

Change in Pathogens Causing Late-onset Sepsis in Neonatal Intensive Care Unit in Izmir, Turkey

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Abstract

Objective: Neonatal sepsis is a common cause of morbidity and mortality among newborns in the developing world. We have investigated the causative agents and their antimicrobial susceptibility of late-onset sepsis (>72 h post-delivery), and determined the possible association between various risk factors and the mortality due to neonatal sepsis in 2008. To view the changes in years, we compared them with the data which we gained in 2004.

Methods: Medical records of all neonates with late-onset sepsis were reviewed for demographic characteristics (birth weight, gestational age, gender, type of delivery, and mortality rate), positive cultures and risk factors of mortality.

Findings: One hundred and forty-seven and 227 neonates had been diagnosed as late-onset sepsis in 2004 and 2008, respectively. Coagulase-negative staphylococcus was the most frequent microorganisms. Gram-negative bacilli, particularly *Pseudomonas aeruginosa* showed a significant increase in years. The mortality rate was 11.5% and 19% in 2004 and 2008, respectively. Birth weight, gestational age, and infection with *Klebsiella* spp. isolates were found to have significant association with sepsis mortality in our neonatal intensive care unit (NICU).

Conclusion: The present study emphasizes the importance of periodic surveys of sepsis encountered in particular neonatal setting to recognize the trend. Increased Gram-negative bacilli rate was possibly related to the widespread use of antibiotics in our NICU.

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Key Words: Sepsis; Risk factors; Neonatal intensive care unit; Neonate; Infection; Antibiotics

Introduction

Neonatal sepsis continues to be an important cause of morbidity and mortality both in term

and in low-birth-weight infants despite advances in healthcare^[1]. There are approximately 7 cases of neonatal sepsis per 1000 live births,

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increasing to 162 per 1000 live births in very low birth weight neonates^[2]. The incidence of the neonatal sepsis varies with the geographical area, the socio-economic structure and various customs and practices in the perinatal period^[3].

Neonates are particularly vulnerable to infection, so any delay in the initiation of therapy or wrong choice of antibiotics is to be avoided^[4,5]. Diagnosing neonatal infection is challenging, since clinical signs and symptoms are often slight and nonspecific for a particular infection.

Therefore, in suspected sepsis, two or three days empirical antibiotic therapy should begin immediately after cultures have been obtained without awaiting the results. In line with this idea the recognition of the epidemiological and antimicrobial susceptibility pattern of common pathogens, and determination of the risk factors for neonatal sepsis mortality is extremely relevant in the clinical setting, since it contributes to the diagnostic reasoning and supports clinical decisions^[4].

The aim of the study was to determine the demographic characteristics of newborns diagnosed as late-onset sepsis and to evaluate their microbiological characteristics (prevalence and antimicrobial susceptibility). To view the changes in years, we compared them with the data which we gained in former years. Also, we investigated the clinical and microbiological characteristics of the survivors and non-survivors to describe the mortality risk factors due to neonatal sepsis.

Subjects and Methods

Setting and Patients Population:

This study was carried out in the neonatal intensive care unit (NICU) and Department of Microbiology at Tepecik Educational and Research Hospital (TERH) in Izmir, Turkey. TERH is a 1000-bed tertiary-care hospital in Izmir, the biggest city in Aegean region in Turkey with a population of 3,400,000 according to the census 2000.

The NICU has a level III nursery with 45 bed capacity. Because there is not an obstetric

service in our hospital, all newborns are out born. There are approximately 1500-1600 admissions per year. The prevalence of sepsis was calculated by dividing the number of newborns with sepsis by the total number of newborns admitted to the NICU. This was a retrospective, hospital-based and single-center study. Data collected from the medical records of each patient were recorded in standardized collection sheets and included demographic information, birth weight, gestational age, gender, outcome, and delivery data (type of delivery, maturity). Gestational age was classified into two distinct groups: 24-29 weeks and ≥ 30 weeks. Birth weight categories were: 600-1499 g and ≥ 1500 g.

