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Evaluation of Antimicrobial Susceptibility Among *Enterococcus* Species by E-Test Method at Khatam-ol-Anbia Hospital During 2013 – 2014

Davood Yadegarynia[1]; Sara Rahmati Roodsari[1] and Zahra Arab-Mazar[1,*]

[1]Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

*Corresponding author: Zahra Arab-Mazar, Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran. Tel: +98-2122439963, Fax: +982122439964, E-mail: arabmazar@sbmu.ac.ir; z.arabmazar@gmail.com

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Abstract

Background: Over the past decade, Enterococci have been shown to be an important cause of nosocomial and community-acquired infections. Inappropriate use of antibiotics led to changes in the pattern of antibiotic resistances in *Enterococcus* species. Unfortunately, no study has been performed in Iran in recent years regarding the antimicrobial resistance of Enterococci using the E-test method as a base. We must gain sufficient knowledge about the regional antibiotic resistances related to *Enterococcus* so that we can monitor the prevalence and antimicrobial resistance of *Enterococcus* by administering appropriate treatments.

Objectives: The objective of this study was to evaluate the antimicrobial susceptibility among *Enterococcus* species by the E-test method at Khatam-ol-Anbia hospital during 2013 - 2014.

Patients and Methods: This descriptive cross-sectional study was carried out during 2013 - 2014. All clinical samples were collected from the intensive care unit (ICU) and general wards of Khatam-ol-Anbia hospital. All *Enterococcus* species were detected via biochemical testing. Antimicrobial susceptibility and minimum inhibitory concentration (MIC) were determined via disk diffusion and the E-test method. We used descriptive statistics to analyze the data.

Results: A total of 53 Enterococci were isolated from clinical samples of blood, urine, wounds, sputum, and cerebrospinal fluid (CSF) over a two-year period from the ICU and general wards. The isolated *Enterococcus* species were 77.35% *E. faecalis*, 18.86% *E. faecium*, and 3.77% other species. Species evaluated by E-test were resistant to imipenem, ampicillin, co-trimoxazole, gentamicin, rifampicin, vancomycin, linezolid, and teicoplanin; 54%, 68%, 100%, 93.8%, 60.4%, 39.6%, 0%, and 29.2%, respectively. Among the strains of enterococci, 90.9% of *E. faecium* and 20% of *E. faecalis* species were resistant to vancomycin.

Conclusions: According to these findings, antibiotic-resistance patterns have changed, and vancomycin resistance, especially among *E. faecium*, is rising because of nosocomial infections. Consequently, it has become a serious subject for patients admitted into a hospital.

Keywords: Microbial Sensitivity Tests, E-test, *Enterococcus*

1. Background

Over the past decade, enterococci have become an important cause of nosocomial and community-acquired infections. Also, in the United States, enterococci are considered to be 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 in rare instances respiratory, osteomyelitis, and cellulitis (1).

In recent years, the extensive use of intravascular devices, prosthesis, cytotoxic chemotherapy, and immune deficiency drugs have increased the importance of these micro-organisms. In recent studies, it has been reported that such infections are transmitted via organ transplantation (2, 3).

Resistance to antibiotics is characteristic of enterococci, whereas some species, such as *E. faecium*, have shown more resistance (4). In the survey, the resistance pattern shows evidence of changing due to inappropriate therapy. In recent studies, antibiotics such as daptomycin and linezolid have been proposed instead of vancomycin (5).

The clinical and laboratory standards institute (CLSI) suggests the E-test method is superior to the antibiotic resistances (6). Unfortunately, in recent years, there have not been any comprehensive studies to assess the resistances of enterococci via E-test.

2. Objectives

This study was performed in order to investigate the antibiotic resistances in enterococci by using E-test at the Khatam-ol-Anbia hospital during 2013 - 2014. To deal with the aforementioned issues, our objective was to learn about the antibiotic resistances in every region, choose the correct treatment, and take the necessary steps to

prevent further resistances, thereby reducing morbidity and mortality.

