RESEARCH ARTICLE

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Identification of the Gram Positive Bacterial Sepsis Agent with Rapid Genotype Test

ABSTRACT

Objective: An irreversible process begins when a systemic infection causes sepsis. Therefore, rapid identification of the agent bacteria in sepsis and its antibiotic resistance is crucially important. In this study, it was aimed to investigate the efficiency of rapid genotype test in detecting sepsis agent Gram positive bacteria and important antibiotic resistance.

Methods: 2132 blood culture samples sent to the laboratory were examined with an automatic blood culture system (BACTEC, BD, USA) between 2018-2019. Blood culture bottles sent to the laboratory were Growing bacteria was identificated by VİTEK (bioMérieux, France) automated bacteria identification / antibiotic susceptibility system. In addition, bacterial species and mecA, vanA, vanB, vanC1, vanC2 / C3 genes in blood cultures with Gram positive bacterial growth were also determined by the "Genotype® BC Gram-positive (Hain Lifesience, Germany)" test.

Results: 72 patients with gram-positive bacteria growth in two or more blood culture bottles were included in the study. In 44 of the samples (61%) the same bacterial species were detected with conventional method (bacteria culture) and BC Gram positive test. In 28 of the samples (39%) differences were detected between results of methods regarding bacterial species name or vancomycin/methicillin resistance rate. Although single agent was isolated with culture method in all of the samples, multiple agents were detected in eight samples with rapid genotype test. Also, it was found that in mecA positive samples, ciprofloxacin resistance was higher than mecA negative ones.

Conclusions: In the study, it was observed that BC Gram positive test could correctly identify sepsis agent bacteria and their resistance genes within 4-5 hours.

Keywords: Drug Resistance, PCR, Rapid test, Sepsis, Staphylococci.

Gram Pozitif Bakteriyel Sepsis Etkenlerinin Hızlı Genotip Test ile Tanımlanması

ÖZET

Amaç: Sistemik bir enfeksiyon sepsise neden olduğunda geri dönüşü olmayan bir süreç başlamaktadır. Bu nedenle sepsisteki etken bakterinin ve antibiyotik direncinin hızlı bir şekilde tanımlanması çok önemlidir. Bu çalışmada, sepsis etkeni Gram pozitif bakterileri ve önemli antibiyotik dirençlerini saptamada hızlı genotip testinin etkinliğinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Laboratuvara 2018-2019 yılları arasında gönderilen 2132 kan kültürü örneği otomatik kan kültürü sistemi (BACTEC, BD, ABD) ile incelenmiştir. Üreyen bakteriler VİTEK (bioMérieux,Fransa) otomatize bakteri identifikasyon/antibiyotik duyarlılık sistemi ile tanımlanmıştır. Ayrıca Gram pozitif bakteri üremesi olan kan kültürlerindeki bakteri türleri ve mecA, vanA, vanB, vanC1, vanC2 / C3 genleri "Genotype® BC Gram-pozitif (Hain Lifesience, Almanya)" testi ile de belirlenmiştir.

Bulgular: İki veya daha fazla kan kültürü şişesinde Gram pozitif bakteri üremesi saptanan 72 hasta çalışmaya dahil edildi. Örneklerin 44'ünde (% 61) konvansiyonel yöntemle (bakteri kültürü) ve BC Gram pozitif testi ile aynı bakteri türü tespit edildi. Örneklerin 28'inde (% 39) bakteri tür adı veya vankomisin / metisilin direnç oranı ile ilgili yöntemlerin sonuçları arasında farklılıklar tespit edildi. Tüm örneklerde kültür yöntemi ile tek etken izole edilmesine rağmen hızlı genotip testi ile sekiz örnekte birden fazla etken tespit edildi. Ayrıca mecA pozitif örneklerde siprofloksasin direncinin mecA negatiflere göre daha yüksek olduğu bulundu.

Sonuç: Çalışmada BC Gram pozitif testinin, sepsis etkeni bakterileri ve direnç genlerini 4-5 saat içinde doğru bir şekilde belirleyebildiği görülmüştür.

Anahtar Kelimeler: İlaç dirençli, PCR, Hızlı test, Sepsis, Stafilokok.

