JOURNAL OF
CONTEMPORARY MEDICINE

DOI:10.16899/jcm.1081770
J Contemp Med 2022;12(6):866-871

Original Article / Orijinal Araştırma

Double Blood Culture Policy is More Effective Than Single in Neonatal Intensive Units

Yenidoğan Yoğun Bakım Ünitesinde Çift Kan Kültürü Politikası Tekliden Daha Etkili

Murat Konak[1], Muhammed Yaşar Kılınç[1], İbrahim Dikmen[2], Saime Sündüs Uygun[1], Hatice Türk Dağı[3], Hanifi Soylu[1]

[1]Division of Neonatology, Department of Pediatrics, Selçuk University, Selçuklu Medical Faculty, Konya, Turkey
[2]Department of Pediatrics, Selçuk University, Selçuklu Medical Faculty, Konya, Turkey
[3]Department of Infectious Diseases, Selçuk University, Selçuklu Medical Faculty, Konya, Turkey

Abstract

Aim: Blood culture (BC) sampling and antibiotic administration are common practices in Neonatal Intensive Care Units(NICUs). However, false positive BC results might affect clinician's decision and lead to inappropriate antibiotic treatments. The aim of this study was to investigate the effect of double culture on clinical application.

Material and Method: The study was conducted retrospectively. The blood culture results of the patients admitted to the NICU between 2016-2019 were analyzed. Considering sepsis before 2017, we took only one sample from the patient. After this period, we started to take double blood cultures. Time frames of BCs were investigated to two groups as early and late onset sepsis fistly, and then subgroups were determined as; a-) Group 1, BCs in the first 24 hours, b-)Group 2, between 24 to 72 hours, and c-)Group 3, after 72 hours. Cultures were taken twice from patients who have central catheter as central and peripheral. Patients who not to have any central line evaluated once times as peripheral blood samples. In this way, it was aimed to evaluate whether the catheter was colonized or the presence of contamination and to determine the true sepsis and sepsis agent.

Results: Total of 1747 BC samples were taken in study. Male/female ratio was 1.3:1. Majority of BCs were in Group 3 (62%). We realized lots of bacterial contaminations in all groups. *Staphylococcus epidermidis* (*S. epi*) was major source for the contamination. But, by taking dBCs, we were able to decided most *S. epi* contamination in Group 2 (11% vs. 3%) and in Group 3 (41%to14%). In addition, we were able to identify some resistant Gr (-) pathogens in one arm although the other arm was negative, by taking dBC. If the double blood cultures results were showed *S. epi*, then it was accepted pathogen microorganism and treatment was continued and arranged for antibiogram results.

Conclusions: Our study indicates that dBC policy in NICUs could help to clinicians for judicious decision in antibiotic use and decrease unnecessary antibiotic exposure of infants. Also it could be enable to detect some highly pathogen microorganism easily.

Keywords: Newborn, septicemia, blood culture, coagulase negative staphylococci, contamination

Öz

Amaç: Yenidoğan Yoğun Bakım Ünitelerinde (YYBB) kan kültürü örneklemesi ve antibiyotik uygulaması yaygın uygulamalardır. Ancak yanlış pozitif kültür sonuçları klinisyenin kararını etkileyebilir ve uygun olmayan antibiyotik tedavilerine yol açabilir. Bu çalışma çift kültürün klinik uygulamaya etkisini araştırmak amacıyla yapılmıştır.

