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RESEARCH ARTICLE

COMPARISON OF VANCOMYCIN AND HIGH LEVEL AMINOGLYCOSIDE SUSCEPTIBILITIES AND VIRULENCE FACTORS ASSOCIATED WITH BIOFILM OF INFECTION AGENT AND FLORA MEMBER ENTEROCOCCUS STRAINS

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ABSTRACT: **Aim**: The aim of this study was to investigate and compare virulence factors of infection agent enterococci (IE) and flora member enterococci (FME). **Methods**: A hundred IE isolated from samples sent to microbiology laboratory and 100 FME isolated from the stools were included in the study. Vancomycin susceptibility was investigated by diffusion gradient method (GDM), high level aminoglycoside resistance and beta-lactamase production were investigated by disk diffusion and nitrocefin disk method respectively. Hemolysin, gelatinase and biofilm production were investigated via phenotypic methods. **Results and Conclusions**: It was found that hemolysin production rate of IE was more than that of FME. Vancomycin MIC values identified by GDM of biofilm-producing strains was found higher than that of biofilm-free strains. It was also revealed that moxifloxacin and ampicillin resistance rates of biofilm producing FME and ciprofloxacin, penicilin susceptibility of those that not produce biofilms were higher. Hemolysin production in the infectious was 0,37 times more than those in the FME. Biofilm production in the FME was 3,67 times more than that in IE. It was found out that the virulence factors affected resistance of the strains against some antibiotics .The hemolysin production was more in IE. Biofilm production was more in FME.

KEYWORDS: Enterococcus, infectious agent, flora, virulence, slime, hemolysis, gelatinase

INTRODUCTION:

The enterococci cause infections mostly in elders who have a severe disease and stay in hospital and in an intensive care unit for a very long time, and the patients who suffer from immune deficiency and whose treatments include invasive devices or broad spectrum antibiotics [1]. In parallel to the increasing resistance against the often used antibiotics, the enterococci turn into an agent causing the infections that are severe and life-threatening. This situation gains much more importance due to the fact that the enterococcus infections are spread to all age groups, and so more people. In this way, the resistance against the often used antibiotics grows into a distinctive feature of the enterococcus species [1].

Multiple antibiotic resistances enable enterococci to live and reproduce in spite of the antibiotic treatments. For that reason, these enterococci mostly

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occur as the factors of superinfection [2]. Although there are almost twenty types of enterococcus, the most common agents causing infection in humans are *Enterococcus faecalis* and *Enterococcus faecium*. These bacteria are one of the most common causes of bacteriemia, and also lead to some severe infections such as nosocomial urinary tract infection, surgical wound infection and endocarditis [2].

According to the searches conducted on *E. faecalis* and *E. faecium*, their antibiotic resistance might be gained [1]. Also, the virulence factors produced by the enterococcus have roles in pathogenesis. The bacterial toxins such as hemolysin, hyaluronidase, gelatinase, and hydrolytic enzymes containing serum protease, and biofilm take part in the virulence of enterococcus species [2]. It is signified that the biofilm, which is a polymeric structure created in relation to the environmental and genetic factors of the bacteria, is the source of many chronic infections [3].

The aim of this study is the comparison of vancomycin and high level aminoglycoside susceptibilities and the virulence factors asssociated with biofilm of the infection agent enterococci (IE) with thoseof flora member enterococci (FME).

MATERIAL AND METHOD:

One hundred IE strains, which were isolated from various clinical samples that were sent to the Microbiology Laboratory from different clinics of Hatay Mustafa Kemal University Hospital and one hundred FME strains, which were isolated from the gaita samples of the medical staffs were included in the study.

Identification and antimicrobial susceptibility of the strains were determined by Vitek 2 automated system (bioMerieux, France). High level aminoglycoside resistance (HLAR) was investigated by disk diffusion test using gentamicin (120 µg) and streptomycin (300 µg) disks (Becton Dickinson, USA), and it was evaluated according to CLSI criteria [4]. Vancomycin MIC values of the strains were determined by gradient diffusion method (GDM) using E-test strips (BioMerieux, France) and evaluated according to CLSI criteria [4]. Nitrocefin method and the nitrocefin disks (Becton Dickinson, USA) were used to investigate the beta-lactamase production of the strains. *Staphylococcus aureus* ATCC 29213strain was used as the positive control strain.

