High-level resistance to aminoglycoside, vancomycin, and linezolid in enterococci strains

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ABSTRACT

Objective: This study aimed to identify antibiotic susceptibility rates of enterococcal strains, and to compare the high-level resistance to aminoglycosides (HLAR) in vancomycin-sensitive enterococcal species (VSE) and vancomycin-resistant enterococcal species (VRE).

Methods: The study included 100 VRE and 100 VSE strains recovered from the samples sent to laboratory from various departments of Haydarpaşa Numune Training and Research Hospital.

Results: All VRE strains were defined as Enterococcus faecium, although of the VSE strains, 53% were identified to be as Enterococcus faecalis, 42% E. faecium, 3% Enterococcus durans, and 2% Enterococcus avium. High-level resistance to vancomycin (MIC, >256 µg/ml) was determined in all VRE strains and when analyzing MIC values for teicoplanin, five strains were found to be moderately susceptible (MIC, 16 µg/ml) and 95 strains were resistant (MIC, >32 µg/ml). Of the VRE strains, one was linezolid-resistant (MIC, 12 µg/ml) and the other was intermediately susceptible (MIC, 4 µg/ml) and remainders were evaluated to be susceptible (MIC, <2 µg/ml). In VRE strains, high-level gentamicin resistance (HLGR) was found to be 83% and high-level streptomycin resistance (HLSR) 89%, association of HLSR with HLGR was 78%. In VSE strains, HLGR was found to be 42% and HLSR 48%, the association of HLSR with HLGR was found to be 36%. HLAR in VRE strains was found to be higher as compared with VSE strains (p <0.005).

Conclusion: Antimicrobial resistance is increasing in enterococci strains. Therefore a follow-up is required resistance pattern including both vancomycin resistance and HLAR.

Key words: Enterococcus spp., vancomycin, linezolid, aminoglycoside, resistance
INTRODUCTION

Enterococci found in the normal flora of the gastrointestinal system have recently become one of the major nosocomial pathogens. These bacteria were isolated from the urinary tract infections, intra-abdominal and pelvic infections as well as the causative agents of endocarditis, surgical wound infections, bacteremia, neonatal sepsis and rarely of meningitis.1,2 The drawback of the control and treatment of enterococcal infections is their intrinsic resistance to various antibiotics, their capabilities to develop new resistance and to live in the external environment for a long time.

The cell wall inhibitors such as penicillin, ampicillin, or vancomycin have been administered in combination with the aminoglycosides such as streptomycin and gentamicin in the treatment of serious infections caused by enterococci.3 A synergistic effect between the cell wall synthesis inhibitors and aminoglycosides disappears in the presence of high-level resistance to aminoglycoside and causes difficulties in the treatment of severe enterococcal infections.4

In this study we attempted to determine antibiotic sensitivity rates of vancomycin-sensitive enterococci (VSE) recovered from a variety of clinical material in Haydarpaşa Numune Training and Research Hospital, Istanbul, Turkey and antibiotic sensitivity rates in vancomycin-resistant enterococci (VRE) by the disk diffusion method and to identify minimum inhibitory concentration (MIC) values for vancomycin, teicoplanin and linezolid in VRE strains by means of E-test method and to compare a high-level aminoglycoside resistance rates in VRE and VSE species.

METHODS

The study was performed in the laboratory of clinical microbiology of Haydarpaşa Numune Training and Research Hospital in 2008. The study was comprised of 200 Enterococcus species recovered from clinical materials and rectal swab samples. Stuart’s transport medium was used in collecting rectal swab samples. The samples were cultivated onto bile esculin agar media containing 100 mg/ml azide and were incubated at 35 °C for 24 hours. Black-colored colonies grown in bile-esculin agar were evaluated in terms of enterococci. The clinical materials were seeded in appropriate media and were incubated at 35 °C for 24 hours. The colonies suspected of enterococci were identified with Gram staining, catalase, PYR and, bacterial growth in 6.5% NaCl and API 20 Strep (bioMerieux®, France).

The antimicrobial susceptibility tests were carried out in accordance with Clinical and Laboratory Standards Institute (CLSI) criteria applying the Kirby-Bauer disk diffusion method by means of penicillin, ampicillin, nitrofurantoin, tetracycline, ciprofloxacin, vancomycin, and teicoplanin discs (Oxoid Ltd, Basingstoke, UK) in all enterococcal species.5 Brain-heart infusion (BHI) agar containing 500 µg/ml of gentamicin and BHI agar containing 2000 µg/ml of streptomycin were used in an attempt to determine high-level gentamicin resistance (HLGR) and high-level streptomycin resistance (HLSR), respectively. The BHI agar containing 6 µg/ml of vancomycin was used to determine resistance to vancomycin. The MIC values for vancomycin, teicoplanin and linezolid in VRE strains were evaluated by the vancomycin agar screen test in accordance with the CLSI recommendations for the E-test method (AB Biodisk®, Sweden).6

Beta-lactamase resistance was examined using nitrocefin disks. (Becton Dickinson®, USA). In our study, standard Enterococcus faecalis ATCC 29212 strain was used as the control. Chi-square test was used for statistical analysis. The results were evaluated at the significance level of p<0.05.

