RESEARCH ARTICLE

Sukru Oksuz[1]
Betul Donmez[1]
Banu Humeyra Keskin[1]
Nagihan Memis[1]
Zeynep Dilara Karamurat[1]
Emel Caliskan[1]
Cihadiye Elif Ozturk[1]
Idris Sahin[1]

[1] Duzce Üniversity, Medical Faculty, Department of Medical Microbiology, Duzce, Turkey

Corresponding Author:
Sukru Oksuz
Duzce Üniversity, Medical Faculty, Department of Medical Microbiology, Duzce, Turkey
Phone:+90 5325723426
mail: droksuz@gmail.com

Received: 12.01.2021
Acceptance: 03.09.2021
DOI: 10.18521/ktd.858764

The study was presented as a poster presentation at the 5th National Clinical Microbiology Congress in Tepekule Congress and Exhibition Center, Izmir, on October 28-November 1, 2019.

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Evaluation of Quality Assurance Indicators and Contamination Rate in Blood Culture

ABSTRACT

Objective: Blood culture are of vital importance in patient follow-up, as they enable the identification and production of sepsis causative microorganisms, initiate antibiotic treatment in a timely manner and reduce mortality and morbidity. In this study, it is aimed to evaluate the microorganisms grown in the automated blood culture in the microbiology laboratory of the hospital in terms of quality indicators.

Methods: In this study, microorganisms grown from automated blood culture BACTEC-9120 (Becton Dickinson, USA) system from the blood culture samples sent to Duzce University Medical Microbiology Laboratory were evaluated retrospectively. For this purpose, the rejection and contamination rate of the samples for which blood culture was requested, the result of Gram staining-final identification compliance, the number of samples sent from a single bottle, and the growth times of microorganisms after incubation were determined.

Results: 5037 blood culture samples were sent to the laboratory from various clinics. 1.7% of these samples were rejected as inappropriate samples. Gram stain-final identification compatibility of blood cultures was investigated and it was determined as 97.8%. The single bottle number of the samples sent was found to be 511. For the 5037 samples included in the study, growth was detected in 20.7%, of which 10.2% were considered as contaminants. In our study, the average breeding time of the factors examined for breeding time was determined to be 30.29 hours.

Conclusions: As conclusion, there is no gold standard to distinguish true pathogens from contaminant agents in blood cultures.

Keywords: Blood Culture, Bacteremia, Quality Indicators.

Kan Kültüründe Kalite Güvence Göstergelerinin ve Kontaminasyon Oranının Değerlendirilmesi

ÖZET

Amaç: Kan kültürü, sepsise neden olan mikroorganizmaların üretilmesini ve tanımlanmasını sağladığı, antibiyotik tedavisini zamanında başlamayı ve mortalite ve morbiditeyi azalttığı için hasta takibinde hayati öneme sahiptir. Bu çalışmada hastanemiz mikrobiyoloji laboratuvarında otomatik kan kültür sisteminde üretilen mikroorganizmaların kalite göstergeleri açısından değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Bu çalışmada, Düzce Üniversitesi Tıbbi Mikrobiyoloji Laboratuvarına gönderilen kan kültürü örneklerinden otomatize kan kültürü BACTEC-9120 (Becton Dickinson, ABD) sisteminde üreyen mikroorganizmalar retrospektif değerlendirilmiştir. Bu amaçla kan kültürü istemi yapılan örneklerin reddedilme ve kontaminasyon oranı, Gram boyama sonucu-son identifikasyon uyum oranı, tek şişe gönderilen örnek sayısı ve inkübasyon sonrası mikroorganizmaların üreme süreleri belirlenmiştir.

Bulgular: Laboratuvara çeşitli kliniklerden gönderilen 5037 kan kültür örneği dahil edilmiştir. Bu örneklerden %1,7'si uygunsuz numune olarak reddedilmiştir. Gram boyama -son identifikasyon uyumu araştırılmış ve %97,8 olarak saptanmıştır. Gönderilen örneklerin tek şişe sayısı 511 olarak bulunmuştur. Çalışmaya alınan 5037 örneğin, %20,7'sinde üreme saptanmış, bunların %10,2'si kontaminant olarak kabul edilmiştir. Araştırmamızda üreme süresi incelenen etkenlerin ortalama üreme süresi 30,29 saat olarak saptanmıştır.

Sonuç: Sonuç olarak, kan kültürlerinde gerçek patojenleri kontaminant ajanlardan ayırt etmek için altın standart yoktur.

Anahtar Kelimeler: Kan Kültürü, Bakteriyemi, Kalite Göstergeleri.

