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A Study on the Pattern of Bacterial Pathogens Isolated in Neonatal Septicemia

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A B S T R A C T

The varying microbiological pattern of septicemia in neonates warrants the need for an ongoing review of the causative organisms and their antimicrobial susceptibility pattern. The incidence of bacteremia in neonates varies widely. As neonatal septicemia is life threatening condition, delay in diagnosis and treatment may lead to adverse complications, hence isolation followed by antibiotic sensitivity are crucial for prompt treatment. The blood-broth media were incubated for 7 days at 37^{o}C under aerobic atmosphere. Blind subcultures were done on blood agar, chocolate agar and MacConkey agar media after 24 hours, 48hours(when no growth was found upon first subculture) and on 7th day(when found sterile upon second subculture) of incubation. The cultures were declared negative only after 7th day of incubation. In our work, of these 49, GPCs were isolated from 26(53%) blood samples which were predominant cause of septicemia in neonates compared to GNBs which were isolated from 21(42.85%) cases followed by non candidaalbicans isolated from 2(4.08%) blood samples.

Introduction

Neonatal sepsis can be defined as a clinical entity occurring in the first month of life associated with systemic signs and symptoms confirmed by a positive blood culture. Sepsis has remained a leading cause of morbidity and mortality worldwide in the neonates despite of careful hygiene practices and the use of broad spectrum antibiotics (Naher and Khamael, 2013).

In developing countries, neonatal infections are estimated to cost 1.6 million annual deaths accounting for about 40% of all neonatal deaths. The incidence of neonatal sepsis according to data from National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live births. (National Neonatal Perinatal Database 2005, 2002-2003). Early diagnosisand aggressive treatment with antibiotics and good supportive care save most cases of neonatal sepsis (Aggarwal *et al.*, 2001). Incidence of neonatal septicemia widely varies from region to region within our country also (Rathod *et al.*, 2012).

Neonatal septicemia clinically can be broadly classified on the basis of onset of symptoms into early onset sepsis (EOS; in first week of life) and late onset sepsis (LOS; after 1 week of life). Risk factors include both maternal and neonatal for the occurrence of septicemia among neonates. Maternal causes include group B streptococcal (GBS) colonization, chorioamniotis, multiple deliveries, prolonged

rupture of membranes (PROM), urinary tract infection and neonatal factors include preterm birth, low birth weight etc. (Raha *et al.,* 2014)

The clinical features are nonspecific and lead to the difficulty indiagnosis of neonatal septicemia on clinical grounds alone. Patients may present with one or more of the following symptoms and signs.

A) Jaundice

B) Apnoea

C) Respiratory distress

D) Meconium aspiration syndrome

E) Umbilical sepsis, conjunctivitis, abscess, pyoderma as foci of infection

F) Meningitis

G) Pneumonia

H) Urinary tract infection

I) Sclerema

J) Otherfeatures such as intestinal obstruction, paralytic ileus, hemolytic disease of newborn (Tallur *et al.,* 2000).

Advances in early diagnosis and treatment have led to constant change in the bacteriological profile of neonatal septicemia with the most common etiologic agents being GBS and Coagulase negative Staphylococci (CONS) in developed industrialized countries. However, in the developing countries, these organisms are rare with an entirely different spectrum of bacteria (Kuruvilla *et al.,* 1986).

The gold standard for diagnosis of septicemia is the isolation of bacterial agent from blood culture (Nwadioha *et al.,* 2010).

The varying microbiological pattern of septicemia in neonates warrants the need for an ongoing review of the causative organisms and their antimicrobial susceptibility pattern. The incidence of bacteremia in neonates varies widely (Karki *et al.,* 2010).

As neonatal septicemia is life threatening condition, delay in diagnosis and treatment may lead to adverse complications, hence isolation followed by antibiotic sensitivity are crucial for prompt treatment (Jain *et al.,* 2003).

Increase in resistance has been noted against many antibiotics in the published medical literature. The investigators (Joshi *et al.,* 2010) reported the predominant resistance of their isolates to cephalosporins, piperacillin and gentamicin. In another work by Mane and others (Mane *et al.,* 2010) most of the clinical isolates had high resistance to 3rd generation cephalosporins, but being susceptible to carbapenems and vancomycin.

Most of the organisms have developed multi-drug resistance (MDR) over the last two decades due to indiscriminate use of antibiotics and lack of legislation to control their use and have shown high antibiotic resistance in bacterial pathogens of neonatal septicemia, clinical features being non-pathognomonic of causative agent. Isolation of the infecting bacterium from the blood specimen followed by antibiotic susceptibility testing are paramount for the institution of effective antibacterial therapy and hence this helps to improve recovery of neonate from septicemia.

Materials and Methods

A total of 122 neonates clinically suspected of neonatal septicemia reported to Hospital, were examined during a study period and the criteria were as outlined below.

Inclusion Criteria

Clinically suspected cases of neonatal septicemia.

Exclusion Criteria

Neonates clinically suspected of septicemia but had received antibiotics were excluded from the study.

Patients presented to department of pediatrics (NICU), were examined clinically by pediatricians and 122 cases of neonatal septicemia were identified on the basis of the signs and symptoms and were included for the study. This is followed by collection of blood for culture after obtaining informed expressed written consent.

