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Defining the Role of the Environment in the Emergence and Persistence of vanA VRE in an Intensive Care Unit: A Molecular Epidemiological Study

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Abstract:	Objective. To describe the transmission dynamics of the emergence and persistence of vanA vancomycin-resistant Enterococcus faecium (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 one group accounting for 38 of 45 (84%) isolates. 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 infection. Of note, 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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Defining the Role of the Environment in the Emergence and Persistence of *vanA* VRE in an Intensive Care Unit: A Molecular Epidemiological Study

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ABBREVIATED TITLE

Role of the environment in VRE transmission

KEYWORDS

vancomycin-resistant *Enterococcus faecium*; VRE; whole genome sequencing; transmission; molecular epidemiology

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ABSTRACT

Objective. To describe the transmission dynamics of the emergence and persistence of *vanA* vancomycin-resistant *Enterococcus faecium* (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 one group accounting for 38 of 45 (84%) isolates. 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 infection. Of note, 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.

INTRODUCTION

Vancomycin-resistant enterococcus (VRE) is endemic in many healthcare facilities, accounting for approximately 60% of bacteremias[1] and over 80% of healthcare-associated infections due to *Enterococcus faecium* in some settings.[2] 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 5.7/10,000 to 3.4/10,000 patient-days).

We therefore undertook this molecular epidemiological study to better characterize the emergence of *vanA* VRE in our Intensive Care Unit (ICU) by 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 was a retrospective cohort study conducted at a 911-bed tertiary referral hospital in Sydney, Australia. The hospital has solid organ transplantation, hematopoietic stem cell transplantation and pelvic exenteration services. The two general ICU wards (ICU-1 and ICU-2) care for both medical and surgical patients. The ICUs are in close proximity 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

ICU patients 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 isolated in single rooms where 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-containing 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 pre-moistening swabs with normal saline then swabbing an area ≥ 5 centimeters in diameter. High-touch areas from bed-spaces (bed-rails, bedside tables, infusion pumps, drawer handles, counters, patient stethoscopes, monitors and computers), waste rooms (door handle, pan sanitizer and taps), bathrooms (light switch, shower taps, rails, call button and

sink taps) and shared equipment (blood gas analyzer, point-of-care coagulation timer, patient slide, patient lifter, air-assisted patient transfer system ["Hovermatt®"], chlorhexidine wipe warmer, ultrasound, intravenous poles, 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 multi-resistant organism other than VRE (e.g. methicillin-resistant *S. aureus*), current occupant not colonized with a multi-resistant organism.

Microbiology methods

Screening and environmental samples were inoculated directly onto selective chromogenic agar (chromID VRE Agar, bioMérieux), incubated at 37^{0}C and read at 24 and 48 hours. Characteristically colored colonies were identified as *E. faecium* by the MALDI-TOF biotyper (Bruker). Presence of *vanA* and *vanB* genes was confirmed by 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 WHO 5 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; while 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 non-parametric variables. For differences in proportions, the $\chi 2$ test was used. Poisson regression was used to calculate differences in rates using 1000 patient-days as the exposure, VRE acquisition count as the dependent variable and time period as the independent variable. All p values were two-tailed and $p<0.05$ was considered statistically significant. Data were analyzed using Stata version 11.0 (StataCorp, College Station, Texas).

Genomic analysis

Isolate sequencing was performed using a bench-top Illumina MiSeq sequencer and MiSeq V3 chemistry following library preparation (Nextera XT kit) as per 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 multi-locus sequence typing obtained from *de novo* assemblies. A maximum-likelihood phylogeny was generated on the SNV matrix using RaxML v8.2.9[15] with clustering determined by hierarchical clustering.[16] Links between isolates were analyzed using the R package *outbreaker*.[12,17] This model determines 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

There were 1,729 patients admitted to the two ICU wards during the study period, of whom 92 (5.3%) were VRE-positive on admission. The majority of patients colonized or infected on admission had *vanB* VRE (55 of 92; 60%), while 36 (39%) patients had *vanA* VRE and one patient was colonized with both *vanA* and *vanB* VRE. VRE acquisition rates in the ICUs rose from 3.1 to 7.0 per 1,000 patient-days between 2013 and 2014 (incidence rate ratio [IRR] 2.2, 95% CI 1.4-3.5, p<0.001), predominantly due to an increase in *vanA* VRE from 0.3 to 3.9 per 1,000 patient-days during this period (IRR 11.2, 95% CI 3.4-36.3, p<0.001). Acquisition of *vanB* VRE remained relatively stable at 2.8 and 3.1 per 1,000 patient-days in 2013 and 2014, respectively (IRR 1.1, 95% CI 0.6-1.9, p=0.69).

Sixty-two (3.6%) patients acquired VRE in the ICUs during the study period, of which 34 (55%) were *vanA* and 28 (45%) were *vanB*. Among the ICU-acquired *vanA* VRE, the majority (74%) were detected in ICU-1. There were 31 patients ~~from whom~~ with ICU-acquired *vanA* VRE from whom isolates had been stored and were therefore available for sequencing. Among these patients, 18 (58%) were male and the median age was 62 (range 26-87) years. Patients with *vanA* VRE were most frequently admitted under gastroenterology/hepatology (10), gastrointestinal surgery (6) or hematology (4) specialties (Table 1). There were 19 (61%) screening and 12 (39%) clinical isolates (Table 1).

