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Original Article

Neonatology Section

Changing Trends of Antimicrobial Resistance in Neonatal Sepsis: Experience from a Tertiary Care Hospital from West Bengal, India

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ABSTRACT

Introduction: Sepsis is the second leading cause of neonatal mortality in India. Emergence of highly resistant microorganisms as an aetiology of neonatal sepsis is a matter of serious concern.

Aim: To study the prevailing aetiological agents in neonatal sepsis and their antimicrobial susceptibility pattern.

Materials and Methods: A prospective observational study was performed in a tertiary care teaching hospital in neonatal care units in North Bengal Medical College and Hospital, West Bengal, India, over a period of two years from March 2017 to February 2019. All the neonates having clinical features suggestive of sepsis were subjected to blood culture using BacT/ALERT® PF Plus. Microbial identification and antibiotic susceptibility testing was done by VITEK-2 automated systems. Chi-square test was done using Epi info software version 7.1 and p-value <0.05 was considered significant.

Results: Out of 403 neonates investigated for suspected sepsis, 156 (38.7%) were found to be culture positive. *Klebsiella pneumoniae* was the most common organism isolated (n=90, 57.7%) followed by *Staphylococcus aureus* (n=24, 15.4%) and *Acinetobacter baumannii* (n=10, 6.4%). *Klebsiella pneumoniae* showed a very high degree of resistance to ampicillin, amoxycillin-clavulenic acid (100% each), cefotaxime (93.4%), ceftazidime (92.3%), gentamycin (94.5%) and tobramycin (94.5%). High resistance of *Staphylococcus aureus* was seen against ampicillin (100%), cloxacillin (62.5%), amoxicillin-clavulenic acid (70.9%), and cefotaxime (79.2%). Few isolates of *Klebsiella pneumonia* (6.6%), *Acinetobacter baumannii* (60%) and *Enterobacter cloacae* (50%) were sensitive only to colistin and tigecycline.

Conclusion: Most of the isolates showed very high degree of resistance against first line of antibiotics recommended by World Health Organisation (WHO) (ampicillin and gentamycin or amikacin) for empirical treatment of neonatal sepsis. Emergence of highly resistant organisms sensitive only to colistin and tigecycline should be considered as an eye opener. Strict adherence to sepsis prevention along with regular surveillance of organisms and their sensitivity patterns is the need of the hour to improve survival by contributing to antibiotic stewardship.

Keywords: *Acinetobacter baumannii*, Antimicrobial resistance, *Klebsiella pneumonia*, Sepsis

INTRODUCTION

Neonatal sepsis is a clinical syndrome characterised by signs and symptoms of infection with or without accompanying bacteremia in the first 28 days of life [1]. The incidence of blood culture proven sepsis in India was reported as 8.5 per 1000 live births in 2002-2003 as per National Neonatal Perinatal Database (NNPD) [2]. It is the second leading cause of neonatal mortality in India, accounting for 18.6% neonatal deaths [2]. The situation has been compounded by emergence of Multidrug Resistant (MDR) organisms, which is defined as resistance to at least 3 classes of antimicrobial agents, among those that are regarded as potentially effective against the respective pathogen [3]. A study from India has showed that 87% of *Escherichia coli* isolates were Extended Spectrum B Lactamases (ESBL) producers, 13% possessed New Delhi B Lactamases-1 (NDM-1) and 6% were Metallo B Lactamases (MBL) producers [4]. Another study from Delhi Neonatal Infection Study (DeNIS) Collaboration has found high rates of multidrug resistance in *Acinetobacter baumannii* (82%), *Klebsiella pneumoniae* (54%), and *Escherichia coli* (38%) isolates [5].

Neonatal units are inherently susceptible to selection and propagation of MDR organisms due to multiple factors like low immunity of sick neonates, multiple opportunities for cross-infection and widespread use of antimicrobials [6]. In addition, other factors like poor infection control, lack of knowledge regarding organisms, and unscrupulous use of fixed drug combinations are the tailwinds in the emergence of MDR strains [7-10]. To prevent the emergence and spread of such antimicrobial resistance, knowledge of the common causative agents and their antimicrobial susceptibility remains pivotal. This helps to draft protocol for rational management of neonatal sepsis. The present study was performed to analyse aetiology and antimicrobial susceptibility of neonatal sepsis in a tertiary care teaching hospital in eastern India.

