 DOI: 10.18231/2455-6807.2016.0009

Bacteriological profile of neonatal septicemia and antibiotic susceptibility pattern of the isolates in western civil hospital India

Sangeeta D. Patel[1,*], Sima Bhatt[2], Piyush A. Patel[3]

[1]Assistant Professor, [2]Professor & HOD, Dept. of Microbiology, GMERS Medical College, Gandhinagar, [3]Associate Professor, Dept. of Pathology, GMERS Medical College, Sola, Gujarat

***Corresponding Author:**
Email: sanginfriend@yahoo.co.in

Abstract

Background: The objective of this study was to isolate pathogenic bacteria in neo-natal septicemia cases, and to know their antibiotic profile.

Material and Method: Under aseptic precautions, blood was drawn from 147 neonates with suspected septicemia and inoculated in BD BACTEC bottle. Isolates obtained were identified as per standard protocol and antibiotic susceptibility was done by Kirby Bauer disc diffusion method (as per CLSI guide-lines).

Results: Total number of 90 (61.2%) patients had positive blood cultures. The most common pathogens isolated were Klebsiella pneumoniae (n=49, 54.4%) followed by coagulase negative staphylococcus (CONS, n=21,23.3%) Staphylococcus aureus (n=10, 11%), Escherichia coli (n=5, 5.5%), and Pseudomonas aeruginosa (n=1%). The Gram negative organisms showed high resistance to commonly used antibiotics and were highly sensitive to Meropenem. The Gram positive bacteria showed high resistance to Ampicillin, Erythromycin and Amoxicillin; but they were highly susceptible to Linezolid and Vancomycin.

Conclusion: As the Gram negative organisms were the most common isolates in neonatal septicemia, their resistance pattern should be considered essential for deciding the empirical treatment. Prompt treatment of neonatal sepsis with judicious use of appropriate antibiotics can minimize the morbidity and mortality, besides reducing the emergence of multi-drug resistant organisms in ICU's.

Keywords: Sepsis, Klebsiella, Blood Culture, Antibiotics.

Introduction

Sepsis is the commonest cause of neonatal mortality and is probably responsible for 30-50% of the total neonatal deaths each year in developing countries.[1,2] It is estimated that 20% of all neonates develop sepsis and approximately 1% die of sepsis related causes.[2] Neonatal septicemia is a common complication for neonates in neonatal intensive care units around the world. The incidence is much higher in developing countries than in developed world. According to World Health Organization estimates, there are about 5 million neonatal deaths a year, with 98%occurring in developing countries.[3,4] Before the discovery of antibiotics, the mortality from septicemia was 90%, but it reduces to 24-58% after antibiotics came to use.[5] Neonatal sepsis is caused by a variety of organisms, ranging from Gram-positive bacteria, Gram-negative bacteria and sometimes yeasts.[6] The major cause of neonatal sepsis in the developing countries is Gram-negative bacteria and these organisms have developed increased resistance over the last 20 years, therefore posing a major problem in managing neonates with sepsis.[7-10] It is essential to conduct periodic review of organisms responsible for neonatal septicemia for the appropriate management of neonates. Proper management and early diagnosis of neonatal septicemia could reduce the mortality and morbidity substantially. Against this background, this study was undertaken to study the bacteriological profile and antibiotic susceptibility pattern of the isolates in septicemic neonates.

Materials and Methods

Study centre, design and period: This study involved 147 clinically suspected cases of neonatal septicemia admitted during the study period January 2016 to July 2016 in a tertiary teaching hospital. The cases were categorized depending upon the time of presentation, within 72 hours of life as early onset septicemia (EOS) and after 72 hours of life as late onset septicemia (LOS).

Institutional Ethics Committee Clearance under study were obtained.

Subject selection

Inclusion criteria: Neonates having features suggestive of systemic inflammatory response syndrome (SIRS), with no localizing source of infection. Neonates with poor activity, fever, refusal of feed, lethargy, tachypnea, tachycardia, birth asphyxia, prematurity, low birth weight, or delivered with PROM, foul smelling liquor, etc.

Exclusion criteria: Neonates having extreme prematurity (less than 30 weeks of gestational age), birth weight less than 1000 gm, gross congenital anomalies, and all children who had received antibiotics before admission, were excluded from the study.

Sample collection and processing: The present study was carried out between January 2016 to July 2016 in the Department of Microbiology, GMERS Medical College, Gandhinagar. Blood for culture was collected from 147 clinically diagnosed septicemia cases following strict aseptic Precautions. Two milliliter blood was collected and inoculated into BD BACTEC bottle.

