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Original Research Article

Microbial Profile of Neonatal Sepsis and its Antibiogram Prevalent in a Tertiary Care Hospital of Bihar

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Abstract:

Objectives: The present study was to evaluate the microbial profile of neonatal septicemia and its antibiogram prevalent and sensitivity pattern in a tertiary care hospital of Bihar.

Methods: A total of 154 neonates with clinical sign of sepsis were admitted in Neonatal Intensive Care Unit during study period. All the clinical assessment and investigation including specimen collection, culture, identification of isolates and antibiotic sensitivity testing were performed.

Results: Out of 154 new born with clinical sepsis, blood culture were positive in 100(64.94%) cases. 82(74%) had EOS and 18(18%) had LOS. Majorities of newborn were males 67(67%). 59(59%) had low birth weight <2500 gram. 58(58%) had gestational age <37 weeks. Out of 64 pathogenic bacteria, klebsiella pneumoniae 23(35.93%), Staphylococcus aureus 19(29.69%) and Escherichia coli 11(17.19%) were the most common isolates. Out of 36 pathogenic candida, Candida tropicalis 13(36.11%) and Candida kruzei 8(22.22%) were the most common. In the gram-negative isolates, the highest sensitivity was reported for Amikacin 42(100%), Colistin 42(100%), Imipenem 41(97.62%), followed by Gentamicin 36(85.71%). Highest resistance was seen in ampicillin (100%). In gram-positive isolates, the highest sensitivity was reported for Vancomycin 24(100%), Teicoplanin 24(100%), Linezolid 23(95.83%) followed by gentamicin 22(91.67%), and Amoxicillin/clavulanic acid 17(70.83%).

Conclusion: Regular monitoring of antibiotic resistance is necessary and depending on the antibiotic sensitivity pattern of the isolates, antibiotic should be used. Blood culture is a Gold Standard for diagnosis of neonatal sepsis and should be done in all the suspected cases of neonatal sepsis.

Keywords: Neonatal Sepsis, Prevalent, Antibiogram, Sensitivity Pattern.

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Introduction

Neonatal sepsis is classified as early onset when it occurs within the first 72 hours of life and late onset when it occurs after 72 hours of life [1]. Early onset sepsis is caused by organisms prevalent in the maternal genital tract, labour room or operating theatre [2] while late onset sepsis usually results from nosocomial or community-acquired infection [3].

Neonatal sepsis continues to be a challenging scenario to paediatricians in developing as well as developed countries. It accounts for a significant proportion of neonatal mortality and morbidity. In India 16.4% neonatal death is associated with neonatal sepsis [4]. Although neonatal sepsis is essentially a systemic infection, the spectrum of sepsis in neonate can vary from subclinical disease to severe systemic infection. The signs of neonatal sepsis viz. Irritability, hyperpyrexia or hypothermia, lethargy, poor feeding, poor perfusion, hypotension, tachycardia, respiratory distress, jaundice are vague and non-specific [5].

According to the World Health Organization (WHO) estimate, there are about 5 million neonatal deaths per year in the world, 98% of these occurring in the developing world. Neonatal mortality rate in developing countries from various causes is about 34 per 1000 live births, most of the deaths occurring in the first week of life. Globally, major causes of the neonatal deaths are due the prematurity (28%), sepsis (26%), and birth asphyxia (23%) [6].

The gold standard for the diagnosis of neonatal sepsis is isolation of bacterial agents from the blood culture [7]. Both gram negative and gram-positive bacteria have been isolated from the blood and predominance of one type over the other varies from place to place and even in the same place over the

time to time [8]. In most of the developing countries, gram negative sepsis remains the major cause of the neonatal septicemia. Commonly isolated organisms include Klebsiella Pneumonia, Escherichia Coli, Enterobacilli, Pseudomonas Aeroginosa, Staphylococcus Aureus, Streptococcal Species, Citrobacter Species and Coagulase Negative Staphylococcus (CONS) [9, 10]. The bacterial susceptibility to different antibiotics varies from time to time over different geographical areas. But there is a rising concern of isolation of highly antibiotic-resistant bacteria [11]. Objective of the present study was to evaluate the microbial profile of neonatal sepsis and its antibiogram prevalent in a tertiary care hospital of Bihar.

Material & Methods

The present study was conducted in the Department of Pediatrics with the collaboration of the Department of Pathology, Jan Nayak Karpoori Thakur Medical College & Hospital, Madhepura, Bihar, India during a period from June 2024 to December 2024.

