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Original Article

Bacteriological profile and antibiotic resistance pattern of isolates from neonatal septicemia patients in tertiary care hospital central India

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Abstract
Background: *Neonatal septicemia still a major cause of mortality and morbidity worldwide. Blood culture is the main stay in the diagnosis of septicemia. Emergence of drug resistant bacterial strains is a major problem in the management of sepsis. Aim of present study is to determine bacteriological profile and antibiotic resistance in neonatal sepsis.*
Method: *The study was carried out in the Department of Microbiology, MGM, Medical College Indore during the period from January 2017 to June 2017.*
450 blood samples collected from clinical suspected cases of bacteremia were studied. Growth from the subcultures were identified by standard biochemical testing and antibiotics resistance pattern were determined as per Clinical Laboratory Standard Institute (CLSI) guidelines.
Result: *During that period, a total of 176 (39%) blood samples were found to be positive for bacterial isolates. Gram negative septicemia (50.5%) was encountered slightly more than gram positive septicemia (49.5%). The common bacteria isolated were Klebsiella spp., CONS, Staphylococcus aureus and Escherichia coli. Antibiotics resistance against gram negative bacilli were cefuroxime, ceftazidime, cefepime and ciprofloxacin. Gram-positive isolates showed excellent sensitivity to linezolid and vancomycin*
Conclusion: *The study concludes that the isolated organism exhibited higher resistance towards commonly used antimicrobials. Healthcare personnel and common population should also be aware of the rising antibiotic resistances to frequently used antibiotics.*
Keywords: *Antibiotic resistance, CONS, S. aureus, Neonatal Septicemia, Blood Culture.*

Introduction

Septicemia in neonates refers to the presence of microbes or their toxins in blood. Early onset and late onset sepsis are defined on the basis of presentation within 72 hours or after 72 hours of life respectively[1] Neonatal sepsis is a global health problem which can be devastating, causing high neonatal morbidity and mortality accounting for 18.6% neonatal deaths.[1] [2] According to the data from National Neonatal Perinatal Database 2002-03, incidence of neonatal sepsis was 30 per 1000 live births.[3] It is one of the leading causes

of neonatal mortality in developing countries like India.[4] The detection of microorganisms from blood culture of patients has significant importance for diagnosis and prognosis.[5] The etiology of septicemia is multi factorial, caused by both gram positive and gram negative organism and predominance of one type over the other varies from place to place and even in the same place over time. The isolation of the microorganism is the gold standard method for diagnosis of septicemia.[6] [7]

Uncontrolled use of various potent and broad spectrum antibiotics has led to emergence of resistant strains. It is a common practice to institute early empirical therapy with broad spectrum antibiotics in patients presenting with clinical features suggestive of septicaemia or bacteraemia. Given the severity of septicaemia, such empirical therapy may be justified, but the specific therapy based on the antibiogram of the isolate will definitely improve the therapeutic outcome.[8]

The present study was conducted to determine the various organisms causing septicemia and their antibiotic resistance pattern in our tertiary care hospital.

Material & Method

The present study was carried out between August 2017 to December 2017 in the Department of Microbiology, MGM, Medical College, Indore. During this period blood samples were received from pediatrics department. Blood samples for culture were collected following strict aseptic precautions. About 1 ml of blood in case of neonates and about 5 ml in case of children was collected in each set. Immediately after collection, the blood was inoculated into BHI broth without switching needles. The culture bottles were incubated at 37°C aerobically. After overnight incubation, Blind Subculture was done on to fresh 5% sheep blood agar and MacConkey's agar. If no growth observed on plates by the next day, subcultures were again repeated from the broth on

day 3, day 4 and finally on day 7. Antibiotic susceptibility tests were performed according to the standard methods.

Final identification of bacterial isolates was done by colony Characteristics, Gram's staining, Motility testing and biochemical testing.

For identification of gram positive isolates catalase and coagulase tests (slide and tube test) were done; for gram negative organisms, Simons's citrate test, motility, indole test, Christensen urease test, sugar fermentation (glucose, lactose, sucrose and mannitol)and triple sugar iron test (TSI) were done.

Antimicrobial susceptibility testing was done by Kirby- Bauer disc diffusion method using Muller Hinton agar media and antibiotics disks (high media) as per CLSI 2017 guidelines[9].