INTRODUCTION

Rapid identification of bacterial pathogens and antibiotic resistance that grow in the blood cultures of patients with sepsis enables the selection of the most effective antibiotic for treatment. With early treatment, morbidity/mortality and hospitalization period decreases, unnecessary use of antibiotics is prevented and hospital costs are reduced. (1-4) Automatized blood culture systems used in clinical microbiology laboratories has significantly decreased the period of identification of bacterial pathogens. (1,2) However, the resulting takes a minimum of 2 days after receiving the positive signals. Faster identification systems are required to start treatment as early as possible. For this purpose, some laboratories use molecular methods to quickly identify the agent and resistance situation, such as DNA microarray, Nested polymerase chain reaction (PCR), multiplex PCR, in addition to non-molecular methods such as matrix-associated laser desorption ionization-time of flight (MALDI-TOF) MS , molecular methods, despite their high costs. (5,6)

In our study, rapid identification of bacterial species and mecA, vanA, vanB, vanC1, vanC2/C3 genes using "Genotype® BC Gram-positive (Hain Lifesience, Germany)" test in blood cultures with Gram positive bacteria growth was aimed.

MATERIAL AND METHODS

This cross sectional study aims identification and resistance detection with rapid genotype test in the patients of Duzce University Health Practice and Research Center who has a growth of gram positive bacteria in blood culture samples. Approval was obtained from Duzce University clinical research ethics committee, dated 21.09.2019 and numbered 2020/201.

Sampling: Among the 2132 blood cultures tested from 14.04.2018 to 26.01.2019, belonging to the patients of Duzce University Health Practice and Research Center. Seventy-two patients with Gram-positive bacteria growth in two or more blood culture bottles were included in the study.

Laboratory Analyses: When the growth signal was received from the automated blood culture system (BACTEC Blood Culture Test--BD, the USA) in our hospital's bacteriology laboratory, Gram staining from blood culture bottle and inoculation to blood agar (Oxoid, England) and eosine methylen blue agara (Oxoid, England) were performed.

Phenotypic Method: VITEK automated bacterial identification system (bioMérieux, France) and conventional methods were used in the phenotypical identification of the growing bacteria. In addition to the susceptibility of the bacteria to methicillin, vancomycin and teicoplanin, susceptibility to ciprofloxacin, gentamicin, trimethoprim / sulfamethoxazole (TMP-SXT) were also examined with VITEK automated system (bioMérieux, France) and with the method of disc diffusion. Antibiotic susceptibilities were evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. (7)

Genotype Test: When gram positive bacteria is detected at least in two samples of the same patient according to the gram staining results, Genotype® BC Gram positive Ver.3.0 (Hain Lifescience Germany) assay kit that detects 17 different Gram positive types (*Streptococcus anginosus / constellatus / intermedius / mutans / sanguinis, Streptococcus mitis / oralis, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus dysgalactiae ssp. equisimilis, Streptococcus bovis, Streptococcus pneumoniae, Staphylococcus aureus, Staphylococcus haemolyticus, Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus warneri, Staphylococcus simulans, Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum ve Enterococcus casseliflavus*) was used in this system, methicillin (mecA) and vancomycin (vanA , vanB, vanC1 and vanC2 / C3) resistance genes can be detected, too.

Genotype test results have not been confirmed by any other molecular method.

Statistical Analysis

Descriptive statistics of all data in the study were estimated. Chi-square and Fisher Exact tests were used in comparison among rates. The compatibility between the test results was examined by Mc Nemar test.

RESULTS

Seventy-two blood culture samples in Duzce University Health Practice and Research Center which were found to have gram positive bacteria growth two times consecutively between April 2018 and January 2019 were included to the study.

The median ages of the patients whose blood samples were taken were 72, (min: 1 max: 91). 36 of them were female (50%) and 36 of them were male (50%). All of the samples were sent from intensive care units.

A total of 45 Coagulase Negative *Staphylococcus* (CNS) (16 of which were *S. hominis*, 15 *S. haemolyticus*, 12 *S. epidermidis*, 2 *S. warneii*), 17 *S. aureus* [9 Methicillin Resistance *S. aureus* (MRSA), 8 Methicillin sensitive *S. aureus* (MSSA)], 9 *Enterococcus* (5 *E. faecalis*, 4 *E. faecium*) a *group D streptococcus* growth was detected with bacteria culture method. In 44 of the samples (61%) the same bacterial species were detected with both methods, while in 28 of the samples (39%) differences were detected regarding bacterial species name or vancomycin / methicillin resistance rate. In the statistical analysis, the results of rapid genotype test CNS, *S. aureus* and bacteria culture for enterococcus species were found to be significantly compatible (respectively p=0,375, p=0,999, p=0,999). However, vancomycin

susceptible *E. faecalis* and MR *S. hominis* species detected in a culture method sample, were found to be undetectable with rapid genotype test. In addition, *S. pneumoniae* species that were not detected in two blood culture samples, were detected with rapid genotype test. Moreover, while single agent was isolated in the samples, multiple agents were detected in 8 (11%) samples with rapid genotype test. In blood culture samples, the distribution of bacteria that were found to be similar or different with rapid genotype test and culture method is shown in Table 1 and Table 2. Results of rapid genotype test are shown in Figure 1.

