RESEARCH PAPER

Healthcare-associated Infections in a Neonatal Intensive Care Unit in Turkey

*†FATIH BOLAT, *SINAN USLU, #GUHER BOLAT, *SERDAR COMERT, *EMRAH CAN, *ALI BULBUL AND *ASIYE NUHOGLU

*From the *Department of Pediatrics, Division of Neonatology, Sisli Children Hospital, Istanbul; †Department of Pediatrics, Faculty of Medicine, Cumhuriyet University, Sivas; and #Goztepe Training and Research Hospital, Istanbul, Turkey. Correspondence to: Dr Fatih Bolat, Cumhuriyet Universitesi, Tip Fakültesi, Çocuk Sagligý ve Hastaliklari Yenidogan Klinigi, Sivas, Türkiye. fatihbolat74@gmail.com*

Received: October 29, 2011; Initial review: December 01, 2011; Accepted: February 10, 2012

Objective: To determine the incidence, risk factors, mortality rate, antibiotic susceptibility and causative agents of healthcare-associated infections (HAIs) in the Neonatal Intensive Care Unit.

Design: Prospective, cohort.

Setting: A 38-bed, teaching, referral, neonatal intensive-care unit.

Participants: All patients in the neonatal intensive care unit who did not have any sign of infection at admission and remained hospitalized for at least 48 hours.

Methods: The study was conducted between January 2009 and January 2011. Healthcare-associated infection was diagnosed according to the criteria of CDC. Risk factors for HAI were analyzed with univariate and multivariate regression analysis.

Results: The incidence of HAI was found to be 16.2%. Blood stream infection was observed as the most common form of HAI (73.2%). The mortality rate was 17.3%. Antenatal steroid use, cesarean section, male gender, low birth weight, parenteral nutrition, percutaneous and umbilical catheter insertion, mechanical ventilation and low Apgar scores were found to be related with HAI (P<0.05). A 10% reduction in infection rate as a consequence of the application of a new total parenteral nutrition guideline was observed. Coagulase negative staphylococci (44. 4%) and *Klebsiella pneumoniae* (25.9%) were the most common etiologic agents isolated from cultures. Methicillin resistance of coagulase-negative staphylococci and ESBL resistance of *Klebsiella pneumoniae* were 72% and 44%, respectively.

Conclusions: Antenatal steroid was found to be associated with HAI. Newly applied total parenteral nutrition guidelines reduced the attack rate of infection. Efforts should be focused on developing more effective prevention strategies to achieve better outcomes.

Key words: *Antenatal steroid, Etiologic agents, Healthcare-associated infections, Mortality, Risk factors.*

Published online: 2012, March 30. PII:S097475591100892–1

Healthcare-associated infection (HAI) is a common complication in intensive care of critically ill patients in neonatal intensive care units (NICUs) in not only developing but also developed countries. It is a major cause of mortality and morbidity in NICUs [1,2]. The incidence ranges from 6-20%, with a wide range of variation according to birth weight and the presence of risk factors [3]. The infections are usually caused by multidrug-resistant organisms. In the literature, the overall mortality rate is reported to vary between 20% and 80%, depending on the underlying risk factors [2]. It is essential to continuously monitor the local epidemiology of HAIs to detect any changes in patterns of infections and susceptibility to various antibiotics.

There are few data about incidence and risk factors for HAIs in neonatal NICUs. The previous studies were retrospectively designed and incidence density was not calculated according to birth weight. In recent years, in a multicenter study conducted by Turkish Neonatal Society (TNS), it was reported that the prevalence of HAI among NICUs varied from 2.6% to 17% [4]. That study had not examined the infection density, invasive device related infections and risk factors for HAIs in these NICUs.

We conducted this study to determine the incidence and risk factors of HAI, the relationship between infection and medical devices, main infection site, common microorganisms, antibiotic susceptibility and mortality.

Accompanying Editorial: Pages 947-8

METHODS

Our NICU is located in Sisli Etfal Education and Research Hospital, one of the largest hospitals of Turkish Ministry of Health, and serves as a teaching facility. Approximately 5000 live births occur annually. The NICU has 38 beds (10 beds for intensive care, 20 beds for

intermediate care and 8 beds for continuous care of neonates) and provide intensive care to about 800-1000 newborn patients annually. This NICU has medical staffing consisting of three full-time neonatologists, three neonatology fellows, four pediatric residents, 35 neonatal nurses. One NICU nurse cares for 2-3 babies in intensive care, 4-5 babies in intermediate care and 6-8 babies in continuous care.

