Epidemiology and molecular typing of VRE bloodstream isolates in an Irish tertiary care hospital

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Objectives: Ireland has the highest rate of vancomycin-resistant Enterococcus faecium (VREfm) isolated from blood of nosocomial patients in Europe, which rose from 33% (110/330) in 2007 to 45% (178/392) in 2012. No other European country had a VREfm rate from blood cultures of >25%. Our aim was to elucidate the reasons for this significantly higher rate in Ireland.

Methods: The epidemiology and molecular typing of VRE from bloodstream infections (BSIs) was examined in a tertiary care referral hospital and isolates were compared with those from other tertiary care referral centres in the region.

Results: The most common source of VRE BSIs was intra-abdominal sepsis, followed by line-related infection and febrile neutropenia. Most of the isolates were positive for vanA; 52% (43/83) possessed the esp gene and 12% (10/83) possessed the hyl gene. Genotyping by SmaI macrorestriction analysis (PFGE) of isolates revealed clonal relatedness between bloodstream isolates and environmental isolates. VRE BSI isolates from two other tertiary care hospitals in the Dublin region showed relatedness by PFGE analysis. MLST revealed four STs (ST17, ST18, ST78 and ST203), all belonging to the clonal complex of hospital-associated strains.

Conclusions: Irish VRE BSI isolates have virulence factor profiles as previously reported from Europe. Typing analysis shows the spread of individual clones within the hospital and between regional tertiary care hospitals. Apart from transmission of VRE within the hospital and transfer of colonized patients between Irish hospitals, no other explanation for the persistently high VREfm BSI rate in Ireland has been found.

Introduction

Ireland has the highest rate of vancomycin resistance among enterococcal bloodstream isolates from humans in Europe. In 2013, 43.1% of Enterococcus faecium isolated from blood were resistant to vancomycin (VREfm). In 2008, 44.1% of Enterococcus faecium isolated from blood were resistant to vancomycin (VREfm). In Enterococcus faecalis, resistance rates of enterococci to glycopeptides (vancomycin, teicoplanin) is an increasingly common problem in E. faecium (as the main reservoir of this resistance) and poses numerous challenges: fewer treatment options, increased morbidity/mortality and increased costs when compared with infection caused by vancomycin-susceptible enterococcal strains. Resistance to linezolid, an important alternative antimicrobial for treating VRE infections, has been reported in enterococci from both outbreaks and individuals on treatment.

While there has been a steady decline in invasive MRSA infections in Ireland over the past 8 years, the opposite is true for VREfm. Invasive infections due to VREfm are an increasing concern. The Irish VREfm bloodstream infection (BSI) rate increased from 33.3% in 2007 to 45.6% in quarter 2 of 2014. Several studies on the epidemiology of VRE have been reported from other European countries. The epidemiology of VREfm BSI in Ireland has not been elucidated and the reasons for the high VREfm rate in Ireland remain unclear. In our hospital, the rate of VREfm BSI has been increasing and was 59.1% in 2013. This study was carried out to characterize epidemiologically the high rates of invasive VRE infection and to determine whether variation in epidemiology or molecular peculiarities are responsible for the difference in Irish data compared with those of other European countries.

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Methods

Setting and study design

St Vincent’s University Hospital (SVUH) is a 479 bed tertiary referral centre and the National Liver Transplant Centre. To identify all patients with VRE BSI, the laboratory records from 1 January 2009 to 30 June 2012 were accessed. A total of 75 patients with VRE BSI were identified in this time period, but only the first isolate per patient was included in the analysis. Source of BSI was identified according to case definitions from ECDC’s document Point Prevalence Survey of Healthcare-Associated Infections and Antimicrobial Use in European Acute Care Hospitals 2011 - 2012. A retrospective review of patient records was carried out and mortality data were taken from the electronic patient information system. In February 2014 an outbreak of linezolid-resistant VRE (LRVRE) occurred, during which isolates were examined and clinical details were collected prospectively.

Routine screening for VRE colonization is undertaken in this hospital for patients admitted to the ICU and those under the care of haematology, oncology and the liver transplant service. In the ICU, VRE-positive patients are routinely isolated in single rooms. The majority of VRE-positive patients on abdominal surgery wards and the hepatology ward are cohorted in rooms with three or four beds with dedicated toilet facilities outside the cohort room along the ward corridor. The haematology/oncology ward was relocated during the study period to a new ward, facilitating single-room isolation instead of cohort isolation for all VRE-positive patients.