Table 1. Distribution of bacteria that were found to be similar rapid genotype test and culture method

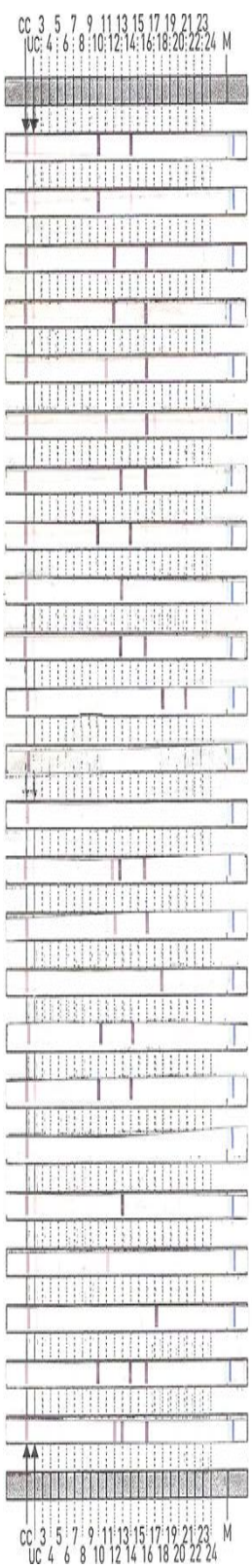
Number (Total=44)	Culture method	Rapid genotype test	
		Bacteria	Resistance gene
11	MR *S. epidermidis*	*S. epidermidis*	Mec A
9	MR *S. haemolyticus*	*S. haemolyticus*	Mec A
6	MR *S. aureus*	*S. aureus*	Mec A
6	MS *S. aureus*	*S. aureus*	-
5	MR *S. hominis*	*S. hominis*	Mec A
1	MS *S. hominis*	*S. hominis*	-
1	MS *S. haemolyticus*	*S. haemolyticus*	-
2	VR *E. faecium*	*E. faecium*	Van A
1	VS *E. faecium*	*E. faecium*	-
2	VS *E. faecalis*	*E. faecalis*	-

MR: Methicillin resistant, MS: Methicillin sensitive, VR: Vancomycin resistant, VS:Vancomycin sensitive

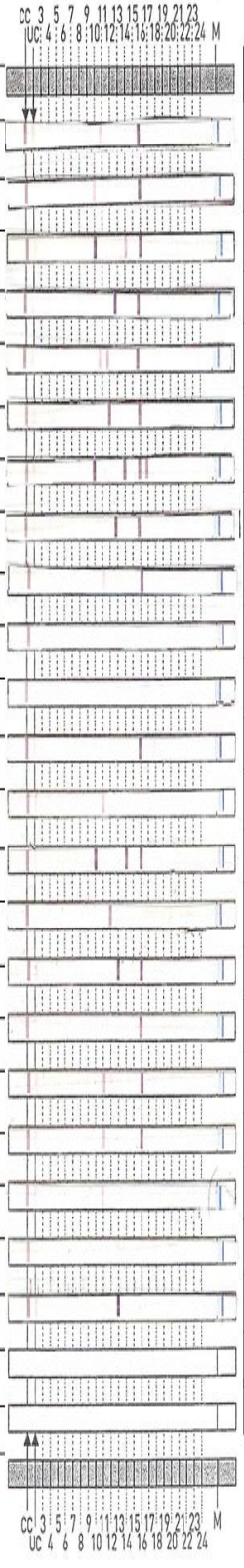
Table 2. Distribution of bacteria that were found to be different with rapid genotype test and culture method

