

Isolation and antibiotic resistance pattern of vancomycin-resistant *Enterococcus fascium* strains in clinical samples in Tabriz hospitals.

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Abstract

Background: In recent decades, *Enterococci* have been identified as an important pathogen. Resistance of these bacteria to antibiotics causes major problems in the therapeutic process. Therefore, the aim of this study was to determine the antibiotic resistance pattern of *Enterococcus fascium* strain in clinical samples.

Methods: In this research, 219 samples of *Enterococci* from clinical specimens including: Blood and urine were studied in Tabriz hospitals. All strains were identified by standard and bacteriological tests, and then antibiotic resistance patterns of bacteria isolated by Kirby-Bauer disc diffusion method were performed. Also, the minimum inhibitory concentration (MIC) was determined by E-Test method.

Results: Of 219 strains of *Enterococcus*, 86 (39.26%) strains belong to the species of *E. fascium*. The highest resistance to antibiotics was tetracycline (83.7%) and erythromycin (65.1%), and lowest compared to linezolid (0%) and teicoplanin (18.6%). Based on the E-Test, 29 (33.7%) sample was resistant to vancomycin.

Conclusion: Considering the increasing number of infectious diseases caused by resistant strains of *Enterococcus* genus and its dissemination in the hospital environment, it is very necessary to use infection control measures to eliminate the potential sources of infection and prevent it from developing.

Keywords: *E. fascium*, Vancomycin, Antibiotic resistance pattern.

Accepted on March 8, 2018

Introduction

Enterococci are optional anaerobic and gram-positive cocci that are considered as the natural flora of the intestines of warm-blooded animals, including humans [1]. Of the more than 20 species of *Enterococcus*, the two most human pathogens of this bacterium are *Enterococcus faecalis* (85-90%) and *Enterococcus fascium* (5-10%) [2]. *Enterococci* are considered as a major cause of hospital infections and acquired infections from the community [3,4]. In the last two decades, *Enterococci* have been identified as one of the most important human pathogens. These organisms are inherently resistant to a large number of antibiotics and, moreover, they are also increasingly able to obtain new resistance [4]. Infections caused by antibiotic-resistant *Enterococci* are considered as a systemic therapeutic system.

These infections are important in many aspects, including mortality and pathogenicity in patients, increasing patient hospital admission life expectancy, increased costs for prolonged stay and diagnostic and therapeutic interventions. One of the sources of infection is the medical equipment and Medical device that the resources of these resources can be transmitted directly or indirectly to a new host [5-8]. Most *Enterococci* are resistant to β -lactam antibiotics and glycopeptide, Therefore their simultaneous use with an aminoglycoside is needed to treat the most serious *Enterococcal* infections, such as endocarditis and meningitis

[9]. Vancomycin is often the last resort to treat infections of gram-positive bacteria, especially *Enterococci* [10].

In recent years, researchers have found that despite public health improvements, there is still a significant increase in bacterial infections, especially *Enterococcus*, the main cause of which is the transfer of these resistors by conjugation, plasmids and transplants are of a genus or other Genus of bacteria [11]. Therefore, it is essential to perform drug sensitivity for proper treatment and prevention of resistant strains. For proper treatment and prevention of resistant strains, it seems necessary. The purpose of this study was to identify antibiotic resistance patterns of *E. fascium* strains.

Materials and Methods

In this descriptive cross-sectional study, 219 samples of *Enterococci* from different clinical specimens including urine, blood from hospitalized sector and outpatients of hospitals and treatment centres in Tabriz during a one-year period from May 2016 to May 2017 were collected by Simple Random Sampling. A questionnaire was prepared for all patients and the required information (age, sex, location of infection) was recorded according to ethical charter. Initially, the strains of *Enterococci* were identified using biochemical tests (Gram stain, catalase, Bacitracin sensitivity, grow in medium containing 6.5% NaCl, L-pyroglutamic and acid- β -naphthylamide) [7,8,12].

Sensitivity test was performed by Kirby-Bauer disk diffusion method to obtain antibiotic resistance patterns of isolated from patients. Antibiotic disks used in this test including: Vancomycin, Erythromycin, Gentamicin, Linezolid, Ciprofloxacin, Teicoplanin, Tetracycline and co-trimoxazole was the Iranian Padtan-Teb medicine Company. For this purpose, the disks were sterilized with appropriate distance from each other on the Muller Hinton Agar medium and then incubated for 24 h at 37°C. Then the diameter of the inhibition zone of bacterial growth was measured and compared with the standard table.

Finally, the results were expressed as sensitive, semi-sensitive, and resistant [13-15] based on the diameter of the growth halo around the discs. The minimum inhibitor concentration of MIC was determined through E-Test method (Himedia Indian Company) based on the Clinical Standards Institute (CLSI) and the findings were interpreted according to CLSI standards guideline [5]. The results were analyzed using SPSS software (version 19) and chi square test. In all cases, $p < 0.05$ was considered significant.

