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Cover Page Footnote

I would like to thank all the staff in the Clinical Microbiology department of the MUH for their assistance during this study. In particular, I would like to thank Siobhan Fitzgibbon (Senior Medical Scientist), Dr. Deirdre O'Brien (Consultant Microbiologist), Sinead Fahy (Medical Scientist), and Joanne Crowley (Infection Control Specialist) for their support throughout. I would also like to thank Shane Whelan and Caoimhe Lynch for their guidance, and the staff in the NMRSARL for providing confirmatory testing of the linezolid-resistant isolate. Finally, I wish to acknowledge Dr. Brigid Lucey for encouraging me to publish this work.

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An Investigation into the Prevalence of Vancomycin-resistant Enterococci within an Irish Hospital

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ABSTRACT

Vancomycin-resistant enterococci (VRE) are well-recognised nosocomial pathogens that pose a significant threat to public health. Associated with poorer clinical outcomes than their vancomycin-sensitive counterparts, the prevalence of VRE in Ireland has increased in recent times, with the European Antimicrobial Resistance Surveillance Network reporting that out of 29 countries, Ireland demonstrated the highest rates of vancomycin resistance among invasive *Enterococcus faecium* isolates between 2011 and 2014 (2011; 34.9%, 2012; 44.0%, 2013; 42.7%, 2014; 45.1%). Herein, we investigate VRE prevalence in the Mercy University Hospital (MUH) – an acute care hospital in southern Ireland. A total of 21 first-time VRE infections were identified over a 40 day period between January and March 2021, representing a prevalence of 24.7%. In addition, one vancomycin-sensitive linezolid-resistant strain was isolated from an Intensive Care patient, and environmental analysis revealed the presence of an extensive VRE reservoir, with isolates identified on a keyboard, bedside desk, and electricity bar of a primary observation unit. We also highlight the actions taken – including staff training and surface decontamination – to curb transmission of the pathogen and prevent a full-scale VRE outbreak developing within the hospital.

KEYWORDS: enterococci, vancomycin-resistance, VRE, nosocomial infection, infection control.

INTRODUCTION

The bacterial genus *Enterococcus*, of the phylum Firmicutes, are a ubiquitous cohort of Gram-positive bacteria that present a formidable challenge to public health (García-Solache & Rice, 2019). While they normally reside in the gastrointestinal tract of humans and other mammals, enterococci have been isolated from many extraenteric habitats, including beach sand, terrestrial vegetation, and bodies of water (Byappanahalli *et al.*, 2012). Indeed, Neely & Maley, (2000) have documented the survival of *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus gallinarum*, and *Enterococcus casseliflavus* on both polyester and polyethylene surfaces, such as may be used in hospital privacy curtains and splash aprons, respectively, for longer than 90 days. Isolates of *E. faecium* have also been shown to remain viable on a 60% cotton-40% polyester blend, as would be encountered on lab coats and scrub suits, for this same time period (Neely & Maley, 2000).

Enterococcal persistence in these environments is of particular concern when the ability of the organism to cause disease is considered; although most species are commensal bacteria, some are implicated in urinary tract infections, wound infections, and infective endocarditis, among others (Byappanahalli et al., 2012; Iaria et al., 2005; Rajkumari et al., 2014; Selleck et al., 2019). Some species, most often *E. faecalis* and *E. faecium*, are capable of causing severe bacteraemia – a condition characterised by high mortality rates (up to 40%) in critically-ill patients (Alqarni *et al.*, 2018; Leibovici, 1995; Rosselli Del

Turco *et al*., 2020). Given that the pathogen is intrinsically resistant to many first-line antibiotics (e.g. β-lactams and aminoglycosides), treatment of enterococcal illness can be complex, often requiring the use of sophisticated, novel antimicrobials (Kristich *et al*., 2014). Vancomycin, a glycopeptide antibiotic that was discovered in the 1950s, initially provided an alternative means of treating multidrug-resistant strains. This success was short-lived however, and vancomycin-resistant enterococci (VRE) were isolated from several patients with end-stage renal failure in 1988 – heralding the birth of a new era in the war against the pathogen (Griffith, 1984; Uttley *et al*., 1988).