Of the 92 environmental samples, 14 (15%) were positive for *vanA* VRE compared with only 1 (1%) positive for *vanB* VRE. In ICU-1, there was widespread environmental contamination, particularly surrounding the VRE-colonized patient (Figure 1). VRE was also

detected, although at fewer sites, around other patients. Of note, 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 one 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 ST types within each cluster. A single cluster (Figure 2) predominated (84% of isolates), within which all isolates were non-typeable as a result of deletion of the *pstS* allele.[19] Cross-transmission events were observed with identical isolates (median SNV between isolate pairs 13 SNVs; range 5-55) between patient and environmental genomes.

Genomic analyses of directionality (of the dominant cluster 1) confirmed the importance of the environment, including shared equipment (Figure 3), as the potential source of ongoing transmission. For example, an infusion pump (labelled "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 in close proximity (within one bed-space) to the contaminated area. In contrast, the majority of transmission events occurring at a distance (greater than one bed-space away) within the same ICU or between the two 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 audit and environmental contamination data. This was 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). There was intensification of cleaning in the unit, with particular attention to ICU-1 and shared equipment, where widespread VRE contamination had been documented.

Hand hygiene compliance rates were lower in ICU-1 compared with ICU-2 during the period of environmental sampling (46% and 75% respectively, $p<0.001$)), but improved to 76% ($p<0.001$) over the following 12 months (Figure 4). *VanA* VRE acquisition 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. There was ongoing VRE spread 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 but also 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 is frequently overlooked, often due to a lack of designated responsible personnel.[22,23] This 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 i~~I~~ncreasing *vanA* VRE incidence may reflect the emergence of a strain with greater ability to persistent in the environment and/or enhanced transmissibility~~ and/or ability to persistent in the environment~~. Although the majority of patients colonized on admission to the ICU harbored *vanB* 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.

~~Our findings also provide indirect information regarding the role of healthcare workers. Environmental sources were largely linked to patient acquisitions in close proximity to the site of contamination. This observation could be explained by cross-transmission related to~~

~~shared equipment in adjacent bed-spaces or indirect spread to neighboring patients via healthcare worker hands contaminated from environmental sources, particularly if adherence to hand hygiene was low, as was the case in ICU-1. In contrast, besides the inter-ICU transmission event related to an item of shared equipment, VRE spread between the two units was predominantly from patient sources. This inter-ICU cross-transmission was potentially related to external medical teams spreading VRE from patient-to-patient across the two units, either on their hands, clothing or equipment such as stethoscopes.~~

Patients with VRE infections were not linked to further transmission events, irrespective of single room isolation. This 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 the risk of transmission from these patients. Other possible explanations include behavioral change (e.g. 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 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 the majority of 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 multi-faceted control strategies. Of note, the environmental data indicates 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 should prompt consideration of specific interventions targeting the environment, particularly shared equipment, an under-appreciated source for healthcare associated infections.

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FIGURE LEGENDS

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. VRE, vancomycin-resistant enterococcus; MRO, multi-resistant organism; ECG, electrocardiogram.

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) with the largest cluster (cluster 1 outlined by the top grey box) all classifying as a single multi-locus sequence type. Further analysis was directed at sequences within the predominant cluster that met inclusion criteria (i.e. isolates with an identifier). Identifiers are shown to allow for cross-referencing between Figures 2 and 3. SNV, single nucleotide variant.

Figure 3. Inter- and intra-Intensive Care Unit 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. Time scale 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 non-isolation area while 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 one bed-space either side of the index isolate) and distant (more than 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 non-isolated 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 (more than one bed-space apart) colonization (patient 24) and an infection event (patient 31) approximately 26 and 41 days later in the same ICU (intra-ICU events). Both patients were isolated at the time of first VRE detection. IV, intravenous; ECG, electrocardiogram; POC, point-of-care.

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. VRE, vancomycin-resistant enterococcus; M, multidisciplinary meeting.

TABLES

Table 1. Characteristics of ICU patients with *vanA* VRE

Characteristic	**ICU-1**	**ICU-2**	**Total**
Total number (%)	23 (74)	8 (26)	31 (100)
Male (%)	13 (57)	5 (63)	18 (58)
Age (median, range)	64 (26-87)	62 (34-68)	62 (26-87)
ICU length of stay in days (median, range)	5 (2-48)	9 (3-61)	8 (2-61)
Admitting specialty (% 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 (non-gastrointestinal)	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

