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Pediatric Blood Culture Resistance Rates from A Single Centre Hospital; How Innocent Are Coagulase-Negative Staphylococci?

Çocuk Kan Kültürü Direnç Oranlarına Tek Odaklı Bir Hastaneden Bakış; Koagülaz Negatif Stafilokoklar Ne Kadar Masum?

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Abstract

Objective: Pediatric blood culture bacteria profile and antibiotic susceptibilities vary for each hospital. In this study, it was aimed to determine the bacterial profile in blood cultures from the pediatric age group and study antibiotic susceptibility. In this context, we want to shed light on rational antibiotic use by determining antibiotic resistance rates in pediatric blood cultures and to reduce the number of samples considered as possible contamination by determining the rates of coagulase-negative *Staphylococci* (CNS).

Material and Methods: In this study, positive 96 samples from a total of 326 samples were brought from the pediatric ward to our laboratory. The cultures were first taken into the growth period on BD Bactec in blood culture bottles. Positive samples were cultivated in suitable media and incubated at 37°C for 24-48 hours. For the suspected specimens of Brucella, the residence time of the bottles was increased to 21 days. The microorganisms were evaluated by biochemical media for identification, and antibiotic susceptibilities were performed by disc diffusion method.

Results: A total of 326 blood samples were cultured. Of the 96 blood cultures with growth, 86 were gram-positive and 10 were gram-negative. While all of the gram-negative bacteria were found to be infectious agents compatible with the patient clinic, the rate of CNS from gram-positive bacteria was found to be about 50% of all bacteria, and the number of patients who had CNS proliferating over the first 12 hours was 48 and positive results were obtained from 27 samples in the first hours. While gram-negative bacteria profiles were found similar to other studies in bacterial susceptibilities, gram-positives were found to be more sensitive. This condition is thought to be based on skin or planting

Öz

Giriş: Pediatrik kan kültürü bakteri profili ve antibiyotik duyarlılıkları her hastane için değişmektedir. Yapılan bu çalışmada hastanemize çocuk yaş grubundan gelen kan kültürlerinde bakteri profilinin saptanarak antibiyotik duyarlılığının yapılması amaçlanmıştır. Bu bağlamda hastanemiz çocuk kan kültürlerinde antibiyotik direnç oranlarının belirlenerek akılcı antibiyotik kullanımına ışık tutulması hedeflenmektedir. Özellikle koagülaz negatif stafilokoklar (KNS)'ın oranlarının belirlenmesi ile muhtemel kontaminasyon olarak düşünülen örneklerin sayısının azaltılması hedeflenmiştir.

Gereç ve Yöntemler: Çalışmamızda iki yıllık süreç içerisinde çocuk servisinden laboratuvarımıza gelen toplam 326 örnekten pozitif 96 örnek değerlendirmeye alınmıştır. Kültürler öncelikle kan kültürü şişelerinde BD Bactec cihazında üreme periyoduna alınmıştır. Pozitif örnekler uygun besiyerlerine ekilerek 37°C'de 24-48 saat bekletilmiştir. Brusella şüpheli örnekler için şişelerin cihazda kalma süreleri 21 güne çıkarılmıştır. Üreyen mikroorganizmalar identifikasyon için biyokimyasal besiyerlerinde değerlendirmeye alınmış disk difüzyon yöntemi ile antibiyotik duyarlılıkları yapılmıştır.

