Original Research Paper Microbiology

MICROBIAL ANALYSIS AND ANTIMICROBIAL RESISTANCE PROFILE OF BLOODSTREAM INFECTIONS IN NEONATAL SEPSIS

Dr. Ramesh Agrawal Assistant Professor, Microbiology, R. D. Gardi Medical College Ujjain, M.P. India
Dr. Yogyata Marothi Professor, Microbiology, R. D. Gardi Medical College Ujjain, M.P. India
Dr. Harshada Shah Professor & Head, Microbiology, R. D. Gardi Medical College Ujjain, M.P. India
Mr. Komal Singh Demonstrator, Microbiology, R. D. Gardi Medical College Ujjain, M.P. India

ABSTRACT **Background:** Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteraemia in the first month of life. The objective of this study was to identify pathogenic bacteria in neo-natal septicemia and their antibiotic resistance pattern. **Material and Method:** 170 blood samples collected aseptically from clinically suspected cases of neonatal septicaemia and cultured in automated BacT/ALERT 3D system. Isolates obtained were identified as per standard protocol and antibiotic susceptibility was done by Kirby Bauer disc diffusion method. **Results:** 110 (64.7%) patients had positive blood cultures. The incidence of early onset neonatal septicaemia (< 72 hrs after birth) was 64(58.2%) & late onset neonatal septicaemia (> 72 hrs to 28 days of birth) were 46 (41.8%). he most common pathogens isolated were Staphylococcus aureus (36.4%) & Klebsiella pneumoniae (30.9%). Gram negative bacteria showed high resistance to amoxy-clav (85%), Ceftazidime (79%) & least resistance to Imipenem. Gram positive bacteria showed high resistance to penicillin (85%), cefoxitin (57.5%), & least resistance to Linezolid and tetracycline. **Conclusion:** We conclude that rate of neonatal septicaemia was very high, constant changing of bacterial isolates recovered from neonatal sepsis and high rate of antibiotic resistance needs constant monitoring & evaluation of NICUs.

KEYWORDS : BacT/alert, blood stream infection, blood samples, Bacterial isolate, neonatal septicaemia, antimicrobial susceptibility.

Introduction

Neonatal sepsis, a systemic infection manifesting in the first month of life, is a leading cause of Mortality in the newborn. Blood culture is the main stay in the diagnosis of neonatal sepsis. Automated blood culture systems are commonly used to identify infectious agents causing septicaemia in human medicine.[1] Risk factors significant for neonatal sepsis are preterm delivery, low birth weight, maternal fever, premature rupture of membrane (PROM) >16hrs.[2] Early onset neonatal sepsis (EOS) presents within 72 hours of birth and late onset neonatal sepsis presents >3-28 days after birth. Source of infection in EOS is the colonized mother's genitourinary tract. The bacteria associated with EOS are *Group B Streptococci, Escherichia coli, Coagulase negative staphylococci, Haemophillus influenzae* and *Listeria monocytogenes*. The bacteria implicated in LOS are from the environment: *Coagulase negative staphylococci, Staphylococcus aureus, E.coli, Klebsiella spp., Pseudomonas spp., Candida spp., Acinetobacter spp. and anaerobes*.[3] Microbial invasion of the bloodstream can have serious immediate consequences i.e., shock, multiple organ failure, disseminated intravascular coagulation (DIC) and death.[4] In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available. Keeping in mind the high mortality and morbidity associated with septicemia, right choice of empiric therapy is of most importance.[5]

The study was undertaken to determine the spectrum of bacteria pathogens associated with neonatal sepsis and their antibiotic susceptibility pattern. It will help in adapting appropriate control measures and antibiotic policy in the NICU in our hospital.

Material & Methods

Study was conducted in Dept. of Microbiology, R.D. Gardi Medical College, Ujjain, Madhya Pradesh, India. Total 170 blood samples were collected from clinically suspected cases of neonatal septicaemia in a five months period (October 2016 to February 2017). Samples were collected aseptically in PF plus aerobic blood culture bottles and cultured in automated BacT/ALERT 3D (bioMerieoux) system. Positive samples were sub cultured on Blood agar and MacConkey agar and incubated at 370C for 24hrs. Final identification of bacterial isolates was done by colony characteristics, Gram's staining, Motility testing and biochemical testing. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method using Muller Hinton agar media as per CLSI guidelines.[6]

Results & observations

In present study out of 170 blood cultures sample 110 (64.7%) were found positive & 60 (35.3%) were negative. Amongst total sample 90 (52.9%) were male & 80 (47.1%) were female neonates patients **(Table 1)**. The incidence of early onset neonatal septicaemia (< 72 hrs after birth) was 64(58.2%) & late onset neonatal septicaemia (> 72 hrs to 28 days of birth) was 46 (41.8%) **(Table 2)**.The microbial profile of neonatal septicaemia is depicted in **Table 3 & Figure 1.** *Staphylococcus aureus* 40 (36.4%) & *Klebsiella pneumonia* 34 (30.9%) were major isolates. Other isolates include *E.coli* 12 (10.9%), *Pseudomonas* 6(5.5%), *Enterococcus* 5(4.5%), *Enterobacter* 4(3.6%), *Citrobacter* 3(2.8%).Non *fermenter* GNB *2*(1.8%) and *Candida albicans* 4(3.6%). We also isolate *Coagulase Negative Staphylococcus* (CoNS) 4(3.6%) & bacillus 3(2.7%) were probably skin contaminants excluded from positive isolates.

