Full Length Research paper

Prevalence of different enterococcal species isolated from blood and their susceptibility to antimicrobial drugs in Vojvodina, Serbia, 2011-2013

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Enterococci are one of the leading causes of nosocomial infections worldwide. Enterococcus faecalis and Enterococcus faecium are the most commonly isolated. The aim of this study was to determine prevalence of these species isolated from blood samples of hospitalized patients and their susceptibility to antibiotics particularly to vancomycin and high concentrations of aminoglycosides. A total of 89 enterococcal strains isolated from blood samples between January 1st 2011 and August 31st 2013 were tested. The species identification and susceptibility to antimicrobial drugs were performed using automated VITEK 2 system. The most common species was E. faecalis (55.05%), followed by E. faecium (41.57%). The enterococcal isolates were multidrug resistant with E. faecium resistance to vancomycin of 54.05%, while resistance in E. faecalis was not found. All vancomycin resistant enterococci had VanA phenotype of resistance. Thirty three (89.18%) isolates of E. faecium were high-level gentamycin resistant and 32 (91.4%) were resistant to high concentration of streptomycin, whereas frequency of resistant E. faecalis was 61.2 and 63.04%, respectively. This study shows that resistance in enterococcal species is a serious clinical problem in our hospital and suggests the need for regular susceptibility test and species level identification of enterococcal isolates.

Key words: Enterococci, blood culture, antimicrobial resistance.

INTRODUCTION

Enterococci are part of normal flora of gastrointestinal tract of humans and animals, but they have also emerged as significant cause of serious infection such as endocarditis, urinary and blood stream infections, intra-abdominal end intra-pelvic abscesses (Moellering, 1992; Teixeira and Facklan, 2003). Among enterococci, Enterococcus faecalis (E. faecalis) and Enterococcus faecium (E. humans. Over the last two decades, they became one of faecium) are responsible for the majority of infections in the most frequent causes of intrahospital infections particularly because of increasing resistance to a wide range of antibiotics (Schaberg et al., 1991; Low et al., 2001; Chou, 2008; Hidron et al., 2008; Bereket, 2012; Sievert et al., 2013).

Enterococci have both an intrinsic and acquired resistance (Murray, 1990, 2000; Leclercq, 1997). They

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Abbreviations: E. faecalis, Enterococcus faecalis; E. faecium, Enterococcus faecium; ATCC, American Type Culture Collection; PBP, penicillin binding protein; HLAR, high-level aminoglycoside resistance; VRE, vancomycin resistant enterococci; SPSS, statistical package for the social sciences; ICU, intensive care unit.
are intrinsically resistant to penicillinase-susceptible penicillins (low level), penicillin-resistant penicillins, cephalosporins, sulphonamides, clindamycin and low-level concentrations of aminoglycosides. Acquired resistance to penicillins, chloramphenicol, tetracyclins, aminoglycosides (high-level) and vancomycin develops either by mutation or transfer of plasmids and transposons. Resistance of many beta-lactams is as a result of overproduction and modification of penicillin-binding-proteins (PBPs), particularly PBPs, with low affinity for antibiotics.

High-level aminoglycoside resistance (HLAR) can be mediated by single mutation within a protein of the 30S ribosome subunit or by production of amino-glycoside-modifying enzymes. Acquired resistance to glycopeptides occurs because of synthesis of modified cell wall precursors with decreased affinity for these drugs. Nine phenotypes of glycopeptide resistance (VanA, B, C, D, E, G, L, M, N) have been detected (Arthur et al., 1993; Perichon et al., 1997; Fines et al., 1999; Xu et al., 2010; Lebreton et al., 2011). The most common and clinically significant are VanA, that determine high-level resistance to both vancomycin and teicoplanin, and VanB with variable resistance to vancomycin only.

Resistance to wide range of antibiotics is a great problem to clinicians because it has seriously affected the treatment of enterococcal infections leaving limited therapeutic options.

Keeping in mind that antimicrobial susceptibility of enterococci is not predictable and that it differs by enterococcal species and changes rapidly over time, species identification and its susceptibility to antimicrobial drugs are important for clinicians for choosing the most effective drug and also useful for epidemiological investigations.

