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Evaluation of microorganisms isolated from blood cultures and their susceptibility profiles to antibiotics in five years period

Beş yıllık sürede kan kültürlerinden izole edilen mikroorganizmaların değerlendirilmesi ve antibiyotiklere duyarlılık profilleri

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Abstract

Aim: In starting antibiotic treatment to know the distribution of infectious agents and the antibiotic resistance rates is vital especially in critically ill patients to prevent disease progression. In this study we aimed to determine the distribution and antimicrobial resistance patterns of blood culture isolates obtained in a tertiary center.

Methods: The microbiological laboratory records between January 2014 and December 2018 were retrospectively reviewed. Each conventional aerobic blood culture bottle per patient with positive results was recorded with the antimicrobial susceptibility profiles of the bacteria isolated. Descriptive statistics (number, percentage, mean and median) were performed. Comparison of descriptive data between groups was performed with cross tables and chi square test. One-way ANOVA test was used to compare the antibiotic resistance rates between spp.

Results: A total of 66004 blood cultures were analyzed in this cross-sectional study. Of the 3882 (21.0%) positive results, 3256 (4.9%) were identified as contamination. The most commonly isolated microorganisms were Coagulase negative staphylococcus, *Escherichia coli, Klebsiella spp., Staphylococcus aureus* and *Acinetobacter spp.* Extended spectrum β-lactamase (ESBL) positivity was determined in 236 (62.4%) isolates of *E.coli,* and 186 (56.8%) isolates of *Klebsiella spp.* Vancomycin resistance showed a significant increase in *Enterococcus spp.* in time. *E.coli* did not show any resistance to imipenem, meropenem, tigecycline or colistin. Methicillin susceptible *Staphylococcus aureus* (MSSA) and methicillin resistant *Staphylococcus aureus* (MRSA) did not show any resistance to vancomycin, teicoplanin, linezolid or daptomycin. In *Enterococcus spp.*, tigecycline resistance was 1.9%, while daptomycin and linezolid resistance were not determined. The most effective resistance agent to *Acinetobacter spp.* and *Pseudomonas spp.* was colistin. Fungal infection was detected in 156 patients. *Candida albicans* and *Candida parapsilosis complex* were the most common isolates.

Conclusion: Antibiotic resistance rates are increasing in all over the world. Rational antibiotic usage may aid the clinicians to overcome this condition. Epidemiological data is important in this regard.

Keywords: Blood culture, Antibiotics susceptibility, Microorganisms

Öz

Amaç: Enfeksiyöz ajanların dağılımını ve antibiyotik direnç oranlarını bilmek, hastalığın ilerlemesini önlemek için antibiyotik tedavisine başlamada hayati öneme sahiptir. Bu çalışmada, Türkiye'de beş yıllık bir dönemde üçüncü basamak merkezimizden elde edilen kan kültürü izolatlarının dağılımını ve antimikrobiyal direnç paternlerini belirlemeyi amaçladık.

Yöntemler: Ocak 2014 - Aralık 2018 arasındaki mikrobiyoloji laboratuvar kayıtları geriye dönük olarak incelendi. Pozitif üreme sonuçları olan hastalar, izole edilen bakterilerin antimikrobiyal duyarlılık profilleri ile kaydedildi. Tanımlayıcı istatistikler (sayı, yüzde, ortalama ve ortanca) yapıldı. Gruplar arasındaki tanımlayıcı verilerin karşılaştırılması çapraz tablolarla ve ki kare testi ile yapıldı. İzolatlar arasındaki antibiyotik direnç oranlarını karşılaştırmak için tek yönlü ANOVA testi kullanıldı.

