African Journal of Microbiology Research Vol. 5(5) pp. 528-531, 4 March, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR10.224
ISSN 1996-0808 © 2011 Academic Journals

Full Length Research Paper

Identification of bacterial agents and antimicrobial susceptibility of neonatal sepsis: A 54-month study in a tertiary hospital

Seyyed Mohammad Hassan Aletayeb[1]*, Azar Dokht Khosravi[2,3], Masood Dehdashtian[4], Farshid Kompani[5], Seyyed Mazyar Mortazavi[1] and Mohammad Reza Aramesh[4]

[1]Neonatology Ward, Abuzar Teaching Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[2]Infectious and Tropical Diseases Research Center, Ahvaz, Iran.
[3]Department of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[4]Neonatology Ward Imam Khomeini Teaching Hospital, Ahvaz Jundishapur University of Medical Sciences, Postal code: 73166 Ahvaz, Iran.
[5]Pediatrics Ward, Golestan Teaching Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Accepted 18 February, 2011

The spectrum of bacteria which cause neonatal sepsis varies in different parts of the world. Antibiotic resistance is an increasing problem of these bacteria. The aim of this study was to detect the most common bacterial causes of neonatal sepsis and determination of their sensitivity to antibiotics. In a descriptive cross-sectional study, records of the neonates suspicious to neonatal sepsis who were admitted to Neonatal Intensive Care Unit (NICU) were assessed. Neonates with positive blood culture along with clinical signs of sepsis were entered in the study. Evaluated data included: age, sex, birth weight, type of infection, type of isolated pathogen, antibiotic sensitivity and disease outcome. Of 3700 screened blood cultures, 153 (4.1%) reported as positive, 76.5% of the evaluated neonates were preterm, 142 (92.8%) had gram negative sepsis and 11 (7.2%) had gram positive sepsis. The most common isolated gram negative bacteria were *Klebsiella pneumoniae* (46.4%), enterobacter spp. (17.6%) and *Escherishia coli* (14.4%). Coagulase negative staphylococci (CONS) were the most prevalent gram positive bacteria (5.9%) in this study. All of the klebsiella and enterobacter strains were resistant to ampicillin and gentamicin. The sensitivity of *K. pneumoniae* and enterobacter to imipenem was: 90 and 92.5%, respectively. Prevalence of early and late-onset sepsis was 64.7 and 35.3% respectively. Mortality rate of sepsis in this study was reported as 53.5%. In conclusion, the most common cause of bacterial sepsis in this study was *K. pneumoniae* which was completely resistant to ampicillin and gentamicin. So empirical treatment of sepsis with ampicillin and gentamicin in Imam Khomeini NICU must be reconsidered.

Key words: Neonatal sepsis, neonates, gram negative bacteria, antibiotic resistance, empirical treatment, mortality rate.

INTRODUCTION

Neonatal sepsis is defined as a disseminated disease with positive blood culture during the first month of life (Edwards, 2006), and is more common in developing countries compared with developed countries (Vergnano et al., 2005). Sepsis neonataroum is an important factor for mortality and morbidity in neonates and its mortality rate is about 20 to 50%. Mortality rate in neonatal sepsis differs according to the type of organism involved. Gram negative bacteria and enterococci cause the highest mortality rate in neonatal sepsis (Karunakaran et al., 2007). Spectrum of organisms which cause neonatal sepsis varies in different countries and sometimes changes from one center to another within the same country (Desinor et al., 2004). The most common causes of neonatal sepsis are group B streptococci (GBS),

*Corresponding author. E-mail: aletayebsmh@yahoo.com. Tel.:+98 611 2236092; Fax: +98 611 2225763.

Escherichia coli (E. coli) and *Listeria monocytogenes* in developed countries and gram negative bacteria and coagulase- negative staphylococci (CONS) in developing countries (Palazzi et al., 2006). Neonatal sepsis is divided into early- onset (from birth to the end of 7 days postnatal) and late onset (from 8 to 28 days postnatal). Due to different pathogens involved in neonatal sepsis of either onset, appropriate management and care depends on our knowledge about the causative organisms and their sensitivity to antibiotics. So results of the studies in other parts of the world are not suitable for the management of neonatal sepsis in the regions that no study has been done (AL-Zwaini, 2000).

