Original Article

Ulutas Med J 2015;1(4):111-115
DOI: 10.5455/ umj.20150917042844
ISSN: 2149-0430 **eISSN:** 2149-388X

Antibiotic Susceptibilities of Enterococci Isolated from Blood Cultures

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Background: The aim of this study was to determine the enterococci species and antimicrobial resistance rates of enteroccus strains isolated from blood cultures of inpatients.

Method: One hundred five Enterococcus spp. strains isolates were identified by conventional methods and Vitek-2 automated system. Antibiotic susceptibility tests were performed by disk-diffusion method and E-test.

Results: 54(51.4%) were identified as E.faecalis and 51(48.6%) as E. faecium. General Intensive Care Unit (GICU) is the section where most frequently isolated species of both enterococci. Other clinics were Dialysis unit, and Internal Medicine clinics. E.faecium strains were found to be resistant to ampicillin, erythromycin, and ciprofloxacin. Resistance rates of E.faecalis strains against ampicillin, erythromycin, ciprofloxacin were 47%, 70.5%, and 11.8%, respectively. High-level resistance to gentamicin and streptomycin were determined in 55.6%, 83.3% of E.faecium strains and in 23.5%, 11.8% of E.faecalis strains, respectively ($p \leq 0.05$). Two of E.faecium strains and one of E.faecalis from GICU were found to be resistant to teicoplanin and vancomycin. Linezolid was found most effective drug and resistance wasn't determined in none of the strains.

Conclusion: Three vancomycin and teicoplanin resistant strains isolated from blood cultures in our hospital. This result showed that the isolates mentioned above might cause significant problems in the future. In addition, linezolid may be a good alternative for the treatment of the resistant enterococcal infections in our hospital.

Key words: Enterococcus facium, Enterococcus faecalis, Antibiotic susceptibility, E-test, Disk diffusion

Introduction

Enterococci become one of the most common nosocomial infection agents that have high mortality rate in recent years. Cause of this situation could be considered as resistance of enterococci to difficult environmental conditions, cephalosporins, lincosamides, trimethoprim-sulfamethoxazole (TMP-SXT), inherent low level of resistance to aminoglycosides (1,2). However, all these properties found always in enterococci, this increase has not fully explained in recent years. The results of the studies showed that real reason for this increase was enterococcal colonization selectively and to become the dominant flora in the gastrointestinal tract as a result of frequent use of antibiotics as 3rd generation cephalosporins, metronidazole, carbapenem, clindamycin that were noneffective or restricted effect on enterococci in the hospitals (3). Enterococci in hospitals can cause infections such as such as urinary tract infections, skin and soft tissue infections, bacteremia, peritonitis, meningitis especially resulting from gastrointestinal flora in immunocomprimized patients (4). It was indicated that

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Received: Sep 10, 2015 **Accepted**: Oct 17, 2015
Published: December 28, 2015

enterococci were second most common cause of skin and soft tissue infections and third most common cause of bacteremia in the studies (5).

Increase in the resistance rate of Enterococci to antibiotics and high mortality rate in bacteremia compared to other clinical situations were shown by the studies (1,6). Initiation of appropriate empiric treatment in the bacteremic patients was stated that change prognosis in the right direction (7,8). In bacteremia caused by enterococci, determination of antibiotic susceptibility for the initiation of the appropriate empirical treatment, follow-up and analysis of the data is very important.

This study was performed to determine *enterococcus spp.* strains patterns of antimicrobial resistance including glycopeptides and linezolid.

Study Design

One hundred five Enterococcus strains isolated from blood cultures sent from clinics at Osmaniye State Hospital Microbiology Laboratory between January 2011-December 2013 were included in this study. The study protocol was reviewed and approved by the secretary general of the Osmaniye association of public hospitals ethics committee. Blood culture were studied with BACTEC 9240 (Becton Dickinson, Sparks, Md., USA) automated blood culture system. The isolates were identified by conventional methods and VITEK-2 system (BioMerieux, France). Antibiotic susceptibility tests were performed by the Kirby-Bauer disk-diffusion method according to the standards of Clinical and Laboratory Standards Institute (9).