VRE screening and identification

FLOQSwabs18 (Copan Flock Technologies) used for rectal screens are inoculated onto a chromogenic agar selective for VRE (bioMérieux, France) and incubated for 48 h at 37°C. This allows the presumptive identification of the two main species involved: E. faecium (violet colonies) and E. faecalis (blue-green colonies). Full identification and susceptibility testing is performed using VITEK MS.

Blood culture isolates were identified and their antibiotic susceptibilities were determined by the VITEK 2 system (bioMérieux, France) according to EUCAST guidelines.9 All invasive isolates were reported to clinicians and advice on treatment was given.

PCR

PCR for detection of glycopeptide resistance genes (vanA, vanB) and virulence genes (esp, hyl) was carried out according to previously described methods.10

SmaI macrorestriction analysis (MRA)

The enterococcal isolates were characterized genotypically by MRA using the restriction endonuclease SmaI (Roche, Basel, Switzerland) and subsequently performed PFGE. The CHEF-DR III apparatus (Bio-Rad Laboratories, Hercules, CA, USA) was used for PFGE, with the following ramped pulse times: 1–11 s for 15 h and 11–30 s for 14 h in a 1.0% agarose gel (Type II, Medium EEO; Sigma, USA) at 14°C. Subsequent staining of the DNA bands was performed with ethidium bromide solution. Bacterial DNA fingerprints were analysed by molecular mass determination of all DNA fragments using the control strain Staphylococcus aureus NCTC 8325 as molecular mass standard and were evaluated as recommended previously.10 The calculations of similarities between fingerprint patterns and their representation in a dendrogram were performed using BioNumerics software version 7.1 (Applied Maths, Sint-Martens-Latem, Belgium).

MLST

Characterization of selected enterococcal strains by MLST was performed according to Homan et al.11 Several isolates from an important MRA cluster were selected for MLST. STs were designated on the basis of sequence analyses of fragments of seven housekeeping genes and according to http://efaecium.mlst.net.

Determination of linezolid resistance mutations

We determined the mutations leading to linezolid resistance by PCR amplification of 23S rDNA alleles and subsequent NheI restriction and fragment analysis in a DNA microarray as described previously.11,12 Part of the cfr gene was amplified using primers described previously.13

Results

Epidemiology of VRE BSI

A total of 75 patients with VRE BSI were identified in the time period from 2009 to 2012, giving a rate of 0.12 VRE BSIs per 1000 bed days. As a percentage of E. faecium BSIs, VREfm BSIs in our centre increased from 40% in 2009 to 59.1% in 2013.1 Patient characteristics and epidemiology are shown in Table 1. There was no

| Characteristics and epidemiology of 75 patients with VRE BSI from 2009 to 2012 |
|---------------------------------|-----------------|-------------------|----------------|-----------------|-----------------|-------------------|
| Total number of patients | 75 | Male, n (%) | 45 (60) | Age (years), median (range) | 65 (21–84) | Days from admission to VRE BSI, median | 23 |
| Admitting specialty, n (%) | | | | | | |
| hepatobiliary and hepatology | 28 (37) | haematology and oncology | 13 (17) | general surgery and colorectal surgery | 7 (9) | orthopaedic surgery | 5 (7) | general medical | 22 (29) |
| Ward at time of VRE BSI, n (%) | | | | | | |
| ICU | 25 (33) | haematology and oncology | 10 (13) | hepatobiliary and hepatology | 16 (21) | other | 24 (32) |
| Probable source, n (%) | | | | | | |
| intra-abdominal sepsis | 28 (37) | line-related infection | 12 (16) | febrile neutropenia | 5 (7) | other | 11 (15) | unknown | 19 (25) |
| Mortality, n (%) | | | | | | |
| in hospital | 40 (53) | 30 day all cause | 31 (41) | within 7 days of VRE BSI | 17 (23) |
| VRE status prior to BSI, n (%) | | | | | | |
| known colonization | 48 (64) | negative screen (rectal swab) | 10 (13) | unknown (not previously screened) | 17 (23) |
| Received broad-spectrum antibiotics prior to VRE BSI, n (%) | 54 (72) |
statistical difference in the sex distribution among VRE BSIs compared with the baseline admissions for this period. The median age among patients with VREfm BSI was 65 years, compared with 58 years in the general hospital population. The median number of days from admission to development of VRE BSI was 23 days, with one-third of patients being in the ICU at the time. Thirty-seven percent of cases (28/75) were inpatients admitted under the hepatobiliary and hepatology (including liver transplant patients) services, 17% (13/75) under haematology/oncology and 9% (7/75) under general surgery and colorectal surgery. The rest of the patients were general medical or orthopaedic surgery inpatients. The most common source was intra-abdominal sepsis (37%), followed by line-related infection (16%) and then febrile neutropenia (7%). Case definitions were derived from ECDC's document *Point Prevalence Survey of Healthcare-Associated Infections and Antimicrobial Use in European Acute Care Hospitals 2011–2012*.²