Since then, VRE have become a well-established source of nosocomial infection, posing a significant threat to patients in high-dependency settings such as transplantation units, oncology wards, and intensive-care units (ICUs) (Austin *et al*., 1999; Uttley *et al*., 1988). Within hospitals, VRE carriage is strongly correlated with an increased average length of stay (LOS) and a notably higher cost of hospitalisation (Jung *et al*., 2014). For these reasons, prevention of nosocomial VRE infection is of paramount importance and due consideration must be given to implement measures that are known to curb transmission of the pathogen. Nonetheless, VRE outbreaks remain a problem in healthcare-settings, with Weterings *et al*., (2021) recently describing a hospital-wide vancomycin-resistant *E. faecium* (VRE*fm*) outbreak in The Netherlands that took over 2 years to control. In addition, a 2014 report by the European Antimicrobial Resistance Surveillance Network (EARS-Net) showed that of the 29 EU countries from which data was provided, only 4 countries – Iceland, Estonia, Malta, and Finland – reported a complete absence of vancomycin resistance among invasive *E. faecium* isolates. Perhaps of more concern, however, was that Ireland, at 45.1%, recorded the highest percentage of invasive VRE*fm* isolates out of all 29 countries. Furthermore, a longitudinal analysis by Mendes *et al*. (2016) showed that between 2001 and 2014, Ireland reported the 3rd highest percentage (5%) of *E. faecium* isolates among blood culture specimens, with only Turkey (5.6%) and Germany (6.1%) reporting a higher mean figure for the 14 year period.

While much research regarding VRE prevalence has been conducted elsewhere in Europe, hospital surveillance of the pathogen is not as prominent in Ireland particularly outside of Dublin. Recent studies by Ryan *et al.* (2015) and Mcdermott *et al* (2018) have investigated VRE trends in Irish hospitals, but these are limited to specific sample types (blood cultures) and wards (ICUs), respectively. Thus, the current study seeks to provide a comprehensive analysis that is not confined to the aforementioned factors, thereby generating an accurate picture – or snapshot – of VRE prevalence over a defined time period. In addition to analysing clinical samples, environmental sampling was conducted in order to elucidate the modes of transmission responsible for VRE infection and provide a means for limiting VRE persistence and spread.

MATERIALS AND METHODS

Clinical analysis

Sample cohort

Between the 28th of January 2021 and the 9th of March 2021 a VRE prevalence study was conducted in the Mercy University Hospital (MUH) (a 330-bed acute general hospital located in the inner city of Cork, Ireland). The sample cohort included those from patients in which a VRE screen was specifically requested (i.e. rectal swabs), in addition to microbiological investigations whereby the identification of an enterococcus warranted a VRE investigation to be subsequently conducted. A total of 98 clinical samples, comprising 18 different sample types, were submitted for analysis (abscess swabs, blood cultures, bone fragments, drain fluids, ear swabs, epigastric fluids, faecal samples, finger swabs, groin swabs, leg swabs, lesion swabs, penile swabs, rectal swabs, stoma swabs, ulcer swabs, urine samples, vaginal swabs, and wound swabs). While most samples (n=78) were obtained from patients within the MUH, some samples were from patients in other Cork-based medical practices, namely South Infirmary Victoria University Hospital (SIVUH) (n=18), Hollyhill Medical Centre (n=1), and Shandon Medical

Centre (n=1). Duplicate positives (i.e. more than one positive result from the same patient) were excluded from prevalence data, but were included in data relating to sample type (as some VRE carriers submitted more than one positive sample type during the study, this data was included). Similarly, where duplicate positives were obtained, antimicrobial susceptibility testing (AST) was only performed on the initial positive sample in line with the hospital policy regarding the frequency of testing of VRE isolates. Given that a large proportion of samples arose from repeated VRE screening of patients, duplicate negatives were included in prevalence data. Patient names and identifiers were omitted in accordance with the ethical approval granted for this study.

VRE screening samples (rectal swabs)

Rectal swabs were directly plated onto chromogenic selective agar (chromID™ VRE Agar, bioMérieux SA, Marcy-l'Étoile, France) and incubated aerobically at 36°C for 48 hours. Plates were analysed twice during this time period (at 24-hour intervals), and presumptive positive VRE were confirmed by identification testing (GP card) and AST analysis (AST-P662 card) performed on the VITEK 2 Compact analyser (bioMérieux SA, Marcy-l'Étoile, France). Plates with no visible growth after 48 hrs. incubation were reported as negative and subsequently discarded. A purity check plate analysis, performed on Columbia Blood Agar (Columbia agar + 5% sheep blood, bioMérieux SA, Marcy-l'Étoile, France), was conducted for all VITEK samples. Purity check plates were incubated for 24 hours in the presence of 5% CO_2 and examined for pure growth to ensure the absence of contaminating organisms that could interfere with VITEK analysis.