Bulgular: Bu kesitsel çalışmada toplam 66004 kan kültürü analiz edildi; 3882 (%21,0) pozitif sonuçtan 3256 (%4,9)'sı kontaminasyon olarak tanımlandı. En sık izole edilen mikroorganizmalar, Koagülaz negatif stafilokok, *Escherichiacoli, Klebsiella spp., Staphylococcus aureus* ve *Acinetobacter spp.*olarak belirlendi. Extended spectrum β-lactamase (ESBL) pozitifliği, 236 (% 62,4) *E.coli* izolatı, 186 (%56,8) *Klebsiella spp.*'de saptandı. Vankomisin direnci zamanla *Enterococcus spp.*'de anlamlı bir artış gösterdi. *E.coli,* imipenem, meropenem, tigesiklin veya kolistine karşı herhangi bir direnç göstermedi. Methicillin duyarlı *Staphylococcus aureus* (MSSA) and methicillin dirençli *Staphylococcus aureus* (MRSA), vankomisin, teikoplanin, linezolid veya daptomisine karşı herhangi bir direnç göstermedi. *Enterococcus spp.*'de tigesiklin direnci %1,9 iken daptomisin ve linezolid direnci saptanmadı. Dirençli *Acinetobacter spp.* ve *Pseudomonas spp.* için en etkili ajan kolistin'di. 156 hastada mantar enfeksiyonu saptandı. *Candida albicans ve Candida parapsilosis complex* en sık izolatlardı.

Sonuç: Antibiyotik direnç oranları tüm dünyada artmaktadır. Akılcı antibiyotik kullanımı klinisyenlerin bu durumu aşmalarına yardımcı olabilir. Bu konuda epidemiyolojik veriler önemlidir.

Anahtar kelimeler: Kan kültürü, Antibiyotik duyarlılığı, Mikroorganizmalar

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Introduction

Bloodstream infections are clearly known to cause mortality and critical illnesses and for that reason prompt diagnosis and treatment is essential [1,2]. The etiological agents of bloodstream infections and their antimicrobial resistance rates differ significantly between countries [3]. Selection and timing of antibacterial treatment seriously affect outcomes in blood stream infections and empiric therapy is based on the antimicrobial sensitivity patterns [4]. For that reason, epidemiological data from different countries on different patient groups gain more importance. Blood culture is the gold standard for the diagnosis of bloodstream infections. Delays in diagnosis and treatment of blood stream infections may result in the septic shock and mortality [5].

To know the distribution of infectious agents and the antibiotic resistance rates is vital in starting antibiotic treatment especially in critically ill patients to prevent disease progression. In this study we aimed to determine the distribution and antimicrobial resistance patterns of blood culture isolates obtained in a tertiary center, in five year period, in Turkey.

Materials and methods

This study was performed in Health Sciences University Okmeydanı Education and Research Hospital, Medical Microbiology Department. The microbiological laboratory records between January 2014 and December 2018 were retrospectively reviewed. Each conventional aerobic blood culture bottle per patient with positive results was recorded with the antimicrobial susceptibility profiles of the bacteria isolated.

Blood cultures were assayed on a fully automated blood culture device, BACTEC 9240 (Becton Dickinson, Diagnostic Instrument System, Sparks, USA). The passage of the detected vials in the automated blood culture device to the MacConkey, chocolate and 5% sheep blood agar was performed. Colonies thought to be effective, were identified at the species level by the Phoenix ™ - 100 (Becton Dickinson, Diagnostic Instrument System, Sparks, USA) automated system and antibiotic susceptibilities were studied. Identification and antibiotic susceptibility tests were performed in Phoenix. In evaluation of susceptibilities was performed by Clinical and Laboratory Standards Institute (CLSI) in January 2014-December 2015, and of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in January 2016- December 2018 [6,7]. Classical methods for identification of fungi and Phoenix ™, Sensititre Yeast One (TREK Diagnostic Systems, USA) kit based on colorimetric method for antifungal susceptibility were used. Due to the high risk of morbidity and mortality in patients with blood stream infections, bacteria with moderate susceptibility were classified as resistant to this antimicrobial agent.

Statistical analysis

Statistical analyses were performed with SPSS 21.0 (IBM Company, Chicago, IL) software. The conformity of the parameters to the normal distribution was evaluated by Kolmogorov-Smirnov test. Descriptive statistics (number, percentage, mean and median) were performed. Comparison of descriptive data between groups was performed with cross tables and chi square test. One-way ANOVA test was used to compare the antibiotic resistance rates between spp. Results with *P*-value <0.05 were considered statistically significant.