Fifty-eight out of 75 patients (77%) were screened for VRE prior to BSI. Overall, 48 patients (64%) were known to be VRE colonized prior to BSI, all 48 having had a positive screen and 14 of those also having VRE in a clinical specimen (6 peritoneal drain fluids, 2 intra-abdominal tissues, 4 superficial wound swabs and 2 other tissues). A total of 54 patients (72%) had been treated with broad-spectrum antibiotic regimens, among which piperacillin/tazobactam, third-generation cephalosporins and carbapenems were the most frequently used, prior to the VRE BSI. Ninety-five percent of VRE isolates were VREfm; the remaining strains were vancomycin-resistant *E. faecalis*. The majority of patients with VRE BSI had previous hospital exposure. Twenty-eight patients (37%) were transferred directly from another hospital in Ireland and 24 of the remaining 47 patients had a hospital admission to this institute within the previous 2 years, the majority within 12 months (20/24). Of the 28 patients who were transferred from another hospital in Ireland, 8 were known to be VRE colonized on transfer, 12 had negative admission screens and 8 had no admission screen taken. Thirty-day all-cause mortality post-VREfm BSI was 41% (31 patients) and seventeen patients (23%) died within 7 days of VRE BSI. Forty-three patients (57%) were still alive at 30 days, but 9 of these died beyond 30 days on the same admission. Information is not available for the remaining patient. In February 2014 an outbreak of LRVREfm occurred in the hepatology unit, encompassing 1 bacteraemia case and 14 cases of rectal colonization followed by 5 further cases of LRVREfm in the following months.

**Analysis of clonal relatedness**

Clonal relatedness of 82 VREfm isolates and 1 vancomycin-susceptible *E. faecium* isolate was analysed by MRA: 43 bloodstream isolates from our institution were analysed in parallel with 5 environmental isolates; 17 rectal swabs; and 18 bloodstream isolates received from two other tertiary hospitals in Ireland. Random bloodstream isolates were requested from other tertiary care referral centres in the city for inter-hospital comparison. Environmental isolates were taken from the ICU (two isolates), the haematology/oncology day ward and the hepatobiliary ward in 2010 (one isolate from each). One additional environmental isolate was taken from the hepatology ward in 2014 during the LRVRE outbreak.

Among the 83 isolates analysed by MRA, only 18 showed a unique typing pattern; all other isolates showed genetic relatedness to at least one other VREfm. Of the 18 isolates with a unique typing pattern, 17 were VREfm BSI isolates (one rectal swab); 11 were from our institution and 6 from two other hospitals. The 11 VREfm BSI isolates from our institution were from patients that were admitted under six different specialties and, of these, 9 had been screened for VRE prior to BSI and 8 were known to be VRE positive. PFGE patterns revealed 15 clusters (Figure 1). In some of the smaller clusters (clusters 5, 6, 11 and 15) no epidemiological link could be established between isolates; the patients were not in hospital at the same time and had not been admitted to the same ward, and there was no sharing of facilities during inpatient stay. Within the larger clusters 12 and 14, there was epidemiological opportunity for both cross-transmission of VREfm between patients and also potential VREfm acquisition from environmental sources.

Cluster 12 consisted of one environmental isolate from the hepatobiliary ward and eight bloodstream samples collected between 2010 and 2012. In this cluster seven patients were in the ICU during or prior to VREfm BSI and four acquired VREfm in the ICU. Only one patient’s admission overlapped with two others: the first was on a different ward under a different specialty and the second patient was in the ICU during the same time (16 days overlapped), but was under a different specialty. All the remaining patients were not in hospital at the same time. Only one patient stayed on the hepatobiliary ward, from which the environmental isolate was taken, for a 2 day period: this patient had a negative VRE screen on discharge from the ward, but a subsequent screen 3 days later in the ICU was positive.

Cluster 14 contained one environmental and four bloodstream isolates. Patients 1 and 2 in this cluster had overlapping admissions in the hepatobiliary ward. Patients 3 and 4 were haematology/oncology patients on the haematology/oncology ward, but their admissions did not overlap temporally. Both of these patients acquired VREfm on the haematology/oncology ward; the environmental isolate was from the same ward.