INTRODUCTION

Despite some limitations in the diagnosis of bloodstream infections, blood culture is still the gold standard. Blood culture samples taken in accordance with the guidelines are of vital importance in patient follow-up, as they enable the identification and production of sepsis causative microorganisms, initiate antibiotic treatment in a timely manner and reduce mortality and morbidity. However, difficulties are encountered in the separation stage of true pathogen and contaminant microorganisms that are reproduced from inappropriate blood culture samples. While detection of a true pathogen may help the clinician to determine the cause of sepsis and provide antibiotic susceptibilities, reporting of contaminant microorganisms as an agent may be detrimental to patient care. Furthermore, it increases the length of hospital stay, hence increases costs for patient, and antimicrobial resistance (1–4).

In this study, it is aimed to evaluate the microorganisms grown in the automated blood culture BACTEC-9120 (Becton Dickinson, USA) system in the microbiology laboratory of the hospital in terms of quality indicators for two years.

MATERIAL AND METHODS

The blood culture results sent to the Medical Microbiology Laboratory of XXXX University Medical Faculty Hospital between April 1, 2017 and May 31, 2019 from departments and emergency services were examined retrospectively. Blood samples which were taken under aseptic conditions and sent in appropriate blood culture bottles were examined on a BACTEC automated blood culture device (Becton Dickinson, USA). All samples giving positive signal were examined by Gram staining, then they were cultured in 5% sheep blood agar, Eosin Methylene Blue agar and Chocolate agar and evaluated after for 24-48 hours of incubation at 37 °C. Microorganisms that grew in culture were identified with VITEK 2 (bioMérieux, France) fully automated identification system and traditional methods (5, 6). Strains of isolated coagulase negative staphylococci, diphteroids, Bacillus spp., Micrococcus spp, Propionibacterium acnes, and Corynebacterium spp were accepted as contaminant (7). It was accepted as a true pathogen when coagulase negative staphylococci growth was detected in two or more samples of one patient (2). The contamination rate was calculated according to the following formula (8). Blood culture samples included in the study were evaluated according to quality indicators.

Number of contaminated blood cultures / Total number of blood cultures = Contamination rate

The time between the loading of blood culture samples sent from the patients to the BACTEC automated blood culture device and the growth signal were measured. In this study, the number of single bottle blood culture samples sent from the services and clinics was determined. After the reproductive signal, cohesiveness of the images of the samples in Gram stain were compared to the final identification and recorded. Simultaneous catheter cultures were not included in the study (3, 9).

XXX University Faculty of Medicine Ethics Committee approval was obtained for the study (15.04.2019, Decision number: 2019/97)

Statistical Analysis: SPSS 17 software was used for statistical evaluation of the data. Categorical data are summarized as frequency and percentage. Pearson chi-square test and Fisher's exact test were used to evaluate the contamination and single bottle sample rates according to the clinics. In the comparison of reproductive time of microorganisms, the significance test of difference between two means were applied and Mann Whitney U Test were applied in cases where there was no agreement to normal distribution. $P < 0.05$ was considered significant.

RESULTS

A total of 5037 blood culture samples of 2767 male and 2270 female patients sent from various clinics to our laboratory were included in the study. According to this evaluation, 90 (1.7%) of the samples were rejected as they were found inappropriate. The Gram stain -Final identification" agreement of the Gram stained blood cultures of samples which showed reproductive signal was 97.8% (23/1045). The distribution of microorganisms that are incompatible with gram staining and final identification are shown in Table 1.

Table1. Gram staining and distribution of discordant microorganisms in final identification

Gram Staining Result (n)	Blood Cultur Result (n)		
	Microorganism1	**Microoragnism2**	**Microorganism3**
No microorganisms (n:2)	CNS* (2)	-	-
Gram positive cocci (n:3)	*A.baumannii* ** (1)	No growth (2)	-
Gram negative cocci (n:1)	*Salmonella* spp (1)	-	
Gram positive bacillus (n:2)	*Kocuria rosea* (1)	*R. planticola* , *** (1)	
Gram negative bacillus (n:4)	CNS	No growth	
Gram positive cocci and Gram negative bacillus (n:6)	CNS (4)	*Klebsiella* spp (1)	*Enterococcus* spp (1)
Gram positive cocci and yeast (n:2)	*A. baumannii* ** (1)	*Enterococcus* spp(1)	
Gram negative cocci (n:1)	*Proteus mirabilis* (1)		
Yeast (1)	CNS (1)	-	-

*CNS:Coagülase negative cocci ** *Acinetobacter baumanii* ****Raoultella planticola*

1045 (20.7%) of the 5037 samples included in the study were found growt. In the study, the number of contaminations was determined as 514 (10.2%). When the contamination rates were examined according to the clinics, it was found that the rate of contamination in the intensive care units was similar to that of the other services (p = 0.662), whereas it was lower in the surgical services compared to the intensive care units and other services (p = 0.030). It was determined that 511 of the samples were single bottles. The rate of sending blood culture in one bottle was found to be highest in neonatal intensive care units (p>0.001) in intensive care units and in neonatal service (p>0.001) in the other services. The most common isolate which is accepted as contaminant was identified as Coagulase negative staphylococci.

