Epidemiology and molecular characterization of vancomycin resistant Enterococci isolates in India

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Abstract
Little attention has been paid to the problem of the spread of vancomycin resistant enterococci (VRE) in India. Between August 2002 to March 2003, faecal and urine samples of patients from various wards of the Postgraduate Institute of Medical Education and Research, Chandigarh, India, were screened for vancomycin resistance. 36 VRE were isolated (18 Enterococcus gallinarum, 9 E. casseliflavus, 7 E. faecium and 2 E. faecalis). These isolates were characterized as low-, moderate- and high-level resistant strains by phenotypic as well as genotypic methods such as minimum inhibitory concentration determination, polymerase chain reaction assays, sequencing of PCR products and multiple sequence alignment of van genes. Correlations established between these results and the vancomycin resistance markers were designated as vanA (783 bp), vanB (635 bp), vanC1 (822 bp) and vanC2 (484 bp) according to the findings of earlier workers as well as comparison with existing databases. Prolonged hospital stay and vancomycin were important risk factors for both VRE UTI and colonization. Renal dialysis, renal failure, prior aminoglycoside and third generation cephalosporin were the other significant factors for VRE UTI. The study also highlights the importance of screening for VRE in clinical samples and recommends the institution of control measures to prevent the further spread of VRE.

Introduction
Vancomycin resistant enterococci are ascendant nosocomial pathogens. From 1989 to 2002 the percentage of nosocomial enterococcal infections reported to the Center for Disease Control and Surveillance System that were due to VRE increased from 0.3 to 27.5% [1]. Six types of glycopeptide resistant enterococci have been described from VanA to VanG that can be distinguished on the basis of transferability, levels of resistance, and the spectrum of glycopeptides to which the strains are resistant [2].

Many risk factors are involved in the acquisition of VRE [3]. Gastrointestinal colonization may precede infection in many patients [4]. Hospitalized patients with gastrointestinal carriage of VRE appear to be the major reservoir of the organisms. Because most colonized patients are asymptomatic, this reservoir can go unnoticed. Early detection of patients colonized or infected with VRE is an essential component of any hospital programme designed to prevent nosocomial transmission of VRE.

The Hospital Infection Control Practices Advisory Committee (HICPAC) of the Center for Disease Control and Prevention has published recommendations for prevention of the spread of glycopeptide resistant enterococci [5]. The recommendations include screening of enterococcal isolates for vancomycin resistance and establishment of a screening programme to detect patients with intestinal colonization with glycopeptide resistant enterococci (GRE). It is also recommended that control efforts be intensified once GRE are known to be present within a hospital, particularly in high-risk areas such as transplantation and oncology units and the ICU.

In India, although enterococci are important agents causing urinary tract infections (UTI), bacteremia and wound infections, very few data are available on the occurrence of VRE and infections
caused by them [6–8], nor has the genotype involved been studied in detail. At our centre, vancomycin is used for treatment of serious infections due to methicillin-resistant Staphylococcus aureus (MRSA), which is endemic in many units [9]. Oral vancomycin is used to treat Clostridium difficile associated colitis. A need was felt to screen for VRE to ascertain the magnitude of the problem and affected units so that a control policy for our hospital could be formulated. Since enterococci are normal inhabitants of the gastrointestinal and genitourinary tracts, urine and stool samples were screened for VRE. The isolates were confirmed and speciated, their genotype confirmed by polymerase chain reaction (PCR), sequencing of PCR products and multiple sequence alignment of van genes was carried out. The risk factors for acquisition of VRE were also studied.

Materials and methods

Hospital setting

The Postgraduate Institute of Medical Education and Research, Chandigarh is a 1359-bed tertiary care referral hospital in north India catering for a population of more than 4 million in the neighbouring states of Punjab, Haryana and Himachal Pradesh. The hospital has 9 ICUs, 36 wards, 32 operation theatres, a renal transplant unit and an advanced paediatric centre.

The study was approved by the ethics committee of PGIMER, Chandigarh. Informed consent was obtained from the patients.

