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# Comparative activity of cefoprazone and ceftriaxone against clinical isolates of Enterococcus : An in vitro study

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**A B S T R A C T**

In the recent years due to antimicrobial resistance to entrococci, especially in debilitated patients, they became problematic. In vitro susceptibility study of *E. faesium* and *E. faecalis* to vancomycin, gentamycin, ampicillin and ceftriaxone with disk diffusion method; and sensitivity comparision of *E. faesium* and *E. faecalis*, separately, to ceftriaxone with two methodes of E-test and disk diffusion; and so detection of MIC 50 and MIC 90 for ceftriaxone and cefoperazone by E-test method; and also importance of these two cephalosporines in emprical therapy of entrococcal infections, and study of emergence of aquired antimicrobial resistance. Sixty isolated entrococci from urine, blood and wound entered in study and differentiated into two species, *E. faesium* and *E. faecalis* by PCR ; and then antibiogram was done for all mentioned antibiotics, exept cefoperazone, by disk diffusion method and for ceftriaxone and cefoperazone again by E-test method. Fermentation of raffinose, sucrose and sorbitol also was done. The most common source of infection was urinary system. Positive fermentation tests of arabinose and raffinose was only seen in *E. faesium*. From all isolates, 20% was *E. faesium*; and in the study of effects of ceftriaxone and cefoperazone without consideration of MIC , the effect of cefteriaxone was 46.6% and cefoperazone was 78.3%; but with consideration of indentified MIC for each species, there was not significant difference between two species (26.6% vs 28.3%, respectively). In comparison of two methodes of antibiogram, E-test was more sensitive than disk diffusion method at detection of sensitive isolates (kappa index: 0.295). By disk diffusion method the most effective antibiotic on *E. faesium* was vancomycin (53.3%) and then respectively were gentamycin (50%), ampicillin (41.6%) and ceftriaxone (16.6%); whereas on *E. faecalis* the most effective antibiotic was ampicillin (93.75%) and then respectively were vancomycin (72.91%), gentamycin (56.25%) and ceftriaxone (27%). Discussion: Superiority of cefoperazone to ceftriaxone on entrococal species was related to high MIC and administration of high doses and possibility of serum level detection. On the other hand, it seems that intrinsic resistance of entrococci on these two cephalosporines is more important than aquired resistance. Also aquired resistance may have some effect.

## Introduction

Enterococci are microorganisms that are found in water, air, soil, vegetable, and wastewater.

The origins of these organisms are human and other homeotherms such as horses. The

most common causes of infection in humans include *E. faesium* and *E. faecalis*.

These organisms were considered non-significant pathogens in the past, but in the past two decades they have found considerable importance in the case of nosocomial infection. The most common infection caused by this organism in humans is urinary infection which is followed by abdominal and pelvic infections in terms of prevalence. Other infections associated with this organism include the following: surgical wound infection; bacteremia; endocarditis; neonatal septicemia; and meningitis (rarely).These bacteria mostly cause infections in severely ill and immunosuppressive patients hospitalized in hospitals. One of the important effects of antibiotics in human intestines is the change in the intestinal colonization dynamics in favor of Enterococci which are naturally resistant to many antibiotic compounds. Antibiotics that are specifically excreted through the bile (such as cephalosporins) increase intestinal colonization with Enterococci. Particularly, VRE contributes to intestinal colonization through down-regulation of intestinal demonstration of the anti-microbial peptide of RegIII $\gamma$ .

Another factor that contributes to the colonization of Enterococci in the intestines is the use of anti-acid drugs in severely ill patients for preventing aspiration pneumonia. Another factor is the presence of an imaginary phosphotransferase system encoder locus in *E. faesium* to increase its ability to undergo colonization in the intestines during antibiotic therapy. The main reason for selection and propagation of this organism, especially in hospitalized patients, is their intrinsic resistance to a number of highly consumed and available antibiotics through mutation or reception of external genetic materials (such as the transfer of plasmids and transposition) (1). Resistance to Vancomycin is the most important microbial resistance with this organism which makes the control of the resulting infections difficult. Enterococci Virulence is not clearly and precisely understood (2).

