Molecular Characterization of Vancomycin-Resistant Enterococci Isolates from Hematology-Oncology Patients

¹Faten M Ali, ¹Rasha A Nasr, ²Tamer M Ahmed and ¹Walaa S Khater.
¹Microbiology and Immunology and ²Internal Medicine and Hematology Departments, Faculty of Medicine, Ain Shams University.

Vancomycin-resistant enterococci (VRE) are important nosocomial pathogens in many countries with the genotype vanA and vanB being the most important is hospital environment. The objectives of this study is the Molecular characterization of VRE isolated from hematology-oncology patients. Fecal/rectal samples from 50 randomly selected patients together with blood samples from the 11 patients who developed bacteremia. Enterococcal isolates were identified and subjected to antimicrobial susceptibility testing to vancomycin by agar screen method. Vancomycin resistance was confirmed by determining its minimum inhibitory concentration by broth dilution method. Susceptibility of the VRE isolates to different antimicrobials was also determined using the disk diffusion method. Multiplex PCR was used to detect vanA and vanB genes among the isolated VRE strains. Fifty enterococcal strains were isolated from the fecal-rectal samples, of which six (12 %) were VRE (3 E. faecium, 2 E. faecalis and one E. gallinarum). On the other hand, blood cultures from patients with bacteremia were all negative for enterococci. The most significant risk factor for colonization with VRE was previous hospitalization. Other factors included prolonged hospitalization, previous ICU admission, febrile neutropenia, current and previous vancomycin administration. 83.3% of the VRE strains were sensitive to nitrofurantoin, while 83.3% were resistant to ampicillin and erythromycin. vanA gene was detected in two isolates while vanB gene was detected in another three isolates. One isolate was found to be devoid of both vanA and vanB. It is concluded that Vancomycin resistance genes are present among the enterococci colonizing the gastrointestinal tract of hematology-oncology patients. This represents a critical risk factor for hospital acquired infection by these pathogens as well as a threat of spread to other pathogens. Surveillance for VRE in high-risk patients together with effective infection control measures and adapted antibiotic policies are important to control transmission of VRE among patients.

Enterococci are normal inhabitants of the gastrointestinal tract and part of the normal intestinal flora which have emerged as significant nosocomial pathogens. They have become far more important in the hospital setting because of the emergence of strains resistant to vancomycin (Calderon-Jaines et al., 2003).

Since 1989, a rapid increase in the incidence of colonization and infection with vancomycin-resistant enterococci (VRE) has been reported. This increase poses important problems, including i) the lack of available antimicrobial therapy for VRE infections, because most VRE are also resistant to drugs previously used to treat such infections (e.g., aminoglycosides and ampicillin), and ii) the possibility that the vancomycin-resistant genes present in VRE can be transferred to other Gram-positive microorganisms e.g., methicillin resistant Staphylococcus aureus (MRSA), creating a highly dangerous pathogen difficult to manage therapeutically (Willems et al., 2005).

So far, six resistant genotypes have already been described among VRE (van A, B, C, D, E and G), three of them are the most common; the vanA gene which encodes inducible high-level resistance to vancomycin as well as teichoplanin, the vanB gene encodes variable (moderate to high) levels of inducible resistance to vancomycin only and the vanC gene that encodes non inducible low level resistance to vancomycin (Teixeira and Facklam, 2003 and Kolar et, al, 2006).
The VanA and VanB phenotypes are acquired and considered the most clinically relevant due to their conjugative transfer, which may occur via plasmids or transposons. They are usually associated with *E. faecalis* and *E. faecium* strains; while VanC mediated resistance is an intrinsic characteristic of *E. gallinarum* (*vanC1* genotype) and *E. casseliflavus* (*vanC2* and *vanC3* genotypes) strains (Clark et al., 1998, DeLisle and Perl, 2003 and Courvalin, 2006).

The most common clinical impact of VRE is intestinal colonization, which does not result in symptoms but may last for long periods serving as a reservoir for transmission of VRE to other patients. Certain VRE colonized patients are at high risk of infection including hematology and oncology patients (DiazGranados and Jernigan, 2005 and Vagnerova et al., 2006). Bacteremia is one of the major infections caused by these pathogens and is a predominant cause of mortality among haematology-oncology patients (Zaas et al., 2002 and Furtado et al., 2006).

