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BACTERIAL ISOLATES, RISK FACTORS AND ANTIBIOGRAM OF NEONATAL SEPTICEMIA

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ABSTRACT

To isolate the bacteria causing neonatal sepsis, study the risk factors and assess their antibiotic sensitivity.Materials and Methods: 143 blood samples from suspected cases were collected, inoculated into BHI broth and cultured on solid media. Microorganisms were isolated, identified and antimicrobial susceptibility testing was performed. Of 143 samples, growth of bacteria was obtained in 45 (31.46%) samples. Klebsiella pneumoniae (46.6%) was the predominant gram negative bacteria and CONS (18.6%) was the commonest gram positive cocci. Neonatal septicaemia was observed in 17 (80.95%) mothers with clinical disease, 16 (48.48%) with premature delivery, 05 (23.8%) with perinatal asphyxia, 03 (6.97%) with Low birth weight, 02 (20%) with Premature rupture of membrane & 02 (13.33%) with fever. 57.14% of Klebsiella pneumoniae was sensitive to Amikacin, 100% Acinetobacter was sensitive to Tetracycline, 100% Pseudomonas was sensitive to Netilmicin, 100% Escherichia coli was sensitive to Amoxyclav, Amikacin, Piperacillin+Tazobactum & Cefoperazone+Sulbactum. 62.5% CONS was sensitive to tetracycline, Piperacillin+Tazobactum & Linezolid. 100% Enterococcus were sensitive to Linezolid. Conclusion Continuous local surveillance studies are required to monitor emerging antimicrobial resistance.

KEYWORDS: **Neonatal septicemia, blood culture, antibiotic susceptibility, antimicrobial resistance, risk factors, Kirby Bauer Disk Diffusion.**

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INTRODUCTION

Inspite of great advances in antimicrobial therapy, neonatal life support measures and the early detection of risk factors, septicemia continues to be a major cause of mortality and morbidity among neonates around the world[1]. It is a life threatening clinical emergency that demands urgent diagnosis and treatment [2]. Moreover several risk factors have been identified both in the neonates and in the mother, which make them susceptible to infections. A very spectrum of organisms has been described for cases of neonatal septicemia and this spectrum is subject to geographical alterations [3] .The rapid emergence of multidrug resistant neonatal sepsis in developing countries is a new potential threat to the survival of new born babies, who are already in a poor condition [4]. So, rapid detection of the infectious cause and immediate and appropriate antimicrobial treatment are very critical for the successful treatment of such patients and also to reduce antibiotic resistance rates. Thus the present study was undertaken to isolate the bacteria causing neonatal septicemia, study the risk factors associated and their antimicrobial susceptibility pattern.

MATERIALS AND METHODS

A cross-sectional retrospective study was conducted on 143 blood samples from suspected neonatal septicemia cases for one year period. Detailed history was recorded from the history sheets to identify the possible risk factors. 1ml – 2ml of blood was collected from each patient using proper aseptic precautions and inoculated immediately into 5ml of Brain Heart Infusion broth with 0.025% Sodium polyanethol sulfonate (HiMedia Laboratories, Mumbai) as anticoagulant[5]. The broth was subcultured after overnight incubation at 37°C aerobically on chocolate agar, MacConkey's agar and blood agar[6]. If there was no growth observed on the plates by the next day, subcultures were again repeated from the broth on day 3, day 4 and finally on day 7 [7, 8]. Any growth was identified by colonial characteristics and standard biochemical tests. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method[9].

Statistical Analysis: was done using Odds Ratio or Chi-square and Fisher's Exact t-test as applicable.

Ethical Clearance and Consent: As it was a retrospective study, ethical clearance and consent was not obtained.

Inclusion Criteria: Neonates with clinical features of septicemia.

Exclusion Criteria: Babies who had received prior antibiotic treatment or chromosomal & congenital anomalies, or surgical problems.