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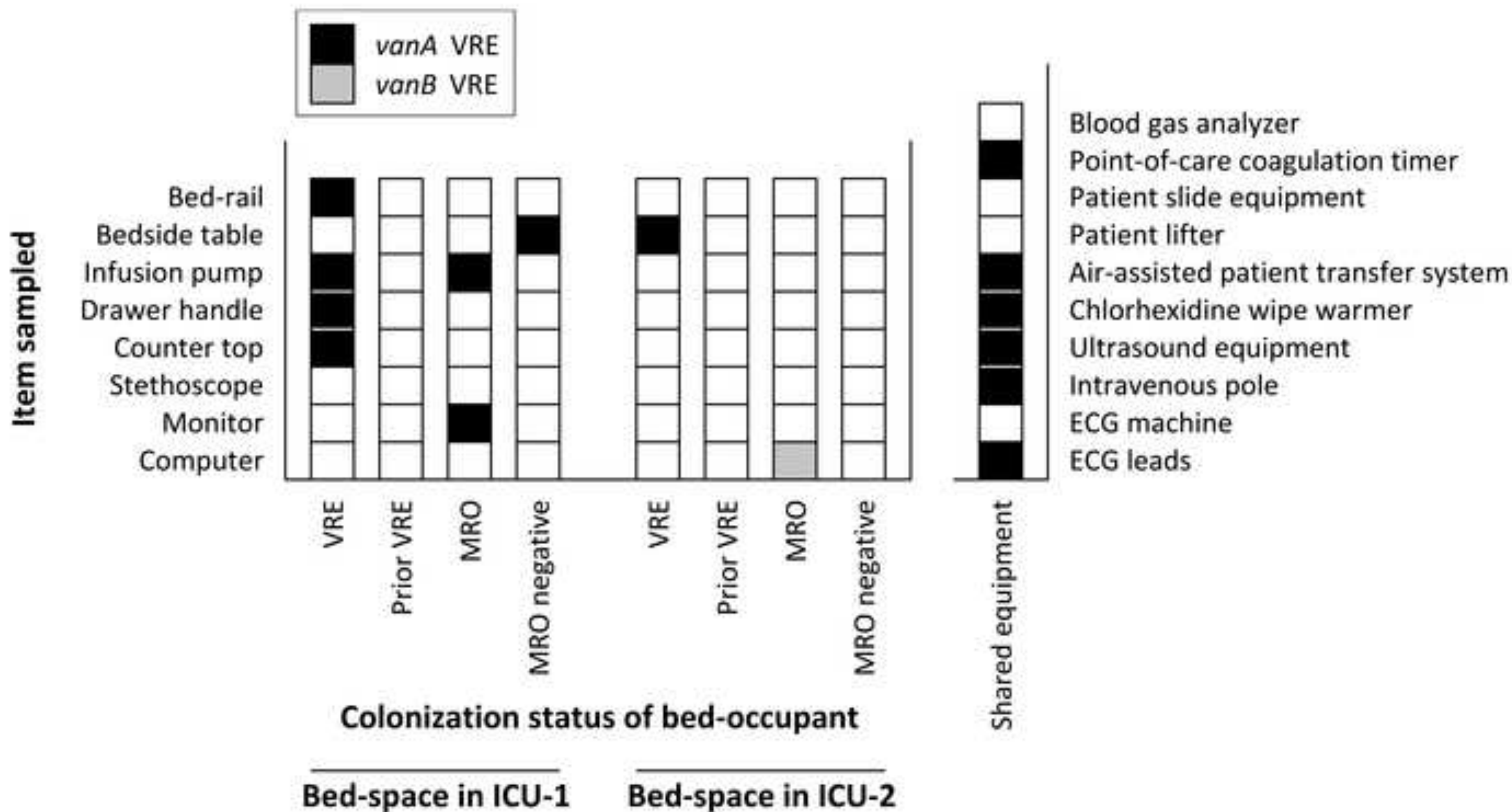

