Prevalence and Antibiotic Resistance of Enterococci Species Isolated from Clinical Samples

Reza Faraji¹, Najma Asadi², Foroozandeh Qasemi Kahtooie³, Mohammadtaghi Sarebanhassanabadi³, Ali Dehghani Firoozabadi³, Masoud Negahdary³ and Adel Eftekhari⁴*

¹Department of Microbiology, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.
²Department of Pathology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
³Yazd Cardiovascular Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
⁴Department of Nursing, School of Nursing and Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

(Received: 06 July 2015; accepted: 10 September 2015)

Enterococcus is an important pathogen all around the world, known as an important nosocomial pathogen. This study determined the strains and antibiotics susceptibility of Enterococci isolated from clinical samples in Imam Ali Hospital in Kermanshah, Iran, from July 2014 to July 2015. This experimental study was performed on 58 Enterococci strains isolated from clinical samples in Imam Ali Hospital in Kermanshah, Iran. Patients' information was collected by a questionnaire. After isolating and identifying 58 strains of Enterococci from clinical samples, complimentary tests were carried out. Antibiogram test was performed by Kirby-Bauer disk diffusion method and CLSI criteria. Statistical analysis was performed using the SPSS 16. The frequencies of the isolated Enterococci species included E. faecalis with 72.41% (42 cases), E. faecium with 13.79% (8 cases), E. hirae with 6.9% (4 cases), E. avium with 3.44% (2 cases), E. gallinarium with 1.73% (1 case), and E. mundtii with 1.73% (1 case). Most of Enterococci strains were isolated from urine samples with 48.27% (28 cases). The highest and lowest resistances were to penicillin (95%) and linezolid (0%), respectively. We found a significant statistical difference between Enterococcus and time of hospital stay (P = 0.03). Based on the results of the present study, E. faecalis was, by far, the most predominant isolate. This suggests that in order to prevent the antibiotic resistance and select an appropriate antibiotic before the treatment, antibiogram test should be done for each patient.

Key words: Enterococcus, nosocomial infections, antibiotic resistance.

The human bowel is the natural habitat of normal flora including various strains of bacteria. Although many of these bacteria are harmless, non-infectious, and useful for human beings, some of them such as enterococci can induce diseases. The gram-positive enterococci are fermentative bacteria seen as single coccus or streptococci. They are the natural inhabitants of the digestive tract of humans, birds, and a group of animals. They may further exist in the human’s oral cavity, intestine, and vagina, earth, water, and foods.1-3 The enterococci can grow in environments with high salinity and temperatures of 10-45º C. The dissemination of enterococci to the healthy members of the community and out-of-hospital population occurs through animal feces, foods of animal origin, superficial waters, and hospital sewage system.4-6 However, the enterococcal
transmission in the hospital milieu occurs via the primary prescription of vancomycin, specially in the patients affected by nosocomial infection that leads to the development of vancomycin-resistant enterococcus, easily transmitted from one patient to another, and also by long residence of enterococci on the hands and feet, gloves, and hospital surfaces. So far, twenty different strains of enterococci have been identified of which the Enterococcus faecalis and Enterococcus feacium are usually the main cause of human infection. In fact, 80-90% of enterococcal infections in humans are induced by Enterococcus faecalis and 10-15% of them are caused by Enterococcus feacium. Other enterococci like Enterococcus avium and Enterococcus raffinosus rarely induce enterococcal infections. These microorganisms possess low potential for creating diseases due to the absence of proteinous secretary toxins and lack of considerable pathogenic factors. Nonetheless, they may lead to the incidence of genito-urinary infections, endocarditis, meningitis, inflammation of bile ducts, septicemia, intra-abdominal and intrapelvic infections, cutaneous infections, and neonatal infections. They are also considered as the common causes of respiratory tract infections since 1970s. This microorganism was first observed in Europe in 1986 and then was widespread in America in 1998 so that the rate of incidence of its related nosocomial infections grew by twenty times during 1989-1998. The rate of incidence of enterococcal infections round the globe is constantly increasing in recent years so that today, the enterococci are rendered as one of the most common nosocomial infections. Indeed, the enterococci are the fourth cause of hospital-acquired infections, the second cause of urinary tract infections (UTI) in hospitals, and the third cause of hospital bacteremia. One of the leading causes of survival of enterococci in hospitals is their intrinsic resistance to several antibiotics used commonly in hospitals. This intrinsic resistance is acquired by mutation, acquisition of the genetic material carrying the resistance gene, and formation of biofilm. The characteristic resistance of enterococci to antibiotics has rendered this microorganism as one of the problematic factors in the field of antibiotic therapy. This is because most enterococci are resistant to the antibiotics beta lactam and glycolipid. For this reason, it is mandatory to use an amino glycoside along with beta lactam and glycolipid antibiotics simultaneously to treat the more serious types of enterococcal infections. However, most of the time; vancomycin is used as the selective antibiotic to treat the infections induced by gram positive antibiotic resistant microorganisms, specifically enterococci. Regarding the prevalence of enterococcal infections in hospitals and also the therapeutic disaster brought about by this antibiotic-resistant organism, the researchers decided to determine strains of enterococci and their resistance to vancomycin isolated from clinical samples of patients in Imam Ali Hospital in Kermanshah, western Iran.