The BACTEC culture bottles were incubated in bactec machine and those bottle which was positive identified by machine and give indication to remove bottle from the machine. Then subculture was done onto Mac Conkey's agar, blood agar and chocolate agar. The growth obtained was identified by conventional biochemical tests like catalase test, coagulase test, oxidase test, tripal sugar iron agar test, citrate test, urease test, indole test etc.

Antibiotic susceptibility test: The standard disk diffusion test for susceptibility to routine antibiotics was done by modified Kirby-Bauer method. Zone sizes were measured and interpreted according to CLSI standards.[11] The drugs for disc diffusion testing(gram positive organisms) were in the following concentrations: Erythromycin(15µg), Tetracycline(30µg), Amoxicillin/Clavulanic acid(30µg), Clindamycin(2µg), Ampicillin(10µg), Amikacin(30µg), Cefoxitin(30µg), Gentamicin(10µg), Vancomycin(30µg), Ciprofloxacin(5µg), Moxifloxacin(5µg), Teicoplanin(30µg), Cefuroxime(30µg), Linezolid(30µg), Piperacillin/ Tazobactam(110µg).

The drugs for disc diffusion testing (gram negative organisms) were in the following concentrations: Tetracycline(30µg), Chloramphenicol(30µg), Tobramycin(10µg), Ampicillin10(µg), Amikacin (30µg), Gentamicin(10µg), Trimethoprim/ Sulfamethoxazole(25µg), Cefotaxime(30µg),Ceftazidime(30µg), Imipenem(10µg), Amoxicillin/Clavulanic acid(30µg), Cefuroxime(30µg), Ciprofloxacin(5µg), Levofloxacin(5µg), Piperacillin/ Tazobactam(110µg), Lomefloxacin(10µg), Polymixin B(300unites). Data analysis was done using Statistical Package for Social Sciences (SPSS) software version 14.0. The level of significance for tests was set at $P < 0.05$.

Results

Of 147 neonates screened, blood culture reports were positive in 90 cases (61.2%).Among this culture positive cases 52 were Male patients (Table 1). Early-onset sepsis cases were found to be about three times higher than late-onset sepsis. Out of 90 cases, 66(73.3%) had early onset sepsis while 24(26.6%) had late-onset sepsis. The most common isolates at the early-onset and late-onset of sepsis were the Gram-negative and Gram-positive organisms respectively (Table 2). Klebsiella spp. (54.4%) and coagulase negative staphylococcus (CONS) (23.3%) were the most common Gram-negative and Gram-positive organisms while staphylococcus aureus (11.1%) Pseudomonas spp. (1.11%) and enterococcus (1.11%) were the least common organisms. Candida sp in 3 cases (3.3%)(Table3). Table 4and 5; show the antibiotic susceptibility pattern of Gram-negative and Gram-positive organisms. Gram-negative isolates were mostly susceptible to Imipenem (90%), followed by Piperacillin tazobactum (86%), Levoflox(75%) and. Best overall susceptibility among Gram-positive isolates was Linezolid (100%) and Vancomycin(100%) Amikacin(95%).

Discussion

In the present study, males were more affected than females, and the male to female ratio was 1.5:1. This is comparable to the other studies by Begum S et al.[12] and Shrestha NJ et al.[13] The reason for male preponderance is unknown, but this could be due to sex-dependent factors16. The synthesis of gamma globulins is probably regulated by X–linked immunoregulatory genes and as males are having one X chromosome, they are more prone for neonatal septicemia than females.[14] Culture-positivity for aerobic organisms in neonates vary from 25% to 60%,[15] In this study, blood culture-positivity rate is 61%. This finding is comparable with the study of Sharma *et al*[16] and Jain *et al*.[17] other reports. In our study, early onset septicemia (66/90, 73%) was more than late onset septicemia (24/90, 27%), which is consistent with the studies of Aletayab et al[18] Wa-seem R et al[19] Al-Shamahy et al.[20]

This could be due to prematurity, low birth weight and unhygienic conditions during labor. On the contrary, other studies done by Kayange N et al[21] reported higher occurrence of late onset septicemia than early onset septicemia.

The etiological agents of neonatal sepsis vary between developed and developing countries .Overall, Gram-negative organisms are more common and are mainly represented by *Klebsiella, Escherichia coli, Pseudomonas*. Of the Gram-positive organisms, *Staphylococcus aureus*, CONS, *Streptococcus pneumonia*, and *S. pyogenes* are most commonly isolated.[22]

Klebsiella pneumoniae and other Gram-negative organisms were the common causes of sepsis in the present study as well other studies from India.[23] In the present study, Klebsiella pneumoniae and CONS were the common etiological agents of sepsis. In an epidemiological study performed to observe the long term trends in the agents causing neonatal sepsis, CONS were showing an increasing trend.[24]