Screening for VRE

In the present prospective study, screening for VRE was carried out for a period of 8 months from August 2002 to March 2003. The samples included all faecal swabs and urine specimens submitted for culture to the Department of Medical Microbiology from admitted patients for suspected UTI or gastrointestinal infection. Enterococci in small numbers, along with normal flora, are found in urine samples from many patients. Their significance in causing UTI is doubtful. Therefore only organisms isolated in pure culture and significant numbers were selected.

Sample collection and identification of VRE

Faecal/rectal swabs were collected in Cary Blair medium, and were inoculated into bile esculin azide (BEA) broth (Difco Laboratories, Detroit, USA) supplemented with 6 μg/ml vancomycin (Duchefa, Netherlands). After incubation at 37°C for 18 h, subculture was performed on BEA agar. Urine samples (midstream and catheter) were cultured on Cystine Lactose Electrolyte Deficient (CLED) medium (Difco Laboratories, Detroit, USA). Suspected Enterococcus colonies from both samples were streaked onto brain heart infusion (BHI) agar (Difco Laboratories, Detroit, USA), supplemented with 6 μg/ml vancomycin and incubated overnight at 37°C. Only the initial isolate from each patient was tested.

Biochemical characteristics of VRE

Tentative identification of bacterial colonies belonging to the genus Enterococcus was performed by culture characteristics, Gram stain, catalase test, ability to grow in BHI agar containing 6.5% NaCl and growth in BHI broth at incubation temperature of 10°C and 45°C as per conventional identification scheme [10]. Definitive identification up to species level was made with the BD-BBL Crystal Identification System, (Gram-Positive ID Kit-Sparks, Maryland, USA), motility test (incubation at 30°C up to 7 d), and colony pigmentation

Bacterial strains

The following vancomycin resistant/sensitive strains were used as control: E. faecium WHO3 (vanC1), E. faecalis WHO14 (vanB) and E. gallinarum WHO11 (vanC1) (kindly gifted by Dr. Arti Kapil, All India Institute of Medical Sciences), E. casseliflavus ATCC 25788 (vanC2), E. faecalis ATCC 29212 vancomycin susceptible enterococci and E. faecium ATCC 19434 vancomycin susceptible enterococci (Qualiswab, Becton Dickinson Microbiology System, Maryland, 21030, USA).

Antimicrobial susceptibility testing

Resistance to vancomycin and teicoplanin was detected by the E-test (AB BIODISK, Solna, Sweden) according to the manufacturer’s instructions. An inoculum with turbidity equivalent to that of 0.5 McFarland standard and Mueller-Hinton (MH) agar (Difco Laboratories, Detroit, USA) was used. The results were read after incubation at 37°C for 48 h. The minimum inhibitory concentrations (MICs) obtained by the E-test were rounded to the nearest higher doubling dilution. All vancomycin resistant enterococci (MICs > 6 μg/ml) were also subjected to susceptibility tests by standard agar dilution method according to the current guidelines of the National Committee for Clinical Laboratory
DNA extraction by boiling procedure

Total DNA of enterococcal strains was extracted by the boiling method [12]. Four to 5 well-isolated colonies obtained after overnight growth on (BHI) agar plates containing 6 μg/ml vancomycin were resuspended in 1 ml of sterile distilled water. The cell suspension was kept for 15 min at 100°C. An equal volume of 24:1 mixture of chloroform/isooamyl alcohol was added to the cell lysate and mixed thoroughly. It was followed by centrifugation at 15,000 rpm for 10 min (Remi-C24 Cooling Microfuge, Remi Instrument Ltd., Mumbai, India). The supernatant containing DNA was stored at −20°C until used as template for PCR.

Detection of vancomycin resistance genes by PCR

To detect genes coding for vancomycin resistance, PCR was performed as described by Clark et al. [13]. The oligonucleotide specific primers selected in this study were synthesized by Metabion GmbH, Germany, and have been shown to be highly specific for amplification and detection of vanA, vanB, vanC1 and vanC2 genes when used in a conventional PCR assay system [14]. The primers used were as follows:

vanA Forw 5'-GGG AAA ACG ACA ATT GC-3', vanA Rev 5'-GTA CAA TGC GCG CTT TA-3';
vbB Forw 5'-ATG GGA AGC CGA TAG TTC TG-3', vanB Rev 5'-GAT TTC GTC CTT CGA CC-3';
vanC1 Forw 5'-GTT ATC AAG GAA ACC TC-3', vanC1 Rev 5'-CTT CCG CCA TCA TAG CT-3';
vanC2 Forw 5'-CTC CTA CGA TTC TCT TG-3', vanC2 Rev 5'-CGA GCA AGA CCT TTA AG-3'.