RESULTS

The study was comprised of 100 VRE and 100 VSE strains. All of VSE strains were isolated from the clinical materials. Of the VRE strains, 57 were collected from rectal swabs, 43 were collected from clinical materials respectively (Table 1). All VRE strains were defined as Enterococcus faecium and had high-level vancomycin resistance (MIC, >256 µg/ml). When the minimal inhibitory concentration of teicoplanin was examined in VRE strains, Five of 100 VRE strains were found to be moderately susceptible (MIC, =16 µg/ml) and 95 strains were determined to be resistant (MIC, >32 µg/ml) against teicoplanin. One of the VRE strains that had high-level resistance to vancomycin and teicoplanin was linezolid-resistant (MIC, =12 µg/ml) and another was moderately susceptible (MIC, =4 µg/ml) the remainders were evaluated as susceptible (MIC, <2 µg/ml) to linezolid. Penicillin, ampicillin, tetracycline, teicoplanin, and nitrofurantoin resistance was found to be 100% and erythromycin and ciprofloxacin resistance was 99% and linezolid resistance was 2% in VRE strains using the disk diffusion method.

Of the 100 VSE strains, 51 were obtained from urine specimens. When VSE strains were examined on the basis of the isolation sites, most of E. faecalis were isolated from the urine, wound and bile culture samples, (63%, 55%, and 63% respectively) and
most of *E. faecium* were isolated from blood culture samples (56%). Of the 100 VSE strains, *E. faecalis* was responsible for 53%, *E. faecium* 42%, *Enterococcus durans* 3%, and *Enterococcus avium* 2%. Penicillin resistance was 33%, ampicillin resistance was 24%, erythromycin resistance was 92%, tetracycline resistance was 78%, ciprofloxacin resistance was 71%, nitrofurantoin resistance was 27% in VSE strains by the disk-diffusion method.

**Table 1.** Distribution of enterococcus strains to isolation sites (n=200)

<table>
<thead>
<tr>
<th></th>
<th>Rectal swap</th>
<th>Blood</th>
<th>Urine</th>
<th>Other samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin-resistant enterococci (n=100)</td>
<td>57</td>
<td>25</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Vancomycin-sensitive enterococci (n=100)</td>
<td>-</td>
<td>25</td>
<td>51</td>
<td>24</td>
</tr>
</tbody>
</table>

Gentamycin and streptomycin resistance in *E. faecium* and *E. faecalis* was established to be 20% and 14% in VSE strains respectively (Table 2). When VRE was compared to VSE, the rate of HLSR was detected to be 89% in VRE, while it was 48% in VSE; the rate of HLGR was noted to be 83% in VRE and it was 42% in VSE. The association of HLGR with HLSR namely high-level aminoglycoside resistance (HLAR) was 78% in VRE, and 36% in VSE strains respectively. HLAR was found to be significantly higher in VRE strains than those in VSE strains (p<0.005; Table 3).

**Table 2.** Distribution of high-level aminoglycoside resistance (HLAR) among vancomycin-sensitive enterococci (n=100)

<table>
<thead>
<tr>
<th>Variable</th>
<th>E. faecalis</th>
<th>E. faecium</th>
<th>E. avium</th>
<th>E. durans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gen R-Strep R</td>
<td>14</td>
<td>20</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gen S-Strep S</td>
<td>31</td>
<td>12</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Gen R-Strep S</td>
<td>1</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gen S-Strep R</td>
<td>7</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Gen R=gentamicin resistant; Strep R=streptomycin resistant; Gen S=gentamicin sensitive; Strep S=streptomycin sensitive.

**Table 3.** Comparison of high-level aminoglycoside resistance (HLAR) between vancomycin resistant enterococci (VRE) and vancomycin-sensitive enterococci (VSE) (n=200)

<table>
<thead>
<tr>
<th>Variable</th>
<th>VRE (n=100)</th>
<th>VSE (n=100)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gen R-Strep R</td>
<td>78</td>
<td>36</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gen S-Strep S</td>
<td>6</td>
<td>46</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gen R-Strep S</td>
<td>5</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>Gen S-Strep R</td>
<td>11</td>
<td>12</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Gen R=gentamicin resistant; Strep R=streptomycin resistant; Gen S=gentamicin sensitive; Strep S=streptomycin sensitive.

