



Bacteriological and Haematological Profile of Neonatal Sepsis in Rural Teaching Hospital in Central India

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ABSTRACT

Aim: This study was aimed to determine the bacteriological profile with their antimicrobial susceptibility as well as haematological profile related to neonatal sepsis.

Materials and Methods: In this prospective study conducted during August 2012 to July 2014, the blood samples collected from total 405 newborns suggestive of suspected sepsis were subjected to blood culture and initial sepsis screen. The identification of causative organisms was carried out by standard microbiological tests and antibiotic sensitivity testing as per Clinical Laboratory Standards Institute (CLSI) guidelines.

Results: Of the 405 neonates with suspected sepsis, 29.38% had positive blood culture among which Gram negative organisms (68.06%) predominated over Gram positive organisms (31.93%) with *Klebsiella* species (35.29%) as the commonest bacterial pathogens. Both Gram negative and Gram positive organisms exhibited high to considerable resistance to commonly used antibiotics and found susceptible to meropenem, piperacillin-tazobactam and cefoperazone-sulbactam, vancomycin, linezolid respectively. The sepsis screen parameters showed varied results among culture positive and culture negative cases.

Conclusions: Blood culture as well as sepsis screen should be carried out in neonates suspected of sepsis. The Gram negative bacterial predominance and high to considerable resistance against commonly used antibiotics in the present study makes it necessary to use antibiotics wisely and continuous surveillance to determine the varied pathogen causing neonatal sepsis with their resistance pattern to revise antibiotic policy accordingly.

Key words: Antimicrobial susceptibility, blood culture, Bacteriological profile, haematological profile, neonatal sepsis, sepsis screen

INTRODUCTION

Neonatal deaths accounts for one third of global child mortality and neonatal sepsis is the leading cause of neonatal mortality^[1]. More recent studies in India ascribe about 40% of neonatal deaths to sepsis in the community (National Neonatology Forum NNPD Network, 2005). It is also important to note that 20-30% of the survivors of neonatal sepsis may exhibit neurological sequelae^[2]. Neonatal Sepsis related mortality and morbidity is however largely preventable with its proper diagnosis and rational antimicrobial therapy^[3]. The clinical diagnosis of neonatal sepsis is difficult because the signs and symptoms are not always specific. Blood culture is a gold standard for diagnosis of neonatal sepsis, but initial sepsis screen like white blood cell count, platelets count, immature to total neutrophil ratio, C-reactive protein, procalcitonin and other serological markers are also important in early diagnosis of clinically suspected cases of neonatal sepsis till the blood culture report is awaited^[4]. The organisms responsible for neonatal sepsis vary across geographical boundaries and with the time of onset of illness^[3]. In addition, one organism or a group of organisms may over time replace another as the leading cause of neonatal sepsis in a particular region^[5]. Microorganisms implicated in neonatal sepsis have developed increased drug resistance to commonly used antibiotics and thus making treatment extremely difficult^[6]. Thus, local profile of neonatal sepsis should be constantly updated to detect changes in the pattern of pathogens of infection and their susceptibility to various antibiotics. The profile of neonatal sepsis and antibiotic resistance patterns of

pathogens may be used to alert clinicians to emerging pathogens that may pose a threat to neonates and also to develop guidelines for management of neonatal sepsis in hospitals including the choice of empiric antibiotic therapy. Therefore, the aim of this study is to determine the bacteriological profile with their antimicrobial susceptibility as well as haematological profile related to neonatal sepsis.

MATERIALS & METHODS

The present prospective study was conducted in the NICU of A.C.P.M. medical college, Dhule, Maharashtra from August 2012 to July 2014 following approval of the local ethics committee. The total 405 newborns aged 0-28 days admitted during the period of study with one or more symptoms or signs suggestive of suspected sepsis with or without risk factors were included in this study. Neonates who had received antibiotics prior to clinical presentation as well as those whose mothers had received antibiotics within one week prior to delivery and at the time of sampling were not included in this study. Also, neonates who had severe congenital abnormalities diagnosed antenatally or at birth and those had clinical features resembling sepsis but attributable to causes other than neonatal sepsis e.g. birth asphyxia, hypoglycaemia, inborn errors of metabolism etc were excluded from the study. Of the 2 ml blood collected from each patient under all aseptic precautions, 1ml was subjected to initial septic screen viz. total leukocyte count (TLC), absolute neutrophil count (ANC) for neutropenia $<1800/\text{mm}^3$, immature to total neutrophils ratio (I/T >0.2), platelets count for

thrombocytopenia $<1.5 \times 10^5$ Cells/mm³, microESR (>15 mm in 1st hour), C-reactive protein (CRP) and remaining 1ml inoculated immediately into 10 ml of brain heart infusion broth (HiMedia Laboratories, Mumbai). After overnight incubation, the broths were subcultured on chocolate agar, MacConkey agar and 5% sheep blood agar. Inoculated subcultured media were considered negative if there was no growth after continuous incubation for up to 7 days, subcultures being made each day. Any growth was identified by colonial characteristics and standard microbiological tests. Antimicrobial susceptibility test was carried out on isolated and identified colonies using commercially prepared antibiotic disks (HiMedia) by the Kirby Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI) guidelines. The results were analysed using Epi Info software system.