The purpose of this study was to detect colonization and associated blood stream infections with VRE among hematology-oncology patients and to determine antimicrobial susceptibility pattern and molecular characterization of *vanA* and *vanB* genes among the VRE isolates.

**Patients and Methods**

**Patients:**

This study was conducted on 50 patients admitted to the Hematology Unit, Ain Shams University Hospital, during the period from December 2005 to the end of June 2006. Eleven of these patients developed bacteremia. The age of the patients ranged from 19 to 64 years (38.4±12.5). They were 27 male and 23 female. The most common reason for admission was immunosuppressive therapy (90%). The most common underlying diseases were acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML) or non Hodgkin lymphoma (NHL) (Figure 1). Full history was taken from each patient including patient’s name, age, sex, duration of hospital stay, underlying disease, type of treatment, current and previous antimicrobial intake and previous hospitalization, surgery or admission to intensive care units (ICU). Complete blood picture was also recorded.

![Figure 1: Diagnosis of the studied hematology-oncology patients.](image)

**Methods:**

- **Sample collection and processing:** Stool samples or rectal swabs were collected from the patients in clean containers and were directly inoculated on bile aesculin azide agar plates (Biolife endomedex, Egypt). The plates were incubated aerobically at 37°C for 48 hours. Blood samples (5 ml) were taken from patients who developed bacteremia and injected directly into monophasic blood culture bottles (50 ml) (DiagSera). The bottles were incubated aerobically at 37°C for 48 hours. Blood samples (5 ml) were taken from patients who developed bacteremia and injected directly into monophasic blood culture bottles (50 ml) (DiagSera). The bottles were incubated aerobically at 37°C and the blood was subcultured on bile aesculin azide agar after overnight incubation and after 48 hours and twice weekly for 2 weeks. Samples were considered negative if there was no growth for two weeks.

- **Identification of enterococci:** Colonies fulfilling the following criteria were identified as enterococci; i) blackening around the colonies due to hydrolysis of aesculin in the presence of 40% bile in the medium, ii) Gram stained smear showing Gram positive cocci arranged singly, in pairs or short chains, iii) negative catalase test, iv) litmus milk decolorization and v) positive PYR test (L-Pyrrolidinyl-β-Naphthylamide) test (Rosco Diagnostica).
• **Identification of enterococcal species:**
  This was carried out using the conventional biochemical reactions as described by Teixeira and Facklam, (2003); fermentation of sugars; arabinose, lactose, mannitol, raffinose and sorbitol and hydrolysis of arginine (Sigma).

• **Antimicrobial susceptibility testing to vancomycin:**
  Using vancomycin agar screen method according to National Committee for Clinical Laboratory Standards (NCCLS, 2004). Any growth was identified as presumptively resistant and was further confirmed by determining the minimum inhibitory concentration (MIC) using broth dilution method according to (NCCLS, 2004). The enterococci were considered sensitive, intermediate or resistant if the vancomycin MICs were \( \leq 4 \) µg/ml, 8–16 µg/ml and \( \geq 32 \) µg/ml, respectively.

• **Antibiotic susceptibility pattern of the VRE isolates:**
  A disk diffusion method was used according to the modified Kirby-Bauer sensitivity testing method (Cheesbrough, 2000). The used antibiotics disks included ampicillin (10µg), chloramphenicol (30µg), ciprofloxacin (5µg), erythromycin (15µg), nitrofurantoin (300µg), rifampicin (5µg), and tetracycline (30µg) (Oxoid-England). The results were interpreted according to (CLSI, 2005).

• **Detection of vanA and vanB genes in the isolated VRE strains using Multiplex Polymerase Chain Reaction (PCR):**
  Vancomycin resistance genotypes (vanA and vanB) were identified by amplifying the respective genes using High Pure PCR Template Preparation kit (Gibco-BRL, France). Amplification was performed using specific oligonucleotide primers chosen for amplification of the vanA, vanB, which was selected from the published sequences according to Clark et al. (1993). Primer sequences were as follows (Gulf Biotech, Egypt):

  **vanA**: \((5'\rightarrow 3')\) CAT GAA TAG AAT AAA AGT TGC AAT A

  CCC CTT TAA CGC TAA TAC GAT CAA

  **vanB**: \((5'\rightarrow 3')\) GTG ACA AAC CGG AGG AGA

  CCG CCA TCC TCC TGC AAA AAA

  DNA purification was done according Kariyama et al. (2000).