Result

Of the 450 Blood cultures received from cases of clinically suspected sepsis, out of which 176 (39%) blood cultures were positive. In which 59% were males and 41% were female neonates. Mixed bacterial growth was obtained from 3 samples. Out of positive bacterial growth the *Klebsiella spp.* 54(30.6%) was most frequently isolated followed by *CONS* 42(23.8%), *Staphylococcus aureus* 35(19.8%), *Escherichia coli* 22(12.5%), *Enterococcus spp.* 10(5.5%), *Pseudomonas aeroginosa* 5(2.8%), *Acinetobacter spp.* 4(2.2%), *Citrobacter spp.* 2(1.1%), and *Proteus spp.* 2(1.1%). *Candida spp.* was isolated from 9 samples considered as contaminant.

Table 1 Isolation of organism from blood culture

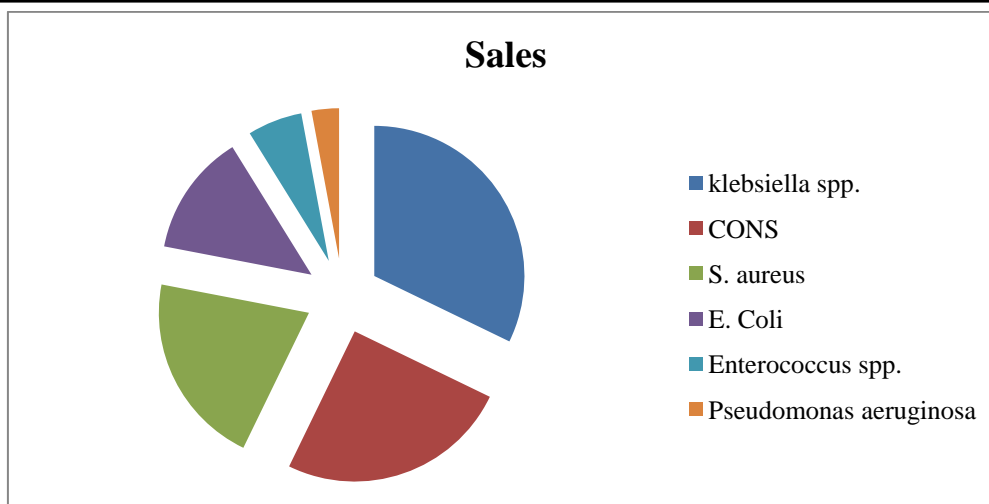| Organism	Number / Percentage
Klebsiella spp	54 (30.6%)
CONS	42 (23.8%)
Staphylococcus aureus	35 (19.8%)
Escherichia coli	22 (12.5%)
Enterococcus spp.	10 (5.6%)
Pseudomonas aeroginosa	5 (2.8%)
Acinetobacter spp.	4 (2.2%)
Citrobacter spp	2 (1.1%)
Proteus spp.	2 (1.1%)
Total	**176**

Table 2 Antibiotics resistance pattern of gram positive microorganism

Antibiotics	*Staphylococcus aureus* (35)	*Coagulase negative staphylococcus* (42)	*Enterococcus spp.* (10)
Penicillin	100% (35)	97% (41)	90% (9)
ampicillin			70% (7)
Azithromycin	54% (19)	48% (20)	
Clindamycin	25% (9)	28% (12)	
Trimethoprim sulfamethoxazole	60% (21)	54% (23)	
Cefoxitin	62% (22)	52% (22)	
Tetracycline	48% (17)	42% (18)	
Chloramphenicol	31% (11)	26% (11)	
Linezolid	5% (2)	2% (01)	10% (1)
Vancomycin	8% (3)	4% (02)	30% (3)
High level Gentamicin			50% (5)

Isolated *Staphylococcus aureus* strains were 100 % resistant to penicillin followed by cefoxitin 62% (MRSA), Trimethoprim sulfamethoxazole 60% (21), Azithromycin 54%, Tetracycline 48%, Chloramphenicol 31%, Clindamycin 25% and least resistant to vancomycin 8% and linezolid 5%. Among coagulase negative *Staphylococcus* strains, high resistance were found against penicillin 97% followed by trimethoprim sulfamethoxazole 54%, cefoxitin 52 %, azithromycin 48 %, tetracycline 42%, chloramphenicol 31% and least resistance with vancomycin 4% and linezolid 2%. The strains of enterococcus was most resistance to penicillin 90%, and ampicillin 70% and least resistance to linezolid 10% and vancomycin 30% Antibiotics resistance pattern of gram positive cocci depicted in table 2.