Bulgular: Toplam 326 kan kültürü ekimi yapılmıştır. Üreme olan 96 kan kültürünün 86'sı gram-pozitiflerden 10 tanesi ise gram-negatiflerden oluşmaktadır. Gram-negatif bakterilerin tümü hasta kliniği ile uyumlu enfeksiyon etkeni olarak saptanırken, gram-pozitif bakterilerden KNS oranı tüm bakterilerin neredeyse %50'si civarında bulunmuş ve ilk 12 saati geçen KNS üreyen hasta sayısının 48 olduğu saptanmıştır, ilk saatler içerisinde 27 örnekten pozitif sonuç alınmıştır. Bakteri duyarlılıklarında gram-negatif bakteri profilleri diğer çalışmalara benzer bulunurken, gram-pozitiflerin

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contaminant CNS. Aminoglycoside resistance was found as 25% (n= 24) and vancomycin resistance as 0% (n= 0) in gram-positive bacteria (vancomycin resistance was not found in the blood culture of any patient). Antibiotic resistance rates were as follows: erythromycin 39% (n= 37), cefoxitin 46% (n= 45), ciprofloxacin 46% (n= 44).

Conclusion: Detection of CNS in up to fifty percent raises the question of whether the bacterium is active or contaminant. In addition to bacterial planting and sensitization determination, patient care and medicines have high costs for hospitals and the government. The aim of our study was to reflect only the laboratory data and the situation. According to the results, it is thought that increasing precautions to be taken during blood collection will cause a decrease in contamination rates.

Keywords: Pediatric blood culture, resistance profile, coagulase-negative *Staphylococci*

daha duyarlı olduğu görülmüştür. Bu durumun cilt ya da ekim kontaminantı KNS'lere dayandığı düşünülmektedir. Gram-pozitif bakterilerde aminoglikozid direnci %25 (n= 24), vankomisin direnci %0 (n= 0) oranında bulunmuştur (hiçbir hastanın kan kültüründe vankomisin direncine rastlanmamıştır). Antibiyotik direnç oranları sırayla; makrolid grubundan eritromisine %39 (n= 37), sefalosporinlerden sefoksitine %46 (n= 45), florokinolonlardan siprofloksasine %46 (n= 44) şeklinde saptanmıştır.

Sonuç: KNS'lerin %50'ye varan oranlarda saptanması bakterinin etken mi, kontaminant mı olduğu sorusunu akıllara getirmektedir. Ayrıca bakteri ekim ve duyarlılıklarının saptanmasının yanı sıra hasta bakım ve ilaçlarının hastanelere yüksek maliyetleri olmaktadır. Çalışmamızın hedefi sadece laboratuvar verileri ile durumun yansıtılmasıdır. Çıkan sonuçlara göre kan alımı sırasında uygulanacak önlemlerin arttırılmasının kontaminasyon oranlarında düşüşe neden olacağı düşünülmektedir.

Anahtar Kelimeler: Pediatrik kan kültürü, direnç profili, koagülaz negatif stafilokoklar

Introduction

During the process of septicemia or sepsis diagnosis and start of antibiotic treatment, blood culture results are of primary importance in the order of laboratory testing (1). Particularly septicemia has a mortality rate of eightfold when compared to other causes of hospitalization and length of hospital stay in septicemia burdens the patient and next-of-kin with a tremendous healthcare expense in the country's economy (2). Along with ranking top in blood culture contaminations worldwide, coagulase negative staphylococci (CNS) can be determined as an agent of infection, and it should be noted that contamination rates are little if any in growths detected in the first 12 hours (3). It is seen that contamination rates worldwide are distributed at a very wide scale between 0.6% and 12.5%. This rate is confirmed higher in developing countries like Turkey (4). As it is known, contaminations in blood cultures are usually caused by the infection of skin bacteria, and bacteria as CNS and diphtheroid are reported specifically at higher numbers (5-7). Since resistance rates in blood cultures can differ for every healthcare center, their detection, recording, and determination of resistant antibiotics as per years are of vital significance (8). Detection of bacteria and resistance rates in blood cultures is also important in terms of comparing contamination rates (9). In this respect, this study aimed at evaluating the bacteria and their resistance found in the blood cultures of pediatric patients within two years.