Ampicillin, Gentamicin, and Vancomycin are the most important antibiotics and usually the clinicians prescribe them with the hope to Enterococci infections. Unfortunately, there is evidence that in the past years the level of resistance to these antibiotics has had an increasing growth. Examples of such evidence include the study by Keryn J.C. et al. in 9 centers in Australia. They studied 1987 *E. faecalis* and 180 *E. faesium* subjects and compared their sensitivity to these antibiotics in 1995, 1999, 2003 and 2005 (3).

Such evidence is used as a warning and guidance for the control of Enterococcus superinfections if broad-spectrum antibiotics that are ineffective for Enterococci are prescribed. The evidence also shows antibiotic synergism. In this regard, the results of in-vitro examinations naturally will form a model useful for subsequent clinical applications.

The aim of this study was to draw a comparison between the activities of two third-generation Cephalosporin, Cefoperazone, and Ceftriaxone and those of the clinical isolates of Enterococcus including *E. faecalis* and *E. faesium*.

## Materials and Methods

In a descriptive cross sectional study that was carried out in Tabriz, the effects of two third-generation cephalosporin, namely Cefoperazone and Ceftriaxone, against the clinical isolates of Enterococcus (including

*E. faecalis* and *E. faesium*) were studied on patients hospitalized in Imam Reza and Sina Hospitals of Tabriz.

About 3200 cultures were obtained in the course of study in each hospital. The positive cultures were studied and all of the Entrococcus-spp. cases were included in the study. A total of 60 strains of the hospitalized patients were isolated. All of the strains were subjected to laboratory differentiation and two important strains of Enterococcus (including Faecalis and Faecium) were subjected to sensitivity tests. For these tests, the Ampicillin, Gentamicin, Vancomycin, and Ceftriaxone were examined using the disc-diffusion method and Ceftriaxone and Cefoperazone were examined using the E.test method. Unfortunately, no Cefoperazone disk was available for the purpose of the disk-diffusion test and the test was not carried out.

The strain of the Enterococci isolated from different clinical samples was identified using the PCR method.

The bacterial isolates were cultured in a broth medium and the DNA extraction procedure was carried out based on the Pakzhen kit instructions. Next, using the special primers of *E. faecalis* and *E. faesium*, which are presented in the following table, the two strains were differentiated in accordance with the following time and temperature plans, using the PCR method.

| | | |
|---|---|---|
| 1 Cycle | 95ºC | 10 min |
| 30 Cycle | 94ºC<br>56ºC<br>72ºC | 1 min<br>1 min<br>1 min |
| 1 Cycle | 72ºC | 10 min |

The reaction occurred in a 20 $\mu l$ and each 100 $\mu l$ of the master-mix included the following elements.

- Primer 15 pm
- X PCR buffer 10µl
- MgCl 2mM
- dNTP 0/2 mM
- Taq polymerase 8 u
- Templet DNA 10µg

The PCR products for *E. faecalis* an *E. faesium* were bp941 and bp658, respectively. The products were subjected to electrophoresis in a 1.5% agar and were assessed against the size of the marker under study.

## Inclusion Criteria

In order to identify the isolates of the Enterococcus bacteria, standard bacteriological and biochemical tests were employed. The process of these tests is described below.

All of the samples were cultured in sheep-blood-agar in a standard loop. The colonies were cultured in a medium containing 6.5% of sodium chloride and were subjected to hemolysis in sheep-blood agar. The colonies were also subjected to esculin hydrolysis in the presence of 40% bile and their growth took place at 10 and 45 temperatures. Colonies lacking such qualities were excluded from the study and the remaining ones were put in 35-37 incubators and were included in the study. In addition, the fermentation of Arabinose, Sucrose, Sorbitol, and Raffinose sugars was also studied.

