Neonatal nosocomial sepsis in a level-III NICU: evaluation of the causative agents and antimicrobial susceptibilities

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Despite advances in supportive care and use of antibiotics, sepsis preserves its importance due to its high mortality and morbidity for neonates. Identifying the causative agents and antibiotic resistance yearly in a neonatal intensive care unit (NICU) helps the physician to choose the most appropriate empirical therapy. In this study we aimed to evaluate positive blood cultures and antibiotic susceptibilities of newborns with proven sepsis during the years 2000-2002 in our NICU. The charts of babies with sepsis were evaluated for clinical characteristics, positive cultures and antimicrobial susceptibilities, retrospectively. Although most of the admitted patients were premature (76.5%), the frequency of proven sepsis was quite low, at 9.1% among 909 newborns. Mortality rate in sepsis was 16%. The most commonly isolated micro-organisms were coagulase-negative staphylococci (CoNS) (31.3%), fungi (19.2%), Staphylococcus aureus (13%) and Klebsiella pneumoniae (10.5%). Methicillin resistance for CoNS was 92.3% and for S. aureus was 72.7%. In the last year, a significant increase in the frequency of Klebsiella pneumoniae (8.3 vs 14.2%), CoNS (27.1 vs 37.1%), Pseudomonas aeruginosa (2.1 vs 8.6%) and fungal infections (18.8 vs 20%) was observed compared to the previous years. An initial empirical antibiotic therapy for late-onset sepsis was designed with teicoplanin + piperacillin-tazobactam/meropenem + antifungal (fluconazole or amphotericin B) as the best combination to cover this spectrum until the culture results arrive. However, this combination is only compatible with our results and may not be applied in all units. Every unit must follow the bacterial spectrum and antibacterial resistance patterns to choose their specific empirical treatment strategy for nosocomial infections.

Key words: *etiology, newborn, sepsis, treatment.*

Nosocomial sepsis, which is a major component of neonatal sepsis, is still an important factor with a high mortality and morbidity rate in spite of new advances in antibiotic therapy[1,2].

The incidence of neonatal sepsis is approximately 1-8 per 1,000 live births. The incidence is increasing to 40-250/1,000 in preterm babies weighing <1500 g. Prenatal factors, the condition of the delivery room, flora of both the delivery room and neonatal intensive care, quality and quantity of the health care personnel, infection control methods of the neonatal intensive care and antibiotics used are the main determining factors for the incidence[1,2].

Nosocomial sepsis is the most frequent type of nosocomial infection in neonatal intensive care units (NICUs). The variety and the antimicrobial sensitivity of the micro-organism causing sepsis can be different for each NICU and it can also change over time for the same unit. For this reason, active surveillance of infected patients and the antimicrobial sensitivity of the responsible pathogenic micro-organisms is important in defining the empiric antibiotic regimens[1,3].

In this study the clinical findings and antibiotic sensitivity of the pathogenic micro-organisms isolated from the patients in our NICU have been evaluated.

Material and Methods

Nine hundred and nine neonates admitted to the NICU between 2000-2002 were included in the study. As nosocomial neonatal sepsis criteria, patients who were younger than three days of lives were excluded. Patients who had clinical signs of septicemia (lethargy, feeding disorder, color changes in skin, petechia, thermal instability, apnea, and bad general appearance) and/or laboratory findings [leukopenia (<5000/mm^3), leukocytosis (>25000/mm^3), thrombocytopenia (<150000/mm^3), immature/total neutrophil ratio >0.2, hypo-hyperglycemia] and were proven to be septic with positive blood cultures were evaluated to determine the micro-organism diversity and antimicrobial sensitivity of the flora. Diagnosis of meningitis was established when there was high leukocyte count (>35/mm^3), high protein concentration (>200 mg/dl) in cerebrospinal fluid (CSF), and growth of bacteria in CSF culture. Culture results were evaluated after inoculation of blood culture media (BactAlert, BioMerieux, France) with appropriate volume of blood samples in appropriate conditions. Antibiograms were studied by disk diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS)[4].

Data was analyzed by Student's t test, Matel Haenzel correlation test and Fischer's exact test using SPSS-10.0 software for Windows®. Statistical significance was described when $p < 0.05$.

Results

Seventy-five patients had 83 septic attacks. Although 76.5% of the hospitalized patients were preterm (<37 weeks), the frequency of proven sepsis was found to be 9.1% (83/909), which is quite low. Mortality rate of these septic babies was 16%. Demographic characteristics of the patients are shown in Table I.