  The multiplex PCR assay was performed in a total volume of 25 µl containing 10mM Tris-HCL (pH 8.3), 50 mM KCL, 1.5 mM MgCl\(_2\), 0.2 mM each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), and 0.625 U of Taq DNA polymerase, 5 pmol of the **vanA** primers, 2.5 pmol of the **vanB** and 2.5 ul of extracted DNA. DNA amplification was carried out with the following thermal cycling profile: initial denaturation at 94°C for 5 minutes, then 30 cycles of amplification (denaturation at 94°C for 1 minute, annealing at 54°C for 1 minute and extension at 72°C for 1 minute), and a final extension at 72°C for 10 minutes in a Gene Amp PCR system (Perkin Elmer 9600 thermocycler).

  The PCR product was visualized by electrophoresis, using 2% agarose gels obtained from (Amersham-Biosciences AB), stained with ethidium bromide and viewed with UV light.

  A 100-2000 bp DNA ladder was run with each gel and VRE genotypes were determined by the size of amplified product (**vanA** gives a band at 1030 bp, **vanB** at 433 bp) and by comparison with positive control. A **vanA** strain (*E. faecalis*, ATCC 51559), a **vanB** strain (*E. faecium* ATCC 51299), and a vancomycin-susceptible *E. faecalis* strain (ATCC 19433) were run with each set of reactions as positive and negative controls (figure 2).
Molecular Characterization of Vancomycin-Resistant Enterococci Isolates

Figure 2: Agarose gel electrophoresis of vanA and vanB by PCR assay. Lane M: is the molecular size marker (100 - 2000 bp). Lane 1: represents vanA positive control strain (E. faecalis ATCC 51559). Lanes 2 & 4: represents vancomycin-susceptible E. faecalis strain (ATCC 19433) (negative control). Lane 3: represents vanB positive control strain (E. faecium ATCC 51299). Lane 5 & 10: represent positive samples for vanA (1030 bp). Lane 6, 7 & 8: represent positive samples for vanB (433 bp). Lane 9: represents negative sample for vanA and vanB.

Statistical Analysis:
Analysis of data was done using SPSS program (Statistical Package for Social Science) using the following tests: Chi square test ($X^2$), Fisher exact probability test, Unpaired t-test, Mann Whitney Wilcoxon U test and Logistic regression analysis which was used to find out the most significant independent predictors of certain outcome which is antibiotic sensitivity, using backward likelihood ratio technique. For all analyses, level of significance was set at 0.05.

Results
All the patients (50) included in the present study were found to be colonized with enterococci. However, the blood samples taken from the 11 patients who developed bacteremia were negative for enterococci. Forty-four (88 %) patients were colonized with VSE while six (12 %) were colonized by VRE. Most of the isolated VSE species were E. faecalis (30 isolates; 68.2 %), followed by six E. faecium (16.3%), four E. durance (9.1%) and the least were E. hircus and E. gallinarum (two isolates; 4.5% each).

E. faecium was the most encountered enterococcal species (three isolates; 50%) among the VRE group while E. faecalis (two isolates) and E. gallinarum (one isolate) represented 33.3% and 16.7%, respectively (Figure 3).

Figure 3: Distribution of enterococcal species among VSE and VRE isolates.

The MIC of vancomycin against the E.faecium isolates was $\geq 64$ µg/ml and against E.faecalis was 64 µg/ml while the MIC against E.gallinarum was 32 µg/ml.
Two of the six VRE isolated from the hematology-oncology patients were from patients with AML (E. faecalis), one from a patient with AML on top of CML (E. faecium), and one from each of a patient with haemolytic anemia (E. faecium), ALL (E. faecium) and biphenotypic acute leukemia (E. gallinarum).

Studying various risk factors for colonization with VRE, revealed that those which were statistically significant included; previous hospitalization, previous ICU admission, current and previous vancomycin administration and febrile neutropenia (Table 1). Prolonged hospitalization was also significantly (P<0.05) related to colonization with VRE, where there was statistically significant difference between length of hospital stay in patients colonized with VRE (19±4.7 days) and those colonized with VSE (11.8±9.2 days). However, there was no statistically significant association between previous surgery, neutropenia, immunosuppressive therapy and vancomycin resistance (Table 1).

Using a logistic regression analysis for risk factors for VRE colonization encountered in this study, it indicated that previous hospitalization is considered an independent predictor of colonization with VRE (Beta coefficient 1.9, 95% CI = 1-3.8, P < 0.05). As regards antibiotic susceptibility of the VRE isolates using disk diffusion method, most of them were sensitive to nitrofurantoin (83.3%) and resistant to ampicillin and erythromycin (83.3% each) (Figure 4).

