Research Article

Study of gram negative septicemia in cases of neonates admitted in neonatal intensive care unit at tertiary care hospital

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Abstract

Background: Septicemia in neonates is one of the four leading causes of neonatal mortality in India. The pattern of causative agents has been constantly changing and there has been a frequent emergence of resistant bacteria. The antibiotic misuse has resulted in emergence of drug resistant bacterial strains in the neonatal units with grave sequelae. **Objectives**: to describe the spectrum of aerobic gram negative bacterial isolates and study the sensitivity pattern in cases of neonatal septicemia. **Methods**: Blood samples were collected aseptically from 200 clinically suspected cases and inoculated into BHI broth and incubated at 37^0 C for 7 days. Repeated subcultures were made on 1st, 3rd and 5th days onto Blood agar and Mac Conkey agar. Any growth was identified by colony characteristics and appropriate biochemical tests. Antibiotic susceptibility was done by disc diffusion method as per CLSI guidelines. **Results**: Of the 200 cases, 31.5% were found to be culture positive. Gram negative isolates were predominant 73% and Gram positive isolates were obtained in 27% of the cases. Out of the Gram negative, Klebsiellaspp was the commonest (44.4%) followed by Pseudomonas aeruginosa (17.5%), Escherichia coli (9.5%), Proteus vulgaris (1.6%). Gram negative isolates were most sensitive to Imipenem and Meropenem (98%), followed by Cefoperazone /Sulbactum (75-80%), Amikacin and Gentamycin (70-75%) and least sensitive to third generation cephalosporins. **Conclusion:** Therefore uncertainty regarding the choice of antibiotics can be minimized by regular survey of etiological agents and their antimicrobial susceptibility pattern.

Keywords: Neonatal septicemia, Klebsiellaspp, Antibiotic sensitivity.

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INTRODUCTION

Septicemia in neonates refers to generalized bacterial infections documented by a positive blood culture in the first 4 weeks of life.[1] It is one of the four leading causes of neonatal mortality in India. The pattern of causative agents has been constantly changing and there has been a frequent emergence of resistant bacteria.[2] A very wide spectrum of organisms have been described for cases of neonatal septicemia and this is subject to geographical alterations.[3] The local microbial flora causing neonatal septicemia needs to be studied in each setting to guide more effective and rational utilization of antimicrobial agents. Moreover the organisms isolated are often resistant to multiple antimicrobials which make the treatment difficult and grave sequelae ensue. The antibiotic misuse has resulted in emergence of drug resistant bacterial strains in the neonatal units with grave sequelae. Thus the successful treatment with favorable outcome of the neonate depends on an ongoing review of the causative organisms and their antibiotic susceptibility pattern.[4]

AIMS AND OBJECTIVES

1. To describe the spectrum of aerobic gram negative bacterial isolates in cases of neonatal septicemia at a tertiary care hospital.
2. To study the gram negative sensitivity pattern in cases of neonatal septicemia.

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MATERIALS AND METHODS

Source of Data

This was a prospective study done at a tertiary care hospital by taking samples from 200 clinically diagnosed cases of neonatal septicemia in Neonatal intensive care unit for a period of one year from Jan 2014 to Dec 2014.

Sample Collection: The skin of venipuncture site was disinfected with 70% alcohol and 1% iodine. Starting in the centre of the circle povidine iodine was applied in widening circles until the entire circle has been saturated with iodine and allowed to dry for at least a minute.

Blood Culture: Brain heart infusion broth was prepared using the commercially available ready to use powder supplied by Hi-Media Laboratories. The broth was distributed into 10 ml quantity in McCartney bottles and sterilized by autoclaving at 121^{o}C for 15 min. 1ml of blood was drawn and immediately inoculated in 10 ml of Brain Heart Infusion broth, thus making dilution of 1 in 10 to nullify the natural bacteriostatic or bactericidal activity of blood. After inoculation, the blood culture bottles were incubated at 37^{o}C under aerobic conditions and carefully examined for any macroscopic evidence of growth like turbidity, hemolysis etc every day for 7 days. The first subculture was done after 6-17hrs. There after on the third and finally on the fifth day. Subcultures were done onto Mac Conkey agar, Blood agar, Nutrient agar and chocolate agar plates. The inoculated plates were incubated at 37^{o} C for 24 hours and observed for any growth. Cultures that did not yield any growth following three subcultures were reported as negative after 7 days.