Number (Total=28)	Culture method	Rapid genotype test	
		Bacteria	Resistance gene
3	MR *S. hominis*	*S. hominis*	-
2	MS *S. hominis*	*S. hominis*	Mec A
1	MS *S. hominis*	*S. aureus*	Mec A
1	MR *S. hominis*	*S. haemolyticus*	Mec A
1	MR *S. hominis*	*S. hominis, S. mitis/oralis, S. pneumoniae*	Mec A
1	MR *S. hominis*	-	-
1	MR *S. hominis*	*S. aureus*	-
3	MR *S. haemolyticus*	*S. haemolyticus*	-
1	MR *S. haemolyticus*	*S. haemolyticus, E. faecalis*	
1	MS *S. haemolyticus*	*S. haemolyticus , E. faecium*	Mec A, Van A
1	MR *S. epidermidis*	*S. epidermidis*	-
1	MS *S. aureus*	*S. aureus*	Mec A
1	MS *S. aureus*	*S. epidermidis, E.faecalis*	Mec A
1	MR *S. aureus*	*S. hominis*	Mec A
1	MR *S. aureus*	*S. aureus, E. faecalis*	Mec A
1	MR *S. aureus*	*S. aureus, S. hominis*	Mec A
2	MR *S. warneii*	*S. haemolyticus*	Mec A
1	VS *E. faecium*	*E. faecium, S. haemolyticus*	Mec A, Van A
1	VR *E. faecalis*	-	-
1	VS *E. faecalis*	*S. aureus*	-
1	VS *E. faecalis*	*S. hominis*	Mec A
1	Streptococcus grup D	*S. mitis/oralis, S. pneumoniae*	

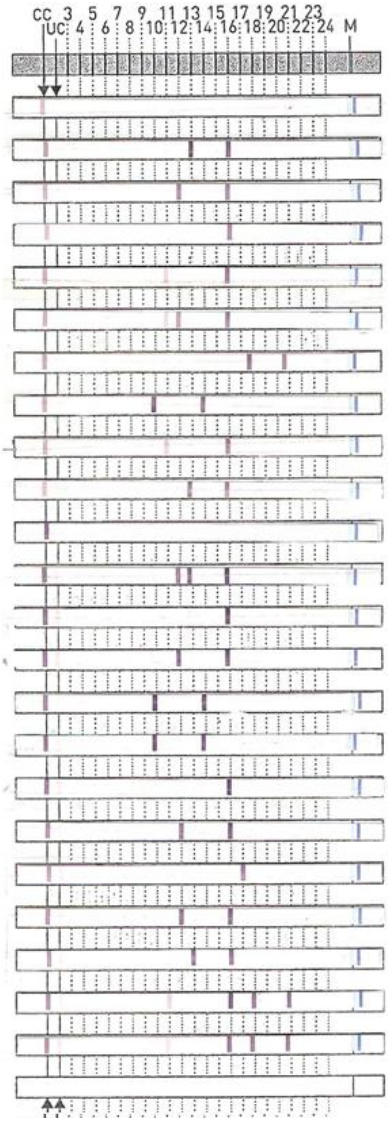
MR: Methicillin resistant, MS: Methicillin sensitive, VR: Vancomycin resitant, VS:Vancomycin sensitive



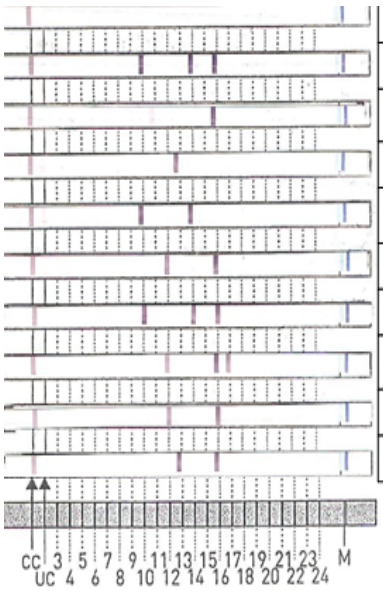
Species of bacteria	Resistance
S. aureus	
S. aureus	
S. epidermidis	Mec A
S. epidermidis	Mec A
S. haemolyticus	Mec A
S. haemolyticus + *E. faecalis*	
S. hominis	Mec A
S. aureus	MecA
S. hominis	
S. hominis	Mec A
E. faecium	Van A
Negative control	
Negative control	
S. epidermidis	Mec A
S. hominis	
E. faecium	
S. aureus	
S. aureus	
Negative control	
S. hominis	
S. haemolyticus	
E. feacalis	
S. aureus	Mec A
S.epidermidis + *S. hominis*	Mec A



