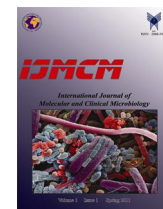


International Journal of Molecular and Clinical Microbiology



Determination of antibiotic sensitivity by E-test in resistant *Enterococcus* species isolated from patients admitted in Khatam- Ol - Anbiya, Hospital, Tehran, Iran, 2013-15

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ARTICLE INFO

Article history:

Received 23 March 2015

Accepted 20 May 2016

Available online 1 June 2016

Keywords:

antibiotic sensitivity,

Enterococcus,

E-test,

Microbial Sensitivity Tests,

linezolid

ABSTRACT

Enterococcus is the second cause of urinary tract infections in hospitals and the third most common cause of nosocomial bacteremia. Overuse of antibiotics for the treatment of nosocomial infections, causes antibiotic resistance in enterococci resistant to antibiotics through their ability to acquire resistance to antibiotics through mutation or acquisition of genetic material carrying a resistance gene by conjugation or other methods. This cross-sectional project, 60 patients with nosocomial infections admitted to the Khatam – Ol- Anbiya Hospital wards during 2013-2015 were studied. Samples were collected and sent to the microbiology laboratory. Drug-resistant of *Enterococcus* and Antibigram test performed by E-Test method. Relevant descriptive variables were analyzed by SPSS software version 21. 34 (56/7%) of patients were male. The patient's mean age was 70.71±18.39 years. Age group of 70-90 years, with 17 (27.9 %) which was the most frequent nosocomial infections. *Enterococcus* dominant species in these patients was 45 (75%) *Enterococcus faecalis*. Antibigram E-Test results showed that 9 cases (18.3%) were resistance to linezolid, 22 (36/7 %) resistance to imipenem, 15 (25 %) resistance to meropenem, 6 patients (10%) resistance to teicoplanin, 9 (15%) were resistant to vancomycin. Identification of common antibiotic resistance in every region has great importance and in addition prevents treatment failure. The result of these studies shows that antibiotic-resistance patterns have changed and vancomycin resistance especially among *E. faecium*, is rising because of nosocomial infections.

1. Introduction

Over the past decade, the decreasing susceptibility of commonly encountered bacterial pathogens has attracted much attention. Enterococci have become an important cause of nosocomial and community-acquired infections. Enterococci contribute to the normal flora of both the human and animal gastrointestinal

tracts. In recent years, it has been reported to be a major cause of nosocomial infections (Kayaoglu and Ørstavik, 2004), and they are among the most common pathogens isolated from infected surgical sites, septicemia and urinary tract infections (Jett et al., 1994).

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In the United States, enterococci are considered as the second most common cause of nosocomial infections. Most infections caused by these pathogens are urinary tract infections, intra-abdominal and pelvic abscesses, biliary infections, surgical wound infections, bacteremia, infections of the central nervous system (CNS), neonatal infection, and rarely respiratory, osteomyelitis, and cellulitis (Delmonico, 2000; Low et al., 2001)

In recent years, the extensive use of intravascular devices, prosthesis, cytotoxic chemotherapy, and immune deficiency drugs have increased the importance of the microorganisms. In recent studies, it has been reported that such infections are transmitted through organ transplantation (Freeman et al., 1999; Mohanty et al., 2005)

The emergence and spread of multidrug-resistant enterococci have been seen in many countries in the past 10 years (Grayson et al., 1991; Murray, 1998). Resistance to antibiotics is the characteristic of enterococci, whereas some species such as faecium has been shown to be more resistance (Sader et al., 2013). In a survey, the resistance pattern has been changing due to inappropriate therapy, and in the recent studies, antibiotics such as daptomycin and linezolid has been proposed as the replacement of vancomycin (Bauer et al., 1966; Rolston et al., 2013).

Clinical and Laboratory Standards Institute (CLSI) suggests that E-test method is superior to antibiotic resistance (Huycke et al., 1998; Emaneini et al., 2008; Feizabadi et al., 2008). Unfortunately, in recent years there have not been any comprehensive studies to assess the resistance in enterococci by E-test.

This aim of this study was to investigate the antibiotic resistance in enterococci by E-test at the Khatam-Ol-Anbia hospital, Tehran, Iran during 2013–2015. To deal with the above-mentioned issues, our objective was to understand about the antibiotic resistance in every region, to choose the correct treatment and to take the necessary steps in preventing further resistance thereby reducing morbidity and mortality.

2. Materials and Methods

This was a cross-sectional study. During the project, samples containing isolated

Enterococcus were sent from different parts of the Khatam-ol-Anbia hospital in Tehran, Iran during 2013 – 2015. Samples collected from blood, urine, wounds, sputum, bronchoalveolar lavage, cerebrospinal fluid or any other samples was sent to the microbiology laboratory. The samples were inoculated onto Enterococcosel agar after 24 hours incubation at 37°C. Isolates were confirmed to be enterococci by Gram stain, pyrrolidonyl arylamidase activity, motility, and catalase. Then they were sub-cultured onto three culture media including Mueller-Hinton agar to determine their growth at 15°C and 45°C, NaCl 6.5%, and bile esculin agar containing 6 µg/mL vancomycin and 64 µg/mL ceftazidime for resistance screening. All media were kept at 37°C for 24 hours. Then, using the Kirby-bauer method and disks of penicillin, ampicillin, gentamicin, erythromycin, chloramphenicol, vancomycin, tetracycline, rifampicin, and clindamycin, the antibiotic resistances were determined based on CLSI protocol. In the next step, the organisms that were multi-drug resistant were evaluated by using E-test strips for vancomycin, linezolid, imipenem and gentamicin antibiotics in order to determine the minimum inhibitory concentration (MIC). Measurement of MIC was performed according to CLSI guidelines. After obtaining *Enterococcus* antibiotic susceptibility and resistance results, SPSS 21 software and descriptive statistical methods (mostly frequency) was used to analyze these results.