Non-VRE screening samples

For non-VRE screening samples (i.e. those in which during the course of another microbiological investigation an enterococcus was isolated), the protocol followed was similar to rectal swabs in that suspect enterococci were cultured onto chromogenic agar and incubated under the same conditions. Presumptive positive VRE colonies were then confirmed *via* identification and AST analysis on the VITEK 2 Compact analyser. In addition, one vancomycin-sensitive isolate, obtained from an ICU patient, also underwent VITEK analysis. For blood culture specimens specifically, initial VRE identification was not performed on chromogenic agar, but rather on the BioFire FilmArray™ analyser (BioFire Diagnostics, LLC, Utah, USA) (as per the hospital policy regarding the investigation of positive blood culture specimens). VITEK confirmatory testing (identification and AST analysis) was conducted on these specimens thereafter.

Confirmation of results

For all sample types, a positive case of VRE was defined as the presence of characteristic VRE growth on chromogenic agar (or a positive FilmArray™ result in the case of blood cultures), in addition to a positive confirmatory result on the VITEK 2 Compact analyser. The genetic mechanism of vancomycin-resistance was determined for VRE isolates by using the VITEK 2 Compact's inbuilt susceptibility-based prediction of *van* genotypes, as described by Abele-Horn *et al*., (2006). For linezolid-resistant isolates, confirmatory PCR testing was conducted in the National MRSA Reference Laboratory (NMRSARL, St. James's Hospital, Dublin, Ireland).

Environmental analysis

Environmental sampling was conducted on the 4$^{th\ of}$ March 2021 in a 5-bedded observation room within the male surgical ward from which an atypically high number of clinical VRE isolates originated (Table 1). Based upon the layout of the room, which was compact with uneven surfaces, swabbing was deemed the most appropriate sampling method (in line with the recommendations of Rawlinson *et al*., (2019)). A total of 30 swabs from three distinct areas of the room were sampled namely the front desk area (n=12), the patient bedding area (n=11), and the toilet area (n=7). All samples were collected using the Copan Liquid Amies Elution Swab (eSwab®) Collection and Transport System (Copan Diagnostics Inc., Murrieta, CA, USA). To standardise the swabbing process, a uniform technique, as described by

Margas *et al*., (2013), was employed on all surfaces. All samples were refrigerated at 3°C for 24 hours, and after this period were streaked onto chromID™ VRE Agar and incubated at 36°C for 48 hours. As for clinical samples, plates were checked for evidence of growth twice during this time period (at 24-hour intervals), and those with no visible growth after 48 hours were reported as negative and subsequently discarded. For plates with mixed (i.e. presumptive VRE colonies in addition to at least one unrelated colony) or sparse growth (i.e. ≤ 3 presumptive VRE colonies) after primary culture, isolated presumptive VRE colonies were subcultured onto fresh VRE plates to eliminate contaminating organisms and enhance VRE retrieval. Presumptive VRE colonies were then confirmed via identification (GP card) and AST analysis (AST-P662 card) on the VITEK 2 Compact analyser. As for clinical samples, a purity check plate analysis was conducted for all environmental samples that underwent VITEK analysis. The gold-standard colony-forming unit count (CFU count) method was used to estimate microbial abundance in primary cultures, with the degree of growth denoted by -, +, ++, +++, and ++++ (where '-' = no growth; '+' = 1-100 CFU; '++' = 101-200 CFU; '+++' = 201-300 CFU; '++++' = too numerous to count (TNTC) (>300 CFU)) (Hazan *et al*., 2012).

Infection prevention and control

The infection control measures that were implemented in response to this studies' findings were based on the guidelines set out by the Hospital Infection Controls Practice Advisory Committee (HICPAC) as regards preventing the spread of vancomycin-resistance in the hospital setting (CDC, 1995).

Statistical analysis

Prevalence was calculated by using the following formula:

$$\text{prevalence (\%)} = \frac{no.\ of\ first\text{-}time\ infections}{no.\ of\ samples\ analysed\ (e.d.p.)}\ x\ \frac{100}{1}$$, where e.d.p. = excluding duplicate positives.