The aim of our study was to detect bacterial pathogens responsible for early and late- onset neonatal sepsis and determination of their antibiotic sensitivity, gender frequency and mortality rate of neonatal sepsis in neonatal intensive care unit (NICU) in Ahvaz Imam Khomeini Hospital, Iran.

MATERIALS AND METHODS

In a cross- sectional descriptive study, over a period of 54 months (from April 2004 to September 2008), 3700 neonates with clinical signs and symptoms suggestive of neonatal sepsis, who were admitted to NICU in Ahvaz Imam Khomeini Hospital, Iran, were assessed inclusion criteria were neonates with respiratory distress, poor feeding, fever, hypothermia, signs of gastrointestinal or central nervous system involvement with a positive blood culture. Exclusion criteria were multiple congenital malformations, chromosomal disorders and newborns who were referred from other wards. Studied neonates were divided into two groups according to timing of clinical signs as early- onset (clinical sings of sepsis from birth to 7- day old) or late-onset (clinical signs of sepsis from 8 to 28- day old) infections. Neonates were also classified into normal birth weight (birth weight≥ 2500 gr) and low birth weight (birth weight< 2500 gr) and also into those with term (gestational age≥ 38 weeks) and preterm (gestational age< 38 weeks) according to gestational age. Blood culture, chest X-ray and laboratory tests including cell blood count (CBC), and blood sugar (BS) and electrolytes were performed for all subjects. For neonates ≥ 3 days, urine analysis and urine and cerebrospinal fluid (CSF) cultures were also performed.

Samples for blood culture were obtained with aseptic method from peripheral veins (2 ml for each blood culture). Blood samples were inoculated into the broth culture media (Trypticase Soy Broth) and incubated for one week in 37ºC (Forbes et al, 2002) and were checked daily for evidence of bacterial growth. For positive broth cultures, subcultures were made on solid media (Blood agar and McConkey agar) and were incubated in 37ºC for 24 to 48 h. The grown bacteria were identified by colony morphology, gram stain and standard biochemical tests (Forbes et al., 2002). The antibiotic susceptibility testing was performed for identified bacteria as per Clinical and Laboratory Standards Institute guideline (CLSI, 2002). The antibiotic disks were purchased from Padtan Teb Co., Tehran, Iran, and their concentrations per disk (µg) comprised:

Penicillin (10), ampicillin (10), cefotaxime (10), gentamicin (10), amikacin (10), ciprofloxacine (30), vancomycin (30), and imipenem (10).

Statistical analysis were done by SPSS version 15. Fisher's exact test was used for comparison of disease outcome between neonates with early and late-onset sepsis. P values less that 0.05 was considered as significant.

RESULTS

Positive blood cultures were obtained for 153 neonates (4.1%). Among these, 142 (92.8%) had sepsis with gram negative bacteria with 121 (79%) positive cultures for species belonging to enterobacteriaceae family and 11 (7.2%) with gram positive bacteria. The most common isolated gram negative bacteria were *Klebsiella pneumoniae* (46.4%), enterobacter spp. (17.6%) and *E. coli* (14.4%). CONS were the most isolated prevalent gram positive bacteria (Table 1). Based on the results from susceptibility testing, all of the *K. pneumoniae* and enterobacter spp. isolates were resistant to ampicillin and gentamicin. *K. pneumoniae* resistance to cefotaxime was 95.8%. However this species showed 90% sensitivity to imipenem (Table 2). Acinetobacters showed the highest antibiotics resistance to imipenem among the isolated gram negative bacteria in this study (69.4%). All of the isolated gram positive bacteria were resistant to ampicillin and penicillin and sensitive to vancomycin. Among neonates with sepsis, 99 patients (64.7%) had early –onset and 54 (35.3%) had late-onset neonatal sepsis. *K. pneumoniae* was the most common pathogen in both early –onset (41 cases- 41.4%) and late-onset (30 cases- 55.6%) sepsis. Among 153 newborns with sepsis, 177 (76.5%) were preterm and 36 (23.5%) were term. There was no significant correlation between gestational age and type of pathogens in this study ($P = 0.139$).