### Discussion

Ampicillin resistance in enterococci may be caused either by change in penicillin-binding proteins or rarely by production of a beta-lactamase enzyme. VRE have multiple antibiotic resistance comprising the aminoglycosides (including high level of resistance) and ampicillin. The penicillin and ampicillin resistant VSE strains were determined to be 33%, 23% and ampicillin resistant VRE strains to be 100% by disk diffusion method in our study. In two different studies penicillin resistance was noted to be 25.7% and 28.8% and ampicillin resistance was noted to be 18.6% and 28.8%. The other study on VRE strains ampicillin resistance consistent with our study.

Glycopeptide resistance in enterococci is one of the most important challenges. VRE takes place among the important nosocomial pathogens, in that the treatment options are limited, it easily spreads in the hospital setting and it is likely to transfer vancomycin resistance to other pathogens. VRE is known to spread in the hospital setting through contaminated hands and surfaces. Centers for Disease Control and Prevention (CDC) suggests that aggressive infection control be implemented and that hospital staff conform to the isolation precautions in order to control and prevent VRE infection. VRE was first reported in 1998 in our country, followed by a multi-hospital VRE outbreaks. The first VRE strain was isolated in our hospital in 2005. After the establishment of VRE, contact isolation was implemented for the patients and rectal swab samples were collected from clinics where VRE was detected particularly in the intensive care unit and screenings were performed.

*E. faecalis* (80-90%) and *E. faecium* (10-15%) in enterococci were the species that were clinically isolated highest. Although *E. faecalis* was frequently isolated as infectious agents, vancomycin resistance was detected at a higher rate in *E. faecium*. We detected all VRE strains as *E. faecium* and *E. faecalis* was the most in VSE strains.

HLAR is caused by the secretions of various aminoglycoside-modifying enzymes. The rate of HLAR in VRE was determined to be higher as compared to that of VSE strains. In the study by Mihajlovic et al. on VRE strains HLGR was identified to be 87.6%, and HLSR to be 95.2%, while in the study by Yildirim et al. HLSR and HLGR in VSE strains were determined to be 19.8%, 9.9%, respectively. The rate of HLAR in VRE strains was significantly higher than VSE strains in our study. Vancomycin resistance in enterococci as well as
HLAR poses difficulty in the treatment of especially severe infections.

HLAR in *E. faecium* strains appears to be higher than that of *E. faecalis*. In the study by Mendiratta et al. of 150 of enterococcal species, 69 (49%) had high-level resistance to gentamicin and/or streptomycin. In their study, HLGR (95.5%) was significantly higher in *E. faecium* species as compared with *E. faecalis* species (37.5%). A study performed in Turkey found that 52% of *E. faecium* were HLGR and 74% were HLSR and 20% of *E. faecalis* species were HLGR, and 31% were HLSR.

In that study no vancomycin resistance was also found in *E. faecium* and *E. faecalis* species. We detected *E. faecium* to be 57.7% in VSE strains where a HLAR was detected. Our results were regarded as compatible with other studies.

Treatment choice in VRE is limited. Linezolid, a ribosomal protein synthesis inhibitor, was approved by FDA in the USA for use in some VRE infections. However, enterococci may be resistant to linezolid, which reduces the treatment options. In our study, one of the VRE species was linezolid-resistant and, the other was identified as moderately sensitive. In our hospital, following isolation of linezolid-resistant enterococci not only were training programs on infection control measures intensified but also policies regarding antibiotic use were revised. It is considered that, although there has been no linezolid resistance after taking these measures, precaution for infection control is necessary to ensure continuity.

The limitation of our study is that no molecular methods have been used. It is of significance to identify the type of vancomycin resistance to enterococci in treatment. The strains containing vanA and vanB genes carry a high level of resistance to vancomycin, while those carrying VanC gene show a low level of resistance to vancomycin. The strains with vanA gene are resistant to teicoplanin as well as vancomycin while those with VanB gene are susceptible to teicoplanin. It is likely to identify microorganisms by molecular methods and to determine the relationship between them. Thus, molecular methods yield results earlier as compared with routine methods, allowing isolation measures in colonized patients without delay. However, molecular techniques are associated with increased cost, limiting its use.

Being familiar with the characteristics of resistance in enterococci, the accurate determination of the results of antibiotic susceptibility will provide the appropriate treatment of infections produced by these pathogens. VRE continues to pose a threat to our hospital. A higher resistance rate to other antibiotics in VRE strains, particularly to aminoglycosides, is also troublesome. Limited treatment options for serious infections caused by resistant enterococci may make it necessary to intensify infection control procedures and a follow-up of resistance.

REFERENCES