Confidence intervals (CI) were calculated by using the binomial proportion confidence interval formula:

$\hat{p} \pm z * \sqrt{\hat{p}\ (1-\hat{p})/_{n}}$, where $\hat{p}$ is the sample proportion, z is the standard normal value for the CI (1.96 for a 95% CI), and n is the sample size.

Ethical approval

Ethical approval for this study was granted by the Clinical Research Ethics Committee, Cork (Review reference number ECM 4 (q)).

RESULTS AND DISCUSSION

Clinical analysis

During the 40 day period between the 28th of January 2021 and the 9th of March 2021, a total of 98 clinical samples were investigated, with 34 positive for VRE (n=21 first-time infections and n=13 duplicate positives) (Table 1). Excluding duplicates (i.e. non-first-time infections), the prevalence of VRE in this study was 24.7% (21 out of 85 specimens) (95% confidence interval (CI): 15.5%-33.9%). The remaining 64 samples were either negative for bacterial growth or contained unrelated organisms or vancomycin-sensitive enterococci. These data reveal a pronounced VRE reservoir within the patient population and our findings are in line with those of Whelton *et al*., (2016) who conducted a similar study in Cork University Hospital (CUH) and observed a VRE prevalence of 19.1% (67 out of 350 specimens) (95% CI: 14.1%-24.1%). Furthermore, of the 18 South Infirmary Victoria University Hospital (SIVUH) samples analysed in our study, 3 were found to be VRE positive, with 2 of these

identified as first-time infections. Thus, in conjunction with the findings of Whelton *et al*. (2016), our findings indicate that VRE carriage likely extends to a number of hospitals within the Cork region.

There was considerable variation in patient location at the time of VRE isolation, with isolates obtained from patients in 7 different wards within the hospital, namely: the female medical ward, the general inpatient ward, the geriatric ward, the ICU, the male medical ward, the male surgical ward, and the semi-private ward (Table 1). The highest number of positive samples were identified in the ICU and male surgical ward, which recorded 10 VRE isolates each. In both of these sample cohorts, 6 of the 10 (60%) positive samples were from patients with no history of VRE colonisation (i.e. first-time infections). Five positive samples were obtained from the general inpatient ward, of which 4 (80%) were first-time infections. Cumulatively, these findings indicate that patients in these wards are at an increased likelihood of VRE colonisation, and that VRE carriage is dispersed throughout the hospital as opposed to being confined to a particular location. Indeed, this observation is in line with Weterings *et al.,* (2021) who describe a VRE*fm* outbreak in the Admiraal De Ruyter Hospital (The Netherlands) that affected all but three wards. The high number of VRE isolates identified in the ICU is particularly concerning given that VRE carriage is invariably associated with medical complications in vulnerable patients (Se *et al*., 2009). However, this is not altogether surprising given that ICUs are at the epicentre of several VRE outbreaks described in the literature (Hughes *et al*., 2019; Marom *et al*., 2020; Peta *et al*., 2006), and while our study does not describe a full-scale VRE outbreak, a similar paradigm appears to have emerged within the MUH.

Variation also existed in terms of the sample types from which VRE isolates were identified. The majority of VRE isolates (16/34 or 47.1%) (95% CI: 30.3%-63.8%) were recovered from rectal swabs, with wound swabs and urine samples accounting for four positive samples each (8/34 or 23.5%) (95% CI: 9.3%-37.8%). Vaginal swabs and drain fluid samples accounted for two VRE isolates each, with the remaining six VRE isolates being isolated from six different sample types (abscess swab, blood culture, epigastric fluid, groin swab, penile swab, and stoma swab). The variety of positive sample types re-emphasises the ubiquitous nature of the pathogen, and upon comparison of our data with Ulrich *et al.*, (2017), some differences can be noted: most importantly, the prevalence of VRE in blood cultures, which in their systematic review accounted for 30.1% (141/468) of isolates – a ten-fold increase on the 2.9% (1/34) observed in our study. In addition to the marked difference in sample size, such discordance may be explained by the variation in patient profiles and while our study involved the general patient population, their review was confined solely to studies involving haematology/oncology patients. Thus, it may be that these patients, often regarded as the most immunocompromised in the hospital setting (Ruhnke *et al*., 2014), are at an inherently higher risk of VRE bloodstream infection (BSI) than the general patient population – an observation that certainly warrants further investigation.