In isolated gram negative bacteria *Klebsiella pneumoniae* was most resistance to Amoxy-clav (85.3%) followed by Ceftazidime (79.4%) & Cefepime (70.6%) and least resistance to Imipenem (26.5%). E.coli was 83.4% resistance to amoxy-clav & 75% resistance to Ceftazidime and least (25%) resistance to Imipenem. Pseudomonas was most resistance to Ceftazidime (83.4%) & least resistance to Imipenem (16.7%).The antibiotic resistance pattern of gram negative bacteria is depicted in **Table 4.** *Staphylococcus aureus* were 85% resistance to Penicillin, 57.5% resistance to Cefoxitin (MRSA), 25% resistance to Vancomycin and 12.5% resistance to Linezolid. *Enterococcus* showed 80% resistance to Penicillin, 60% resistance to Ampicillin & Vancomycin, 40% resistance to High level Gentamycin and 20% resistance to Linezolid. Antibiotic resistance pattern of gram positive bacteria depicted in **Table 5**

Table.1 –Blood culture positive and negative in total blood samples

Blood culture	Male	Female	Total
Culture positive	62	48	110
Culture negative	28	32	60
Total	90	80	170

Table .2 Distribution of microbial isolates according to age

Age	Positive isolate No.	Percentage (%)
Early onset septicaemia	64	58.2%
Late onset septicaemia	46	41.8%

Table.3- Microbial profile of neonatal septicaemia

Organism	Number of positive cases	Percentage %
Staphylococcus	40	36.4
Enterococcus	5	4.5
E. coli	12	10.9
Klebsiella pneumoniae	34	30.9
Pseudomonas	6	5.5
Enterobacter	4	3.6
Citrobacter	3	2.8
Candida	4	3.6
Non-ferment GNB	2	1.8
Total	110	100%

Figure 1: Number of isolated organism from blood culture

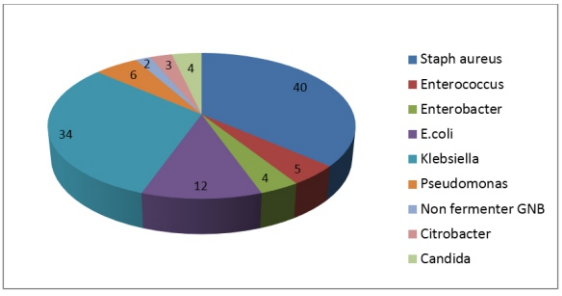

Table .3 Antibiotic resistance patterns of gram negative bacteria

Antibiotic drugs	E.coli (N=12)	Klebsiella (N=34)	Pseudomonas (N=6)
	No &percentage of resistance isolates	No &percentage of resistance isolates	No & percentage of resistance isolates
Amikacin	5 (41.7%)	19 (55.8%)	2 (33.3%)
Aztronem	-	-	3 (50%)
Piperacillin	8 (66.7%)	21 (61.7%)	3 (50%)
Cefepime	6 (50%)	24 (70.6%)	4 (66.6%)
Ceftazidime	9 (75%)	27 (79.4%)	5 (83.3%)
Ciprofloxacin	4 (33.3%)	13 (38.3%)	2 (33.3%)
Imipenem	3 (25%)	9 (26.5%)	1 (16.6%)
Amoxy-clav	10 (83.3%)	29 (85.3%)	-
Piperacillin tazobactum	5 (41.7%)	18 (52.9%)	2 (33.3%)

Table .4 Antibiotic resistance patterns of gram positive bacteria

Antibiotic drugs	Staph aureus (N=40)	Enterococcus (N=5)
	No & percentage of resistance isolates	No & percentage of resistance isolates
Penicillin	34 (85%)	4 (80%)
Cefoxitin	23 (57.5%)	-
Cotrimoxazole	15(37.5%)	-
Erythromycin	21 (52.5%)	-
Clindamycin	9 (22.5%)	-
Tetracycline	7 (17.5%)	-
Vancomycin	10 (25%)	3 (60%)
Linezolid	5 (12.5%)	1 (20%)
Ampicillin	-	3 (60%)
High level Gentamycin	-	2 (40%)