Identification of Isolate: Positive growth was identified on the basis of colony characteristics, gram's staining, hanging drop preparation, Catalase test, Oxidase test, OF test, Indole test, Methyl Red test, Voges Proskauer test, Citrate utilization test, Carbohydrate fermentation tests, Urease production, Amino acid decarboxylase test, Coagulase test (for Staphylococcus).

Antibiotic Susceptibility Testing: Antibiotic susceptibility testing was done for the isolates on Muller-Hinton agar using commercially available discs (Hi Media) by Kirby-Bauer disc diffusion method, using CLSI guidelines for interpretation. Every batch of Mueller-Hilton agar and antibiotic discs were tested by using ATCC control strains

Inclusion Criteria: Neonates admitted with the clinical diagnosis of neonatal septicemia were included in the study i-e on the basis of H/O maternal fever, H/O prolonged labour, Birth asphyxia, convulsions, symptoms of respiratory distress, temperature >37.7°C or <35.5°C, refusal of feeds and lethargy, were included under the study.

Exclusion Criteria: OPD patients, neonates on antibiotic therapy were excluded from the study.

Table 1: Antibiotics that were used for testing

Gram negative organisms			
Sl no	Antibiotics	Disk content (µg/disc)	Abvv
1	Amikacin	30	AK
2	Gentamycin	10	GEN
3	Netilmicin	30	NET
4	Ofloxacin	5	OF
5	Ciprofloxacin	5	CIP
6	Ceftazidime	30	CAZ
7	Ceftriazone	30	CTR
8	Cefotaxime	30	CTX
9	Ceftizoxime	30	CXZ
10	Cefoperazone –sulbactum	75/10	CFS
11	Piperacillin- Tazobactum	100/10	PIT
12	Imipenem	10	IPM
13	Meropenem	10	MRP

RESULTS

Table 1: Distribution of Cases According to Gender

Gender	Clinically suspected		Culture positive	
	No	%	No	%
Males	115	57.5	34	**53.97**
Females	85	42.5	29	46.03
Total	**200**	**100**	**63**	**100**

Of the 200 cases, 63(31.5%.) were found to be culture positive. Of these 63 culture positive cases, 34 (53.97%) were males and 29 (46.03%) were females. Males were higher than females in culture positivity with a ratio of 1.17:1

Table 2: Distribution of cases according to results of Blood Culture

Blood culture results	EOS	LOS	Total
Positive	57 (90.47%)	6 (9.53%)	63 (31.5%)
Negative	73 (53.3%)	64 (46.7%)	137 (68.5%)
Total	**130 (65%)**	**70 (35%)**	**200**

Of the 200 clinically suspected cases, 63 (31.5%) were culture positive and 137 (68.5%) were culture negative. Of the 63 culture positive cases, early onset septicemia was seen in 57 (90.47%) cases and late onset septicemia was seen in 6 (9.53%) cases.

Table 3: Distribution of culture positive cases according to Spectrum of Bacterial Isolates

Bacterial isolates	Culture positive	
	No	%
Gram Negative isolates	46	73
Klebsiellaspp	28	44.4
Pseudomonas aeroginosa	11	17.5
Escherichia coli	6	9.5
Proteus vulgaris	1	1.6
Gram Positive isolates	17	27
Coagulase negative staphylococcus	8	12.7
Staphylococcus aureus	7	11.1
Enterococcus spp	2	3.2
Total	**63**	**100**

Above table shows that, majority of the isolates 46 (73%) were Gram negative, out of which Klebsiellaspp was the commonest 28 (44.4%) followed by Pseudomonas aeruginosa 11(17.5%), Escherichia coli 6 (9.5%), Proteus vulgaris 1 (1.6%). Gram positive isolates were obtained in 17 (27%) of the cases, coagulase negative staphylococcus was the most common isolate 8 (12.7%). The other organisms isolated were Staphylococcus aureus 7 (11.1%), Enterococcus spp 2 (3.2%)

Table 4: Association of culture positive gram negative isolates with antibiotic Sensitivity Pattern