The PCR reaction mixture consisted of PCR Master Mix (Banglore Genei, KT-77) containing 10 mM Tris-HCl buffer (pH 9.0), 50 mM KCl, 1.5 mM MgCl2, a total of 1 mM deoxynucleotide triphosphates (dATP, dCTP, dGTP, and dTTP), 0.5 mM of each primer, 1 U of Taq DNA polymerase, 0.01% gelatin and 10 μl purified DNA solution in a total volume of 50 μl. The amplification of DNA was performed on a BIO-RAD (CA, USA) thermocycler that was programmed for an initial denaturation step of 94°C for 2 min, followed by 30 cycles of 94°C, 55°C and 72°C each for 1 min and a final cycle of 72°C for 10 min. The amplicons were analysed by electrophoresis on agarose gels (1.5% w/v) (Sigma-Aldrich, Poole, UK) in TAE buffer for 45 min at 100 V. A 100 bp DNA ladder (Bangalore Genei, India) was used as molecular size marker. The gels were stained with ethidium bromide (0.5 μg/ml), viewed by UV transillumination and photographed.

Sequencing of PCR products

The PCR products were purified from agarose gel using Qiagen gel extraction kit (Qiagen Inc., Hilden, Germany) and then subjected to forward and reverse DNA sequencing by the fluorescent dideoxyterminator method using an ABI PRISM 3100 capillary sequencer (ACTG, Inc., USA) at the DNA Sequencing Facility of the Department of Biochemistry, University of Delhi South Campus, Delhi, India.

Multiple sequence alignment of van genes

Multiple sequence alignment of vanA, vanB, vanC1 and vanC2 genes of Enterococcus isolates and 3 previously reported Enterococcus sequences was performed using ClustalW software (www.ebi.ac.uk/clustalw).

Risk factor analysis

Clinical details of all patients whose sample grew VRE were noted by regular visits to the patient and chart reviews by a trained hospital infection control nurse. For each VRE positive patient, 3 age and gender matched control patients were selected from the same clinical unit and their data similarly analysed. Risk factors for analysis included prolonged hospital stay (>15 d), urinary catheter, renal failure, transplant surgery, dialysis, and use of antimicrobial agents such as vancomycin, third generation cephalosporin and aminoglycoside. Nosocomial UTI was defined as UTI developing in patients 72 h after hospitalization in a patient whose urine culture was sterile or when a patient was asymptomatic at time of admission.

Statistical analysis

Statistical analysis was performed using Clara Sisa Software (http://home.clara.net/sisa/index.htm). Bivariate analyses were performed separately for each of the variables. ORs, p-value and 95% CIs were calculated for binomial variables. p-values were calculated by use of the χ² test. Variables for which the p-value was <0.05 were considered significant.
Results

During the (8 months) period of the study, 485 faecal samples yielded 28 VRE (18 E. gallinarum, 9 E. casseliflavus and 1 E. faecium). 193 enterococcal isolates were obtained in pure culture from 10, 280 urine samples. On screening, 8 VRE were obtained (E. faecium 6, E. faecalis 2).

Clinical details of VRE from stool samples

28 VRE were isolated and confirmed from 485 samples. The affected units from where faecal VRE were isolated included the gastroenterology unit (8), paediatric oncology unit (8), renal transplant unit (6), emergency wards (3) and medical wards (3 strains); age of the patients (17M, 11F) ranged from 7 months to 65 y with an average of 23.9 y and median of 26.5 y. 14 patients had diarrhoea, 7 had chronic renal failure, 4 had ulcerative colitis, whereas 1 each had cancer, HIV infection, typhoid fever and intussusception. E. gallinarum carrying sunC1 was isolated from 18 patients, E. casseliflavus carrying sunC2 was isolated from 9 patients, and E. faecium carrying sunB was isolated from 1 patient who was admitted in the male medical ward (MMW).

