

Study of the bacterial agents in nosocomial and acquired infections based on the blood culture in neonatal intensive care unit of a hospital, north east of Iran

Mahbobeh Naderi-Nasab, PhD, Ahmadshah Farhat, MD, Parasto Tajzadeh, MSc, Setareh Sourosh, MSc, Mehvar Amiri, BSN.

ABSTRACT

Objective: To study the distribution of bacterial agents in bloodstream infections among hospitalized patients in the neonatal intensive care unit (NICU).

Methods: From March 2003 to February 2004, 1341 blood samples were taken from hospitalized infants in NICU of Imam Reza Hospital in Mashhad north east of Iran. In this study, 6 ml of blood was extracted from the patients having septicemia, which was then added to the bottle containing the blood culture broth. The bottles were labeled and incubated at 35°C for maximum of 7 days.

Results: The result shows that out of the 202 patients with positive blood cultures 109 had gram positive organisms while 93 had gram negative organisms. In those with gram-positive bacteria, 73 cases were acquired infection and 36 cases were nosocomial infection. These results for gram negative bacteria showed that 51 cases were due to acquired infection and 42 cases due to nosocomial infection. In determining the relation between the rate of death and the type of infection, we found that out of 202 patients 83 (41%) died. Of these cases 48 (39%) were due to acquired infection and 35 (45%) were due to nosocomial infection.

Conclusion: We conclude that nosocomial bloodstream infection is an important target for the most aggressive strategies for prevention and control.

Saudi Med J 2007; Vol. 28 (5): 723-726

From the Department of Microbiology (Naderi-Nasab, Tajzadeh, Sourosh), Neonatal Intensive Care Unit (Farhat), Department of Nursing (Amiri), Imam Reza Hospital, Medical School, University of Mashhad, Mashhad, Iran.

Received 17th September 2006. Accepted 20th December 2006.

Address correspondence and reprint request to: Dr. Mahbobeh Naderi-Nasab, Microbiology Lab, Imam Reza Hospital, Medical School, University of Mashhad, Mashhad, Iran. Tel. +98 (511) 7685785. Fax. +98 (511) 7636185. E-mail: mnaderinasab@yahoo.com

Although nosocomial infections have been identified and their importance has been known for many years, they are still considered as a significant world problem. Considering the high infection rate among patients, their high treatment expenses, the high rate of mortality and the resistance of these hospital pathogen microorganisms to antibiotics, specific attention and proper measurements especially in the prevention of nosocomial infections is quite necessary.^{1,2} Consequently, we decided to study the causes of nosocomial infections in neonatal intensive care unit (NICU) in Imam Reza Hospital using sample of blood cultures.

Bloodstream infections cause substantial morbidity and mortality. Increasing numbers of antimicrobial resistance, changing patterns of antimicrobial usage and the wide application of new medical technologies (such as, indwelling catheters and other devices) may change the epidemiology and outcome of bloodstream infection.

The role of the environment, hospital staff and the patient population in a hospital ward in preventing infection or making nosocomial infection into endemic or epidemic form has been proven.³⁻⁵

Coagulase-negative staphylococci (CONS) and among them especially *Staphylococcus epidermidis* have recently been considered as a serious pathogen in nosocomial infections in NICU and have been isolated from blood culture.⁶⁻⁹

In this study, we have studied the rate of nosocomial infection, bacterial causes and the distribution of the bacterial in the bloodstream infection among hospitalized patients in NICU ward of Imam Reza Hospital. The aim of this research is to study the distribution of bacterial agents in bloodstream infections among hospitalized patients, ratings and assessment of bacterial infections, which cause nosocomial and acquired infections in these patients in NICU.

Methods. This retrospective study was carried out on March 2003 to February 2004 using the blood culture results of hospitalized infants in NICU of Imam Reza Hospital, Mashhad, Iran. The NICU of Imam Reza Hospital has 25 beds, of which 15 beds are dedicated to receiving inborn babies and 10 beds to out born babies.

Blood culture are sent for all infants suspected to have systemic infection. The bottles used for the blood culture held 30 ml of brain heart infusion broth, which were sent to the ward for sampling. Six ml of blood was extracted from the patients having septicemia, which was then added to the bottle containing the blood culture broth. The bottles were labeled and then transferred to the lab; the samples were incubated at 35°C for a maximum of 7 days, except for those cultures that became positive at an earlier stage.