The definition of sepsis was based on a positive blood culture in the presence of clinical signs and symptoms of infection; Tachypnea (respiratory rate >60 bpm) plus either grunting/retraction or desaturation, temperature instability ($<36^{\circ}\text{C}$ or $>38^{\circ}\text{C}$), heart rate $>SD$ above normal for age (≥ 180 beats/min, ≤ 100 beats/min), lethargy/altered mental status, systolic pressure <65 mm Hg (infants ≤ 1 month), capillary refill time >3 sec, WBC count ($<4000 \times 10^9/\text{L}$ or $>34000 \times 10^9/\text{L}$, immature/total neutrophil ratio >0.2 , CPR >10 mg/ml, IL-6 or IL-8 >70 pg/ml) was diagnosed septicemia in this study^[6]. Late-onset infection was defined when positive blood cultures were obtained after first 72 hours of life. Newborns that were younger than three days were excluded. When coagulase-negative staphylococci (CoNS) were isolated the definition of true infection was based on the presence of clinical symptomatology strongly suggestive of infection together with at least two blood cultures positive for CoNS strains, drawn on different occasions; otherwise, isolated CoNS was defined as contamination. Cultures positive for organisms that are generally considered to be contaminants including *Corynebacterium*, *Propionibacterium*, and diphtheroids were excluded from analysis. Copy isolates, defined as the same organism isolated from multiple blood cultures of the same patient within 48 h and contaminant bacteria were excluded from this study.

During the study period, standard initial empirical treatment protocols in our NICU

included ampicillin and cefotaxime or teicoplanin and meropenem in serious clinical conditions.

Microbiology:

Blood culture was done for all neonates suspected to have sepsis. All blood cultures were collected from peripheral vein under proper aseptic precautions before starting any antibiotic therapy. Blood culture samples were incubated in the BacT/ALERT 3D (bioMerieux, France) and isolates identified by standard laboratory techniques^[7] or automated system (VITEK2, bioMerieux, France), as required. Eosin Methylene blue and blood agars were used for sub-culture and incubated at 37 °C aerobically.

Antibiotic susceptibility testing was performed by Kirby-Bauer disk diffusion method according to the Clinical Laboratory Standard Institute (CLSI) criteria^[8]. Extended-spectrum β -lactamase (ESBL) activity was detected by phenotypic confirmation with ceftazidime/ceftazidime-clavulanate and cefotaxime/cefotaxime-clavulanate disks recommended by the CLSI. In this test, a ≥ 5 mm increase in the zone diameter for disks ceftazidime-clavulanate or cefotaxime-clavulanate versus ceftazidime or cefotaxime disks alone demonstrates that the strain has ESBL activity. When susceptibility was intermediate, the bacteria were considered resistant.

Data were analyzed using SPSS software (Version 10.0; SPSS Inc., Chicago). The comparison of categorical variables was performed by using Chi-square test or Fisher's Exact test. Univariate analysis was done to identify the risk factors of mortality. To find out

the overall effects of significant variables, logistic regression analysis was done. The standard significance level, $P < 0.05$, was used and all tests of statistical significance were two-tailed.

Findings

Patients:

Demographic characteristics of the groups are shown in Table 1. There is no significant difference between these two groups ($P > 0.05$).

Comparison of pathogens in 2004 and 2008:

Microorganisms causing late-onset sepsis were significantly increased in years ($P < 0.001$).

As shown in Table 2, Gram-positive microorganisms were more frequently isolated than Gram-negative microorganisms. Among Gram-positive pathogens CoNS was the leading cause of late-onset sepsis. It was responsible for half of sepsis cases in all years. CoNS showed a significant increase from 2004 to 2008 (76 vs 115, $P = 0.04$).

During the years 2004 to 2008, the number of sepsis caused by GNB showed an increase of more than 3-fold ($P < 0.001$). *Klebsiella* spp. and *Escherichia coli* (*E. coli*), together, were responsible for 77% and 67% of late-onset sepsis which was caused by GNB in 2004 and 2008, respectively. The most important finding of our study is that a considerable proportion of *Pseudomonas aeruginosa* isolates increased in recent years. While 21 non-fermentative bacteria were isolated from blood cultures in

Table 1: Demographic characteristics of the study groups

Characteristics	2004	2008
No of patients	147	227
Prevalence (%)	11.9	14.3
Birth weight (g)*	2303 \pm 1040	2229 \pm 974
Gestational age (week)*	34.5 \pm 5	32.5 \pm 4
Gender (M/F)	89 / 58	127 / 100
Preterm/Term	84 / 63	127 / 100
Type of delivery (CS/Normal)	84 / 63	137 / 90
Mortality, n (%)	17 (11.5)	42 (19)