Sixty-seven septic attacks (80.8%) were caused by bacterial pathogens, of which 59% were Gram-positive and 41% Gram-negative bacterial septicemia. The distribution of the responsible bacteria was as follows: coagulase negative staphylococcus (CoNS) 31.3%, fungal infections 19.2% (of which Candida spp. was 94%), S. aureus 13%, Klebsiella pneumoniae 10.5% and Enterobacter spp. 8.2%. Three bacterial septic attacks were observed in a preterm newborn, two bacterial septic attacks were observed in two preterm newborns, and four preterm newborns had Candida sepsis following bacterial sepsis (Table II).

Table I: Characteristics of Neonates with Nosocomial Sepsis

	All (n=75)	Preterm† (n=64)	Term‡ (n=11)	p († vs. ‡)
Gestational age (weeks)	30.2 ± 4.8 (23–40)	29.8 ± 4.1 (23–35)	39.2 ± 1.0 (38–40)	0.01
Sex (F/M)	31/44	27/37	4/7	0.071
Birth weight (g)	1774.5 ± 582.3 (540–4150)	1298 ± 427.2 (540–3050)	3238.7 ± 691.2 (2100–4150)	0.008
Delivery type (C/S) (n, %)	63 (84%)	59 (92.1%)	4 (36.3%)	0.001
Referred neonates (n, %)	12 (16%)	10 (15.8%)	2 (18.1%)	0.023
Time of sepsis (d)	8.45 ± 3.68 (3–37)	8.3 ± 5.35 (3–37)	9.5 ± 4.1 (3–22)	0.060
Duration of hospitalization (d)	33.6 ± 10.5 (18–116)	34.47 ± 11.3 (21–116)	22.9 ± 2.5 (18–36)	0.041
Duration of TPN (d)	15.9 ± 10.5 (2–44)	22.3 ± 12.1 (2–44)	9.9 ± 4.3 (2–22)	0.025
Duration of mechanical ventilation (d)	14.4 ± 12.9 (0–40)	13.3 ± 12.1 (0–40)	5 ± 9.2 (0–20)	0.010
Meningitis (n, %)	11 (13.6%)	10 (15.6%)	1 (9%)	0.011
Mortality (n, %)	12 (16%)	11 (17.2%)	1 (9%)	0.013

TPN: Total parenteral nutrition.

Table II: Spectrum of Micro-organisms Regarding Maturity

	All	Preterm	Term
No of septic attacks	**83**	**72**	**11**
No of septic newborns	**75**	**64**	**11**
Gram (+) bacteria	**41 (49.4%)**	**36 (50%)**	**5 (45.5%)**
CoNS	26 (31.3%)	23 (32%)	3 (27.2%)
S. aureus	11 (13%)	9 (12.5%)	2 (18.2%)
Enterococcus spp.	3 (3.5%)	3 (4.1%)	0
S. pyogenes	1 (1.2%)	1 (1.4%)	0
Gram (-) bacteria	26 (31. 4%)	21 (29.2%)	5 (45.5%)
Enterobacter spp.	7 (8.2%)	7 (9.7%)	0
Klebsiella pneumoniae	9 (10.5%)	7 (9.7%)	2 (18.2%)
E. coli	2 (2.3%)	1 (1.4%)	1 (9%)
Acinetobacter spp.	2 (2.3%)	1 (1.4%)	1 (9%)
Aeromonas spp.	1 (1.2%)	1 (1.4%)	0
P. aeruginosa	4 (4.7%)	4 (5.5%)	0
S. maltophilia	1 (1.2%)	0	1 (9%)
Fungal infections	**16 (19.2%)**	**15 (20.8%)**	**1 (9%)**
Candida albicans	15 (18%)	14 (19.4%)	1 (9%)
A. strictum	1 (1.2%)	1 (1.4%)	0

Note : Rates for micro-organism/septic attack number. CoNS: Coagulase-negative staphylococci.

ANTIMICROBIAL SUSCEPTIBILITIES of the MICRO-ORGANISMS

Coagulase-negative Staphylococcus (n: 26)

Although all the isolated bacteria were sensitive to glycopeptides (teicoplanin and vancomycin), 13 micro-organisms showed susceptibility to gentamicin (50%). Resistance rates to penicillin and methicillin were 96.1% and 92.3%, in order.

S. aureus (n: 11)

All the micro-organisms were sensitive to glycopeptides, but only six of them were also sensitive to clindamycin (54.5%) and six were sensitive to gentamicin (54.5%). Nine of the micro-organisms (91%) were resistant to penicillin, while methicillin resistance was determined in eight (72.7%).