The aim of this study was to determine prevalence of *E. faecalis* and *E. faecium* isolated from blood and their susceptibility to antibiotics particularly, vancomycin and high concentration of aminoglycosides.

**MATERIALS AND METHODS**

This study was conducted at the Centre for Microbiology of Institute of Public Health, Vojvodina. A total of 89 enterococcal strains that were isolated from blood samples of hospitalized patients between January 1st 2011 and August 31st 2013 were included in the study. All blood samples were routinely cultured in blood culture bottles (Bio Merieux, France) using semi-automated blood culture system (Bact/Alert, Bio Merieux, Marcy’ Etoile, France). The positive samples were inoculated onto blood and MacConkey agar plate (HiMedia, India) that were incubated aerobically for 24 h at 37°C.

Enterococcal genus identification was based on colony morphology, Gram staining results, catalase reaction, growth on bile-esculin agar and tolerance to 6.5% NaCl. The species identification was done using automated VITEK 2 system (BioMerieux). Susceptibility to ampicillin, gentamycin (high-level), streptomycin (high-level), ciprofloxacin, vancomycin, teicoplanin, linezolid, tigecycline, chloramphenicol and quinupristin/ dalfopristin was determined using VITEK 2 system according to Clinical Laboratory Standards Institute guideline (2010, 2011, 2012). *E. faecalis* American Type Culture Collection (ATCC) 29212 was used as control.

**Statistical analysis**

The statistical differences were analyzed using x² test. The test was performed using SPSS (statistical package for the social sciences). A p-value of < 0.05 was considered significant.

**RESULTS**

Among 89 enterococci isolated from blood samples, the most common species was *E. faecalis* (55.05%), followed by *E. faecium* (41.57%). There was no significant difference between prevalence of these two species (p value = 0.196). Prevalence of all enterococcal species isolated from blood is given in Table 1. Susceptibility of *E. faecium* and *E. faecalis* to antibiotics is shown in Table 2.

Resistance of *E. faecium* isolates to all antibiotic tested, except to chloramphenicol, was higher as compared to *E. faecalis* strains. A high percentage of *E. faecium* resistant to vancomycin (54.05%) was detected, while resistance in *E. faecalis* was not found. All vancomycin resistant enterococci (VRE) were resistant to teicoplanin also, belonging to VanA phenotype of resistance. Thirty three (89.18%) isolates of *E. faecium* were high-level gentamycin resistant and 32 (91.4%) were high-level streptomycin, whereas the number of resistant *E. faecalis* was 30 (61.2%) and 29 (63.04%), respectively. The difference in resistance to gentamycin and streptomycin in these two species was statistically significant (p = 0.004 and p = 0.003, respectively). The most effective antibiotics were linezolid and tigecycline against all isolates tested. Unlike *E. faecium*, all isolates of *E. faecalis* were susceptible to vancomycin and teicoplanin also. *E. faecium* isolates were susceptible to quinupristin/dalfopristin. Resistance to other antibiotics range from 37.2% for chloramphenicol to 83.3% for ciprofloxacin in *E. faecalis* isolates. In *E. faecium* it was between 9.3% for chloramphenicol and 97.2% for ampicillin. All *E. faecium* 89.7% isolates of *E. faecalis* were resistant to three or more groups of antibiotics and the most common multidrug resistant profile in *E. faecium* was resistance to ampicillin, high-level concentrations of aminoglycosides, vancomycin, and ciprofloxacin. Resistance to ampicillin, high-level concentrations of aminoglycosides, quinupristin/ dalfopristin and ciprofloxacin was the most commonly found pattern in isolates of *E. faecalis*. The rates of VRE isolates from different wards are given in Table 3.

