

1-1-2018

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Citation

McDermott H, Skally M, O'Rourke J, Humphreys H, Fitzgerald-Hughes D. Vancomycin-Resistant Enterococci (VRE) in The Intensive Care Unit in a Nonoutbreak Setting: Identification of Potential Reservoirs and Epidemiological Associations Between Patient and Environmental VRE. *Infection Control and Hospital Epidemiology*. 2018;39(1):40-45

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Vancomycin-resistant enterococci (VRE) in the intensive care unit in a non-outbreak setting: Identification of potential reservoirs and epidemiological associations between patient and environmental VRE.

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Running Title: VRE in ICU patients and the environment

Word Count =2495

Abstract

Objective Among nosocomial bloodstream infections caused by enterococcal species, Ireland has the highest proportion caused by vancomycin-resistant enterococci (VRE) in Europe at 45.8%. The contribution of the near-patient environment to VRE transmission outside of outbreaks was investigated. **Design and Methods** A prospective observational study was conducted during seven sampling periods. VRE recovery from the near-patient environment and patients in the ICU was investigated, to identify; reservoirs, clinical and molecular epidemiological associations and the success of active surveillance cultures (ASC). **Results** Of 289 sampling occasions involving 157 patients and their bed-space, VRE was recovered from the patients bed-space, their clinical sample, or both on 114/289 (39.4%) of sampling occasions. The patient and their bed-space was positive for VRE on 34/114 (29.8 %) of VRE-associated sampling occasions. Of 1647 environment samples, 107 sites (6.5%) were VRE-positive, with significantly greater VRE recovery from isolation rooms than open plan area (9.1% Vs 4.1 %, $p < 0.0001$). The most frequently VRE-contaminated sites were the drip stand, bed control panel, and chart holders, together accounting for 61% of contaminated sites. The use of ASC resulted in a 172% increase in identification of VRE-colonized patients. Molecular typing revealed two environmental clusters, one cluster involving three patients and generally, greater heterogeneity of patient isolates compared to environmental isolates **Conclusion** Even outside of outbreaks, near-patient ICU environmental contamination with VRE is common. Better infection control policies that limit environment transmission of VRE in the ICU and that are supported by molecular epidemiological studies, in real time, are necessary.

Introduction

Vancomycin-resistant enterococci (VRE) are important causes of healthcare-associated infection (HCAI) and are associated with increased mortality, lengthened hospital stays and significant economic burden.^{1,2} In 2013, cases of VRE in hospitalized patients in the USA numbered 20,000 and were associated with 1300 deaths. Of these, 77% were *Enterococcus faecium* (VRE_{fm}) and the remainder *Enterococcus faecalis* (VRE_{fl}).³ In Europe, invasive *E. faecium* isolates reported to the European antimicrobial resistance surveillance network EARS-net in 2015 numbered 9123, with glycopeptide resistance ranging from 0 to 45.8%.⁴ Of 29 reporting countries, Ireland had the highest rate of VRE at 45.8%.

VRE colonization and environmental contamination are associated with transmission to other patients.^{5,6} and infection prevention and control (IPC) measures, including active surveillance cultures (ASC), isolation of VRE-positive patients and contact precautions are recognized as important.¹ In Ireland, ASC for VRE is recommended for intensive care unit (ICU) admissions. The reasons for Irelands high VRE rate are unknown and may be better informed by epidemiological investigations of VRE_{fm} in an Irish setting, which here has been limited to date⁷. The aims of this study were; to identify potential reservoirs of VRE_{fm} in an ICU, to investigate the clinical and molecular epidemiology of VRE_{fm} outside of outbreaks in the ICU and to assess the role of VRE ASC in this setting.

Materials and Methods

Setting

Beaumont Hospital, Dublin, is an 820 bed tertiary referral teaching hospital. It is the national referral centre for neurosurgery, cochlear implantation, neurology, and renal transplantation and is a regional referral centre for many specialties for North-East Ireland. The study took

place in the 12-bedded general ICU which has six beds in an open plan area, four single rooms and two air-controlled isolation rooms (rooms 11 and 12, Figure 1). The two isolation rooms are occupied less frequently (e.g. due to requirements for additional nursing staff) unless clinically required for logistical reasons. As per national guidelines, patients are screened for VRE on ICU admission and weekly thereafter and are isolated with contact precautions if positive. Previously known VRE positive patients are isolated on admission. Cleaning of the ICU environment is performed by a dedicated member of the cleaning staff who is rostered from 07.00 to 19.00h and an on-call service is available outside these times. Bed spaces are cleaned one at a time using 1000 ppm sodium dichloroisocyanurate (Precept, Advanced Sterilization products, Ontario, Canada). Ethical approval was obtained from the Beaumont Hospital Ethics Committee.