Bacteria were inoculated on gelatin agar (Sigma, Switzerland) and incubated at 37 °C for 48 hours. The halo which was formed around colonies after dropping the prepared Frazier solution onto the colonies on the gelatin agar, was considered as the gelatinase positivity[5]. The production of hemolysin was searched with the Columbia blood agar (Becton Dickinson, USA) containing 5% sheep blood. After incubation at 37 °C for 24 hours, the hemolysin zone which was formed around the colonies on the medium was considered as hemolysin positivity.

To investigate the biofilm production; enterococcal strains were inoculated into 10 ml of tryptic soy broth (TSB) and incubated at 37 °C for 24 hours. After the prepared suspensions were centrifuged at 2000 r/min for 10 minutes, they were washed by 10 ml physiological saline. 20 µL bacterial suspension prepared with 0.5 McFarland turbidity density equivalents ($1x10^5$ cfu/ml) was added into the each well of a 96-well microplate. By adding 180 µL TSB into the wells , bacterial concentration was set to the $1x10^4$ cfu/ml. After the microplates were incubated at 37 °C for 48 hours and were washed twice with phosphate-buffered water, the microplates were kept 30 minutes in the room temperature by adding 200 µL 0.1% Congo red. Their absorbance at 492 nm was measured three times in spectrophotometer (Thermo Scientific, USA) and the averages of the values were calculated. The measurements were evaluated in accordance with the criteria below; [6,7]

OD (492) ≤ ODc (OD negative control); negative
ODc < OD ≤ (2xODc); weak
ODc < OD ≤ (4xODc); intermediate
4xODc < OD; powerful

Those which have negative and weak biofilm production were considered as negative, those which

have intermediate and powerful biofilm production as positive.

Statistical Analysis

Data were analyzed using IBM Statistical Package for Social Sciences. The continuous variables were examined in terms of normal distribution and equality of variances. The Mann-Whitney U tests were used for the variables which were measured in the intergroup comparisons, and the Fisher exact χ^2 tests were used for the numerical variables. For the times when the value of P was less than 0.05, the test result was considered as meaningful. To examine the effective virulence factors within the infection-influencing group, the logistic regression analysis (multivariate analysis) was made. For the values with no standard distribution, it was given as the median value (minimum-maximum).

RESULTS:

It was determined that 42% of IE were isolated from the clinical samples sent from the internal science clinics, 35% from the surgical science clinics, 23% from the intensive care units. IE were isolated from urine (63%), wound (21%), and blood (13%) at most. The samples which IE were isolated from, were shown in **Figure 1**.

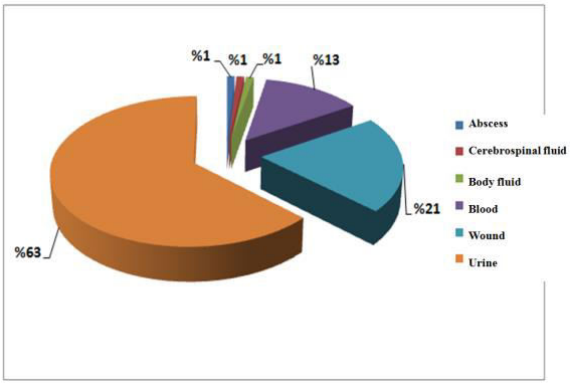


Figure 1. The samples which IE were isolated from

In the study 50.5% of the strains was *E. faecalis*, 42.5% was *E. faecium*, 4.5% was *E. gallinarum*, 1.5% was *E. casseliflavus,* and 1% was *E. durans*.

It was found that the antibiotics to which FME were most resistant were erythromycin (90%), clindamycin (87%), and ampicillin (63%), and the antibiotics to which these species were most susceptible were teicoplanin (90%), linezolid (94%), and vancomycin (89%). It was identified that the antibiotics to which IE were most resistant, were clindamycin (95%), erythromycin (86%), and ciprofloxacin (79%), and the antibiotics to which these species were most susceptible were teicoplanin (95%), linezolid (90%), and vancomycin (87%).