Table 3 Antibiotics resistance pattern of gram negative bacilli

Antibiotics	*Klebsiella* 54	*E. coli* 22	*Pseudomonas* 5	*Acinetobacter* 4	*Citrobacter* 2	*Proteus* 2
Amikacin	*15 (27.7%)*	*1 (4.5%)*	*2 (40%)*	*2 (50%)*	*1 (50%)*	*1 (50%)*
Aztreonam			*2 (40)*	*2 (50%)*	*1 (50%)*	*0 %*
Ceftazidime	41 (76%)	16(72.7%)	2 (40%)	3 (75%)	*1 (50%)*	2 (100 %)
cefuroxime	49 (90%)	18 (81.8%)	5 (100%)	3 (75%)	*1 (50%)*	2 (100%)
cefepime	32 (59%)	9 (40.9%)	3 (60%)	*2 (50%)*	(0 %)	2 (100%)
ceftriaxone	31 (57%)	11 (50%)		*2 (50%)*	*1 (50%)*	2 (100%)
Gentamicin	10 (18%)	3 (13.6%)	2 (40%)	*2 (50%)*	*1 (50%)*	*1 (50%)*
Trimethoprim sulfamethoxazole	40 (74%)	6 (27.2%)		3 (75%)	(0 %)	*1 (50%)*

ciprofloxacin	41 (75%)	9 (40.9%)	3 (60%)	4 (100%)	*1 (50%)*	*1 (50%)*
Piperacillin	33 (61%)	9 (40.9%)	2 (40%)	*2 (50%)*	*1 (50%)*	*1 (50%)*
Piperacillin tazobactam	10 (18%)	3 (13.6%)	1 (20%)	1 (25%)	*1 (50%)*	*1 (50%)*
Imipenem	20 (37%)	2 (9%)	1 (20%)	1 (25%)	(0 %)	(0 %)
Meropenem	7 (12%)	1 (4.5%)	1 (20%)	(0 %)	(0 %)	*1 (50%)*
colistin	1 (1.8%)	(0 %)	1 (20%)	(0 %)	(0 %)	(0 %)

Among gram negative bacterial isolates, *Klebsiella pneumonae* was most resistance to cefuroxime 90%, ceftazidime 76%, ciprofloxacin 75% and cotrimoxazole and least resistance meropenem 12%. *E. coli* was most resistance to cefuroxime 81.8%, ceftazidime 72.2% and ceftriaxone and least resistance to meropenem 4.5% and amikacin 4.5%. *Pseudomonas aeruginosa* was most resistance to cefuroxime and least resistance to colistin, imipenem, meropenem and piperacillin tazobacam 20%. Antibiotics resistance pattern of gram negative bacilli depicted in table 3.

Discussion

Blood culture is still a gold standard method for diagnosis of septicemia and information provided by antibiotic susceptibility report can help the clinician to choice of empiric therapy.

In this study blood culture positivity was found 39%, previous studies reported blood culture positivity rate ranging from 20% to 45%. Mahapatra [10], Khanal[11] and DS Murthy et al[8] have reported 40%, 44% and 24.3% respectively. In India, the variation of blood culture positivity might be due to the fact that most of the patients received antibiotics before they come to the tertiary care hospital & other reason is that in most of the cases self-medication as the medicines are easily available at the counter.

The common isolates in blood culture in this study were *Klebsiella* 54 (30.6%), coagulase negative staphylococci 42 (23.8%)*S. aureus* 35 (19.8%) and *E coli* 22 (12.5%). Sangeeta et al[10] also reported Klebsiella spp. (54.4%) and coagulase negative staphylococcus (CONS) (23.3%) as most common Gram-negative and Gram-positive organisms respectively. Whereas, many studies

reported *Klebsiella and Staphylococcus aureus* to be the commonest organism.[12] [13] [5]. In an epidemiological study performed to observe the long term trends in the agents causing neonatal sepsis, CONS were showing an increasing trend.[18]

Antibiotic resistance is today a global problem. Reports of multi-resistant bacteria causing neonatal sepsis in developing countries are increasing.

The high resistance against penicillin and cotrimoxazole, exhibited by most of the Gram positive bacterial isolates tested in this study was comparable with the findings of Roy I et al[14]. 62% of *S.aureus* were methicillin resistance comparable to study by Michael B et al 85%.[5] Our study showed that, among gram-positive *isolates*, maximum numbers were sensitive to linezolid and vancomycin This is in accordance with other studies[15] [16] [17].