Gereç ve Yöntem: Çalışma retrospektif olarak yapıldı. 2016-2019 yılları arasında YYBÜ'ye başvuran hastaların kan kültürü sonuçları incelendi. 2017 öncesi dönemde sepsis düşünüldüğünde hastalarımızdan sadece bir örnek alıyorduk. Bu süreden sonra çift kan kültürü almaya başladık. Kan kültürleri alım zamanına göre; ilk olarak erken ve geç başlangıçlı sepsis olmak üzere iki gruba, ardından alt gruplara; a-) Grup 1, ilk 24 saatte kültür, b-)Grup 2, 24-72 saat ve c-)Grup 3, 72 saatten sonra kan kültürü alınanlar olarak gruplandırıldı. Santral kateteri olan hastalardan santral ve periferik olmak üzere iki kez kültür alındı. Santral katateri olmayan hastalar periferik kan örneği olarak bir kez değerlendirildi. Bu şekilde kateterin kolonize olup olmadığı veya kontaminasyon varlığının değerlendirilmesi ve gerçek sepsis ve sepsis etkeninin belirlenmesi amaçlandı.

Bulgular: Çalışmada toplam 1747 kültür örneği alındı. Kan kültürlerinin çoğu Grup 3'teydi (%62). Erkek/kadın oranı 1.3:1 idi. Tüm gruplarda çok sayıda bakteri kontaminasyonu tespit ettik. *Staphylococcus epidermidis* (*S. epi*), kontaminasyon için ana etkendi. Ancak, çift kan kültürü alarak, Grup 2'deki (%11'e karşı %3) ve Grup 3'teki (%41 ila %14) çoğu *S. epi* kontaminasyonunu ortadan kaldırmayı başardık. Ayrıca çift kan kültürü alarak bir kolda bazı dirençli Gr (-) patojenleri diğer kol negatif olmasına rağmen tespit edebildik. Çift kan kültürü sonuçlarında *S. epi* saptanması durumunda patojen mikroorganizma kabul edildi ve tedaviye devam edilerek antibiyogram sonucuna göre düzenlendi.

Sonuçlar: Çalışmamız, YYBB'lerde çift kan kültürü politikasının klinisyenlere antibiyotik kullanımında mantıklı karar vermelerine yardımcı olabileceğini ve bebeklerin gereksiz antibiyotik maruziyetini azaltabileceğini göstermektedir. Ayrıca kan kültürünün çift olarak alınması bazı patojen mikroorganizmaların kolaylıkla tespit edilmesini sağlayabilir.

Anahtar Kelimeler: Yenidoğan, septisemi, kan kültürü, koagülaz negatif stafilokoklar, kontaminasyon

Corresponding *(İletişim)*: : Murat Konak, Division of Neonatology, Department of Pediatrics, Selcuklu Medical Faculty, Selcuk University, Konya, Turkey.
E-mail *(E-posta)*: drmkonak@hotmail.com
Received *(Geliş Tarihi)*: 03.05.2022 **Accepted *(Kabul Tarihi)*:** 24.06.2022

INTRODUCTION

Neonatal sepsis is a clinical syndrome that involves systemic signs and symptoms related to infection and growth of a specific causative agent in blood culture in the first month of life. Newborns are extremely susceptible to infection, and sepsis is a significant cause of morbidity and mortality in this population.[1] The incidence of sepsis in neonates is lower in developed countries but it is reported to be between 1-8.1 per 1000 live births.[2-4] The most common agents in early-onset sepsis include GBS and *Escherichia coli* (*E. Coli*). In late-onset sepsis (LOS), coagulase-negative staphylococci (CoNS) including mainly *Staphylococcus epidermidis* (*S. epi*) are observed most commonly with a rate of 53.2-77.9% in developed countries. On the other hand, there are also countries and clinics in which Gram-negative bacilli including *E. Coli*, *Klebsiella*, and *Pseudomonas* species are in the forefront. *Staphylococcus aureus* (*S. aureus*) and *Candida* species are among the other agents.[5]

The gold standard in the diagnosis of sepsis is the production of the agent in blood culture. The sensitivity of the blood culture is 50-80%. If the culture is not taken under appropriate conditions, the risk of contamination is high. Lack of reproduction in culture may be due to the low amount of blood taken (<1 ml), the initiation of antibiotics without culture samples, the use of antibiotics by the mother, the low bacterial density in the blood, the intermittent and short-term bacteremia.[3,6]