3. Patients 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 - 2014. The samples were inoculated onto Enterococcosel agar after 24 hours of incubation at 37°C. Isolates were confirmed to be enterococci by Gram stain, pyrrolidonyl arylamidase activity, motility, and catalase, and were then subcultured onto three culture media: 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, organisms that were multi-drug resistant were evaluated by using E-test strips for ampicillin, vancomycin, linezolid, imipenem, gentamicin, co-trimoxazole, and teicoplanin 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, we used SPSS software and descriptive statistical methods (mostly frequency) to analyze these results.

4. Results

In this study, the mean age of patients was 73.5 ± 1.5 years, of which 29 (54.7%) patients were men and 24 (45.3%) were female. The percentage of samples obtained from the ICU and general wards was 24.5% and 75.5%, respectively. Distribution of clinical samples were urine, 27 (53%); sputum, 3 (5.7%); blood, 5 (9.4%); wounds, 7 (13.2%); CSF, 1 (1.9%); and bronchoalveolar lavage (BAL), 2 (3.8%).

A total of 53 isolates of *Enterococcus* from clinical samples were obtained: 77.35% *E. faecalis*, 18.86% *E. faecium*, and 3.77% other species. Antibiotic susceptibility was evaluated by the disk-diffusion method and the results were as follows:

Nitrofurantoin 11 (22.9%), vancomycin 19 (39.6%), ampicillin 22 (45.8%), penicillin 45 (93.8%), tetracycline 42 (87.5%), imipenem 26 (54.2%), chloramphenicol 6 (12.5%), erythromycin 46 (95.8%), clindamycin 47 (97.9%), reifampicin 28 (58.3%), gentamicin 42 (87.5%), and ciprofloxacin 45 (93.8%).

All samples were resistant to co-trimoxazole, and in the same method, no resistance to linezolid was observed.

In the disk-diffusion method, two cases of other enterococci species were resistant to vancomycin, and the *E. faecium* strain was resistant to ampicillin, penicillin, ciprofloxacin, tetracycline, imipenem, erythromycin, and clindamycin. An *Enterococcus* species survey by the E-test method revealed 54%, 68%, 100%, 93.8%, 60.4%, 66.7%, 0%, and 29.2% resistances to imipenem, ampicillin, co-trimoxazole, gentamicin, rifampicin, vancomycin, linezolid, and teicoplanin, respectively. Among the strains of enterococci, *E. faecium* 90.9% and *E. faecalis* 20% were resistant to vancomycin.

5. Discussion

Vancomycin-resistant enterococci species are considered one of the most important factors regarding nosocomial infection in susceptible patients (7, 8). In this study, like other studies, the prevalence of *Enterococcus faecalis* infection is higher than other strains (73% vs. 23%). Also, the resistances of *E. faecalis* are greater than *E. faecium* (9). Based on the results of 53 clinical specimens infected with *Enterococcus*, 22 (66.7%) were resistant to vancomycin; *E. faecium* 20 (90.9%), and *E. faecalis* 2 (9.1%) cases, respectively. A study by Emaneini et al. in Tehran hospitals during 2006 (10) mentioned a 12% incidence of vancomycin-resistant enterococci species. In this study, we found more incidence of resistance than in Amani and colleagues' research. This study showed that the increasing upward trend toward resistance can be attributed to the indiscriminate use of antibiotics.

A 2008 report by Feizabadi et al. in Labbafinejad, Tehran, showed 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 (11-13). The results of this study are somewhat different from the results of our study statistically, which could 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 (14, 15).

In our study, *Enterococcus* species by the E-test method showed the highest resistance to co-trimoxazole (100%), gentamicin (93.8%), and ampicillin (68%). All *E. faecium* samples were resistant to gentamaicin, ampicillin, and imipenem. 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 synercid 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 (16). In our disk-diffusion method results, *Enterococcus* species were sensitive to linezolid in all cases, with chloramphenicol and nitrofurantoin observed as having the least impact.

In Rahimi et al. study, similar to our study, the most clinical samples of isolated *E. faecium* were urine samples (17). 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 (18). 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 (19, 20).

5.1. Conclusions

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 mandatory 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.

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Footnotes

Authors' Contribution: The core idea of this work came from Davood Yadegarynia. Zahra Arab-Mazar collected the data and acted as technical and material support.

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