MATERIALS AND METHODS

A prospective observational study was carried out in neonatal care units in North Bengal Medical College and Hospital, West Bengal, India over the past two years (from March 2017 to February 2019). Clearance from Institutional Ethical Committee was taken (No: PCM/2014-15/IEC/45 dated 23/12/2014).

Inclusion criteria: All the neonates (postnatal age within 28 days) delivered in our institution or outside who had clinical features suggestive of neonatal sepsis were included in the study.

Exclusion criteria: Neonates with gross congenital malformation and suspected metabolic disorder were excluded from the study.

Written consent for participation in the study was taken from parents of each neonate. All the consecutive cases of suspected neonatal sepsis during the study period were included in the study. Detailed history and clinical examination were done. Sepsis screen was performed in each neonate. For interpretation of sepsis screen, total leukocyte count <5000/mm^3, low absolute neutrophil count as per Manroe chart for term neonates and Mouzinho's chart for very low birth neonates, immature to total neutrophil ratio >0.2, micro-Erythrocyte Sedimentation Rate (ESR) >15 in 1st hour and C-reactive protein >1 mg/dL were considered abnormal. Sepsis screen was considered positive when two or more of the above mentioned parameters were abnormal [11]. Diagnosis of sepsis was suspected on the basis of clinical features and results of sepsis screen; and was confirmed by observing growth on blood culture. Early onset

sepsis and late onset sepsis were defined as infection within first 72 hour of life and after 72 hour of life respectively. Blood culture was done for all the neonates with suspected sepsis. Blood samples (1-2 mL) were collected by aseptic precautions before starting antibiotics. Blood cultures were performed using BacT/ALERT® PF Plus. Microbial identification and antibiotic susceptibility testing were done by VITEK-2 automated systems.

STATISTICAL ANALYSIS

Chi-square test was done using Epi info software version 7.1 to compare different groups. All the statistical analyses were carried out at 5% level of significance and p-value <0.05 was considered significant.

RESULTS

During the study period, 403 neonates were investigated for suspected sepsis. Sepsis screen was positive in 223 (55.3%) neonates. Total 156 cases (38.7%) showed growth in blood culture and were included in the study. A 126 (80.7%) inborn and 30 (19.2%) outborn neonates showed positive blood culture. Early onset sepsis was documented among 48 (38%) inborn neonates and 7 (23.3%) outborn neonates. Details of demographic data and other baseline characteristics have been given in [Table/Fig-1]. Various risk factors associated with sepsis among inborn neonates, and clinical presentations of the study cohort have been detailed in [Table/Fig-2,3], respectively.

Parameters	Inborn (n=126)	Outborn (n=30)	Total (n=156)
Male/Female	70/56	17/13	87/69
Gestational age	**n (%)**	**n (%)**	**n (%)**
<34 weeks	45 (35.7)	8 (26.7)	53 (34)
34-37 weeks	54 (42.9)	12 (40)	66 (42.3)
>37 weeks	27 (21.4)	10 (33.3)	37 (23.7)
Birth weight			
≥2500 g	36 (28.5)	10 (33.3)	46 (29.5)
1500-2499 g	60 (47.7)	12 (40)	72 (46.2)
1000-1499 g	21 (16.7)	6 (20)	27 (17.3)
≤999 g	9 (7.1)	2 (6.7)	11 (7)
Mode of delivery			
Normal	52 (41.3)	20 (66.6)	72 (46.2)
Instrumental/Cesarean	74 (58.7)	10 (33.3)	84 (53.8)
Early onset sepsis	48 (38)	7 (23.3)	55 (35.3)
Late onset sepsis	78 (62)	23 (76.6)	101 (64.7)

[Table/Fig-1]: Demographic data and other baseline characteristics of culture positive cases.