Species of bacteria	Resistance
S. haemolyticus	Mec A
S. aureus	Mec A
S. aureus	Mec A
S. hominis	Mec A
S. epidermidis	Mec A
S. epidermidis	Mec A
S. aureus + *E. faecalis*	Mec A
S. hominis	Mec A
S. haemolyticus	Mec A
Negative control	
Negative control	
S. haemolyticus	Mec A
S. haemolyticus	
S. aureus, S. hominis	Mec A
S. epidermidis	
S. hominis	Mec A
S. haemolyticus	Mec A
S. haemolyticus	Mec A
S. haemolyticus	
S. haemolyticus	
non-working	
S.mitis/oralis+S.pneumoniae	
non-working	-
non-working	-



Species of bacteria	Resistance
Negative control	
S. hominis	Mec A
S. epidermidis	Mec A
S. haemolyticus	Mec A
S. haemolyticus	Mec A
S. epidermidis	Mec A
E. faecium	Van A
S. aureus	
S.haemolyticus	Mec A
S. aureus	Mec A
Negative control	
S.hominis+S.pneumoniaeS.mitis/oralis	Mec A
S. hominis	Mec A
S. epidermidis	Mec A
S. aureus	
S. aureus	
S. hominis	Mec A
S. epidermidis	Mec A
E. faecalis	
S.epidermidis	Mec A
S.haemolyticus	Mec A
S. aemolyticus + E. faecium	Mec A+VanA
S. haemolyticus + E. faecium	Mec A+VanA
Non-working	-



Negative control	
S. aureus	Mec A
S. haemolyticus	Mec A
S. hominis	
S. aureus	
S. epidermidis	Mec A
S. aureus	Mec A
S. epidermidis + E. faecalis	Mec A
S. epidermidis	Mec A
S. hominis	Mec A

Figure 1. Results of rapid genotype test

When the resistance rates of antibiotics other than beta lactam group in staphylococcus species were examined by VITEK automated system (bioMérieux, France) and disc diffusion method, it was found that the resistance rate of ciprofloxacin among the antibiotics included in the study was

higher than the mecA negative ones (p <0,001). Gentamicin and TMP-SXT were found to have similar susceptibility in mecA positive and negative samples (p = 0.447, 0.601, respectively). The susceptibility of staphylococcus species to ciprofloxacin, gentamicin and TMP-SXT is shown in Table 3.

Table 3. The susceptibility of staphylococcus species to ciprofloxacin, gentamicin and TMP-SXT (n/%)

	Ciprofloxacin,	TMP-SXT	Gentamicin
MecA positive staphylococci (n=44)	34 (77)	8 (18)	19 (43)
MecA negative staphylococci (n=18)	2 (11)	3 (17)	6 (33)
p değeri	<0,001	0,999	0,473

TMP-SXT: trimethoprim / sulfamethoxazole

DISCUSSION

Sepsis is a syndrome that is caused by uncontrollable inflammatory response against the infection in the host and organ failure as a result. The most common triggers in sepsis are bacterial infections. Early diagnosis of sepsis is crucially important in order for it to be treated before the patient's condition deteriorates and results in death. (8) Worldwide, 31 million cases of sepsis and 6 million deaths are reported regarding sepsis, each year. In one out of ten patients that has access to health services, an infection with sepsis characteristic develops. (9) When only culture-oriented microbiological analyzes are used in sepsis treatment, physicians do not have time to wait for microbiological bacteria culture results. (10) Time is crucially important in the treatment of sepsis and antimicrobial treatment is recommended to start in 60 minutes after the diagnosis. In the conducted studies, it is found that every hour of delay in antibiotic treatment after the diagnosis of sepsis, causes 8.4% mortality in patients. (11-13) Until the bacteria culture results of patients diagnosed with sepsis are received, physicians should empirically start the most extensive antimicrobial treatment which contains antibiotics that can affect all possible agent bacteria pathogens. As a result of this approach, not only costs increase but also antimicrobial resistance development is caused. (13)

Blood culture made in the diagnosis of sepsis is accepted as the "gold standard". (14-17) In the modern blood culture systems, positive signal can be received within few days after culturing in the bottles. It is necessary to wait a minimum of five days before the culture bottle can be detected to be negative. Microorganisms causing sepsis give a positive signal in automated blood culture systems in 90% of the cases within the first 48 hours. However, identifying microorganisms in blood culture that signals growth and making tests for their antibiotic susceptibility requires an additional few days. (16,17) Due to this crucial disadvantage in automatized blood culture systems, use of molecular diagnostic methods has become dominant on the agenda. The most practical and the most common of these is the PCR method. (18) There are studies indicating that mortality decreases and various molecular methods allow early antimicrobial treatment with rapid identification/antibiotic susceptibilities among microorganisms growing in blood cultures. (1,3,4,19-27)