In cluster 4 there were four bloodstream isolates from hepatology and hepatobiliary patients, three of whom spent time in the ICU, but none of their admissions overlapped temporally. The isolates spanned a 9 month period.

Cluster 3 had three bloodstream isolates from our institution. None of the patients’ admissions overlapped temporally, but they had been admitted to the same wards under the same specialty (hepatobiliary). These isolates spanned a 13 month period.

Cluster 13 represented isolates from one of the other institutions for which clinical and epidemiological information was not available.

Cluster 7 was the largest cluster, with 20 isolates. It included 16 LRVRE isolates, which were included in the typing analysis: 15 rectal screens from LRVREfm-colonized patients and 1 environmental sample from the liver transplant unit during the outbreak. The remaining four isolates in this cluster were all linezolid susceptible: one VREfm isolate from a rectal screen (Figure 1; UW11818) from a patient who later developed an LRVREfm line-related BSI with a unique PFGE type (Figure 1; UW11825) and three linezolid susceptible VREfm bloodstream isolates, from patients in two different hospitals who had never been inpatients in our institute. One of the LRVREfm rectal screen isolates taken as part of the outbreak investigation had a unique typing pattern (UW11825).
Figure 1. Genotyping by SmaI MRA of 83 VREfm isolates (74 vanA isolates, 3 vanB isolates, 5 vanA+vanB isolates and 1 glycopeptide-susceptible, vanA/vanB-negative isolate) from patients and a few environmental samples in different units of three hospitals in Dublin, Ireland. Different clusters could be detected; 16 of 18 linezolid-resistant (indicated by *) vanA-positive E. faecium isolates are present in the MRA cluster 7.
Of the 16 LRVREfm isolates in this cluster, 11 were part of an outbreak in February 2014 in the hepatology unit, which is described below. Worryingly, the other 5 LRVREfm isolates were taken in July, August and December 2014, and although there were potential opportunities for cross-infection amongst these patients, they had no obvious epidemiological link to the outbreak on the hepatology unit.

The presumed index patient in the hepatology unit LRVREfm outbreak was 6 months post-orthotopic liver transplant when admitted in January 2014 with a large intra-abdominal collection and was treated empirically with linezolid, as VREfm had been detected previously on rectal screening, and meropenem. On admission a month later, a bile sample cultured LRVREfm; at this point the patient had already been cohorted on admission with other VRE patients and this is deemed to have facilitated the outbreak of LRVREfm as well as other contributing factors (C. O’Driscoll, V. Murphy, O. Doyle, C. Wrenn, A. Flynn, N. O’Flaherty, L. Fenelon, K. Schaffer and S. FitzGerald, unpublished data). Worryingly, there were an additional five cases of LRVREfm detected over the following months in patients from different wards in the hospital that had no obvious epidemiological link to the outbreak on the hepatology unit.

All environmental isolates investigated by MRA showed genetic relatedness to VREfm BSI isolates from patients, highlighting the capacity of VREfm to spread from patients into the environment.

Eighteen blood culture isolates from two other Dublin teaching hospitals were analysed in parallel. Only six of them showed a unique typing pattern. Of the remaining 12 isolates, 8 clustered with isolates from the same institution and 8 clustered with isolates from one of the other two teaching hospitals. Four of 18 VREfm BSI isolates from other institutions clustered with isolates from our hospital. To our knowledge, none of the patients had been an inpatient in our institution. There was no shared residence or connection between these patients.

**PCR analysis**

Out of 83 isolates tested, 74 (89%) were positive for vanA, 5 (6%) were positive for both vanA and vanB, 3 (4%) were positive for vanB and 1 (1%) was susceptible to vancomycin and teicoplanin (vanA and vanB negative by PCR); 43 (52%) possessed the esp gene and 10 (12%) possessed the hyl gene. We investigated the molecular basis of linezolid resistance in 13 LRVREfm. Amplification of 23S rDNA alleles and subsequent NheI restriction and fragment analyses revealed that all LRVREfm possessed the common G2576T mutation, mainly in a ratio of WT to mutated allele variants of 4:2. None of the isolates was positive for plasmid-encoded linezolid resistance mediated by cfr. The G2576T ribosomal DNA mutation has been frequently associated with linezolid resistance among enterococci.14,15

**MLST**

Several isolates from important MRA clusters were selected for MLST (Figure 1) and revealed four STs: ST17, ST18, ST78 and ST203 (the last of which was exhibited by LRVREfm). All these STs belong to the clonal complex of hospital-associated strain types.16–19

