Nosocomial blood stream infections in a neonatal intensive care unit in Ankara, Turkey

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Nosocomial blood stream infections continue to be a cause of high mortality and morbidity in newborn intensive care units (NICUs). Identification of the causative microorganisms and their antimicrobial sensitivities will guide the selection of appropriate empirical treatment. We prospectively evaluated culture-proven nosocomial sepsis cases and antibiotic sensitivity patterns seen in the NICU of Dr. Sami Ulus Children's Hospital, in Ankara, Turkey during a six-year period (2000-2006). A total of 106 nosocomial sepsis attacks were found in 100 patients, with 72 of them preterm. Gram-negative bacteria were isolated at a rate of 70.8%, gram-positive at 22.6% and Candida species (spp.) at 6.6%. The most commonly isolated microorganisms were, in order of frequency, Klebsiella spp. (39.6%), *Pseudomonas aeruginosa* (11.3%) and Coagulase-negative staphylococci (9.4%). During the study, 12 of the 28 term babies (42.9%) and 26 of the 72 preterm babies (36.1%) died due to nosocomial sepsis, with a mortality rate of 38%. Resistance to ampicillin was 100%, to cefotaxime 88%, to gentamicin 73%, and to amikacin 23% in gram-negative bacteria. No carbapenem resistance was found except for *P. aeruginosa* (25%). Resistance to penicillin was 100% and clindamycin 58.3% in gram-positive bacteria. No glycopeptide or carbapenem resistance was found. In conclusion, nosocomial sepsis still has a high mortality rate. Gram-negative bacteria were the most commonly isolated microorganisms with Klebsiella spp. being dominant. All gram-negative species were resistant to ampicillin, and all gram-positive bacteria were resistant to penicillin. No glycopeptide or carbapenem resistance was found in gram-positive bacteria. In gram-negative bacteria, low amikacin and high gentamicin and cephalosporin resistances were found. No carbapenem resistance was found except for *P. aeruginosa*. Restricted and alternate antibiotic usage policies seem important for the resistance problem.

Key words: nosocomial blood stream infection, nosocomial sepsis, neonatal sepsis, bacteremia, newborn.

The survival rates of low birth weight and very low birth weight children in newborn intensive care units (NICUs) have increased in recent years with technological advancements, but the increased inpatient duration of hospitalization and number of invasive procedures may increase nosocomial infections[1].

The nonspecific findings of sepsis in the newborn period and the rapid and fatal course make it necessary to diagnose the disease as early as possible and start appropriate antibiotic treatment. However, the widespread use of antibiotics has caused a resistance problem. Periodic review of the nosocomial infections in the newborn unit is important to determine the etiological agents and their antibiotic sensitivity so that suitable treatment protocols can be developed[2].

The aims of our study were 1) to determine the microorganisms responsible for nosocomial sepsis in our unit and their antibiotic sensitivity patterns, 2) to determine the choice of antibiotics for patients thought to be developing nosocomial sepsis based on these results,

and 3) to determine the nosocomial sepsis mortality rate.

Material and Methods

We evaluated blood culture-proven nosocomial sepsis cases in babies admitted to the NICU of Dr. Sami Ulus Children's Hospital between 1 January 2000 and 1 January 2006.

The distribution of the organisms isolated from the blood and the ratio of patients who died due to nosocomial sepsis to all patients were recorded according to the gestation period together with the antibiotic sensitivity patterns.

***Characteristics of the unit*:** There were no labor wards in our hospital during the study period, and our newborn unit was accepting out-born newborn babies from the Central Anatolian region.

Nosocomial sepsis: Patients with no sepsis on admission and who had microorganisms isolated in blood cultures taken 48-72 hours after birth on suspicion of sepsis according to the clinical signs and/or laboratory findings were diagnosed as nosocomial sepsis[3].

For blood culture, 0.5-1 ml of venous blood was inoculated into Bact Alert (Organon) pediatric bottles, and the antibiotic sensitivity tests suggested by the National Committee for Clinical Laboratory Standards were performed[4].

Strains of *Staphylococcus epidermidis* were judged to be involved with infection if they represented the only microorganisms isolated from the blood specimen in the presence of clinical signs or symptoms of infection. Most authorities recommend obtaining two independent cultures to work up an episode of suspected bloodstream infection[5].

Extended spectrum ß lactamase (ESBL) presence to determine whether *Escherichia coli* and *Klebsiella* spp. were resistant species was investigated with the double disc synergy test. Inducible *ß lactamase* (IBL) presence for *Pseudomonas aeruginosa, Enterobacter* spp. and *Acinetobacter* spp. was investigated with the double disc synergy method.

The microbiology laboratory of our hospital is unable to carry out typing and antibiograms for fungal infections. It can only specify *Candida* spp. among the fungus types.

