ARAŞTIRMA MAKALESİ / Research Article

Baseline Evaluation of Causative Agents and Their Susceptibility Patterns of Late-Onset Blood Stream Infections in A NICU; Gram-Negative Domination

Bir Yenidoğan Yoğun Bakım Ünitesinde Geç Başlangıçlı Kan Akımı Enfeksiyonlarına Neden Olan Ajanlar ve Antibakteriyel Duyarlılıklarının Değerlendirilmesi; Gram Negatifler Baskın

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Abstract

Background Blood stream infections (BSI) are the most common hospital-acquired infections in neonatal intensive care units (NICU). Despite the improvement of medical capabilities with neonatal care, diagnosis of BSI in neonates is still problematic. Blood cultures are main confirmatory tool for neonates with clinical signs and symptoms of BSI. They are also useful for determination of pathogen and their antimicrobial susceptibilities for surveillance purposes. In this retrospective study, we aim to determine the causative bacteria of late-onset neonatal sepsis and their antimicrobial susceptibilities to create a baseline data for active surveillance and empiric antimicrobial regimens.

Materials and Methods: The study was conducted at NICU during three-year period from opening date 2013 to 2015. We retrospectively evaluated the blood cultures of the neonates with the proven diagnosis of late-onset blood stream infection.

Results: Total number of 1245 blood culture samples of 516 neonates with suspected blood stream infection was evaluated during the three-year study period. Among 516 neonate included in the study, 35 of them (6.8%) suffered BSI. Causative agent of BSI was reported in 2.8% (n=35) of all the samples. When the results of the blood cultures were evaluated through the years, we did not determine any significant difference (p>0.05). Gram-negative bacteria caused late-onset neonatal sepsis cases (88.9%) more commonly than gram positive ones (17.1%). K. pneumoniae was identified as the most predominant bacterium (45.7%), followed by E. cloaca (11.4%) and other enteric bacilli. Coagulase negative staphylococci and Enterococcus faecalis were the Gram-positives determined in 17.1% of the cases. Carbapenems for the Gram-negative bacteria and glycopeptides for the Gram-positive bacteria were the most susceptible antimicrobials as expected.

Discussion and Conclusion: The study was conducted at NICU during three-year period from opening date 2013 to 2015. We retrospectively evaluated the blood cultures of the neonates with the proven diagnosis of late-onset blood stream infection.

Key words: Neonate, Blood stream infections, blood cultures, surveillance, empirical treatment.

Özet

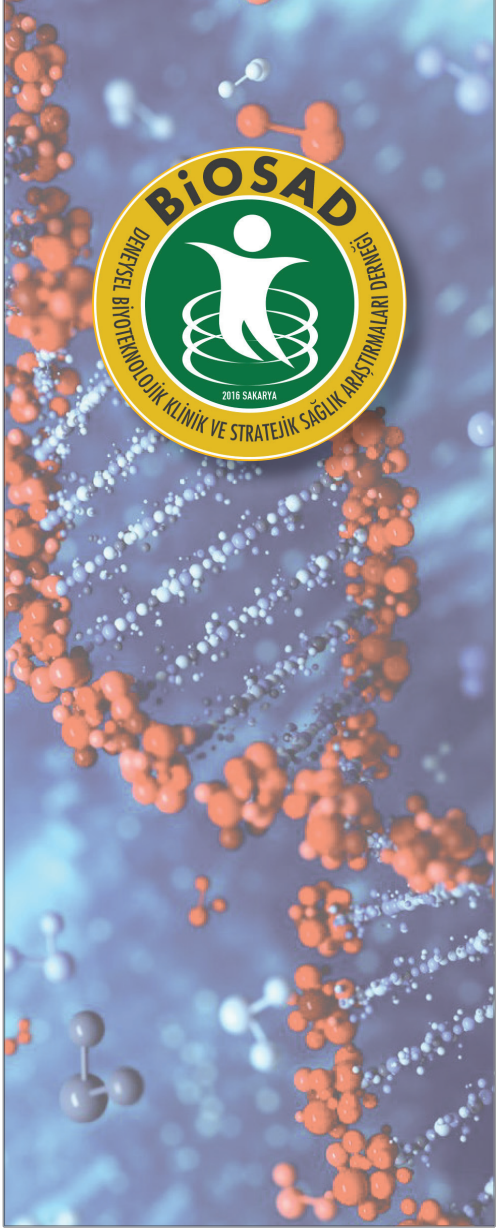
Giriş: Kan akışı enfeksiyonları (KAE), yenidoğan yoğun bakım ünitelerinde (YYBÜ) hastane kaynaklı enfeksiyonlardır. Yenidoğan bakımında tıbbi tekniklerdeki gelişmeye rağmen yeni doğanlarda KAE tanısı hala sorunludur. Kan kültürleri, BSI'nın klinik bulguları ve belirtileri olan yenidoğanlar için temel doğrulayıcı yöntemlerdir. Kan kültürleri ayrıca patojenlerin ve bunların sürveyans amaçlı antimikrobiyal duyarlılıklarının belirlenmesinde de yararlıdırlar. Bu retrospektif çalışmada, aktif gözetim ve ampirik antimikrobiyal rejimler için başlangıç verileri oluşturmak için geç başlangıçlı yenidoğan sepsis etkeni bakterileri ve bunların antimikrobiyal yatkınlıklarını belirlemeyi amaçladık.