Blood culture (BC) and empirical antibiotic use in NICU are common procedures to protect babies from life-threatening infections. However, some complications, such as unnecessary antibiotic initiation and prolonged hospitalization, occur as a result of a false positive culture.[7-9] Routine double blood culture is recommended for adults to increase their culture reliability and to facilitate their interpretation.[10] This practice for newborn babies continues to be a subject of debate because of the need for a large amount of blood. However, the confusion of single culture results (colonization, contamination, etc.) in the NICUs may still cause difficulties in decision making. In order to prevent infants from taking unnecessary antibiotics in our clinic, if the first time is being examined in terms of sepsis, a policy of taking blood culture from two different places is followed. This study was carried out in order to better examine the benefits and harms of this policy.

MATERIAL AND METHOD

The study was carried out with the permission of ..... Ethics Committee (Date: ..... , Decision No: 2019/103). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study was conducted retrospectively. The blood culture results of the patients admitted to the NICU between 2016-2019 were analyzed. Considering sepsis before 2017, we took only one sample from the patient. We realized there were too many colonisation of CoNS and those caused unnecessary use of antibiotics. After this period, we started to take double blood cultures to exclude colonisation and display real infection. Fetal distress, low APGAR score and resuscitation requirement at birth, presence of maternal fever, chorioamnionitis, prolonged (early) rupture of membranes (>18 hours), frequent vaginal examination, bad smelling discharge in the mother, early-onset of delivery urinary tract infection were considered risk factors for EOS; invasive procedures such as intubation, mechanical ventilation, catheter/catheter insertion, inadequate breast milk, long-term parenteral nutrition, reduction of gastric acidity, and need for surgical intervention were considered as risk factors for LOS. Clinical signs of sepsis in the absence of any other pathology to explain the patient's situation; respiratory distress, rejection or intolerance of feeding, abdominal distension, vomiting, diarrhea, hypothermia, hyperthermia, lethargy, unexplained hyperglisemia and hemorrhage were accepted. If two or more of the following parameters were positive, it was considered as a positive sepsis screening: Total leukocyte count <4000/mm³, >20000/mm³, immature/total neutrophil ≥0.2; C reactive protein >10 mg/l, platelet count <100000 mm³ and clinical presentation consistent with sepsis. Criteria for inclusion were infants with at least one clinical feature with two or more risk-factors for sepsis who were admitted to the service. The exclusion criteria were patients who received antibiotics prior to blood culture sampling.

Early neonatal sepsis (EOS) (first 72 hours) and LOS (> 72 hours) were evaluated. Time frames of BCs were stratified to three subgroups as; BCs in the first 24 group 1, hours, between 24 to 72 hours group 2, after 72 hours group 3.

The cultures of our study were carried out in Selcuk University Medical Faculty Microbiology Laboratory. Whether the single (sBC) or double (dBC) blood culture was taken from the baby was determined from the date of receipt. Cultures were taken twice from patients who have central catheter as central and peripheral. Patients who not to have any central line evaluated once times as peripheral blood samples. The single BC was taken from either the intravenous catheter during initial insertion or from a peripheral vein, under aseptic precautions. Double BC was taken from two peripheral veins by asepsis rules with less than 15 min intervals from patients without catheter. If the patient had catheter, one of the blood cultures was taken from the catheter and the other was taken from the peripheral vein. In this way, it was aimed to evaluate whether the catheter was colonized or the presence of contamination and to determine the true sepsis and sepsis agent. At least 1 ml of blood was targeted to the culture bottles. Blood cultures were monitored on a fully automated blood culture device BACTEC 9240 (Becton Dickinson, Diagnostic Instrument System, Sparks, USA).[11]

In these cultures, microorganisms were evaluated separately as gram positive, negative and fungi. Gram-positive CoNS was examined in a separate group. Single blood culture (sBC) and double blood culture (dBC) results from patients were separated according to positivity or negativity. However, the dBC results were subclassified as one sample positive and one negative. In addition, Gram (+) and Gram (-) agents were categorized separately. But, *S. epi* was further analyzed because of its major contribution to false positive results. Growth of gram-negative organisms or *S. aureus* in any culture was considered the true positive. For CoNS, it was accepted as positive if growth was detected in two cultures. While CoNS was positive in the cultures taken from the catheter, the peripheral culture negative was considered as colonization.