Intermediate susceptible strains were considered as resistant. For high-level resistance to aminoglycoside, gentamicin 120 mcg and 300 mcg of streptomycin discs were used. High-level resistance to aminoglycoside was also studied by the agar screening test. Minimum inhibitory concentrations (MIC) were determined by E-test (AB Biodisk, Sweden) for all isolates showing decreased susceptibility to vancomycin and teicoplanin by disk diffusion method. The test was quality controlled by using *E. fecalis* ATCC 29212. Statistical Analysis was performed using SPSS software. The chi-square test was used to test the statistical differences.

Results

Enterococcus strains evaluated, and among of 105, 54 (51.4%) were identified as *E. faecalis* and 51 (48.6%) as *E. faecium.* General Intensive Care Unit (GICU) is the section where the most frequently isolated species of both enterococci. Other clinics were Dialysis unit and Internal Medicine clinics (*Table-1*).

Table-1. Distribution of clinics and species of enterococci isolated from blood cultures

Clinics	***E. faecium*** *(n:54)*	***E. faecalis*** *(n:51)*	***Total*** *(n:105)*
Intensive Care Unit	51 (%94.5)	27 (%52.8)	78 (%74.3)
Dialysis Unit	-	15 (%29.5)	15 (%14.2)
Internal Medicine Unit	3 (%5.5)	9 (%17.7)	12 (%11.5)

All of *E. faecium* strains were found to be resistant to ampicillin, erythromycin, ciprofloxacin. Resistance rates of *E. faecalis* against ampicillin, erythromycin, ciprofloxacin were as 47%, 70.5%, 11.8%, respectively. High level resistance to gentamicin and streptomycin were determined in 55.6%, 83.3% of *E. faecium* strains and in 23.5%, 11.8% of *E. faecalis* strains ($p \leq 0.05$).

Table-2. Susceptibility of E.faecalis and E.faecium to 9 antibiotics

	E.faecium (n:54)		*E.faecalis* (n:51)		Total (n:105)		Signif.
Antibiotics	***Rn***	***%***	***Rn***	***%***	***Rn***	***%***	***p***
Ampicillin	54	100	24	47	78	74.3	0.015
Erythromycin	54	100	36	70.5	90	85.7	0.230
Tetracycline	45	83.3	45	88.2	90	85.7	0.842
Gentamycin[h]	30	55.6	12	23.5	42	40	0.0001
Ciprofloksasin	54	100	6	11.8	60	57.1	0.026
Streptomycin[h]	45	83.3	6	11.8	51	48.6	0.0001
Teicoplanin	2	3.7	1	1.9	3	2.8	0.942
Vancomycin	2	3.7	1	1.9	3	2.8	0.942
Linezolid	-	-	-	-	-	-	

Rn: Number of isolates that are resistant to antibiotics. **(h)** High level Gentamycin and Streptomycin Chi-square test was used. $p \leq 0.05$ was considered as significant.

Resistance rate of both strains against tetracyclin was similiar, but found to be higher in E.faecalis strains. Both 2 of E. faecium strains and 1 of E. faecalis from GICU were found to be resistant to teicoplanin and vancomycin. MIC values of all the strains for vancomycin was determined as ≥256 µg/mL, for teicoplanin was determined as ≥64 µg/mL. Linezolid was found most effective drug and resistance wasn't determined in none of the strains (*Table-2*).

Discussion

Gram-negative bacteria were frequently isolated in the 1970s, while after the 1990s gram-positive bacteria has come to the forefront in bacteremia. Enterococci found in normal flora are isolated from bacteremia at an increasing rate. In studies from England in 2005,

enterococci in bacteremic agents were isolated more than 8% compared to the previous year (3,10). Besides, being naturally resistance of enterococci to many antibiotics, gaining new resistance mechanisms and transferring it to the new generation can be an indication of a more important issue in the future. Enterococci are naturally resistant to aminoglycosides (except for high level of aminoglycosides), cephalosporins, clindamycin and trimethoprim/sulfamethoxazole. Beta-lactams has limited clinical efficacy on enterococci due to the low affinity PBPs. In addition, enterococci may acquire resistance to high levels aminoglycosides, macrolides, streptogramin, tetracycline, rifampin, chloramphenicol, fluoroquinolones and glycopeptides (10).