The distribution of the samples identified as contaminants and sent as single bottles according to the clinics is shown in Table 2.

Table 2. Distribution of isolates identified as contaminants sent as single bottles according to services

Intensive Care Units	**Total number (n)**	**Number of single Bottles**		**Contamination**	
		n	**%**	**n**	**%**
İnternal medicine	1178	31	2,6	119	10,1
Neonatology	319	**190**	**59,6**	29	9,1
Anesthesia	315	6	1,9	31	9,8
Pediatry	91	30	33	12	13,2
Coroner	90	5	5,6	13	14,4
Neurosurgery	64	5	7,8	7	10,9
Cardiovascular surgeon	27	1	3,7	3	11,1
Total	**2084**	**268**	**12,9**	**214**	**10,3**
Internal Units					
Internal medicine	767	39	5,1	75	9,8
Emergency medicine	518	22	4,2	71	13,7
Chest diseases	384	23	6	40	10,4
Infection disease	266	30	11,3	24	9
Nephrology	139	10	0,7	11	7,9
Pediatry	137	36	26,3	15	10,9
Hematology	129	10	7,8	13	10,1
Neurology	127	10	7,9	19	15
Oncology	72	4	5,6	3	4,2
Neonatology	37	**26**	**70,3**	6	16,2
Cardiology	30	6	20	0	0
Gastroenterology	27	1	3,7	3	11,1
PM&R *	6	2	33,3	1	16,7
Dermatology	5	1	20	-	0
Forensic medicine	1	1	100	1	100
Total	**2645**	**221**	**8,4**	**282**	**10,7**
Surgery Units					
Urology	104	9	8,7	6	5,8
General surgery	96	2	2,1	3	3,1
Orthopedics	37	5	13,5	4	10,8
Gynecology	34	4	11,8	0	0
Neurosurgery	15	-	0	-	0
Otorhinolaryngology	10	2	20	4	40
Thoracic surgery	8	0	0	-	0
Cardiovascular surgeon	2	0	0	1	50
Ophthalmology	2	0	0	-	0
Total	**308**	**22**	**7,1**	**18**	**5,8**
General Total	**5037**	**511**	**10,1**	**514**	**10,2**

PM&R: Physical medicine and rehabilitation

The growth span of 1045 samples with true pathogen and contaminant growth was examined and the average growth span was found to be 44.45 hours. *Streptococcus pneumoniae* was found to

reproduce more rapidly than other microorganisms. While it was found that the growth span of the contaminant bacteria is statistically similar to yeasts, it is significantly longer than Gram-positive cocci and Gram-negative bacilli (p = 0.897, p = 0.005, p = 0.025, respectively). The growth span of Gram-positive cocci and Gram-negative bacilli was found to be statistically similar (p = 0.147). The growth spans of bacteria and fungi from isolated microorganisms are shown separately and on average in Table 3.

Table 3. Isolated microorganisms and growth times

Microorganism		Growth time (h)	Avarage growth time (h)
Gram positive cocci	*Streptococcus pneumoniae*	11,5	
	Streptococcus agalactia	22,3	
	Staphylococcus aureus	22,6	
	Enterococcus spp	23,7	22,2±6,9
	CNS*	30,85	
Gram negative bacillus	*Acinetobacter* spp	16,7	
	E. coli	19,2	
	Enterobacter spp	19,5	
	Klebsiella spp	22,3	
	Pseudomonas spp	27,3	
	Salmonella spp	28,5	
	Other	28,6	30,7±11,5
	Burkholderia spp	29,3	
	Citrobacter spp	35,3	
	Proteus spp	42,7	
	Morganella morganii	45,5	
	Serratia spp	53,5	
Yeast	*Candida tropicalis*	22,3	
	Candida parapsilosis	34,4	
	Candida albicans	60	59,2±27,6
	Stephanoascus ciferrii	60,2	
	Candida glabrata	84,5	
	Blastoschizomyces capitatus	93,6	
Contaminant	Alfa hemolitic streptococcus	27,4	
	CNS*	32,7	
	Veillonella spp	33,4	
	Other	35,2	
	Corynebacterium spp	56,6	65,8±47,7
	Micrococcus spp	85,6	
	Kocuria spp	87,7	
	Propionebacterium spp	168	