To control the growth of bacteria, some blood was extracted from the blood culture bottle using a sterile syringe and was cultured in the blood agar, eosin methylene blue agar and chocolate agar media plates and then blood culture bottles and solid media were incubated. Only the chocolate agar was incubated under 10% CO₂. If the results of the culture in the solid media were positive, the gram smears were prepared from the colonies and then the bacteria were identified to the species level and antimicrobial susceptibility test were performed, the result of the culture and antimicrobial susceptibility test were sent to the ward after being prepared.

The antimicrobial susceptibility test was determined using the disk diffusion method according to the recommendation of the National Committee for Clinical Laboratory Standards. The antibiotic discs were chosen based on gram positive and negative bacteria and after 18 to 24 hours of the incubation of antibiogram plates, the results were assessed following the recommended NCCLS table and were reported as R (resistance), S (sensitive), and I (intermediate).

Results. During the study, 1341 blood samples were sent from NICU to the diagnostic microbiology lab. Out of 1341-blood culture, 7 isolates were gram-positive non-pathogen bacilli and *micrococci*. After contact with the ward and studying the clinical symptoms, it became evident that their blood cultures were contaminated and they were omitted from the study. A total of 248 samples were positive for bacteria that belong to 233 patients, this means that 15 patients had 2 positive blood cultures. Out of 233 patients, 31 patients were either discharged from the NICU or we could not trace their record so they were omitted from our study. Consequently, the number of patients that had positive blood cultures was 202.

The bacteria isolated from blood cultures according to their gram stain and type of infection is summarized in **Table 1**. Coagulase-negative staphylococci was the highest organism isolated from 79 cases, whereas *Citrobacter* was the lowest organism isolated from only one case. Gram positive pathogens constituted the majority of acquired infection, infection with CONS being the most common pathogen in both acquired and nosocomial infection (**Table 2**).

Table 1 shows that one third of the studied patients (39%) who had septicemia and positive blood culture had nosocomial infection.

The type of bacteria from acquired and nosocomial infection is summarized in **Table 2**. Gram-positive bacteria were seen mainly with acquired infections, while the gram-negative bacteria were seen mainly with nosocomial infections. However, CONS caused the

Table 1 - Summary of bacteria isolated from blood culture according to gram stain and type of infection.

Type of microorganisms	No. of bacteria isolated (%)		Total
	Acquired infection	Nosocomial infection	
Gram positive bacteria	73 (67)	36 (33)	109
Gram negative bacteria	51 (55)	42 (45)	93
Total	124 (61)	78 (39)	202

Table 2 - Summary of pathogens detected from acquired and nosocomial bloodstream infection.

Type of microorganisms	No. of pathogens (%)		Total
	Acquired infection	Nosocomial infection	
Coagulase-negative staphylococci	55 (70)	24 (30)	79
<i>Klebsiella pneumoniae</i>	19 (46)	22 (54)	41
<i>Staphylococcus aureus</i>	12 (52)	11 (48)	23
<i>Enterobacter aerogenes</i>	11 (52)	10 (48)	21
<i>Escherichia coli</i>	11 (58)	8 (42)	19
<i>Acenitobacter</i> species	5 (71)	2 (29)	7
<i>Enterococcus</i>	4 (100)	-	4
<i>Pseudomonas aeruginosa</i>	4 (100)	-	4
<i>Streptococcus pyogenes</i>	2 (67)	1 (33)	3
<i>Citrobacter</i> species	1 (100)	-	1
Total	124	78	202

majority of both nosocomial and acquired bloodstream infections (Table 1 & 2).

The types of the selected microorganisms are seen by their rate of distribution in Table 2. The CONS had the highest incidence of acquired infections whereas *Klebsiella pneumoniae* (*K. pneumoniae*) had the highest incidence of nosocomial infections among gram negative organisms (54%) (Table 2).

In determining the relation between the rate of death and the type of infection, we found that out of 202 patients, 83 (41%) died. Of these cases 48 (39%) were due to acquired infection and 35 (45%) were due to nosocomial infection. Gram positive bacteria were the cause of 36 cases of death of which 22 (61%) were due to acquired infection and 14 (39%) cases were due to nosocomial infection. Forty seven cases of death were related to gram negative bacteria, in which 26 (55%) cases were acquired infection and 21 (45%) cases of nosocomial infection was the cause of death. Fifty five cases (70%) of CONS were the cause of acquired infection of which 18 (32%) cases died while 24 cases (30%) was the cause of nosocomial infection of which 8 cases (32%) died. The mortality rate relating to nosocomial infections are higher than that relating to acquired infection (Table 2 & 3).

Table 3 shows *Acinetobacter* than *E. aerogenes* follows *E. coli* as the main cause of mortality in both groups.