Statistical Analysis

The data were analyzed with the SPSS 11.5 package program. The Mann-Whitney U test was used to determine the presence of a difference between the measured features between the groups. Chi-square of Fisher's exact test was used for categorical comparisons.

Results

A total of 5,165 babies consisting of 1,497 preterm and 3,668 term babies were admitted to the Dr. Sami Ulus Children's Hospital Newborn Unit between 1 January 2000 and 1 January 2006.

Demographic Evaluation

A total of 106 culture-proven nosocomial sepsis attacks were found in 236 cases of suspected nosocomial sepsis. The ratio of culture positivity in blood was 45%. Culture-proven nosocomial sepsis attacks were found in 100 patients admitted to the unit during the six years and these patients were included in the present study. The percentage of males was 52% and of females 48%, with 72% preterm. The percentage of babies weighing 1500 g or less with culture-proven nosocomial sepsis was 41%.

Microbiological Evaluation

The causative agent was gram-negative bacteria in 75 (70.8%) of the nosocomial sepsis cases, gram-positive bacteria in 24 (22.6%) and *Candida* spp. in 7 (6.6%) (Table I).

Gram-negative bacteria consisted mostly of *Klebsiella* spp. followed by *P. aeruginosa* and *E. coli*. **Gram-positive bacteria** consisted mostly of coagulase-negative staphylococci (CoNS) followed by *Streptococcus viridans, Enterococcus* spp. and *S. aureus*. **Candida infections:** The nosocomial sepsis agent isolated was *Candida* spp. in 6.6% of the cases (Table I).

Gram-positive bacteria growth was significantly higher in term patients compared to preterm patients ($p=0.02$). However, there was no significant difference between term patients and preterm patients for gram-negative and candida sepsis ($p>0.05$). Table II presents the isolated microorganisms by gestational period.

In our study group, a total of 23 nosocomial meningitis cases were diagnosed during the

Table I. Distribution of Pathogens Associated with Episodes of Nosocomial Bloodstream Infection

Pathogen	Infection episodes: 106
Gram (+) microorganisms	24 (22.6%)
CoNS	10 (9.4%)
S. aureus	3 (2.8%)
S. viridans	6 (5.7%)
Enterococcus spp.	5 (4.7%)
Group D streptococci	0 (0%)
Gram (-) microorganisms	75 (70.8%)
Klebsiella spp.	42 (39.6%)
P. aeruginosa	12 (11.3%)
E. coli	8 (7.6%)
Serratia spp.	3 (2.9%)
Acinetobacter spp.	4 (3.8%)
Enterobacter spp.	3 (2.9%)
Salmonella spp.	0 (0%)
Proteus spp.	1 (0.9%)
S. maltophilia	2 (1.8%)
Candida species	7 (6.6%)

CoNS: Coagulase-negative staphylococci.

six-year period, with 22.4% (17/76) preterm and 20% (6/30) term. The causative agent was gram-negative bacteria in 75% and gram-positive bacteria in 25%.

In our study group, 34.3% of preterm and 40% of term infants were mechanically ventilated. Ventilator-associated pneumonia occurred in 15.3% of these patients. During the study period, umbilical and percutaneous central venous catheterization was not being performed.

Mortality

Of the 5,165 patients admitted to the unit, 516 died and 38 (7.4%) of these deaths were in nosocomial sepsis patients. Of the 100 culture-proven nosocomial sepsis patients, 38 (38%) died.

Among the exitus patients, 68.4% were preterm and 31.6% term. Mortality was observed in 14 of the 41 (34.1%) patients weighing 1500 g or less and developing nosocomial sepsis.

When the association between the micro-organism causing the nosocomial sepsis and mortality was assessed, gram-negative bacteria were isolated from 78.9% of the cases who died, gram-positive bacteria from 15.8% and *Candida* spp. from 5.3%. The most commonly isolated agents, in order of frequency, were *Klebsiella* spp. (36.8%), *P. aeruginosa* (18.4%) and *Enterococcus* spp. (7.9%). Gram-negative microorganisms were isolated from 85.7% of the patients weighing 1500 g or less who died.

When evaluating the fatality due to the microorganisms causing the sepsis, no statistical difference was found between gram-negative and gram-positive bacteria and candida sepsis mortality ($p>0.05$). When all causative agents were evaluated together, the mortality was significantly higher in patients in whom *P. aeruginosa* was isolated ($p<0.05$).

Antibiogram Results

Antibiogram profiles of gram-negative bacteria causing nosocomial sepsis: Resistance to ampicillin was 100%, to cefotaxime 88%, to gentamicin 73%, and to amikacin 23% in gram-negative bacteria. No carbapenem resistance was found except for *P. aeruginosa* (25%). Table III presents the antibiogram resistance patterns of gram-negative bacteria.