CNS: Coagulase negative staphylococci

DISCUSSION

Contamination of blood cultures is the source of chain of mistakes in health care (10). In this study, blood culture contamination rate was calculated as 10.42%. This ratio was considered high when compared with the literature. Although the target rates for blood culture contamination are below 3%, the actual rates range from 0.6% to 6% among institutions. In a prospective study focusing on blood culture contamination caused by coagulase negative staphylococci, Souvenir et al. reported that almost half of the patients who received false positive results were treated with antibiotics, usually vancomycin (11, 12). Ramli et al. found a significant relationship between pre- and post-corrective action in their study to reduce blood culture contamination. They stated that a multidisciplinary approach that includes various strategies on raising awareness, lifelong training, technical improvement and monitoring of contamination rate may provide an important solution to decrease the contamination rate in the long term (13). Veranyurt et al. found the average contamination rate as 4.30% in their study comparing the years 2016-2018. They stated that as the contamination rate is above the quality standards, in-service trainings are needed, and each laboratory needs to establish a quality assurance program that includes quality control (14). The 10.42% contamination rate found in our study is within unacceptable limits. The reason is thought to be lack of in-service training, non-compliance

with disinfection rules, non-continuous monitoring of contamination rate and lack of personnel.

It was reported in different studies that the growth span of microorganisms isolated in blood cultures had a critical role in the separation of agent and contamination. Balıkçı et al. suggested that the growths detected in the first 12 hours would be interpreted as the causative agent, the ones in 24 hours as highly likely causative agent and the growths in the first 48 hours and more would be interpreted as contaminant agent. However, they reported that the growth span of methicillin-resistant staphylococci, causative agent or not, is longer than 24 hours (3). Durmaz et al. found average growth span of Gram-positive bacteria, Gram-negative bacteria and yeasts as 18.83, 15.67 and 23.87 hours, respectively, in their study (15). In their study, Dierig et al. stated that bacterial growth in blood cultures in children with sepsis is positive in 90% of the children within the first 36 hours of incubation, so it is not valid to treat all children for at least 48 hours. On the contrary, they suggested that the decision to continue empirical antibiotic treatment in the absence of blood culture would be reviewed after 24 and 36 hours and antibiotic treatment would be discontinued if the children are not diagnosed with sepsis (16). In this study, the average growth span was 22.2 hours for Gram-positive bacteria, 30.7 hours for Gram-negative bacteria, 65.8 hours for contaminants, and 59.2 hours for yeasts. This finding shows that bacteria identified as true agents grow in the first 36 hours. However, the fact that the growth span of yeast fungi is similar to contaminant agents should be taken into consideration especially in patients with fungemia.

Single blood cultures are often collected during suspected bacteremia events. Guidelines for obtaining blood culture guide clinicians to determine if patient needs blood culture or not and how many cultures they will get from the patient (17). Taking too many sets is not cost effective and not having enough number of sets and the lack of anaerobic or aerobic vials in one set may delay accurate diagnosis. Of the 5037 blood cultures included in the study, 511 were sent as single bottles (Table 2). Single-bottle samples are commonly sent from neonatal intensive care units and pediatric services. Although it is not recommended to send a single bottle sample, when evaluating post-reproductive factors in pediatric units where amount of blood is low and colleting blood is difficult, these difficulties should be taken into consideration. This reveals the importance of the communication between the clinic and the laboratory.

In our study, noncompliant samples such as leaky, insufficient or incompletely identified, with quality processes and guidelines were preanalitically rejected and this rate is 1.7%. Even if some of the preanalytical errors that constitute a significant part of laboratory errors are possible to prevent by rejecting these kinds of samples, it increases the cost of the patient and causes delays in diagnosis.

In their study, Sogaard et al. found the sensitivity of Gram staining as 91.3-99.7% and specificity as 98.9-100% in positive blood culture samples and they reported that the interpretation and evaluation of Gram staining performed by experienced personnel gives fast, cheap and high accuracy results (16). In our study, Gram staining and final identification agreement rate was found as 97.55% which is high. Assuming that delays in bacterial isolation and identification in blood cultures increase the mortality rate by 1.2% for each day of delay (17), it is suggested that Gram staining performed with well-trained experienced personnel would be more effective together with molecular methods which are being used more frequently nowadays.

