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Research Article

Bacteriological profile of neonatal septicemia cases and the antimicrobial resistance pattern in a tertiary care hospital of central Nepal

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Abstract

Background: Neonatal septicemia is an important cause of morbidity and mortality. Knowledge of bacteriological profile and antimicrobial susceptibility is very important for management of such infection.
Objective: To determine the bacteriological profile of neonatal septicemia and the antimicrobial resistance pattern.
Materials and Methods: A total of 377 neonatal blood cultures samples were processed in the Department of Microbiology, Chitwan Medical College Teaching Hospital, Nepal in one year period. Isolation, identification and antimicrobial susceptibility was determined by standard microbiological methods.
Results: Of 377 specimens studied, bacterial growth was obtained in 80 specimens (2.1%). Gram-positive organisms were isolated in 35 (43.7%) and Gram-negative in 45 (56.3%) specimens. *Staphylococcus aureus* was the most common organism (23/70 isolates) isolated in early-onset septicemica followed by *Acinetobacte*r species (18/70 isolates) and *Klebsiella* species. However, late-onset septicemia was primarily associated with *Acinetobacte*r species (4/10 isolates).
On performing antimicrobial susceptibility testing, Gram-positive organisms exhibited maximum resistance to Cotrimoxazole (100%) followed by Penicillin (75%) and Cephalexin (50%) while Gram-negative organism to Norfloxacin (100%) followed by Cefixime (90.6%), Cotrimoxazole (80%) and Ceftriaxone (78.5%) among all antibiotics tested.
Conclusion: A wide spectrum of antimicrobial resistant bacterial agents are responsible for neonatal septicemia in our set up. A longitudinal surveillance program coupled with good infection control practices and rational use of antibiotics is important to reduce infection rate and ensure better therapeutic success.
Keywords: Neonatal septicemia, bacteriological profile, antimicrobial resistance, Nepal

1. Introduction

Neonatal septicemia is defined as a disseminated disease with positive blood culture during the first month of life [1], and is more common in developing countries compared with developed countries[2]. Neonatal septicemia is one of the leading causes of hospital admission in Nepal and one of the leading causes of neonatal deaths[3]. It is estimated that 20% of all neonates develop sepsis[4] and it is responsible for 30-50% of total neonatal deaths in developing countries.

Neonatal sepsis is classified as "early-onset" if it occurs within the first week of life and as "late-onset" if occurring after the first week until the end of the neonatal period[5]. Early-onset sepsis is conventionally regarded as maternally-acquired, with causative organisms, such as *Escherichia coli* and Group B *Streptococcus* (GBS) usually found in the maternal genital tract, whereas late-onset sepsis is most commonly caused by Coagulase-negative staphylococci (CONS),

Staphylococcus aureus, *Escherichia coli, Klebsiella* and *Pseudomonas* and is usually acquired in the hospital or in the community[5,6].

Changing bacterial flora and emergence of resistant strains make it imperative to have knowledge on the prevailing pattern of antimicrobial susceptibility of the etiological agents of septicemia for the appropriate management of the patient. Hence, the study was conducted to determine the bacteriological profile of neonatal septicemia cases and the antimicrobial resistance pattern in Chitwan Medical College Teaching Hospital, Chitwan, Nepal.

2. Materials and methods

Blood specimens for culture, collected from 377 neonates suspected of having sepsis, in Chitwan Medical College Teaching Hospital during one year period (August 2010-July 2011) were evaluated.

About 1-1.5 ml of venous blood was collected from each patient following strict aseptic precautions. Blood culture was performed by inoculating the collected blood aseptically into 9 ml of Brain Heart Infusion (BHI) broth contained in the paediatric blood culture bottle as per the recommended procedure[7].

The culture bottles were incubated aerobically at 37˚C for seven days. Routine subculturing was done on 5% sheep blood agar and MacConkey agar after 24 hours, 48 hours and then at 7 days. In between these time points, subculturing was done only if there was visible turbidity. The bacterial colonies grown on either of the media were indentified as per the standard methods (colony morphology, Gram stain and biochemical tests)[8] . The culture specimens not showing any growth after the final subculture were regarded as sterile and discarded. Antimicrobial susceptiblity testing was performed by Kirby-Bauer disk diffusion method on Muleller-Hinton agar method as per CLSI guidelines[9].