Table 1: Risk factors associated with colonization with VSE and VRE.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients colonized with VSE (No.= 44)</th>
<th>Patients colonized with VRE (No.= 6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Previous hospitalization</td>
<td>13</td>
<td>29.5</td>
<td>6</td>
</tr>
<tr>
<td>Previous ICU admission</td>
<td>9</td>
<td>20.5</td>
<td>4</td>
</tr>
<tr>
<td>Previous surgery</td>
<td>15</td>
<td>34.1</td>
<td>3</td>
</tr>
<tr>
<td>Current vancomycin intake</td>
<td>19</td>
<td>44.2</td>
<td>5</td>
</tr>
<tr>
<td>Previous vancomycin intake</td>
<td>14</td>
<td>34.1</td>
<td>3</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>35</td>
<td>79.5</td>
<td>6</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>7</td>
<td>5.9</td>
<td>6</td>
</tr>
<tr>
<td>Immunosuppressive therapy</td>
<td>39</td>
<td>88.6</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 4: Antibiotic susceptibility test results for VRE isolates
Molecular Characterization of Vancomycin-Resistant Enterococci Isolates

Of the six VRE isolates, vanA gene was detected in two isolates while vanB gene was detected in another three isolates. One isolate was found to be devoid of both vanA and vanB. The resistant genes were not specifically distributed among the VRE species as illustrated in figure 5.

![Diagram of Enterococcal species and van genes distribution](image)

**Figure 5: Distribution of van genes among the VRE isolates**

**Discussion**

Enterococci have become increasingly responsible for serious clinical and nosocomial infections encountering the third most common cause of hospital-acquired infections (Cookson, 2006). They are also recognized as the third leading cause of nosocomial blood stream infections (Koch et al., 2004 and DiazGranadoz et al., 2005) and recent data indicated that the rate of vancomycin resistance in bloodstream-infecting enterococcal isolates has been one of the highest (Stampone et al., 2005 and Furtado et al., 2006).

The emergence and spread of vancomycin resistance in enterococci has become a significant clinical concern, and vancomycin-resistant enterococci (VRE) have been recognized as an increasingly important universal problem in hospitals worldwide (Palladino et al, 2003).

Molecular methods such as PCR have been increasingly used for the detection and identification of VRE, having several advantages over phenotypic methods. They have overcome the limitations of phenotypic methods for the detection of low-level glycopeptide resistance. Additionally, these methods allowed distinguishing between the different Van types, while they reduce the time required to identify these organisms (Coombs et al, 1999).

In the present study, 50 enterococcal strains were isolated from stool or rectal swabs from the patients who have been admitted to the hematology ward. Six isolates (12 %) were found to be resistant to vancomycin.

Van der Auwera et al (1996), Ieven et al, (1999) and Littvik et al., (2006) reported a similar rate accounting for 12%, 12.8% and 12.2%, respectively. A higher rate of VRE colonization was reported by Khan et al., (2004) where VRE were detected among 25% of all enterococcus species isolated from hematological malignancy patients. Gambarotto et al., (2000) also demonstrated a higher rate among hematology patients accounting for 37%. However this high rate was explained by the fact that the latter study was undergone in a cattle rearing area in France; where avoparcin and vancomycin-related antibiotics have been used in animal husbandry. They also used an enrichment broth step which required a bile aesculin supplemented with 4 mg of vancomycin/liter. On the other hand, Kolar et al. (2006) detected VRE among 4.6% of enterococcal isolates colonizing the GIT of hematological patients.

Van der Auwera et al (1996), Ieven et al, (1999) and Littvik et al., (2006) reported a similar rate accounting for 12%, 12.8% and 12.2%, respectively. A higher rate of VRE colonization was reported by Khan et al., (2004) where VRE were detected among 25% of all enterococcus species isolated from hematological malignancy patients. Gambarotto et al., (2000) also demonstrated a higher rate among hematology patients accounting for 37%. However this high rate was explained by the fact that the latter study was undergone in a cattle rearing area in France; where avoparcin and vancomycin-related antibiotics have been used in animal husbandry. They also used an enrichment broth step which required a bile aesculin supplemented with 4 mg of vancomycin/liter. On the other hand, Kolar et al. (2006) detected VRE among 4.6% of enterococcal isolates colonizing the GIT of hematological patients.