This study was conducted over two years between January 2009 to January 2011 in NICU of Sisli Etfal Education and Research Hospital. This is a prospective cohort study. All patients admitted to the NICU without any sign of infection, who remained hospitalized for at least 48 hours were eligible for inclusion. Neonates discharged before 48 hours or those who had perinatal and community-acquired infections were excluded from the study. The study protocol was approved by institutional review board.

Demographic, clinical and microbiological data were prospectively collected and recorded on standardized form 5 times a week until discharge from the hospital or death. The data consisted of patient information about antenatal history (including betamethasone administration), APGAR scores, need for resuscitation and the procedures applied such as respiratory support and catheter insertion.

Healthcare-associated infection was defined as an infection not present and without evidence of incubation at the time of hospitalization. The diagnosis of infection based on clinical symptoms (fever, hypothermia, apnea, bradycardia, lethargy, hypotonia, unstable vital signs, and feeding intolerance, etc.), laboratory findings (leukocytosis or leukopenia, thrombocytopenia, elevated C reactive protein and immature / total neutrophil ratio) and positive blood cultures [5].

In all suspected patients, blood cultures were taken. When needed, urine and tracheal aspirate cultures were added. If the patient had a device or an operation, application or incision site cultures were obtained. Lumbar puncture and CSF culture were performed in all patients who had bacterial growth in blood culture or clinical signs of meningitis.

A blood stream infection (BSI) was defined as isolation of at least one positive peripheral-blood culture, except coagulase-negative staphylococcus, for which isolation of two positive blood cultures was required [6]. Ventilator-associated pneumonia (VAP) was defined as a pneumonia developing after 48 hours of mechanical ventilation with radiological, clinical and microbiological findings consistent with a positive tracheal aspiration fluid culture [7]. Catheter related bloodstream infection (CRBSI) was defined as the isolation of the same microbe from blood cultures that is shown to be significantly colonizing the catheter of a patient with clinical features of BSI in the absence of any other local infection caused by the same microbe [8]. Urinary tract infection was diagnosed if the bacterial pathogens were detected at $\geq 10^5$ CFU/mL of no more than two isolated species and 10^2 CFU/mL if associated with consistent symptoms and pyuria [9]. Postoperative wound infections were defined as surgical wounds occurring less than 30 days after surgery (i.e. superficial/ deep surgical site infections) [10].

To assess the effect of birthweight on infection rate, all newborns were stratified to four categories: <1000 g, 1000–1500 g, 1501–2500 g and >2500 g.

Microbiological data and infection rates: Blood specimens were inoculated into a blood culture media: BACTEC 9240 (Becton Dickinson, USA). Isolates of bacteria were identified by conventional biochemical and serological methods. Isolation of *Bacillus* spp., *Corynebacterium* spp. and coagulase-negative staphylococcus recovered from a single culture were considered as contaminants. The antibiotic susceptibility for isolated pathogens was tested by the disk diffusion, employing the criteria of the National Committee for Clinical Laboratory Standards [11]. Isolates were screened for ESBL production using MacConkey agar with cefotaxime.

The incidence was calculated as number of infections per 100 patients admitted, and incidence density as number of infections per 1000 days. Invasive device (mechanical ventilation and catheter) related infections were calculated as CRBSI per 1000 central venous catheterization exposure days and VAPs per 1000 ventilator days.

Chi-square and Fisher's exact chi-square tests were used for categorical variables. Student *t* test or Mann-Whitney U test were used for continuous variables. Univariate and multivariate logistic regression models analysis were performed to compare risk factors associated with HAI. Analysis of mortality was performed using Cox's proportional hazards modeling. *P* value <0.05 was considered statistically significant.