RESULTS

Out of 143 cases studied, 42(95.5%) neonates were < 1week & only 02 (4.44%) were > 1 week of age. Growth of bacteria was obtained in 45 (31.46%) samples and Candida spp. was isolated in 2 (1.4%) samples. Of the bacterial isolates the most frequent bacteria isolated was Klebsiella pneumoniae (46.6%) followed by CONS (17.7%), Acinetobacter spp. (15.5%), Pseudomonas (11.1%) and other less frequent isolates (Table 1).In early onset disease (age < 1wk), the most common isolate was Klebsiella pneumoniae (46.5%) and CONS (18.6%) followed by Acinetobacter spp. (13.9%). In late onset illness (age>1wk) also Klebsiella pneumoniae (50%) and Acinetobacter spp (50%) were isolated from one sample each. (Table 1) Neonatal septicaemia was observed in 17 (80.9%) mothers with clinical disease, 16 (48.4%) cases with premature delivery, 05 (23.8%) with perinatal asphyxia, 03 (6.97%) with LBW, 02 (20%) with PROM & 02 (13.3%) with fever. (Table 2) 57.14% of Klebsiella pneumoniae was sensitive to Amikacin, 38.09% to tetracycline & 38.09% to Ciprofloxacin. 100% of Acinetobacter was sensitive to Tetracycline, 71.4% to Netilmicin. 100% of Pseudomonas was sensitive to Netilmicin, 80% to Amikacin, 80% to Ofloxacin & 80% to Cefoperazone+Sulbactum. 100% of Escherichia coli was sensitive to Amoxyclav,

Amikacin, Piperacillin+Tazobactum & Cefoperazone+Sulbactum. (Table 3) 62.5% of CONS was sensitive to Tetracycline, Piperacillin+Tazobactum & to Linezolid. Majority CONS were resistant to Penicillin, Ist & IInd generation Cephalosporins.100% Enterococcus were sensitive to Linezolid, 66.6% to Amoxyclav, 66.6% to Tetracycline & 33.3% to Cefoperazone+Sulbactum. Enterococcus was Resistant to Penicillin, Erythromycin, Gentamicin, Ist & IIndgeneration Cephalosporins,Ciprofloxacin,Piperacillin+ Tazobactum. (Table 4)

Table 1
Age wise distribution of Isolates

Organism	Total Isolates (%)	Age ≤ 1 wk (%)	Age ≥ 1wk (%)
Escherichia coli	1(2.2)	1(2.3)	00
Klebsiella pneumoniae	21(46.5)	20(46.5)	1(50)
Acinetobacter spp.	7(15.5)	6(13.9)	1(50)
Pseudomonas aeruginosa	5(11.1)	5(11.6)	00
CONS	8(17.7)	8(18.6)	00
Enterococcus	3(6.66)	3(6.97)	00
Total	45 ()	43(95.5)	2(4.44)

Table 2
Risk factors associated with neonatal septicaemia.

Risk factors	Total No of Cases	No of Positive cases	ODDS Ratio (CI)	X^2 Test – P value	Z test	P value
PROM	10	02(20%)	78.52 (3.4865 to 157.435)	0.0044	2.746	0.0060
Fever	15	02(13.3%)	9.0714 (0.5373 to 153.1485)	0.1981	1.529	0.1262
Perinatal asphyxia	21	05(23.8%)	30.25 (3.180 to 287.72)	0.0014	2.967	0.0030
Premature delivery	33	16(48.4%)	23.183 (6.0514 to 88.816)	0.0001	4.587	< 0.0001
LBW	43	03(6.9%)	23.183 (6.0514 to 88.816)	0.0299	4.587	0.0001
Clinical Disease	21	17(80.9%)	150 (27.7346 to 811.2601)	0.0001	5.818	<0.0001

PROM- Prolonged rupture of membrane
LBW- low birth weight

Table 3
Sensitivity pattern of gram negative bacilli

Drug	Escherichia coli n=1	Klebsiella pneumoniae n=21	Acinetobacter n=7	Pseudomonas n=5
Ampicillin	00	00	00	1(20)
Amoxyclav	1(100)	00	1(14.2)	3(60)
Tetracycline	00	8(38.09)	7(100)	3(60)
Gentamicin	00	2(9.5)	4(57.1)	4(60)
Co-trimoxazole	00	4(19)	2(28.5)	3(60)
Amikacin	1(100)	12(57.1)	2(28.5)	4(80)
Netilmicin	00	4(19.04)	5(71.4)	5(100)
Ciprofloxacin	00	8(38.09)	2(28.5)	3(60)
Ofloxacin	00	7(33.3)	2(28.5)	4(80)
Piperacillin+Tazobactum	1(100)	00	00	00
Cefoperazone+Sulbactum	1(100)	00	00	4(80)
Cephalexin	00	1(7.7)	00	1(20)
Cefuroxime	00	1(14.2)	00	1(20)

Table 4
Sensitivity pattern of gram positive bacteria

Drug	CONS n=8	Enterococcus n=3
Amoxyclav	2(25.0)	2(66.6)
Penicillin	1 (12.5)	1(33.3)
Erythromycin	3(37.5)	1(33.3)
Tetracycline	5(62.5)	2(66.6)
Gentamicin	3(37.5)	1(33.3)
Cephalexin	1(12.5)	1(33.3)
Cefuroxime	1(12.5)	1(33.3)
Ciprofloxacin	3(37.5)	1(33.3)
Piperacillin+Tazobactum	5(62.5)	1(33.3)
Cefoperazone+Sulbactum	3(37.5)	2(33.3)
Linezolid	5(62.5)	3(100)

Conflict of interest: Conflict of interest declared none.