Discussion: The present study was conducted to determine the predominant organisms responsible for neonatal septicaemia and their antibiotic resistance pattern in our hospital setup. Neonatal sepsis is associated with high morbidity and mortality. Knowledge of the bacterial profile and the antibiotic susceptibility pattern of the isolates play an important role in the management of sepsis. In the present study incidence of neonatal septicaemia was high (64%) which was accordance with the Garg A et al.[7] &Tallur et al.[8]Early onset sepsis was more in number (58.2%) compared to the cases with late onset sepsis which is consistent with the findings of Agrawal A et al.[9] & Chung et al.[10] In the present study Gram negative organisms (55.4%) constitutes the major group of isolates in neonatal septicaemic cases which was accordance with the khinchi et al.11& Nwadioha et al.[12] Amongst the gram negative organism dominant pathogen isolated was Klebsiella pneumonia (30.9%) similar results have been reported by Agrawal A et al.[9] & Kotgire et al.[13] Staphylococcus aureus was the predominant isolate among gram positive organism this was accordance with the Murty et al.[14] Anwer et al.[15] In the present study, GNB were highly resistance to Amoxy-clav (85%), Ceftazidime (80%) & least resistance to Imipenem (25%). Among Gram-positive isolates Staphylococcus aureus was high resistance to penicillin (85%), cefoxitin (MRSA) (57.5%), erythromycin (52.5%) & least resistance was seen to linezolid (12.5%), tetracycline (17.5%) & Vancomycin (25%). Enterococcus was high resistance to Penicillin (80%) & least resistance to linezolid (20%). In this study maximum sensitivity was observed in Imipenem & linezolid which was accordance with the Patel et al.[16]

Conclusion: We conclude that rate of neonatal septicaemia was very high, Klebsiella pneumoniae and Staphylococcus aureus were the commonest isolates from cases of neonatal septicemia. Constant changing of bacterial isolates recovered from neonatal sepsis and high rate of antibiotics resistance needs constant monitoring & evaluation of NICUs. 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.

References

1. Horvath L L, Hospenthal D R, Murray C K, Dooley D P 2003 Detection of simulated candidemia by the BACTEC 9240 system with plus aerobic/f and anaerobic/f blood culture bottles. Journal of Clinical Microbiology 41: 4714–4717
2. Rao PS, Baliga M, Shivananda PG. Bacteriology of neonatal septicemia in a rural referral hospital in south India. J Trop Pediatr 1993; 39:230-33
3. Zaidi AK, Thaver D, Ali SA, Khan TA. Pathogens associated with sepsis in newborns and young infants in developing countries. Pediatr Infect Dis J 2009; 28:S10–8.
4. Kamga HLF, Njunda AL, Nde PF. Prevalence of septicemia and antibiotic sensitivity pattern of bacterial isolates at the University Teaching Hospital, Yaoundae, Cameroon. African Journal of Clinical and Experimental Microbiology 2011; 12(1):2–8.
5. Kavitha P, Sevitha B, Sunil R, Bacteriological profile and Antibiogram of blood culture isolates in a pediatric care unit. Journal of Laboratory Physicians 2010; (2):85–8.
6. A. Mathew et al (2006) "Performance standards for antimicrobial susceptibility testing," Clinical and Laboratory Standards Institute, vol. 26, supplement 16.
7. Garg A, Kumar D, Kumar S.Microbiological study of neonatal sepsis in a tertiary care hospital of western Uttar Pradesh, India. Int J Res Med Sci 2016;4:2701-4.
8. Tallur SS, Kasturi AV, Nadgir SD, Krishna BV. Clinico-bacteriological study of neonatal septicemia in Hubli. Indian J Pediatr. 2000;67:169-74.
9. Avinika Agarwal 1, Sevitha Bhat2 Clinico-microbiological study of neonatal sepsis Journal of International Medicine and Dentistry 2015; 2(1): 22-29.
10. Chugh K, Aggarwal BB, Kaul VK, Arya SC. Bacteriological profile of neonatal septicemia. Indian J Pediatr 1988; 25: 961- 65.
11. Khinchi YR, Kumar Anil, Yadav Satish. Profile of neonatal sepsis. Journal of college of Medical Sciences-Nepal 2010;6(2):21-6.
12. Nwadioha SI, Nwokedi EOP, Kashibu E. A review of bacterial isolates in Blood culture of children with suspected septicaemia in a Nigerian tertiary hospital.African journal of Microbiology 2010 Feb 1;4(4):222-225.
13. Santosh Kotgire1, Sunil Hatkar2 Neonatal septicaemia: Its bacteriological profile and antibiogram, International Journal of Medical Microbiology and Tropical Diseases, October-December, 2016;2(4):148-152.
14. Murty DS., Gyaneshwari M. Blood culture in Paediatric Patients: a study of clinical impact. Indian journal of medical microbiology (2007);25(3):220-4.
15. Anwer SK, Mustafa S, Pariyani S, Ashraf S, Taufiq KM. Neonatal sepsis an etiological study.J Pak Med Asso 2000;50:91-4.
16. Sangeeta D. Patel1, Sima Bhatt2, Piyush A. Patel3 Bacteriological profile of neonatal septicemia and antibiotic susceptibility pattern of the isolates in western civil hospital India International Journal of Medical Microbiology and Tropical Diseases, October-December, 2016;2(4):157-160.