**Discussion**

The epidemiology of VREfm BSI in this Irish tertiary care hospital is different from that described in previous reports in the literature. VREfm BSIs worldwide (Spain, France, South Korea, North America) are commonly reported in patients from haematology and oncology units, while we found preponderance among hepatobiliary and hepatology/general surgery and colorectal surgery patients (47%) and only 17% among haematology/oncology patients.20–23 This may represent the case mix in this hospital rather than true disease prevalence, as hepatobiliary, colorectal and general surgery accounted for 13.2% of overall bed days, whereas haematology and oncology accounted for only 3.7% of bed days and liver transplant/hepatology accounted for 3.4%.

The breakdown of bed days indicates that there is a higher percentage of hepatobiliary and surgical patients than haematology and oncology patients and this may be the reason for the preponderance of VRE BSIs we have seen amongst these patients. VREfm colonization in Ireland may be more widespread across all inpatient populations and no longer merely associated with traditional high-risk patients. High-risk groups in our hospital include not only haematology and oncology patients, but also hepatobiliary and hepatology patients, as many patients are referred from other centres in Ireland to the National Liver Transplant Unit and the National Centre for Pancreatic and Hepatobiliary Surgery and 29% of patients transferred from other hospitals who were screened on admission had positive VRE admission screens. Screening for VRE has been performed in traditional at-risk groups, like haematology/oncology, ICU and liver transplantation patients, in this hospital for many years. Screening was expanded to hepatobiliary patients in 2014. In 2010, a publication from a different Irish university hospital reported that the majority of new VRE isolates were from renal, vascular and haematology/oncology patients as well as from neurosurgical patients, which is not surprising as it is the National Centre for Renal Transplant Surgery and Neurosurgery.7 The authors reported rates of new VRE broken down by specialty (presented per 1000 bed days). Renal medicine accounted for the highest rates of new VRE (screens and clinically significant isolates) followed by vascular surgery and then haematology over the study period. Local patient mix may dictate the groups who are most affected in each hospital, as described above for our institution.

An anonymous screen of 100 consecutive faecal samples from patients attending the emergency department and from hospital inpatients in 2009 revealed that 1% and 38%, respectively, of the samples contained VRE (C. W., SVUH). In our centre, intra-abdominal collections are a major source of VREfm BSIs, which frequently require drainage and a prolonged antibiotic course if not adequately drained, which is a risk for the bacteria acquiring resistance during the treatment course. This may have been implicated in the index case in the LRVREfm outbreak described above.

VREfm BSI isolates that had been selected for typing from the 42 month period from 2009 to 2012 showed a polyclonal pattern with a number of clusters and some unique types, implying horizontal transmission as well as influx of new clones. Epidemiological data from within clusters and the presence of environmental isolates of the same PFGE type as BSI isolates strongly indicate opportunities for in-hospital transmission of VREfm clones. Single outbreaks are often reported to have clonally related VRE types, indicating horizontal transmission or clonal dissemination.12,14–16 Horizontal transmission on the general surgical wards and the liver transplantation ward may be fuelled by the limited capacity for patient isolation and lack of single-patient toilet facilities. Although patients with VRE are routinely isolated in...
and pass it on in a second institution, and the circulation of certain clonally related to isolates from our institution. Potential explanations for this finding are that patients acquire VRE in one institution and pass it on in a second institution, and the circulation of certain VREfmr strains in the Irish community.

Previous studies have analysed VRE isolates over prolonged time periods in single institutions and found a small number of predominant clones and a number of minor ones. Other authors have observed identical clones found in numerous institutions across a country in outbreak settings, suggesting dissemination of clones and inter-hospital transfer.

The prevalence of the virulence genes esp and hyl found in our isolates was 52% and 12%, respectively. These figures are similar to those reported across Europe, China and South Korea and offer no explanation for our outstandingly high rates of invasive infection.

Molecular typing of our VREfmr by MLST revealed four predominant types (ST17, ST18, ST78 and ST203). ST17 and ST18 represent classical outbreak strain types prevalent in many countries for many years. ST78 and ST203 are comparatively new STs, but also already known to be prevalent in a number of European countries, like Spain, Italy, the Netherlands and Germany, but also in Asian countries, like South Korea, China and Australia.

MLST data obtained in this study suggest that Ireland does not have unique VREfmr STs in circulation compared with the situation in other European countries, thus not explaining its high VREfmr BSI rates.

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Transparency declarations
None to declare.

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