3. Results

Of 377 specimens studied, bacterial growth was obtained in 80 specimens (2.1%): 47 from male patients and 33 from female patients. Gram-positive organisms were isolated from 35 specimens (43.7%) and Gram-negative from 45 (56.3%) specimens. In all culture positive cases, the cause of septicemia was found to be monomicrobial.

Early-onset septicemia was observed in 87.5% cases while late-onset septicemia in 12.5% cases. *Staphylococcus aureus* was the most common organism (23/70 isolates) isolated in early-onset septicemica followed by *Acinetobacter* species (18/70 isolates) and *Klebsiella* species. However, late-onset septicemia was primarily associated with *Acinetobacte*r species (4/10 isolates) [table 1].

Table 1: Organism isolated in early-onset and late-onset septicemia

Organisms isolated		**Early-onset (Upto 7days)**	**Late- onset (˃7 days to 1 month)**	**Total no. of organism**
Gram-positive organisms (n=35)	CONS	2	1	3
	Enterococcus species	7	1	8
	Staphylococcus aureus	23	1	24
Gram-negative organisms (n=45)	*Acinetobacter* species	18	4	22
	Enterobacter species	2	0	2
	Escherichia coli	3	0	3
	Klebsiella species	10	2	12
	Proteus species	1	0	1
	Pseudomonas species	2	0	2
	Salmonella Paratyphi A	1	1	2
	Salmonella Typhi	1	0	1
Total no. of organisms		**70 (87.5%)**	**10 (12.5%)**	**80**

On performing antimicrobial susceptibility testing, Gram-positive organisms exhibited maximum resistance to Cotrimoxazole (100%) followed by Penicillin (75%) and Cephalexin (50%) [table 2].

Table 2: Antibiotic resistance pattern of Gram-positive organisms

Antibiotics	Organisms							
	CONS (n=3)		*Staphylococcus aureus* (n=24)		*Enterococcus species* (n=8)		Total Gram Positive organisms (n=35)	
	Tested	Resistant	Tested	Resistant	Tested	Resistant	Tested	Resistant (%)
Amikacin	3	0	18	1	7	4	28	5 (17.8)
Ampicillin	0	0	2	0	8	4	10	4 (40)
Cephalexin	2	1	20	9	4	3	26	13 (50)
Ciprofloxacin	3	0	7	0	2	2	12	2 (16.6)
Cotrimoxazole	2	2	7	7	1	1	10	10 (100)
Erythromycin	0	0	1	0	0	0	1	0 (0)
Gentamicin	3	0	23	8	8	3	34	11 (32.3)
Nalidixic acid	0	0	1	0	0	0	1	0 (0)
Ofloxacin	3	0	23	4	6	2	32	6 (18.7)
Penicillin	0	0	24	18	0	0	24	18 (75)
Tetracycline	1	0	0	0	0	0	1	0 (0)
Vancomycin	0	0	7	0	0	0	7	0 (0)

Gram-negative bacteria also showed variation in their resistance pattern. Higher rate of resistance was observed for Norfloxacin (100%), Cefixime (90.6%), Cotrimoxazole (80%) and Ceftriaxone (78.5%) among all antibiotics tested (table 3).

Table 3: Antibiotic resistance pattern of Gram-negative organisms

Antibiotics	Organisms																	
	Acinetobacter spp (n=22)		*Enterobacter* spp (n= 2)		*E. coli* (n= 3)		*Klebsiella* spp (n= 12)		*Pseudomonas* spp (n=2)		*Proteus spp* (n= 1)		*S.* Paratyphi A (n= 2)		*S.* Typhi (n=1)		Total Gram negative organisms (n=45)	
	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R (%)
Ak	21	4	2	2	2	1	10	3	2	0	1	0	2	1	1	1	41	12(29.2)
A	18	12	2	2	1	1	10	10	1	1	1	1	2	1	1	1	36	29(80.5)
CA	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0 (0)
CB	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0 (0)
CE	16	4	1	1	3	3	11	10	0	0	1	1	1	0	1	1	34	20(58.8)
COT	0	0	2	2	1	0	9	8	0	0	1	1	2	1	0	0	15	12 (80)
CTR	22	16	2	2	3	2	11	9	1	0	1	1	2	1	2	2	42	33(78.5)
CFX	19	18	2	2	2	2	7	5	0	0	1	1	1	1	0	0	32	29(90.6)