All patients include our study had septic clinical suspections. As a septic work out was examined CRP, total blood count and blood culture. We started ampiric antibiotic. Patients who applied single blood culture and positive of results were accepted septic and treatments were continued. Patients who applied double blood culture and positive of double cultures results If there were no comtaminant microorganism were accepted septic and treatments were continued. When double blood culture results showed one of contaminant and other gram negative, these patients were accepted septic and treatments were continued and arranged with antibiogram. In the other hand, when double blood culture results showed one of contaminant or *S. epi* and other blood culture results negative, these patients were accepted non-septic/contaminations of blood cultures and treatments were canseled and for explain the patients clinic status were investigated another etiological factors. If the double blood cultures results were showed *S. epi*, then *S. epi* was accepted pathogen microorganism and treatment was continued and arranged for antibiogram results.

Statistical Analysis

Statistical evaluation was performed using IBM SPSS Statistics for Windows, Version 20.0 (Armonk, USA) computer program. Categorical variables were analyzed using chi-square/Fisher's exact test.

RESULTS

Total of 1340 BC procedures were done and 1747 BC samples were taken in this time frame (**Figure 1**). Majority of BCs were in Group 3 (62%) (**Figure 1**). Overall, male to female ratio was 1.3:1. A total of 918 sBC were performed and detected 30% positive. 830 dBC were done and 29.2% growth was detected and there was no statistically significant difference between both group (p>0.05). The general analysis of culture results are shown in **Table 1**. In all cases, the most common microorganism detected was coagulase negative staphylococcus (CoNS) and the second was *Klebsiella*. In Group 1, while CoNS was positive in 15 patients in sBC, CoNS grew from both cultures in those with dBC in 4 out of 151 cases. In Group 2, while CoNS was positive in 7 patients in sBC, CoNS grew from both cultures in those with dBC in 2 out of 33 cases. In Group 3, while CoNS was positive in 105 patients in sBC, CoNS grew from both cultures in those with dBC in 38 out of 223 cases. In Group 3, CoNS ratio decreased from 42.6% to 14% by dBCs. Although no significant decrease was observed in the groups 1 and 2, the rate of contamination and colonization detection was statistically significant in Group 3 (p<0.05) (**Figure 2**) (**Table 2**). In Group 3, 23.1% of sBCs detected *Klebsiella* compared to 33.6% of dBCs (**Table 2**). The number of microorganisms determined according to EOS or LOS is shown in **Table 3**. In the dBC group, 81 (9%) were positive only in a single culture while 166 (20%) were positive in both cultures. Results according to groups are summarized in **Table 1** and **2**.

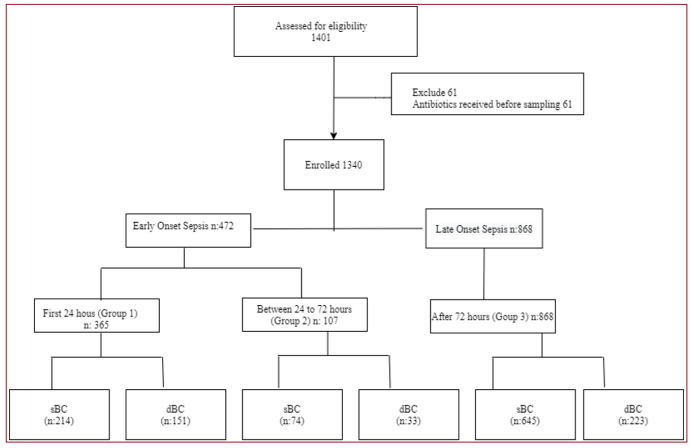

Figure 1. Flowchart showing study participants.