MATERIALS AND METHODS

Sample collection
This experimental study was conducted on 58 enterococcal strains obtained from clinical samples of urine, wound, sputum, blood, and feces of patients hospitalized in Imam Ali Hospital in Kermanshah, western Iran, during July 2014 to July 2015. Demographic information of each patient including gender, age, length of hospitalization, previous use of antibiotics, and underlying diseases was collected via a questionnaire and recorded. There were no limitations for inclusion into the study regarding these variables.

Isolation and identification of strains
First, all samples were cultured in the differential media of Bile Esculin Agar and incubated for 24 hs at 37°C. After 24 hs, formation of colonies and the change in the environment color into black indicating hydrolysis of Esculin were investigated. Next, the colonies were identified on the basis of gram staining, catalase and oxidase tests, growth in salinity of 6.5%, growth in temperature of 45°C, tellurite reduction, hydrolysis of arginine amino acid, and fermentation of sugars of arabinose, mannitol, sorbitol, lactose, and sorbose. The antibiogram of the obtained strains was studied using disk diffusion (Kirby-Bauer). The antibiogram of the obtained strains was studied using disk diffusion (Kirby-Bauer). To do so, a suspension equal to 0.5 McFarland was prepared from the grown bacteria and cultured in the Muller Hinton agar using the sweeping
The culture media plates were placed in the room temperature for 10-15 min so that the bacteria would acclimatize with the new environment. Then, dependent on the type of the isolated bacteria, the disks of vancomycin antibiotic (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), teicoplanin (30 µg), erythromycin (15 µg), amikacin (30 µg), tetracycline (30 µg), penicillin (10 IU), linezolid (30 µg), gentamicin (fabricated by Mast)(10 µg) were placed in the culture media so that the disks were 1.5 cm distant from the plate rims and 2.5 cm from each other. The plates were subsequently incubated for 18-24 hs. Following this, the surroundings of the disks were investigated for the presence of no-growth halo. The diameter of the area around the disks was measured using Antibiotic Zone Scale Ruler and the sensitivity and bacterial antibiotic resistance were determined using Clinical and Laboratory Standards Institute (CLSI).25

**RESULTS**

This study was carried out in Imam Ali Hospital in Kermanshah during one year from July 2014 to July 2015. The mean age of the patients participating in the study was 51.16 ± 4.27 years. Thirty-one patients (53.44%) were male and 27 patients (46.56%) were female. The mean of hospital

<table>
<thead>
<tr>
<th>Samples</th>
<th>Urine</th>
<th>Blood</th>
<th>Wound</th>
<th>Sputum</th>
<th>Feces</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>20</td>
<td>17</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>42 (72.41)</td>
</tr>
<tr>
<td><em>E. feacium</em></td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>8 (13.79)</td>
</tr>
<tr>
<td><em>E. hirea</em></td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4 (6.9)</td>
</tr>
<tr>
<td><em>E. avium</em></td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2 (3.44)</td>
</tr>
<tr>
<td><em>E. gallinarium</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1 (1.73)</td>
</tr>
<tr>
<td><em>E. mundtii</em></td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (1.73)</td>
</tr>
<tr>
<td><strong>Total (%)</strong></td>
<td>28 (48.27)</td>
<td>20 (34.49)</td>
<td>6 (10.34)</td>
<td>3 (5.17)</td>
<td>1 (1.73)</td>
<td>58 (100)</td>
</tr>
</tbody>
</table>