One of the most common problems encountered in identification with molecular methods from blood cultures is PCR inhibition with inhibitors in the structure of the sample and blood culture bottles. (1,16,28-31)

In our study, the results of bacterial culture and genotyping tests were found to be compatible. However, it has been observed that streptococci and enterococci can be overlooked or misidentified in bacterial culture, especially in mixed infections. These factors can be important causes of mortality and morbidity. Correct identification of these overlooked bacteria and detection of resistance genes can allow early treatment and inhibit spreading in the hospital. Similar to our study, Gülhan et al. found in their study that the results found with the genotypic method were 83.6% compatible with phenotypic results. They also reported that mecA and vanA resistance genes can be detected with genotypic method and this method can be used practically in routine diagnosis for rapid diagnosis and treatment of sepsis caused by gram positive cocci. (1)

Moreover, while single agent was isolated in the samples, multiple agents were detected in 8 (11%) samples with rapid genotype test. Rapid genotype testing was found to be more susceptible in detecting mixed infections than bacteria culture methods with these results. Gülhan et al. found similar results in their study and stated that genotyping method is more susceptible in detecting mixed infections.

In our study, for staphylococci, which are considered causative agents since they are grown in both blood culture sets, methods of detecting methicillin resistance in culture and detecting presence of mecA with rapid genotype test methods were found compatible. However, incompatible results have been encountered in some samples. MecA gene detection is considered the gold standard in detecting methicillin resistance. (15-17) Rapid genotype testing is much faster than the culture method. Bacterial culture-antibiogram analysis is closely related to the personal experience

and attention of the laboratory worker. Therefore, it is thought that mecA detection can be used more frequently with rapid genotype testing to minimize errors. In similar studies, it is reported that the detection of the presence of the mecA gene with methicillin resistance and genotyping in culture in staphylococci has been found to be compatible and that mecA gene can be detected rapidly by the genotyping method. (1,3,27,31)

In our study, when glycopeptide resistance and van genes were examined in enterococci; the vanA gene was found positive in two species of *E. faecium* detected as susceptible to glycopeptides and culture antibiogram (Table 2). In two species of *E. faecium*, antibiotic susceptibility testing revealed glycopeptide resistance and vanA gene was detected positive (Table 1). In the rapid genotype test, glycopeptide resistance was not detected in two species in which the vanA gene was detected positive with VITEK2 and other conventional antibiogram methods. Considering that the presence of the vanA gene is the gold standard in the glycopeptide resistance, it was found that with genotyping method, resistance detection was easier and quicker than bacterial culture antibiogram methods and that major errors could be prevented. Since the glycopeptide resistance could be detected 4-5 hours after receiving a positive signal from the blood culture and making the gram staining, the empirical broad spectrum antibiotic treatment will be very short. In this case, unnecessary drug use and glycopeptide resistance can be prevented. This will decrease morbidity/mortality as well as patient care costs. In the conducted studies, compatibility was detected in enterococci with genotypic and phenotypic analyzes. It was reported that genotyping method can be used in the detection of van genes. (1,3,27,31)

In recent years, there has been an increase in staphylococcus bacteremia. With this increase, CNS and *S. aureus* species are isolated more than the blood cultures. Increasing rate of methicillin resistance in staphylococci causes major problems in treatment. (21) Therefore, resistance of antibiotics other than beta lactam group has been gaining importance. In our study, the resistance rates of staphylococci to antibiotics other than the beta lactam group were examined, and the resistance to ciprofloxacin was significantly higher in those with positive mecA gene than negative ones (Table 3).

CONCLUSION

Genotyping method can accurately and rapidly identify the infection agents in sepsis, which can be overlooked by the bacterial culture method. Early treatment is provided with rapid detection of the agent and resistance genes. Thus, the success of treatment increases and morbidity/mortality, the empirical treatment period, patient hospitalization time and the cost decreases. Since the treatment does not start until the suscepibility pattern is provided, meticillin and glycopeptide resistances will be prevented as unnecessary antibiotic treatment is not performed. In addition, patients with positive mecA appear to have a high resistance to ciprofloxacin. Therefore, use of this antibiotic in the empirical treatment for patients who are mecA positive is not advisable. In addition to these advantages of genotype tests, it should be used with bacterial culture methods.

Conflict of interest None to declare.

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