Antibiotics	Organisms											
	Klebsiellaspp			E.Coli			Pseudomonas			Proteus		
	S	I	R	S	I	R	S	I	R	S	I	R
Amikacin	54.4	4.0	41.6	75	10	15	65	12	23	100	0	0
Gentamycin	50	0	50	85	5	10	65	12	23	100	0	0
Netilmicin	30	3.3	66.7	85	5	10	74.7	10	15.3	0	0	100
Ofloxacin	71.1	8.1	20.8	61	6	33	72.2	20.1	7.7	0	0	100
Ciprofloxacin	70	5	25	95	5	0	70.2	14.4	15.4	0	0	100
Ceftazidime	5.3	3.0	91.7	23.3	10	66.7	4.3	3.3	92.3	0	0	100
Ceftriazone	5.3	3.0	91.7	23.3	10	66.7	12.3	3.1	84.6	0	0	100
Cefotaxime	20	5	75	51.2	15.5	33.3	42.3	13.5	44.2	0	0	100
Ceftizoxime	3.2	1	95.8	23.3	10	66.7	27.7	3.1	69.2	0	0	100
Cefoperazone/Sulbactum	70	0	30	73	0	27	71.9	5.03	23.07	100	0	0
Piperacillin/Tazobactum	65	5	30	73	0	27	74	0	26	0	0	100
Imipenem	92	0	8	100	0	0	100	0	0	100	0	0
Meropenem	92	0 +-	8	100	0	0	100	0	0	100	0	0

The above table shows that, maximum sensitivity for Klebsiellaspp was seen with Imipenem and Meropenem (92%) followed by Ofloxacin (71.1%), Ciprofloxacin (70%), Cefoperazone/Sulbactum(70%), Piperacillin/Tazobactum (65%). Most of the isolates were found to be resistant to third generation cephalosporins. Pseudomonas aeruginosa (n=11, 17.5%) was the second most common isolate with 100% sensitivity to Imipenem and Meropenem, Piperacillin /Tazobactum (74%), Netilmicin (74.7%), Ofloxacin (72.2%), Cefoperazone/Sulbactum (71.9%), Ciprofloxacin (70.2%), Amikacin and Gentamycin (65% each) and lowered sensitivity to third generation cephalosporins. Escherichia coli isolates showed 100% sensitivity to Imipenem and Meropenem, followed by ciprofloxacin (95%), Gentamycin and Netilmicin (85% each) , Amikacin (75%), Cefoperazone /Sulbactum (73%), Piperacillin /Tazobactum (73%).

DISCUSSION

Neonatal septicemia remains a challenging problem even with modern drug therapy. It is associated with considerable morbidity and mortality. The timely detection of bacteremia can have a profound influence on the final clinical outcome. In the present study, an attempt has been made to know the various etiological agents responsible for neonatal septicemia, study of bacteriological profile and their changing pattern of antibiotic sensitivity. In the present study, the percentage of Gram negative septicemia was 73% which outnumbered the Gram positive cocci (27%). Similarly Gram negative isolates exceeded the Gram positive in many studies conducted by Ghanshyam *et al* (60%)[5], Agnihotri *et al* (58.5%)[1], Movahedian *et al* (72.1%)[6], Bhattacharjee *et al* (73.04%)[7], AtulGarg *et al* (67.5%)[3], Vishwanathan R *et al* (71.6%)[8], Tsering D C *et al* (61%)[9]. However our results differed from the study done by Prabhu K *et al*[10] 64% who reported a higher incidence of Gram positive cocci. In the present study Klebsiellaspp (44.4%) was the predominant isolate. Similar higher proportion of Klebsiellaspp were isolated by Vishwanathan R *et al* (71.6%)[8], Tallur *et al* (53.5%)[11], Roy *et al* (24.5%)[4], Zakariya B P *et al* (66%)[12], Madhu Sharma *et al* (61.4%)[13] Pseudomonas aeruginosa as a commonest isolate was reported by Betty Chacko *et al* (60%)[14], Movahedian A H *et al* (36%)[6], Tsering D C *et al* (20%)[9], AtulGarg *et al* (16%)[3].