Results

In five years period, totally 66004 blood cultures were analyzed and among those, 13882 (21.0%) were having positive results. In detailed analyses of those isolates, 3256 were defined as contamination (mainly coagulase negative staphylococci (CoNS), *Corynebacterium spp., Gemella spp.,* non-pneumococcal alpha hemolytic streptococci, *Micrococcus spp.* etc.). Contamination rate was determined as 4.9%. Reproduction in single blood culture bottle, reproduction of different coagulase negative staphylococci or skin flora elements in the same patient's blood culture bottles obtained at the same time and inconvenience with the clinical features are regarded as contamination [8].

In a total of 10626 reproductions of 3116 patients (1715 female and 1401 male) were examined. Repetitive reproductions of the same patient were excluded. 95% (n=2960) of the reproductions were bacterial and 5% (n=156) were the yeasts. Among patients with bacterial growth, 52.5% were gram positive and 47.5% were gram negative. The mean age of the patients was 56.42 (22.19) years while the median age was 54 (range: 0-109) years. Of the patients, 3007 (96.5%) were hospitalized patients and 109 (3.5%) were outpatients. These patients were admitted to the emergency departments and then transferred to inpatient services.

The distribution of most commonly isolated microorganisms according to years is summarized in Table 1. Although the number of total cultures increased in time, the percentage of positive cultures in years was decreasing. The most commonly obtained microorganism was methicillin resistant coagulase negative staphylococci in all years. *E.coli* and *Klebiella spp.* were increasing in time.

Table 1: The distribution of most commonly isolated microorganisms according to years

	2014	2015	2016	2017	2018	Total
MRSA	22	22	17	32	21	114
MSSA	45	26	40	42	52	205
MRCoNS	180	130	157	110	133	710
MSCoNS	42	32	55	35	37	201
Enterococcus spp.	35	55	56	57	65	268
Escherichia coli	44	90	77	85	81	377
Klebsiella spp.	40	60	74	77	76	327
Acinetobacter spp.	32	61	68	59	81	301
Pseudomonas spp.	20	36	44	25	54	179
Others	36	49	57	76	60	278
Total	496 (5.2%)	561 (4.8%)	645 (4.6%)	598 (4.2%)	660 (3.9%)	2960
Number of blood cultures	9568	11730	13938	14210	16558	66004

MRCoNS: Methicillin Resistant Coagulase negative staphylococci, MSCoNS: Methicillin Susceptible Coagulase negative staphylococci, MRSA: Methicillin Resistant *Staphylococcus aureus*, MSSA: Methicillin susceptible *Staphylococcus aureus*, Others: Beta hemoliytic streptococcus [A, B, F, G], *Streptococcus pneumoniae, Listeria monocytogenes, Brucella spp., Morganella morganii, Serratia spp., Salmonella spp., Enterobacter spp., Proteus spp., Burkholderia cepacia, Stenotrophomonas maltophilia*

Regarding the blood cultures obtained from inpatients, *Acinetobacter*, *Klebsiella*, *Enterococci* and *Pseudomonas spp.* were most commonly isolated from intensive care unit followed by internal medicine wards, while *E.coli* and *S. aureus* were most commonly isolated from internal medicine wards. *Candida spp.* were also most commonly isolated from intensive care unit.

Extended spectrum β-lactamase (ESBL) positivity was determined in 236 (62.4%) isolates of *E.coli*, 186 (56.8%) isolates of *Klebsiella spp.* Distribution of ESBL positivity in time is summarized in Table 2.

Distribution of methicillin resistance in *S.aureus* and coagulase negative *staphylococcus spp*. is summarized in Table

3. Distribution of Vancomycin resistance in *Enterococcus spp.* is summarized in Table 4.

Table 2: Distribution of ESBL positivity in time

	2014	2015	2016	2017	2018	*P*-value
ESBL (+) *E.coli*	39.10%	47.70%	71%	69.40%	75%	0.001
ESBL(+) *Klebsiella spp.*	23%	33.30%	51.30%	79.20%	72.30%	0.001

Table 3: Distribution of MRSA and MRCoNS

	2014	2015	2016	2017	2018	*P*-value
MRSA	48.8%	44.4%	42.5%	43.2%	41.7%	0.105
MRCoNS	81%	80.20%	74%	76%	78%	0.242