Usually, enterococci that cause infections in patients with impaired host defense lead to difficulty in the treatment due to their ability to acquire resistance to very large groups of antibiotics. Although enterococci are the members of the gastrointestinal system flora, *E. faecalis* are dominant than *E. faecium*. Therefore, *E. faecalis* isolated more frequently as an infectious agent in clinical samples. However, it is stated that isolation of *E. faecium* is seen especially from the blood in enterococcal infection in the recent years studies (1,11).

Recent studies were reported that 42-67% of enterococ isolated from bacteremia was *E. Faecium* in our country (1,2,11). In the study, E.faecium rate is 51.4% that supports *E. faecium* more frequently isolated in bacteremia.

It was reported antibiotic resistance with increasing rates in enterococcus species and these rates varies among species. Ampicillin that antibiotics can be preferred in enterococcal infection resistance have been reported in 39-93% and 4-52% as for *E. faecium* isolates and *E.faecalis* studies in studies from Turkey, respectively (1,2,11). In this study, ampicillin resistance were determined in *E. faecium* isolates as 100%, for *E.faecalis* isolates as 47%. Rate of ampicillin resistance in *E.faecium* isolates were found higher than *E.faecalis* isolates that was statistically significant.

Erythromycin was another most preferred antibiotic in enterococcal infection. Due to resistance to erythromycin that was one of the macrolide has similar resistance mechanism with clindamycin and streptogramin B, resistance in one of these antibiotics appear as resistance to other antibiotics (12). Studies have shown that erythromycin resistance was found higher in E. faecium isolates than in *E. faecalis* isolates. Berktas et al(1), detected erythromycin resistance in the isolates of *E. faecalis* and *E. faecium* as 47% and 88%, Gozubuyuk et al(11) 56.3% and 80.3%, Turkdağı et al(2) detected in 79% and 99%. In this study, resistance rate was detected 70.5% in *E. faecalis* isolates, 100% in *E. faecium* isolates. Our results were found to be compatible with literature.

Tetracycline resistance occurs with different genes as tetL, tet M, tetN ve tetO in enterococci and with spreading of these genes by conjugation (13). Tetracycline resistance rate in enterococci strains isolated from various clinical specimens in Turkey was found between 28-52% (14,15). Gozubuyuk et al detected tetracycline resistance as 62.5% in the *E. faecalis* strains isolated from blood cultures, and 22.9% of *E. faecium* strains (11). Tetracycline resistance in our study, was 88.2% for *E. faecalis*, and 82.3% for *E. faecium*. These results showed that high tetracycline resistance for both types of enterococcus and should be avoided from use of tetracycline for empirical treatment.

Quinolones in combination therapy with other antibiotics for the treatment of enterococcus often preferred (8,12). Meric et al(16) found ciprofloxacin resistance rates for *E. faecalis* strains as 21% and as 78% for E. *faecium* strains, Berktas et al(1) found that 21% and 60%, Turkdagı et al (2) found that 61% and 85% respectively. In this study, 11.8% in *E.faecalis* strains and 100% in *E.faecium* strains. Resistance rate of ciprofloxacin in *E. faecium* strains were found statistically significantly higher than *E. faecalis*. Too high ciprofloxacin resistance in *E. faecium* strains was considered worrisome on behalf of hospital. Quinolones was thought to be appropriate in the treatment of enterococci infections with determining quinolone antibiotic susceptibility and identification of the species.