Risk factors	No. of cases (%)
Low birth weight or preterm	88 (69.8)
Perinatal asphyxia (APGAR score <4 at 1 minute)	18 (14.3)
Prolonged or difficult delivery with instrumentation	13 (10.3)
Foul smelling or meconium stained liquor	11 (8.7)
Premature rupture of membrane >24 hours	8 (6.3)
≥3 vaginal examination during labour	5 (3.9)
Maternal fever within two weeks prior to delivery	2 (1.6)

[Table/Fig-2]: Various risk factors for sepsis among inborn neonates (n=126).

Out of 156 culture positive cases, 118 (75.6%) were gram-negative (103/126 inborn, 15/30 outborn), 32 (20.5%) were gram-positive (19/126 inborn, 13/30 outborn), and 6 (3.84%) (4/126 inborn, 2/30 outborn) were due to *Candida albicans*. *Klebsiella pneumonia* was the most common organism isolated (90, 57.7%), followed by *Staphylococcus aureus* 24 (15.4%) and *Acinetobacter baumannii* 10 (6.4%). The details of aetiological agents are given in [Table/Fig-4].

Among 90 *Klebsiella* isolates, all were sensitive to colistin and tigecycline. 85 (94.4%) isolates were sensitive to cefoperazone-salbactum,

Presenting features	No. of cases (%)
Lethargy	124 (79.5)
Refusal to feed	107 (68.6)
Respiratory distress	55 (35.3)
Abdominal distension	44 (28.2)
Gastrointestinal bleeding	31 (19.9)
Convulsion	25 (16)
Conjugated hyperbilirubinemia	24 (15.4)
Purpura	21 (13.5)
Temperature instability	17 (10.9)
Sclerema	15 (9.6)
Hepatomegaly	9 (5.8)
Diarrhoea	6 (3.8)
Splenomegaly	5 (3.2)

[Table/Fig-3]: Presenting features of the study cohort (n=156).

Organism	Inborn (n=126) (%)	Outborn (n=30) (%)	Total (n=156) (%)	p-value
Klebsiella pneumonia	81 (64.3)	9 (30)	90 (57.7)	<0.001
Staphylococcus aureus	13 (10.3)	11 (36.7)	24 (15.4)	<0.001
Acinetobacter baumannii	6 (4.8)	4 (13.3)	10 (6.4)	0.26
Escherichia coli	8 (6.4)	-	8 (5.1)	0.33
Enterobacter cloacae	4 (3.2)	-	4 (2.6)	0.72
Pseudomonas aeruginosa	4 (3.2)	2 (6.6)	6 (3.8)	0.71
Enterococcus sp	4 (3.2)	2 (6.6)	6 (3.8)	0.71
Candida albicans	4 (3.2)	2 (6.6)	6 (3.8)	0.71
CONS	2 (1.6)	-	2 (1.3)	0.83

[Table/Fig-4]: Aetiology of neonatal sepsis.
CONS: Coagulase negative staphylococcus; p-value <0.05 was considered significant

84 (93.3%) were sensitive to meropenem, and 65.6% isolates to ciprofloxacin. Very high resistance to cefotaxime (93.4%), ceftazidime (92.3%), gentamycin (94.5%) and tobramycin (94.5%) was noted. Six (6.6%) *Klebsiella* isolates were sensitive to only colistin and tigecycline.

All *Escherichia coli* isolates were highly sensitive to meropenem, cefoperazone-salbactum (100% each), ciprofloxacin, and ofloxacin (87.5% each). *Acinetobater baumanii*, third most common isolate in our study, was highly resistant to commonly used antimicrobials ranging from 60-100%. However, these isolates were sensitive (100%) to colistin and tigecycline. Similarly, *Enterobacter cloacae* isolates showed high resistance to common antimicrobials and 50% of them were exclusively sensitive to colistin and tigecycline. All gram-negative isolates are universally resistant to ampicillin (100%) and amoxycillin-clavulenic acid (100%). *Staphylococcus aureus* was isolated from 24 cultures and they were highly sensitive to meropenem (100%), vancomycin (100%), linezolid (100%), piperacillin-tazobactum (91.7%), gentamycin (70.8%), and amikacin (70.8%). *Staphylococcus aureus*, Coagulase Negative Staphylococci (CONS) and *Enterococcus* are universally resistant to ampicillin (100%). Detail of sensitivity pattern is given in [Table/Fig-5].