**Table 2.** Frequency distribution of predisposing factors for infection with enterococci resistant to vancomycin isolated from clinical samples

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50 years</td>
<td>34 (58.63)</td>
<td>0.441</td>
</tr>
<tr>
<td>≤ 50 years</td>
<td>24 (41.37)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31 (53.44)</td>
<td>0.431</td>
</tr>
<tr>
<td>Female</td>
<td>27 (46.56)</td>
<td></td>
</tr>
<tr>
<td>Hospital stay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 7 day</td>
<td>12 (20.69)</td>
<td>0.03</td>
</tr>
<tr>
<td>≤ 7 day</td>
<td>46 (79.31)</td>
<td></td>
</tr>
<tr>
<td>Antibiotic usage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22 (37.94)</td>
<td>0.423</td>
</tr>
<tr>
<td>No</td>
<td>36 (62.06)</td>
<td></td>
</tr>
<tr>
<td>Underlying disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26 (44.83)</td>
<td>0.252</td>
</tr>
<tr>
<td>No</td>
<td>32 (55.17)</td>
<td></td>
</tr>
</tbody>
</table>
stay was 9 days. On the whole, of all the collected samples, 58 isolates were identified as enterococcus. The highest rates of isolates in the descending order of magnitude, belonged to urine, 28 cases (48.27%), blood, 20 cases (34.49%), wound, 6 cases (10.34%), sputum, 3 cases (5.17%), and feces, 1 case (1.73%). Performance of biochemical tests on the isolated strains indicated that Enterococcus faecalis with 42 cases had the highest percentage of the isolates (72.41%)(Table 1). Determination of sensitivity model of the isolated enterococci to the antibiotic disks demonstrated that among the samples under study, the highest resistance existed against penicillin (95%) and the lowest resistance was against linezolid (0%)(Figure 1). Comparison of the results obtained from the predisposing factors of infection with enterococcus resistant to vancomycin isolated from the clinical samples revealed that there was a statistically significant correlation between the time of hospital stay and prevalence of enterococcal infection (P = 0.03)(Table 2).