Materyal ve Metod: Çalışma, YYBÜ'de 2013'ten 2015'e kadar üç yıllık süre boyunca gerçekleştirildi. Geç retinopterol gelişimi tanısı ile yenidoğanların kan kültürlerini retrospektif olarak değerlendirdik.

Bulgular: Üç yıllık çalışma süresi boyunca Kan akımı şüphesi bulunan 516 yenidoğanın toplam 1245 kan kültür örneği, değerlendirildi. Çalışmaya dahil edilen 516 yenidoğanın 35'inde (% 6.8) KAE vardı. BSI'nın etken ajanı tüm örneklerin % 2.8'inde (n = 35) bildirildi. Kan kültürlerinin sonuçları yıllara göre değerlendirildiğinde anlamlı fark tespit etmedik (p> 0.05). Gram negatif bakteriler geç başlangıçlı yenidoğan sepsis vakalarına (% 88.9) gram pozitiflere (% 17.1) göre daha sık neden oldu. K. pneumoniae en baskın bakteri (% 45.7), ve ardından E. cloaca (% 11.4) diğer enterik basil olarak tanımlandı. Olguların% 17.1'inde koagülaz negatif stafilokoklar ve Enterococcus faecalis gram pozitif olarak bulundu. Beklenildiği gibi Gram negatif bakteriler için karbapenemler ve Gram pozitif bakteriler için glikopeptitler en duyarlı antimikrobik maddelerdi.

Tartışma ve Sonuç: Bu çalışmada yenidoğanların kan kültürü örneklerinden ağırlıklı olarak ve dikkat çekici derecede Gram-negatif bakteriler izole edilmiştir. Bu çalışmadan elde edilen antimikrobiyal yatkınlık test sonuçlarına göre, daha dirençli izolatlar için karbapenem püskürtmeyle birlikte alternatif olarak amikasin ve piperasilin-tazobaktam kullanımı ampirik tedaviyi planlamak için şimdilik mantıklı bir yaklaşım gibi gözükmektedir.

Anahtar kelimeler: Yenidoğan, Kan akımı enfeksiyonları, kan kültürleri, sürvelans, ampirik tedavi



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Introduction

Blood stream infections (BSI) are the most common hospital-acquired infections in neonatal intensive care units (NICU). All premature infants possess the risk for blood stream infection at a rate of 21-43%[1]. Despite all the improvements and the interventions related with neonatal care are not enough to diminish the rates of morbidity and mortality related to the BSI in NICU[2,3]. Patients followed in a NICU are very vulnerable to hospital-acquired infections since they do not have a mature and fully functional immune system and their poorly developed skin/mucous barriers do not prevent transmission of potentially pathogenic infectious agents. Very low birth weight and low gestational age are determined as additive risk factors for infections in neonates. Other documented risk factors are previous exposure to broad-spectrum antimicrobials, extereme invasive procedures, male sex, use of steroids and intravenous lipids[4,5].