DISCUSSION

For effective management of sepsis, study of bacteriological profile along with the antimicrobial sensitivity pattern plays a pivotal role [12,13]. Bacteria are the leading causes of neonatal sepsis; but it can also be caused by viruses (adenovirus, enterovirus, coxsackie virus, rubella virus etc.,), protozoa (e.g., toxoplasma), and fungi (e.g., *Candida albicans*) [12] resulting in low yield of blood cultures. A negative blood culture does not exclude sepsis as about 26% of all neonatal sepsis might be due to anaerobes, and anaerobic culture is not routinely performed [12]. Administration of antimicrobial agents before collecting blood samples for culture also attenuates

Antimicrobials drugs	*Klebsiella* (n=90)	*Acinetobacter* (n=10)	*E coli* (n=8)	*Pseudomonas aeruginosa* (n=6)	*Enterobacter cloacae* (n=4)	*Staph aureus* (n=24)	*Enterococci* (n=6)	CONS (n=2)
Ampicillin	0	0	0	0	0	0	0	0
Cloxacillin	-	-	-	-	-	9 (37.5)	0	0
Amoxycillin/Clavulanic acid	0	0	0	0	0	7 (29.1)	1 (16.7)	1 (50)
Cefotaxime	6 (6.6)	0	2 (25)	4 (66.7)	0	5 (20.8)	-	1 (50)
Ceftazidime	7 (7.7)	0	2 (25)	6 (100)	0	-	-	-
Piperacillin/Tazobactum	68 (75.6)	4 (40)	6 (75)	6 (100)	1 (25)	22 (91.7)	6 (100)	2 (100)
Cefoperazone/Salbactum	85 (94.4)	3 (30)	8 (100)	2 (33.3)	1 (25)	-	-	-
Meropenem	84 (93.3)	1 (10)	8 (100)	5 (83.3)	2 (50)	24 (100)	6 (100)	2 (100)
Gentamycin	5 (5.5)	0	4 (50)	2 (33.3)	0	17 (70.8)	-	1 (50)
Amikacin	28 (31.1)	1 (10)	2 (25)	6 (100)	0	17 (70.8)	-	1 (50)
Netilmycin	26 (28.9)	1 (10)	4 (50)	3 (50)	0	18 (75)	-	1 (50)
Tobramycin	5 (5.5)	1 (10)	4 (50)	3 (50)	0	-	-	-
Ciprofloxacin	59 (65.6)	1 (10)	7 (87.5)	1 (16.7)	0	16 (66.7)	3 (50)	1 (50)
Ofloxacin	67 (74.4)	2 (20)	7 (87.5)	1 (16.7)	1(25)	15 (62.5)	3 (50)	2 (100)
Vancomycin	-	-	-	-	-	24 (100)	6 (100)	2 (100)
Rifampicin	-	-	-	-	-	24 (100)	6 (100)	2 (100)
Linezolid	-	-	-	-	-	24 (100)	6 (100)	2 (100)
Tigecycline	90 (100)	10 (100)	8 (100)	6 (100)	4 (100)	-	-	-
Colistin	90 (100)	10 (100)	8 (100)	6 (100)	4 (100)	-	-	-

[Table/Fig-5]: Antimicrobial susceptibility pattern of isolated organisms.
E coli: Escherichia coli, *Staph aureus*: Staphylococcus aureus, CONS: Coagulase negative staphylococcus. Figures in the parentheses indicate percentages

the proportion of culture positivity. In the present study, bacterial growth could be isolated in 38.7% cases.