## The Disk-Diffusion Method

The antimicrobial sensitivity of all of the isolates was determined using the Kirby-

Bauer-Disk-diffusion method in accordance with the CLSI (2003) proposed standards. The cultured bacteria were used after 24 hours of incubation to prepare a suspension based on the 0.5%Mac Farland suspension standard. Plates containing the Mueller-Hinton agar were prepared beforehand based on the recommendations by the manufacturing company and were cooled to a temperature of 45-50% and prepared for use after the autoclave process.

Using a sterile swab the aforementioned suspension was spread over plates containing the Mueller-Hinton agar and the disks were placed over the plates with 1.5-2 cm intervals within 15 minutes. Antibiogram disks were made by Himedia Company of India. For each strain two plates were prepared, one for the disk-diffusion test and one for the E.test.

The mediums were put for about 20 hours in an incubator at a temperature of $37\,^{\circ}C$. At the end of the incubation, the diameter of the halo around the disks, which reflected the lack of growth of the bacteria, was measured and the measurements were interpreted based on the CLSI (2003) criteria.

| | Disk Diffusion agar | | |
|---|---|---|---|
| | Sensitive | Intermediate | Resistant |
| Ampicillin | ≥17mm | 16-17mm | ≤16mm |
| Vancomycin | ≥17mm | 15-16mm | ≤14mm |
| Ceftriaxone | ≥27mm | 25-26mm | ≤24mm |
| Gentamicin | ≥10mm | 7-9mm | ≤6mm |

The results were classified as follows based on the corresponding numbers. No disk diffusion test was carried out for Cefoperazone as it was not possible to prepare the required materials.

## The E-Test Method

The bacterial suspension in physiological serum was obtained based on the 0.5% Mac-Farland standard and the cultures were prepared using a sterile swab on the Mueller-Hinton agar.

Plates were preserved at room temperature for 15 minutes to provide for the absorption of the excess moisture. The E.test strips were kept at room temperature 20 minutes prior to use and were then placed on the surface of the incubated medium so that no bubbles remained beneath the E.test strips. Finally, the strips were put in an incubator at a temperature of $37\,^{\circ}C$ for about 20 hours. The E.test equipment used for testing Ceftriaxone and Cefoperazone were made by Liofilchem (Italy) Company.

After 16 to 20 hours of incubation, the antimicrobial regions formed around the test strips on the agars were examined on the plates and the MIC levels were determined based on the area intersecting the ellipse resulting from the lack of growth of bacteria on the strips. The results were interpreted in accordance with the EUCAST criteria and were put into one of the following categories: resistant, intermediate, and sensitive.

The Enterococci criterion for Ceftriaxone and Cefoperazone were also determined based on the related criteria for streptococcus pneumonia (4).

## Ethical Considerations

The study was not indirectly carried out patients and no expense or adverse effect was also imposed on the patients. Moreover, most of the patients were released from the hospital during the study and there was no need to obtain their informed consent.

## Statistical Analysis

The collected data were analyzed by SPSS-17 statistical software. The collected data

were expressed as percentage and mean ± SD. Continuous (quantitative) variables were compared by Independent samples and Paired t test. Categorical (qualitative) variables were compared by contingency tables and Chi-square test or Fisher's exact test. P-value ≤0.05 was considered statistically significant.

## Result and Discussion

As seen, the most common place of Enterococcus infection is the urinary tract.
In the analysis of the fermentation of sugars to clarify the properties of Enterococci, four sugars namely Arabinose, Raffinose, sucrose and Sorbitol, were examined. Results of this analysis are presented in Table (1).

As seen, the sucrose fermentation test was the most sensitive sugar test with a sensitivity of 98.3%. However, when the *E. faecalis* and *E. faesium* strains were studied separately, it was found out that the positive fermentation results for Arabinose and Raffinose tests only belonged to the *E. faesium* strain. The related results are shown in Table (2).

Therefore, it is possible to use the two tests to differentiate the two strains. In addition, the results of examination of the growth of bacteria in the medium containing 6.5% sodium chloride and 40% bile-esculine were 100% for both strains.

The PCR results used for differentiating the *E. faecalis* and *E. faesium* strains are presented in Table (3). As seen in this table, 80% of the isolates were of the *E. faecalis* strain. The frequencies of resistance and sensitivity of each of the antibiotics were obtained using the disk-diffusion method and are presented in Table (4).