Figure 2

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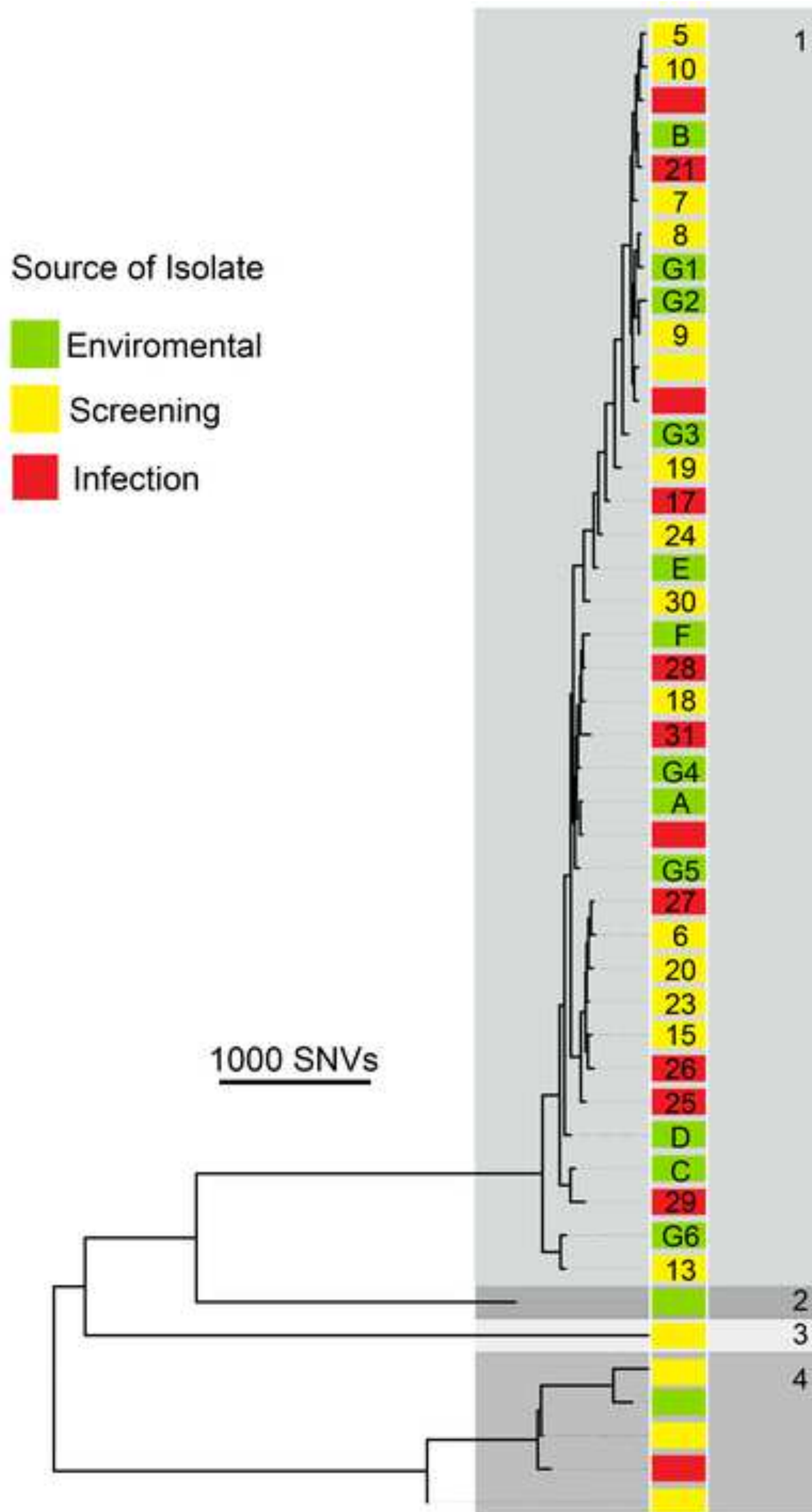

Figure 3

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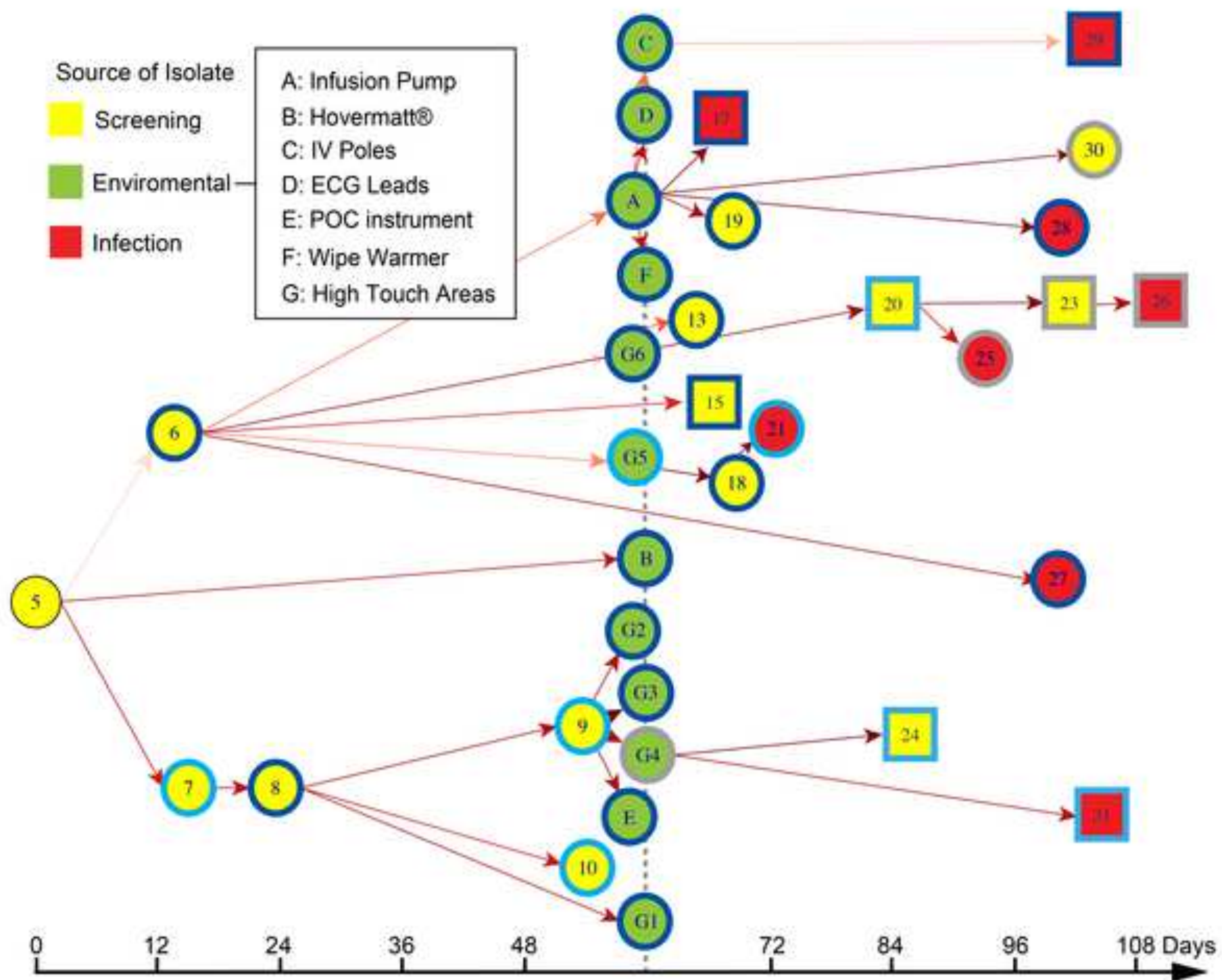