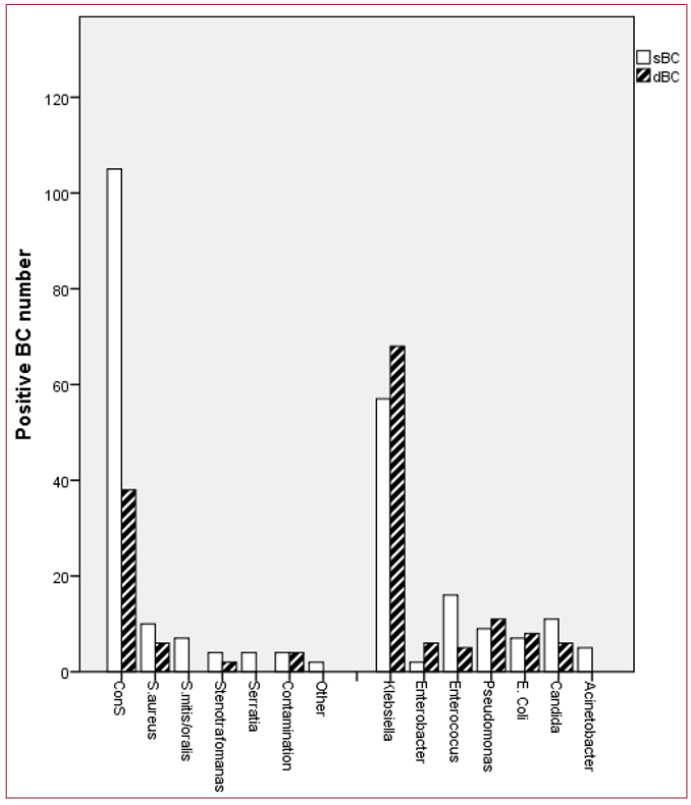

Figure 2. Effect of dBC on detection of pathogens in late-onset neonatal sepsis

Table 1. Analysis of Culture Results and Determination of *S. epi*

	Group 1 (n:365)		Group 2 (n: 107)		Group 3 (n: 868)	
	sBC (n:214)	dBC (n:151)	sBC (n:74)	dBC (n:33)	sBC (n:645)	dBC (n:223)
No growth	87%[b]	93%[b]	86%[b]	82%[b]	61%[b]	55%[b]
Single arm growth	NA	5%	NA	6%	NA	13%
Full growth	13%	3% [a,b]	14%[b]	12%[b]	39%[b]	32% [a,b]

aP<0.05 between in group: bp>0.05: between in whole group NA: not applicable.

When evaluated in terms of EOS, 656 cultures were taken. In 59 (8.9%) reproduction was detected. The most frequently detected microorganism is 54% CoNS, second frequency is *E. Coli* (6.7%) and Group B Streptococcus (GBS) was the third frequent Group (5%). When evaluated for LOS, the incidence of CoNS was 43.2%, whereas the actual CoNS infection rate was 16.5%, if DBC was taken. The most common cause of infection in LOS was *Klebsiella* with a rate of 30.8%) (**Figure 2**) (**Table 3**).