Discussion. Up to the 1970s, the major cause of nosocomial infections has been gram negative microorganisms but at the present time, there has been a

shift to a predominance of gram positive microorganisms as the cause of nosocomial bloodstream infections.¹⁰

Most epidemiology studies of bloodstream infection focus on nosocomial infection alone. Our study examines both acquired and nosocomial bloodstream infections, allowing us to estimate the proportion of all bloodstream infection mortality that is associated with nosocomial versus acquired infection.

In this study, 202 episodes of bloodstream infection were identified of which 39% were due to nosocomial infection. Diekema et al¹⁰ reported 52% of all isolates were nosocomial infection.

Hsueh et al¹¹ reported the increase in nosocomial methicillin resistant *Staphylococcus aureus* bloodstream infection from 26.3% in 1986 to 77% in 2001.¹¹ More frequent reports on *Staphylococcus epidermidis* (*S. epidermidis*) sepsis began to appear in the early 1980, with Goldman et al¹² finding that although gram negative bacilli were still the pre dominant cause of nosocomial infection at Children's Hospital in Boston from 1974 to 1978, *S. epidermidis* was the principal gram positive organism recovered during this period.

Hoogkamp-Korstanje et al¹³ in 1977 in Holland reported that coagulase-negative staphylococcus infection occurred in 33% of cases in the NICU. Krediet et al⁸ and Burnie et al¹⁴ too, have considered the CONS as the highest cause of hospital infections. According to the report by Miragaia et al¹⁵ in Denmark, the infection caused by *Staphylococcus aureus* had a lower epidemic due to their precise control but *S. epidermidis* were the more recurrent cause of hospital infection in these areas.

Table 3 - Summary of mortality by organisms for both acquired and nosocomial bloodstream infection.

Type of microorganisms	Number of patients with acquired infection		Number of patients with nosocomial infection	
	Recovery (%)	Death (%)	Recovery (%)	Death (%)
Coagulase-negative staphylococci	37 (67)	18 (32)	16 (67)	8 (33)
<i>Klebsiella pneumoniae</i>	11 (58)	8 (42)	13 (59)	9 (41)
<i>Staphylococcus aureus</i>	10 (83)	2 (17)	6 (55)	5 (45)
<i>Enterobacter aerogenes</i>	5 (45)	6 (55)	5 (50)	5 (50)
<i>Escherichia coli</i>	3 (27)	8 (73)	3 (37)	5 (63)
<i>Acinetobacter</i> species	2 (40)	3 (60)	-	2 (100)
<i>Enterococcus</i>	3 (75)	1 (25)	-	-
<i>Pseudomonas aeruginosa</i>	4 (100)	-	-	-
<i>Streptococcus pyogenes</i>	1 (50)	1 (50)	-	1 (100)
<i>Citrobacter</i> species	-	1 (100)	-	-

Villari et al⁹ reported the rate of nosocomial infection as 18.7% at the University of Federico II, Italy they also confirmed that CONS are becoming increasingly important nosocomial pathogens in NICU especially *S. epidermidis* as the principal organism recovered (39.8%). In this study, *K. pneumoniae* was the third cause of nosocomial infection (16.3%). While in our study, *K. pneumoniae* had the highest rate (54%) of nosocomial infection.

In a study carried out in the NICU of the Mackay hospital in Taiwan, CONS (20.1%) was the most common organism isolated from blood cultures and the *Enterobacteriaceae* including *E.coli*, *K. pneumoniae* were the most recurrent.¹⁶

In the present study, gram positive bacteria were the most common cause of septicemia infections in the NICU and among them CONS had the highest rate.

Among all of the patients with positive blood culture in this study, nosocomial and acquired bloodstream infection, the mortality rate had been 41%, 45% of which had nosocomial infection and 39% had acquired infection. This shows that the mortality rate among patients with nosocomial infection was more than the patients with acquired infection. The same result has been reported by Diekema et al¹⁰ in patients with nosocomial infection, the mortality rate among the patients with septicemia infection at the NICU was 24% and the mortality rate among the patients with nosocomial infection was 34% while patients with acquired infection was 14%.

Escherichia coli was the most common cause of mortality in nosocomial and acquired infection. After *E. coli*, *Enterobacter* and then *K. pneumoniae* had a high mortality rate in both group. Jaing et al¹⁶ reported bloodstream infection caused by *Pseudomonas aeruginosa* had a high mortality rate (55%).

According to Schabery et al,¹⁷ the gram positive bacteria were recognized as the most important cause in nosocomial infections and mortality. Also in our study, we found the highest rate of nosocomial infections were gram positive bacteria but the highest mortality rate in nosocomial infections was with gram negative bacteria (45%).