Enterobacter spp. (n: 7)

All of the isolated bacteria were sensitive to carbapenem (meropenem) and piperacillin-tazobactam. Quinolone sensitivity was seen in six bacteria (86%) and amikacin sensitivity in five bacteria (72%).

Klebsiella pneumoniae (n: 9)

In five bacteria (55.5%), extended spectrum β-lactamase (ESBL) was detected. All of the isolated bacteria were sensitive to carbapenem (meropenem) and isepamicin. Eight micro-organisms (89%) were sensitive to quinolones, but only four bacteria (44.5%) were sensitive to amikacin and three bacteria (33.3%) to piperacillin-tazobactam. In 2002, a significant increase in the frequency of this micro-organism was detected compared to the previous years (Table III, p=0.041).

E. coli (n: 2)

Both were sensitive to aminoglycoside and 3rd generation cephalosporins. ESBL was not detected in either of the isolated bacteria.

Enterococcus spp. (n: 3)

β-lactam and glycopeptide resistance was not detected in micro-organisms; however, one of them had high resistance against aminoglycoside.

Acinetobacter spp. (n: 2)

Sensitivity to carbapenem and quinolones was demonstrated, but they were resistant to 3rd generation cephalosporins and aminoglycosides.

Aeromonas spp. (n: 1)

Sensitivity was demonstrated to amikacin among aminoglycosides and to 3rd generation cephalosporins.

Streptococcus pyogenes (n: 1)

Resistance to penicillin was not detected.

Table III: Spectrum of Micro-organisms According to Time Period

	2000-2002 No of septic attacks (n=83)	2000≠-2001† No of septic attacks (n=48)	2002‡ No of septic attacks (n=35)	p (≠,† vs.‡)
No of septic newborns	**75**	**44**	**31**	**0.036**
Gram (+) bacteria	41 (49.3%)	23 (48%)	18 (51.4%)	0.040
CoNS	26 (31.3%)	13 (27.1%)	13 (37.1%)	0.945
S. aureus	11 (13%)	7 (14.6%)	4 (11.4%)	0.065
Enterococcus spp.	3 (3.5%)	2 (4.1%)	1 (2.85%)	0.078
S. pyogenes	1 (1.2%)	1 (2.1%)	0	
Gram (–) bacteria	**26 (31.3%)**	**16 (33.2%)**	**10 (28.5%)**	**0.075**
Enterobacter spp.	7 (8.2%)	7 (14.6%)	0	
Klebsiella pneumoniae	9 (10.5%)	4 (8.3%)	5 (14.2%)	0.041
E. coli	2 (2.3%)	2 (4.1%)	0	
Acinetobacter spp.	2 (2.3%)	1 (2.1%)	1 (2.85%)	0.095
Aeromonas spp.	1 (1.2%)	1 (2.1%)	0	
P. aeruginosa	4 (4.7%)	1 (2.1%)	3 (8.6%)	0.031
S. maltophilia	1 (1.2%)	0	1 (2.85%)	0.082
Fungal infections	**16 (19.2%)**	**9 (18.8%)**	**7 (20%)**	**0.044**
Candida albicans	15 (18.8%)	9 (18.8%)	6 (17.1%)	0.071
A. strictum	1 (1.2%)	0	1 (2.85%)	

Note: Rates for micro-organism/septic attack number. CoNS: Coagulase-negative staphylococci.

***Pseudomonas aeruginosa* (n: 4)**

Sensitivity rates for this micro-organism were as follows: amikacin 100%, quinolone 100%, carbapenem 75%, piperacillin-tazobactam 75% and ceftazidime 75%. Similar to Klebsiella pneumoniae, a significant increase in the frequency of this micro-organism was detected in 2002 compared to the previous years (Table III, p=0.03).

***Stenotrophomonas maltophilia* (n: 1)**

This micro-organism was only sensitive to ceftazidime and resistant to carbapenems, quinolones, piperacillin and amikacin.

***Fungal infections* (n: 16)**

Susceptibility tests were not performed for technical reasons. Last year, the rate of fungal infections was increased compared to the previous years (p=0.044). Interestingly, one of isolated species was very unusual. Causative agent was Acremonium strictum, which is an opportunistic micro-organism very likely to be mistaken for Candida spp. We have recently reported this case as the second newborn with A. strictum fungemia in the English literature[5].

Discussion

Life expectancy of neonates is increasing with the help of advances in neonatology, new life support techniques, and new treatment modalities. As a result of increased life expectancy, duration of hospitalization is also increasing, and in spite of new antibiotics and new supportive measures, nosocomial sepsis and its treatment has become a major problem in NICUs. To overcome this problem, it is important to know the distribution of etiological agents and their antimicrobial sensitivity[1-7].

