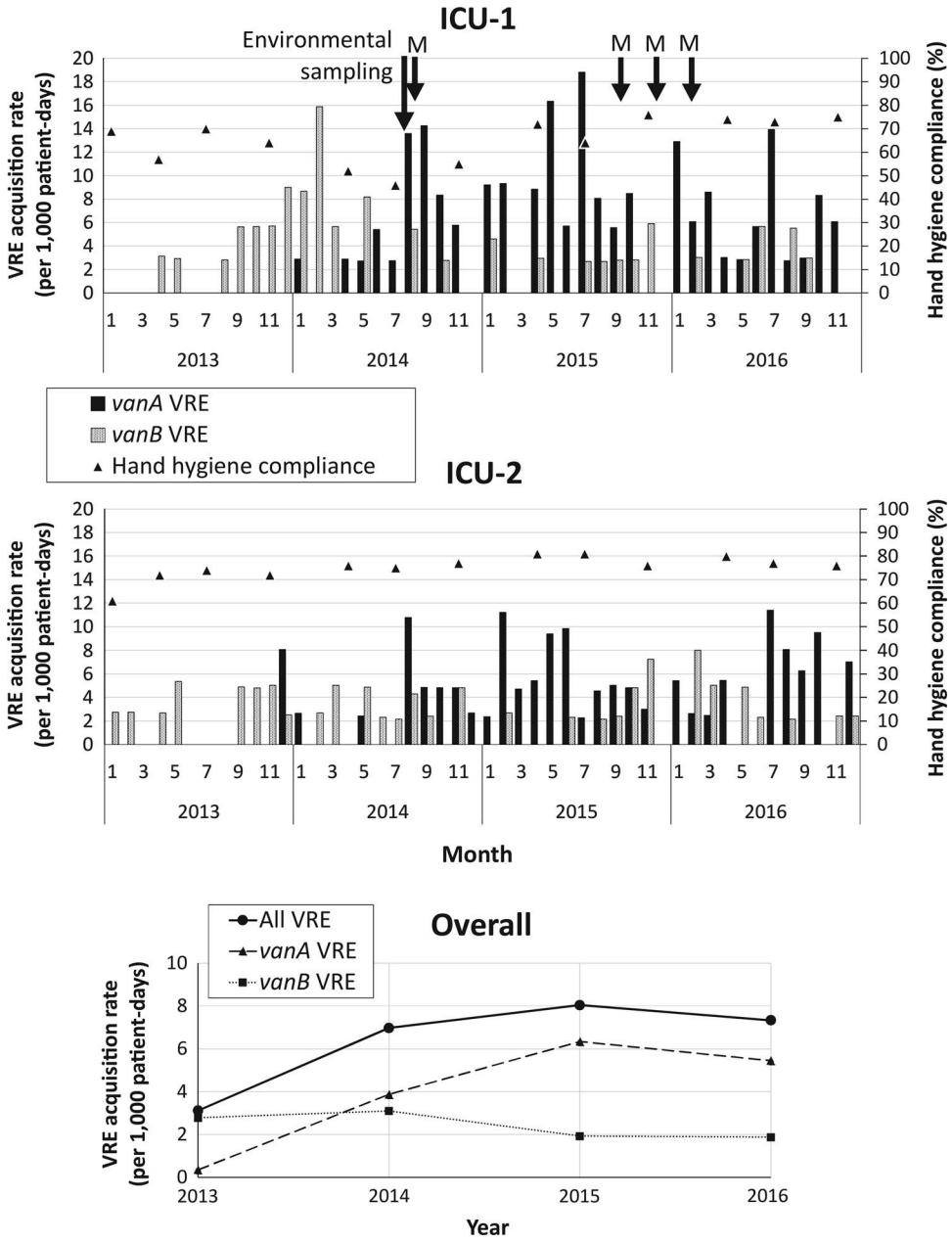

FIGURE 4. Vancomycin-resistant enterococcus (VRE) acquisition and hand hygiene compliance rates. The long arrow indicates the time point at which environmental sampling occurred in the intensive care units (ICUs). The short arrows labelled with "M" indicate the timing of multidisciplinary meetings between ICU, executive, environmental services, infection control and infectious diseases staff. NOTE. VRE, vancomycin-resistant enterococcus; M, multidisciplinary meeting.

VRE, acquisition in the unit and environmental contamination was predominantly with *vanA* VRE. These data support the hypothesis that the emerging *vanA* VRE strain possessed characteristics enabling its long-term survival in the environment. Interestingly, in contrast to previous studies,[24,25] VRE was not detected in bed spaces where the prior bed occupant had been VRE positive, suggesting that terminal cleaning had been adequately performed in the ICU. Furthermore, it is possible that intensification of daily cleaning of VRE-positive patient bed spaces may have a significant impact on environmental burden and potentially reduce cross-transmission.

Patients with VRE infections were not linked to further transmission events, irrespective of single-room isolation. This finding is contrary to the expectation that infected patients (with higher VRE burden) would lead to a greater intensity of environment contamination compared to asymptomatically colonized individuals. VRE-specific antimicrobial therapy may have reduced VRE shedding and consequently lowered

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the risk of transmission from these patients. Other possible explanations include behavioral change (eg, greater adherence to hand hygiene and contact precautions), enhanced cleaning of bed spaces, and dedicated equipment for infected patients. Cessation of such interventions may increase VRE burden, as has occurred in settings where VRE control measures were discontinued.[26,27]

This study used whole-genome sequencing, a powerful epidemiological tool, to provide a deeper understanding of the transmission dynamics of VRE, including extensive environmental sampling, to characterize the contribution of this reservoir to VRE spread. Weekly, in addition to admission and discharge, screening enabled more accurate classification of acquisition events. We used culture-based rather than nucleic acid detection methods for VRE screening, using direct inoculation of a chromogenic medium. Although they are less sensitive, culture-based methods may more closely reflect a patient's ability to transmit VRE, as positive cultures correlate with higher density of stool and in turn with skin colonization.[28] It is expected that ICU patients would have a high load of VRE carriage,[29] and cultures were incubated for 48 hours, which increases the sensitivity of VRE detection.[30] It is therefore likely that most VRE carriers in the ICU were identified. In addition, nucleic acid detection assays have been associated with high rates of false-positive results related to fecal carriage of non-enterococcal species harboring *van* genes.[31] We did not sample healthcare workers. Screening of this group could be incorporated into future research to enhance our understanding of transmission chains. This study is limited by its small sample size and residual confounding inherent in its retrospective nature. However, these data can be used to provide the basis for future prospective studies aimed at evaluating the utility of specific environmental interventions.

In conclusion, the transmission dynamics of VRE in the ICU were complex, emphasizing the importance of multifaceted control strategies. Notably, the environmental data indicate that hospital cleaning inadequacies, especially of equipment, can contribute to continuing VRE spread. However, infected patients were not linked with further transmission, suggesting that the interventions instituted for them were effective and providing ongoing support for such measures for VRE control. Our findings are likely generalizable to many healthcare facilities where VRE is now endemic, and they should prompt consideration of specific interventions targeting the environment, particularly shared equipment, as an underappreciated source of healthcare-associated infections.

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Address correspondence to Andie S. Lee, Department of Microbiology, Royal Prince Alfred Hospital, Missenden Road, Camperdown NSW 2050, Sydney, NSW, Australia (andie.lee@health.nsw.gov.au).

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit https://doi.org/10.1017/ice.2018.29

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