Antibiotic Sensitivity of the Commonest Isolates

In the present study, the percentage of Gram negative septicemia was 73% with Klebsiellaspp (44.4%) being the predominant isolate followed by Pseudomonas spp (17.5%). Maximum sensitivity for Klebsiellaspp was seen with Imipenem and Meropenem (92%) followed by Ofloxacin (71.1%), Ciprofloxacin (70%), Cefoperazone /Sulbactum (70%), Piperacillin /Tazobactum (65%), Amikacin (54.4%), Gentamycin (50%). Most of the isolates were found to be resistant to third generation cephalosporins.

In a study done by Tallur *et al*[11], Klebsiella pneumonia was found to be more susceptible to Gentamycin, Amikacin and third generation cephalosporins. Kumhar GD*et al*[5] reported that 50% of the Klebsiella and E coli isolates were sensitive to cefotaxime, 50% to Amikacin. Most of the isolates were sensitive to ciprofloxacin (50-75%). Another study by Madhu Sharma *et al*[13] showed Gram negative organisms as the predominant isolates (88.8%) with the commonest isolate being Klebsiellaspp (47.1%). They found that Cefoperazone/Sulbactum (97.4%), followed by ceftizoxime (66.47%) and Amikacin (65.6%) were the most effective antibiotic against these isolates. A study done by Movahedian *et al*[6] revealed a very high degree of resistance in gram negative organisms not only to commonly used antibiotics, but also predominantly to broad spectrum cephalosporins. A study done by Bambala *et al*[12]in 2011 reported 33 isolates of Klebsiellapneumonaie which were 100% sensitive to Meropenem , 82% to Amikacin, varying susceptibility to chloramphenicol (24%), ciprofloxacin (18%), ceftriazone (3%), ceftazidime (3%). Most of the gram negative isolates in the present study were multi-drug resistant. Other workers also have reported majority of gram negative isolates in their study as multi–drug resistant.

We found Pseudomonas aeruginosa (n=11, 17.5%) as a second most common isolate with 100% sensitivity to Imipenem and Meropenem, Piperacillin /Tazobactum (74%), Netilmicin (74.7%), Ofloxacin (72.2%), Cefoperazone/Sulbactum (71.9%), Ciprofloxacin (70.2%), Amikacin and Gentamycin (65% each) and lowered sensitivity to third generation cephalosporins. Similar results were observed by Bhattacharjee *et al*[7] who reported susceptibility of P.aeruginosa to Piperacillin/tazobactum (94%), Imipenem (86%), Cefoperazone (78%), Ceftazidime (79%), Ciprofloxacin (63%), Amikacin (68%), Gentamycin (53%) and Tobramycin (38%). Study by A H Movahedian *et al*[6] observed 70% sensitivity of Pseudomonas to Amikacin followed by Gentamycin (53%), and lower sensitivity to Ampicillin, Cephalexin, Ceftriazone and Ceftizoxime. Ramesh Bhat *et al*[2]observed that Pseudomonas isolates were susceptible to Amikacin, moderately to Gentamycin and ciprofloxacin and less susceptible to Ceftazidime and Piperacillin.

In our study, Escherichia coli isolates (n=6,9.5%) showed 100% sensitivity to Imipenem and Meropenem, followed by Ciprofloxacin (95%), Gentamycin and Netilmicin (85% each), Amikacin (75%), Cefoperazone /Sulbactum (73%), Piperacillin /Tazobactum (73%), Ofloxacin (61%). The resistance to third generation cephalosporins was found to be 66.7%. Also in our study, 1 isolate of Proteus vulgaris, was sensitive to Amikacin, Gentamycin, Cefoperazone/Sulbactum, Imipenem and Meropenem. The isolate showed resistance to Netilmicin, Ofloxacin, Ciprofloxacin, 3rd generation cephalosporins, Piperacillin/Tazobactum. Gram negative organisms continue to be a threat to the debilitated newborns. Among these, Klebsiella septicemia continues to be a challenge to the neonatologists and Microbiologists. One of the reasons for the predominance of an organism in causing septicemia in the neonatal units is the selective pressure of antimicrobial agents, so that resistant microorganisms tend to colonize and proliferate in the neonatal units.[10]

CONCLUSION

.It is therefore necessary to generate hospital data on antimicrobial sensitivity of common isolates, provide timely sensitivity report and guide the clinicians regarding the judicious use of antibiotics. The uncertainty regarding the choice of antibiotics can be minimized by regular survey of etiological agents and their antimicrobial susceptibility pattern.

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