CPM	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0 (0)
C	16	4	2	2	3	1	11	2	2	1	1	0	2	1	1	0	38	11(28.9)
G	19	10	2	2	3	1	9	8	1	0	1	0	1	0	1	1	37	22(59.4)
NX	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	2	2 (100)
OF	22	6	1	0	3	2	10	2	2	1	1	0	1	0	1	1	41	12(29.2)
PT	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0 (0)
PC	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	1	0 (0)
TB	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	1	0 (0)

Abbreviations: Spp-species, Ak-Amikacin, A-Ampicillin, CA-Ceftazidime, CB-Carbenicillin, CE-Cephotaxime, COT-Cotrimoxazole, CTR-Ceftriaxone, CFX-Cefixime, CPM-Cefepime, C-Chloramphenicol, G-Gentamicin, NX-Norfloxacin, OF-Ofloxacin, PT-Piperacillin-Tazobactum, PC-Piperacillin, TB-Tobramycin, T-tested, R-resistant

4. Discussion

Neonatal septicemia is a life threatening condition that requires prompt treatment and management. Knowledge of isolation pattern of organism and their antibiotic susceptibility pattern would help clinician for effective management of the condition.

In the present study, culture positivity in the suspected neonatal septicemia was 2.1%, the isolation rate being much lower than the rate observed by Rahman et al[10] (62.8%), Karki et al[11] (59.7%), Bhattacharjee et al[12] (48%) and Desai *et al*[13] (46.2%). Lower prevalence of documented neonatal sepsis with positive blood culture in our study might have different reasons such as antibiotic administration in mother or neonate prior to sample collection, difficulty in sampling, blood culture technique[14], sepsis due to anaerobic, viral or fungal pathogens[15] and misdiagnosis because of some similarities between the clinical signs of sepsis with other diseases like metabolic disorders[16].

In current study, early-onset sepsis was more common than late-onset sepsis (87.5 versus 12.5%). This finding was consistent with the results of the Vinodkumar et al[17] which reported that prevalence of early-onset neonatal sepsis was 73%. On the contrary, Kuruvilla et al[18] reported the higher prevalence of late-onset sepsis compared with early-onset sepsis (77.1 versus 22.9%).

We have observed that the cause of septicemia was monomicrobial in all the cases. However, 6.8% of polymicrobial cause of sepsis have been reported by Kumhar et al[19] who noted that in culture-positive neonates, the cause of septicaemia was monomicrobial in 718 (93.2%) cases and polymicrobial in 52 (6.8%) cases.

Unlike in western countries where Group B streptococci are mainly isolated, in the present study Gram-negative septicemia (56.3%) was more common than Gram-positive septicemia (43.7%) with *Acinetobacter* being the most common isolate. The role of *Acinetobacter* in cases of neonatal septicemia has already been stressed[20]. The isolation pattern of the organisms has been found to differ from study to study: for example, Karki *et al*[11] and Shrestha et al[21] isolated *E. coli* as the as the most common organism while Kumhar et al[19] and Kaistha et al[22] isolated *Klebsiella* species.

Nowadays, antibiotic resistance is a widespread global problem causing ineffectiveness of current empirical treatment. In the present study, a large number of Gram-positive and Gram- negative bacteria exhibited resistance, though variable, to almost all the clnically usefull antibiotics. Resistance being developed against those antibiotics may be primarily due to their indiscriminate and over use.

Gram-positive organisms showed maximum resistance to Cotrimoxazole (100%) followed by Penicillin (75%) and Cephalexin (50%). However, all Gram-positive organisms were sensitive to vancomycin, indicating its usefullness as a reserved drug.

In case of Gram-negative organisms, highest rate of resistance was observed for Norfloxacin (100%). High level of resistance against Ampicillin (80.5%) and Cephalosporins (Cefixime-90.6%, Ceftriaxone-78.5%, Cephotaxime- 58.8%) could be due to β- lactamases expression in these bacteria. The Gentamicin resistance (59.4%) was quite higher than the

Amikacin resistance (29.2%). Thus, Amikacin can be considered to have better efficacy than Gentamicin. Newer combination drug like Piperacillin-Tazobactam tested for 5 isolates of *E. coli* was quite effective. Though tested for only single isolate, Ceftazidime, Carbenicillin, Piperacillin and Tobramycin were also found to be useful for treating *pseudomonas* infection.

5. Conclusion:

A wide spectrum of antimicrobial resistant bacterial agents are responsible for neonatal septicemia in our set up. Therefore, a longitudinal surveillance program along with clean and safe deliveries, strict infection control practices and rational use of antibiotics are emphasized to reduce the infection rate and ensure better therapeutic success.