*Data are presented as mean \pm standard deviation

Table 2: Distribution of pathogens of late-onset sepsis in 2004 and 2008

Pathogen	Pathogen of sepsis			
	2004	2008	P value	
Gram-positive microorganism	Coagulase-negative Staphylococcus	76	115	0.04
	<i>Staphylococcus aureus</i>	33	18	NS
	Group B Streptococcus	7	12	NS
	<i>Enterococcus</i> spp.	2	2	NS
	<i>Streptococcus</i> spp.	2	1	NS
	<i>Listeria monocytogenes</i>	1	1	NS
	Subtotal	121 (82.3%)	149 (65.5%)	NS
	Gram-negative microorganism	<i>Escherichia coli</i>	7	18
<i>Klebsiella</i> spp.		10	29	NS
<i>Enterobacter</i> spp.		1	1	NS
<i>Serratia marcescens</i>		2	1	NS
<i>Stenotrophomonas maltophilia</i>		0	6	NS
<i>Pseudomonas aeruginosa</i>		1	14	0.01
<i>Acinetobacter baumannii</i>		0	1	NS
Other		1	0	NS
Subtotal		22 (15%)	70 (31%)	P<0.001
Candida spp.	4 (2.7%)	8 (3.5%)	NS	
Total	147	227	P<0.001	

NS: Not significant

2008, only one non-fermentative pathogen (*P. aeruginosa*) isolate was responsible for late-onset sepsis in 2004 ($P<0.05$).

Evaluation of Antibiotic Resistance Patterns:

Table 3 shows the antimicrobial susceptibility of CoNS, *Staphylococcus aureus* (*S. aureus*), *Klebsiella* spp. and *E. coli* that made up 79-85% of all isolates. Gentamicin and trimethoprim-sulfamethoxazole (SXT) showed increased resistance rate for CoNS and *S. aureus* in years (NS). There were no detected vancomycin and teicoplanin resistance to the Gram-positive bacteria.

Although Gram-negative strains showed increase in resistance rate, there was no statistically significant difference between years.

Especially for *E. coli*, gentamicin and SXT resistance rate had increased in years. *Klebsiella* spp. isolates showed an apparent increase in resistance from 2004 to 2008 (NS). No carbapenem resistance for all tested Gram-negative strains was observed. Although number of *P. aeruginosa* isolates was very low, high

resistance rates were detected in recent years (NS).

Generally, there was a trend of increasing resistance rate of all isolates to tested antibiotics in 2008, compared to those in 2004. However, the difference was not statistically significant.

Mortality risk factors:

Table 4 shows clinical characteristics of the survivors and non-survivors of the study groups. Preterm neonates, smaller gestational age (<30 weeks) and low birth weight (<1500 g) were found significantly associated with mortality ($P<0.05$). Mortality risk factors of neonates were evaluated in 2008 (Table 5). The variables associated with mortality according to the univariate analyses were: birth weight ($P<0.001$), gestational age ($P<0.001$), and infected with *Klebsiella* spp. ($P=0.002$). Logistic regression analysis showed birth weight ($P=0.000$, OR: 1.001, 95%CI: 1.0009-1.002) and infected with *Klebsiella* spp. ($P=0.005$, OR: 2.472, 95%CI: 1.003-6.092) associated with high mortality rate in neonates.

Table 3: Antimicrobial resistance pattern of isolates in 2004 and 2008, No (%)

Parameter	CoNS		<i>S. aureus</i>		<i>E. coli</i>		<i>Klebsiella spp.</i>		<i>P. aeruginosa</i>	
	2004	2008	2004	2008	2004	2008	2004	2008	2004	2008
Year	2004	2008	2004	2008	2004	2008	2004	2008	2004	2008
No	76	115	33	18	7	18	10	29	1	14
Methicillin	61 (80%)	83 (72%)	20 (60%)	10 (55%)						
Ampicillin					4 (57%)	15 (83%)	10 (100%)	29 (100%)		
Cefazolin					2 (28%)	13 (72%)	2 (20%)	26 (89%)		
Cefaclor					1 (14%)	12 (66%)	3 (30%)	23 (79%)		
Ceftazidime					1 (14%)	7 (38%)	3 (30%)	16 (55%)	0	5 (35%)
Cefepime					1 (14%)	7 (38%)	3 (30%)	15 (51%)	0	5 (35%)
Gentamicin	36 (47%)	77 (66%)	6 (18%)	6 (33%)	1 (14%)	11 (61%)	2 (20%)	19 (65%)	0	3 (21%)
Amikacin					0	0	0	8 (27%)	0	0
Trimethoprim-sulfamethoxazole	34 (44%)	60 (52%)	8 (24%)	6 (33%)	0	11 (61%)	1 (10%)	13 (44%)		
Piperacillin-tazobactam					2 (28%)	6 (33%)	3 (30%)	20 (69%)	0	3 (21%)
ESBL					1 (14%)	6 (33%)	3 (30%)	13 (44%)		

CoNS: coagulase-negative staphylococci; *S. aureus*: *Staphylococcus aureus*; ESBL: Extended-spectrum β -lactamase; *E. coli*: *Escherichia coli*

Discussion

Early diagnosis and therapy are essential for the prevention of morbidity and mortality of neonatal sepsis in NICU^[9]. In the vast majority of cases, antimicrobial therapy must be initiated empirically. Therefore, the accurate prediction of likely pathogens and antimicrobial resistance patterns is crucial for successful therapy.

