

Comparison of resistance in isolates of *Enterococcus faecalis* and *Enterococcus faecium*

Fırat Zafer Mengeloğlu¹, Derya Çakır², Hüseyin Agah Terzi²

¹Mikrobiyoloji ve Klinik Mikrobiyoloji Uzmanı, Siirt Devlet Hastanesi, Siirt, Türkiye

²Zonguldak Karaelmas Üniversitesi Tıp Fakültesi Mikrobiyoloji ve Klinik Mikrobiyoloji AD, Türkiye

ABSTRACT

Objectives: Enterococci are Gram positive cocci causing hospital- and community-acquired infections even though they are found in normal flora and they have low-virulence. Their most common species detected as the causative agent are *Enterococcus faecalis* and *Enterococcus faecium*. This study aimed to evaluate the differences between the antibiotic susceptibilities of the isolates of these two species isolated from various clinical specimens.

Materials and methods: In this study, 68 enterococci strains (40 *E.faecalis* and 28 *E.faecium*) isolated from the specimens sent to the clinical microbiology laboratory of Zonguldak Karaelmas Practice and Research Hospital in 2007-2008 were evaluated. Identification at species level and the susceptibilities were performed by using MicroScan WalkAway system (Dade Behring, USA).

Results: No resistance to glycopeptides were detected among these isolates. High rates of resistance to ciprofloxacin, rifampicin and erythromycin were observed. Resistance rates to penicillin, ampicillin, ciprofloxacin, rifampicin, erythromycin and high-level gentamicin and streptomycin in *E.faecium* isolates were found statistically higher than the rates of *E.faecalis* group ($p<0.05$).

Conclusions: As concordant with the literature, this study observed that *E.faecium* isolates were significantly more resistant. In enterococcal infections, performing the identification at the species level and determining the antibiotic susceptibility will be helpful in managing of the treatment. *J Microbiol Infect Dis* 2011;1(1):10-13.

Keywords: *Enterococcus faecalis*, *Enterococcus faecium*, resistance, MicroScan

Enterococcus faecalis ve Enterococcus faecium izolatlarında direnç karşılaştırması

ÖZET

Giriş: Enterokoklar normal florada bulunmalarına ve virulansları düşük olmasına rağmen hastane ve toplum kaynaklı infeksiyonlara neden olan Gram pozitif koklardır. Etken olarak en sık saptanan iki türü *Enterococcus faecalis* ve *Enterococcus faecium*'dur. Bu çalışmada çeşitli klinik örneklerden soyutlanan bu iki türe ait izolatların antibiyotik duyarlılıklarının değerlendirilmesi amaçlanmıştır.

Gereç ve yöntem: Çalışma dahilinde 2007-2008 yıllarında Zonguldak Karaelmas Üniversitesi Uygulama ve Araştırma Hastanesi klinik mikrobiyoloji laboratuvarına gönderilen örneklerden soyutlanan 40'ı *E.faecalis*, 28'i *E.faecium* olmak üzere toplam 68 enterokok izolatı değerlendirilmiştir. İzolatların tür düzeyinde identifikasyonları ve antimikrobiallere duyarlılıkları MicroScan WalkAway sistemi (Dade Behring, ABD) ile yapıldı.

Bulgular: Enterokok izolatları arasında glikopeptidlere direnç saptanmadı. Siprofloksasin, rifampisin ve eritromisine yüksek oranlarda direnç gözlemlendi. *E.faecium* izolatlarında ampisilin, penisilin, siprofloksasin, rifampisin, eritromisin ile yüksek doz gentamisin ve streptomisine karşı direnç oranları *E.faecalis* izolatlarına göre anlamlı olarak yüksek bulundu ($p<0,05$).

Sonuçlar: Çalışmamızda önceki çalışmalara benzer şekilde *E.faecium* izolatları anlamlı olarak daha dirençli olduğu görüldü. Enterokok infeksiyonlarında mikroorganizmanın tür düzeyinde adlandırılması ve antibiyotik duyarlılığının saptanması tedavinin düzenlenmesinde faydalı olacaktır.

Anahtar kelimeler: *Enterococcus faecalis*, *Enterococcus faecium*, direnç, MicroScan

Correspondence: Dr. Fırat Zafer Mengeloğlu

Microbiology and Clinical Microbiology Department, Siirt State Hospital, Siirt, Turkey Email: mengeloglu@gmail.com

Received: 15.05.2011, Accepted: 12.06.2011

Copyright © Journal of Microbiology and Infectious Diseases 2011, All rights reserved

INTRODUCTION

Enterococci are catalase-negative Gram positive cocci which are components of normal flora of bowel, and despite their low virulence, they are frequently detected as the causative factors in hospital and community acquired infections.¹ Enterococci are stable to environmental conditions and they have intrinsically resistance to some antimicrobials and capabilities to develop new resistance.¹

In general, the most common species of enterococci detected as the causative agent are *Enterococcus faecalis* and *Enterococcus faecium*.¹ This study aimed to evaluate the differences between the antibiotic susceptibilities of the isolates of these two species isolated from various clinical specimens. The resistance rates were compared previous Turkish studies as well.