RESULTS

Of the 405 neonates with suspected sepsis included during the study period, 29.38% had positive blood culture with preponderance of Gram negative organisms (68.06%) over Gram positive organisms (31.93%). *Klebsiella species* (35.29%) were the commonest bacterial pathogens isolated followed by *Staphylococcus aureus*

(21.84%), *Escherichia coli* (14.28%), *Pseudomonas aeruginosa* (12.6%) and coagulase negative *Staphylococcus* (10.08%) while the least common organism isolated was *Acinetobacter baumannii* (5.88%) as illustrated in Table 1.

Table 1. Bacterial isolates frequency in neonatal sepsis

Isolates	Frequency N (%)
Gram positive isolates	38 (31.93)
<i>Staphylococcus aureus</i>	26 (21.84)
CONS	12 (10.08)
Gram negative isolates	81 (68.06)
<i>Klebsiella species</i>	42 (35.29)
<i>E. coli</i>	17 (14.28)
<i>Pseudomonas aeruginosa</i>	15 (12.6)
<i>Acinetobacter baumannii</i>	07 (5.88)
Total	119 (29.38)

CONS: Coagulase Negative *Staphylococcus*

All the Gram positive bacterial isolates were showed high resistance to penicillin, considerable resistance to cotrimoxazole, gentamycin, ciprofloxacin, erythromycin and relatively lower resistance to netilmycin, cephalixin, amikacin, amoxicillin-clavulanic acid. All these isolates were found to be susceptible to cefoperazone-sulbactam, vancomycin and linezolid (Table 2).

Table 2. Resistance pattern of the Gram positive bacterial isolates

Antibiotics	<i>Staphylococcus aureus</i> (n=26) N (%)	CONS (n=12) N (%)
Penicillin	24(92.30)	10(83.33)
Erythromycin	12(46.15)	05(41.66)
Ciprofloxacin	11(42.30)	06(50.00)
Cotrimoxazole	15(57.69)	08(66.66)
Gentamycin	14(53.84)	06(50.00)

Amikacin	05(19.23)	02(16.66)
Amoxicillin-Clavulinic acid	04(15.38)	01(8.33)
Netilmycin	09(34.61)	04(33.33)
Cefoperazone-Sulbactam	00 (0.00)	00 (0.00)
Cephalexin	08(30.76)	03(25.00)
Vancomycin	00 (0.00)	00 (0.00)
Linezolid	00 (0.00)	00 (0.00)

All the Gram negative bacterial isolates showed high resistance to cotrimoxazole, gentamycin and comparatively moderate resistance to ciprofloxacin, amikacin, ceftazidime, cefotaxime. All these isolates tested were found to be susceptible to meropenem. Additionally, *Klebsiella species* and *E. coli* isolates were also

reported high resistance to ampicillin, considerable resistance to cefoperazone, and relatively lower resistance to colistin, aztreonam. None of the *Pseudomonas aeruginosa* isolates were exhibited resistance to piperacillin-tozabactam (Table 3).

Table 3. Resistance pattern of Gram negative bacterial isolates

Antibiotics	<i>Klebsiella species</i> (n=42) N (%)	<i>E. coli</i> (n=17) N (%)	<i>Pseudomonas aeruginosa</i> (n=15) N (%)	<i>Acinetobacter baumannii</i> (n=7) N (%)
Ampicillin	40 (95.23)	16 (94.11)	NT	NT
Gentamycin	34 (80.95)	13 (76.47)	11 (73.33)	05 (71.14)
Amikacin	22 (52.38)	08 (47.05)	06 (40.00)	03 (42.85)
Ciprofloxacin	25 (59.52)	10 (58.82)	09 (60.00)	04 (57.14)
Cotrimoxazole	30 (71.42)	13 (76.47)	13 (86.66)	07 (100.00)
Piperacillin	NT	NT	07 (46.66)	NT
Ceftazidime	17 (40.47)	06 (35.29)	06 (40.00)	03 (42.85)
Cefotaxime	15 (35.71)	05 (29.41)	05 (33.33)	02 (28.57)
Colistin	09 (21.42)	03 (17.64)	NT	NT
Aztreonam	07 (16.66)	02 (11.76)	NT	NT
Cefoperazone	23 (54.76)	09 (52.94)	NT	NT
Meropenem	0 (00.00)	0 (00.00)	0(00.00)	0 (00.00)
Piperacillin-Tozabactam	NT	NT	0(00.00)	NT

NT: Not Tested

Among the 119 culture positive cases, increased total leukocyte count was found in 65.54% of cases, neutropenia noted in 19.32%, thrombocytopenia in 32.77%, I/T ratio >0.2 in

70.58% , CRP positive in 80.67% and microESR >15mm in 30.25% cases as shown in table 4 with their respective Sensitivity, Specificity, positive predictive values and negative predictive values.