Striking variations in different geographical areas have been clearly observed, making the emergence and spread of antibiotic-resistant enterococci not fully understood (Coque et al., 2005).
The rate of VRE colonization in this study was high among patients with AML. This finding comes in agreement with that of Suntharam et al. (2002) where AML was the most frequent underlying disease in hematology-oncology patients colonized with VRE.

Five different enterococcal species were isolated from the studied hematology-oncology patients. The most common species detected among VSE was *E. faecalis* (68.2%), *E. faecium* (16.3%), *E. durance* (9.1%) and the least common were *E. hirae* and *E. gallinarum* (4.5% each). Perlada et al. (1997) reported that the majority of the isolates were *E. faecalis* (69%) in a study done to determine the antimicrobial susceptibility and molecular epidemiology of enterococci. However, Parkash et al. (2005) reported an alarming increase in the prevalence of unusual enterococcal species of which *E. gallinarum*, *E. hirae* and *E. durans* accounted for 6.2%, 2.5% and 0.8% of all enterococcal isolates, respectively.

Some in vitro studies have suggested that enterococcal virulence determinants (gelatinase, aggregation substance, cytolysin/hemolysin, lipase, extracellular superoxide, and extracellular surface protein) are found more frequently in *E. faecalis* isolates than in *E. faecium* isolates (Elsner et al. 2000b). However, *E. faecium* has been reported to be more often resistant to phagocytosis than *E. faecalis* (Arduino et al. 1994).

*E. faecium* had the highest rate among all VRE isolates of this study, accounting for 50%. Kolar et al. (2006) and Littvik et al., (2006) also found that *E. faecium* was the most common isolate (78%, 94.4%, respectively) among the VRE colonizing the GIT of hemato-oncological and ICU patients. Despite that *E. faecium* has been more frequently associated with vancomycin resistance than *E. faecalis* (Mundy et al. 2000 and DiazGranados et al., 2005), yet, epidemiological studies failed to show a significant association between enterococcal species and relevant clinical outcomes (Garbutt et al., 2000 and DiazGranados and Jernigan, 2005).

Enterococci were not isolated from blood of the 11 patients who developed bacteremia in the current study. Padiglione et al., (2003), reported that there was no blood stream infections caused by enterococci, yet the latter study was designed in hospitals which employ strict infection control procedures. However, Vagnerova et al. (2006) also reported that no enterococcal isolates were obtained from blood samples taken from hemato-oncological patients. Saied (2006) stated that enterococcal blood stream infection rate was less than one percent and no VRE were detected among surgical and ICU patients in two major hospitals in Egypt. Montecalvo et al. (1995) deduced that colonization is at least 10-fold more prevalent than infection among oncology patients. This was also emphasized by Zirakzadeh and Patel (2006) who stated that the ratio of colonized to infected patients may reach as high as 10:1.

The risk factors associated with VRE colonization are often complex and confounded; depending on whether the patient acquires VRE by nosocomial transmission or by primary in vivo emergence (e.g., gene transfer to previously susceptible enterococci) (Harbarth et al, 2002).

Colonization by VRE among the studied hematology-oncology patients has been shown to be significantly associated with prolonged hospitalization, thus it could be considered as a risk factor for colonization with VRE. Montecalvo et al. (1995) also recorded high colonization rates in long
hospitalized patients. Suntharam et al. (2002) included length of hospital stay (31 days) as a risk factor for colonization with VRE, among hematology-oncology patients. However, Metwally (2003) deduced that hospitalization increases the prevalence of faecal colonization with VRE despite its duration.

Most of the patients colonized with VRE (66.7%) had previous history of ICU admission giving significant statistical relation between hospital admission and colonization with VRE. This comes in accordance with Suntharam et al (2002) who found that previous admission to medical ICU was associated with VRE colonization.

Although the association between vancomycin treatment and VRE has been investigated in numerous studies, the true effect of vancomycin on the colonization of VRE remains controversial. In the present study antecedent and current treatment with vancomycin has been found to increase the risk VRE colonization where about 83% of the patients colonized with VRE were receiving vancomycin at the time of the study, and 50% had history of receiving vancomycin. Many reports also describe an association between prior vancomycin use and VRE colonization (Tokars et al., 1999, D'Agata et al., 2001 and Trick et al., 2004) whereas others did not find such effect (Gambarotto et al., 2000, Ostrowsky et al., 2001 and Carmeli et al., 2002).