Figure 4

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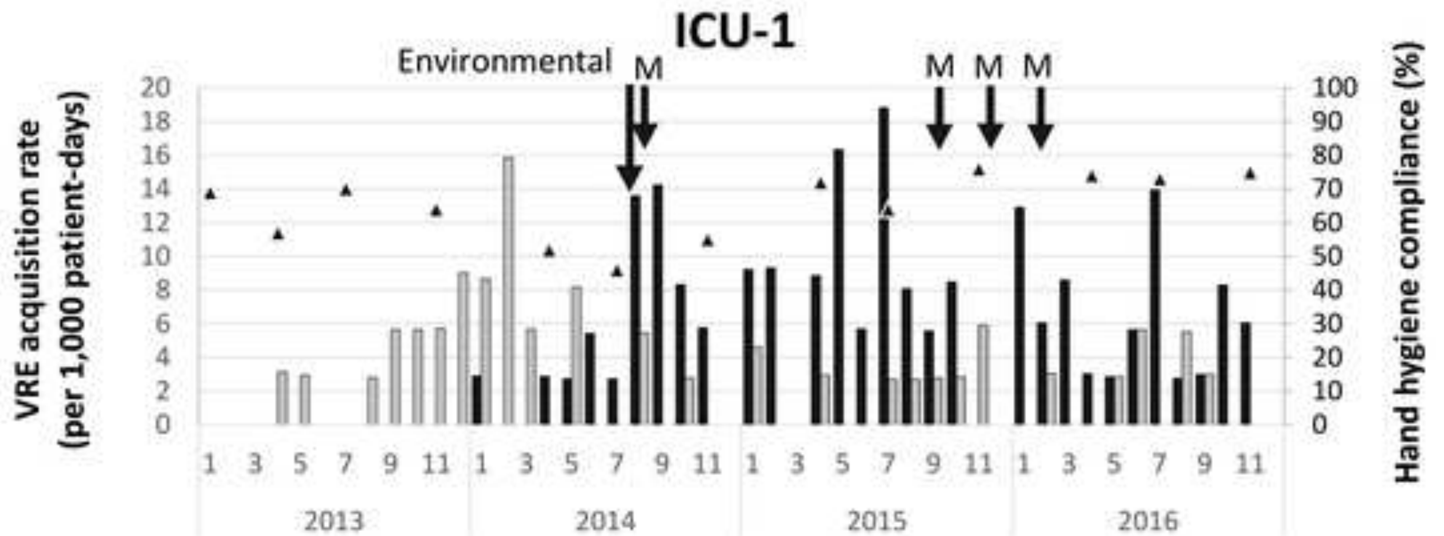