Extended spectrum ß lactamase (ESBL) production was 90.5% in *Klebsiella* spp. and 75% in *E. coli*. IBL production was 100% in *Enterobacter* spp., 91.7% in *P. aeruginosa* and 75% in *Acinetobacter* spp.

Antibiogram profiles of gram-positive bacteria causing nosocomial sepsis: Resistance to penicillin was 100% and to clindamycin 58.3% in gram-positive bacteria.

Table II. Distribution of Pathogens Associated with Duration of Gestation

Duration of gestation (weeks)	Klebsiella spp.	*E. coli*	*S. aureus*	CoNS	Enterococcus spp.	*P. aeruginosa*	Proteus spp.	Enterobacter spp.	Acinetobacter spp.	*S. viridans*	Candida spp.	Serratia spp.	*S. maltophilia*	Total no. of isolates
≤30	12	1	1	3	2	2		1	1	1	3	2		29 (27.3%)
31-34	18	3		1	2	6		2	2	1	2		1	38 (35.8%)
35-37	6	1				1	1							9 (8.6%)
> 37	6	3	2	6	1	3			1	4	2	1	1	30 (28.3%)
Tol no. of isolates	42	8	3	10	5	12	1	3	4	6	7	3	2	106 (100%)

No glycopeptide or carbapenem resistance was found.

Oxacillin resistance was 100% in *S. aureus* and 80% in CoNS, while penicillin resistance was 100% in *S. viridans*. There was no vancomycin resistance in the *Enterococcus* spp. (Table IV).

Discussion

The survival rate of low birth weight, very low birth weight and ill newborns in NICUs has increased in recent years with technological advancements but the increased duration of hospitalization and number of invasive procedures may increase nosocomial infections[1]. Resistant hospital infections are an important problem especially in preterm babies[6]. Knowing the nosocomial sepsis rate of each unit, the causative microorganisms and their resistance patterns enables the development of antimicrobial treatment strategies.

The most common pathogen in hospital-based neonatal sepsis is reported to be CoNS in developed countries[7]. Gram-negative sepsis is more a problem in developing countries[8]. A study from Germany found gram-negative microorganisms responsible for 22.7% of nosocomial infections in the NICU, while this rate is much higher in developing countries and reported as 60.5%[9]. The rate of gram-negative bacteria was 70.8% in our study. Gram-negative bacteria were the most frequent causative agent in our study with the most common being *Klebsiella* spp. The prominence of *Klebsiella* spp. in our study was consistent with the data from our country and other developing countries [10]. We isolated *P. aeruginosa* at a rate of 11.3% and *E. coli* at a rate of 7.6% among all causative microorganisms, which are similar to rates in the previous reports in developing countries[11-13].

Coagulase-negative staphylococci (CoNS) are reported as the most common agent of neonatal nosocomial sepsis in developed countries and were also the most commonly grown gram-positive microorganism in our study.

S. aureus is reported as the most common cause of nosocomial sepsis in term babies in developed countries[3]. A multi-center study from Europe determined it at a rate of 7.5% in bloodstream infections in pediatric patients[14]. It is reported to be responsible for 8-22% of all nosocomial sepsis cases in developing countries[8]. *S. aureus* was isolated at a rate of 2.8% in our study, and all the babies with *S. aureus* were term babies except one.

Most enterococcal infections during the neonatal period are in the form of nosocomial infections[3]. *Enterococcus* spp. was responsible for 4.4% of nosocomial bloodstream infections in a Taiwan study[15]. The rate was 4.7% in our study. Of these patients, 80% were preterm and 60% had necrotizing enterocolitis (NEC).

Table III. Antimicrobial Resistance Rates Among Gram-Negative Microorganisms (%)

Organism	Ampicillin	Cephazolin	Cefotaxime	Ceftazidime	Cefepime	Piperacillin	Amikacin	Gentamicin	Aztreonam	Carbapenem	Ciprofloxacin
Klebsiella spp	100	92.8	95.2	92.8	80.9	92.8	28.5	71.4	88.0	0	0
P. aeruginosa	100	100	83.3	41.7	58.3	50.0	33.3	75.0	66.6	25.0	8.3
E. coli	100	100	87.0	75.0	100	100	0	62.0	100	0	62.0
Enterobacter spp.	100	100	66.6	66.6	66.6	66.6	33.3	100	66.6	0	33.3
Acinetobacter spp.	100	100	50	50	75	75	25	75	100	0	0
Serratia spp.	100	100	100	100	100	100	0	100	0	0	0
S. maltophilia	100	100	50	50	50	100	0	50	100	0	0

We thought that this microorganism, present in the gastrointestinal flora, led to an increased risk of sepsis due to NEC.

Candida albicans is reported as a cause of nosocomial infection, especially in low birth weight babies[2]. Patients with a gestational age of 30 weeks or less made up 42.8% of the candida sepsis cases in our study.