RESULTS

A total of 1713 patients were admitted to NICU during the two year period. Three hundred and eighteen were excluded for the following reasons: 114 died or were discharged from the NICU in the first 48 hours, 204 had perinatal or community-acquired infections. One

hundred and fifty-six infants developed 227 HAI episodes. Most of these infections were detected in newborns admitted to intensive and intermediate care. Episodes of HAI which were 127 (55.9%) between January 2009 and 2010, decreased to 100 (44.1%) between January 2010 and 2011. Number of episodes was shown to decrease by 10% within one year.

Total length of hospital stay in NICU was 21884 days and HAI incidence rate was 10.3:1000 days. Newborns with and without HAI are evaluated and compared for presence of risk factors (***Table* I**). The most common HAI was blood stream infection (66.7%), followed by ventilator-associated pneumonia (16%), catheter related bloodstream infection (14.7%), urinary tract infection (1.3%) and surgical wound infection (1.3%).

One hundred and four patients had episodes of 166 BSI. The mean overall BSI rate was 4.7:1000 days. Coagulase-negative staphylococcus was the most commonly isolated agent in hemocultures of patients with BSI (***Table* II**). All of six newborns with *Candida* infection had birth weight <1500 g.

Birthweight, umbilical and percutaneous catheter, mechanical ventilation, and total parenteral nutrition were significantly associated with BSI in univariate analyses ($P<0.05$). Total parenteral nutrition [RR: 3.3 (95% CI; 1.3-8.4 $P<0.0001$)], percutaneous catheter use [RR: 6.0 (95% CI;3.4-10.6 $P<0.001$)] and mechanical ventilation [RR: 4.0 (95% CI; 2.2-7.0) $P<0.001$)] achieved significance with multivariate logistic regression analysis. Among 104 infants with BSI, 15 died and mortality rate was 14.4%.

TABLE II MICROORGANISMS CAUSING BLOOD STREAM INFECTION

Microorganisms	*Number (%)*
Coagulase-negative staphylococcus	87 (52.4)
Coagulase- positive staphylococcus	11 (6.6)
Klebsiella pneumonia	32 (19.3)
Pseudomonas aeruginosa	4 (2.4)
Enterococcus	2 (1.2)
Acinetobacter	10 (6)
Escherichia coli	3 (1.8)
Enterobacter	11 (6.6)
Candida albicans	6 (3.6)

Figures in parentheses indicate percentages.

Presence of clinical and laboratory features of 48 patients were consistent with meningitis. Twelve isolates were detected from cerebrospinal fluid cultures. Coagulase-negative staphylococcus was the most common pathogen, accounting for 4 of 12 isolates, followed by *Klebsiella pneumonia* (*n*=3), *Pseudomonas aeruginosa* (*n*=2), *S. aureus* (*n*=2), *Acinetobacter* (*n*=1).

Of 1395 patients, 92 required only intubation, 140 only CPAP, 143 both intubation and CPAP. In 25 patients, 28 VAP episodes developed. Birth weights of 11 patients with VAP (44%) were less than 1000 g, 7 (28%) were 1000 to 1500 g, 7 (28%) were above 1500g. The total

TABLE I UNIVARIATE ANALYSIS OF RISK FACTORS

Characteristics	*HAI(-)(n=1239)*	*HAI(+)(n=156)*	*RR 95% CI*	*P value*
Antenatal steroid use (%)	89 (7.2)	50 (32.1)	3.36 (1.6-4.3)	<0.001
Cesarean delivery (%)	719 (58)	137 (87.8)	2.0 (1.3-4.4)	0.02
Male gender (%)	673 (54.3)	100 (64.1)	1.56 (1.1-2.38)	0.04
Birthweight (g) (%)				
<1000	26 (2.1)	33 (21.2)	30.6 (16.5-56.7)	<0.001
1000-1500	60 (4.8)	38 (24.4)	15.2 (8.9-26)	<0.001
1501-2500	333 (26.9)	51 (32.7)	3.6 (2.3-5.8)	<0.001
> 2500	820 (66.2)	34 (21.8)	Reference	
TPN (%)	340 (22.4)	138 (88.5)	8.4 (4.8-14.7)	<0.001
Percutaneous catheter (%)	82 (6.6)	106 (67.9)	7.1 (4.3-12.5)	<0.001
Mechanical ventilation (%)	129 (10.4)	106 (67.9)	18.2 (12.4-26.7)	<0.001
Umblical venous catheter (%)	425 (34.3)	122 (78.2)	6.8 (4.6-10.2)	<0.001
5 min APGAR scores, means±SD	8.6 ±1.4	7.7±1.7		