Table 4: Distribution of Vancomycin resistance in *Enterococcus spp.* (VRE)

	2014	2015	2016	2017	2018	*P*-value
VRE	5.7 %	6.9 %	8.7 %	9.5 %	9.1 %	0.001

Antibiotic resistance profiles of *E.coli and Klebsiella spp.* were summarized in Table 5, antibiotic resistance profiles of MSSA and MRSA were summarized in Table 6, antibiotic resistance profiles of *Enterococcus spp.* in Table 7 and antibiotic resistance profile of *Pseudomonas* and *Acinetobacter spp.* were summarized in Table 8.

Table 5: Antibiotic resistance profiles of *E.coli* and *Klebsiella spp.* (%)

	E.coli	*Klebsiella spp.*
Ampicillin	81.1	-
Amoxicillin-Clavulanate	70.8	84.7
Piperacillin-Tazobactam	21.3	66.8
Cefazolin	67.3	88.1
Cefuroxime Axetil	75	85.6
Ceftazidime	58.6	80.9
Ceftriaxone	63.9	82.7
Cefepim	65	76.9
Amikasin	2.1	20.4
Gentamycin	27.7	51.9
TMP-SMZ	52.9	76.9
Ciprofloxacin	55.3	71.5
Ertapenem	4.7	57.1
Meropenem	0	42.2
Imipenem	0	41.2
Tigecycline	0	41.9
Colistin	0	28.8

TMP-SMZ: Trimethoprim-sulfamethoxazole

Table 6: Antibiotic resistance profiles of *MSSA* and *MRSA* (%)

	MSSA	MRSA
Erythromycin	11.6	45.2
Clindamycin	4.4	38.1
Tetracycline	7.7	62.1
Rifampicin	20	74.6
Ciprofloxacin	11.9	67.4
Levofloxacin	3.3	56.3
Quinupristin-dalfopristin	0	4.7
Daptomycin	0	0
Vancomycin	0	0
Teicoplanin	0	0
Linezolid	0	0
Tigecycline	0.6	16.9
TM*P*-SMZ	2.8	11.1

Table 7: Antibiotic resistance profile of *Enterococcus spp.* (%)

	Enterococcus spp.
Tigecycline	1.9
Vancomycin	9.6
Teicoplanin	9.6
Ampicillin	39.5
Daptomycin	0
Linezolid	0
Gentamycin (high level)	45.8
Streptomycin (high level)	62.1

Table 8: Antibiotic resistance profile of *Pseudomonas spp.* and *Acinetobacter spp.* (%)

	Pseudomonas spp.	*Acinetobacter spp.*
Imipenem	45	94.9
Meropenem	43.4	94.9
Piperacillin-Tazobactam	37.9	93.2
Ceftazidime	40.7	-
Ciprofloxacin	36.5	94.9
Levofloxacin	26.1	93.3
Colistin	2	3.4
TMP-SMZ	-	75

The number of fungal infections reproduced in the blood cultures of patients was 156 in five years. Among those, 36.5% (n=57) were *C.albians* and 63.5% (n=99) were non-albicans yeasts. The frequency of non-albicans yeasts, *C.parapsilosis complex*, *C.tropicalis*, *C.glabrata* and others (*C.keyfr*, *C.krusei*, *C.lusitaniae*, *C.dubliniensis etc.*) was 34%, 10.3%, 10.3% and 8.9%, respectively. Resistance to echinocandins was detected in one *C.parapsilosis* complex strain and Fluconazole resistance was detected in eight *C.parapsilosis* complex, two *C.albicans* and two naturally resistant *C.krusei* strains. The MIC value in amphotericin B (0.25 g/ml -1 µg/ml) was the highest in four *C.krusei, two C. albicans, two C.parapsilosis complex, one C.kefyr* and one *Trichosporon asahii.*