Table 1: VRE prevalence data obtained from clinical samples received in the Mercy University Hospital.

Patient location	No. of samples analysed	No. of VRE isolated	No. of first-time VRE infections	Prevalence (%)†
A&E	3	0	0	0
CCU	2	0	0	0
Female medical ward	3	1	1	33.3
General inpatient ward	7 (6)	5	4	66.7
Geriatric ward	2	1	1	50.0
ICU	37 (33)	10	6	18.2
Male medical ward	1	1	1	100
Male surgical ward	13 (9)	10	6	66.7
OPD	2	0	0	0
Other*	3	0	0	0
Paediatric ward	1	0	0	0
Semi-private ward	4 (1)	3	0	0
SIVUH	18 (17)	3	2	11.8
Urology ward	2	0	0	0
Total	**98 (85)**	**34**	**21**	**24.7**

Where applicable, numbers in parentheses following the no. of samples analysed indicates the no. of samples analysed excluding duplicate positives.

Note: A&E = Accident and Emergency; CCU = Coronary Care Unit; ICU = Intensive Care Unit; OPD = Outpatient Department; SIVUH = South Infirmary Victoria University Hospital

† where prevalence (%) is the no. of first time VRE infections expressed in terms of the no. of samples analysed (excluding duplicate positives (e.d.p.)) i.e. prevalence (%) = $\frac{no.of\ first\ time\ infections}{no.of\ samples\ analysed\ (e.d.p.)}$ x $\frac{100}{1}$

* includes samples from Mercy Private Corridor, Hollyhill Medical Centre, and Shandon Medical Centre.

Environmental analysis

Owing to the high number of VRE isolated from patients in the male surgical ward, environmental swabbing of the ward's primary observation room was conducted in order to investigate the presence of an environmental reservoir (Table 2). Three areas of the observation room were swabbed, resulting in a total of 30 swabs submitted for analysis. The front desk area, from which 12 swabs were obtained, was found to harbour VRE contamination in one site, namely the keyboard. Seven environmental swabs were taken from the toilet area, with none of these showing evidence of VRE contamination. Of the 11 sites swabbed in the patient bedding area, two were positive for VRE, namely the electricity bar and bedside desk of bedding area 2. In total, three of the 30 (10%) environmental swabs analysed in this study were VRE contaminated. These findings are strongly suggestive of an active environmental VRE reservoir within the primary observation room of the male surgical ward.

In contrast to the sparse VRE growth observed upon primary culture of the keyboard and electricity bar swabs (1 CFU each), a near-confluent lawn of VRE growth was demonstrated upon primary culture of the bedside desk swab (CFU = too numerous to count (TNTC)) (Table 2). Although the CFU method may not be as accurate as a microscopic cell count, for example, it does allow for an estimate of microbial abundance in an environmental sample (Andrés Christen & Parker, 2020; Cundell, 2015). In this regard, it can be assumed that of the three positive environmental swabs, the bedside desk of

bedding area 2 carried the highest VRE burden. The significance of this observation is underpinned by the fact that at the time of sample collection, this patient bedding area was vacant and presumably awaiting the admission of a new patient, who, given the high environmental VRE load, would have been at an increased likelihood of becoming colonised by the pathogen. Coupling this with the identification of VRE isolates on the electricity bar, it is evident that the cleaning protocol utilised following the discharge of the previous patient failed to eradicate residual VRE. Indeed, it is plausible to hypothesise that this VRE reservoir existed for a considerable period of time and may have been a source of infection prior to this study. As such, our results do not represent a novel find and VRE contamination of these sites is to be expected when prevalence rates among patients are high.

The identification of VRE isolates on the keyboard is noteworthy given the frequency of its use by hospital staff. Indeed, keyboard contamination is somewhat unsurprising as Schultz *et al.(2003)* report that of the 100 keyboards tested in their hospital-wide study, 95 (95%) were positive for microorganisms. Moreover, while most of these isolates were commensal-skin flora, four of the isolates were enterococci, of which one was vancomycin-resistant. Similar rates of keyboard contamination have also been reported by Nazeri *et al*., (2019), with microbes being identified on more than two-thirds (76%) of the keyboards and electronic devices analysed in their cross-sectional study. Thus, it is clear that keyboards represent a potential source of nosocomial infection in many hospitals and our findings suggest the MUH cannot be considered an exception. Furthermore, while the keyboard analysed in our study is confined to the primary observation room, the frequency of its use by doctors and nurses, estimated to be 8.6 times/hour in one study, could facilitate VRE dissemination to distant areas within the hospital (Hong *et al*., 2012).

