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Neonatal Sepsis Caused by Gram-negative Bacteria in a Neonatal Intensive Care Unit: A Six Years Analysis

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Abstract ***Objective:*** To analyse the Gram-negative bacteriological profile of nosocomial sepsis and antibiotic susceptibility patterns. ***Methods:*** Neonates clinically diagnosed with sepsis and whose blood cultures tested positive for Gram-negative microorganisms from 2002 to 2008, retrospectively. ***Results:*** 143 cases of neonatal sepsis caused by Gram-negative microorganisms were identified. Eighty-one percent (n=116) of these had nosocomial sepsis. In nosocomial sepsis, the most common isolated Gram- negative micro-organism was *Serratia marcescens* (16.4%). Levofloxacin (97.4%), meropenem (97.1%), imipenem (95.6%), ciprofloxacin (95.4%) and amikacin (94.8) were the most sensitive antibiotics to Gram-negative micro-organisms. Sepsis-related mortality rate was 16% (n=23) in nosocomial sepsis caused by Gram-negative microorganisms. ***Conclusions:*** Carbapenem seems to be the best option for nosocomial sepsis caused by Gram-negative micro-organisms in our neonatal intensive care unit. Every unit must evaluate causative agents and antimicrobial susceptibilities in order to select the appropriate empirical therapy for nosocomial sepsis.

Key words Antibiotic susceptibility; Gram-negative bacteria; Newborn; Nosocomial sepsis

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Introduction

Despite advances in healthcare, neonatal sepsis, and especially that caused by Gram-negative rod bacteria, is a significant cause of morbidity and mortality among neonates. The incidence of neonatal sepsis is 1-4 per 1000 live births in developed countries.[1] Preterm and low birth weight infants especially very low birth weight (VLBW) have a 3- to 10-fold higher incidence of infection than full-term newborns. Prematurity, VLBW, exposure to invasive procedures, receiving parenteral nutrition with lipid emulsions, alterations in the skin and/or mucous membrane barriers, frequent use of broad-spectrum antibiotics and prolonged hospital stay are the most common risk factors for nosocomial sepsis in newborns.[1] An increase in sepsis caused by Gram-negative organisms has been reported in recent years.[2,3] Neonatal sepsis caused by Gram-negative

microorganisms is responsible for 18%-78% of all neonatal sepsis.[4-8] We aimed to determine the Gram-negative bacteriological profile of nosocomial sepsis and antibiotic susceptibilities in our neonatal intensive care unit (NICU).

Methods

This retrospective study was performed to determine the Gram-negative bacteriological profile of nosocomial sepsis and antibiotic susceptibilities in neonates clinically diagnosed with nosocomial sepsis and whose blood cultures tested positive for Gram-negative microorganisms at the Karadeniz Technical University Medical Faculty Farabi Hospital Neonatal Intensive Care Unit from October 2002 to December 2008. Blood culture registers from the Microbiology Laboratory were reviewed and all blood culture positive cases were selected and their records evaluated in terms of sepsis criteria, gestational age, age at onset, gender, birth weight, microorganisms and antimicrobial susceptibilities. A nosocomial infection was defined as an infection not present or incubating at the time of NICU admission and occurring >48 hours after NICU admission.[9] Cases with nosocomial sepsis caused by Gram-negative microorganisms constituted the study population. Sepsis was determined if two or more of the following criteria associated with positive blood culture were met: (a) fever or hypothermia, (b) tachycardia, (c) tachypnea or apnea and (d) abnormal white blood cells or an increase in immature forms.[10,11] Leukopenia was defined as <5000/mm^3, leukocytosis >20,000/mm^3, thrombocytopenia <150,000/mm^3, hypoglycaemia <2,8 mmol/L and hyperglycaemia >10 mmol/L.[11-13]

Nosocomial sepsis was empirically treated with imipenem/meropenem (20-25 mg/kg per dose every 12 hours) + vancomycin (10 mg/kg per dose given intravenously every 8 or 12 or 18 hours depending on gestational and postnatal age) + amikacin (15 or 18 mg/kg given intravenously every 24, 36 or 48 hours depending on gestational and postnatal age) or netilmicin (4 or 5 mg/kg given intravenously every 24, 36 or 48 hours depending on gestational and postnatal age).[14] Mortality due to nosocomial sepsis was defined as death occurring within seven days of episode onset.