Similarly, no significant relationship was found between birth weight and type of neonatal sepsis. There were 112 (73.2%) neonates with low birth weight and 41 newborns (26.7%) with normal birth weight. Considering sex preponderance there were more cases of sepsis in male neonates compared with female neonates (102 male and 51 female with 2:1 ratio). The mortality rate was 53.5 % (82 cases: 52 male neonates and 30 female neonates) in this study (Table 3). There was no significant correlation between mortality rate and type of causative pathogen, early or late-onset sepsis and neonate sex. However, significant relationship was observed between gestational age and mortality rate in this study ($P = 0.2$).

DISCUSSION

In this study, the prevalence of documented neonatal sepsis with positive blood culture was 4.1%. This incidence was much lower than the prevalence of positive blood cultures in Rahman et al. (2002) study (62.8%) and Bhttacharjee et al. (2008) study (48%). The lower prevalence of documented neonatal sepsis with positive blood culture in our study had different reasons such as antibiotic administration in mother or neonate, difficulty in sampling, blood culture technique (Bansal et al., 2004) or

Table 1. Type and number of bacterial isolates in neonates with sepsis based on the sepsis onset.

Microorganism	Onset of sepsis		Total (%)
	Early-onset (%)	Late-onset (%)	
Klebsiella pneumoniae	41 (41.4)	30 (55.6)	71 (46.4)
Enterobacter spp.	16 (16.2)	11 (20.4)	27 (17.6)
E. coli	16 (16.2)	6 (11.1)	22 (14)
Pseudomonas aeruginosa	6 (6.1)	3 (5.6)	9 (5.9)
Acinetobacter spp.	9 (9.1)	3 (5.6)	12 (7.8)
Citrobacter spp.	1 (1)	0 (0)	1 (0.7)
CONS	9 (9.1)	0 (0)	9 (5.9)
Staphyloccus aureus	0 (0)	1 (1.9)	1 (0.7)
Enterococcus	1 (1)	0 (0)	1 (0.7)
Total	99 (100)	54 (100)	53 (100)

CONS: coagulase negative staphylococci.

Table 2. Antimicrobial susceptibility pattern of isolated bacteria to commonly used antibiotics in present study.

Organism (no.)	Antibiotics no. (%)					
	AM	AMK	CTX	CIP	GM	IMP
K. pneumonia (71)	0(0)	22(30.9)	3(4.2)	32(45)	0(0)	64(90)
Enterobacter (27)	0(0)	14(51.8)	0(0)	13(48.1)	0(0)	25(92.5)
E. coli (22)	0(0)	10(45.4)	0(0)	11(50)	4(18.1)	18(81.8)
Acinetobacter (13)	0(0)	4(30.6)	0(0)	7(53.8)	2(15.3)	4(30.7)
P. aeruginosa (12)	0(0)	7(58.3)	0(0)	3(25)	10(83.3)	9(75)

Key: AM: ampicillin; AMK: amikacin; CTX: cefotaxime; CIP: ciprofloxacine; GM: gentamycin; IMP: imipenem.

Table 3. Treatment outcome (No. of cases) according to sex, birth weight and type of sepsis.

	Female/male	Weight ≥ 2500	Weight < 2500	Late onset	Early onset
Recovery	21/50	23	48	26	45
Death	30/52	18	64	28	54
Total	51/102	41	112	54	99

sepsis due to anaerobic, viral or fungal pathogens (Agnihotri et al., 2004) and misdiagnosis because of some similarities between the clinical signs of sepsis with other diseases like metabolic disorders (Lund et al., 2002). In current study, early onset sepsis was more common than late-onset sepsis (64.7 versus 35.3%).This finding was similar to the results of the Vinodkumar et al. (2008) study which the reported prevalence of early onset neonatal sepsis was 73%. On the contrary, Kuruvilla et al. (1998) reported the higher prevalence of late-onset sepsis compared with early-onset (77.1 versus 22.9%).