The absence of VRE contaminants in the toilet area was largely unexpected, particularly given that the toilet within the observation room is a communal facility; at any one time up to 5 patients may share its use. In other studies, VRE isolates have been identified in hospital toilets, with Ulrich *et al.*, (2017) stressing that a special emphasis need be placed on toilets with respect to environmental VRE disinfection. Thus, our findings may indicate that the shared toilet facility within the observation room is, unlike other areas of the room, adequately cleaned as to prevent VRE persistence.

Table 2: Environmental sampling data obtained from the primary observation room of the male surgical ward.

Site of swab	VRE present (Y/N)	Degree of VRE growth in primary culture†
Front desk area		
Door handles	N	-
Telephone	N	-
Keyboard	Y	+
Chair armrest	N	-
Computer mouse	N	-
Bloodtrack devices	N	-
Light switches	N	-
Sink taps	N	-
Soap dispensers	N	-
Drawer handles	N	-
Monkey bar (4)	N	-
Remote control	N	-
Toilet area		
Sink taps	N	-
Door handles	N	-
Toilet handle	N	-
Soap dispensers	N	-
Support rail	N	-
Toilet bowl surface	N	-
Paper towel holder	N	-
Patient bedding area		
Chair armrest (1)	N	-
Table at end of bed (1)	N	-
Walking frame (1)	N	-
Windowsill	N	-
IV fluid holder (1)	N	-
Blood pressure monitor (1)	N	-
Electricity bar (2)	Y	+
Bed front railings (1)	N	-
Bed back railings (1)	N	-
Bedside desk (1)	N	-
Bedside desk (2)	Y	++++

Where applicable, numbers in parentheses following a swabbing site indicate which patient bedding area the swab was taken from.

† where '-' = no growth; '+' = 1-100 CFU; '++' = 101-200 CFU; '+++' = 201-300 CFU; '++++' = too numerous to count (TNTC) (>300 CFU)

Antimicrobial susceptibility testing

For all first-time VRE infections and positive environmental specimens, identification testing was performed to provide a species-level resolution of the isolated enterococci. Antimicrobial susceptibility testing (AST) was also conducted in order to elucidate the patterns of antimicrobial resistance present within the VRE population (Table 3). In addition to the 24 VRE, one vancomycin-sensitive strain (subsequently found to be linezolid-resistant), isolated from a wound swab of an ICU patient, was also subject to identification and AST testing. All 24 presumptive positive VRE identified by culture (21 clinical and 3 environmental) were confirmed to be vancomycin-resistant by AST testing, with minimum inhibitory concentration (MIC) values of >16μg/mL reported for all isolates. Furthermore, in agreement with Whelton *et al*. (2016), all isolates (n=24 VRE and n=1 VSE) investigated in our study were identified as *E. faecium* (with VITEK identification probabilities ranging from 86%-98%), implicating this species as the predominant nosocomial strain. As regards the mechanisms of vancomycin-resistance, all isolates demonstrated the *vanA* genotype. Although confirmatory polymerase-chain reaction (PCR) testing of *van* genotypes was not performed in our study, Abele-Horn *et al., (2006)* report that 98.5% (65/66) of PCR-confirmed *E. faecium vanA* strains are correctly characterised by the VITEK 2 system. Thus, it can be assumed that the majority, if not all, of the tested VRE isolates were accurately characterised as *vanA*. In line with this, a key characteristic of *vanA* subtypes, high-level teicoplanin resistance (MIC >16μg/ml), was observed in all VRE isolates (Qu *et al*., 2009).