Study Group: The study group comprised 154 neonates admitted to Neonatal Intensive Care Unit with clinical signs of septicemia.

Inclusion Criteria: All the neonates who were admitted with clinical signs of Sepsis.

Exclusion Criteria: Neonates born to mothers who had received Antenatal Antibiotic Therapy within 48 hours prior to delivery and symptomatic neonates who had been started on antibiotics. The diagnosis of Neonatal Septicemia was based on clinical criteria like poor activity, lethargy, inability or poor sucking, hypothermia, abdominal distension, and diminished or absent Neonatal Reflexes. Media preparation: Brain Heart Infusion Biphasic Media (BHIBPM) culture bottles were used for performing Blood Culture. Commercially available Brain Heart Infusion (BHI) dehydrated media obtained from Himedia, (India) was used as per instruction17.

Specimen collection and Transport: Any peripheral vein was chosen as the venepuncture site. The site was cleaned and disinfected with povidoneiodine followed by 70% Isopropyl alcohol and allowed to dry. Two millilitres of blood was collected with the help of a sterile disposable scalp vein (24G) and syringe (2 ml.) and immediately transferred to BHIBPM. Then the bottles were shaken gently to mix the blood with the broth homogeneously and transferred to the Microbiology laboratory in an upright position for incubation and culture [45].

Culture: In the laboratory the, bottles were incubated at 37°C for a maximum period of 7 days. The bottles were observed daily for signs of growth like turbidity, air bubbles and colonies on the surface of the sedimented red cells or over the solid slant portion of the biphasic medium. As soon as growth was observed, subculture was done on Blood agar and CLED agar plates and incubated at 37°C [42]. The bottles that showed no growth for 7 days were discarded.

Identification: Isolates were identified by their characteristic appearance on the respective media, Gram staining, and confirmed using standard biochemical tests [42]. For Gram-positive bacteria catalase, coagulase bile aesculin and other tests were performed. Gram-negative isolates were identified by motility test, indole production, citrate utilization, oxidase, sugar fermentation, and other tests. In the case of Candida isolates first Germ tube test was done to differentiate Candida albicans from Nonalbicans. Carbohydrate Assimilation and Carbohydrate Fermentation test was done for further speciation [43].

Antibiotic Sensitivity Testing: The antimicrobial sensitivity testing was done for all pure bacterial isolates in Mueller- Hinton agar plate by using Kirby-Bauer disk diffusion technique. The antibiotic discs used were obtained from Himedia (India) Laboratories. The sensitivity pattern was studied depending on the zone of inhibition as per the standard CLSI guidelines [44].

The antibiotic discs used in the study were: Ampicillin (10µg), Amikacin (30µg), Amoxycillin/ Clavulanic acid (20/10µg), Ciprofloxacin (5µg), Cefuroxime (30µg), Cefotaxime (30µg), Cefoxitin (30µg), Colistin (10µg), Gentamicin (10µg), Imipenem(10µg), Linezolid (30µg), Piperacillin/ Tazobactam (100/10µg), Teicoplanin (30µg), Vancomycin (30µg).

Statistical Analysis: Data was analysed by using simple statistical methods with the help of MS-office software. All data was tabulated and percentages were calculated.

Results

A total of 154 new born clinical sepsis was admitted. Among them, blood culture was positive in 100 cases (64.94%). Out of 100 positive cases 82(74%) had EOS. 18(18%) had LOS. Majorities of newborn were males 67(67%). Majorities of sepsis newborn 59(59%) had low birth weight <2500 gram.

Table 1: Age at onset of Septicemia in neonates of both sexes

Age at onset of sepsis	Total number of cases (%)	Number of male babies	Number of female babies
0-3 days (EOS)	82(82%)	52 (52%)	26 (26%)
4-28 days (LOS)	18 (18%)	15 (15%)	06 (06%)
Total	100 (100%)	67 (67%)	33 (33%)

Table 2:Distribution of culture-positive neonates in relation to Gestational age.

Birth weight	No. of cases	Percentage
<2500gm (LBW)	59	59%
>2500gm (Normal)	41	41%
Total	100	100%

Most of the newborn 58(58%) had gestational age <37 weeks.

Table 3: Distribution of culture-positive neonates in relation to Gestational age

Gestational age in weeks	No. of cases	Percentage
<37 weeks (Preterm)	58	58%
>37 weeks (Term)	42	42%
Total	100	100%

Pathogenic bacteria were isolated in 64 cases. Among them, klebsiella pneumoniae 23(35.93%), Staphylococcus aureus 19(29.69%), Escherichia coli 11(17.19%) and Coagulase negative staphylococcus 7(10.93%) were found in majorities of cases.