Figures in parentheses indicate percentages; TPN: Total parenteral nutrition; HAI (+): neonates with healthcare-associated infection;HAI(-) neonates without healthcare-associated infection;RR: relative risk and CI: confidence interval.

duration of CPAP and intubation were 2002 and 1818 days, respectively. Duration of nasal CPAP was longer in patients with VAP (nCPAP median 17 *vs*. 2 days $P<0.001$). The duration of endotracheal intubation was longer in patients with VAP (intubation median days: 19 *vs*. 3 $P<0.001$). Ventilator-associated pneumonia rate was 15.4:1000 intubation days. Birth weight, endotracheal intubation, presence of bronchopulmonary dysplasia and duration of hospital stay were found to be associated with increased risk of VAP on univariate analysis ($P<0.05$). The most common pathogen isolated from VAP cases was *K. pneumoniae*. The distribution of pathogens causing VAP is shown in ***Table* III**. Eight infants died due to VAP and mortality rate was 32%. Of these infants, birth weight of 5 were <1000 g, 2 were 1000 to 1500 g and 1 was >1500 g.

The catheter was inserted to five hundred and seventy-nine patients. Of these patients, 390 (28%) had only umbilical venous catheter, 32 (2.3%) had only percutaneous catheter, 157 (11.2%) had both umbilical venous catheter and percutaneous catheter. Total catheter days were 7132 days. Twenty-six CRBSI episodes developed in 23 patients. All of the patients with CRBSI were born less than 2500 g. Catheter-related blood stream infection rate was 3.64:1000 device days. Coagulase-negative staphylococcus (46.1%) was the most common catheter related bloodstream infection isolate, followed by *K. pneumoniae* (19.2%), coagulase-positive staphylococcus (15.5%), *Enterobacter* (15.4%), *Candida albicans* (3.8%). There were colonizations with coagulase-negative staphylococcus and *C. albicans* in two of patients with catheters. Three infants, whose birth weights were less than 1000 g, one of whom had bronchopulmonary dysplasia and the other two had hydrocephalus, died. The mortality rate was 11.5%.

TABLE III ETIOLOGIC AGENTS ISOLATED FROM CULTURES IN VENTILATOR ASSOCIATED PNEUMONIA CASES

Microorganisms	*TAC (n=28)*	*Blood culture (n=28)*
Klebsiella pneumoniae, n (%)	13 (46.4)	9 (32.1)
Pseudomonas aeroginosa, n (%)	5 (17.9)	2 (7.1)
Acinetobacter, n (%)	2 (7.1)	2 (7.1)
Coagulase-negative staphylococci, *n* (%)	–	6 (21.4)
Coagulase-positive staphylococcus, *n* (%)	–	1 (3.6)
Enterococcus species, *n* (%)	–	1 (3.6)
No growth, *n* (%)	8 (28.6)	7 (25.0)

Figures in parentheses indicate percentages; TAC: tracheal aspirate culture.

According to birth weight, distribution of CRBSI, VAP and BSI were shown in ***Table* IV**.

Fifteen patients had a urinary catheter. Out of the 15 patients, birth weights of 9 were 1500 to 2500 g, 6 were above 2500 g. Twenty-eight urine specimens were obtained for culture from 25 patients with suspected healthcare urinary tract infections. Positive urine cultures were found in only four of the patients. In all positive urine cultures *Klebsiella pneumonia* was isolated. A total of urinary catheter day was 65 days. Urinary tract infection related catheter rate was 62.5:1000 catheter days.

Forty-eight surgical interventions were performed during the study. Types of surgical procedures were neurosurgical (66.7%), cardiovascular (12.5%), gastrointestinal (12.5%), and other surgery types (8.3%). Three postsurgical infections developed in two patients. In three wound swab culture, methicillin resistant *Staphylococcus aureus* were isolated.