It was identified that there was no beta lactamase production in any of the enterococcal strains involved in the study. When the strains which had intermediate susceptibility were considered resistant, IE were found more resistant to clindamycin ($p=0.048$), ciprofloxacin ($p< 0.001$), and moxifloxacin ($p< 0.001$) than FME. However, FME were found more resistant to the ampicillin ($p= 0.003$).

It was found that *E. faecalis* species were more resistant to the tetracycline ($p< 0.001$) and erythromycin ($p= 0.015$) than *E. faecium* species. On the other hand, it was confirmed that *E. faecium* species were more resistant to the penicillin ($p< 0.001$), ampicillin ($p< 0.001$), vancomycin ($p= 0.014$), teicoplanin ($p= 0.006$), and linezolid ($p= 0.036$) than *E. faecalis* species.

When the susceptibilities of the strains causing infection to the antibiotics was analyzed, it was identified that their susceptibility and resistance status didn't vary according to the clinics from which the samples were sent ($p> 0.05$).

The high level streptomycin resistance (HLSR) ratio was found more in IE than FME ($p< 0.001$). No difference was found within these two groups in terms of high level gentamycin resistance (HLGR)

(p> 0.05). It was found that the HLSR ratio in FME was 16%, and the HLGR ratio was 22%. In IE, the HLSR ratio was determined as 55%, and the HLGR ratio as 25%.

When those with intermediate susceptibility were considered as resistant, four FME and five IE were found resistant to the vancomycin by the GDM (p> 0.05).

The HLSR ratio (40%) and HLGR ratio (41.2%) within the *E. faecium* strains were found more than those in the *E. faecalis* strains (p<0.001).

While seven of those strains were found resistant to the vancomycin by the gradient diffusion method and automated system, two strains were found intermediately susceptible by the GDM and found susceptible by the automated system. 16 strains, which were found susceptible by the GDM, were considered as resistant, whereas one strain was found intermediately susceptible.

It was determined that the vancomycin MIC median value of the strains was 1 µg/ml (min 0.50-max 32 µg/ml) by the automated system, and was 1 µg/ml (min 0.19-max 256 µg/ml) by the GDM.

The vancomycin MIC median value of IE was found as 1 µg/ml (min. 0.50, max. 32) by the automated system, and of FME as 1mg/ml (min. 0.50, max. 32). The MIC value of IE was found more than the MIC value of FME (p=0.019) (**Figure 2**).

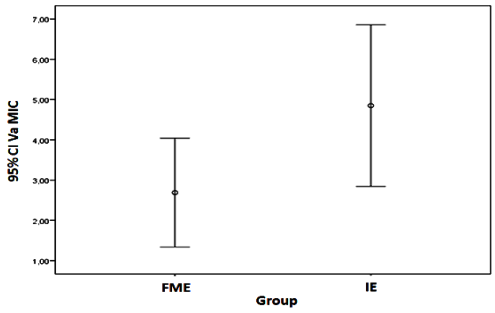
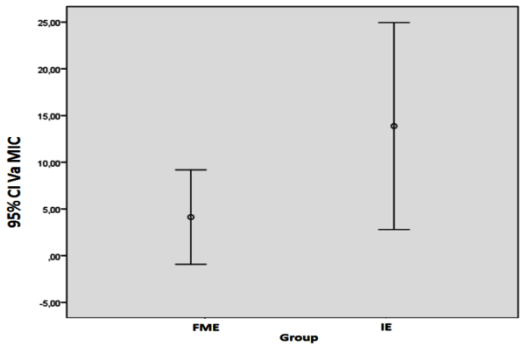


Figure 2. The comparison of the vancomycin MIC median values measured by the automated system

It was determined that the vancomycin MIC median value of IE is 1 µg/ml (min 0.38-max 256) by the GDM, and of the FME is 1µg/ml (min 0.19-max 256). No difference could be found between these two groups (p=0.116) (**Figure 3**).