As seen in this table, the highest level of sensitivity belonged to Ampicillin (sensitivity=83.3%) which was followed by Gentamicin, Vancomycin, and Ceftriaxone. In the study of the effect of Ceftriaxone and Cefoperazone using the E-test method it was found out that Cefoperazone affects 78.3% Enterococcus strains whereas Ceftriaxone affects 46.7% of the isolates. These results are presented in Table (5).

As seen, 32 Enterococcus isolates (53.33%) showed no in-vitro reaction to Ceftriaxone and 13 isolates (21.7%) showed no in-vitro reaction to Cefoperazone when examined using the E-test method. In other words, no zone activity was observed in any of the above cases (NZ).

Results of the examination of sensitivity to Ceftriaxone and Cefoperazone using the E-test method are shown in Table (6).

In Table (7), the levels of sensitivity to Ceftriaxone obtained from the disk-diffusion and E-test methods were compared. Hence, the E-test method showed a higher sensitivity than the disk-diffusion method in determining the sensitive strains. The kappa index measured using SPSS was 0.295, which reflects a moderate level of agreement. In the following table(8), the sensitivities of the *E. faecalis* and *E. faesium* strains to antibiotics are also evident.

As seen in this table, the most effective treatment for the Faecium strain is Vancomycin and the second most effective medications include Gentamicin and Ampicillin. However, in the case of *E. faecalis*, Ampicillin was the most effective treatment with a sensitivity level of 93.75% and was followed by Vancomycin, Gentamicin, Cefoperazone and ceftriaxone.
Of the *E. faesium* strain isolates, only two isolates were sensitive to Ampicillin and two isolates were not sensitive to any of the medications. One isolate was also sensitive to all antibiotics.

**Table.1** Sugar Fermentation

| | Sorbitol | Sucrose | Raffinose | Arabinose |
|---|---|---|---|---|
| Non-Fermentation | 12(20%) | 1(1.66%) | 51(85%) | 50(83.33%) |
| Fermentation | 48(80%) | 59(98.33%) | 9(15%) | 10(16.66%) |

**Table.2** Raffinose and Arabinose Tests in *E. faecalis* and *E. Faecium*

| | *E. faecalis* | *E. Faecium* |
|---|---|---|
| Positive Raffinose test | 0 | 9(75%) |
| Positive Arabinose test | 0 | 10(83.33%) |

**Table.3** PCR results

| | *E. Faecium* | *E. faecalis* |
|---|---|---|
| Frequency | 12 | 48 |
| Percent | 20% | 80% |

**Table.4** Sensitive and Resistant with Disk Diffusion methods

| | Ceftriaxone | Vancomycin | Gentamicin | Ampicillin |
|---|---|---|---|---|
| Sensitive | 50(83.33%) | 33(55%) | 42(70%) | 5(8.3%) |
| Resistant | 10(16.66%) | 27(45%) | 7(11.6%) | 44(73.33) |
| Intermediate | - | - | 11(18.33%) | 11(18.33%) |

**Table.5** Efficacy rate of Cefoperazone and Ceftriaxone on isolated Enterococcus

| | Cefoperazone | Ceftriaxone |
|---|---|---|
| Frequency | 28 | 47 |
| Percent | 46.66% | 78.33% |

**Table.6** Sensitivity rate of Cefoperazone and Ceftriaxone with E-Test methods

| | Cefoperazone | Ceftriaxone |
|---|---|---|
| Sensitive | 17(28.33%) | 16(26.66%) |
| Resistant | 38(63.33%) | 38(63.33%) |
| Intermediate | 5(8.33%) | 6(10%) |

**Table.7** Sensitivity rate of Ceftriaxone with E-Test and Disk Diffusion methods

| | E-test | Disk Diffusion |
|---|---|---|
| Sensitive | 5(8.33%) | 16(26.66%) |
| Resistant | 44(73.33%) | 38(63.33) |
| Intermediate | 11(18.33%) | 6(10%) |
| Total | 60(100%) | 60(100%) |