Microbiological characteristics: Of 278 pathogens isolated from cultures, 121 (43.5%) were coagulase-negative staphylococcus, 71 (25.5%) *Klebsiella pneumonia*, 25 (9%) coagulase-positive staphylococcus, 19 (6.8%) *Enterobacter,* 15 (5.4%) *Acinetobacter,* 13 (4.7%) *Pseudomonas aeruginosa*, 3 (1.1%) *Escherichia coli, 3 (1.1%) Enterococcus spp.* and 8 (2.9%) *Candida albicans*. All the isolates of coagulase-negative staphylococcus, coagulase-positive staphylococcus, *Enterococcus spp.* were sensitive to vancomycin. ESBL

TABLE IV INFECTION RATES ACCORDING TO HEALTH-CARE ASSOCIATED INFECTION TYPES

	<1000 g	*1000-1500 g*	*1501-2500 g*	*>2500 g*
BSI				
Episodes, *n*	47	44	48	27
Hospitalization, d	3548	3887	7836	6613
BSI rate, per 1000 d	13.2	11.3	6.1	4.08
VAP				
Episodes, *n*	11	7	8	2
Intubation days	718	377	521	202
VAP rate, per 1000 d	15.3	18.5	15.3	9.9
CRBSI				
Episodes, *n*	5	9	12	–
Catheter days	2161	1488	2322	1161
CRBSI rate, per 1000 d	2.3	6	5.1	–

BSI: Blood stream infection; VAP: ventilator associated pneumonia; CRBSI: Catheter related blood stream infection.

WHAT IS ALREADY KNOWN?

- Healthcare-associated infections are associated with an increased risk of neonatal morbidity and mortality in neonatal intensive care units.

WHAT THIS STUDY ADDS?

- Coagulase negative staphylococcus was the most frequent pathogen in the NICU.

production of *Klebsiella pneumonia* was found as 44%. Ceftazidime resistance for *P. aeruginosa* and cefotaxime resistance for *Enterobacter* were 27.2% and 22.2%, respectively. The seven *Candida* isolates were also susceptible to amphotericin B, fluconazole and itraconazole.

A total of 58 infant died. The overall mortality in our study sample was 4.1% (58/1395). Of these, 31 (2.5%) did not have HAI and 27 (17.3%) had HAI. Mortality tended to be higher in patients who had at least one HAI than those without HAI (*P*: 0.02). In the Cox proportional hazards models, the other factors significantly associated with mortality were total parenteral nutrition, percutaneous catheter use and mechanical ventilation (***Table* V**).

DISCUSSION

In our study, the incidence of HAI was 10.3:1000 patient days and 16.2 infections per admissions, which was consistent with the results of the below mentioned studies. Incidence of HAI was reported to vary between 6.2 and 50.7 infections per 100 admissions, and between 4.8 and 62 infections per 1000 patient days at various centers in the previous studies [12-15]. It is stated that this discrepancy between neonatal units could be possibly due to underlying differences in patient populations studied, care practices, surveillance methods and study designs.

We observed that neonates, exposured to antenatal steroids for pulmonary maturation, had high risk of developing HAI. In several studies, it is identified to be associated with an increased number of hospital admissions due to infectious diseases not only in the neonatal period but also in early childhood, possibly demonstrating some proof of significant immune system suppression in offspring due to antenatal betamethasone exposure [16-19].

As a Health Improvement Project, great efforts have been made by Health Ministry to reduce the neonatal mortality and morbidity rates in recent years. A circular, consisting of a guideline about safe parenteral nutrition was sent to all academic, private and state hospitals [20]. A 10% reduction in the number of episodes of HAI between 2010 and 2011 can be attributed to the application of this new consensus guideline.

In some studies, umbilical catheterization was observed to be the most important risk factor for the development of HAI [21-24]. Yet, we observed that MV had the highest calculated risk for developing HAI. The umbilical catheterization was found to be the least risky

TABLE V FACTORS AFFECTING MORTALITY

Variables	*Survival (n=1329)*	*Non-survival (n=58)*	*Hazard Ratio (95% CI)*	*P value*
Birthweight, *n* (%)				
<1000 g	31 (2.3)	28 (48.3)	5.4 (2.2-13.0)	<0.001
1000-1500 g	84 (6.3)	14 (24)	0.8(0.3-2.1)	0.6
1501-2500 g	381 (28.7)	3 (5.2)	0.1 (0.03-0.4)	<0.001
>2500 g	833 (62.7)	13 (22.6)	Reference	1
HAI n, %	129 (9.7)	27 (46.6)	2.5 (1.1-5.8)	0.02
TPN n, %	423 (31.8)	55 (94.8)	6.1 (1.5-24.3)	0.009
Percutaneous catheter, *n* (%)	166 (12.5)	22 (37.9)	7.8 (3.0-20.8)	<0.001
Mechanical ventilation, *n* (%)	179 (13.5)	51 (87.9)	8.4 (3.3-21.7)	<0.001