Enterococci are naturally resistant to low-level aminoglycoside but high levels of aminoglycosides (HLA) can be used in treatment.12 HLA resistance in Turkey according to data of multicenter study were found to be 48.1% and this rate was reported to be the second highest value in the European countries.17 Gales et al.18 found high level gentamycin resistance (gentamycin) as 29% in *E. faecalis* strains, 11% in *E. faecium* strains, a high level streptomycin resistance (streptomisinh) as 25%, 77% respectively. Meric et al(16) reported that resistance rate of gentamycinh as 13% and 41%, streptomisinh as 22%, 67% in *E.faecalis* and *E.faecium* strains respectively. In another study, gentamisinh resistance of *E. faecalis* and *E. faecium* strains were detected as 14% and 52%, streptomisinh resistance were found to be 11%, 62%, respectively (19). Gentamisinh resistance in our study was 23.5% and 55.6%, streptomisinh resistance was 11.8% and 83.3% in *E.faecalis* and *E.faecium* strains respectively. According to the results of our study, HLA in *E. faecium*

strains were detected significantly higher than *E.faecalis* strains and also HLA observed higher than from the other studies in the literature.

Glycopeptides have been effectively used for a long time due to emergence and gradually increase of enterococci resistance to the beta-lactam antibiotics and amino glycosides. For first time in 1987, reporting vancomycin resistant enterococci (VRE) have been reported in France and in the UK, this should be thought that VRE would be cause serious problems in the future (9). In our country, the first VRE were reported in 1998 (20). In multicenter study SENTRY conducted in 2005, VRE rate was 0% in France, Sweden and Switzerland, 66.7 % in the UK, 71.4% in Ireland. In this study, the resistance rate of *E.faecalis* was 0%, while in *E.faecium* was 8.6% in Turkey (1). In studies from Turkey in recent years, vancomycin and teicoplanin resistance rate in *E. faecium* strains as 0-24 %, while the rate of resistance in *E. faecalis* strains have been reported in 0-5% (1,2,11). In our study of vancomycin and teicoplanin resistance has been seen in the same strains and detected in two (3.7%) E. faecium and one (1.9%) in *E. faecalis* strains. All of the strains isolated from GICU and *E. faecium* strains isolated at the same time. This result was thought that transmission of infection between the patients. In this case, more attention should be paid to our hospital infection control measures, the implementation of rapid and accurate diagnostic methods to prevent possible outbreaks and surveillance studies.

Linezolid is effective against gram-positive bacteria, including VRE, MRSA, multidrug resistant staphylococci, penicillin-resistant Streptococcus pneumoniae (21,22). Multicenter study conducted in United States, vancomycin resistance was detected in 10% of *E. faecalis*, only a strain was found intermediate susceptible to linezolid. The vancomycin resistance was detected in 76.9%, linezolid resistance was found to be 1.5% in *E. faecium* strains in the same study (23). Linezolid resistance has been reported in studies from Turkey between 0-4 % in both species (1,2,11). In our study, linezolid resistance was not observed in any strain. Monitoring antibiotic susceptibility pattern of each center has an important role in the infection control for early diagnosis and initiation of appropriate empirical treatment in bacteremia that has high mortality rate and caused by enterococci.

Made of *E. faecalis*-*E.faecium* species distinction will be an important guiding empiric therapy due to statistically significant differences between species in the resistance rate to antibiotics, as in our study.

Conclusion

Vancomycin was still found to be an effective agent against both enterococci species, although more frequently encountered every day with VRE infections. Isolation of three VRE strains from blood cultures in our hospital shows that infections related to enterocci could lead to bigger problems in the future. In this study, it was determined that linezolid was found to be effective against all strains including VRE strains and to be a good alternative in enterococcal infections. It was considered that to follow susceptibility patterns of linezolid which was one of the rare option in the treatment of VRE infections and to limit unnecessary clinical use would be more appropiate.

Conflict of Interest

There is not any commercial associations, contractual relations, or proprietary considerations that might pose a conflict of interest related or unrelated with the article, and no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

Acknowledgements

This study was presented as oral presentation at VI. Eurasia Congress of Infectious Diseases (EACID) Congress in Belgrad/Serbia at 24-27 September 2014.