Results

Of the 219 samples of *Enterococcus*, 152 (69.4%) samples were isolated from the urine and 67 (30.6%) samples were isolated from the blood. 45 (20.5%) of samples were collected from hospitalized sector and 174 (79.5%) of them were collected from outpatients. The mean age of patients was 48.4 ± 27 and variation range was between 10 months to 85 years old. 168 (65.63%) belonged to men and 88 (34.37%) belong to women. 86 (39.26%) of the samples belonged to *fascium* species. There was no significant difference in the distribution of *fascium* species between age groups ($p > 0.05$). 52 (60.5%) samples were isolated from the urine and 34 (39.5%) samples were isolated from the blood. No statistically significant differences were found regarding frequency of *fascium* species in Sample type ($p > 0.05$). 20 (23.3%) of samples were collected from hospitalized sector and 66 (76.7%) of them were collected from outpatients.

There was no significant difference in the distribution of *fascium* species between hospitalized sector and outpatients ($P > 0.05$). 53 (61.63%) belonged to men and 33 (38.37%) belong to women. There was no significant difference in the distribution of *fascium* species between male and female groups ($P > 0.05$). Based on antibiotic susceptibility and resistance test, *E. fascium* has the highest resistance to antibiotics tetracycline (83.7%) and erythromycin (65.1%), and the lowest resistance linezolid (0%) and ticoplanin (18.6%) (Figure 1).

There was no statistically significant difference between the place of infection and resistance to antibiotics ($P > 0.05$). Of the 86 samples of *E. fascium* using the E-test method 7 (31.4%) strains were $256 \mu\text{g/ml}$, 2 (2.3%) strains were more than $32 \mu\text{g/ml}$, 73 strains were between $4 \mu\text{g/ml}$ and $32 \mu\text{g/ml}$.

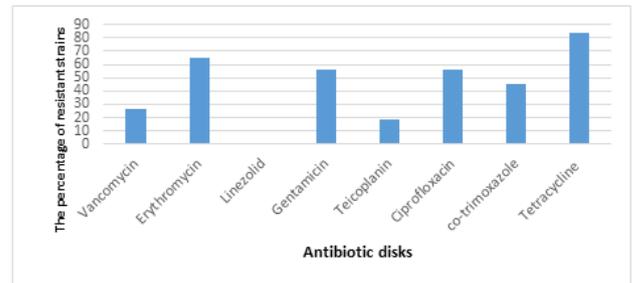


Figure 1. Antibiotic resistance rates of isolated *E. fascium* strains

Discussion

VRE is known as one of the most important causes of hospital infections in patients with a weakened immune system. In addition, the presence of resistant *Enterococci* in human societies acts as a source for hospital infections [16]. In this study, like several other studies, resistance to *E. fascium* is more than other *Enterococci* [17]. It has been proven that in most cases, due to the excessive use of antibiotics, we have seen many drug resistances in pathogens. This leads to failure in treatment and the emergence of many complications, despite the high cost of treatment [18]. Drug resistance to antibiotics in different regions of Iran and the world due to genetic variation, the difference in the consumption of antibiotics and difference in the availability of new extended spectrum are different. Until 1981-86, ciprofloxacin resistance to *enterococci* was not common, but in 1990, resistance significantly increased to 15.2%. [19,20]. According to a study by Seifi in 2008, high resistance (about 40%) to ciprofloxacin was observed among clinical isolates and sewage, which can be used as the first treatment option for the treatment of various types of urinary tract infections [21]. The reasons for increasing the role of *Enterococci* in hospital infections can be attributed to the use of antibiotics that are resistant to them [5]. In 2006, Fatholazadeh and his colleagues reported that the prevalence of *E. fascium* was 30% in urinary tract infections in three hospitals in Tehran. Which is consistent with the findings of the present study [14]. In the present study, the prevalence of vancomycin resistant *Enterococci* was 33.7%. The prevalence of vancomycin-resistant *Enterococci* was reported as 6.8%, 10%, and 7.7%, respectively [22-24]. Also in domestic studies, the prevalence of vancomycin-resistant *Enterococci* was reported to be 4.7%, 5.6%, 12%, and 23%, respectively [25-28]. However, Salah et al. [29], Loza et al. [30], And Guiney [31] did not find any *Enterococci* resistant to vancomycin. The study of 414 *Enterococci*, 19(4.6%) isolates of vancomycin resistant *Enterococci* were identified. By specifying the species, all 19 samples were resistant to vancomycin *E. faecium* [32]. The prevalence of erythromycin resistance in *Enterococcal* isolates in this study was 65.1%, which was lower, higher and close to the results obtained in Tabriz (91.24%), Lebanon (59%), and India (85%) [33-35]. In the current study, resistance to ciprofloxacin was 56%, and the results were higher than those of similar studies in France (14.3%), Japan (10%), Lebanon (34%), Pakistan (11.57%), and Tabriz (60.58%) [33,34,36,37]. In this study, the least resistance to Linezolid antibiotics was observed Which

consistent with the study of Shafiyabi et al in 2013 [38]. But Loza et al. reported 100% sensitivity to linezolid and ampicillin antibiotics [31].

Conclusion

Based on the results of the present study, a significant percentage of *Enterococcal* strains show resistance to various antibiotics, Therefore, proper treatment requires strategies to control and prevent the release of resistant strains, as well as continuous monitoring for the detection of emerging resistance traits in these bacteria.

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