Materials and Methods

The ethical approval for the study was received from Kafkas University Medical School Ethics Board on April 30, 2019 with the number 80576354-050-99. A total of 326 blood cultures reached our laboratory between 2016 and 2019. Recurrent patients were ruled out. The study included 96 samples. Samples in the blood culture pediatric bottle were first taken to a growth period on the BD Bactec device. Positive samples were cultivated in jars with candles and in incubators two for each in blood, EMB and Chocolate media and incubated at 37°C for 24-48 h. For Brucella suspected specimens, the residence time of the bottles in the device was increased to 21 days. Microorganisms that grew were taken under evaluation for identification. Morphology of the colonies and a preliminary evaluation with Gram staining were carried out. For the typing of gram-positive microorganisms, catalase, coagulase in tube and PYR tests were used first, and esculin hydrolysis and growth features in a 6.5% NaCl were examined. In the identification of gram-negative bacteria, TSI agar, Simmon's citrate agar, urease medium, and MIO medium for the reactions in indole medium. *Candida* spp. diagnosis was made for cultures found to have yeast in Gram staining. In order to evaluate antibiotic susceptibilities of the microorganisms in the bottles with growth, cultivation was performed on Müller-Hinton agar preparing the bacteria suspension at McFarland 0.5 turbidity in bouillon with the Kirby Bauer Disk Diffusion method In line with Eucast recommendations (5). Tables recommended by Eucast were made use of in the selection of disks according to bacteria. Efficiency levels of the antibiotics were again evaluated as per Eucast criteria.

Results

Table 1 shows the distribution of the specimens reaching our laboratory within 2 years. The rate of the number of patients in whom gram-positive bacteria grew to the total number of patients in whom bacteria grew was 90%. Again, CNS rate among the gram-positive patients was found as high as 87%.

Table 2 shows antibiotic resistance rates of patients in whom gram-positive bacteria grew. The highest resistance rates in MRCNS, MSCNS, enterococci and streptococci were found against cefoxitin and ciprophloxacin, erythromycin, tetracycline, and cefoxitin and tetracycline, respectively.

Table 3 summarizes gram-negative bacteria and their resistance numbers. Gram-negative growth numbers are

Table 1. The number and percentage of gram-positive and gram-negative bacteria

Bakteria		**n**	**%**
Gram-positive	MRCNS	45	47
	MSCNS	30	31
	Enterococcus spp.	4	4
	Streptococcus spp.	5	5
	Staphylococcus aureus	1	1
	Other (*Micrococcus* etc)	1	1
Gram-negative	*Pseudomonas* spp.	1	1
	Proteus mirabilis	1	1
	Escherichia coli	1	1
	Acinetobacter baumanii	1	1
	Brucella spp.	6	6
Total		96	100

MRCNS: Methicillin-resistant coagulase-negative *Staphylococcus*, MSCNS: Methicillin-susceptible coagulase-negative *Staphylococcus*.

Table 2. Antibiogram-results of gram-positive specimens

Antibiotics	**MRCNS (n= 45)**		**MSCNS (n= 30)**		***Staphylococcus aureus* (n= 5)**		***Enterococcus* (n= 4)**		***Streptococcus* (n= 5)**	
	n	%	n	%	n	%	n	%	n	%
AMC	3	7	0	0	0	0	3	75	1	20
FOX	45	100	0	0	0	0	0	0	2	40
CIP	44	100	1	3	0	0	0	0	1	20
DA	24	53	5	17	0	0	0	0	0	0
E	37	82	9	30	0	0	0	0	0	0
CN	17	38	0	0	0	0	0	0	0	0
LEV	15	33	1	3	0	0	0	0	0	0
LNZ	0	0	0	0	0	0	0	0	0	0
NET	7	16	0	0	0	0	0	0	0	0
TEC	1	2	1	3	0	0	0	0	1	20
TE	17	38	4	13	0	0	2	50	2	40
SXT	25	56	2	7	0	0	0	0	0	0
VA	0	0	0	0	0	0	0	0	0	0

* n represent resistant strains, % represent the percentage of these strains.

lower than the number of specimens, which indicates that gram-positive bacteria rank higher in blood cultures.