The blood samples were inoculated at a volume of 1 to 2 ml into BACTEC Peds Plus/F culture vials and loaded into a Bactec 9240 blood culture instrument (BD Diagnostic Systems, Sparks, MD). All study bottles were incubated for five days. Bottles with a positive signal were subcultured directly onto trypticase soy agar with 5% sheep blood on chocolate agar and onto eosin-methylene blue (EMB) agar plates. Bacteria identification and antimicrobial susceptibility testing were performed using a BD Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) according to the manufacturer's recommendations.

Statistical Analysis

Data were analysed using Chi-Square and Fischer's exact test. $p<0.05$ was regarded as statistically significant.

Results

Our NICU is a single institution providing level III intensive care in the Eastern Black Sea region of Turkey. Approximately 30,000 births per year take place in our region. Our NICU has seven mechanical ventilation units, 16 incubators, two open beds, a monitor for each bed, and an isolation room, and is equipped with modern devices. It houses seven tertiary level and 11 secondary level intensive care beds. Space per incubator is 4.86 m^2, distance between incubators is 2 m, there are three hand-washing units, and patient care is provided by three doctors and three nurses daily.

During the six-year study period from October 2002 to December 2008, 3,061 neonates were admitted to the NICU. Of these 482 (15.8%) had culture-proved sepsis. Four hundred forty-six sepsis episodes (92.5%) were caused by bacterial pathogens, of which 68% (n=303) were Gram-positive and 32% (n=143) Gram-negative bacterial septicaemia. 81.1% (n=116) of Gram-negative bacterial septicaemia had including criteria for nosocomial sepsis. Thirty-six (7.5%) neonates had fungal septicaemia. The incidence of Gram-negative nosocomial bloodstream infections (BSIs) in our NICU was 5.5 infections per 1000 patient days.

The male-to-female ratio was 1.2:1. The majority of cases of sepsis occurred in <2500 g (66%), 62% of their birth weights were <1500 g, and preterm babies (66%). Demographic characteristics and laboratory findings of newborns with nosocomial sepsis are shown in Table 1.

The most common Gram-negative pathogens causing nosocomial bloodstream infection were *Serratia marcescens* (16.4%), followed by *Klebsiella pneumoniae* (14.7%), *Pseudomonas aeruginosa* (12%) (Table 2). Receiving parenteral nutrition (PN) (100%) with lipid emulsions, a long stay in hospital (100%), H_2-blockers (73%), mechanical ventilation (61%) and central venous

Table 1 Demographics and laboratory findings of patients with nosocomial sepsis

	Nosocomial sepsis (n=116)
Postnatal age (d)	18.7±18.1
Gestational age (w)	33.4±4.6 (23-42)
≥37, n (%)	40 (34)
<37, n (%)	76 (66)
Birth weight (g)	2080±941 (670-4200)
≥2500, n (%)	39 (34)
2499-1500, n (%)	29 (25)
<1500	48 (41)
Gender	
Male, n (%)	63 (54)
Female, n (%)	53 (46)
Delivery type	
Vaginal, n (%)	53 (46)
C/S, n (%)	63 (54)
Leukopenia, n (%)	18 (16)
Leukocytosis, n (%)	19 (17)
Thrombocytopenia, n (%)	49 (42)
Hypoglycaemia, n (%)	10 (9)
Hyperglycaemia, n (%)	13 (11)

catheterization (42%) were the most important risk factor for nosocomial sepsis.

Antibiotic susceptibility in all Gram-negative microorganisms causing nosocomial BSI is shown in Table 3. Sensitivity to levofloxacin (97.4%), meropenem (97.1%), imipenem (95.6%), ciprofloxacin (95.4%) and amikacin (94.8). Resistance to cefotaxime was 37.8%, to gentamicin 33.1% and to ceftazidime 31.5% in all Gram-negative microorganisms causing nosocomial BSI.