This difference may be due to more referral of preterm labors and preterm newborns to our center. Neonatal sepsis with gram negative bacteria was more common than gram positive bacteria in our study and these results were in concordance with some of other studies (Isaacs and Royal, 1999; Sundaram et al., 2009). These results may be because of the colonization of gram negative bacteria in the skin of the neonates and the personnel of neonatal wards and use of less invasive procedures. In our study *K. pneumoniae* was the most common microorganism isolated from the blood cultures. This finding was similar to the results of Kumar et al. (2002), and was dissimilar to a recent study from Iran showing the CONS as the most common isolated bacteria (Gheibi et al., 2008). No GBS colonies were isolated from cultures in our study as previously reported from both developed and developing countries (Ahmed et al., 2002; Aurangzeb and Hameed, 2003). This may be due to lower colonization of pregnant mothers with GBS or weak virulence of these bacteria. A large number of gram negative and gram positive bacteria, were resistant to

one or more type of antibiotics which was in agreement to similar studies (Lund et al., 2002; Vinodkumar et al., 2008). Nowadays antibiotic resistance is a widespread global problem that has been caused ineffectiveness of current empirical treatment against gram negative bacteria. As we observed in the current study, all of the klebsiella and enterobacters which were the most common cause of bacterial sepsis were completely resistant to current empirical treatment protocol (ampicillin+ gentamicin). These are the first line treatment for sepsis according to World Health Organization (WHO) recommendation. Antibiotic resistance can cause many difficulties in the treatment of sepsis such as increase in mortality rate, duration of hospitalization and treatment expenses. So it is necessary that antibiotic treatment program is reevaluated continuously (Goossen, 2000).

In this study male to female ratio in sepsis was 2 to 1 which was close to the results of Mosayebi et al. (2003) study. Higher susceptibility to sepsis has been shown in male sex and its reason is unknown but it may be related to sex –dependent factors (Llorens, 2004). In our study mortality rate in sepsis with gram negative bacteria was 53.5% which was higher compared with that reported in Khassawneh et al. (2009) study which was reported as 30.9%. According to the result of this study, we can conclude that 1. gram negative bacteria were the main cause of early and late-onset neonatal sepsis in our center and *K. pneumoniae* was the most common pathogen; 2.many of the isolated bacteria from sepsis were resistant to the used antibiotics; and 3. the mortality rate due to neonatal sepsis was higher compared with the other studies. So reconsideration in empirical antibiotic therapy seems necessary in our hospital.

REFERENCES

Agnihotri N, Kaistha N, Gupta V (2004). Antimicrobial susceptibility of isolates from neonatal septicemia. JPN. J. Infect. Dis., 57(6): 273-275.

Ahmed AS, Chowdhury MA, Hoque M, Darmstadt GL (2002). Clinical and bacteriological profile of neonatal septicemia in a tertiary level pediatric hospital in Bangladesh. Indian Pediatr., 39(11): 1034-1039.

AL-Zwaini EJK (2000). Neonatal septicaemia in the neonatal care unit . Al-Anbar governorate, Iraq . East Mediterr. Health J., 8: 509-514.

Aurangzeb B, Hameed A (2003). Neonatal sepsis in hospital-born babies:bacterial isolates and antibiotic susceptibility patterns. J. Coll. Physicians Surg. Pak., 13(11): 629-632.

Bansal S, Jain A, Agarwal J, Malik GK (2004). Significance of coagulase negative staphylococci in neonates with late onset septicemia. Indian J. Pathol. Microbiol., 47(4): 586-568.

Bhttacharjee A, Sen MR, Prakash P, Gaur A, Anuprba S (2008). Increased prevalence of extended spectrum β lactamase producers in neonatal septicaemic cases at a tertiary referral hospital. Indian J. Med. Microbiol., 264(4): 356-360.

Clinical and Laboratory Standards Institute (2002). Performance standards for antimicrobial susceptibility testing. 12th Informational Supplement. CLSI Document M100-S12, Pennsylvania, USA. 22: 1.

Desinor OY ,Silva JL, Menos MJ (2004). Neonatal sepsis and meningitis in Haili. J. Trop. Pediatr., 50(1): 48-50.

Edwards MS (2006). Postnatal infections. In : Fanaoff and Martins Neonatal-perinatal Medicine, 8thed . Philadelphia: Mosby Elsevier, pp. 791-804.