Enterococcal species and VRE were most commonly found in patients from intensive care unit (ICU), followed by those from haematology/oncology ward. There was no significant difference between proportion of enterococcal isolates and VRE found in different hospital wards (p value=0.068 and p value=0.894, respectively). Demographic characteristics of patients with
Table 1. Prevalence of enterococcal species isolated from blood.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. (%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecalis</td>
<td>49 (55.05%)</td>
</tr>
<tr>
<td>E. faecium</td>
<td>37 (41.57%)</td>
</tr>
<tr>
<td>E. gallinarum</td>
<td>2 (2.24%)</td>
</tr>
<tr>
<td>E. caseiflavus</td>
<td>1 (1.12%)</td>
</tr>
<tr>
<td>Total</td>
<td>89 (100%)</td>
</tr>
</tbody>
</table>

Table 2. Susceptibility of E. faecium and E. faecalis to antibiotics.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>E. faecium</th>
<th>E. faecalis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) of resistant isolates</td>
<td>No. (%) of resistant isolates</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>37</td>
<td>36 (97.2)</td>
</tr>
<tr>
<td>Gentamycin (hl)</td>
<td>37</td>
<td>33 (89.1)</td>
</tr>
<tr>
<td>Streptomycin (hl)</td>
<td>35</td>
<td>32 (91.4)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>26</td>
<td>25 (96.1)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>37</td>
<td>20 (54.05)</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>37</td>
<td>19 (53.1)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>37</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>37</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>32</td>
<td>3 (9.37)</td>
</tr>
<tr>
<td>Quinupristin/</td>
<td>29</td>
<td>0 (0)</td>
</tr>
<tr>
<td>dalofpinprin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

hl- High-level.

Table 3. Distribution of E. faecium, E. faecalis and VRE within hospital ward.

<table>
<thead>
<tr>
<th>Ward</th>
<th>No. of E. faecium and E. faecalis</th>
<th>No. (%) of VRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensive care unit (ICU)</td>
<td>32</td>
<td>8 (25)</td>
</tr>
<tr>
<td>Haematology/oncology</td>
<td>20</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Internal</td>
<td>17</td>
<td>4 (23)</td>
</tr>
<tr>
<td>Other</td>
<td>17</td>
<td>3 (17)</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>20</td>
</tr>
</tbody>
</table>

positive blood cultures and VRE are showed in Table 4.

Proportion of E. faecalis and E. faecium isolates in males (62.7%) was higher than in females (32.2%), \( p = 0.018 \). Prevalence of VRE also was higher in males (25.9%) than in females (18.8%), but the difference was not statistically significant \( p = 0.619 \). The number of enterococcal isolates found in patients above 60 years of age was higher than in other age groups \( p = 0.004 \). There was no significant difference in proportion of VRE between different age groups \( p = 0.737 \).

DISCUSSION

Enterococci are one of the leading causes of nosocomial infections worldwide because of increasing resistance to a wide range of antibiotics. Until 1990s, glycopeptides were antibiotics of choice in treating serious infections caused by enterococci, but occurrence of strains resistant to these drugs significantly decreased their efficiency. VRE were first detected in France and United Kingdom in 1986, a year later similar strains were isolated in hospitals in United States and since then VRE have been discovered throughout the world (Uttley, 1988; Murray, 2000; Edmond et al., 1999; Treitman et al., 2005; Goossens, 1998). The main reason for emergence of resistant isolates is widespread use of vancomycin. Broad spectrum antibiotics, such as third-generation cephalosporins and fluoroquinolons, also contributed to the selection of resistant isolates (Sood et al., 2008; Heath et al., 1996). In some European countries the outbreaks of VRE were related to the extensive use of avoparcin, as growth
promoter in farm animals (McDonald et al., 1997; Bates, 1997; Bager et al., 1997). Avoparcin causes bacterial cross-resistance to vancomycin and teicoplanin.

Resistant enterococcal isolates are reported all over the world, but prevalence of enterococcal species and their resistance vary widely in different geographic regions.

In this study, *E. faecalis* (55.04%) was the most common enterococcal species isolated from blood samples, followed by *E. faecium* (41.57%). *E. faecalis* was predominant isolate in studies from India (62.2%) by Ajay et al. (2012), 76% by Sreeja et al. (2012) and 82% by Bose et al. (2012), UK (62% by Brown et al. (2008) and 63% by Fisher and Phillips (2009) and Turkey (76%) by Shah et al. (2012). Until the early to mid-1990s this species was the most frequent isolated enterococci in US hospitals (Treitman et al., 2005). Since then there has been an important increase in the incidence of *E. faecalis* as a cause of nosocomial infections (Hidron et al., 2008).