Environmental sampling

Environmental sampling took place during seven sampling periods within a 32 month time-frame (October 2012-June 2014). During each sampling period, the environment of occupied ICU bed-spaces was sampled twice-weekly between 10.00-12.30h for three consecutive weeks. A patient bed-space was defined as the near-patient environment in isolation rooms or open-plan area from which six 'high touch' sites were sampled. Each area was swabbed using Copan eSwabs™ (Copan Diagnostics, Italy). A 5cm² area (approximately) was swabbed on flat surfaces. A sampling occasion refers to the sampling of multiple surfaces of an occupied patient bed-space on a single day. As only occupied bed-spaces were sampled, the number of sampling occasions in each time-period varied with ICU occupancy levels.

Identification of VRE from patient swabs.

Rectal swabs, taken from patients on admission to the general ICU as part of an ASC programme, were processed for identification of VRE by the Clinical Microbiology

Laboratory, Beaumont Hospital. Swabs taken were plated onto selective ChromeID VRE and incubated for 24h at 37°C. Organism identity was confirmed by matrix-assisted laser desorption/ionization- time-of-flight mass spectrometry (MALDI-TOF) using a MALDI Biotyper (Bruker). The minimum inhibitory concentration (MIC) for vancomycin was determined using E-test strips (Biomérieux). *E. faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 were negative and positive control strains.

Recovery of VRE from environmental samples

Swabs were transferred to brain heart infusion (BHI) broth (2ml) for enrichment and incubated overnight (16-18h) at 37°C in a shaking incubator (Gallenkamp, Leicester, UK) at 150-200 rpm. A 10 µl loop of enriched suspension was streaked onto UTI Brilliance agar (Oxoid, UK) and turquoise colonies (presumptive enterococci) were sub-cultured onto VRE ChromeID and confirmed using MALDI-TOF MS. Vancomycin MICs were determined using E-tests. Patient clinical details were collected at the time of sampling.

Pulsed field gel electrophoresis (PFGE) for VRE

SmaI-digested genomic DNA from patient and environmental VRE_{fm} and *E. faecalis* ATCC 29212 were subjected to PFGE based as described by Turabelidze *et al*⁸ but with modifications as outlined in supplemental file S1.

Statistical analyses

Fisher's exact test was used to analyze categorical variables using GraphPad QuickCalcs on-line software. The significance of differences between the groups was expressed as two-tailed *p*-values, *p* values of ≤ 0.05 were considered significant.

Results

Characteristics of VRE patients in the ICU

Of 157 patients sampled, 30 (19 %) were VRE colonised. Clinically relevant patient details for VRE-colonized patients are summarised in Table 1. Eighteen patients (60%) were admitted from another ward in the hospital. Not all VRE positive patients had contemporaneous viable isolates of VRE. Of the VRE positive patients included, two were treated for invasive VRE infection, one for a catheter-related bloodstream infection (BSI) and the other for VRE surgical-site infection. They had both initially been treated with vancomycin.

Potential reservoirs of VRE in the ICU

Of 1647 swabs taken from the environment of 157 patients in the ICU, 107 sites (6.5%) were positive for VRE. These were recovered from the six bedded open-plan area (35), four single rooms (67) and two negative pressure isolation rooms (5). Significantly more VRE were recovered from the environment of single rooms where the majority of VRE positive patients were located (beds 7-10), compared to the open plan area (beds 1-6), $p < 0.0001$, (Figure 1). Based on the number of environmental swabs taken, rates of contamination with VRE were 4.1% and 9.1% in open-plan and isolation rooms, respectively. During the study, beds 11 and 12 were used infrequently to isolate VRE-patients (two patients). Sixty nine of 157 patients (44 %) occupied beds 7-10 at least once over the study duration and 24/69 (34.8%) were colonised with VRE. In total 26/30 (86 %) of VRE-positive patients were isolated at the time of sampling.