BSI in neonates are mainly classified into two as early-onset neonatal sepsis and late-onset neonatal sepsis. This classification aids to differentiate potential pathogens. Early-onset neonatal sepsis is defined as sepsis occurring in the first 24-72 hours after the birth6,7. Group B streptococci and Escherichia coli are common cause of early-onset neonatal sepsis, which are transmitted vertically. Neonatal sepsis arising after 24-72 hours after the birth is called as late-onset neonatal sepsis. Gram-negative enteric bacilli (mainly Klebsiella pneumoniae and Escherichia coli) and Gram-positive cocci (mainly coagulase-negative staphylococci) are commonly isolated from the patients with late-onset neonatal sepsis. Candida species are also increasingly isolated in neonates with gestational age lower than 28 weeks and very low birth weight[4,5,8,9].

Despite the improvement of medical capabilities with neonatal care, diagnosis of BSI in neonates is still problematic. Blood parameters are not dependable to set accurate diagnosis. Blood cultures are main confirmatory tool for neonates with clinical signs and symptoms of BSI[10]. Blood cultures are also useful for determination of pathogen and their antimicrobial susceptibilities for surveillance purposes. Possible pathogens may differ for each NICU and their distribution and resistance patterns may change over time. Appropriate empiric antimicrobial treatment schemes can only be suggested by evaluating the data from blood cultures. In this retrospective study, we aim to determine the causative bacteria of late-onset neonatal sepsis and their antimicrobial susceptibilities to create a baseline data for active surveillance and empiric antimicrobial regimens.

Materials and Methods

Setting-Neonatal intensive care unit

NICU of Sakarya University Training and Research Hospital is a level III NICU and has 12 incubators and cradles. It was first begun serving in the year 2013. There are 3 neonatologist, 2 assistant doctors, 25 nurses and 5 other staff working. Approximately 172/year newborns are admitted to the unit. The unit serves not only the obstetric clinic of the hospital but also serves as a regional center for critically ill neonates from the other hospitals in the city and neighboring cities.

Patients

The study was conducted at NICU during three-year period from opening date 2013 to 2015. We retrospectively evaluated the blood cultures of the neonates with the proven diagnosis of late-onset blood stream infection. Patients' data were gathered from the NICU files, laboratory and hospital records.

Determination of the isolates

BacT/ALERT® PF Plus bottles were used for sampling blood from the suspected neonates. The bottles were incubated in BacT/Alert 3DTM automated blood culture system (bioMerieux, Marcy l'Etoile, France). Incubation period was set as 5 days. Subsequent cultures from positive samples were inoculated onto the Tryptic Soy agar containing 5% sheep blood and Eosin Methylene Blue agar, and chocolate agar plates. The plates were incubated at 35° C for 18-24 hours. VITEK® 2 automated system (bioMerieux, Marcy l'Etoile, France) was used for identification and antimicrobial susceptibility testing. When the same microorganism was isolated from the consecutive samples of the same individual patient, only one isolate was included in the study. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2015 criteria.

Statistical analyses

Comparisons between groups were made with Chi-square or Fisher's exact test for categorical variables. A p value <0,05 was considered as significant. Comparisons between the blood culture results and the years were analysed. Commercial statistical software SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) was used to perform statistical evaluations.

Results

Total number of 1245 blood culture samples of 516 neonates with suspected blood stream infection was evaluated during the three-year study period. The mean gestational age of patients with late-onset neonatal sepsis was 26.2 weeks and the gestational ages ranged between 24 and 30 weeks. Among 516 neonate included in the study, 35 of them (6.8%) suffered BSI. Blood cultures yielded no growth in 93.5% (n=1165) of the whole cultures included in the study. Contamination (microbiological and clinical contamination) was determined in 3.6% (n=45) of the blood cultures. Causative agent of BSI was reported in 2.8% (n=35) of the samples. When the results of the blood cultures were evaluated through the years, we did not determine any significant difference.