Out of 78 cases of nosocomial infection, *K. pneumoniae* had the highest percentage (54%) of infection. Our study shows that the mortality rate of patients with nosocomial infection are between 33-100% while the mortality rate among the acquired infection was between 17-100%. This shows the importance of nosocomial infections.

We conclude that nosocomial bloodstream infection is an important target for the most aggressive strategies for prevention and control.

References

1. Wachino J, Doi Y, Yamane K, Shibata N, Yagi T, Kubota T, et al. Nosocomial spread of ceftazidime resistant strains producing a novel class A β -lactamase, GES-3, in a neonatal intensive care unit in Japan. *Antimicrob Agents Chemother* 2004; 48: 1967-1960.
2. Yan J, Kasi S, Chuang C. Epidemiological investigation of bloodstream infections by extended spectrum cephalosporin resistant in a Taiwanese teaching hospital. *J Clin Microbiol* 2004; 42: 3332-3329.
3. Huebner J, Pier GB, Maslow JN, Muller E, Shiro H, Parent M, et al. Endemic nosocomial transmission of *Staphylococcus epidermidis* bacteremia isolates in a neonatal intensive care unit over 10 years. *J Infect Dis* 1994; 169: 526-531.
4. Lyytikäinen O, Valtonen V, Sivonen A, Ryhänen R, Vuopio-Varkila J. Molecular epidemiology of *Staphylococcus epidermidis* isolates in a hematological unit during a 4-month survey. *Scand J Infect Dis* 1995; 27: 575-580.
5. Vermont CL, Hartwing NG, Fleer A, de Man P, Verbrugh H, van den Anker J, et al. Persistence of clones of coagulase negative staphylococci among premature neonates in neonatal intensive care units: two center study of Bacterial genotyping and patient risk factors. *J Clin Microbiol* 1998; 36: 2485-2490.
6. Gaynes RP, Edwards JR, Jarvis WR, Culver DH, Tolson JS, Martone WJ, et al. Nosocomial infections among neonates in high - risk nursery in the United States. *Pediatrics* 1996; 98: 357-361.
7. Hall SL. Coagulase negative staphylococcal infection in neonates. *Pediatr Infect Dis J* 1991; 10: 50-67.
8. Krediet TG, Mascini EM, van Rooij E, Vlooswijk J, Paauw A, Gerards LJ, et al. Molecular epidemiology of coagulase negative staphylococci causing sepsis in a neonatal intensive care unit over an 11-year period. *J Clin Microbiol* 2004; 42: 992-995.
9. Villari P, Sarnataro C, Lacuzio L. Molecular epidemiology of *Staphylococcus epidermidis* in a neonatal intensive care unit over a three year period. *J Clin Microbiol* 2000; 38: 1740-1746.
10. Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and outcome of nosocomial and community-onset bloodstream infection. *J Clin Microbiol* 2003; 41: 3655-3660.
11. Hsueh PR, Teng LJ, Chen WH, Pan HJ, Chen ML, Chang SC, et al. Increasing prevalence of methicillin resistant *Staphylococcus aureus* causing nosocomial infections at a university Hospital in Taiwan from 1986 to 2001. *Antimicrob Agents Chemother* 2004; 48: 1361-1364.
12. Goldman DA, Durbin WA, Freeman J. Nosocomial infections in a neonatal intensive care unit. *J Infect Dis* 1981; 144: 449-458.
13. Hoogkamp-Korstanje JAA, Cats B, Senders RC, van Ertbruggen I. Analysis of bacterial infection in a neonatal intensive care unit. *J Hosp Infect* 1982; 3: 275-284.
14. Burnie JB, Naderi-Nasab M, Loudon KW, Matthews RC. An epidemiological study of blood cultures isolates of coagulase negative staphylococci demonstrating hospital acquired infection. *J Clin Microbiol* 1997; 35: 1746-1750.
15. Miragaia M, Cauto I, Pereira SE, Kristinsson KG, Westh H, Jarlov JO, et al. Molecular characterization of methicillin resistant *Staphylococcus epidermidis* clones: evidence of geographic dissemination. *J Clin Microbiol* 2002; 40: 430-438.
16. Jiang JH, Chiu Nc, Huang FY, Kao HA, Hsu CH, Hung JH, et al. Neonatal sepsis in the neonatal intensive care unite: characteristics of early versus late onset. *J Microbiol Immunol Infect* 2004; 37: 30-36.
17. Schabery D R, Culver D, Gaynes RP. Major trends in the microbial etiology of nosocomial infection. *Am J Med* 1991; 91: 72-75.