Consistent with the findings of Engin *et al*. (2013) and Whelton *et al*. (2016), ampicillin-resistance was observed in all isolates (MIC >16 μg/mL), indicating the widespread prevalence of an ampicillin-resistant VRE phenotype in the patient population. As regards antimicrobial synergism, the synergy between a cell wall active agent (e.g. ampicillin) and an aminoglycoside (e.g. streptomycin) is best predicted by screening for high level resistance to the aminoglycoside (Torres *et al.*, 1995). As such, the susceptibility of isolates to gentamicin, kanamycin, and streptomycin was investigated, with results showing considerable variation between the three agents. With respect to gentamicin, all but two isolates were shown to exhibit high-level resistance to the antibiotic. Interestingly, one of the susceptible isolates was recovered from a drain fluid sample of a patient located in the male surgical ward and further analysis revealed the entire AST profile of this isolate to be an exact replicate of that obtained from both the keyboard and electricity bar (n=2) isolates however, more sophisticated methods, such as the gold standard pulsed-field gel electrophoresis (PFGE) (Salipante *et al.*, 2015), would be required to conclusively determine the relatedness of these three strains.. Kanamycin resistance was observed in all but one VRE isolate, indicating that synergistic combinations which incorporate kanamycin as an aminoglycoside no longer represent an option for VRE treatment in the MUH. In contrast, susceptibility to streptomycin varied among isolates, with 37.5% (9/24) observed to be resistant, and 62.5% (15/24) remaining susceptible. Hence, of the three aminoglycosides investigated in this study, streptomycin offers the greatest potential as a therapeutic against VRE, and may still be considered for treatment of VRE infection against which other treatments have been exhausted.

Linezolid has been approved by the FDA for treatment of VRE*fm* infections which are unresponsive to first/second-line antimicrobial therapy (Hashemian *et al*., 2018). Commonly referred to as a 'last-resort antibiotic', the use of linezolid is typically reserved for cases of severe infection whereby the causative agent is multi-drug resistant (Sadowy, 2018). Given this status, it is concerning that one of the enterococci identified in our study was linezolid-resistant. An MIC value of >4 μg/mL was reported, with the specimen subsequently being referred to a specialist centre (National MRSA Reference Laboratory (NMRSARL)) for confirmatory MIC testing and PCR analysis. MIC testing conducted in the NMRSARL revealed an MIC value of 8 μg/mL (*via* broth dilution), and PCR analysis did not detect the presence of the transferrable resistance genes *cfr*, *optrA*, and *poxtA*. These resistance determinants, which are often nested within plasmid-borne mobile genetic elements (MGEs), are easily transferred between bacteria and have the potential to mediate the widespread dissemination of linezolid-resistance (Bender *et al*., 2019). The failure to detect these genes *via* PCR indicates that linezolid-resistance in this isolate is governed by an alternative mechanism: most likely a mutation in bacterial ribosomes and/or associated ribosomal proteins (Ruiz-Ripa *et al*., 2021). Given the non-transferable nature of these

mutations, it is unlikely that the isolate represents a real threat in terms of resistance dissemination. Furthermore, while a clear linezolid-resistant phenotype was identified, the isolate was susceptible to vancomycin, demonstrating an MIC value of <0.5µg/mL. Nonetheless, the identification of this isolate highlights the need for strict antimicrobial stewardship to prevent a vancomycin-resistant linezolid-resistant phenotype emerging in the patient.

Tigecycline and quinupristin-dalfopristin were the only two antibiotics against which all of the isolated VRE were susceptible. While these data are encouraging, it is important to note that the high rate of quinupristin-dalfopristin susceptibility is likely a reflection of the sole isolation of *E. faecium* species in our study. More precisely, although the rates of resistance to quinupristin-dalfopristin are low among strains of VRE*fm*, the converse is true for vancomycin-resistant *E. faecalis*, with only 3.2% of isolates remaining susceptible according to one study (in contrast to 90.6% of VRE*fm*) (Eliopoulos, 2003). Thus, while quinupristin-dalfopristin therapy may be indicated for most VRE infections arising within the MUH, this may not be the case with respect to infections in patients travelling from areas such as Northern and Eastern Europe where vancomycin-resistant *E. faecalis* is more prominent (Ayobami *et al*., 2020).

Table 3: VITEK 2 Compact antimicrobial susceptibility testing (AST-P662 card) on presumptive positive vancomycin-resistant enterococci recovered from first-time infections and environmental samples.