Figure 3. The comparison of the vancomycin MIC median values measured by the gradient diffusion method



The hemolysin, gelatinase and biofilm production ratios in FME, were found as 11%, 10% and 54% respectively. In the other group (IE), the ratios of the hemolysin, gelatinase, and biofilm production were found respectively as 26%, 15%, and 23%.

The hemolysin production was found more in IE than the other group (p=0.006). The gelatinase production was also found more in IE, but no difference was found between these two groups statistically. The biofilm production was detected more in FME (p<0.001) (**Table 1**).

Table 1. The distribution of the hemolysin, gelatinase and biofilm production of enterococcal strains

Virulence Factors		Group		Total	P
		FME* N (=%)	IE** N(=%)	N (%)	
Hemolysin	Negative	89	74	163 (81,5)	**0.006**
	Positive	11	26	37 (18,5)	
Gelatinase	Negative	90	85	175 (87,5)	0.285
	Positive	10	15	25 (12,5)	
Biofilm	Negative	46	77	123 (61,5)	**<0.001**
	Positive	54	23	77 (38,5)	

***FME; flora member Enterococci **IE; Infectious Enterococci**

The hemolysin (p<0.01) and gelatinase (p=0.035) production of *E. faecalis* strains was found more than *E. faecium strains.* It was seen that there was no distinction within the biofilm production of these two groups (p=0.217) (**Table 2**).

Table 2. The virulence factors of the strains

Virulence Factors		*E. faecalis*	*E. faecium*	Total	P
		N (%)	N (%)	N (%)	
Hemolysin	Negative	39 (61,9)	35 (94,6)	74 (37)	**<0.001**
	Positive	24 (38,1)	2 (5,4)	26 (13)	
Gelatinase	Negative	52 (82,5)	33 (89,2)	85 (42,5)	0.369
	Positive	11 (17,5)	4 (10,8)	15 (7,5)	
Biofilm	Negative	46 (73,0)	31 (83,8)	77 (38,5)	0.217
	Positive	17 (26,9)	6 (16,2)	23 (11,5)	

It was determined that the production of the hemolysin, gelatinase, and biofilm did not differ in the enterococcal strains according to the clinics (p>0.05).

When the relation between the virulence factors (hemolysin, gelatinase and biofilm) and the MIC values of the strains were analyzed, only one statistically significant relation among the virulence factors. This virulence factor was biofilm production. The vancomycin MIC median value, which was determined by the GDM, was found 1.5 µg/ml in the strains, whose biofilm production was positive. The MIC median value of the strains whose biofilm production was negative, was found 1 µg/ml higher than biofilm producing strains (p<0.001) (**Figure 4**).

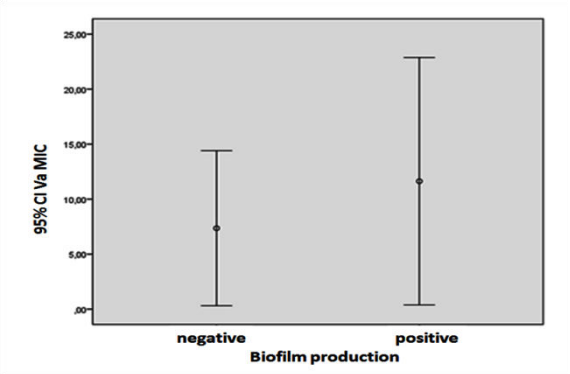


Figure 4. The comparison of the vancomycin MIC values of the strains measured by the gradient diffusion method

It was found that the hemolysin-free FME were more susceptible to the moxifloxacin (p=0.042) and the ciprofloxacin (p=0.033). The hemolysin producing strains was identified more resistant to the tetracycline (p<0.001).

It was seen that the biofilm producing FME were more resistant to the moxifloxacin (p=0.037) and ampicillin (p=0.001). Biofilm-free FME was identified more susceptible to the ciprofloxacin (p=0.046) and the penicillin (p=0.027). Nothing statistically significant was found between the biofilm production of IE and their antibiotic resistance (p>0.05). With no relation found between the other virulence factors and the resistance, merely the gelatinase-free strains which were identified more susceptible to the penicillin (p=0.042).