Among Gram negative isolates high resistance was noted against cefuroxime (97.5%) and ceftazidime (73.03%). Ciprofloxacin(66.29%), cotrimoxazole(56.17%), ceftriaxone(55.05%) also showed a higher level of resistance. A combination of a third generation cephalosporin (cefotaxime or ceftazidime) with amikacin may be appropriate for GNB showed multiple drug resistance but some studies suggest that 60–70% of the Gram negative organisms are resistant to these antibiotics and routine use of these antibiotics might increase the risk of infections with ESBL positive organisms.[8]

The wide availability of over-the-counter antibiotics and the inappropriate use of broad-spectrum antibiotics in the community may explain this situation.[10]

Conclusion

In our study *Klebsiella*, CONS, and *S. aureus* were found to be a leading cause of neonatal sepsis, and most of them were resistant to multiple antibiotics. Therefore, we suggest that surveillance of antimicrobial resistance is necessary. Institute should also formulate its own antibiotic policy and on the basis of sensitivity pattern empirical therapy should be decided. Furthermore, we advise to spread awareness among healthcare personnel and common population regarding 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.

References

1. Lesser CF, Miller SI. Septicemia. In: Braunwald E, Hauser SL, Fauci AS, Longo DL, Kasper DL, Jameson JL, eds. Harrison's Principles of Internal Medicine. 15th ed. McGraw-Hill Company, 2001; vol.1: 970-974.

2. Tsai MH, Chu SM, Lee CW, Hsu JF, Huang HR, Chiang MC, et al. Recurrent late-onset sepsis in the neonatal intensive care unit: incidence, clinical characteristics and risk factors. *Clin Microbiol Infect*. 2014; 20(11): O928 – O935.

3. Report of the National Neonatal Perinatal Database (National Neonatology Forum) 2002-03. New Delhi: National Neonatology Forum NNPD Network; 2005:70.

4. Kumaravel K S, Rameshbabu B. A Study of the Bacteriological Profile and Antibiotic Sensitivity in Neonatal Septicemia. International Journal of Contemporary Medical Research. Volume 3 | Issue 6 | June 2016 | ICV: 50.43 |

5. Meenakshi Kante*, Muni Lakshmi P, Sreenivasulu Reddy P Bacterial profile of blood stream infections and their antibiograms. *Int J Res Med Sci. 2015 Mar;3(3):698-704*.

6. Kenneth C. Iregbu, Olufumilayo Y. Elegba, Iretiola B. Babaniyi Bacteriological profile of neonatal septicaemia in a tertiary hospital in Nigeria *African Health Sciences* 2006; 6(3): 151-154

7. Kavitha Prabhu, Sevitha Bhat, Sunil Rao Bacteriologic Profile and Antibiogram of Blood Culture Isolates in a Pediatric Care Unit Journal of Laboratory Physicians / Jul-Dec 2010 / Vol-2 / Issue-2

8. Murty D S, Gyaneshwari M. Blood cultures in paediatric patients: A study of clinical impact. Indian J Med Microbiol 2007;25:220-4

9. Clinical and Laboratory Standards Institute [CLSI]. Performance for Antimicrobial disk Susceptibility Testing-Approved Standard. 27th information supplement. Wayne, PA. M100-S27; 2017.

10. Mahapatra, A., Ghosh, S.K., Mishra, S., *et al*. 2002. Enterobacter cloacae: a predominant pathogen in neonatal septicaemia. *Indian J. Med. Microbiol.,* 20: 110–12.

11. Khanal B, Harish BN, Sethuraman KR, Srinivasan S. Infective endocarditis: report of prospective study in an Indian hospital. Trop Doct. 2002;32:83-5.

12. Shrestha S, Shrestha NC, Singh S, Shrestha RPB, Kayestha S, Shrestha M et al. Bacterial isolates and its antibiotic susceptibility pattern in NICU. Kathmandu Univ Med J. 2013;41:66-70.

13. Patel D, Nimbalkar A, Sethi A, Kungwani A, Nimbalkar S. Blood culture isolates in neonatal sepsis and their sensitivity in Anand district of India. Indian J Pediatr. 2014;81:785-90.

14. Roy I, Jain A, Kumar M , Agarwal SK.Bacteriology of neonatal septicemia in a tertiary care hospital of northern India.IJMM 2002; 20:156-9.

15. Bhat YR, Lewis LES Vandana KE. Bacterial isolates of early-onset neonatal

sepsis and their antibiotic susceptibility pattern between 1998 and 2004: an audit from a center in India. Ital J Pediatr. 2011;37:32-7.

16. Shrestha S, Shrestha NC, Singh S, Shrestha RPB, Kayestha S, Shrestha M et al. Bacterial isolates and its antibiotic susceptibility pattern in NICU. Kathmandu Univ Med J. 2013;41:66-70.

17. Kumhar GD, Ramachandran VG, Gupta P. Bacteriological analysis of blood culture isolates from neonates in a tertiary care hospital in India. J Health Popul Nutr. 2002;20:343-7.

18. 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.