

# Frequency and Antibiogram of Vancomycin Resistant Enterococcus in a Tertiary Care Hospital

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## ABSTRACT

**Objective:** To determine the frequency of Vancomycin Resistant Enterococcus (VRE) in a tertiary care hospital of Rawalpindi, Pakistan.

**Study Design:** Observational, cross-sectional study.

**Place and Duration of Study:** Department of Microbiology, Army Medical College, Rawalpindi, from May 2011 to May 2012.

**Methodology:** Vancomycin resistant Enterococcus isolated from the clinical specimens including blood, pus, double lumen tip, ascitic fluid, tracheal aspirate, non-directed bronchial lavage (NBL), cerebrospinal fluid (CSF), high vaginal swab (HVS) and catheter tips were cultured on blood agar and MacConkey agar, while the urine samples were grown on cystine lactose electrolyte deficient agar. Later the antimicrobial susceptibility testing of the isolates was carried out using the modified Kirby-Bauer disc diffusion method on Mueller Hinton agar.

**Results:** A total of 190 enterococci were isolated. Of these, 22 (11.57%) were found to be resistant to vancomycin. The antimicrobial sensitivity pattern revealed maximum resistance against ampicillin (86.36%) followed by erythromycin (81.81%) and gentamicin (68.18%) while all the isolates were 100% susceptible to chloramphenicol and linezolid.

**Conclusion:** The frequency of VRE was 11.57% with the highest susceptibility to linezolid and chloramphenicol.

**Key Words:** *Enterococcus. Glycopeptide resistance. Linezolid. Vancomycin resistant Enterococcus.*

## INTRODUCTION

The Enterococcus as a genus was described in the 1980s to house Gram positive cocci agglutinating with the Group-D antiserum used for the Lancefield grouping of Streptococci.<sup>1-3</sup> The two most commonly isolated species are *Enterococcus faecalis* (80 – 90%) and *Enterococcus faecium* (5 – 15%).<sup>3-6</sup> They are the streptococci that grow at both 10-45°C, pH 9.6, and 6.5% NaCl. They survive at 60°C for 30 minutes and hydrolyze esculin in the presence of bile.<sup>1</sup>

Normally present in the human intestine and the female genital tract, they were hardly considered a challenge in the clinical practice.<sup>4,5</sup> However, the acquisition of the glycopeptide resistance has suddenly made them important, not only because of limiting the therapeutic options, but also the possibility of transferring this resistance to other organisms e.g. methicillin resistant *Staphylococcus aureus* (MRSA).<sup>7</sup> Nosocomial infections being a global phenomenon, our hospitals are witnessing more and more patient turnover with heavy empirical antibiotic usage.<sup>8</sup>

Until recently, the effective antibiotics against enterococci were the glycopeptides (vancomycin and

teicoplanin), but the resistance against them has considerably reduced their use.<sup>9</sup> Vancomycin Resistant Enterococci (VRE) were first isolated in USA in 1980s and in 1986 in Europe.<sup>9,10</sup> It has been reported that 60% of the enterococcal infections are nosocomial, with half of them occurring in the intensive care units (ICUs).<sup>11</sup> They are being reported as the second most frequent cause of wound and nosocomial urinary tract infections and the third most frequent cause of blood stream infections.<sup>10</sup> The occurrence of VRE is associated with the injudicious use of glycopeptides, immunosuppression and prolonged hospitalization.<sup>12,13</sup> Avoparcin, a glycopeptide used as a growth promoting and prophylactic drug for the live stock in Europe till 1997, may also have contributed in the development of VRE.<sup>14</sup>

The purpose of this study was to assess the frequency of the infections caused by Vancomycin Resistant Enterococcus (VRE) in a tertiary care hospital of Pakistan and also to ascertain the activity of various antibiotics against different isolates from routine samples.

## METHODOLOGY

This descriptive, cross-sectional study was carried out in the Department of Microbiology, Army Medical College, National University of Sciences and Technology Islamabad, from May 2011 to May 2012. Received clinical specimens like blood, pus, double lumen tip, ascitic fluid, tracheal aspirate, non-directed bronchial lavage (NBL), cerebrospinal fluid (CSF), high vaginal swab (HVS) and catheter tips were cultured on Blood

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and MacConkey agar, while the urine samples on Cystine lactose electrolyte deficient agar. Later the isolates were identified by a positive Gram reaction, a negative catalase test and a positive bile aesculin hydrolysis test followed by their serological confirmation by agglutination with Group-D antiserum. Duplicate samples of the same patient during the same episode of illness, vancomycin sensitive *Enterococcus* species and enterococci from faecal samples were excluded.

Antimicrobial susceptibility testing of the isolates was carried out using the modified Kirby-Bauer disc diffusion method. Bacterial suspensions equivalent to 0.5 McFarland turbidity standard were prepared and inoculated on Mueller Hinton agar plates. Antibiotic discs of Linezolid (30 µg), Minocycline (30 µg), Vancomycin (30 µg), Chloramphenicol (30 µg), Tetracycline (30 µg), Erythromycin (30 µg), Ampicillin (25 µg) and Gentamicin (120 µg) (Oxoid, UK) were applied followed by incubation at 35°C for 24 hours. The results were interpreted following the Clinical and Laboratory Standards Institute (CLSI) guidelines. The isolates were considered resistant to vancomycin if the zones of inhibition around the vancomycin disc were ≤ 14 mm and susceptible if ≥ 17 mm. American Type Culture Collection (ATCC) *Enterococcus faecalis*-29212 was used as the quality control strain.