Table 3. Pathogens detected in early and late onset sepsis

Pathogens	sBC (n:918)		dBC (n:830)			
			Single arm growth (n:80)		Full growth (n:750)	
	EOS (n:288)	LOS (n:630)	EOS (n:24)	LOS (n:56)	EOS (n:8)	LOS (n:742)
CoNS (n:197)	25	105	4	15	6	41
Klebsiella (128)	1	56	1	3	0	67
E. Coli (n: 21)	3	7	1	0	0	10
Enterococcus (n:28)	2	15	1	2	0	8
S. aureus (n:31)	2	10	1	2	0	16
Enterobacter (n:10)	1	2	0	1	0	6
Pseudomonas (n:26)	1	9	0	2	0	14
Candida (n:18)	1	11	0	0	0	6
Serratia (n:6)	0	4	0	0	0	2
GBS (n:4)	1	0	0	0	1	2
Acinetobacter (n:7)	1	5	1	0	0	0
Others	4	10	2	1	1	2

CoNS: Coagulase-negative staphylococci

DISCUSSION

In neonatal infants, the symptoms and signs of various diseases (congenital heart disease, metabolic disease, RDS, sepsis, etc.) are often similar, and until the diagnosis of sepsis is evaluated in the direction of the patients and the initiation of empiric antibiotics is a common approach all over the world. The use of long-term or unnecessary antibiotics does not contribute to the patient's current picture; on the contrary, the risk of necrotizing enterocolitis, sepsis, bronchopulmonary dysplasia and death is increased for premature babies, and evidence for increasing the incidence of obesity, atopy, etc. in the later period continues to increase.[12] Although all this information is well known by neonatal physicians, physicians dealing with patients with very sensitive and inadequate resistance can be prolonged with the suspicion of infection, even if the pathology is detected, or antibiotic therapy can be prolonged or the drug is being stopped.[13] Detection of blood cultures, which are definitive evidence of infection, facilitates the management of cases, but difficulties can arise in deciding if the pathogens which are not frequently encountered or are the normal flora elements of the skin. Therefore, various methods have been tried to increase the efficiency of blood cultures. These include inoculation of high volume of blood into culture bottles, automated continuous blood culture monitoring systems and use of 2 or more blood cultures, maintaining blood-broth ratio of 1:5 to 1:10 and avoiding samples from indwelling catheters for risk of contamination.[14,15]

In 2017, Tomar et al. In the studies evaluating 475 babies, double culture was taken from all patients. The positive rate was 185 (38.9%) based on a culture, and 221 (46.5%) were found to be positive when evaluated with the second culture. This increase was statistically significant.[16] In our study, a statistically significant higher rate of *Klebsiella* infection was detected in the patients who were evaluated for late sepsis in the case of double culture (p <0.05). In addition, we were able

Table 2. Distribution Of Identified Pathogens According To Groups

Pathogens	Group 1			Group 2			Group 3		
	sBC	dBC single growth	dBC full growth	sBC	dBC single growth	dBC full growth	sBC	dBC single growth	dBC full growth
CoNS	15	2	4	7	2	2	105	14	38a
Klebsiella	1	1	0	0	0	0	57	3	68a
E. Coli	3	1	0	0	0	0	7	0	8
Enterococcus	1	1	0	0	0	0	16	2	5
S. aureus	1	0	0	0	1	0	10	2	6
Enterobacter	0	0	0	0	0	0	2	1	6
Pseudomonas	1	0	0	0	0	0	9	2	11
Candida	1	0	0	0	0	0	11	0	6
Serratia	0	0	0	0	0	0	4	0	0
GBS	1	0	0	0	0	2	0	0	0
Acinetobacter	0	0	0	1	0	0	5	1	0
Mitis/Oralis	2	1	0	0	1	0	7	1	0
Stenotrafomanas	0	0	0	0	0	0	4	0	2
Contamination	0	2	0	1	0	0	4	2	4
Others	1	0	0	0	0	0	2	0	0

CoNS: Coagulase-negative staphylococci aP<0.05

to identify some resistant pathogens in one arm although the other arm was not positive and the proven sepsis rate was detected higher (**Figure 2**). The most commonly detected gram negative organisms were *Klebsiella*, *E. Coli* and *Psoudomanas*, and these results were consistent with the literature.[3,5]