Discussion

In this study we reported the five years blood culture results with the susceptibility patterns of a tertiary center in Turkey. Culture positivity was 21.0% in this period of time. The five most commonly isolated microorganisms were coagulase negative staphylococcus, *E coli, Klebsiella spp., S aureus* and *Acinetobacter spp.* There was a significant increase in time in ESBL positivity in *E. coli* and *Klebsiella spp.* methicillin resistance did not increase significantly in time in *S. aureus* or coagulase negative staphylococcus. Vancomycin resistance showed a significant increase in *Enterococcus spp.* in time. *E.coli* did not show any resistance to imipenem, meropenem, tigecycline or colistin. MSSA and MRSA did not show any resistance to vancomycin, teicoplanin, linezolid or daptomycin. In *Enterococcus spp.*, tigecycline resistance was 1.9%, while Daptomycin, Linezolid resistance were not determined. *Acinetobacter spp.* was highly resistant to many antibiotics. The most effective agent for *Acinetobacter spp.* and *Pseudomonas spp.* was colistin.

Social, economical and environmental factors may affect the distribution of pathogens isolated from blood stream infections [9]. Culture positivity was 21.0%. The positivity rate was 16.1% when isolates considered to be contaminated were removed. In previous literature culture positivity was ranging between 12-50%. Our results were compatible with the previous data. Since our hospital is a tertiary center and the data was obtained from all of the departments of hospital, this data is crucial [10,11].When we look at the frequency of contamination detected in blood culture samples, this rate was 8.7% in the study of Yılmaz et al. [12] and 10.5% in the study of Sevim et al. [13]. On the other hand, Bentley et al. [14] found the rate of contamination as 4.74%. In our study, this rate was found to be 4.9%. Ideally, the contaminated blood culture rate should not exceed 2-3% [15]; however the rate of contamination in our hospital was higher. As a result, problems related to blood collection techniques, education level of the personnel taking blood, the place or region where the culture is taken are among the reasons that should be considered in the first place.

In this study, the most commonly isolated microorganisms were coagulase negative staphylococcus, *Klebsiella spp., S aureus, and E coli*. Similarly, in a study of Tian et al. *E. coli, S. aureus* and *K. pneumoniae* were reported as the most common pathogens in bloodstream infections in China [16]. Banik et al. [17] reported the contamination rate as 1.63% in 1895 blood specimens. They also reported the most common organisms as *S aureus*, Coagulase negative staphylococcus, and *Acinetobacter spp*.

ESBL production is an important resistance mechanism for bacteria causing a global health burden. It is an important cause of poor outcomes and increased hospital expenses [18,19].

Fennell et al. [20] reported that there was an increase in numbers of ESBL-producing *E. coli* isolated from urine and blood cultures between 2004 and 2008. In Ireland, in a surveillance performed with blood culture isolates, an increase in the prevalence of ESBL in *E. coli* has been reported [21]. In this study, we determined that ESBL positive *E.coli* was still highly susceptive to imipenem, meropenem, tigecycline and colistin but the same condition was not the case for *Klebsiella spp*. Carbapenem-resistance is one of the main problems in *K. pneumoniae. The rate of* carbapenem-resistant *K. pneumoniae* isolates was reported to be over 50%, which is a very serious condition [22]. In our country, ertapenem resistance in *K. pneumoniae* isolates was reported as 43% while imipenem/meropenem resistance was reported as 38% [23]. Due to the rapidincrease of carbapenem-resistance, colistin has been defined as the only treatment choice in resistant enteric bacteria [24]. In a recent study, Rojas et al. [25] reported the colistin resistance as 13% in 246 patients infected with *K. pneumonia.* Colistin resistance was also determined to be associated with high mortality. In our study, we found carbapenem resistance as 40-50% and colistin resistance as 28.8%. This high resistance rates significantly reduce the chance of treatment in *Klebsiella* infections.