Sepsis-related mortality was 16% (n=23) in nosocomial sepsis caused by Gram-negative microorganisms. Sepsis-

Table 2 Microorganisms caused by nosocomial sepsis

Microorganisms	**n (%)**
Serratia marcescens	19 (16.4)
Klebsiella pneumoniae	17 (14.7)
Pseudomonas aeruginosa	14 (12.0)
Sfingomonas paucimobilis	13 (11.2)
Burkholderia cepacia	11 (9.5)
Enterobacter cloacae	9 (7.8)
Acinetobacter baumanii	8 (6.9)
Other Gram-negative microorganisms	25 (21.5)
Total n (%)	**116 (100)**

Table 3 Antibiotic susceptibility of microorganisms

	Serratia marcescens	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Sfingomonas paucimobilis***	***Burkholderia cepacia***	***Enterobacter cloacae***	***Acinetobacter baumanii***	**The other Gram-negative microorganisms**	**Total**
	n=19	n=17	n=14	n=13	n=11	n=9	n=8	n=25	n=116
Amikacin	89	100	93	100	100	100	88	88	94.8
Ciprofloxacin	89	94	100	100	100	100	88	92	95.4
Cefotaxime	32	53	–	–	–	100	50	76	62.2
Ceftazidime	21	53	64	100	82	89	75	64	68.5
Gentamicin	5	59	64	100	–	89	63	88	66.9
Imipenem	100	100	93	100	100	100	88	84	95.6
Levofloxacin	100	94	100	100	–	100	88	100	97.4
Meropenem	100	100	93	100	100	100	100	84	97.1
Piperacillin	5	35	–	–	55	78	38	76	47.8
Piperacillin/ tazobactam	53	65	86	100	–	100	50	76	75.7
TMP/SMX	89	71	29	–	100	89	75	100	79.0

TMP/SMX: Trimethoprim/Sulfamethoxazole

related mortality was 33% (16/48) in very low birth weight (VLBW) infants, 24% (6/25) in low birth weight (LBW) and 3% (1/39) in ≥2500 g. Sepsis-related mortality was higher in VLBW than in ≥2500 g ($p<0.0005$). It was also higher in LBW than in ≥2500 g ($p<0.05$). Sepsis-related mortality rate was higher in VLBW than LBW infants, but there was no statistical difference ($p>0.05$). Sepsis-related mortality was 0.3% (n=1) in nosocomial sepsis caused by Gram-positive microorganisms. Sepsis-related mortality was statistically higher in nosocomial sepsis caused by Gram-negative microorganisms than caused by Gram-positive microorganisms ($p<0.0001$).

Discussion

This retrospective study presents important data on nosocomial sepsis caused by Gram-negative bacteria, all of them monomicrobial infections, in a NICU over a six-year period. Sepsis is one of the main causes of neonatal morbidity and mortality. Nosocomial sepsis frequency and microorganism profiles vary widely from center to center and from country to country. The frequency of infections in NICUs varies from 6% to 25% in the United States and from 8% to 10% in Europe.[15] In our country, neonatal sepsis frequency ranges from 2.1% to 17%.[16] In our study, sepsis frequency was 15.8%.

Despite advances in supportive care and use of antibiotics, nosocomial sepsis is one of the most important causes of neonatal mortality and morbidity. Increased survival rates among newborns have led to a rise in the frequency of nosocomial infections. Newborns, especially preterm and <1500 g infants, are vulnerable to nosocomial infections due to their immature immune systems, prolonged hospital stay, exposure to invasive procedures and their receiving total parenteral nutrition in the NICU.[17] In our study, sepsis was more common in <1500 g (41%), males (54%), and premature infants (66%).

Nosocomial sepsis often occurs by Gram-positive bacteria (58-70.2%) in developed countries.[5,18] In our study, 63% of neonatal sepsis (303/482) was caused by Gram-positive bacteria. Although distance between incubators and numbers of medical personnel per patient are lower than in developed countries, the microorganism profiles are similar. We thought this might be associated with strict observance of the rules for hand washing, our NICU having modern equipment and the close cooperation between the hospital infection control committee and our unit.