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References

1. Edwards MS. Postnatal infections. In: Fanaoff, Martins editors. Neonatal-perinatal Medicine, 8th ed. Philadelphia: Mosby Elsevier, 2006. p. 791-804.
2. Vergnano S, Sharland M, Kazembe P, Wansambo CM, Heath PT. Neonatal sepsis. An international perspective. Arch Dis Child Fetal Neonatal Ed 2005;90: F220-F224.
3. National neonatal health strategy. Family health division, Department of health services, Ministry of health, Government of Nepal, 2004;1-4**.**
4. Stoll BJ. Infections of the neonatal infant. In: Behrman RE, Kliegman RM, editors. Nelson textbook of pediatrics. 18th ed. Philadelphia: WB Saunders Company; 2008, p.794-8.
5. Klein JO. Bacterial sepsis and meningitis. In: Remington JS, Klein JO, editors. Infectious Diseases of the Fetus, Newborn, and Infants. 5th ed. Philadelphia: WB Saunders Company; 2001:943–84.
6. Stoll BJ. Infections of the Neonatal Infant: Pathogenesis and Epidemiology. In: Behrman RE, Kliegman RM, editors. Nelson Textbook of Pediatrics. 17th ed. Philadelphia: Saunders; 2004: 623–40.
7. Colle JG, Duguid JP, Fraser AG, Marmion BP editors. Mackie & McCartney Practical Medical Microbiology. 13 th ed. New York: Churchil Livingstone; 1989.
8. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. 12th ed. St. Louis: Mosby Elsevier; 2007. Chapter 13, Overview of Bacterial Identification Methods and Strategies; p.216-247.
9. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests. 9th ed. Approved standard. Wayne, PA: Clinical and Laboratory Standards Institute, 2006. (CLSI document no. M2-A9).
10. Rahman S, Hameed A, Roghani MT, Ullah Z. Multidrug resistant neonatal sepsis in Peshhawar, Pakistan. Arch Dis Child Fetal Neonatal Ed 2002;87(1): F52-F54.
11. Karki BMS, Parija SC. Analysis of blood culture isolates from hospitalized neonates in Nepal. Southeast Asian J Trop Med Pub Health 1999;30(3):546-8.
12. Bhattacharjee A, Sen MR, Prakash P, Gaur A, Anuprba S. Increased prevalence of extended spectrum β-lactamase producers in neonatal septicaemic cases at a tertiary referral hospital. Indian J Med Microbiol, 2008;264(4):356-60.
13. Desai KJ, Malek SS. Neonatal Septicemia: Bacterial Isolates & Their Antibiotics Susceptibility Patterns. NJIRM 2010;1(3):12-5.
14. Bansal S, Jain A, Agarwal J, Malik GK. Significance of coagulase negative staphylococci in neonates with late onset septicemia. Indian J Pathol Microbiol 2004; 47(4): 586-8.
15. Agnihotri N, Kaistha N, Gupta V. Antimicrobial susceptibility of isolates from neonatal septicemia. JPN J Infect Dis 2004;57(6):273-5.

16. Lund AM, Christensen E, Skovby F. Diagnosis and acute treatment of inborn metabolic diseases in infants. Ugeskrift for Laeger 2002. 164(48):5613-9.
17. Vinodkumar CS, Neelagund YF, Suneeta K, Sudha B, Kalapannavar NK, Basavarajapa KG. Perinatal risk factors and microbial profile of neonatal septicemia: A multicentred study. J Obstet Gynecol India 2008;58(1): 32-40.
18. Kuruvilla KA, Pillai S, Jesudason M, Jana AK. Bacteriological profile of sepsis in a neonatal unit in south India. Indian Pediatr 1998;35:851-8.
19. Kumhar GD, Ramachandran VG, Gupta P. Bacteriological Analysis of Blood Culture Isolates from Neonates in a Tertiary Care Hospital in India. J Heath Popul Nutr 2002;20(4):343-7.
20. Mondal GP, Raghavan M, VishnuBhat B, Srinivasan S. Neonatal Septicemia Among Inborn and Outborn Babies in a Referral Hospital. Indian J Pediatr 1991; 58: 529-33.
21. Shrestha NJ, Subedi KU, Rai GK. Bacteriological Profile of Neonatal Sepsis: A Hospital Based Study. J Nepal Paediatr Soc 2011;31(1):1-5.
22. 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 2010; 4(1):055-057.