As shown in Table III, prolonged hospital stay and nosocomial UTI; 4 patients developed sepsis and died. All patients were catheterized, 5 had renal failure for which patients underwent dialysis, 4 had surgery on the urinary tract, including renal transplant surgery in 2 patients. Prior treatment with vancomycin, aminoglycosides, third generation cephalosporins and fluoroquinolones were present in 2, 5, and 1 patients, respectively. Four patients had hospital stay exceeding 4 d.

Antimicrobial susceptibility

Three of E. faecium isolates were highly resistant to vancomycin (MIC >512 µg/ml) as well as resistant to teicoplanin (MIC, 32–128 µg/ml). The other 4 E. faecium isolates showed low to intermediate level resistance to vancomycin (MIC, 6–16 µg/ml) and were sensitive to teicoplanin (MIC, 0.5–1 µg/ml). Two E. faecalis isolates exhibited low-level resistance to vancomycin (MIC 8 µg/ml) and were sensitive to teicoplanin (MIC, 0.5 µg/ml). 18 E. gallinarum isolates showed low to intermediate level resistance to vancomycin (MIC values 8–16 µg/ml) and were susceptible to teicoplanin (MIC values 0.5–1 µg/ml). Two E. casseliflavus isolates were intermediately resistant to vancomycin (MIC 16 µg/ml), while the remaining 7 exhibited low level resistance to vancomycin (MIC, 6–8 µg/ml) and low level resistance to teicoplanin (MIC, 0.5–1 µg/ml) as shown in Table II.

Detection of vancomycin resistance genes by PCR

Amplification of vanA, vanB, vanC1, vanC2 genes produced distinct bands corresponding to their respective molecular size (Figures 1 and 2). The entire procedure for the PCR assay was carried out with a negative control containing all the reagents without a DNA template. Vancomycin resistant genotypes were determined by using PCR assays that contained a single primer set. No multiplexing was used. PCR products corresponding to vanA (783 bp), vanB (635 bp), vanC1 (822 bp) and vanC2 (484 bp) targets were obtained. From the 7 isolates of E. faecium, 3 were positive for vanA and 4 were positive for vanB (Figure 1). Two E. faecalis isolates also generated PCR products corresponding to vanB gene. 18 E. gallinarum and 9 E. casseliflavus strains were positive for vanC1 and vanC2 genes, respectively (Figure 2).

DNA sequencing results and GenBank submitted

Only 4 PCR products were sequenced, 1 from each genotype. The PCR product for the vanA gene was 614 bp long and corresponds to nucleotide 235-848 of the complete vanA gene of Enterococcus faecium (1032 bp) as reported by Arthur et al. (1993) (GenBank accession number M97297) with 99% identity.

The vanB PCR product (502 bp) corresponds to nucleotide 259-740 of the complete vanB gene (1029 bp) of Enterococcus faecium as reported by Ballard et al. (2005) (GenBank accession number AY655711) with 99% identity.
Table 1. Clinical details of 8 patients with significant isolation of VRE from urine samples.