Within all enterococcal strains included in the study, it was detected that hemolysin and biofilm production of the strains within two groups were found similar (p=0.112). It was also detected that the hemolysin, and gelatinase production of the strains did not affect the biofilm production (p=0.249).

No difference was found in the enterococcal strains in terms of the production of the biofilm, hemolysin, and gelatinase, and in terms of the HLGR and HLSR (p>0.05).

It was seen in the multivariate analyses that the hemolysin production was effective in the infection pathogenesis. It was detected that the hemolysin production of IE was 0.37 times more than that of FME. However, it was also detected that the biofilm production of FME was 3.67 times more than that of IE (OR: 3.67 (95% CI:1.97-6.83)) (**Table 3**).

Table 3. The evaluation of the virulence factors of the strains by the multivariate analysis by groups

Virulence Factors	B	S.E	Wald	p	OR	%95 CI
Biofilm	1,30	0,32	16,91	**<0,001**	3,67	1,98-6,83
Gelatinase	-0,47	0,46	1,03	0,31	0,63	0,25-1,55
Hemolysin	-0,99	0,41	5,78	**0,02**	0,37	0,17-0,83
Constant	0,42	0,63	0,45	0,50	1,52	

The variables entering the model: the production of the biofilm, gelatinase, and hemolysin

DISCUSSION:

The enterococci, which cause the urinary tract infections, endocarditis, intra-abdominal and pelvic infections, catheter-related infections, surgical wound infections, and central nervous system infections are the most important causes of the infections related to the health services. The enterococci, which are the natural member of the oral cavity, bowel, and female genital system within humans and animals are known as the opportunistic pathogen [3].

Although many enterococcus species have been identified, *E. faecalis* is the most dominant species in the human infections [8]. In the studies conducted in the other countries, *E. faecalis* was found in different ratios between 56% and 76%, and the *E. faecium* between 24% and 43.1% [9,10,11]. In this study, in IE group and in FME group *E. faecalis* (63%) and *E. faecium* (48%) was the most isolated species. The beta lactam resistance in the enterococci occurs by the decrease in the affinity of the low affinity penicillin-binding protein (PBP5) to the beta lactams, and this situation is widely seen. It is also known that the other mechanism of the beta lactam resistance is the beta lactamase production [8]. In most studies conducted in our country, beta lactamase-producing strains didn't found [12,13]. Also in our study, similar to other studies, it was detected that there was no beta lactamase production by the nitrosephine disk method.

The first choice for the treatment of the peritonitis, the wound, and the urinary system infection which do not require bactericidal effect is the penicillin or the ampicillin [2]. Combined treatments are recommended for severe systemic infections such as endocarditis and bacteremia. In the study of Sreeja et al. [11] with 128 enterococcal strains, the penicillin and ampicillin resistance was detected respectively as 47% and 45%. In the studies fromour country, Sirin and Adiloglu found the penicillin and ampicillin resistance as 28% [12]. Yildirim et al. [13] identified the penicillin resistance as 27.2% and the ampicillin resistance as 18.5%. In our country, there are also studies which the researchers have detected ampicillin and penicillin resistance in all species [14,15]. In our study, the penicillin and ampicillin resistance of the strains were detected respectively as 38% and 62% for IE, 33% and 63% for FME. While the penicillin resistance ratio in IE was higher than in FME, the opposite was valid for the ampicillin resistance. In IE, the both of the penicillin and ampicillin resistance ratios were more in the *E. faecium* than the *E. faecalis*, which were respectively detected as 32% and 35%.

Erythromycins, which inhibit the protein synthesis by binding to bacterial ribosomal 50S subunits, are narrow-spectrum antibiotics effective to Gram-positive cocci and bacilli. Protonotariou et al. [16] demonstrated in their study that erythromycin resistance ratio of *E. faecalis* and *E. faecium* was 67.6% and 85.4% respectively. Fernandes and Dhanashree showed that the antibiotic to which enterococcal strains were most resistant was

erythromycin; they reported that 81% of 84 *E. faecalis* strains and 90% of 51 *E. faecium* strains were resistant to erythromycin in their study [9]. In our study similar to these studies, it was found that there was 86% erythromycin resistance in IE and 90% in FME. When the erythromycin resistance ratio (69%) in our another study conducted by Kurtgoz Ozarslan et al. in our hospital was considered, the ratio has been found to be higher over the years [17]. Surprisingly, the erythromycin resistance in FME was found higher than in IE [17]. It was concluded that the reason why this was the case might be that the flora members were isolated from the health workers, and that making such studies with environmental isolates would have more meaningful consequences. It was found that there was 50% erythromycin resistance in IE strains. *E. faecalis* strains were seen that they were more resistant to erythromycin than *E. faecium* strains in similar way to the study done in our hospital before [17].