Gram-negative bacteria caused late-onset neonatal sepsis cases (88.9%) more commonly than gram positive ones (17.1%). K. pneumoniae was identified as the most predominant bacterium (45.7%), followed by E. cloaca (11.4%) and other enteric bacilli. Coagulase negative staphylococci and Enterococcus faecalis were the Gram-positives determined in 17.1% of the cases. All the pathogens, isolated as the causatives of the late-onset neonatal sepsis, were listed in Table 2.

The majority of the susceptible antimicrobials were determined to be carbapenems for the Gram-negative bacteria and glycopeptides for the Gram-positive bacteria as expected according to the antimicrobial susceptibility testing results (Table 3) Ampicillin, Amoxicillin-clavulonate and cefazolin were the least effective antimicrobials for the Gram-negative bacteria. Extended spectrum beta-lactamase (ESBL) production, which limited the use of certain cephalosporins as treatment choice, was determined in 15 out of 29 (51.7%) of the Gram-negatives. No significant resistance was detected for the Gram-positive bacteria expect for an isolate of S. haemolyticus, which was resistant to cefoxitin-representative antibiotic for resistance to penicillins, Beta lactam/beta lactamase inhibitor combinations, cephems, and carbapenems.

Discussion

BSIs are the most common cause of morbidity and mortality among neonates and it is estimated that neonatal BSIs cause for more than one million deaths annually throughout the world[6]. Approximately 95 % of the neonates followed in a NICU receive empirical antimicrobials and antimicrobials are being the most prescribed drugs in NICU[11,12]. Among these patients only 1-5% of them has a culture-proven BSI[6]. In most NICU units empirical antimicrobial treatment schemes mainly depend on experience of health care provider, data from other age groups and international guides, however such guidance may not represent the actual status of the pathogens. Each institute should have dependable epidemiological data about causative agents of BSI to set appropriate empirical treatment schemes.

We determine 35 culture-proven late onset BSI attacks in NICU during three-year period. The frequency of neonatal BSI in NICU is determined as 6.8%. Wide ranges of frequencies from 1.8% to 39.8% regarding to neonatal BSI are given in various papers[13]. Although not significant, percentage of reports resulted as contamination tend to have a gradual decrease throughout the years. When sampling difficulties are taken into consideration, decrease in contamination rates is interpreted as a favorable condition.

Gram-negative bacteria predominantly and remarkably are isolated from the blood culture samples of the neonates in this study (82.9%). In developed countries gram positives, especially coagulase negative Staphyloccus spp., Enterococcus spp. and S. aureus are commonly isolated as the agents of BSI in neonates[7]. However, members of Enterobacteriaceae cause more BSI compared to the Gram-positives in developing countries[7,14]. We do not identified any fungal agent from the blood samples, despite the fact that the high ratio of Gram-negatives and high prevalence of ESBL producers force the use of carbapenems, which in turn may cause fungal colonization and infection.

Table 1. Evaluation of the blood culture results according to years in neonatal intensive care unit.

Result of the Blood Cultures	2013		2014		2105		Total		p
	n	%	n	%	n	%	n	%	
No Growth	551	92.0	353	94.6	261	95.6	1165	93.6	>0.05
Contamination	28	4.7	13	3.5	4	1.5	45	3.6	
Blood stream infection	20	3.3	7	1.9	8	2.9	35	2.8	
Total Blood Cultures evaluated	599	100	373	100	273	100	1245	100	

Table 2. Distribution of pathogens causing blood stream infections in patients followed in neonatal intensive care unit.

Isolate	n	%
Gram Negatives	29	82.9
Gram Positives	6	17.1
Total	35	100
Klebsiella pneumoniae	16	45.7
Enterobacter cloaca	4	11.4
Escherichia coli	2	5.7
Klebsiella oxytoca	2	5.7
Pseudomonas aeruginosa	2	5.7
Serratia marcescens	2	5.7
Enterobacter aerogenes	1	2.9
Enterococcus faecalis	2	5.7
Staphylococcus epidermidis	2	5.7
Staphylococcus haemolyticus	2	5.7
Total	35	100

Table 3. Antibiotic susceptibilitiy test results of isolates causing blood stream infections in neonatal patients.