**Table.8** Sensitivity of *E. faecalis* and *E. Faecium*

| | Ceftriaxone | Ampicillin | Vancomycin | Gentamicin |
|---|---|---|---|---|
| *E. faecalis* (12(20%)) | 13(27%) | 45(93.75%) | 35(72.91%) | 27(56.25%) |
| *E. Faecium* (48(80%)) | 2(16.66%) | 5(41.6%) | 7(58.33%) | 6(50%) |

**Table.9** MIC 50-90 rate of Cefoperazone and Ceftriaxone

| | Range µg/ml | MIC 90 µg/ml | MIC 50 µg/ml |
|---|---|---|---|
| Ceftriaxone | 0.87 | 2.1 | 0.097-7.5 |
| Cefoperazone | 8 | 48 | 2-64 |

Results of the comparison between the *E. faecalis* and *E. faesium* strains in the table indicate that, generally, the *E. faesium* strain shows less antibiotic sensitivity to *E. faecalis* and the difference is considerable with Beta-lactams including Ampicillin and cephalosporin.

Although CLSI introduces the microdilution testing method as the recommended method for determining MIC-50 and MIC-90, due to impossibility of preparing the required materials for this test the E-test method was used in this research. Results of this test are also presented in the following table(9).

Enterococci are anaerobic facultative gram-positive bacteria that are found in the environment, human body, and the body of other homeotherms such as horses. These bacteria tolerate difficult environmental conditions, which cannot be tolerated by other bacteria, and grow in such conditions. They are oval bacteria that are seen individually, in pairs, in short chains, or even in very long chains. They can grow in mediums containing 6.5% NaCl, hypotone mediums, acid and alkaline mediums, and aerobic and anaerobic mediums. They also can grow with temperatures ranging from 10 to 45 degrees (5) and are resistant to freezing. Two common types of Enterococci (i.e. Faculis and Fecium) can hydrolyze esculin in the presence of 40% bile salts to produce one LAP and one PYR. They have alpha hemolytic and gamma hemolytic properties in 5% sheep blood agar and trypticase-soy. However, the blood of horses, rabbits and humans has a beta hemolytic property (6).

The traditional methods for separating different species of Enterococci (such as production of acid and arginine hydrolysis) are not practiced anymore due to their difficulty. Today, automatic or rapid biochemical methods (such as API) are used. These methods are applied to *E. faecalis* studies and do not apply to other species of Enterococci. Since *E. faesium* is capable of Arabinose fermentation, *E. faecalis* can be distinguished from other species of Enterococci (7). In addition, several molecular methods have been developed that are not routinely employed in laboratories. These methods include the following.

Moreover, resistance to Ampicillin usually reveals *E. faesium* and resistance or lack of sensitivity to quinupristin-dalfopristin is usually seen in *E. faecalis*.

The most common species that cause illness in humans are *E. faecalis* and *E. faesium*. Usually, there is no need to make a

distinction between these two species but in some epidemiological studies and some clinical scenarios, due to the discrepancies in virulence and antibiotic resistance it is necessary to make a distinction between these two species.

In the past two decades, these organisms have played an important role in the development of nosocomial infections. The resulting antibiotic resistance also makes treatment difficult. Particularly, in severely ill patients and patients with weak immune systems who are hospitalized for a long time, these organisms cause infection. They can be transferred to patients in hospitals through the hands of the health system personnel or through the environment. In each gram of human colon there are about $10^{12}$ bacteria with more than one hundred types of cultivable bacteria that are mostly anaerobic (8). Enterococci form a small part of this population in relation to the anaerobic bacteria. Enterococci have a symbiotic relationship with the immune system and other bacteria. These organisms show intrinsic resistance to some antibiotics and acquired resistance to some other antibiotics. Antibiotics that are mostly used for treatment purposes include Ampicillin, Gentamicin, and Vancomycin. In many references it is stated that Enterococci show an intrinsic resistance to cephalosporin (9) but in some references, Cefoperazone is introduced as the most effective form of cephalosporin for Enterococci species (10-11). Even ceftriaxone, which is introduced as an ineffective drug in many articles, is used for some particular forms of endocarditic caused by Enterococci, due to the synergy between this drug and some antibiotics (especially with amino glycosides and Ampicillin) and the effect of this synergy on Enterococci (12-13). The primary objective of this study was to compare the effects of Cefoperazone and ceftriaxone on these microorganisms. This is because Cefoperazone is not available and there is no emergence of resistance for Cefoperazone whereas ceftriaxone is widely used both in inpatient and outpatient conditions and the possibility of acquired resistance in addition to intrinsic resistance is high with ceftriaxone.