Reference

1. Berktas M, Cıkman A, Parlak M, Guducuoğlu H, Ozkacmaz A. The Antibiotic resistance of Enterococcus strains isolated from blood cultures. Sakaryamj 2013;3:76-79.
2. Turk Dagı H, Arslan U, Tuncer EI. Antibiotic resistance in Enterococci isolated from blood cultures. Turkish Society Microb 2011;41:103-106.
3. Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. Microbiology 2009; 155: 1749-1757.
4. Sava IG, Heikens E, Huebner J. Pathogenesis and immunity in enterococcal infections. Clin Microbiol Infect 2010;16:533-540.
5. De Fatima Silva Lopes M, Ribeiro T, Abrantes M, Figueiredo Marques JJ, Tenreiro R, Crespo MT. Antimicrobial resistance profiles of dairy and clinical isolates and type strains of enterococci. Int J Food Microbiol 2005;103:191-198.
6. Fraser A, Paul M, Almanasreh N, Tacconelli E, Frank U, Cauda R, et al. Benefit of appropriate empirical antibiotic treatment: thirty-day mortality and duration of hospital stay. American journal of medicine 2006;119:970-976.
7. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest Journal 2000;118:146-155.
8. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective

antimicrobial therapy is the critical determinant of survival in human septic shock. Critical care medicine 2006;34:1589-1596
9. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement M100-S18, CLSI, Wayne, Pa (2008).
10. Tunger O. The old and new treatment options for vancomycin-resistant enterococcal infections. Ankem Journal 2012;26:215-227.
11. Gozuboyuk G, Uyanik MH, Hancı H, Aktas O, Ozbek A. Antimicrobial susceptibility of Enterococcus strains isolated from blood cultures. Ankem Journal 2013;27:107-112.
12. Durmaz S, Kiraz A, Ozer TT, Percin D. Macrolide-lincosamide-streptogramin B resistance phenotypes in Staphylococcus aureus. Eur J Gen Med 2014;11:217-220.
13. Murray BE. The life and times of the Enterococcus. Clin Microbiol Rev 1990;3:46-65.
14. Eksi F, Gayyurhan ED. Antibiotic susceptibility of Streptococcus and Enterococcus strains isolated from clinical specimens. Ankem Journal 2008;22:53-58.
15. Kalayci O, Yurtsever SG, Gungor S, Uzun B, Kurultay N. Evaluation of in vitro antibiotic sensitivity of Enterococci isolated from urine samples. Klimik Journal 2011;24:105-107.
16. Meric M, Ruzgar M, Gundes S, Willke A. Enterococcus species isolated from hospitalized patients and their antibiotic resistance patterns. Ankem Journal 2004;18:141-144.
17. Sumerkan B. Antibiotic resistance in Streptococcus pneumoniae and Enterococci: Map of Turkey (2003-2004). Ankem Journal 2005;19:61-65.
18. Gales AC, Sader HS, Ribeiro J, Zoccoli C, Barth A, Pignatari AC. Antimicrobial susceptibility of Gram-positive bacteria isolated in Brazilian hospitals participating in SENTRY Program (2005-2008). Braz J Infect Dis 2009;13:90-98.
19. Mert Dinc B, Aykut Arca E, Yağcı S, Karabiber N. In-vitro antibotic susceptibility of Enterococcus faecalis and Enterococcus faecium strains isolated from various clinical samples. Turk Hij Den Biyol Derg. 2009;66: 117-121.
20. Vural T, Sekercioglu AO, Ogunc D, Gultekin M, Colak D, Yesilipek A, et al. Vancomycin resistant Enterococcus faecium strain. ANKEM Journal 1999;13:1-4
21. Barrett JF. Linezolid Pharmacia Corp. Curr Opin Invest Drugs 2000;1:181-187.
22. Diekema DJ, Jones RN. Oxazolidinone antibiotics. Lancet 2001;358:1975-1982
23. Jones RN, Ballow CH, Biedenbach DJ and ZAPS Study Group Medical Centers. Multi-laboratory assessment of the linezolid spectrum of activity using the Kirby-Bauer disk diffusion method: report of the "Zyvox Antimicrobial Potency Study" (ZAPS) in the United States. Diagn Microbiol Infect Dis 2001; 40:59-66.

DOI: **dx.doi.org/10.5455/umj.20150917042844**
***Cite article* as**: Durmaz S, Ozer TT. Antibiotic Susceptibilities of Enterococci Isolated from Blood Cultures. Ulutas Med J. 2015;1(4):111-115.