The specific high touch ICU sites most frequently contaminated with VRE were the drip stand, bed control panel, and chart holders, together accounting for 61% of contaminated sites (Figure 2). The difference in the proportional recovery of VRE from any one surface was not statistically significant.

The positive impact of ASC on VRE recovery from ICU patients

The use of ASC resulted in the identification of an additional 19/157 (11.6 %) VRE-colonized patients compared to 11/157 (7%) identified from non-screening or clinical samples (e.g. urine samples) in the absence of ASC. This represents a 172 % increase in VRE detection rates with ASC. Of the 19 new cases, 14 were VRE positive on ICU admission, (within 48h) and five acquired VRE in the ICU. These patients were isolated once VRE was recovered from screening swabs, as per local IPC guidelines.

Clinical epidemiological associations between patient and environmental VRE

Over the seven sampling periods, there was a total of 289 ICU sampling occasions (defined as the sampling of multiple touch sites in a single bed-space on a single day) involving 157 ICU patients. On 114/289 (39.4 %) sampling occasions, VRE was recovered from the patient bed-space, the patient clinical sample, or both. For 34/114 (29.8 %), both the patient and their bed-space was positive at the time of sampling. For the remainder of sampling occasions where VRE was recovered, either the patient (44/114, 38.6%) or their environment (36/114, 31.6%) was positive for VRE.

To investigate potential transmission events related to the movement of patients within the ICU, 189 unique patient and bed number associations involving 157 patients were identified (i.e. some patient's occupied more than one ICU bed over the study period).

Tracking the pattern of recovery of VRE with respect to time and bed-space revealed six

possible VRE transmission events. Four of these were from patient to environment (recovery of VRE from an environmental site (bed-spaces 7, 8 and 9) which was previously negative but became positive within two to four days of a VRE-positive patient occupying the bed-space). A further two possible transmissions of VRE from environment to patient were identified. In one case the patient became VRE-positive nine days after admission and placement in an environment which sampled positive for VRE. The other involved a patient acquiring VRE having spent 48 h in a room where the environment was VRE-positive.

Molecular epidemiology of VRE_{fm} from the ICU

In total, 137 VRE isolates were recovered during this study comprising *E. faecium*, *E. faecalis*, *E. gallinarum* and an isolate of *Paenibacillus* spp. Of these, 71 VRE_{fm} isolates were typed using PFGE which included 49 environmental and 22 patient isolates from 17 patients (some patients had more than one isolate). Analysis revealed distinct 32 PFGE types and three PFGE clusters. Clusters (A, B and C) and their association with bed-spaces and patients are summarised in Figure 3. Clusters A (eight isolates) and B (nine isolates) were exclusively environmental isolates from multiple bed-spaces during separate sampling periods (Periods 1 and 5). Cluster A isolates were recovered within a two-day period (sampling period 1) from five bed-spaces including isolation rooms and open-plan area (beds 2, 6, 7, 8 and 9). Cluster B included nine isolates, eight recovered on a single day (sampling period 5) from three bed-spaces (beds 4, 8, 9). The ninth isolate was recovered two days later from bed 8. Cluster C contained five isolates, four patient isolates from three patients and the fifth an environmental isolate. The three patients had occupied two separate bed-spaces at different times. The environmental isolate was unrelated in space and time to the patient isolates. This patient was re-admitted to ICU having been previously VRE-colonized and

developed BSI twenty days later. The two other patient isolates in this cluster were from patients who were in ICU at the same time as the first patient (sampling period 2) and one of these developed a VRE BSI one month later (this patient rectal swab contained vancomycin-susceptible *E. faecium*). A further two patients, in bed-spaces 9 and 11, in different sampling periods had genetically indistinguishable VRE. A detailed dendrogram of VRE_{fm} isolates is provided in supplemental file S2.