As seen in this study, the most prevalence source of infection is the urinary system. However, there are no records of the biliary tract which can be caused by the lack of demand for cultivation of biliary excretions or the physician's failure to obtain the required samples. In different studies, the degrees differ based on the study population and other factors such as history of hospitalization, presence of catheter affluent, and existence of background diseases (6). For instance, in less than 5% of urinary infections the pathogen is Enterococcus. On the other hand, distinguishing between a disease and colonization is difficult even when the number of colonies exceeds $10^5$. Hence, a positive culture does not solely determine the infection and it is important to consider the clinical signs and background conditions as well (6). In a study that was carried out by Ruoff et al. to distinguish Enterococcus isolates and the isolation source, the level of *E. faecalis*, *E. faesium* and E.Raffinosu was 87.1%, 8.6% and 0.3%, respectively (14).

As seen, the results are somewhat different but the most common source in both studies is the urinary tract. In the present study sugar, the most sensitive test for Enterococcus is the sucrose fermentation test (with sensitivity of over 98%), but the most applicable test is the Arabinose and Raffinose fermentation tests which were particularly carried out for *E. faesium*.
The same finding was reported for Arabinose in other studies (7). In a study by

Ford et al. (1994) it was indicated that Arabinose fermentation is achieved by both *E. faesium* and E.Raffinosus, but since the prevalence of Arabinose fermentation is very low (0.3%), it is of slight importance (14). On the other hand, the use of a CAA-specific medium also helps distinguish *E. faecalis* from *E. faesium*.

In other words, the negative predictive value of this test for making a distinction between the Faecalis and Faecium species is about 100%. Hence, these two tests can be used instead of PCR (which is highly costly) to distinguish between these two species.

In order to distinguish the two species using the PCR method, 12 of the 60 samples were of the *E. faesium* kind. In other words, 80% of the species were of the *E. faecalis* type. In our study, 20% of the isolates were *E. faesium* and this percentage is more than double the percentage obtained by Ruoff in 1989 (20% vs. 8.6%) (14). *E. faesium* is a resistant species and its isolations is followed by subsequent prognosis (6). Therefore, isolation of a higher percentage of *E. faesium* is an important warning sign. In analyzing the distinction between the sensitivities of these species it was found out that beta-lactams (Ampicillin and cephalosporin) have slight effect on *E. faesium* whereas Vancomycin and Gentamicin were the most effective drugs. However, in the cast of *E. faecalis*, Ampicillin was the most effective drug and cephalosporin had a better effect compared to *E. faesium*.

In the comparison between the sensitivities of the two species of Faecalis and Faecium differences were also observed. Although the most effective antibiotics for Enterococcus Faecium were Vancomycin (58.3%) and Gentamicin (50%), Ampicillin also showed desirable results with a share of 41.6%. On the other hand, in the aforementioned sources, the resistance to Ampicillin is usually seen in the Faecium species and is indicator of this species. Similarly, resistance to quinupristin-dalfopristin is usually only seen in *E. faecalis* (6). In Enterococcus Faecalis this sensitivity is mostly shown to Ampicillin (93.75%) which is followed by Vancomycin and Gentamicin. Even ceftriaxone and Cefoperazone are also more effective for the Faecalis species than the Faecium species (27% and 31.2% vs. 16.6%).