Antibiotic resistance is today a global problem. Reports of multi-resistant bacteria causing neonatal sepsis in developing countries are increasing. The wide availability of over-the-counter antibiotics and the inappropriate use of broad-spectrum antibiotics in the community may explain this situation. It is difficult to compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is extremely variable.[22]

Antibiotic susceptibility pattern was studied for all isolates causing neonatal sepsis. The analysis of drug resistance pattern showed that, among Gram-negative isolates, maximum numbers (95.7%) were resistant to ampicillin and lowest to imipenem (7.6%). Resistance

was observed to be against commonly used antibiotics such as ampicillin, amoxiclav, cephotaxime, and Gentamycin. Among Gram-positive isolates, high resistance was seen to penicillin (99%), erythromycin (80%), Piperacillin tazobactum(57%)and amoxiclav (47%),. Least resistance was seen to linezolid (0%), Vancomycin(2%), Levofloxacin(14.3%) followed by cefoxitin(34%) tetracycline (28.6%). The greater prevalence of resistance to commonly used antibiotics has also been reported by other studies.[25]

In this study, maximum sensitivity (93%) was observed in imipenem and linezolid (100%). Sensitivity to imipenem and linezolid was much higher than that to other antibiotics and the difference was statistically significant ($P < 0.05$), but these two drugs should not be used indiscriminately and be kept as a reserve drugs, otherwise resistance to these drugs may develop, thereby threatening the treatment.

Table 1: Blood culture positivity

Blood culture	Male	Female	Total
Culture positive	52	38	90
Culture negative	25	32	57

Table 2: Effect of Age on the distribution of microbial isolates

Age	Number of Gram-positive isolates	Number of Gram-negative isolates
(<72hrs)	14	47
(≥72hrs-28days)	18	8

Table 3: Number of microbial isolates from culture positive neonates (n=90)

Organisms	Frequency of isolation (%)
Klebsiella spp	49
Escherichia coli	5
Pseudomonas spp	1
Coagulase negative staphylococcus (CONS)	21
Staphyloccus aureus	10
Enterococci	1
Candida spp	3

Table 4: Gram negative organisms (Number of isolates = 55)

Antibiotics	Resistant (%)	Sensitive (%)
Tetracycline	65.7	34.3
Chloramphenicol	15	85
Tobramycin	80	20
Ampicillin	95.7	4.3
Amikacin	73.1	26.9
Gentamicin	80	20
Trimethoprim/ Sulfamethoxazole	16.4	83.6
Cefotaxime	85.5	14.5
Ceftazidime	81.4	18.6
Imipenem	7.6	90.3
Amoxicillin/ Clavulanic acid	87.9	12.1
Cefuroxime	86.4	13.6
Ciprofloxacin	77.9	22.1
Levofloxacin	25.5	74.5
Piperacillin/Tazobactam	11.7	86.2
Lomefloxacin	72.4	27.6
Polymixin B	6.9	93.1

Table 5: Gram positive organisms (Number of isolates = 32)

Antibiotics	Resistant (%)	Sensitive (%)
Erythromycin	80.4	19.6
Tetracycline	28.6	71.4
Amoxicillin/ Clavulanic acid	46.4	53.6
Clindamycin	21.3	78.7
Ampicillin	50	50
Amikacin	4.3	95.7
Cefoxitin	14	86
Gentamicin	17	83
Vancomycin	0	100
Ciprofloxacin	57.1	42.9
Moxifloxacin	7.1	92.9
Teicoplanin	0	100
Cefuroxime	36.2	63.8
Linezolid	0	100
Piperacillin/ Tazobactam	42.9	57.1

Conclusion

It is evident from this study that *Klebsiella*, CONS, and *S. aureus* are the leading cause of neonatal sepsis, and most of them are resistant to multiple antibiotics. Therefore, the authors suggest that surveillance of antimicrobial resistance is necessary. Also, an antibiotic policy should be formulated in the hospital. Depending on the antibiotic sensitivity pattern of the isolates, antibiotics should be used. Furthermore, we advise that health education be provided to the public on the dangers of indiscriminate use of antibiotics, which is currently considered to be a menace in our society and which has been responsible for the ineffectiveness of most commonly used antibiotics such as penicillin and ampicillin, as observed in our study.