Tetracyclines are wide-spectrum antibiotics showing bacteriostatic effects on Gram-positive and Gram-negative bacteria by inhibiting the extension of the peptide chain in their proteins. Tetracycline resistance is the most typical example of the resistance gained through genetic material transfer in enterococci [18]. Within the study held in Greece between 2002 and 2007, where the antimicrobial resistance status of 2123 strains (1498 *E. faecalis* and 625 *E. faecium*) were investigated, the tetracycline resistance of *E. faecalis* was found as 0.1% and of *E. faecium* as 8.2% [16]. Within the study made by Rathnayake et al., the tetracycline resistance of clinical isolates was declared as 72.9% [18]. In the studies conducted in our country, the tetracycline resistance rate of enterococcal strains was found 8.3% in the study of Yildirim et al [13]. Within the study of Sirin and Adiloglu the antibiotic susceptibilities was determined via disk diffusion method , the tetracycline resistance was found 51% [12]. It has been observed that tetracycline resistance of enterococcal strains tends to increase over time in all these studies. In accordance with these studies, tetracycline resistance was found in 63% of strains in our study. In the study conducted between 2008 and 2011 with the enterococci isolated from the clinical samples in our hospital, tetracycline resistance was detected 64% [17]. Also in our study, it was found that tetracycline resistance did not change, while the rates were 67% in IE and 59% in FME. In our study, tetracycline resistance of IE was found higher than that of FME. 54% resistance was detected in *E. faecalis* strains in IE group, and it was seen that they were more resistant to tetracycline than the strains of *E. faecium*.

Low level of aminoglycoside resistance in enterococcus is dependent on the reduced permeability of the cell wall, whereas high level of resistance is by means of ribosomal or inactivating enzymes. The synergistic bactericidal effect of beta lactam-aminoglycoside combination is removed in the presence of HLAR. The enterococci with HLAR are significant because of the fact that they may be more resistant to other antibiotics. While in other studies HLSR rate was found between 14% and 53%, and HLGR rate between 13% and 76%, the rates were 65% and 40% respectively in our another research conducted in our hospital [17,20,21].

With the recent increase of glycopeptide resistance in enterococci, the resistance to antibiotics, especially in *E. faecium*, has been complicating the treatments enterococcal infections. The enterococci resistant to glycopeptide, penicillin, and aminoglycoside group-antibiotics cause severe infections. Especially the number of strains with inducible resistance to glycopeptide group-antibiotics such as vancomycin and teicoplanin has increased steadily [3].

Protonotariou et al. detected, with automated system, 0.5% vancomycin resistance for *E. faecalis* and 9.6% for *E. faecium* as a result of the study composed of 2123 strains including *E. faecalis* (1498) and *E. faecium* (625) [16]. Fernandes and Dhanashree found 13 strains resistant to vancomycin in their study [9]. They specified that at 11.7%, *E. faecium* strains