The distribution of the causative pathogens

for sepsis showed that these infections are caused mainly by Gram-positive microorganisms, mostly CoNS in our NICU. CoNS had increased in years ($P < 0.05$). Our results are in agreement with other studies that reported CoNS as the most common bacteria causing late-onset sepsis in neonates, accounting for more than 50 % of cases^[1,5,10]. These infections are often associated with use of indwelling vascular catheters or central lines^[3,10,11]. Hensey et al^[12],

Table 4: Clinical characteristics of the survivors and non-survivors of the study group

Parameter	2004		2008		<i>P. value</i>
	Survivors (n=130)	Non-survivors (n=17)	Survivors (n=185)	Non-Survivors (n=42)	
Birth weight (gram)*	2413±1036	1462 ± 598	2414± 949	1411 ± 589	0.000
Gestational age (week)*	35 ± 4.9	30.7 ± 4	33.1 ± 3.9	29 ± 3	0.000
Gender (M/F)	101 /29	10/7	106/79	21/21	NS
Type of delivery (CS/Normal)	74/56	10/7	113/72	24/18	NS

*Data are presented as mean ± standard deviation/ NS: Not significant

Table 5: Distribution of 227 patients according to demographic variables and microbiologic characterization and occurrence of mortality in 2008

Parameter		No of isolates (n=227)	Survivors (n=185)	Non-Survivors (n=42)	P value
Birth weight (g)	600-1499	75	47 (63)	28 (37)	<0.001
	≥1500	152	138 (91)	14 (9)	
Gender	Male	127	106 (83)	21 (17)	NS*
	Female	100	79 (79)	21 (21)	
Gestational age (week)	24-29	67	42 (63)	25 (37)	<0.001
	≥30	160	143 (89)	17 (11)	
Type of delivery	Normal	90	72 (80)	18 (20)	NS
	CS	137	113 (82)	24 (18)	
Microorganisms	CoNS	114	103 (90)	11 (10)	0.001
	<i>S. aureus</i>	18	16 (89)	2 (11)	NS
	GBS	15	13 (87)	2 (13)	NS
	<i>E. coli</i>	18	13 (72)	5 (28)	NS
	<i>Klebsiella</i> spp.	29	17 (59)	12 (41)	0.002
	<i>P. aeruginosa</i>	14	9 (64)	5 (36)	NS

NS: Not significant/ CoNS: Coagulase-negative staphylococci; *S. aureus*: *Staphylococcus aureus*; *E. coli*: *Escherichia coli*; ESBL: Extended-spectrum β -lactamase; GBS: Group B Streptococcus

described the rise in incidence as coincident with change in skin disinfection usage and general use of 3rd generation cephalosporins to which the CoNS were resistant. Also, CoNS are among the most common causes of blood culture contaminants, since they are the most common microorganisms found colonizing the skin and mucous membranes of neonates^[11]. Although CoNS were the leading pathogen of sepsis in NICU, it remains difficult to determine which blood isolates of CoNS represent true infection and which are contaminants. The virulence factor of CoNS may well be a biofilm formation, which aids colonization of not only intravascular devices but also tissues, and may also allow the organism to persist in hospital environments.

Thus, CoNS strains must be tested for biofilm formation or its genetic markers and if proven so, the offending central lines must be removed^[13,14].

Historically, the predominant organisms associated with neonatal sepsis have changed in years. Although Gram-positive bacteria dominate over GNB in all years, GNB increased in the recent years^[15]. In this study, 15% and 31% of isolates were related to GNB in 2004 and 2008 ($P<0.05$) respectively, very similar to that found by others, with reports varying between 10%-67%^[5,10]. Although *Klebsiella* spp. were the most

common microorganisms among Gram-negative germs, *P. aeruginosa* was significantly increased in our NICU in recent years ($P<0.05$).