Discussion

Bacteria profile and antibiotic susceptibilities of pediatric and adult blood cultures differ for each hospital, which necessitates every healthcare institution to detect its own bacteria distribution (10-12). This study aimed at determining bacteria distribution and antibiotic susceptibilities of the blood cultures from pediatric outpatient and inpatient clinics. Data of the last three years showed that the rate of gram-positive bacteria was 90% and the rate of gram-negative bacteria was 10%. Misuse of cephalosporins, beta-lactam antibiotics, and fluoroquinolones in small-scale healthcare institutions and fully-equipped hospitals causes resistant microorganisms to emerge as hospital infections (13,14). Compared to other hospitals, our hospital has lower resistance rates, which is an indicator of the attention paid to antibiotic use and the care given to not form resistant bacteria. In gram-positive bacteria, aminoglycoside resistance was found as n= 24 (25%) and vancomycin resistance was found as n= 0 (0%) (Vancomycin resistance was not confirmed in any blood cultures of the patients). Other antibiotic resistance rates from the highest to the lowest were as follows: erythromycin from the macrolide

Tablo 3. Antibiogram-results of gram-negative specimens

Antibiotics	*Pseudomonas* spp. (n= 1)	*Proteus mirabilis* (n= 1)	*Escherichia coli* (n= 1)	*Acinetobacter baumannii* (n= 1)
AK	0	0	0	1
AMC	0	0	1	1
SAM	0	0	0	1
AM	0	0	1	1
ATM	0	0	1	1
FEP	0	0	1	1
CAZ	0	0	1	1
CRO	0	0	1	1
CIP	0	0	0	0
LEV	0	0	0	0
CN	0	0	0	1
PIP-TAZO	0	0	0	0
SXT	0	0	0	0
IPM	0	0	0	1
MEM	0	0	0	1

group as n= 37 (39%), cefoxitin from cephalosporins as n= 45 (46%), ciprophloxacin from fluoroquinolones as n= 44 (46%). It was seen that resistance rates both for gram-negative and gram-positive bacteria were lower than those reported from other healthcare institutions in Turkey in the last few years. This is a positive condition for both our hospital and city. In some studies from our country, CNS and *S. aureus* rates have been reported as follows: Aktaş et al. 33.0% and 28.7%; Öksüz et al. 52.7% and 37.8%; Yurtsever et al. 49.6% and 15.0%, respectively (14-16). The general consensus is that blood culture contamination rate must be held below 3% in all cultures taken (17-19). In our study, 50% of this rate comprised of CNS. This situation leads us to consider that sterilization rules are not fully followed during blood drawing, and skin flora is also drawn and that appropriate disinfection rules are not abided by during blood transfer and cultivation processes of the transported blood culture bottles. In both scenarios, it is evident that the training of the nurse drawing blood and the technician working in the laboratory where cultivation is performed is of vital importance (20-23).

When we compared our study with the studies conducted in other regions, it was seen that gram-positive bacteria were largely detected as the contaminant agent in blood cultures (24). While the rate of gram-positive bacteria in our study was confirmed as n= 86 (90%), the rate of gram-negative bacteria was determined as n= 4 (42%). Total CNS was 75, making up 3% of the patients. While the results were examined, it was seen that the number of CNS among the bacteria growing within the first 12 hours was 27. While all of the gram-negative bacteria were established as the infection agent compatible with the patient's clinical status, the rate of CNS from gram-positive bacteria was found as nearly 50% of all bacteria, and the number of patients in whom CNS grew after 12 hours was 48 (positive results were received from 27 specimens within the first 12 hours). Again, it is known that contamination is nearly zero in bacteria growing within the first 12 hours (25). For bacteria resistance, while gram-negative bacteria profiles were found similar to other studies, gram-positive bacteria were determined to be more susceptible, whose skin or cultivation contaminant is considered to be due to CNS. Specimens with brucella were sent to the Public Health Institution for species detection, and the patients were detected to be infected with *Brucella melitensis*. When family histories of the patients were investigated, *Brucella* cases were found in children over the age of 8 and their families. Ours is a 233-bed hospital. Pediatric clinic has 20 beds, and the newly opened intensive care unit provides healthcare services in 10-bed secondary and 6-bed primary care levels.