Discussion

The microbiome of the ICU is variable and factors including the patient cohort, changes in staff, cleaning regimens, IPC policies and compliance, impact on microbial population dynamics. Here, the overall contamination of the ICU environment with VRE was 6.5 % of environmental sites sampled. A similar rate of 6.0 % contamination based on six high-touch surfaces in 37 ICU rooms was previously reported.¹⁰ Notably, we did not limit our study to the bed-spaces of patients with VRE, an approach taken by others, which may yield greater numbers over longer sampling periods. For example, 21% of environmental VRE contamination was reported in the rooms of VRE-colonised patients at baseline in a US intervention study in a similar setting with similar sampling methodologies.¹¹ However, one intervention study, comparing multiple surfaces from rooms housing VRE-colonised and non-colonised patients, reported 23.6 % and 5% contamination, respectively,¹² higher than the rates found here of 9 % and 4.1%.

Our study confirms previous reports that surfaces close to patients and frequently touched by staff, harbour the majority of VRE.¹³ The majority of VRE positive samples were recovered from isolation rooms 7-10, where VRE colonised patients were accommodated once identified as VRE-positive, and this is supported by the literature. Prior room contamination increases the risk of patient acquisition of VRE.¹⁴ Isolation rooms 7-10 were

significantly more contaminated than the open plan area. Standard size recommendations exist for ICU isolation rooms.¹⁵ The cramped conditions in smaller sized rooms (such as beds 7-10 here), may hamper proper cleaning of the environment, which may contribute to VRE persistence.

Patients in our ICU are screened on admission and weekly thereafter for VRE and a colonization rate of 30/157 (19.1 %) was found. A recent meta-analysis reported an average VRE colonization rate of 8.8 % across 37 studies including 62959 patients at risk. Furthermore VRE colonization on ICU admission was higher across US studies (12.3%), compared to studies from Europe (2.7%) and elsewhere.¹⁶ While Ireland has the highest VRE BSI rate in Europe, data on ICU colonization rates are not widely available. In this study, the positive effects of ASC on VRE detection were evident. A new finding of VRE colonization was confirmed in 19/30 patients, who were subsequently isolated within the unit representing a 172% increase in detection rate with this approach. A recent Canadian longitudinal multicentre study indicated no significant impact on clinical outcomes following removal of all VRE controls, including screening.¹⁷ While other challenges such as MRSA have received significant attention, the significant complications of VRE colonization and infection in vulnerable ICU patients in addition to the high rate of VRE invasive infection reported in Ireland, warrants the implementation of effective IPC strategies.

Molecular typing by PFGE revealed genetic diversity among patient isolates, whereas the environmental isolates showed more clonal relationships. The acquisition of resistant determinants by susceptible enterococci in the gut, under antibiotic pressure¹⁹ may contribute to the heterogeneity observed here among patient isolates. Acquisition of *vanB* associated with the transposon Tn1549 by susceptible enterococci from anaerobes of the gut flora has been shown previously.^{20,21} The more clonal pattern of environmental isolates found here suggests that the environment may select for certain clones.

Our study identified potential transmission of VRE within the unit based on the movement of patients between bed-spaces. Furthermore, molecular epidemiological analysis, identified two patients who were in the unit at the same time, in single rooms at either end of the unit, with closely related VRE suggesting transmission facilitated by poor hand hygiene and/or environmental hygiene. The heterogenic nature of colonizing isolates reported in this and other studies suggests horizontal rather than clonal transmission. While this pattern of transmission highlights the importance of implementing effective IPC measures, in a non-outbreak setting, evaluation of which methods (eg patient isolation, environmental cleaning) are most effective in reducing transmission to patients require well-designed intervention studies supported by detail epidemiological investigations.

There were limitations to this study. Because VRE transmission dynamics were monitored discontinuously for logistical and cost reasons some transmission events were not captured. Hand carriage of VRE by healthcare workers was not investigated and may contribute to VRE transmission. Genetic relatedness of a subset of study isolates (66%) was conducted by PFGE. While this was discriminatory for the purpose of identifying clusters or confirming heterogeneity, there is no standard protocol for VRE PFGE or for data interpretation. More discriminatory but expensive approaches (e.g. multi-locus sequence typing, whole genome sequencing) might have provided more robust characterization of isolates. However, we demonstrated that the application of molecular typing (PFGE) could potentially, in real-time, provide indications of VRE transmission that could assist improved IPC measures in a setting where the physical infrastructure, i.e. limited space and too few isolation rooms, is inadequate.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. HH has received research support from Pfizer and Astellas in recent years. No conflict of interest is reported by the other co-authors. The authors thank Ms Mary O'Connor and Ms Margaret Fitzpatrick Department of Microbiology, Beaumont Hospital and ICU Beaumont staff and patients for facilitating this study.