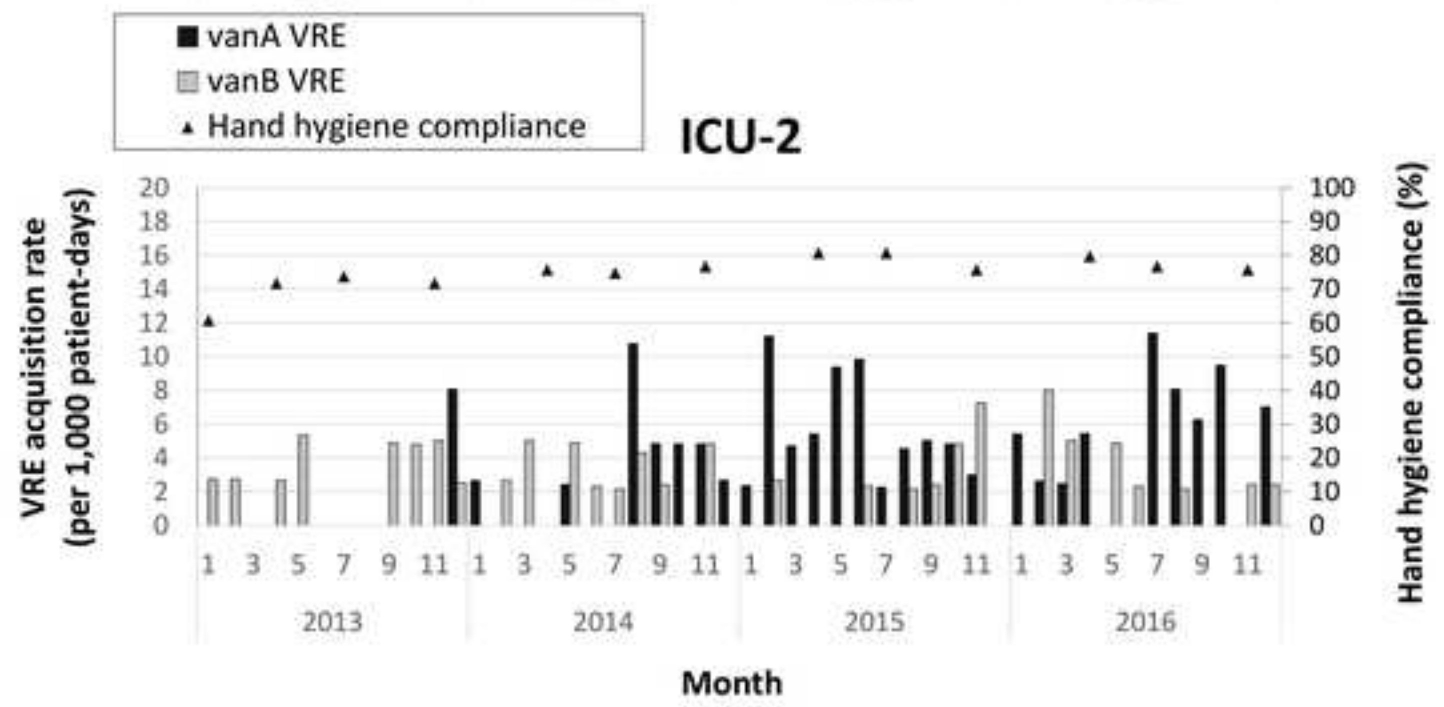

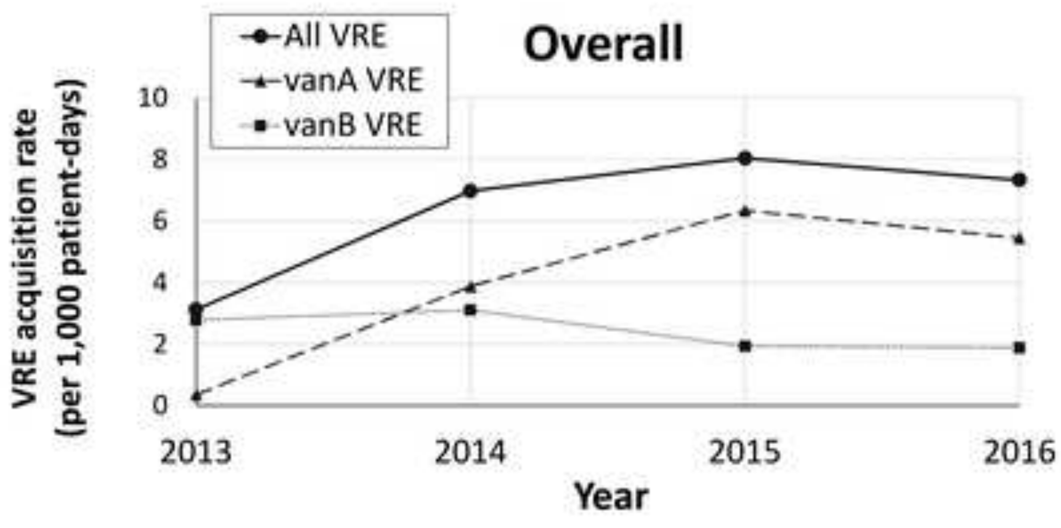

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SUPPLEMENTARY APPENDIX

Table S1. Intensive Care Unit (ICU) vancomycin-resistant enterococcus (VRE) Action Plan

Action	Purpose	Comments
Meetings with Stakeholders	To engender support and commitment from leadership and key stakeholders. To discuss actions required, formulate an action plan, identify potential barriers and monitor progress.	Including the Director of the Intensive Care Services, Nursing Unit Managers of the ICU areas, Executive representative, Environmental Services Manager, Infection Control Practitioners and Infectious Diseases staff.
Education	To increase awareness regarding VRE incidence in the Unit and promote VRE control activities.	Education sessions for ICU staff providing information regarding VRE incidence, environmental contamination and infection control audits within the ICU.
Environmental Cleaning	To reduce microbial contamination associated with the environmental reservoir contributing to ongoing VRE transmission.	Regular cleaning inspection "rounds" in the ICU. Review and revision of cleaning schedules. Dedicated cleaning team in ICU for all cleaning including terminal cleaning.

		Use of sodium hypochlorite disinfectant for cleaning. Terminal cleaning for all discharges from ICU. Special attention to high-touch surfaces and dedicated cleaning of shared equipment (including ultrasound probes and blood gas analyzer). Additional cleaning of pan-rooms. Reduction of clutter in the ICU to facilitate cleaning and reduce contamination of equipment and supplies. Better separation of clean and dirty areas.
Single patient use equipment	To reduce transmission associated with shared equipment.	Including blood pressure cuffs.
Hand hygiene promotion	To reduce VRE cross-transmission.	New posters with key clinicians from within and outside the ICU to encourage hand hygiene.