MATERIAL AND METHODS

Within the study, 68 enterococci strains (40 *E.faecalis* and 28 *E.faecium*) isolated from the specimens sent to the clinical microbiology laboratory of Zonguldak Karaelmas Practice and Research Hospital in 2007-2008 are evaluated. For this purpose, clinical specimens were inoculated in 5% sheep blood agar. At the end of the incubation at 35-37°C for 18-24 hours, colonies of Gram positive cocci which were catalase negative were processed in MicroScan WalkAway system (Dade Behring, USA) according to the manufacturer's recommendations in order to perform either the identification at the species level or the determination of the antibiotic susceptibilities. At

the end of the incubation, minimum inhibitor concentrations determined for each antibiotic by the system were interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria.

Statistical analysis was carried out using SPSS software (version 11.0). Descriptive statistics were expressed as the numbers and percentages. Chi-square and Fisher's Exact tests were used to analyse the differences between the groups according to the categorical variables and to evaluate the associations between the variables. Results were evaluated in 95% confidence interval and p value of <0.05 was considered as significant.

RESULTS

In total, 68 enterococci isolates were studied. The microorganisms were identified at species level. At same time, antibiotic susceptibility patterns of these isolates were investigated

In the both of study groups, no glycopeptide resistance was detected. High rates of resistance to ciprofloxacin, rifampicin and erythromycin were observed. The resistance rate against Erythromycin was found the highest among *E.faecalis* isolates. All of the *E.faecium* isolates were resistant to Erythromycin. Resistance rates in *E.faecium* isolates to ampicillin, penicillin, ciprofloxacin, rifampicin, erythromycin and high-level gentamicin and streptomycin were significantly higher than the rates in *E.faecalis* isolates (p<0.05). No significant difference was detected between the resistance rates to chloramphenicol (p=0.577) (Table 1).

Table 1. Resistance rates of isolates*

Antibiotics	Total (n=68)		<i>Enterococcus faecalis</i> (n=40)		<i>Enterococcus faecium</i> (n=28)		P
	n	%	n	%	n	%	
Erythromycin	62	91	34	85	28	100	0,032
Rifampicin	54	79	26	65	28	100	<0,001
Ciprofloxacin	50	74	22	55	28	100	<0,001
Streptomycin (High-level)	42	62	15	38	27	96	<0,001
Penicillin	35	51	8	20	27	96	<0,001
Gentamicin (High-level)	33	49	12	30	21	75	<0,001
Ampicillin	32	47	5	13	27	96	<0,001
Chloramphenicol	22	32	14	35	8	29	0,577
Vancomycin	0	0	0	0	0	0	-
Teicoplanin	0	0	0	0	0	0	-

* The percentages are expressed as even numbers due to the low number of isolates.

Table 2. Comparison of the high-level aminoglycosides resistance rates in previous studies from Turkey

Authors	Year	Number of isolates*	HLGR †		HLSR ‡	
			<i>E.faecium</i>	<i>E.faecalis</i>	<i>E.faecium</i>	<i>E.faecalis</i>
This study	2011	68	75	30	96	38
Mert Dinç et al. (5)	2009	100	52	14	62	11
Çaylan et al. (6)	2004	291	64	29	45	18
Meriç et al. (7)	2004	105	41	13	67	22
Gazi et al. (8)	2004	123	23	21	-	-
Kaçmaz et al. (9)	2003	56	80	4	-	-
Karadenizli et al. (10)	2002	136	49	26	35	16

* Total numbers of just *E.faecalis* and *E.faecium* isolates included in the study. † High-level gentamicin resistance ‡ High-level streptomycin resistance

DISCUSSION

Enterococci are intrinsically resistant to cephalosporins, clindamycin, penicillin and low-level aminoglycosides. In addition, enterococci could gain resistance to other antibiotics. In this study, the susceptibility patterns of the species most commonly identified two enterococcal as the causative agent were examined *E.faecium* isolates were found more resistant to majority of antibiotics than *E.faecalis*.^{2,3}

It has been reported that resistance in enterococci to aminoglycosides is caused by the difficulty of penetration of this agent through the cell membrane and the secreting of the enzymes modifying aminoglycosides as a result of genes acquired by plasmids and transposons.⁴ In our study, resistance rate in *E.faecium* group to high-level gentamicin (75%) was found significantly higher than the rate in *E.faecalis* isolates (30%) ($p<0.001$). Similarly, resistance rate to high-level streptomycin was detected significantly higher in *E.faecium* isolates (96% vs. 38%) ($p<0.001$). In Table 2, resistance rates to high-level aminoglycosides in these two species detected in some Turkish studies (Table 2).^{5,10} Similarly previous studies showed higher resistance rates among *E.faecium* isolates.