In the present study, gram-negative bacilli predominated over gram-positive organisms. *Klebsiella pneumoniae* was the overall predominant cause of sepsis, which is comparable with studies from India and other developing countries [2,14]. *Escherichia coli* was the second commonest gram-negative organism and *Staphylococcus aureus* was commonest gram-positive isolate. Moreover, we found *Staphylococcus aureus* as the overall second most common aetiology of sepsis, which is similar to findings of NNPD. The predominant causes of sepsis in the developed countries are Group B Streptococcus (GBS) and CONS [13]. GBS was not isolated in our cohort. Similarly, GBS is an uncommon isolate of neonatal sepsis in India; mostly attributed to prevalence of less virulent strains, high titers of transplacentally acquired antibodies and also due to non-detection of cases among stillbirths [15]. *Enterococci* was the second most common gram-positive organism isolated and was found to be highly resistant to commonly used antimicrobials. However, it showed 100% sensitivity to rifampicin, linezolid and vancomycin. CONS was the least common gram-positive organism in the present study. A previous study [14] has proposed to consider two positive blood cultures to confirm CONS as aetiological agent, which was followed in the present study.

WHO has recommended the use of penicillin or ampicillin and one aminoglycoside for empirical therapy in neonatal sepsis [16]. In the present study, high degree of resistance was found to commonly used antimicrobial agents in neonatal sepsis. High resistance to antimicrobial agents recommended by World Health Organisation (WHO) as empirical therapy (ampicillin and aminoglycosides) is particularly alarming [16]. In the present study, sensitivity pattern shows that there is very high resistance against ampicillin (100%) and gentamycin (29.2-100%) which are recommended for empirical use in neonatal sepsis by WHO. Third generation cephalosporins are used as the first line antimicrobials in many units which also face high degree of resistance similar to this study. This high resistance may be related to prolonged exposure to these antibiotics during antenatal period [17]. Resistance pattern of organisms towards empirical antimicrobials suggested by WHO, as found in various recent studies [5,18-23] is given in [Table/Fig-6]. The other striking observation in the present study was emergence of some highly resistant isolates of *Klebsiella* and *Acinetobacter* which are sensitive only to colistin and tigecycline. Similar resistance pattern has also been reported from other units in India [22]. Considering the sensitivity pattern observed in our study, piperacillin-tazobactum and ciprofloxacin or ofloxacin may be a good combination for empirical therapy which covers most of the organisms. Although ciprofloxacin is not approved for use in neonates, still it is used as empirical treatment in some neonatal units with favorable outcome [23]. After receiving the sensitivity report, further treatment should be guided by results of sensitivity pattern of the isolate.

Country/WHO region [Ref]	Ampicillin resistance	Aminoglycoside resistance
SEARO [18]	97%	83%
AFRO [18]	93%	43%
EURO [18]	64%	23%
Nepal [19]	0-50%	43-100%
Pakistan [20]	ND	0-36.6%
China [21]	72.6-100%	0-40.7%
India [5]	ND	16.7-84.1%
India [22]	ND	75%
India [23]	33.3-100%	14-75%
India (Present study)	100%	29.2-100%

[Table/Fig-6]: Antimicrobial resistance to ampicillin and gentamycin as reported in various studies [5,18-23].
SEARO: WHO South East Asia region, AFRO: WHO Africa region, EURO: WHO Europe region

Limitation(s)

The present study has its own share of limitations. It was a single center-based study, so the results cannot be extrapolated to the whole country. Also, larger sample size would have given better results.

CONCLUSION(S)

Authors noticed a very high degree of resistance to commonly used antimicrobials in the neonatal unit. Most gram-negative organisms are highly resistant to commonly used antimicrobial agents, especially those recommended by WHO. These isolates are sensitive to piperacillin-tazobactum, meropenem, 4th generation cephalosporin or fluoroquinolone to variable extent. Few isolates of *Klebsiella, Acinetobacter* and *Enterobacter* are resistant to all antimicrobial agents except colistin and tigecycline which is a matter of great

concern. Likewise, gram-positive organisms are only sensitive to piperacillin-tazobactum, meropenem, vancomycin or linezolid. To avoid exponential increase of this problem we should emphasise more on preventive aspects of sepsis. Regular surveillance of organisms and their sensitivity patterns is the need of the hour to improve survival by contributing to antibiotic stewardship.

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