We did not determine an increase in Methicillin resistance in *S.aureus* or in coagulase negative staphylococcus. Methicillin resistance is also a global health problem increasing morbidity and mortality and reaching 70% in *S.aureus* isolates in some southern countries [26,27]. We also determined that approximately half of *S. aureus* isolates and more than three fourth of coagulase negative staphylococcus isolates were Methicillin resistant. In a recent study performed on pediatric and adolescent patients, in a tertiary referral center, the most frequently identified microorganisms causing bacteremia were *S epidermidis* (26.1%), and *S aureus* (14.9%). They defined the Methicillin resistance in 60.3% of *S aureus* isolates [28]. As expected, MRSA isolates were more resistant to many antimicrobial agents than MSSA isolates. However, MRSA was completely susceptible to Vancomycin, teicoplanin, linezolid and daptomycin but was having a resistance rate of 4.7% to Quinupristin-dalfopristin. Vancomycin, linezolid and daptomycin are the first line recommended agents in MRSA treatment; which is also compatible with our results. Teicoplanin is another alternative in MRSA cases [29,30].

Enterococcus spp. are also an important cause of blood stream infections. In resistant cases the best alternative are the daptomycin and linezolid. Tigecycline resistance was also very low (1.9%) in *Enterococcus spp*. The number of vancomycin resistance in *Enterococcus spp*. was also increasing significantly in time. VRE were also highly resistant to high level gentamycin or streptomycin. In a recent study daptomycin treatment was reported to be associated with a higher rate of clinical failure as compared with linezolid treatment [31].

Another important finding of this study was also the high antibiotic resistance rates of *Acinetobacter spp*. This was also compatible with the previous literature [32]. In a study of El Mekes et al. [33], the most commonly isolated multi-drug resistant bacteria in the ICU were *Acinetobacter baumannii*. Multi-drug resistance in *Acinetobacter spp*. is also an imperative issue in hospitals.

Unfortunately, Candida species are increasingly causing hospital-acquired infections, which can lead to serious mortality, especially in immunocompromised patients. The most common *Candida species* in the world are *C.albicans* in the USA, Northern and Central Europe; while it is reported as non-albicans *Candida* in Asia, Southern Europe and South America [34]. In a study conducted in South Korea [35], *C.albicans* was found to be 38%, *C.parapsilosis* 26%, *C.tropicalis* 20% while in a study of Oztürk et al. [36], the rates of these fungi were reported as 53%, 30%, 5.5%, respectively. In our study, *C. albicans, C. parapsilosis complex* and *C. tropicalis* were the first three candida species in accordance with the previous data.

Varying proportions (0-20%) of amphotericin B resistance have been reported in previous literature [37-39]. Aydın et al. [40] reported *C.kefyr* and *C.lusitaniae* strains having MIC values >1 μg/ml for amphotericin B.

In our study, the MIC value in Amphotericin B was between 0.25 μg / ml -1 μg / ml and the highest MIC values were found in 4 *C. krusei*, 2 *C.albicans*, 2 *C.parapsilosis complex*, 1 *C.kefyr* and 1 *Trichosporonasahii*. Oztürk et al. [36] did not determine Fluconazole resistance in non-albicans *Candida species* other than intrinsic resistant *C. krusei*, whereas resistance was observed in 6 (32%) of *C.albicans* isolates. Aydın et al. [40] did not detect Fluconazole resistance except *C. glabrata* strains. In a study of Karabicak et al. [41], Fluconazole resistance rate was found to be 3.5% in *Candida species*. In our study, Fluconazole resistance was 6.4% for all candida species.

Echinocandin resistance is quite variable among *Candida* isolates. Diekema et al. [42] found micafungin resistance as 0.8% and Etiz et al. [43] found caspofungin resistance, anechinocandin derivative, as 11%. In our study, we detected echinocandin resistance in only one *Candida parapsylosis complex* isolate. This value was significantly lower than the data in the previous studies. Perhaps it may be due to the lack of intensive use of echinocandin in our hospital.

Limitations

The main strength of this study was the high number of blood cultures isolated. There are also some limitations of this study that should be mentioned. First, this is the report of a single center. Secondly, the treatment outcomes and antibiotic responses of patients were not analyzed since the number of blood cultures analyzed was very high. Lastly, chronic disease or conditions predisposing these infections were not recorded and analyzed in the study, which may be the topic of another study.

Conclusion

For rational antibiotic usage, epidemiological data is important. All tertiary centers should know the distribution of microorganisms and their susceptibility patterns in detail, in initiating treatment promptly to improve outcomes.

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