Gram-negative	Number of susceptible isolates												
Bacteria	n	AMP	CZ	GN	AK	AMC	CFX	CRO	TZP	IMP	MEM	FEP	ESBL
Klebsiella pneumoniae	16	0	9	3	16	8	5	5	15	16	16	6	12
Enterobacter cloaca	4	0	2	3	3	0	1	1	2	4	4	2	1
Escherichia coli	2	0	1	1	1	1	1	1	2	2	2	1	1
Klebsiella oxytoca	2	0	2	1	1	1	0	1	2	2	2	2	1
Pseudomonas aeruginosa	2	0	0	2	2	0	0	2	1	2	2	2	-
Serratia marcescens	2	2	2	2	2	0	1	1	2	2	2	2	-
Enterobacter aerogenes	1	0	1	1	1	0	1	1	1	1	1	1	-
Gram-positive	Number of susceptible isolates												
Bacteria	n	P	AMP	FOX	E	CIP	GN		DA	SXT	LNZ	VA	TEC
Enterococcus faecalis	2	NA	2	NA	0	2	2	2	2	NA	2	2	2
Staphylococcus epidermidis	2	0	NA	0	2	2	1	NA	2	2	2	2	2
Staphylococcus haemolyticus	2	0	NA	1	0	2	1	NA	2	2	2	2	2

AMP: Ampicillin, KZ: Cefazolin, GN: Gentamicin, AK: Amikacin, AMC: Amoxicillin-clavulonate, CFX: Cefuroxime, CRO: Ceftriaxone, CIP: Ciprofloxacin, IMP: Imipenem, MEM: Meropenem, FEP: CefepimeSXT: trimethoprim-sulfamethoxazole, NF: Nitrofurantain, P: Penicillin, FOX: Cefoxitin, E: Erythromycin, HLAR: High level aminoglycoside resistance, FOS: Fosfomycin LNZ: Linezolid, VA: Vancomycin, TEC: Teicoplanin

According the antimicrobial susceptibility testing results obtained from this study, alternately use of amikacin and piperacillin-tazobactam with spearing the carbapenems for more resistant isolates appears to be logical approach for planning empirical treatment for the time being. This will avoid the selection pressure and development of carbapenem resistant isolates, which are not uncommon in our hospital and country. In the last three years experienced two K. pneumoniae epidemics, one of which was occurred by NDM-1 producing, carbapenem resistant K. pneumoniae[15,16]. Vancomycin, the life-saving drug against Gram-positive bacteria, can be added to the empirical treatment in case of infections with suspected such pathogens. However, despite staying in the range of susceptible values, increase in MIC levels of vancomisin for the Staphyloccus spp. is worrying concern recently[17]. The development of resistance is inevitable in case of inappropriate use of broad-spectrum and last choice antimicrobials[17,18]. Antibiotic stewardship policies, effective implantation of infection control measures and continuous education will slow down the unavoidable upcoming era of pan-resistant bacteria.

In conclusion, it was determined the Gram-negative bacteria as the main cause of BSI in our newly opened level III NICU. Then, we have provided a baseline data about the causative agents and have proposed empirical antimicrobial treatment scheme which is alternately use of amikacin and piperacillin-tazobactam, when needed, in combination with vancomisin. The knowledge of causative agents of BSI and their antimicrobial susceptibility properties is one of the corner stone of the efforts to decrease the BSIs in NICU and is one of the major components of neonatal infection surveillance programs to discover epidemiology of the disease. Further efforts to identify BSI in timely manner and close following of NICU patients for surveillance purposes are the future obligations to be done.

Conflict of Interest:
The authors declare that they have no conflict of interest.

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Ethical Committee Approval:
Sakarya University Non-Invasive Ethics Committee, 050.01.04.87
Declaration: All the authors declare that they have obeyed the rules in “Helsinki Declaration”, “Good Medical Practice Guidelines”, and “Good Laboratories Practice Guidelines”.

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