Prevalence of vancomycin resistant *E. faecium* (50.05%) in this study was very high, much higher than that reported in most of the other countries. Some European countries (Bulgaria, Cyprus, Estonia, Iceland, Malta, Slovenia and Sweden) reported an absence or very low prevalence (Denmark-1.3%, Norway-1.8%, Netherlands-1% and France-1.4%) of vancomycin resistance (European Centre for Disease Prevention and Control, 2012). Similar results were reported from India (Sreeja et al., 2012) and Turkey (Shah et al., 2012). Among European countries, the highest rates of VRE were obtained from Ireland (34.9%), Greece (23.1%) and Portugal (20.2%). In other parts of the world the highest frequency of vancomycin resistant *E. faecium* was found in US (60%) by Bearman et al (2005), 67% by Forrest (2008), 80% by Arias et al. (2010).

Resistance to vancomycin is still very rare in *E. faecalis* isolates. In this study all *E. faecalis* were susceptible to vancomycin. Bose et al. (2012), Sunilkumar and Karthika (2012) and Bearman and Wenzel (2005) also reported low prevalence of resistant isolates (0, 1 and 2%, respectively). All *E. faecium* in this study had VanA phenotype of resistance. This type is widely distributed and predominant type of resistance in Iran (Talebi et al., 2007), Czech Republic (Kolar et al., 2006), Poland (Sadowy et al., 2013), Serbia (Mihajlovic-Ukropina et al., 2011) and other European countries (Werner et al., 2008).

Enterococcal infections develop usually in patients with some risk factors, such as severe underlying disease, long hospital stay, particularly in ICU, immune-suppression and haematological malignance. The largest number of vancomycin resistant *E. faecium* in this study was found in samples of patients from ICU and haematology/oncology ward that is in agreement with results of other authors (Kolar et al., 2006; Brown et al., 2008).

In addition to intrinsic resistance to low-level resistance to aminoglycosides, enterococci can acquire resistance to high concentrations of aminoglycosides. Very high percentages of HLAR in *E. faecium* (~90%) and *E. faecalis* (~60%) were found in this study, similar to the results reported in India (Sood et al., 2008; Jain et al., 2011), Iran (Talebi et al., 2007) and Turkey (Baldir et al., 2008). HLAR in *E. faecalis* was reported from all European countries except Iceland. The majority of countries reported frequency of resistant isolates between 25 and 50%. The highest percentages were detected in Italy (50%), Slovakia (49.5%), Hungary (48.6%) and Poland (48.4%). (European Centre for Disease Prevention and Control, 2012).

Combinations of aminoglycosides with beta-lactam antibiotics or glycopeptides are the treatment of choice for serious enterococcal infections, such as endocarditis, due to their synergistic activity. Occurrence of resistance to these antibiotics and loss of their synergistic bactericidal activity significantly limited therapeutic options.

Newer antibiotics, such as linezolid, tygycyclin, daptomycin and quinopristin/dalfopristin have been suggested as an alternative option for treating infections caused by multidrug resistant enterococci (Aksoy and Unal, 2008; Yemisen et al., 2009; Arias et al., 2010; Tsai et al., 2012). The results obtained in this study confirmed good activity of linezolid and tygycyclin against both enterococcal species tested and quinopristin/dalfopristin against *E. faecium* only. Unfortunately, their clinical use

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) of <em>E. faecium</em> and <em>E. faecalis</em></th>
<th>No. (%) of VRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>54 (62.7)</td>
<td>14 (25.9)</td>
</tr>
<tr>
<td>Female</td>
<td>32 (37.2)</td>
<td>6 (18.8)</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-19</td>
<td>23 (26.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>20-39</td>
<td>11 (12.7)</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td>40-59</td>
<td>18 (20.9)</td>
<td>7 (38.9)</td>
</tr>
<tr>
<td>≥60</td>
<td>34 (39.5)</td>
<td>10 (29.4)</td>
</tr>
</tbody>
</table>
may be limited by emergence of resistance that is reported in several studies (Werner et al., 2008; Berdal and Eskesten, 2008; Bonora et al., 2006; Kainer et al., 2007).

In conclusion, this study shows a very high prevalence of multi drug resistant enterococci isolated from blood samples. These results suggest that regular susceptibility test and species level identification of enterococcal isolates are important in order to treat enterococcal infections effectively and implement appropriate infection control measures to limit the spread in nosocomial settings.

REFERENCES