References

1. Bang AT, Bang RA, Bactule SB, Reddy HM, Deshmukh MD. Effect of home based neonatal care and management of sepsis on neonatal mortality: field trial in rural India. Lancet 1999;354:1955-61.
2. Stoll BJ. The global impact of neonatal infection. Clin Perinatol 1997;24:1-21.
3. P. Jyothi, C. B. Metri, and V. B. Peerapur, "Bacteriological profile of neonatal septicemia and antibiotic susceptibility

pattern of the isolates," *J Nat Sci Biol Med*, vol. 4, no. 2, pp. 306–309,2013.
4. N. Kaistha, M. Mehta, N. Singla, R. Garg, and J. Chander, "Neonatal septicemia isolates and resistance patterns in a tertiary care hospital of North India," *J Infect Dev Ctries*, vol. 4, pp. 55–57,2009.
5. S. L. Kaushik, V. R. Parmar, N. Grover, P.S. Grover, and R. Kaushik, "Neonatal sepsis in hospital born babies," *J Commun Dis*, vol. 3, pp. 147-152,1998.
6. H. H. Gomaa, E. E. Udo, and U. Rajaram, "Neonatal septicemia in Al-Jahra hospital,14 Kuwait: Etiologic agents and antibiotic sensitivity patterns," *Med Princ Pract*, vol.10, pp. 145–50,2001.
7. Dawodu, K. Al Umran, and K. Danso, "A case study of neonatal sepsis in very low birth weight infants," *N Engl. J. Med*, vol. 347,pp. 240-247,2002.
8. F. Motara, D. E. Ballot, and O. Perovic, "Epidemiology of neonatal sepsis at Johannesburg Hospital," *Southern Afr. J. Epidemiol. Infect*, vol. 20, pp. 90-93,2005.
9. Z. A. Bhutta and K. Yusuf, "Early-onset neonatal sepsis in Pakistan: a case control study of risk factors in a birth cohort," *American Journal of Perinatology*, vol. 14, pp. 577-581,1997.
10. R. N. Musoke and G. Revathi, "Emergence of multi-drug resistant gram negative organisms in a neonatal unit and the therapeutic implications," *Journal of Tropical Pediatrics*, vol. 46, pp. 86-91,2000.
11. NCCLS performance standards for antimicrobial susceptibility testing. Twelfth international supplement. NCCLS document M100-S12 [ISBN 1-56238-454-6]. NCCLS, 940 WEST VALLEY Road, suite 1400, Wayne Pennsylvania 19087-1898 USA,2013].
12. Begum S, Baki MA, Kundu GK, Islam I, Kumar M, Haque A. Bacteriological profile of neonatal sepsis in a tertiary hospital in Bangladesh. J Bangladesh Coll Phys Surg 2012;30:66-70).
13. Shrestha NJ, Subedi KU, Rai GK. Bacteriological profile of neonatal sepsis: A hospital based study. J. Nepal Paediatr. Soc. 2011;31(1):1-5.
14. Khatua SP, Das AK, Chatterjee BD, Khatua S, Ghose B, Saha A. Neonatal septicemia. Indian J Pediatr 1986;53(4):509-514.
15. Neonatal morbidity and mortality; report of the National Neonatal-Perinatal Database. Indian Pediatr. 1997;34:1039–42. [PubMed]].
16. Sharma PP, Halder D, Dutta AK, Dutta R, Bhatnagar S, Bali A, et al. Bacteriological profile of neonatal septicemia. Indian Pediatr. 1987;24:1011–7.[PubMed].
17. Jain NK, Jain VM, Maheshwari S. Clinical profile of neonatal sepsis. Kathmandu Univ Med J. 2003;1:117–20. [PubMed].
18. Aletayeb SMH, Khosravi AD, Dehdashtian M, Kompani F, Mortazavi SM, Aramesh MR. Identification of bacterial agents and antimicrobial susceptibility of neonatal sepsis. African Journal of Microbiology Research 2011;5(5):528-531.
19. Waseem R, khan M, Izhar TS, Qureshi AW. Neonatal sepsis. Professional Med J 2005;12(4):451-456.
20. Al-Shamahy HA, Sabrah AA, Al-Robasi AB, Naser SM. Types of bacteria associated with neonatal sepsis in Al-Thawra University Hospital, Sana, Yemen and their anti-microbial profile. SQU Med J 2012;12(1):48-54.
21. Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC Pediatrics 2010;10:39.
22. Vergnano S, Sharland M, Kazembe P, Mwansambo C, Heath PT. Neonatal sepsis: An international perspective. Arch Dis Child Fetal Neonatal Ed. 2005;90:F220–4. [PMC free article] [PubMed].
23. Kaistha N, Mehta M, Singla N, Garg R, Chander J. Neonatal septicemia isolates and resistance patterns in a tertiary care hospital of North India. J Infect Dev Ctries. 2009;4:55–7].
24. van den Hoogen A, Gerards LJ, Verboon-Maciolek MA, Fleer A, Krediet TG. Long-term trends in the epidemiology of neonatal sepsis and antibiotic susceptibility of causative agents. Neonatology. 2010;97:22–8.
25. Tsering DC, Chanchal L, Pal R, Kar S. Bacteriological profile of septicemia and the risk factors in neonates and infants in Sikkim. J Global Infect Dis. 2011;3:425. [PMC free article] [PubMed].