In the results obtained from intensive care units in our country, Dursun et al. have confirmed *Pseudomonas aeruginosa* growth in 68 specimens [blood: 31, endotracheal aspirate (ETA: 37)], *Acinetobacter baumannii* growth in 42 specimens (blood: 22, ETA: 20), and *Klebsiella pneumoniae* growth in 25 specimens (blood: 18, ETA: 7). Pediatric intensive care unit of our hospital has just gone into service, and a couple of patients could be included into our study in terms of gram-positive bacteria, and no gram-negative growth was encountered (26).

Considering patient distribution, it was seen that the distribution of CNS was 90% and as mentioned before, CNS presence in blood cultures in many patients is considered as a contaminant agent. CNS are encountered at a wide range from 61% to 85% in blood cultures (27-29). In order to differentiate whether the bacterium is the agent or the contamination, identification of the specimens, clinical findings like fever and leukocytosis, follow of the specimen after reaching the laboratory and the duration when the growth takes place are of paramount importance. Fastidious growing bacteria must be kept in mind or contamination of the bottle must be suspected especially in growths taking place on the sixth or seventh day. The point to be considered is that CNS can be regarded as the contaminant if there are clinical findings like fever and leukocytosis suggestive of sepsis in the patient, if there is a primary infection caused by the same microorganism, if the same bacteria growth is observed in all of the blood cultures taken, or if the individual does not have a predisposing condition like immune suppression (30). It is seen in some foreign-based studies that only 24.2% of the CNS are only detected as agents, which leads us to consider that gram-negative agents are rarely contaminants in patients without any symptoms and that contamination must be suspected in one of three specimens for gram-positives (4). This is frequently encountered in gram-positives. Particularly CNS and diphtheroid bacilli are seen in the blood cultures of patients showing no symptoms, which is indicative of the fact that these are found as contaminant agents in the blood cultures (12). Sudden fever and leukocytosis were respectively detected in 10 and 27 of the 75 CNS culture patients detected in our study, and results compatible with our findings were determined. The fact that rises in temperature are not detected in patients in whom CNS is thought to be the agent makes us consider that treatment has been started early. Taking into account that the most important point in detecting contamination is the follow-up and culture result of the patient, the harm CNS does to hospitals and country's economy will be understood better and it will be clearly seen that more attention should be given to sterilization during blood drawing, bottle transfer, and cultivation in laboratories. When viewed from this aspect, in the event of overlooking CNS as an agent by the clinician, sepsis and mortality might occur. Taking into consideration high-cost damages like hospital care, follow-up, and compulsory culture, it is seen that CNS are not as innocent as they are believed to be and that contaminant CNS should be fought with. It is understood that a major part of this fight can be handled by personnel training. In a study by Özcan et al., attention has been drawn to high number of contaminations generated in blood cultures, and they have emphasized that these may have resulted from pre-clinic errors (31). Blood culture training should be absolutely provided and issues such as carrying out skin asepsis, wiping the region for blood drawing with iodine or povidone, waiting for the region to dry for asepsis, and no palpation following this stage should be mentioned (32).

To conclude, as specified in the present and previous studies, binary blood cultures are recommended to be taken to decrease numbers of contamination. We, as the infection control committee, provide training twice a year for this topic at hand. Even so, it is to our belief that laying emphasis on the continuity of training will contribute to decrease the contaminations (33).

Ethics Committe Approval: Ethics board approval for the study was obtained from Kafkas University Medical School Ethics Board (Decision number: 80576354 050-99/129, Date: 30.04.2019).

Informed Consent: Patient consent was obtained

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