<table>
<thead>
<tr>
<th>Pt. No</th>
<th>Age</th>
<th>Gender</th>
<th>Ward</th>
<th>Clinical diagnosis</th>
<th>Isolate</th>
<th>Risk factors</th>
<th>Outcome</th>
<th>Hospital d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>M</td>
<td>DMW*</td>
<td>DM-type II, CRF, ESRD, stone, bilateral pyelonephritis, nosocomial UTI* with sepsis</td>
<td>E. faecium Van A</td>
<td>Urinary catheterization, renal failure, 3&lt;sup&gt;rd&lt;/sup&gt; generation cephalosporin, aminoglycoside</td>
<td>Died</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>M</td>
<td>TSCU*</td>
<td>DM* with nephropathy with ESRD* with retinopathy and nosocomial UTI*</td>
<td>E. faecium Van A</td>
<td>Urinary catheterization, renal failure, renal transplant, ceftriaxone, augmentin</td>
<td>Discharged</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>M</td>
<td>NMW*</td>
<td>Acute intestinal obstruction with acute renal failure after resection and upper GI bleed and DVT* with sepsis (blood culture +ve for Enterococcus) with hospital acquired pneumonia</td>
<td>E. faecium Van B</td>
<td>Urinary catheterization, renal failure, ciprofloxacin, amoxicillin, quinolone, vancomycin</td>
<td>Died</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>F</td>
<td>FMW*</td>
<td>DM-type II, diabetic retinopathy, anasarca and dyspnoea, nosocomial UTI</td>
<td>E. faecium Van B</td>
<td>Urinary catheterization, cefotaxime</td>
<td>Improved</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>3m</td>
<td>F</td>
<td>APC*</td>
<td>Enteric fever encephalopathy, no symptoms of UTI</td>
<td>E. faecalis Van B</td>
<td>Urinary catheterization, ciprofloxacin</td>
<td>Discharged</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>73</td>
<td>M</td>
<td>MSW*</td>
<td>Bilateral renal stone, hydronephrosis with chronic renal failure, nosocomial pyelonephritis with sepsis</td>
<td>E. faecium Van A</td>
<td>Urinary catheterization, renal failure, surgery (stenting), percutaneous nephrostomy, haemodialysis, ceftriaxone, timentin, augmentin, vancomycin</td>
<td>Died</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>38</td>
<td>M</td>
<td>TSCU*</td>
<td>Renal and bladder calculi with obstructive uropathy and chronic renal failure</td>
<td>E. faecium Van B</td>
<td>Urinary catheterization, renal transplant surgery, 3&lt;sup&gt;rd&lt;/sup&gt; generation cephalosporin, renal failure</td>
<td>Survived</td>
<td>15</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>F</td>
<td>FMW*</td>
<td>Post partum fulminant hepatic failure and sepsis</td>
<td>E. faecium Van B</td>
<td>Urinary catheterization, cefotaxime, amikacin</td>
<td>Died</td>
<td>12</td>
</tr>
</tbody>
</table>

#Pt.no. patient's number.

*DM: male medical ward; DMW: diabetes mellitus; CRF: chronic renal failure respectively; ESRD: end stage renal disease; UTI: urinary tract infection; TSCU: transplant intensive care unit; GI bleed: gastrointestinal bleed; DVT: deep vein thrombosis; FMW: female medical ward; APC: advanced paediatric center; MSW: male surgical ward.
The PCR product corresponding to vanC1 gene was 705 bp long and corresponds to nucleotide 205-909 of the complete vanC gene (1032 bp) of Enterococcus gallinarum as reported by Dutka-Malen et al. (1992) (GenBank accession number AF162694) with 99% identity.

Similarly, BLAST results of the vanC2 PCR product (380 bp) showed an identity of 93% between our sequence (nucleotide 6-379) and the reported nucleotide sequence of the vanC2 of Enterococcus casseliflavus (543-916 of a complete gene of 1086 bp) as reported by Navarro and Courvalin (1994) (GenBank accession number L29638).

Discussion

In our study, the majority (75%) of VRE isolates were identified as E. gallinarum (n = 18) and E. casseliflavus (n = 9). Both of these species were from stool samples. This finding is not consistent with the studies conducted by other workers [15,16]. Of all enterococci, the global incidence of E. faecalis is the highest followed by E. faecium, these 2 species accounting for approximately 85% and 10% of clinical isolates, respectively [17]. Screening for VRE excludes sensitive strains of these 2 species, hence their low occurrence in our study. On the other hand, the mechanism of selection by vancomycin favours the selection of higher number of strains of E. gallinarum followed by E. casseliflavus because the VanC phenotype is an intrinsic property of these species. By using a selective broth containing vancomycin, it is very likely to pick up low-level constitutively resistant strains that are generally less pathogenic and less likely to be encountered in clinical samples. Although these low-level vancomycin resistance strains can be clinically important [17], they have not been associated with wide dissemination of vancomycin resistance [15]. Ability of enterococci to colonize the gastrointestinal tract, together with intrinsic and acquired resistance allows these organisms to cause disease despite relatively low intrinsic resistance [18]. Moreover, these results may indicate transfer of VRE by other means, as colonization rates for transferable drug resistance are low. In a recent study from an acute kidney unit where only rectal colonization is being studied, we
have found that colonization with both Van A and van B has become higher (unpublished data). There could also be a sample selection bias as we did not include those isolates from urine that were growing in mixture with other commensals.