		Intensification of audits. Regular feedback of hand hygiene compliance rates in real-time and discussion in Departmental meetings.
Isolation of patients	To reduce transmission from VRE patients.	Reinforce adherence to contact precautions for patients colonized or infected with VRE.
Antimicrobial Stewardship	To reduce emergence of resistance associated with inappropriate antibiotics use.	Review of glycopeptide and broad-spectrum antibiotic use. Feedback of antibiotic use data to ICU clinicians.

Table S2. Vancomycin-resistant *Enterococcus faecium* (VRE) acquisition rates in the Intensive Care Unit

	Overall					ICU-1					ICU-2				
Year	No.	Patient-days	Rate (per 1000 patient-days)	IRR (95%CI)[a]	p value	No.	Patient-days	Rate (per 1000 patient-days)	IRR (95%CI)	p value	No.	Patient-days	Rate (per 1000 patient-days)	IRR (95%CI)	p value
All VRE															
2013	27	8642	3.12	-	-	12	3996	3.00	-	-	15	4646	3.23	-	-
2014	63	9038	6.97	2.23 (1.42-3.50)	**<0.001**	37	4109	9.01	3.00 (1.56-5.75)	**0.001**	26	4929	5.28	1.63 (0.87-3.08)	0.130
2015	71	8830	8.04	1.15 (0.82-1.62)	0.409	38	4011	9.47	1.05 (0.67-1.65)	0.826	33	4819	6.85	1.29 (0.78-2.17)	0.320
2016	70	9554	7.33	0.91 (0.66-1.27)	0.581	32	4148	7.72	0.81 (0.51-1.30)	0.392	38	5406	7.03	1.05 (0.64-1.64)	0.913
vanA VRE															
2013	3	8642	0.35	-	-	0	3996	0	-	-	3	4646	0.65	-	-
2014	35	9038	3.87	11.16 (3.43-36.27)	**<0.001**	21	4109	5.11	[b]		14	4929	2.84	4.40 (1.26-15.31)	**0.020**
2015	56[c]	8830	6.34	1.64 (1.07-2.50)	**0.022**	31[c]	4011	7.73	1.51 (0.87-2.63)	0.143	25[c]	4819	5.19	1.83 (0.95-3.51)	0.071
2016	52	9554	5.44	0.86 (0.59-1.25)	0.427	25	4148	6.03	0.78 (0.46-1.32)	0.355	27	5406	4.99	0.96 (0.56-1.66)	0.891
vanB VRE															
2013	24	8642	2.78	-	-	12	3996	3.00	-	-	12	4646	2.58	-	-
2014	28	9038	3.10	1.12 (0.65-1.92)	0.694	16	4109	3.89	1.30 (061-2.74)	0.496	12	4929	2.44	0.94 (0.42-2.10)	0.885
2015	17[c]	8830	1.93	0.62 (0.34-1.14)	0.122	8[c]	4011	1.99	0.51 (0.22-1.20)	0.122	9[c]	4819	1.87	0.77 (0.32-1.82)	0.548

2016	18	9554	1.88	0.97 (0.50-1.90)	0.949	7	4148	1.69	0.85 (0.31-2.33)	0.747	11	5406	2.04	1.09 (0.45-2.63)	0.849

NOTE. IRR, incidence rate ratio; CI, confidence interval; VRE, vancomycin-resistant enterococcus.

[a]Compared with prior calendar year.

[b]Unable to calculate - denominator zero.

[c]There were two isolates carrying both the *vanA* and *vanB* genes, one each in ICU-1 and ICU-2.

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ORION Checklist of items to include when reporting an outbreak or intervention study of a nosocomial organism