DISCUSSION

In the present study, early onset septicaemia was observed in 95.5% cases which is similar to the study of Shah AJ et al (95.0%) [2], While it was 49.6% in the study by Shaw CK et al[10], 53.3% in Vinodkumar et al[11], 73% in Movahedian et al[12], 64.7% in Aletayeb et al[13]. Studies from western countries have reported lower incidence of neonatal sepsis (3.5-4.3/1000LB), early onset representing 58% of cases.[14] This difference probably reflects variations in population characteristics & in predisposing factors. In the present study gram negative bacilli constituted the major group of isolates. Klebsiella pneumoniae 21(46.6%) was the predominant, followed by Acinetobacter species 7 (15.5%) & Pseudomonas 5 (11.1%). CONS 8 (17.7%) was the commonest among the gram positive cocci followed by Enterococcus 3 (6.97%). This is similar to the findings of Joshi SG et al (67.2%) [15]& Shah AJ et al (52%) [2]. This could be because; new born most probably acquire these GNB from the vaginal and faecal flora of the mother and the environment where delivery occurs. Importance of both vertical transmission from the mother and postnatal acquisition of infection from the environment has been suggested in literature for pathogenesis of neonatal sepsis[2]. The CONS, previously considered as a contaminant, has been recognized increasingly as a cause of bacteremia. The ascendance of this group of Staphylococci has created increased interpretative difficulties for the clinicians, since the great majority of CONS isolates represent contamination rather than true bacteremia[16]. In the present study there was a significant association between premature delivery, clinical disease in mother like UTI, low birth weight, perinatal asphyxia and premature rupture of membrane with neonatal sepsis. Similar to Chacko et al [14], Tallur et al[17], Oddie et al.[18]

Chacko et al[14] & Soman et al[19] found LBW in 83% cases, Bhat et al[20] found prematurity & obstetric factors in 54.6%, Tallur et al[17] found association of PROM > 24 hrs in 14% & perinatal asphyxia in 22%. Kuruvilla et al[21] found an association of meconium stained amniotic fluid with sepsis. Agarwal et al[22] found association of EONS more frequently in neonates with perinatal asphyxia. Sepsis is generally considered to be the result of various risk factors both maternal and neonatal such as prematurity, low birth weight & asphyxia. In the present study, majority of the gram negative bacilli were resistant to Ampicillin, Amoxyclav, Piperacillin+Tazobactum, Cefoperazone+sulbactum, Ist & IInd generation Cephalosporins. Amikacin, Ciprofloxacin, Ofloxacin & Netilmicin were more effective. Similar results were observed by Bhat et al[20], Tallur et al[17], Karthikeyan et al.[23] Agarwal et al [22] found low susceptibility to Amikacin (45%). Shah AJ[2] et al found increased resistance to Amikacin & Gentamicin. Multiple drug resistance of the causative organisms of sepsis is a rapidly emerging, potentially disastrous problem[24, 25]. In fact, the situation is worst in developing countries because of the lack of control of the use of antibiotics, the non-existence of legislation on antibiotic prescription, over the counter sale of antibiotics, poor sanitary washing, lack of

surveillance of the standards of maternity homes, and the practices of traditional birth attendants, who deliver almost 80% of all the babies[26]. Sensitivity of CONS was good to tetracycline (62.5%), Piperacillin+Tazobactum (62.5%) & Linezolid (62.5%). Majority CONS were resistant to Penicillin, Ist & IInd generation Cephalosporins. Enterococcus showed better sensitivity to Linezolid (100%), Amoxyclav (66.6%), Tetracycline (66.6%), Cefoperazone+Sulbactum (33.3%). Enterococcus was Resistant to Penicillin, Erythromycin, Gentamicin, Ist & IInd generation Cephalosporins,Ciprofloxacin,Piperacillin+Tazobactum. Similar results were obtained by Roy I et al[3], Shah AJ et al.[2]

CONCLUSION

Continuous surveillance is required in a hospital setting to know the causative bacterias of neonatal septicemia and their antibiotic sensitivity pattern. This data can be helpful in empirical treatment of the neonates which in turn can reduce the mortality in the neonates.

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