Table 4: Pathogenic bacteria isolated from Blood culture

Bacteria	No. of cases	Percentage
Klebsiella pneumoniae	23	35.93%
Staphylococcus aureus	19	29.69%
Escherichia coli	11	17.19%
Coagulase negative staphylococcus	7	10.93%
Enterococcus species	2	3.12%
Serratia species	1	1.56%
Acinetobacter species	1	1.56%
Citrobacter species	1	1.56%
Total	64	64%

Pathogenic candida species was found in 36(36%) cases. Among them, Candida tropicalis, Candida kruzei, Candida albicans, Candida parapsilosis and Candida glabrata were found in 13(36.11%), 8(22.22%), 6(16.67%), 5(13.89%) and 4(11.11%) cases respectively.

Table 5: Pathogenic Candida species isolated from blood culture

Candida species	No. of cases	Percentage
Candida tropicalis	13	36.11%
Candida albicans	6	16.67%
Candida kruzei	8	22.22%
Candida parapsilosis	5	13.89%
Candida glabrata	4	11.11%
Total	36	100%

In the present study, Antibiotic susceptibility pattern was studied for all bacterial isolates causing Neonatal Sepsis. For the Gram-negative isolates, the highest sensitivity was reported for Amikacin 42(100%), Colistin 42(100%), Imipenem 41(97.62%), followed by Gentamicin 36(85.71%), Ciprofloxacin 37(88.09%), Piperacillin/Tazobactam 35(83.33%) Amoxycillin/Clavulanic acid 20(47.62%). The highest resistance was observed to Ampicillin (100%).

Table 6: Antimicrobial sensitivity pattern of Gram-negative bacilli (N=42)

Antibiotic	No. of cases	Percentage
Amikacin	42	100%
Gentamicin	36	85.71%
Cefotaxime	3	7.14%
Cefuroxime	2	4.76%
Ciprofloxacin	37	88.09%
Piperacillin/Tazobactam	35	83.33%
Amoxycillin/Clavulanic Acid	20	47.62%
Imipenem	41	97.62%
Ampicillin	0	0%
Colistin	42	100%

For Gram-positive isolates, the highest sensitivity was reported for Vancomycin 24(100%), Teicoplanin 24(100%), Linezolid 23(95.83%) followed by gentamicin 22(91.67%), and Amoxicillin/clavulanic acid 17(70.83%).

Tabe 7: Antimicrobial sensitivity pattern of Gram-positive isolates (N=24)

Antibiotic	No. of cases	Percentage
Gentamicin	22	91.67%
Ciprofloxacin	18	75%
Amoxycillin/Clavulanic Acid	17	70.83%
Cefoxitin	15	62.5%
Vancomycin	24	100%
Linezolid	23	95.83%
Teicoplanin	24	100%

Discussion

The World Health Organization has called for urgent action to avoid an antimicrobial resistance crisis, placing antimicrobial stewardship (AMS) programs at the centre of attention to help physicians optimize antibiotic prescription and improve patients' outcomes [12]. In addition to increasing rates of antimicrobial resistance, [13,14] early-life antibiotic exposure disrupts the developing microbiome, which may contribute to numerous diseases later in life, including diabetes, obesity, inflammatory bowel disease, asthma, and allergy [15]. Furthermore, neonatal antibiotic treatments are associated with mother-newborn separation, longer duration of hospital stay, reduced breastfeeding rates, and increased health care costs [16]. AMS is, thus, of crucial importance at the start of life. Antibiotics are the most prescribed medication in neonatal units, and their prompt initiation can be life-saving in neonatal early-onset sepsis (EOS) [17]. However, for late-preterm and full-term neonates, the incidence of EOS has decreased over the last decades [18]. Limited precision of current diagnostic tools and resulting concern of missing sepsis in conjunction with unchanged management strategies for suspected EOS are the main factors associated with antibiotic overuse in early life [42-44]].

In the present study, out of 154 clinically suspected cases of neonatal Sepsis, 100 were Culture positive with a Blood Culture positivity rate of 64.94%. This finding correlates well with the study of other workers like Premlatha DE et al, 72.3% [21]. EOS culture-positive cases were 82% and 18% were LOS. A higher prevalence of EOS was also reported by another study like Galhotra S et al, [22]. The ratio of culture-positive Male to Female babies was 2:1. Similarly, Buch et al found a Male to Female ratio of 1.8:1 [23]. Several explanations had been laid down for higher male susceptibility, it may be due to a gene located on the X Chromosome involved in the synthesis of immunoglobulins in the male infants thus conferring less immunological protection compared to females [24].