Frequency of neonatal nosocomial sepsis is reported as between 1.8 and 39.8% in various reports[8,9]. Especially neonates are more susceptible to infections because of their weak immune system and frequent invasive procedures[1-3,6]. In our series, although 76.5% of neonates were preterm, the frequency of nosocomial sepsis was found to be 9.1%, which is quite low.

Mortality rate in neonatal nosocomial sepsis has been reported to range from 10-50%[3,6,10]. Among the intrinsic medical risk factors for neonatal sepsis, several studies confirmed low gestational age as an important risk factor[11,12]. In our study, the majority of the patients were preterm and the mortality rate was 16%.

In developed countries L. monocytogenes, E. coli, S. aureus, S. epidermidis (CoNS) and Gram-negative enteric bacilli are the most common causes of nosocomial sepsis in the NICU; however, in developing countries, these bacteria are replaced by Gram- negative

enteric bacilli (e.g. Klebsiella spp., Enterobacter spp. and Serratia spp.), CoNS, E. coli and S. aureus[9,10,13]. Recent studies from developing countries show that the most common micro-organisms in nosocomial sepsis are CoNS, Klebsiella spp., Enterobacter spp., Serratia spp., E. coli, Candida spp., S. aureus and Pseudomonas spp.[14,15]. In our unit, an earlier study[16] revealed Gram-negative bacteria as the most common etiological agents in nosocomial sepsis, but in the last three years, if fungal infections are excluded, Gram-positive bacteria are more common (41/67; 61%) as etiological agents. Especially staphylococcal sepsis (37/41; 90%) is important among other Gram-positive causes of sepsis. Staphylococcal sepsis is associated with birth weight, presence of central venous catheter, duration of parenteral nutrition and duration of mechanical ventilation ($p<0.05$ for each, not shown on the Table).

Klebsiella, Pseudomonas and Candida infections increased markedly in 2002 compared to the earlier years (2000, 2001 vs. 2002; $p<0.05$ for each). Significant association has been found between Candida infection and birth weight and duration of antibiotic therapy ($p<0.05$ for each, not shown the Table). In addition, the reason for increased incidence of Candida spp. infection in 2002 is thought to be discontinuation of our routine fluconazole prophylaxis together with the antibiotic treatment of late-onset neonatal sepsis. Supporting information comes from a study reporting that oral nystatin prophylaxis is effective in preventing fungal infection in the neonatal period[17,18].

In the last three years, CoNS, S. aureus, Klebsiella pneumoniae, Enterobacter spp. and Candida spp. were found as the major constituents of etiological agents of nosocomial sepsis in neonates. Thus, it seems wise to change the earlier empiric antibiotic therapy of suspected nosocomial sepsis to cover the most common etiological agents. Since staphylococci are mostly resistant to methicillin, teicoplanin may be empiric coverage of the Gram-positive pathogens. The most common Gram-negative pathogens, Klebsiella pneumoniae and Enterobacter spp., are resistant to most antibiotics, but sensitive to carbapenem and piperacillin-tazobactam. Increasing fungal infection rate shows the necessity of antifungal coverage in late-onset septicemia. Therefore, the appropriate empiric regimen seems to consist of teicoplanin + piperacillin-tazobactam / meropenem + antifungal (fluconazole or amphotericin B) until culture results arrive. Furthermore, it also seems appropriate to initiate oral nystatin prophylaxis in neonates receiving vigorous, wide spectrum antibiotic treatment which may lead to increased incidence of endogenous Candida infection.

In conclusion, sepsis, especially nosocomial sepsis, carries high mortality and morbidity risk for neonates. Gram-positive bacteria, especially CoNS and S. aureus, together with Klebsiella pneumoniae and fungi are the leading causative agents of nosocomial sepsis in our unit and they are resistant to commonly used antibiotics. Due to antibiotic sensitivity results, appropriate initial empirical antibiotic therapy for late-onset sepsis has been determined to be teicoplanin + piperacillin-tazobactam / meropenem + fluconazole / amphotericin B. Because of the high fungal infection rate, oral nystatin prophylaxis for babies receiving wide spectrum antibiotics may be considered. However, this antibiotic regimen is only compatible with our own culture results. Every unit must carefully follow the bacterial spectrum and resistance patterns of micro-organisms responsible for nosocomial infections to design a specific empirical antibiotic regimen for their unit.

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