Increased GNB rate was possibly related to many possibilities: Primarily the widespread and injudicious use of antibiotics, also "selection pressure" on the ESBL producing multi-resistant clones by the use of higher antibiotics, and catheter related bloodstream infections especially by "biofilm" forming organisms (like *P. aeruginosa*, and *S. epidermidis*^[2,15]). Jain et al^[5], found that Gram-negative rods were responsible for the 67.7% of septicemic neonates and authors detected ESBL in 87% of *Klebsiella* spp isolates. Authors indicate that this high rate may be due to the selective pressure imposed by extensive use of antimicrobials in the intensive care unit. In our study, ESBL was detected 14% and 33% for *E. coli*; 30% and 44% for *Klebsiella* spp. in 2004 and 2008, respectively (NS). In this study the resistance rate of β -lactam antibiotics and aminoglycosides to GNB showed increase in years. There may be different explanations for increased resistance rate in our NICU; our NICU serves very wide area in Aegean and patients were usually premature, received more mechanical ventilation and were more exposed to antibiotic treatment, which in turn may lead to selection of resistant microbes.

Some data suggest that the recent introduction of intrapartum antibiotic prophylaxis programs, through which selected women are administered penicillin or ampicillin during labor to prevent vertically transmitted Group B streptococcal disease, has increased the incidence and resistance of GNB sepsis in the newborn^[2,16]. Cordero et al^[16], assessed the enteric GNB sepsis during 17-years period and showed that antepartum antibiotic prophylaxis may have increased antibiotic resistance in *E. coli* isolates. In our study, Group B streptococcal infections showed increase in the last years (NS), but we do not have any data on antibiotic prophylaxis during pregnancy.

Rapid treatment with antibiotics is essential for favorable outcome for neonatal sepsis. In the present study, staphylococcus and Gram-negative strains were frequently found to be resistant to ampicillin, thus indicating that the use of these drugs alone may be ineffective. Since staphylococci are mostly resistant to methicillin, teicoplanin may be empirical coverage of the Gram-positive pathogens. The most common Gram-negative pathogens, *K. pneumoniae* and *E. coli* are resistant to most antibiotics but susceptible to amikacin and carbapenem. Therefore, the appropriate empiric regimen seems to consist of teicoplanin and carbapenem until culture results arrive.

Neonatal sepsis is associated with high mortality and morbidity^[3]. Mortality rate in neonatal sepsis has been reported 7-55% in many studies^[10,17]. In our study, mortality rate was 11.5% and 19% in 2004 and 2008, respectively. A number of antenatal and intrapartum factors have been associated with perinatal and neonatal mortality. Although, neonatal sepsis is an important risk factor for mortality in itself, low birth weight, prematurity, and infection with *Klebsiella* spp. were associated with high mortality rate in this study. Prematurity and low birth weight brings many health problems. Many studies showed that high mortality rate is related to low birth weight. Basu et al^[18] and Locatelli et al^[19] showed that shorter gestational age and low birth weight were independent predictors of survival.

In the present study, neonates infected with *Klebsiella* spp had higher mortality than with

other pathogens. Cordero et al^[16], also found high mortality rate related to *K. pneumoniae* and *E. coli* in neonates. Multi-resistant GNB, including ESBL producing bacteria are associated with increased mortality.

In this study, ESBL was higher in *Klebsiella* spp isolates. This might have affected the mortality rate. Lautenbach et al^[20] showed that patients with ESBL-positive strains had significantly higher average hospital costs, longer median hospital stays after the onset of bacteremia, and higher mortality compared with control individuals.

There are several limitations to this study. This study was limited by its retrospective nature. In addition, mortality risk factors (major neonatal co-morbidities such as presence or absence of underlying congenital abnormalities, bronchopulmonary dysplasia, necrotizing enterocolitis, duration of mechanical ventilation, the need for invasive procedure, etc.) were omitted. Moreover, we could only analyze culture-proven BSI data. Nevertheless, large-scale multicentric prospective studies are necessary to identify the changing patterns of the prevailing flora and mortality risk factors of neonatal sepsis.

Conclusion

Knowledge of prevailing strains and antibiotic sensitivity patterns in the region is essential to meet the challenge of neonatal sepsis with equal competence and opposing force.

Observed decline in susceptibility of pathogens to common antibiotics in this study calls for increased efforts to ensure monitoring, restricting and rationalizing the use of these antimicrobials. Prevention measures should therefore be employed with an emphasis on proper hand hygiene and evaluation of other potential reservoirs as sources of bacterial acquisition and transmission. Low birth weight and infection with *Klebsiella* spp were found related to high mortality rate in our NICU. These results raise two important tasks, first, providing a better antenatal care to minimize low birth

weight and second, importance of infection control practices.

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Conflict of Interest: None

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