showed higher resistance than *E. faecalis* strains (4.7%). Oluwole et al. found that all of *E. faecium* strains included in the study were resistant to cotrimoxazole, ampicillin, and chloramphenicol, but none of these strains had vancomycin resistance [5]. They found similar results to the findings of the study of Chayakul et al [22]. When Rathnayake et al. compared the antibiotic susceptibilities of the strains isolated from clinical specimens and water, they identified that clinical isolates had higher resistance than the enterococcal strains isolated from water and that multiple antibiotic resistance was detected more in the clinical isolates [19]. Rathnayake et al. encountered that all of the strains of *E. faecium* and *E. faecalis* isolated from water were resistant to vancomycin [19]. Also, the clinical isolates were found 3.4 percent resistant to vancomycin. In the study done by Kafil et al., the vancomycin resistance in *E. faecalis* strains was found 16.3%, 33.8% in those of *E. faecium* [10]. In the results of antibiotic susceptibilities, the antibiotic resistance of *E. faecalis* isolates was found higher than *E. faecium*. The vancomycin resistance ratio measured by automated system in our study was found 12%. IE were found to be more resistant than FME. The vancomycin resistance ratio of IE measured by automated system was 9% in *E. faecium* and 4% in *E. faecalis*. By GDM, vancomycin resistance ratio were found 4.5%. The vancomycin resistance was found more in strains with the automated system. In a previous work in our hospital, the vancomycin resistance was detected in ten strains (10%) by automated system and in five strains (5%) by GDM [17]. It was concluded that vancomycin resistant strains required to be verified by GDM in such cases.

Linezolid is an oxazolidine antibiotic that inhibits protein synthesis by binding to the ribosomal subunit 50S and has a bacteriostatic effect. The linezolids with good activity against Gram-positive pathogens are used in the treatments of VRE [18]. Akhter et al. detected the linezolid resistance ratio as 4%, Protonotariou et al. as 0.3% in *E. faecalis* and 1.6% in *E. faecium* [16,23]. In another study conducted abroad by Rathnayake et al., no linezolid resistance was found [19]. In the study of Kurtgoz Ozarslan et al., the linezolid resistance ratio was found 14.3% in the strains of *E. faecalis,* 71.5% in those of *E. faecium* [17]. In our study, the strains of *E. faecium* were found to be more resistant to linezolid than those of *E. faecalis.* The linezolid resistance in IE was found more than FME. *E. faecalis* in IE was found to be more susceptible than *E. faecium*.

The role of biofilms formed by microorganisms in infectious diseases in recent years is quite remarkable [24]. Biofilm infections may appear both in the natural regions of human body and on the implanted prosthetic surfaces, and may cause chronic infections [25]. The prevalence of biofilm production varies globally.

When analyzed in terms of virulence factors, the strains of *E. faecalis* and *E. faecium* were found to have different patterns In Japan, Seno et al. reported that all of the strains of *E. faecalis* isolated from the urinary tract infections included by the study were found to produce biofilm [26]. In Poland, 59% of *E. faecalis* isolates collected from clinical samples was found to produce biofilm [2]. Baldassari et al. reported that 96% of *E. faecalis* strains isolated from orthopedic infections produced biofilm [28]. When these findings are considered, it seems plausible to state that *E. faecalis* produced more biofilms than *E. faecium*, and the biofilm formation or production is a significant factor in the pathogenesis of enterococcal infection. Also in our study, it was determined that biofilm production did not differ according to enterococci species. Vancomycin MIC value in the strains producing biofilm, which was determined by GDM, was found to be higher than the strains which did not produce biofilms. The resistance of biofilm-producing FME to moxifloxacin and ampicillin was found to be higher, whereas the susceptibility of FME with negative biofilm production to ciprofloxacin and to penicillin was found to be higher. In addition to this, it was notified that biofilm production in FME was 3.67 times higher than the other group. In this

way, it was concluded that the biofilm production affects the resistance of strains to some antibiotics, and that these bacteria found in flora may lead infection when a suitable host environment is found.

Kristich et al. reported that gelatinase increased the *E. faecalis* biofilm formation, but Tendolkar et al. defended that neither gelatinase nor enterococcal surface protein (ESP) had a synergystic effect on biofilm formation [29,30]. Kafil and Mobarez identified that the presence or absence of hemolysin and gelatinase did not have a significant effect on biofilm formation [31]. In a study with animal and human origins of enterococci, conducted by Tsikrikonis et al., the biofilm production of *E. faecalis* and *E. faecium* isolates were compared and detected that unlike other researchers, isolates of human origin produced more biofilm than other kinds of isolates [32]. In addition to this, they identified that ESP gene was not necessary for biofilm production, but might be related to biofilm production ratio. Also, in the same study, it was determined that hemolysin production of human clinical samples was more common than animal-origin *E. faecalis* isolates.