Antimicrobial	No. of isolates tested*	No. of isolates susceptible	No. of isolates resistant	Likely to be effective †
Ampicillin (AMP)	24	0	24	✗
Gentamicin (GEN)	24	4	20	✗
Kanamycin (KAN)	24	1	23	✗
Linezolid (LZD)	24	24	0	✓
Quinupristin-Dalfopristin (Q/D)	24	24	0	✓
Streptomycin (STM)	24	15	9	✓
Teicoplanin (TEI)	24	0	24	✗
Tigecycline (TIG)	24	24	0	✓
Vancomycin (VAN)	24	0	24	✗

† where the majority of isolates were found to be susceptible an antibiotic was considered 'likely to be effective'

* the linezolid-resistant isolate also underwent AST analysis but was vancomycin-sensitive and so was excluded from the above-shown data. This isolate had the following resistotype: AMP-GEN-KAN-LZD.

Infection prevention and control

In the context of pathogen containment, an emergency meeting was conducted in April 2021 in order to address the findings of this study. Many of the topics discussed are in line with the guidelines set out by the Hospital Infection Controls Practice Advisory Committee (HICPAC) as regards preventing the spread of vancomycin-resistance in the hospital-setting (CDC, 1995). These guidelines, which require a coordinated interdisciplinary approach, are comprised of four core elements: a) the prudent use of vancomycin by clinicians, b) education of all hospital staff regarding the impact of VRE, c) early detection and reporting of VRE by laboratory staff, and d) the prompt implementation of infection control measures to prevent person-person VRE transmission (CDC, 1995).

Regarding vancomycin usage, it has been estimated that in an estimated 34-67% of cases, its use could be considered inappropriate, with methicillin-resistant *Staphylococcus aureus* (MRSA) being cited as a contributory factor in many hospitals (i.e. use of vancomycin to treat MRSA often favours the emergence of VRE) (Junior *et al*., 2007). While neither the prevalence of MRSA nor the frequency of

vancomycin use were investigated in our study, our findings highlight the need for prescribing clinicians to adopt a cautious approach moving forward.

Education of hospital staff, in particular nurses and other ward-level personnel, on the significance of VRE was an obvious requirement given the high number of first-time infections arising in many wards. The importance of proper hygiene was relayed to the relevant staff, with an emphasis on handwashing technique, use of personal protective equipment (PPE), and surface disinfection (Mutters *et al*., 2013). As regards the environmental reservoir, the seriousness of the issue was highlighted and the use of a plastic keyboard cover in tandem with frequent disinfection was implemented (Hong *et al*., 2012).

Given that the microbiology laboratory serves as the first-line of defense against VRE spread in the hospital setting (CDC, 1995), laboratory staff play a central role in breaking the chains of pathogen transmission. With a view to ensuring patient-patient and patient-staff VRE transmission is minimised within the hospital, the need for prompt identification and reporting of VRE-positive patients was reiterated to microbiology staff. In addition, the continued monitoring of VRE prevalence was recommended to allow for the early-identification of VRE clusters emerging at the ward-level.

Limitations

This study had a number of limitations. Firstly, although the study was conducted over a considerable time period, the sample size (n=98) was relatively small. Secondly, owing to a lack of resources and financial constraints, molecular typing of VRE isolates was not performed in this study. Although an attempt was made to determine the relatedness of strains by comparison of AST profiles, this cannot be considered an adequate substitution for molecular methods such as PFGE and multilocus sequence typing (MLST).

Using PFGE, Ryan et al. (2015) determined the clonal relatedness of VRE strains obtained from patients within St. Vincent's University Hospital (SVUH), allowing them to decipher the epidemiological links between many of the isolates and establish the likely starting points for several clusters of infection. Thus, it is clear that molecular typing adds an extra dimension to prevalence data and should be incorporated into future studies. Finally, given that only three areas in the primary observation room of the male surgical ward were sampled, it is likely that the VRE burden within this area, if not the entire ward, is greater than our results imply. In addition, environmental sampling of other wards from which high numbers of first-time infections were reported, such as the general inpatient ward and the ICU, is needed to determine if VRE reservoirs exist in these areas.

CONCLUSION

In summary, the findings described herein reveal high rates of VRE carriage among patients within the MUH, while also pointing towards the presence of an active environmental reservoir in the male surgical ward. In line with other Irish studies (Morris-Downes *et al.*, 2010; Ryan *et al*., 2015; Whelton *et a*l., 2016), *vanA* VRE*fm* appears to be the predominant nosocomial strain, with linezolid-resistance still a cause for concern. It is hoped that this study highlights the ongoing threat posed by VRE in the hospital setting and may, at least in part, help to explain why Ireland continues to surpass its neighbours in terms of VRE prevalence.

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