Those studies that compared VRE colonized patients with those colonized with VSE found a stronger association than those that compared them to whom no VRE were isolated. In addition, the length of stay (LOS) has a confounding effect on the association between vancomycin and VRE, owing to the strong correlation between LOS and VRE colonization and between LOS and vancomycin use. Studies that were adjusted LOS found a non-significant association between vancomycin treatment and VRE colonization (Harbarth et al., 2002). Treatment with other antibiotics, especially third-generation cephalosporins and metronidazole might also influence the correlation between vancomycin intake and VRE colonization as was stated by Carmeli et al. (2002). Thus the reported association between vancomycin use and risk for VRE colonization may be distorted by the selection of inappropriate control groups (i.e., patients colonized with VSE), the effect of duration of hospitalization and other antibiotic intake.

All patients colonized with VRE had febrile neutropenia providing a statistically significant association between febrile neutropenia and VRE colonization. Although the rate of neutropenia was higher in VRE colonized patients than those colonized with VSE yet the difference did not attain statistical significance. The relation between neutropenia and VRE colonization among haematology patients was studied by Suntharam et al (2002) and neutropenia was also not identified as a risk factor. This could be explained by the fact that neutropenia is typically associated with a haematology oncology diagnosis. On the other hand, neutropenia was found to be risk factor for infection with VRE in other previous studies, as that done by Husni et al. (2006), who reported that colonized neutropenic patients with cancer are prone to VRE infection.

History of previous hospital admissions was found among all patients colonized with VRE versus 29.5 % of patients colonized with VSE. This suggested significant association between previous hospital admission and colonization with VRE. Moreover, previous hospitalization was found to be an independent predictor of vancomycin resistance using a logistic regression analysis for risk factors of VRE.
colonization encountered in this study. Dan et al. (1999) reported that previous hospitalization was the main risk factor for VRE colonization in high risk population. Padiglione et al. (2003), in a study conducted upon patients admitted to high risk units (hematology, renal, transplant and intensive care units), also deduced that the rate of colonization with VRE increased with previous hospital admission.

Most of the VRE strains isolated in the present study were resistant to ampicillin and erythromycin (83.3%). High resistance rates among different enterococcal species to erythromycin were reported by Elsner et al. (2000a) and d’Azavedo et al (2004). This indicates that these antimicrobials cannot be recommended in empiric therapy for enterococcal infections. On the other hand, 83.3% of VRE isolated in this study were sensitive to nitrofurantoin. d’Azavedo et al (2004), as well found that most of enterococci isolated form different clinical samples were sensitive to nitrofurantoin. This supports the assumption that the use of this antimicrobial could be effective in the treatment of uncomplicated urinary tract infections caused by this organism (Murray and Bartlett, 2004).

From an epidemiological point of view, the most important genotypes among VRE are vanA and vanB, as they represent acquired and transferable resistance (DeLisle and Perl, 2003). In this study, vanA was detected in two isolates and vanB in another three isolates, whereas one isolate (E.gallinarum) was found to be devoid of both vanA and vanB. This might be attributed to the presence of low level resistance genes such as vanC which was reported to be an intrinsic characteristic of E.gallinarum strains (vanC1 genotype) (Clark et al., 1998 and Cetinkaya et al., 2000).

Perlada et al. (1997) reported the majority of the enterococcal isolates in a citywide survey harboured the vanB genotype. Lee et al. (1998) also found that most of the VRE strains isolated from different clinical samples carried the vanB genotype. On the other hand, Yoo et al. (2005) reported that although vanB was detected in many cases yet vanA was the major genotype among VRE isolates from hematology patients. In addition, the vanA genotype was the predominant type (63%) of the VRE isolated from hemato-oncological patients in the study done by Vagnerova et al., (2006).

The findings of the presented study bring an important issue, which is the occurrence of vanA and vanB genes among the enterococci colonizing the gastrointestinal tract. This provokes a serious problem due to the possibility of spread of these resistance genes to other enterococci and to other pathogens as MRSA. Thus testing the presence of vancomycin resistant genes in MRSA isolated from clinical specimens is recommended.