Baldassari et al. compared the gelatinase production with hemolysin production in enterococci strains with susceptibility to VRE and vancomycin, and showed that there was no difference between them [28]. However, in our study, the hemolysin production of IE was found to be higher than that of FME . When examined by species, hemolysin and gelatinase production in *E. faecalis* strains were found to be higher than in *E. faecium* strains. The resistance of the hemolysin-producing strains to tetracycline was found to be higher, whereas FME with negative hemolysin showed higher susceptibility to moxifloxacin and ciprofloxacin. Also, FME with negative gelatinase showed higher susceptibility to penicillin. Fernandes and Dhanashree observed that 82% of strains produced hemolysin and detected that there was gelatinase production in 40.6% [9]. While hemolytic activity was observed in all species, no gelatinase production was detected in *E. durans* and *E. avium*. The identified ratio of hemolysin production was 43.9% in *E. faecalis*, and 29.5% in *E. faecium*. Almost 44 percent of *E. faecalis* strains were identified as producing both hemolysis and gelatinase. The study of Tsikrikonis et al. presented that 34.4% of clinical *E. faecalis* isolates produced gelatinase but none of clinical *E. faecium* isolates produced it [32]. This very same study found similar results to the study of Di Rosa et al. and declared that 37% of clinical *E. faecalis* isolates produced gelatinase [33]. In our study, merely hemolysin production from virulence factors was found to be higher in the strains of *E. faecalis* than of *E. faecium*, and no variation was detected by species in terms of gelatinase and biofilm production.

Seno et al. ascertained in their study that there was no difference between gelatinase positive and gelatinase negative *E. faecalis* isolates, which were obtained from clinical and fecal sources, and that gelatinase production was not related to biofilm production [26]. In another research, which was conducted by Di Rosa et al. and included 83 *E. faecalis* strains and 45 *E. faecium* strains, it was identified that gelatinase was not necessary for biofilm formation [33]. Even though the genetic studies supported that gelatinase was necessary for biofilm formation, epidemiological studies gave the results showing that there was no connection between gelatinase and biofilm production among the tested clinical isolates [3]. Also, our study found similar results and indicated that biofilm production in strains was independent of hemolysin and gelatinase production.

In another study comparing normal flora members with infection agents, similar to ours, normal flora members were found to produce more biofilm than *E. faecalis* strains isolated from patients with infective endocarditis [34]. Our study found compatible results with this study. Johansson and Ramussen showed that while the ratio of biofilm formation in flora member strains was 54%, it was 23% in IE [34]. The strains of *E. faecalis* were found to produce more biofilm than the strains of *E. faecium*. The hemolysin

production of the strains included in their studywas found 26% in IE , and 11% in FME. In IE, hemolysin production of *E. faecalis* strains was found more than *E. faecium* strains. Gelatinase production was found 15% in IE, and 10% in FME. In IE, gelatinase production of *E. faecalis* strains was found more than *E. faecium* strains as in hemolysin and biofilm production.

CONCLUSION:

The antibiotics to which IE and FME were most resistant were erythromycin and clindamycin. IE were more resistant to clindamycin, ciprofloxacin and moxifloxacin than FME and on the other hand, FME were more resistant to ampicillin than IE. The ampicillin resistance in FME was thought to be due to much use of ampicillin in society.

E. faecium species were more resistant to the antibiotics than *E. faecalis* species. And also HLSR rate of *E. faecium* species were higher than *E. faecalis* species. Although there was no statistically difference in the two groups in terms of HLGR rate was found to be higher in IE.

The vancomycin MIC values of IE were higher than those of FME.

It was detected that the hemolysin production of IE was more than that of FME so it was concluded that the hemolysin production was effective in the infection pathogenesis. It was found that the hemolysin-free FME were more susceptible to the moxifloxacin and the ciprofloxacin. The hemolysin producing strains was identified more resistant to the tetracycline. However, it was also detected that the biofilm production of FME was more than that of IE. The MIC median value of the biofilm-free strains was found higher than biofilm producing strains. It was concluded that the biofilm production affects the resistance of strains to some antibiotics, and these biofilm producing FME may lead infection when a suitable host environment is found.

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