The widespread use of antibiotics in NICUs is reported to cause the spread of bacteria with multiple antibiotic resistance[8]. The microorganisms isolated and their resistance status have been monitored with the active surveillance study carried out in our hospital since 2000.

Resistance to ampicillin was 100%, to cefotaxime 88%, to gentamicin 73%, and to amikacin 23% in gram-negative bacteria in our study. We found no vancomycin resistance in gram-positive bacteria and no carbapenem resistance in gram-negative bacteria except *P. aeruginosa*. The low amikacin resistance and high gentamicin and cephalosporin resistance may be due to the preference for cefotaxime and gentamicin for empirical treatment of neonatal sepsis in our unit before the present study. The alternate use of amikacin and gentamicin during various periods may decrease the resistance rate.

The incidence of bacteria producing wide-spectrum β lactamase varies between countries and also between hospitals in the same country. The ESBL rate was 52.8% in a report from Malaysia[16]. The ESBL incidence in *Klebsiella* spp. was 90.5% in our study while another study reported 55-70%[10]. The high resistance may be due to the use of wide spectrum antibiotics in the empirical treatment of neonatal sepsis in the period before our study.

A study from India found the resistance rate in *P. aeruginosa* for ceftazidime as 55%, for ciprofloxacin as 32.3% and for amikacin as 45.2%[17]. In our study, the resistance rates were 41.7% for ceftazidime, 8.3% for ciprofloxacin, 33.3% for amikacin, and 50% for piperacillin, while the IBL production rate was 91.7%. We recognize that the IBL production rate and high antibiotic sensitivity rates of *P. aeruginosa* are important problems for our unit.

Ampicillin resistance was 100%, 3rd-generation cefotaxime resistance was 66.6% and amikacin resistance was 33.3% for *Enterobacter* spp. Our results seem to emphasize that cephalosporins should not be the first-choice antibiotics for the empirical treatment of infections that are not nosocomial.

Data from South Asia show resistance among *E. coli* for amikacin as 27%, for 3rd- generation cephalosporins as 51% and for gentamicin as 55%[8]. In our study, the amikacin and carbapenem resistance among *E. coli* was 0%, while this rate was 87% for cefotaxime and 62% for gentamicin, while the ESBL incidence was 75%.

Data from South Asia reported the methicillin resistance of *S. aureus* as 56%[8]. A study from our country reported clindamycin resistance as 54.5%, methicillin resistance as 96.1% and glycopeptide resistance as 0%[18]. All *S. aureus* strains in our study were sensitive

Table IV. Antimicrobial Resistance Rates Among Gram-Positive Microorganisms (%)

Organism	Penicillin	Ampicillin	Oxacillin	Clindamycin	Cefotaxime	Vancomycin	Teicoplanin
CoNS	100	100	80.0	60.0	100	0	0
S. aureus	100	100	100	100	100	0	0
S. viridans	100	100	-	33.3	66.6	0	0
Enterococcus spp.	100	100	-	60	80	0	0

CoNS: Coagulase-negative staphylococci.

to glycopeptide antibiotics. There was 100% resistance to penicillin, clindamycin and oxacillin. We found the CoNS oxacillin resistance as 80% while there was no glycopeptide resistance.

All enterococcus strains were resistant to both penicillin and ampicillin and there was no vancomycin resistance. It should be kept in mind that choosing glycopeptide antibiotics as the treatment for neonatal sepsis cases that are not nosocomial in origin can lead to the development of enterococci resistant to vancomycin.

The rank of isolated microorganisms and antibiotic resistance patterns did not show any difference from 2000 to 2006. Taking into account the patient profile and resistance pattern in our unit, we believe the best empirical treatment approach for the initial treatment of nosocomial sepsis is carbapenem and/or vancomycin.

The nosocomial sepsis mortality was found to be 38% in our unit, with a rate of 34.1% for very low birth weight babies. The nosocomial sepsis mortality rate in other reports ranges between 20-60%[19].

The morbidity, mortality and increased treatment cost of nosocomial infections have made it necessary to employ infection-control strategies. A surveillance system for nosocomial infections in the NICUs in our country and the development of data collection methods concerning the whole country may be useful to decrease the incidence of nosocomial infections in NICUs.

Careful attention to infection-control procedures, including proper hand-washing, increasing the number of nurses/staff per patient and the distance between the beds and devices in our unit, preparing the fluids and parenteral solutions in sterile/closed systems, and establishing a laminar flow system in the unit, thus increasing compliance with standardization suggestions, may lead to a further decrease in the nosocomial sepsis incidence and high antibiotic resistance.

In conclusion, active surveillance studies and logical antibiotic use principles will make it easier to control the microorganisms responsible for nosocomial sepsis in our unit and their antibiotic resistance.

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