Sarkar et al. in the 2006 study, they obtained a double blood culture in 216 patients who were hospitalized in NICU and found that culture positivity was 20 (9.2%) in the case, but double culture did not provide additional benefit and did not increase the rate of positivity.[16] One year later, the same researchers concluded that double blood cultures after 3 days later of antimicrobial therapy in neonates for sepsis would lead to better ascertainment of the clearance of bacteremia than single cultures.[17]

Detection of microorganisms, which are normally a skin flora element, in culture can cause confusion. In a study of 69 babies with sepsis clinical findings, a double culture was performed; In 16 patients, they found double culture reproductive and a single culture in 10 patients. They stated that they had accepted a single positive as a contamination and stopped the antibiotherapy at 48th hour and thus reduced the use of antibiotics (vancomycin) by 8.2%.[18] In another study, it was determined that 460 babies were evaluated with dBC for EOS and 10 of 18 patients who detected reproduction were found to have skin flora contamination.[15] In our study, we couldn't find any statistically different when we evaluated to effect of taking double blood culture in EOS.

Not surprisingly, in our study *S. epi* was major source for the contamination. When all cases were examined with respect to CoNS, culture positivity was determined as 43.2% with sBC and the rate of true CoNS infection was 16.5% with dBCs. In the literature, the frequency for LOS is reported to be 11.5% to 32.4%, and CoNSs are responsible for 53.2-77.9% of these.[5,19] Struthers et al. in case of the use sBC instead of dBC indicated that 31% more CoNS infections would be diagnosed, but they were evaluated with dBC and used shorter antibiotics.[18] Another study reported that CoNS and fungal infections are common in recent years and it may be beneficial to obtain double culture to confirm that these identified pathogens are the true infectious agents.[15] Moreover, the retrospective study of Wisswel et al. was one of the first double-culture studies in the literature. They have taken 2 sets of blood cultures in 1 week. In 8 cases of 18, contamination from skin flora was documented.[20]

The prevalence of proven EOS in high-income countries is between 0.01 and 0.53 per 1000 live births in Europe, while in low-income countries this figure ranges from 0.01 to 3.06 per 1000 live births.[4] The lack of consensus in the existing guidelines for suspected EOS management and the low threshold of pediatricians to assess and treat a newborn with suspected EOS has been found to cause unnecessary treatment and hospitalization in Europe of approximately 395,000 newborns (7.9% of all births) per year.[21] In infants who are admitted to the hospital for sepsis assessment, false positivity or growth in culture will increase unnecessary interventions. In our study, it was suggested that the false positive rate of CoNS, which is a frequent factor for LOS, was reduced by approximately 26.7% with the addition of dBC. We couldn't find same results for EOS. However, In our results, Positivity rate of blood culture in EOS was 8.9%.

Because newborns have limited symptoms and are similar for many diseases, it is known that most of the poorly treated infants evaluated for sepsis are not infected and there is also a significant number of viral causes even in infected infants. Therefore, by taking dBC, it is easier to remove bacterial infection and the underlying causes can be revealed earlier.[13,22]

Limitations of our study were retrospective and also colony count and time to positivity could not be documented.

CONCLUSION

Our study indicates that dBC policy in NICUs could help to clinicians for judicious decision in antibiotic use and decrease unnecessary antibiotic exposure of infants. In addition, it could increase chance of identification of some dangerous Gr (-) pathogens. It is applicable, reliable and cost/time saving strategy.

Abbreviations: Neonatal Intensive Care Units NICU. *Staphylococcus epidermidis S. epi*, Blood culture BC, Blood cultures BCs, Single BC sBC, Double BC dBC, Group B Streptococcus GBS, *Escherichia coli E. Coli*, Coagulase-negative staphylococci CoNS, Early neonatal sepsis EOS, Late neonatal sepsis LOS

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of ..... Ethics Committee (Date: ..... , Decision No: 2019/103).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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