Figures in parentheses indicate percentages; HAI: healthcare-associated infection; TPN :Total parenteral nutrition; CI Confidental interval.

intervention. This difference may be attributed to the sterile practices during catheter insertion, the microenvironment or colonization of NICU and the infant, presence of co-morbidities and duration of catheter use but especially to our principle of early shifting from umbilical catheter to percutaneous catheter whenever possible and assigning a well-educated and experienced team which is responsible for insertion and optimal care of the catheter.

We found coagulase-negative staphylococcus as the most common pathogen causing HAI. Compared with the results of a previous study conducted in our NICU two years ago [25], there seems to be no change in etiologic agents. This is unlike the report of Richards, *et al*. [26], which shows a microbiological shift in their NICU from gram-positive to gram-negative organisms during the six years surveillance period.

Along with previously stated limitations, this is a clinical microbiological study rather than an epidemiological study. Molecular epidemiologic analysis of microorganism types could not be performed due to limited facilities of our institution. The subtypes, and antibiotic resistance genes detected with PCR could have offered more information. Due to the lack of routine screening for every infant admitted to the NICU, the organisms responsible for clinical infection did not allow for a reliable assessment of the ratio colonized in infected patients and any causal association between colonization and infection. Since this is a single-center study with a limited number of patients, the results are difficult to apply directly to other hospitals. Further studies preferably multi-centric are needed to confirm our results.

While blood stream infection and catheter-related blood stream infection rates were similar to the results from developed countries, VAP rate was higher than previously reported from, [24,27]. Coagulase-negative staphylo-coccus was the most frequent pathogen in the NICU. Methicillin resistance rate of coagulase-negative staphylococcus have also increased recently. In neonates who are already prone to infection because of premature immune system, the use of corticosteroids may contribute to infection rate. However, we need to know much more about antenatal steroid-HAI interactions, and further experimental studies are required. A 10% reduction in infection rate as a consequence of application of a new total parenteral nutrition guideline was observed. These data provide valuable information for control and prevention of healthcare-associated infections in the future.

Contributors: FB and SU designed the study. FB, EC and AB collected data. FB, SU, GB, SC, AN revised the manuscript for important intellectual content. The final version of the manuscript was approved for publication by all authors.

Funding: None; *Competing interests:* None stated.

REFERENCES

1. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, *et al*. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics. 2002;110:285-91.
2. Zaidi AK, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA. Hospital-acquired neonatal infections in developing countries. Lancet. 2005;365:1175-88.
3. Adams-Chapman I, Stoll BJ. Prevention of nosocomial infections in the neonatal intensive care unit. Curr Opin Pediatr. 2002;14:157-64.
4. Turkish Neonatal Society; Nosocomial Infections Study Group. Nosocomial infections in neonatal units in Turkey: epidemiology, problems, unit policies and opinions of healthcare workers. Turk J Pediatr. 2010;52:50-7.
5. Garner JS, Favero MS. CDC guidelines for the prevention and control of nosocomial infections. Guideline for handwashing and hospital environmental control, 1985. Supersedes guideline for hospital environmental control published in 1981. Am J Infect Control. 1986;14:110-29.
6. Macharashvili N, Kourbatova E, Butsashvili M, Tsertsvadze T, McNutt LA, Leonard MK. Etiology of neonatal blood stream infections in Tbilisi, Republic of Georgia. Int J Infect Dis. 2009;13:499-505.
7. Apisarnthanarak A, Holzmann-Pazgal G, Hamvas A, Olsen MA, Fraser VJ. Ventilator-associated pneumonia in extremely preterm neonates in a neonatal intensive care unit: characteristics, risk factors, and outcomes. Pediatrics. 2003;112:1283-9.
8. Tavora AC, Castro AB, Militao MA, Girao JE, Ribeiro Kde C, Tavora LG. Risk factors for nosocomial infection in a Brazilian neonatal intensive care unit. Braz J Infect Dis. 2008;12:75-9.
9. Su BH, Hsieh HY, Chiu HY, Lin HC. Nosocomial infection in a neonatal intensive care unit: a prospective study in Taiwan. Am J Infect Control. 2007;35:190-5.
10. Raymond J, Aujard Y. Nosocomial infections in pediatric patients: a European, multicenter prospective study. European Study Group. Infect Control Hosp Epidemiol. 2000;21:260-3.
11. Wayne PA. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing: 12th International Supplement: National Committee for Clinical Laboratory Standards. 2002.
12. Brito DV, de Brito CS, Resende DS, Moreira do OJ, Abdallah VO and Gontijo Filho PP. Nosocomial infections in a Brazilian neonatal intensive care unit: a 4-year surveillance study. Rev Soc Bras Med Trop. 2010;43: 633-7.
13. Tian LY, Hamvas A. Risk factors for nosocomial bloodstream infections in a neonatal intensive care unit. Zhongguo Dang Dai Er Ke Za Zhi. 2010;12:622-4.
14. Auriti C, Ronchetti MP, Pezzotti P, Marrocco G, Quondamcarlo A, Seganti G, *et al*. Determinants of