In this study, the effect of Cefoperazone was about 78.3% whereas the effect of ceftriaxone was 46.6%. However, in the study that was carried out by J.M. Blondaou et al. in Canada (1997) the effects of Cefoperazone and ceftriaxone were 76% and 10%, respectively (15).

This finding reflects the better performance of ceftriaxone in the study area (Iran) as compared to countries such as America, Canada and perhaps Europe. Since the increase in the consumption of ceftriaxone started about 10-14 years ago in Iran, the better performance of this drug in this area can be perhaps explained by the fact that ceftriaxone has been available in Iran less than European and American countries. Another possible reason is the lack of development of acquired resistance in Enterococci under consumption pressure. Moreover, the resistance to cephalosporin is only intrinsic unlike the resistance to Vancomycin.

The comparison between the disk-difussion and E-test methods for determining the sensitivity of Enterococci to ceftriaxone it was found out that the E-test method shows more sensitivity in identifying the species that are sensitive to ceftriaxone. Therefore, in the E-test method, 26.6% of the species

were sensitive whereas in the disk-diffusion method only 8.3% were sensitive. That is to say, a number of species that were identified as resisting and intermediate species in the disk-diffusion method showed to be sensitive by the E-test method. Therefore, the disk-diffusion method cannot be a suitable substitute for the E-test method (it has a kappa index of 0.295). In other words, the correlation and relationship between the two tests is moderate (i.e. there is a moderate agreement between the two tests).

Concerning MIC-50 and MIC-90, our findings differed from previous findings. That is to say, for Cefoperazone the values for MIC-50 and MIC-90 were 8 and 48, respectively. However, in the study carried out by J.M. Blondaou et al. in Canada the values for MIC-50 and MIC-90 were 16 and 32, respectively. Concerning ceftriaxone, the values for MIC-50 and MIC-90 were 0.87 and 2.1, respectively. However, in the aforementioned study, the levels of MIC-50 and MIC-90 were $\geq 64$. The difference can be ascribed to two reasons: the difference between the methods and the high resistance to ceftriaxone in the geographical region under study.

## Conclusion

In the present study, the E-test method was used as a method for analyzing the sensitivity of Enterococcus isolates to ceftriaxone and Cefoperazone. The lack of formation of an inhibition zone (no-zone) in the presence of ceftriaxone as compared to Cefoperazone was higher (53.33% vs. 21.7%) but concerning MIC-breakpoint, no difference was observed in the in-vitro sensitivity of the samples to these two antibiotics (36.7% vs. 36.7%). This result indicates that the advantage of Cefoperazone over ceftriaxone for the treatment of Enterococcus isolates is caused by the high MIC values and administration of high clinical dosages. In other words, Cefoperazone probably be superior to ceftriaxone in important clinical uses such as for the treatment of Enterococci provided that it is used in high dosages and the chance of monitoring the serum level is provided.

On the other hand, considering the considerable in-patient and out-patient uses of ceftriaxone in our area and the lack of absolute use of Cefoperazone, based on the experimental results of this research, it can be said that the significance of intrinsic resistance to these two forms of cephalosporin is higher than that of the acquired resistance caused by consumption pressure.

Moreover, it is worth noting that from the theoretical point of view, since excretion of these antibiotics mainly occurs through the bile, there is a possibility of development of acquired resistance in addition to intrinsic resistance as a result of excessive consumption.

## Suggestions

Enterococci are of great importance because of their intrinsic and acquired resistance to antimicrobial drugs. In order to control this organism prevention of increased prevalence and resistance and prevention of the transfer and pathogenesis of Enterococcus are more valuable and important than antimicrobial measures.

Due to the increased antibiotic resistance to Enterococci, the necessity of rational prescription of antibiotics is sensed more than before. This is also important for antibiotics that are excreted through the bile and change the digestive system flora in favor of Enterococci. On the other hand, standard preventive measures (such as hand

washing) are also highly important for preventing the nosocomial transmission of the resisting species.

In addition, considering the extent of resistance to some bacteria, the stress on the isolation of the organism from the focus of infection and sensitivity tests shall be doubled in clinical treatment of Enterococcus infection.

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