Forbes BA, Sahm DF, Weissfeld AS (2007). Bailey and Scott's Diagnostic Microbiology (12th ed.). St. Louis: Mosby Inc, pp. 478-509.

Gheibi S, Fakoor Z, Karamyyar M, Khashabi J, Ilkhanizadeh B, Asghari-Sana F, Mahmoodzadeh H, Majlesi AH (2008). Coagulase Negative Staphylococcus; the Most Common Cause of Neonatal Septicemia in Urmia, Iran. Iranian J. Pediatr., 18(3): 237-243.

Goossen H (2000). Antibiotic resistance and policy in Belgium. Verh. K. Acad. Geneeskd. Belg., 62: 439-469.

Httacharjee A, Sen MR, Prakash P, Gaur A, Anuprba S (2008). Increased prevalence of extended spectrum β lactamase producers in neonatal septicaemic cases at a tertiary referral hospital. Indian J. Med. Microbiol., 264(4): 356-360.

Isaacs D, Royal JA (1999). Intrapartum antibiotics and early-onset neonatal sepsis caused by group B streptococcus and by other organisms in Australia. Australasian Study Group for Neonatal infections. Pediatr. Infect. Dis. J., 18: 524-528.

Karunakaran RN, Raja S, Ng KP, Navaratnam P (2007). Etiology of blood culture isolates among patients in a multidisciplinary teaching hospital in Kuala Lumpur. J. Microbiol. Immunol. Infect., 40: 432-437.

Khassawneh M, Khader Y, Abuqtaish N (2009). Clinical features of neonatal sepsis caused by resistant Gram-negative bacteria. Pediatr. Inter., 51(3): 332-336.

Kumar GD, Ramachandran VG, Gupta P (2002). Bacteiological Analysis of Blood Culture Isoltes from Neonates in a Tertiary Care Hospital in India. J. Health Popul. Nutr., 20(4): 343-347.

Kuruvilla KA, Pillai S, Jesudason M, Jana AK (1998). Bacteriological profile of sepsis in a neonatal unit in south India. Indian Pediatr., 35: 851-858.

Liorens XS (2004). Perinatal bacterial diseases In: Feigin RD,Chery JD, Demmler GJ, Kaplan SL. Textbook of Pediatric Infectious Diseases, 5th ed. Philadelphia: Saunders, p. 930.

Lund AM, Christensen E, Skovby F (2002). Diagnosis and acute treatment of inborn metabolic diseases in infants. Ugeskrift for Laeger. 164(48): 5613-5619.

Mosayebi Z, Movahedian AH, Moniri R (2003). Profile of Bactrial Sepsis in Neonates from Kashan in Iran. J. Infect. Dis. Antimicrob. Agents., 20: 97-102.

Palazzi D, Klein J, Baker C (2006). Bacterial sepsis and meningitis. In : Remmington JS, Klein J (eds) Infectious Diseases of the Fetus and Newborn Infant. 6th ed. Philadelphia: Elsevier Saunders, pp. 247-295.

Rahman S, Hameed A, Roghani MT, Ullah Z (2002). Multidrug resistant neonatal sepsis in Peshhawar, Pakistan. Arch. Dis. Child. Fetal Neonatal Ed., 87(1): F52-F54.

Sundaram V, Kumar P, Dutta S, Mukhopadhyay K, Ray P, Vikas Gautam V, Narang A (2009). Blood Culture Confirmed Bacterial Sepsis in Neonates in a North Indian Tertiary Care Center: Changes over the Last Decade Jpn. J. Infect. Dis., 62(1): 46-50.

Vergnano S, Sharland M, Kazembe P, Wansambo CM, Heath PT (2005). Neonatal sepsis. An international perspective. Arch. Dis. Child. Fetal Neonatal Ed., 90: F220-F224.

Vinodkumar CS, Neelagund YF, Suneeta K, Sudha B, Kalapannavar NK, Basavarajapa KG (2008). Perinatal risk factors and microbial profile of neonatal septicemia: A multicentred study. J. Obstet. Gynecol. India, 58(1): 32-40.