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 19. Qualitative variables for example clinical specimens and antimicrobial susceptibility were expressed as frequency and percentages.

## RESULTS

A total of 190 enterococci were isolated during the study period. The maximum number of enterococci were from urine (79) followed by double lumen tip (41), blood and pus (24 each) and 11 were isolated from HVS, ascitic fluid, tracheal aspirate, non-directed bronchial lavage and CSF. Out of these, 22 (11.57%) were found to be resistant to vancomycin. Among VRE, maximum resistance was seen against ampicillin 86.36% followed by erythromycin 81.81% and gentamicin 68.18% while all the isolates were 100% susceptible to chloramphenicol and linezolid (Table I). Maximum number of VRE was isolated from double lumen tip followed by blood and pus while the minimum from body fluids (Table II).

**Table I:** Antibiogram of vancomycin resistant *Enterococci* (n=22).

| Antibiotic disc used | Number of resistant isolates | Resistance (%) |
|----------------------|------------------------------|----------------|
| Linezolid            | 0                            | 0              |
| Chloramphenicol      | 0                            | 0              |
| Ampicillin           | 19                           | 86.36          |
| Erythromycin         | 18                           | 81.81          |
| Gentamicin           | 15                           | 68.18          |
| Tetracycline         | 8                            | 36.36          |
| Minocycline          | 6                            | 27.27          |

**Table II:** Frequency of the VRE in different clinical samples (n=22).

| Sample                        | Frequency | Percentage |
|-------------------------------|-----------|------------|
| Double lumen Tip              | 07        | 31.81      |
| Blood                         | 05        | 22.72      |
| Pus                           | 04        | 18.18      |
| Urine                         | 03        | 13.63      |
| Ascitic fluid                 | 01        | 4.54       |
| Tracheal aspirate             | 01        | 4.54       |
| Non-directed bronchial lavage | 01        | 4.54       |

## DISCUSSION

Infections due to VRE are being increasingly reported worldwide.<sup>4</sup> Vancomycin resistance is mediated primarily by those strains that have the ability to synthesize the cell wall by using D-alanine-D-lactate.<sup>4</sup> The first report of vancomycin-resistant *Enterococcus faecium* isolated in Pakistan was published in December 2002, from a patient admitted to the intensive care unit (ICU) of the Aga Khan University Hospital, Karachi.<sup>15</sup>

This study revealed a high frequency of VRE (11.57%) in a tertiary care hospital of Rawalpindi. A comparison with studies from different parts of the world including Pakistan revealed contrasting results. Majeed *et al.* reported a frequency of 1.7%,<sup>16</sup> which is much lower as compared to the present study. However, no VRE were isolated in a previous study from Rawalpindi, conducted by Aamer *et al.*<sup>17</sup> Another study by Abdulla *et al.* from Karachi, showed a frequency of VRE of only 0.9% which is quite low in comparison to the present study.<sup>18</sup> Akhi *et al.* on the other hand, reported a frequency of 4.38%.<sup>19</sup> The contrast observed in the frequencies of the above mentioned studies in comparison to ours indicates the fact that the frequency of VRE is increasing with time. A surveillance study conducted in United States of America from 1996 – 2002 revealed a frequency of 2% which is lower as compared to ours because ours is a hospital based study.<sup>20</sup>

In a study conducted only on the urinary isolates by Zhanel *et al.*, in the University of Manitoba, USA, 3% of VRE isolates were found to be resistant to linezolid, 2.4% to chloramphenicol, 47.6% to gentamicin and 85.8% to ampicillin.<sup>21</sup> In contrast to their findings our VRE isolates were found to be 100% susceptible to both linezolid and chloramphenicol, 32% susceptible to gentamicin and the susceptibility to ampicillin was the same (Table I). The prevalence of VRE associated infections and colonization is still very low in Canada (< 1%). Because of this low prevalence, some researchers consider these VRE as colonizers rather than pathogens.<sup>22,23</sup>

The increasing incidence of VRE is a big threat as they appear to be able to transfer vancomycin resistance to unrelated bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA). This can make the situation even worse by creating vancomycin inter-

mediate(VISA) and vancomycin resistant strains of *Staphylococcus aureus* (VRSA).<sup>23</sup> Twelve such cases have been reported in the United States, 8 of which occurred in southeast Michigan.<sup>24,25</sup>

The injudicious use of broad spectrum antimicrobial agents and improper hygienic measures are important contributors to this increased frequency of VRE in the local set up. This high frequency of VRE is adding to the morbidity of patients because of the high treatment cost and prolonged duration of illness. This study has revealed highest susceptibility of VRE to linezolid which costs almost half as compared to vancomycin thus, suggesting an economical empirical antimicrobial agent for the management of enterococcal infections. Without extensive control measures, emergence and spread of VRSA may be the next stage in the global crisis of antimicrobial resistance. The knowledge of frequency and antibiogram of VRE, reported in this study may help clinicians in planning the control measures for its spread to reduce the associated mortality and morbidity.

### CONCLUSION

The frequency of VRE in this study was 11.57% with the highest susceptibility to linezolid and chloramphenicol.

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