nosocomial infection in 6 neonatal intensive care units: an Italian multicenter prospective cohort study. Infect Control Hosp Epidemiol. 2010;31:926-33.
15. Sarvikivi E, Karki T, Lyytikainen O. Repeated prevalence surveys of healthcare-associated infections in Finnish neonatal intensive care units. J Hosp Infect. 2010;76:156-60.
16. MacArthur BA, Howie RN, Dezoete JA, Elkins J. Cognitive and psychosocial development of 4-year-old children whose mothers were treated antenatally with betamethasone. Pediatrics. 1981;68:638-43.
17. Schmand B, Neuvel J, Smolders-de Haas H, Hoeks J, Treffers PE, Koppe JG. Psychological development of children who were treated antenatally with corticosteroids to prevent respiratory distress syndrome. Pediatrics. 1990;86:58-64.
18. Doyle LW, Kitchen WH, Ford GW, Rickards AL and Kelly EA. Antenatal steroid therapy and 5- year outcome of extremely low birth weight infants. Obstet Gynecol. 1989;73:743-6.
19. London TD. Prediction of fetal lung maturity. Am J Obstet Gynecol. 1980;137:152.
20. Turkish Ministry of Health, Nosocomial Infection Prevention Scientific Committee Consensus on Guidelines of safe practices for parenteral nutrition. Available from: URL: http://www.saglik.gov.tr/TR/1-10553/ Guideline of safe practices for parenteral nutrition.html. Accessed June 17, 2010.
21. Gaynes RP, Edwards JR, Jarvis WR, Culver DH, Tolson JS, Martone WJ. Nosocomial infections among neonates in high-risk nurseries in the United States. National Nosocomial Infections Surveillance System. Pediatrics. 1996;98:357-61.
22. Pessoa-Silva CL, Richtmann R, Calil R, Santos RM, Costa ML, Frota AC, *et al*. Healthcare-associated infections among neonates in Brazil. Infect Control Hosp Epidemiol. 2004;25:772-7.
23. Perlman SE, Saiman L, Larson EL. Risk factors for late-onset health care-associated bloodstream infections in patients in neonatal intensive care units. Am J Infect Control. 2007;35:177-82.
24. Babazono A, Kitajima H, Nishimaki S, Nakamura T, Shiga S, Hayakawa M, *et al*. Risk factors for nosocomial infection in the neonatal intensive care unit by the Japanese Nosocomial Infection Surveillance (JANIS). Acta Med Okayama. 2008;62:261-8.
25. Bulbul A, Tasdemir M, Pullu M, Okan F, Bulbul L, Nuhoglu A. Nosocomial infection in the neonatal intensive care unit. Medical Bulletin of Sisli Etfal Hospital. 2009;43:27-32.
26. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in pediatric intensive care units in the United States. National Nosocomial Infections Surveillance System. Pediatrics. 1999;103:e39.
27. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control. 2004;32:470-85.