DISCUSSION

The enterococcus is an opportunistic bacterium considered as one of the most important factors involved in nosocomial infections round the globe since the last two decades due to increasing prevalence and constantly increasing resistance to antibiotics. They may lead to serious disorders such as urinary tract infections (UTI), endocarditis, and bacteremia.26, 27 In the present study, 58 isolates of enterococci were obtained from clinical samples. Regarding the frequencies of varieties, the most frequent were Enterococcus faecalis with 42 cases (72.41%), Enterococcus faecium with 8 cases (13.79%), Enterococcus hirae with 4 cases (6.9%), Enterococcus avium with 2 cases (3.44%), Enterococcus gallinarium with 1 case (1.73%), and Enterococcus mundtii with 1 case (1.73%), respectively. In the study by Schouten et al. conducted on 27 European countries, the prevalence of enterococci showed more variety. These strains included Enterococcus faecalis with 83%, Enterococcus faecium with 13.6%, Enterococcus gallinarium with 1.20%, Enterococcus durans 0.71%, Enterococcus casseliflavus with 0.53%, Enterococcus avium with 0.46%, Enterococcus hirae with 0.12%, Enterococcus mundtii with 0.05%, and Enterococcus raffinosus with 0.02%.28 Another study conducted in Kuwait during 1999-2001 reported that of 415 isolated enterococci, 85.3% were Enterococcus faecalis, 7.7% were Enterococcus faecium, and 7% were other miscellaneous enterococci.29 Furthermore, other studies carried out in Australia30, Sweden31, India32, Switzerland33, and USA34 reported the rate of prevalence of Enterococcus faecalis and Enterococcus faecium to be greater than other enterococcal strains. As it is clear, in this study and those mentioned above, the Enterococcus faecalis and Enterococcus faecium have been the most prevalent strains isolated from clinical samples. There are two reasons for this: 1) These strains are usually found in feces as the normal flora of the alimentary canal, vagina, mouth, and skin while other strains are often found in the environment; and 2) The higher ability and capability of these two strains for acquiring resistance to antimicrobial agents and being equipped with more variable virulent factors compared to other enterococcal strains.35 In this study, the highest amount of enterococcal strains was isolated from urine samples with 28 cases (48.27%) followed by blood with 20 cases (34.49%), wound with 6 cases (10.34%), sputum with 3 cases (5.17%), and feces with 1 case (1.73%). The study by Leblank et al., reported the urinary enterococci to be 36.6%, feces 12%, wound 11%, and blood 10.4%.29 Additionally, in the study by Sharifi et al., of 220 enterococcal samples under study, 85.5% were obtained from urine, 7.7% from blood, and 4.1% from body electrolytes.36 Also, in another study by Sedaghat et al., 17 strains (89%) were isolated from urine, and 1 strain (11%) from blood and the respiratory system.37 The findings of the present study and those mentioned above regarding isolation of enterococcal strains obtained from clinical samples indicate the importance of the residence of enterococci in the urinary tract passageways. This can be due to the point that the enterococci are the prime factor of infection among the gram positive infectious enterococcus in the urinary tract and the third cause of bacterial infections in women’s urinary tract after Escherichia coli and Cabecilla pneumonia.36, 37 In this study, there was no significant association between the prevalence of
enterococcal infection, and age, gender, previous consumption of antibiotics, and underlying diseases. As it is the case in this study, in the studies by Javadi et al. and Pourakbari et al., there was no correlation between age and gender, and affliction with enterococcal infection.38,39 However, there was a statistically significant correlation between prevalence of enterococcal infection and hospital stay (P=0.03) so that the rate of prevalence of enterococcal infection in patients with hospital stay of 7 days or more with 46 cases (79.31%) was greater than those with less than 7 days of hospital stay with 12 cases (20.69%). It should be kept in mind that the predisposing factors including long hospital stay, the use of antibiotics, surgical operations, diabetes, leukemias, use of metronidazol, underlying disease, affliction with cancer, organ grafts, and immunosuppressive drugs can be among the probable predisposing factors for enterococcal infection.40 Moreover, in this study, the resistance of isolated enterococci to vancomycin antibiotic was 33%, ciprofloxacin 45%, chloramphenicol 73%, teicoplanin 5%, erythromycin 53%, amikacin 86%, tetracycline 23%, penicillin 95%, gentamycin 61%, and linezolid 0%. As can be observed, the highest resistance (95%) was against penicillin and the lowest resistance (0%) was against linezolid. In the study conducted in southern India in 2013 by Shafiyabi et al., the rate of resistance of chloramphenicol to cephalaxin, gentamycin, co-trimoxazole, vancomycin, and linezolid were 100%, 90%, 85%, 5%, and 0%, respectively.41 Also, in the study by Bhatt, the rate of resistance to penicillin was 95%, ampicillin 95%, co-trimoxazole 90%, and linezolid 2%.32 The studies by Hällgren and O’Driscoll also reported the lowest rate of resistance against the antibiotic linezolid.31,42 These findings along with our results indicate that the lowest resistance belongs to linezolid.42 Perhaps, the limited use of linezolid as an antibiotic in therapeutic cases is the main cause of the low resistance of this enterococcus to the antibiotic linezolid.42

CONCLUSION

Regarding the point that today the enterococcal infection is rendered as one of the most important and major disasters of hospitalized patients, the need is felt for further research into the prevalence of this strain in the hospital setting and among the hospital personnel. Moreover, since the enterococci are instinctively resistant to a wide spectrum of antibiotics, this issue is an alarm for the authorities involved to reduce the risk of antibiotic resistance by prescribing the right kind and the correct dosage of antibiotics for treatment purposes and to perform the required antibiogram tests.

REFERENCES


J PURE APL MICROBIO. 9(SPL. EDN.), NOVEMBER 2015.