In the present study, it was observed that low birth weight babies were more prone to Sepsis, observed frequency in the present study was 59%, comparable to the study done by Tallur SS et al, 55% [25]. The immature cellular immunity and low level of immunoglobulin (IgG), excessive handling and contaminated incubators expose them to infecting organisms, thus increasing infections rate. Second, to low birth weight, prematurity is the most important predisposing factor for Septicemia. In the study, preterm neonates accounted for 58% of the cases. This coincides with, a study by Khinchi YR et al, 54.6% [26]. It has been established that several phagocytic functions are impaired including chemotaxis, phagocytosis and bacterial killing in preterm neonates. Also, there is impaired opsonic activity of the serum in pre-terms which is attributed to low levels of complement factors and partly to antibody deficiency. Stoll BJ [27].

In the present study, out of the 100 culture-positive cases, 64% were Bacteria and 36 % were Fungal isolates. Similar studies done in the past by workers like R Rani et al reported 62.3% Bacterial and 37.7% Fungal agents [28]. Changing trends of higher incidence of candidemia may be due to indiscriminate use of broadspectrum antibiotics in neonatal septicemic cases.

In the present study, Klebsiella pneumonia 23(35.93%) is most commonly isolated. This Gram-negative preponderance was also reported by Sriram R [29]. Amongst Gram positive isolates, our study reported Staphylococcus aureus (29.69%) as the most common one. This is in accordance with the study done by Agnihotri N, et al, who reported 35% as Staphylococcus aureus [30].

In the present study, out of the total 36(36%) Candida species, the highest was Candida tropicalis 13(36.11%). This was similar to the findings of Jain A et al, who reported Candida tropicalis as the most common isolate [31]. Regarding the Antibiotic susceptibility pattern of the Gram-negative isolates in our study: 100% sensitivity was reported for Amikacin, Colistin followed by Imipenem (97.62%). These isolates reported 100% resistance to Ampicillin. This is in concordance with the finding of other workers like Rasool KH, et al, [32]. This was mostly due to the indiscriminate use of third-generation cephalosporins. Gram-positive isolates in our study were 100% sensitive to Vancomycin, Teicoplanin and 97.4% sensitive to Linezolid. These results are very much similar to the study done by Shaw CK [33]. Out of the 19 Staphylococcus aureus isolated, 31.58% were found to be Methicillin-resistant Staphylococcus aureus (MRSA). This finding is comparable to the findings of Kayenge N, et al, where 28% MRSA was reported [34].

Sepsis is one of the most common causes of neonatal hospital admissions [35]. Newborns are particularly susceptible to sepsis as a result of their immature immune system, the decreased phagocytic activity of their white blood cells and their incompletely developed skin barriers [36]. Common risk factors for neonatal sepsis in Northern India have been identified as low birth weight, perinatal asphyxia, preterm labour and premature rupture of membranes [37].

Neonatal sepsis is a medical emergency which presents with subtle, diverse and nonspecific symptoms and signs. Delay in diagnosis and commencement of appropriate treatment may result in high morbidity and mortality rates [38]. Blood culture, which is the gold standard for the diagnosis of sepsis, takes at least 48 hours to obtain preliminary results [39]. It is therefore necessary to initiate an empirical choice of antibiotics based on the epidemiology of causative agents and antibiotic sensitivity patterns in a locality [40]. Periodic bacterial surveillance is a necessity in every unit because the organisms responsible for neonatal sepsis have been shown to vary across geographical boundaries and with time of onset of illness [41].

Conclusion

The present study concluded that the low birth weight, preterm labour are the most common risk factors of neonatal sepsis. klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli and Candida tropicalis are the most common pathogens isolates from blood culture. Amikacin, colistin and imipenem are the highest sensitivity for gram negative isolates. While, ampicillin is the highest resistance. Vancomycin, teicoplanin, linezolid are the highest sensitivity for gram positive isolates. Hence, regular monitoring of antibiotic resistance is necessary and depending on the antibiotic sensitivity pattern of the isolates, antibiotic should be used. Blood culture is a Gold Standard for diagnosis of neonatal sepsis and should be done in all the suspected cases of neonatal sepsis.

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