Moreover, colonization by VRE is considered a critical risk factor for infection by these pathogens especially in high risk patients. This necessitates strict infection control measures and adapted antibiotic policies to control transmission of VRE among patients. Elimination of patient’s carriage would also be beneficial for controlling the spread of VRE. In addition, nosocomial infections caused by VRE can be greatly reduced through routine surveillance for colonization in high-risk patients and contact isolation of colonized or infected patients.

For better understanding of VRE emergence, spread and control, further studies are required to clarify the epidemiology of VRE, to detect low level resistance genes and to determine the susceptibility pattern of
enterococci for newly developed antimicrobial agents.

References


الوصف الجزيئي للمكورات المعوية المقاومة للفانكوميسين المعزولة من مرضى أمراض و أورام الدم

فان علي، ُرشا نصر، نادر أحمد، واء خاطر

فرض الكائنات الديفية والمناعية وفرض أمراض الدم الباطنة، وحدة أمراض الدم، كلية الطب، جامعة عين شمس

اصبحت المكورات المعوية المقاومة للفانكوميسين [VRE] من أهم مسببات عددًا من المستشفيات في بلدان عديدة مما يمثل عائقًا لعلاج العدوى المزمنة التي تسببها هذه المكورات. ولقد تم تحديد ستة أنواع جينية مختلفة للمكورات المعوية المقاومة . 

vanB و vanA للفانكوميسين هما الـ [VRE] واناليتيك ميتوما وتمكن من استخدام الأجزاء الموزعة في الفانكوميسين. 

وذلك استهدفت هذه الدراسة تحديد حدوث الإستطان وتجنح الدم بالمكورات المعوية المقاومة للفانكوميسين [VRE] في مرضى أمراض و أورام الدم و اختبار استجابة المكورات المعوية المقاومة للفانكوميسين التي تم عزلها للمضادات الحيوية المختلفة وكذلك الكشف الجزيئي عن جينات الـ vanB and vanA في هذه المكورات. 

تضمنت هذه الدراسة 50 حالة من مرضى أمراض و أورام الدم تم أخذ عينات تراز أو مسحات من المستقيم و زراعتها. كما تم عمل مزدوجة دم لنفس المريض في حال حدوث تجاثر للدم و تم عزل المكورات المعوية و التعرف عليها ثم تحديد مقاومتها للفانكوميسين عن طريق استخدام الأجزاء الموزعة بالفانكوميسين و التأكد من النتائج عن طريق تعريض الأغلفة المتنوعة. 

وقد اظهرت نتائج هذه الدراسة جوهرًا لاستيان حساسية للعوامل المقاومة للفانكوميسين. التلقيح الجيني عن طريق اختيار مريض. كما تم الكشف عن جينات مختلفة عن طريق اختبار تشكي الفنون. كما أن التلقيح على الفنون عدى عن vanB و vanA.

وأظهرت النتائج عزل خصوصا من المكورات المعوية من عديد البراز بينما لا يتم عزل أي مكورات معوية من مزارع الدم للمرضى المصابين بعديد الدم البكتيري المصاحبة. كما وجد أن معدل الاستطان بالمكورات المعوية المقاومة للفانكوميسين في مرضى أورام و أمراض الدم هو 12% و أن أغلب المكورات أظهرت حساسية للتروفوكلوتين و مقاومة للأمبيسيلين في vanB و إيرثانيوس. كما أظهرت نتائج الفنون السلبية وجد جين vanA في أثلاث مختبرات و برائه في مختبرات الفانكوميسين و وجد أن أهم الهدف هو أن يكون الفنون قد سبق له خروج المضيف و شملت الانتشار المتعدد الأخرى الأقامة. 

وقد استخلصت الدراسة وجود جينات مراقبة للفانكوميسين في المكورات المعوية التي تستوطن أمعاء مرضى أمراض و أورام الدم مما قد يمثل مشكلة خطيرة لأنه قد يسبب عديده من المكورات في هؤلاء المرضى. بالإضافة إلى امكانية انتقال هذه الجينات إلى مرضى أورام الدم، والمكورات المعوية المقاومة للميثيسيلين أ. كما استقصت هذه الدراسة تحريроغونى عن المكورات المعوية المقاومة للفانكوميسين بين المرضى ذوي الاختبار الالعالي و عزل المرضى الحاملين أو المصابين بهذه المكورات بالإضافة إلى ضرورة تنفيذ إجراءات مكافحة العدوى في المستشفيات والاستخدام الحذر لمضادات المكورات المختلفة من أجل تقليل انتقال المكورات المعوية المقاومة للفانكوميسين بين المرضى.