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Table 1. Demographics and clinical details of patients colonized with the VRE in the ICU

Patient characteristics	VRE positive n=30
Colonized with VRE fm , n (%)	22 (73)
Colonized with VRE fl , n (%)	8 (27)
Male gender, n (%)	16 (53)
Mean age in years (range)	64 (34-85)
Mean length of stay in days (range)	11 (1-42)
Mean APACHE II score (range)	24 (12-38)
Exposure to antibiotics in ICU ^a , n (%)	30 (100)
Vancomycin exposure	6 (20)
Specialty, n (%)	
Medicine	15 (50)
Surgery	15 (50)
Co-morbidities n (%)	
Diabetes mellitus	1 (3)
Malignancy	11 (37)
Chemotherapy	6 (20)
Steroids	4 (13)
Immunosuppression	7 (23)
Abdominal surgery	12 (40)
Devices, n (%)	
Central venous catheter	25 (83)
Urinary catheter	25 (83)

Mechanical ventilation	18 (60)
Surgical drain	7 (23)

VRE Vancomycin resistant enterococci, APACHEII Acute Physiological and Chronic Health Score, ICU intensive care unit. ^a antibiotics received included piperacillin/tazobactam, co-amoxiclav, vancomycin, clarithromy

Figure Legends

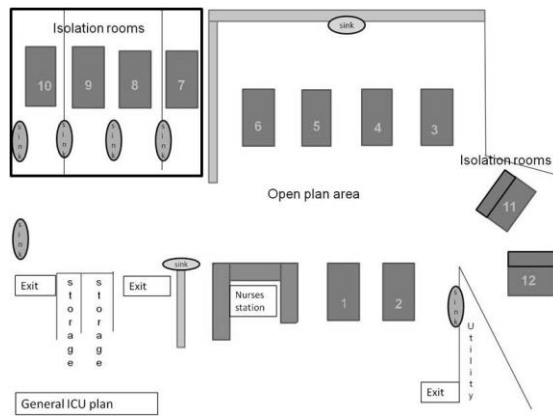
Figure 1. Detection of VRE in the ICU. The ICU layout indicating beds in the six-bed open plan area and isolation rooms (a) and the total number of sites and patients positive for VRE in isolation rooms 7-10 compared to the open plan area (beds 1-6) during seven sampling periods (b). The negative pressure isolation rooms (11 and 12) were excluded from the comparison due to their low frequency use (2 patients over study periods). *** indicates statistical significance, $p < 0.0001$.

Figure 2. Distribution of VRE among high touch surfaces adjacent to patients in the ICU Data expressed as % of each type of site sampled from which VRE was recovered. Total surfaces sampled = 1647, total surfaces VRE positive = 107. Chart holders were replaced with keyboards during the study. Control buttons were sampled on patient monitors.

Figure 3. Clusters identified by PFGE analysis and patient bed-space associations. From 71 *VRE_{fm}* isolates investigated, three clusters (A, B and C) were identified. Cluster A and B (light grey and dark grey bars) were exclusively environmental isolates recovered in sampling periods 1 and 5). Cluster C (patterned bars) contained four patient isolates (P) and one environmental isolate (E).