Table 4. Haematological profile of neonatal sepsis

Septic screen	Culture (+ve) (119) N (%)	Culture (-ve) (286) N (%)	SN (%)	SP (%)	PPV (%)	NPV (%)
Increased TLC	78(65.54)	114(39.86)	66	60	41	81
Neutropenia	23(19.32)	31(10.83)	19	89	43	73
Thrombocytopenia	39(32.77)	13(04.54)	33	95	75	77
I/T ratio >0.2	84(70.58)	28(09.79)	71	90	75	88
CRP positive	96(80.67)	115(40.20)	81	60	45	88
MicroESR	36(30.25)	27(09.44)	30	91	57	76

SN-Sensitivity, SP-Specificity, PPV-Positive Predictive Value, NPV-Negative Predictive Value

DISCUSSION

The causative organisms in neonatal sepsis vary from place to place and the frequency of the causative organisms is different in different hospitals and even in the same hospital at different time [7]. The culture positivity rate of 29.38% found in our study was comparable with Shah et al [8] (31.57%) and Komolafe et al [9] (31.4%). In contrast to our study, some Indian studies reported high culture positivity of (56%) [10], (47.5%) [11] and some found low culture positivity of (7.9%) [12], (5.6%) [13]. The predominance of Gram negative bacterial isolates (68.06%) over Gram positive bacterial isolates showed in the present study was similar to that reported by Garg et al (67.5%) [14] and Desai K J et al (67.85%) [15]. The report of the National Neonatal Perinatal database 2003 showed *Klebsiella* as the predominant (29%) pathogen in neonatal sepsis [16]. *Klebsiella species*

(35.29%), the overall predominant pathogen isolated in this study, was in accordance with other Indian studies Kumar GD et al [17] and Roy I et al [18]. The second most common pathogen isolated in our study was *Staphylococcus aureus* (21.84%) followed by *Escherichia coli* (14.28%), *Pseudomonas aeruginosa* (12.6%) and CONS (10.08%) while the least common organism isolated was *Acinetobacter baumannii* (5.88%). The high resistance against penicillin and considerable resistance to cotrimoxazole, gentamycin, ciprofloxacin, erythromycin exhibited by all the Gram positive bacterial isolates tested in this study was comparable with the findings of Roy I et al [11]. The same isolates showed relatively less resistance against netilmycin, cephelexin, amikacin, amoxicillin-clavulinic acid tested. In our study, all the Gram positive bacterial isolates found sensitive to

cefoperazone-sulbactam, vancomycin and linezolid was correlated with the findings to those of Roy I et al ^[11] and Mehta M et al ^[18], Mustafa M et al ^[19]. Among all the Gram negative bacterial isolates, high resistance to cotrimoxazole, gentamycin and comparatively moderate resistance to ciprofloxacin, amikacin, ceftazidime, cefotaxime noticed was comparable with Roy I et al ^[11] and Desai K J et al ^[15]. All those same isolates found susceptible to meropenem was in accordance with those reported by Mustafa M et al ^[19]. High resistance to ampicillin and relatively lower resistance to colistin, aztreonam showed by *Klebsiella species* and *E. coli* isolates in our study can be also comparable with that of Khatua et al ^[20] and Mustafa M et al ^[19]. Our study noted none of the *Pseudomonas aeruginosa* isolates resistant to piperacillin-tozabactam. Among the 119 culture positive cases in our study, increased total leukocyte count was found in 65.54% of cases, neutropenia noted in 19.32%, thrombocytopenia in 32.77% , I/T ratio >0.2 in 70.58% , CRP positive in 80.67% and microESR >15mm in 30.25% cases. Different studies had reported variable results for these parameter which may be due to the variations in time of onset of infection (early or late), age of the neonates, severity of infection, blood sampling time, diagnostic criteria followed ^[21,22].

CONCLUSION

Blood culture along with sepsis screen should be carried out in neonates suspected of sepsis. The Gram negative bacterial predominance and high to considerable resistance against commonly used antibiotics in the present study makes its

necessary to wise use of antibiotics and continuous surveillance at regular interval to determine the varied pathogen causing neonatal sepsis with their resistance pattern to revise antibiotic policy accordingly. Each and every hospital must have its own local antibiogram mentioning empirical therapy options.

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