3. Results

In this study, the mean age of patients was 70.71 ± 18.39 years, of which 34 (56.7%) patients were men and 26 (43.3%) were female. Age frequency distribution of participants showed that the frequency of patients increased with age with age groups of 70-80 years and 80-90 years have the highest rates (Table 1).

A total of 60 isolates of *Enterococcus* from clinical samples were obtained: 75% *E. faecalis*, 21.7% *E. faecium*, and 3.3% *E. agglomerous* species. Distribution of clinical samples were urine, 31 (51.7%); sputum, 3 (5 %); blood, 6 (10%); wounds, 7 (11.7%) and 13(21.7%) were unknown samples. Antibiotic susceptibility was evaluated by the E-test method (Table 2).

Table 1. Age frequency distribution of patients

	Frequency	Percentage
10-20	1	1.7
20-30	1	1.7
30-40	4	6.8
40-50	2	3.4
50-60	5	8.5
60-70	6	10.2
70-80	17	27.9
80-90	17	27.9
90-100	7	11.9

Table 2. Results of the antibiogram of cultivations using E-Test.

Antibiotics	Sensitive	Intermediate	Resistance
linezolid	49	2	9
Imipenem	23	1	22
Meropenem	4	4	15
Teicoplanin	28	23	9
Vancomycin	28	0	32

4. Discussion

Enterococci, leading nosocomial bacteremia, surgical wound infection, and urinary tract infection, are becoming resistant to many and sometimes all standard therapies (Huycke et al., 1998). Despite the fact that enterococci have been considered to have relatively low virulence, in the past few years these organisms, among all nosocomial pathogens, have emerged as a significant concern (Linden et al., 1996). Vancomycin-resistant enterococci species are considered one of the most important factors regarding nosocomial infection in susceptible patients (Yadegarynia et al., 2016).

In this study, like other studies, the prevalence of *Enterococcus faecalis* infection was shown to be higher than other strains (75% vs. 21.7%). Also, the resistances of *E. faecalis* are greater than *E. faecium* (Huycke et al., 1998). Based on the results of 60 clinical specimens infected with *Enterococcus*, 32 (53.3%) were resistant to vancomycin. A study by Emaneini and colleagues in Tehran hospitals during 2006 found incidence of 12% vancomycin-resistant enterococci species. In this study, we found more rate of resistance than in Emaneini and colleagues' (Emaneini et al., 2008) research. This study showed that the increasing upward trend toward resistance can be attributed to the indiscriminate use of antibiotics.

It was reported that 100% of *E. faecalis* isolates from urine samples were susceptible to vancomycin by disk-diffusion method, whereas *E. faecium* samples were 71% resistant to vancomycin (Feizabadi et al., 2008; Lozano et al., 2015; Smith et al., 2015). The results of this study are somewhat different from the results of our study statistically, which may be due to the type of study, or the increasing trend of antibiotic resistance and resistance patterns in hospitals. Another possible reason for this difference could be related to the hospital itself, in that a teaching hospital's antibiotic prescription process is more logical (Sreeja et al., 2012; Khalili et al., 2012).

In our study, *Enterococcus* species by the E-test method showed the highest resistance to vancomycin (53%) and imipenem (36.7%). In Feizabad's study, *E. faecium* species were resistant to ampicillin (86%), gentamicin (71%), and imipenem (100%). Zhanel et al. during a 2002 study in the United States, reported that linezolid, nitrofurantoin, chloramphenicol, and synergic antibiotics are the most effective drugs for the treatment of urinary tract infections caused by a vancomycin-resistant *E. faecium* strain, while ampicillin and ciprofloxacin were the least effective (Zhanel et al., 2003).

In a study by Rahimi et al, in which the results was similar to ours, the most clinical samples of isolated *E. faecium* were urine samples (Rahimi et al., 2007). It would seem that the intensive care and nephrology wards have a greater risk of becoming infected with resistant strains. The studies carried out in this country and abroad have shown a pattern of increased antibiotic resistance, and we face the emergence of multi-drug resistant (MDR) strains. This could be because of prolonged hospital stays, irrational prescription of antibiotics, weakened immune systems because of underlying factors, and immune-system depressant drugs (Mikalsen et al., 2015). Some studies have even shown that the use of growth stimulant medications in livestock farms, such as avoparcin, has increased vancomycin-resistant species of animals, humans, and the environment (Wegener et al., 1999; Mourand et al., 2014).

Conclusions

Technological advances reduce human resistance, the emergence of new drugs and thus reduce immune resistance has to be added variety and number of nosocomial infections. It seems that the only way to deal with this changing pattern is to approach it with rational broad-spectrum drug prescriptions and treatment of Enterococcal infections. This can be done by giving proper instructions, such as having broad-spectrum drugs prescribed only by specialists in infectious diseases, and have to consultations or requests required by other health groups to prescribe antibiotics and control the ascending rate of resistance. In this regard, it seems that proper health education and information are both necessary. Effective control of multiple-drug resistant enterococci will require better understanding of the interaction between enterococci, the hospital environment, and humans, and also prudent antibiotic use, better contact isolation in hospitals and other patient care environments, and in addition improved surveillance.

Acknowledgments

The authors would like to acknowledge their gratitude to Dr. Saeed Azad, who participated in data collection in Khatam-ol-Anbia hospital laboratory.

Authors' Contribution:

This article is extracted from Dr. Maryam Dadgar thesis for the degree specialist infectious diseases and tropical medicine.

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