By susceptibility studies alone, we could not differentiate between VanA, VanB, VanC1 and VanC2 phenotypes. There is an overlap between the MICs at the bottom of the range for VanB strains and those at the top of the range for VanC1 and VanC2 strains (Table II). Moreover, intrinsic vancomycin resistant E. gallinarum and E. casseliflavus isolates have been found to exhibit a high level of resistance to glycopeptide owing to the presence of both vanA and vanC1 genes or both vanA and vanC2 genes [19,20]. Thus, the vancomycin resistance genotype of an isolate cannot always be inferred from its phenotype, and vice versa. This has led to the increased use of PCR methods for confirmation of the presence of isolates of VRE. PCR analysis has also indicated that MIC determination alone is not sufficient for the unambiguous classification of isolates of VRE. Also, difficulties continue to occur with commercial identification systems, and the not infrequent occurrence of non-motile E. gallinarum and E. casseliflavus isolates and non-pigmentation E. casseliflavus isolates compounds the problem [19]. However, in the present study, species identification correlated well with the genotype.

There are very limited data available from India on VRE [6—8]. The results of the present study also reflect that transposon or plasmid mediated phenotypes such as VanA and VanB, conferring moderate to high level resistance to vancomycin, are not frequently encountered in Indian patient samples compared to intrinsic phenotypes such as VanC1 and VanC2. Until now there are very few patients in our hospital who carry transferable drug resistant enterococci in the intestine, whereas 4% of urine isolates were VRE. In the majority of these patients, UTI was catheter related and nosocomial in origin. It is known that VRE may be transferred through contaminated hands of health care workers and fomites. Four of the 8 patients developed sepsis and died. Therefore, all significant clinical isolates of enterococci should be screened for vancomycin resistance.

Patient risk factors for the acquisition of VRE include prolonged hospital stay, admission to an ICU, exposure to antibiotics and transplantation [21]. Renal dialysis patients are at risk because of their dependency on hospital facilities, the requirement on occasions for admission to an ICU when requiring acute dialysis, and the need for courses of antibiotics especially to treat device-associated infections. Others have cited parenteral vancomycin use and receipt of third generation cephalosporins as risk factors for colonization or infection with VRE [3,22].

The most serious infections in our study group involved patients who had renal failure, urinary catheter, renal transplant, haemodialysis, renal transplant surgery and transplant surgery. This result supports the previous finding of Beltrami et al. [23].

Small numbers of patients with VRE may lead to large confidence intervals in statistical analysis. We identified prolonged hospital stay as an important risk factor for both infection and rectal colonization.

<table>
<thead>
<tr>
<th>Gene (s) detected</th>
<th>Enterococci spp.</th>
<th>No. of isolates</th>
<th>Vancomycin</th>
<th>Teicoplanin</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>vanA</td>
<td>E. faecium</td>
<td>3</td>
<td>&gt;512</td>
<td>128</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;512</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>0.5</td>
<td>2</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>6</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>vanB</td>
<td>E. faecium</td>
<td>4</td>
<td>8</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>2</td>
<td>8</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16</td>
<td>0.5</td>
<td>7</td>
</tr>
<tr>
<td>vanC1</td>
<td>E. gallinarum</td>
<td>18</td>
<td>8</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>0.5</td>
<td>1</td>
</tr>
</tbody>
</table>