Neonatal sepsis caused by Gram-negative bacteria is more frequent in developing countries.[4,6,8] Macharashvili et al[8] demonstrated that 78% of BSIs in the Republic of Georgia were caused by Gram-negative bacteria, with *K. pneumoniae* accounting for 37%, *E. cloacae* for 19% and *E. coli* 11%. Kamath et al[6] reported that 71.8% of BSIs in India were caused by Gram-negative bacteria, with *Klebsiella* species accounting for 16.4%, *Pseudomona*s spp. 13.6%, *E. coli* 11.8%, *Enterobacter* spp. 11.4% and *Acinetobacte*r spp. 10%. Couto et al[4] reported that 51.6% of BSIs in Brazil were caused by Gram-negative bacteria, with *Klebsiella* spp. accounting for 26.6%, *E. coli* 9.7% and *Pseudomona*s spp. 6.4%. Bizzarro et al[18] reported that 32.8% of BSIs in the USA were caused by Gram-negative bacteria, with *E. coli* accounting for 37%, *K. pneumoniae* 17% and *P. aeruginosa* 12%. Stoll et al[5] reported that 17.6% of BSIs were caused by Gram-negative bacteria in late-onset sepsis in very low birth weight neonates in USA. The most frequent microorganisms grown in blood cultures were *E. coli, Klebsiella, Pseudomonas,* and *Enterobacter* spp. In our country, the most frequent Gram-negative microorganisms grown in blood cultures are *Klebsiella* spp. and *Serratia* spp.[16] Neonatal sepsis in our study population was caused in 29.6% (143/482) of cases by Gram-negative bacteria, 81.1% (n=116) of Gram-negative bacterial septicaemia fulfilled criteria for nosocomial sepsis, and *Serratia marcescens (16.4%), K. pneumoniae* (14.7%), and *P. aeruginosa* (12%) were the most commonly isolated pathogens in nosocomial sepsis. Our findings are in agreement with national findings for our country.

Risk factors, clinical presentations and empiric antibiotic selections vary according to type of sepsis. When sepsis is suspected, empiric antimicrobial therapy should be initiated immediately once suitable cultures have been obtained. Blood culture is the gold standard for the confirmation of sepsis. For empiric therapy vancomycin and gentamicin are commonly used for initial treatment of nosocomial sepsis.[10] It is recommended that every unit evaluate the causative agents and antimicrobial susceptibilities in order to select an appropriate regime for nosocomial sepsis.[19] In our unit, since the most common Gram-negative pathogens are sensitive to carbapenems, quinolones and aminoglycosides, we use carbapenem + vancomycin + aminoglycoside with prophylactic antifungal therapy for nosocomial sepsis until the culture results arrive. Depending on the causative agents and antimicrobial susceptibility pattern, imipenem and/or amikacin seem to be the best choice empirical therapy of Gram-negative nosocomial sepsis in our unit.

Gram-negative microorganisms have high degree of resistance to commonly used antibiotics. Couto et al[4]

reported a high resistance of *K. pneumoniae, S. marcescens*, and *E. coli* to third-generation cephalosporins (19%-64.1%). Aurangzep and Hameed[20] showed that Gram-negative microorganisms have high resistance to ceftazidime (71.6%), cefotaxime (55.2%), gentamicin (43.2%), imipenem (23.6%) and amikacin (22.3%). In our study, Gram-negative microorganisms were highly resistant to third-generation cephalosporins [cefotaxime (37.8%), ceftazidime (31.5%)] and gentamicin (33.1%) but had low resistance to amikacin (5.2%), meropenem (2.9%) and imipenem (4.4%).

The empirical antibiotic treatment administered may not always be effective for all microorganisms. Culture results should therefore be obtained as soon as possible and appropriate antibiotics applied. Some of the *Pseudomonas* and *Acinetobacter* spp. and *Stenotrophmonas maltophilia* were resistance to imipenem/meropenem and amikacin in our study.

Although Gram-positive organisms are the most common causes of nosocomial BSIs, Gram-negative bacteremia carries higher risks of severe sepsis, septic shock and death. Sundaram et al[21] reported a neonatal mortality rate due to Gram-negative sepsis of 34% to 55%. A sepsis related mortality rate of 24.3 per 100 sepsis cases has been reported in our country.[16] The equivalent figure in our study was 16% in nosocomial sepsis caused by Gram-negative microorganisms and 0.3% caused by Gram-positive microorganisms.

Every unit must evaluate the causative agents and antimicrobial susceptibilities in order to select the appropriate empirical therapy for nosocomial sepsis. Firm standard infection control procedures such as hand hygiene and to avoid from unnecessary invasive procedures are the best methods of preventing nosocomial infection. Further large-scale multicenter studies are required to generalise the data for Turkey as a whole.

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