	Item No	Descriptor	Section or Page No and Comments
Title & Abstract	1	Description of paper as outbreak report or intervention study. Design of intervention study (eg Randomised Controlled Trial , Cluster Randomised Controlled Trial, Interrupted Time Series, Cohort study etc). Brief description of intervention and main outcomes.	This is not quite an outbreak report but describes changes in VRE epidemiology and interventions over a prolonged period of time. The study design has been included in the title and abstract as a molecular epidemiological study using a retrospective cohort design. (pages 1 and 3)
Introduction Background	2	Scientific and/or local clinical background and rationale. Description of organism as epidemic, endemic or epidemic becoming endemic.	Background and rationale are presented on page 4. Described as endemic (page 4, line 77)
Type of paper	3	Description of paper as Intervention study or an Outbreak Report. If an outbreak report, report the number of outbreaks.	See comment for Item 1
Dates	4	Start and finish dates of the study or report.	This has been included in the Methods section (page 5)
Objectives	5	Objectives for outbreak reports. Hypotheses for intervention studies	See last paragraph of the Introduction (page 4)
Methods Design	6	Study design. Use of EPOC classification recommended (RCT or CRCT, CBA, or ITS) Whether study was retrospective, prospective or ambidirectional. Whether decision to report or intervene was prompted by any outcome data. Whether study was formally implemented with predefined protocol and endpoints.	This was a retrospective cohort study (page 5) Enhanced infection control interventions as a result of increasing VRE are described (page 10)
Participants	7	Number of patients admitted in study or outbreak. Summaries of distributions of age and lengths of stays. If possible, proportion admitted from other wards, hospitals, nursing homes or from abroad. Where relevant, potential risk factors for acquiring the organism. Eligibility criteria for study. Case definitions for outbreak report.	See Table 1 for patient characteristics. Study eligibility are noted in the first paragraph of the Methods (page 5). Case definitions are outlines in the "Data collection" section of the Methods (page 6-7)
Setting	8	Description of the unit, ward or hospital and, if a hospital, the units included. Number of beds, the presence and staffing levels of an infection control team.	See Study setting and VRE screening and infection control precautions sections (page 5). There are no dedicated infection control personnel in the ICU.
Interventions	9	Definition of phases by major change in specific infection control practice (with start and stop dates). A summary table is strongly recommended with precise details of interventions, how and when administered in each phase.	See Figure 4 for interventions and Table S1 in Supplementary Appendix
Culturing & Typing	10	Details of culture media, use of selective antibiotics and local and /or reference typing. Where relevant, details of environmental sampling.	See "Environmental sampling" (pages 5-6), "Microbiology methods" (page 6) and "Genomic analysis" (page 7) sections
Infection-related outcomes	11	Clearly defined primary and secondary outcomes (eg incidence of infection, colonisation , bacteraemia) at regular time intervals (eg daily, weekly, monthly) rather than as totals for each phase, with at least three data points per phase and, for many two phase studies, 12 or more monthly data points per phase. Denominators (eg numbers admissions or discharges, patient bed days). If possible, prevalence of organism and incidence of colonisation on admission at same time intervals. Criteria for infection, colonisation on admission and directly attributable mortality. For short studies or outbreak reports, use of charts with duration patient stay & dates organism detected may be useful (see text)	Outcomes are described in the "Data collection" section (pages 6-7) and presented monthly (Figure 4) or yearly (Table S2 Supplementary Appendix). This was not an interrupted time series analysis with clearly defined phases. Denominators used are described in the "Statistical analysis" section (page 7).
Economic outcomes	12	If a formal economic study done, definition of outcomes to be reported, description of resources used in interventions, with costs broken down to basic units, stating important assumptions.	Not applicable
Potential Threats to internal validity	13	Which potential confounders were considered, recorded or adjusted for (eg: changes in length of stay, case mix, bed occupancy, staffing levels, hand-hygiene compliance, antibiotic use, strain type, processing of isolates, seasonality). Description of measures to avoid bias including blinding & standardisation of outcome assessment & provision of care.	This was a retrospective descriptive study with small numbers so we did not perform multivariable analysis to adjust for potential confounders. Bias was minimised by including all potential cases, using prospectively collected, standardised definitions of VRE outcomes by trained infection control

			personnel and hand hygiene data by trained healthcare workers (see “Data collection” section – pages 6-7).
Sample size	14	Details of power calculations, where appropriate	This was a descriptive retrospective observational cohort study including all patients over the study period with no statistical calculation of associations of a particular exposure(s) with outcome(s) so a power calculation was not performed.
Statistical methods	15	Description of statistical methods to compare groups or phases. Methods for any subgroup or adjusted analyses, distinguishing between planned and unplanned (exploratory) analysis. Unless outcomes are independent, statistical approaches able to account for dependencies in the outcome data should be used, adjusting, where necessary, for potential confounders. For outbreak reports statistical analysis may be inappropriate.	We focused our statistical analysis on descriptive statistics with limited comparisons as there were no distinct phases in the study ((page 7).
Results Recruitment	16	For relevant designs the dates defining periods of recruitment and follow-up. A flow diagram is recommended to describe participant flow in each stage of study.	Time period of the study is presented in the Methods section (page 5). As this was a retrospective cohort study, recruitment and follow-up data are not presented.
Outcomes & estimation	17	For the main outcomes, the estimated effect size and its precision (usually using confidence intervals). A graphical summary of the outcome data is often appropriate for dependent data (such as most time series).	Graphical summaries of data are presented in the Figures.
Ancillary analyses	18	Any subgroup analyses should be reported and it should be stated whether or not it was planned (specified in the protocol) and possible confounders adjusted for	Not applicable.
Adverse events	19	Pre-specified categories of adverse events and occurrences of these in each intervention group . This might include drug side effects, crude or disease specific mortality in antibiotic policy studies or opportunity costs in isolation studies.	Not applicable.
Discussion Interpretation	20	For intervention studies an assessment of evidence for/against hypotheses, accounting for potential threats to validity of inference including regression to mean effects and reporting bias. For outbreak reports, consider clinical significance of observations and hypotheses generated to explain them.	This was not an intervention study. Significance of an environmental reservoir on VRE transmission discussed (pages 10-13).
Generalisability	21	External validity of the findings of the intervention study i.e. to what degree can results be expected to generalise to different target populations or settings.	Addressed in conclusion (last paragraph of Discussion – page 13)
Overall evidence	22	General interpretation of results in context of current evidence.	See second paragraph of Discussion (page 11) and conclusion (last paragraph – page 13)

Abbreviations: RCT: randomised controlled trial CRCT : Cluster Randomised Controlled Trial CBA: controlled before and after study ITS: interrupted time series