*Screening for vancomycin resistant enterococci MIC ≥6 μg/mL.
Table III. Odds ratio calculation with 95% CI and p-value for stool sample.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>State</th>
<th>Control n = 84 (%)</th>
<th>Cases n = 28 (%)</th>
<th>p value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolonged hospital stay (&gt; 15 d)</td>
<td>−</td>
<td>81 (96.4)</td>
<td>18 (64.3)</td>
<td>0.00</td>
<td>15 (3.75-60.08)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>3 (3.6)</td>
<td>10 (35.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary catheter</td>
<td>−</td>
<td>66 (78.6)</td>
<td>21 (73)</td>
<td>0.70</td>
<td>1.22 (0.45-3.33)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>18 (21.4)</td>
<td>7 (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal failure</td>
<td>−</td>
<td>66 (78.6)</td>
<td>22 (78.6)</td>
<td>N.S</td>
<td>1.00 (0.35-2.84)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>18 (21.4)</td>
<td>6 (21.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transplant surgery</td>
<td>−</td>
<td>66 (78.6)</td>
<td>22 (78.6)</td>
<td>N.S</td>
<td>1.00 (0.35-2.84)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>18 (21.4)</td>
<td>6 (21.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dialysis</td>
<td>−</td>
<td>66 (78.6)</td>
<td>22 (78.6)</td>
<td>N.S</td>
<td>1.00 (0.35-2.84)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>18 (21.4)</td>
<td>6 (21.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third generation cephalosporin</td>
<td>−</td>
<td>80 (95.2)</td>
<td>24 (85.7)</td>
<td>0.09</td>
<td>3.33 (0.77-14.34)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>4 (4.8)</td>
<td>4 (14.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>−</td>
<td>80 (95.2)</td>
<td>24 (85.7)</td>
<td>0.09</td>
<td>3.33 (0.77-14.34)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>4 (4.8)</td>
<td>4 (14.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>−</td>
<td>74 (88.1)</td>
<td>24 (85.7)</td>
<td>0.74</td>
<td>1.23 (0.35-4.29)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>10 (11.9)</td>
<td>4 (14.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>−</td>
<td>83 (98.8)</td>
<td>24 (85.7)</td>
<td>0.00</td>
<td>13.83 (1.48-129.67)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1 (1.2)</td>
<td>4 (14.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance:
OR: ≥ 1
p < 0.05 = with 0.00 means extreme significance.
As C% = > 1 lower limit.
N.S. = not significant.

This is consistent with a study from the USA [3] in which longer hospital stay, in a tertiary care hospital, was found to be a significant risk factor for both rectal colonization and infection. Third generation cephalosporins and aminoglycosides emerged as significant risk factors for UTI, but were not significant for rectal colonization. Prior treatment with vancomycin was a significant risk factor for rectal colonization but not for UTI. For VRE UTI, renal failure and dialysis [95% CI 2.39–140.40, p = 0.00] emerged as other significant risk factors; transplant surgery was without significant value. Humphreys et al. [24] have shown that, among renal patients, those who are severely ill and receive multiple and prolonged courses of antimicrobials are at greatest risk for acquiring VRE infection or colonization. However, others found no association between prior intravenous vancomycin use and VRE colonization or infection [25,26].

Urinary catheter did not emerge as a significant risk factor. Studies on the epidemiology of nosocomial UTI caused by vancomycin susceptible enterococci have shown urinary catheterization to be an important predisposing factor present in 82 to 95% of patients [27]. However, in another study it was found that only 31% of patients with nosocomial UTI due to VRE were catheterized. Although all our patients with VRE bacteriuria were catheterized, on statistical analysis it did not appear to be significant compared to the control group.

Molecular typing of the isolates by PFGE will shed more light on the clonal relatedness. However, the study highlights the emergence of VRE as a significant threat in future y in Indian hospitals. Since the problem is just beginning, it is important to institute control measures. The following control measures are recommended:

- Early detection of VRE colonization in patients admitted in high-risk areas such as transplant units, ICUs and oncology units.
- Since vancomycin is used in the gastroenterology ward for treating antibiotic associated colitis, there is a need to watch this reservoir of infection.
- Isolation of a patient who has VRE colonization and develops diarrhoea to restrict further spread of infection.
- Strict barrier precautions and improved hand hygiene practices for a VRE colonized/infected patient.
- Improved cleaning and disinfection of high risk areas to decrease the spread of VRE.
- Excessive usage of broad-spectrum antibiotics to be discouraged.
Acknowledgements
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References