As conclusion, there is no gold standard to distinguish true pathogens from contaminant agents in blood cultures. There is no one and only reason for blood culture contamination; but reasons may be various such as inadequate sampling, sampling with inappropriate technique, insufficient number of set or insufficient number of bottles in a set and patient's disease profile. Although coagulase negative staphylococci are the most frequently isolated contaminant, it should be kept in mind that these microorganisms are the most common causative agent of nosocomial infection. Hospitals and / or units should monitor blood cultures by evaluating contamination rates and quality indicators reported in the guidelines in order to reduce patient costs and prevent inappropriate antibiotic therapy and the associated antibiotic resistance. Furthermore, it is thought that regular training and continuous feedback on personnel compliance will ensure continuity in this regard; contamination rates will decrease, and quality indicators will improve.

Disclosure statement

No potential conflict of interest was reported by the authors.

REFERENCES

1. Pan F, Zhao W, Zhang H. Value of time to positivity of blood culture in children with bloodstream infections. Can J Infect Dis Med Microbiol. 2019(1):1-6.
2. Lamy B, Dargere S, Aredrup MC, Parienti JJ, Tattevin P. How to optimize the use of blood cultures for the diagnosis of blood stream infections? A state of the art. Front Microbiol. 2016; 7: 697.
3. Balıkçı A, Belas Z, Topkaya AE. Blood culture positivity: is it pathogen or contaminant? Mikrobiyol Bul. 2013;47(1):135–40.

4. Hopkins K, Huynh S, McNary C, Walker A, Nixon R, Craighead JE. Reducing blood culture contamination rates: a systematic approach to improving quality of care. Am J Infect Control. 2013;41(12):1272–74.
5. Ombelet S, Barbé1 B, Affolabi D, Ronat JB, Lompo P, Lunguya O, et al. Best practices of blood cultures in low- and middle-income countries front. Front Med. 2019; 6: 131.
6. Sogaard M, Norgaard M, Schonheyder HC. First notification of positive blood cultures and the high accuracy of the gram stain report. J Clin Microbiol. 2007;45(4):1113–7.
7. Dempsey C, Skoglund E, Muldrew KL, Garey KW. Economic health care costs of blood culture contamination: a systematic review. Am J Infect Control. 2019;47(8):963–67.
8. Garcia RA, Spitzer ED, Beaudry J, Beck C, Diblasi R, Gilleeny-Blabac M, et al. Multidisciplinary team review of best practices for collection and handling of blood cultures to determine effective interventions for increasing the yield of true-positive bacteremias, reducing contamination, and eliminating false-positive central linee associated bloodstream infections. Am J Infect Control. 2015;43(11):1222-37.
9. Klinik Örnekten Sonuç Raporuna Uygulama Rehberi [Internet].Ankara: Çağhan Ofset Matbaacılık; 2017 [cited 2019 Jun 23]. Available from: https://www.klimud.org/public/uploads/content/files/Kan%20Dola%C5%9F%C4%B1m%C4%B1%20%C3%B6rnekleri.pdf
10. Hughes A, Cabilan CJ, Williams J, Ray M, Coyer F. The effectiveness of interventions to reduce peripheral blood culture contamination in acute care: a systematic review protocol. Syst Rev. 2018;7(1):216.
11. Hall KK, Lyman JA. Updated review of blood culture contamination. Clin Microbiol Rev. 2006;19(4):788–802.
12. Dawson S. Blood culture contaminants. J Hosp Infect. 2014;87(1):1–10.
13. Ramli SR, Zahari S, Sadri A, Aziz ZF, Francis A. Reducing blood culture contamination rate: a quality assurance project in a Malaysian tertiary hospital. Int J Infect Control. 2014;10(2).
14. Veranyurt Ü, Ertürk A, Veranyurt O, Akalın B. Contamination rates of blood culture samples. Journal of Current Researches on Health Sector. 2018;8(2):365–70.
15. Durmaz G, Us T, Aydinli A, Kiremitci A, Kiraz N, Akgun Y. Optimum detection times for bacteria and yeast species with the BACTEC 9120 Aerobic Blood Culture System: evaluation for a 5-year period in a Turkish University Hospital. J Clin Microbiol. 2003;41(2):819–21.
16. Dierig A, Berger C, Agyeman PKA, Bernhard-Stirnemann S, Giannoni E, Stocker M, et al. Time-to-positivity of blood cultures in children with sepsis. Front Pediatr. 2018;6:222.
17. Friedman ND, Braun T, Fallach N, Carmeli Y. Blood culture sampling practices among internal medicine inpatients. Clin Microbiol Infect Dis. 2017;2(1):1–6.