Figure 1

a



b

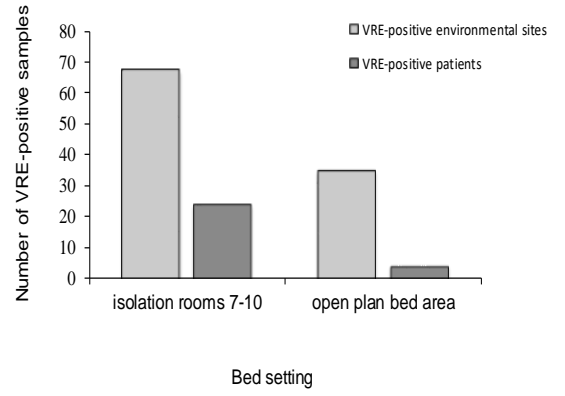


Figure 2

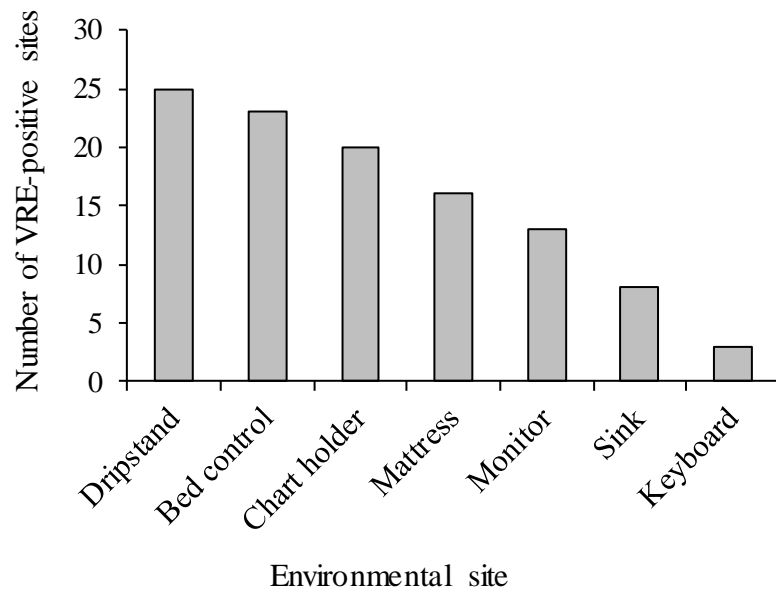
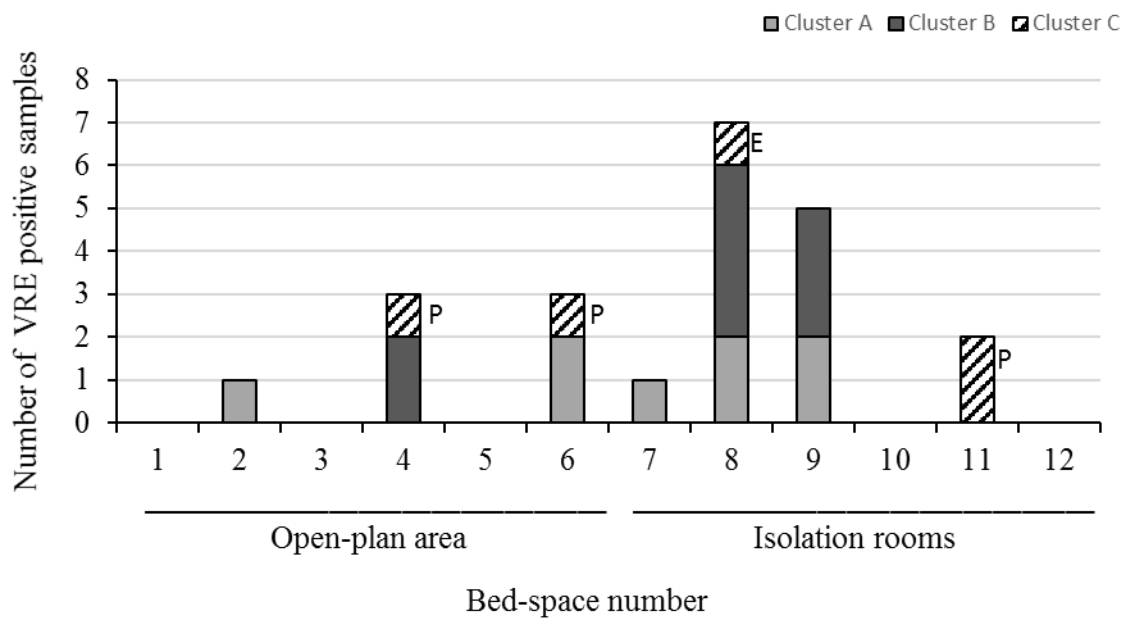
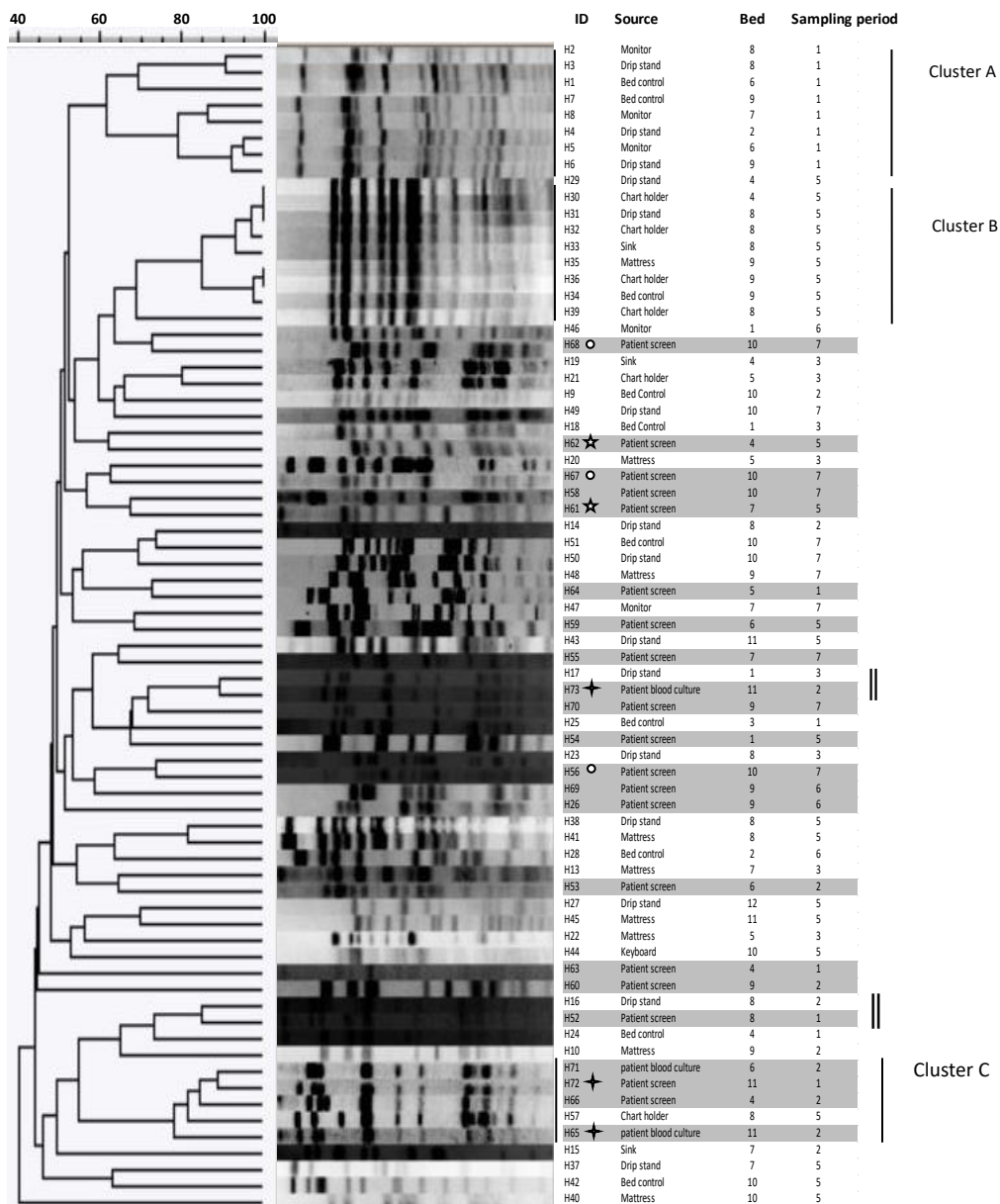


Figure 3



Supplemental Figure S1



S2. Dendrogram of patient and environmental *VREfm* isolates. PFGE analysis of 71 *VREfm* isolates (49 environmental and 22 patient (grey background)) from the ICU in Beaumont Hospital, Dublin. Analysis was performed using GelCompare®II software (version 6.5, Applied Maths). The extent of variability was determined by the Dice coefficient using a 1% tolerance. Three clusters (A, B and C) were identified. || indicates

closely related patient and environmental isolates. Symbols * ◦ † indicate multiple isolates from the same patient.