Penicillin resistance in enterococci is due to the development of tolerance to beta-lactams as a result of production of low-threshold penicillin-binding protein.¹ In this study, it was observed that penicillin resistance in *E.faecium* group (96%) was significantly higher than the rates in *E.faecalis* isolates (20%) ($p<0,001$). Resistance

rate to ampicillin in *E.faecium* isolates was also significantly higher in *E.faecium* group (96% vs. 13%) ($p<0,001$). In several studies, penicillin and ampicillin resistance in *E.faecium* isolates have been reported to be significantly higher.^{3,5,7,9,11,15}

In our study, all *E.faecium* isolates were resistant to ciprofloxacin, but the resistance rate in *E.faecalis* group was 65% ($p<0,001$), the difference was found to be statistically significant ($p<0,001$ as similar to some previous reports.^{17,20}

As concordant with the literature, in our study it is observed that *E.faecium* isolates were significantly more resistant than *E.faecalis* isolates. In the infections which enterococci are isolated as the causative agent, performing the identification at the species level and determining the antibiotic susceptibility will be helpful in managing of the treatment.

REFERENCES

1. Soysal A. Enterokoklar. J Pediatr Inf 2007; 1: 39-42.
2. Ünlü M, Vardar Ünlü G, Bakıcı MZ, Şahin A. Resistance of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from clinical samples to various antibiotics. İnfek Derg 2002; 16: 471-475.
3. Hoşgör M, Çavuşoğlu C, Tünger A, Özinel MA. High-level aminoglycozide resistance in enterococci. İnfek Derg 1997; 11: 7-9.
4. Shepard BD, Gilmore MS. Antibiotic resistant enterococci: the mechanisms and dynamics of drug introduction and resistance. Microb Infect 2002; 4: 215-224.
5. Mert Dinç B, Aykut Arca E, Yağcı S, Karabiber N. In-vitro antibiotic susceptibility of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from various clinical samples. Türkiye Hijyen ve Deneysel Biyoloji Dergisi 2009; 66: 117-121.

6. Çaylan R, Üstünakın M, Kadımov V, Aydın K, Köksal İ. Antibiotic susceptibilities of Enterococcus strains isolated from stool and clinical samples. *Türk Mikrobiyol Cem Derg* 2004; 34: 24-28.
7. Meriç M, Rüzgar M, Gündeş S, Willke A. Enterococcus species isolated from hospitalized patients and their antibiotic resistance patterns. *ANKEM Derg* 2004; 18: 141-144.
8. Gazi H, Kurutepe S, Sürücüoğlu S, Ecemiş T, Özbakkaloğlu B. Antimicrobial resistance in nosocomial *Enterococcus faecalis* and *Enterococcus faecium* strains. *ANKEM Derg* 2004; 18: 49-52.
9. Kaçmaz B, Akça G, Çağlar K, Sultan N. Antimicrobial susceptibility of enterococci. *ANKEM Derg* 2003; 17: 28-32.
10. Karadenizli A, Kolaylı F. Enterococcus species and their antibiotic susceptibilities isolated in Kocaeli University Medical Faculty. *Türk Mikrobiyol Cem Derg* 2002; 32: 212-5.
11. Moaddab SR, Töreci K. Species level identification and investigation of resistance to vancomycin and some other antibiotics in Enterococcus strains. *Türk Mikrobiyol Cem Derg* 2000; 30:77-84.
12. Öztürk R, Eroğlu C, Köksal F, Mert A, Aygün G. Antibiotic resistance and high-level gentamicin resistance in enterococci. *ANKEM Derg* 1995;9: 351-354.
13. Ulusoy S, Hoşgör M, Özkan F, Özinel M, Tokbaş A. Investigation of antibiotic resistance of *Enterococcus faecalis* and *Enterococcus faecium*. *ANKEM Derg* 1995; 9: 12-16.
14. Çınar T, Lelebicioğlu H, Sünbül M, Eroğlu C, Esen Ş, Günaydın M. Determination of high-level gentamicin and streptomycin resistance in enterococci. *Flora* 1999; 4:114-119.
15. Gülsoy Ö, Kocazeybek B, Arıtürk S. Investigation of the susceptibility to various antibiotics and high level aminoglycosides resistance in enterococci isolated from surgical intensive care unit. *Ankem Derg* 2002; 16:96-99.
16. Akıncı E, Balık İ, Tekeli E. Antimicrobial susceptibility of Enterococcus species collected from clinical specimens. *Flora* 1999; 4: 40-45.
17. Schouten MA, Voss A. Hoogkamp-Korstanje JAA. The European VRE Study Group. Antimicrobial susceptibility patterns of enterococci causing infections in Europe, *Antimicrob Agents Chemother* 1999; 43: 2542-2546.
18. Simonsen GS, Smabrekke L, Monnet DL et al. Prevalence of resistance to ampicillin, gentamicin and vancomycin in *Enterococcus faecalis* ve *Enterococcus faecium* isolates from clinical specimens and use of antimicrobials in five Nordic hospitals, *J Antimicrob Chemother* 2003; 51: 323-331.
19. Doern GV, Jones RN, Pfaller MA, Kugler K, Beach ML. Bacterial pathogens isolated from patients with skin and soft tissue infections: frequencies of occurrence and antimicrobial susceptibility patterns from the SENTRY Antimicrobial Surveillance Program (United States and Canada, 1997), *Diagn Microbiol Infect Dis* 1999; 34: 65-72.