Blood Specimen Collection

1-2mL of blood was collected from the peripheral veins following all standard aseptic precautions as per CLSI [33] guidelines. The collected blood specimen was immediately inoculated onto 5mL (when 1mL was obtained) or 10mL (when 2mL was obtained) of liquoid broth (BHI broth with SPS) culture medium and mixed gently immediately.

Processing of sample and approach to identification

The blood-broth media were incubated for 7 days at 37^{o}C under aerobic atmosphere. Blind subcultures were done on blood agar, chocolate agar and MacConkey agar media after 24 hours, 48hours (when no growth was found upon first subculture) and on 7th day(when found sterile upon second subculture) of incubation. The cultures were declared negative only after 7th day of incubation. The colony characteristics including results of microscopic morphologic features of the colony such as Gram-staining and hanging drop preparation were noted. Finally the bacterial pathogen was identified by subjecting the growth for standard biochemical and other necessary tests. The culture media, chemicals, and oxidase discs for the study were procured from HIMEDIA, Mumbai, India.

Results and Discussion

Of 122 blood cultures carried out, 49 yielded the growth. In our work, of these 49, GPCs were isolated from 26(53%) blood samples which were predominant cause of septicemia in neonates compared to GNBs which were isolated from 21(42.85%) cases followed by non-candida albicans isolated from 2(4.08%) blood samples.

Includes 6 MRSA

Among 47 bacterial culture positive blood samples, *S.aureus* were 14(29.78%), of which 6(42.8%) tested MRSA. CONS were from 9(19.14%) samples, followed by Streptococcus species from 2(4.25%) and Pneumococcus from 1(2.12%) sample. 14(29.78%) blood samples yielded *K.aerogenes*, followed by isolation of Acinetobacter species in 4(8.51%), and *E.coli*, *C. freundii* and *P.aeruginosa*, were isolated from 1(2.12%) blood sample each. 23 cases of EOS showed GPCs followed by GNBs in 20. But GPCs were detected only in 3 cases of LOS followed by GNB in 1 case. However, statistically the difference was not found significant ($\chi 2=0.13$; $p> 0.05$).

The present prospective study, bacterial culture positivity from blood samples was noted in 47(38.52%) among the 122 cases studied. (Tallur *et al.*, 2000) reported 156(64.87%) blood samples positive for bacterial isolates of total 242 cases studied which is higher than the present study. Whereas, (Kaistha *et al.*, 2009) recorded

296(13.17%) blood culture positivity for bacterial isolates among 2247 cases in their retrospective study (Agnihotri *et al.*, 2004). found 588(19.2%) culture positive cases among 3064 cases studied which is less when compared to our study. The differences in the culture positivity rates noticed in different studies could be attributed to geographical distribution and also type of study- for instance retrospective or prospective and also whether the patients were on antibiotics or not before obtaining blood sample for culture.

Table.1 Distribution of 49 culture positive isolates from blood cultures of 122 neonatal septicemia cases studied

Broad type of organism	No (% of cases)
GPC	26(53.0)
GNB	21(42.85)
Non candida albicans	02(4.08)
Total	49

Table.2 Distribution of 47 bacterial isolates (organism wise) obtained

Organism	No (%) of isolates
S.aureus	14 *(29.78)
CONS	9(19.14)
Streptococcus species	2(4.25)
Pneumococcus	1(2.12)
K.aerogenes	14(29.78)
Acinetobacter species	4(8.51)
E. coli	1(2.12)
C. fruendii	1(2.12)
P. aeruginosa	1(2.12)
Total	47

Table.3 Distribution of 47 bacterial isolates of neonatal septicaemic cases in association with onset of septicemia

Onset of septicemia	GPC	GNB
EOS	23	20
LOS	3	1
Total	26	21

Table.4 Distribution of bacterial culture positivity rates in different studies

Authors and reference	No. of cases studied	No(%) of culture positive cases
Tallur*et al.,* [6]	242	156(64.87)
Kaistha*et al.,* [13]	2247	296(13.17)
Agnihotri *et al.,*[14]	3064	588(19.2)
Present study	122	47(38.52)

Table.5 Distribution of the most commonly isolated bacteria (Klebsiella) from blood cultures of clinically suspected neonates in different studies

Authors and reference	Organism	No (%) of isolates
Tallur*et al.,* [6]	Klebsiella species (*K.pneumoniae, K.aerogenes, K.oxytoca*)	84(33.76)
Kaistha*et al.,* [13]	Klebsiella species	84(28.3)
Mustafa and Ahmed [15]	Klebsiella (*K.pneumoniae*)	22(35)
Present study	Klebsiella(*K.aerogenes*)	14(29.78)

Spectrum of bacterial pathogens observed in causing neonatal septicemia in various studies

In the present work, GPCs (26; 53%) were principal bacterial agents of neonatal septicemia than GNBs (21; 42.85%) which closely matches with the observations made by Stoll *et al.,* where in predominant isolations were GPCs accounting for 73%, GNBs being 27% only. Reports of Gram-positive bacteremias, however, are accumulating in the last three decades. (Kuruvilla *et al.,* 1986)

However, (Joshi *et al.,* 2000) and coworkers reported isolation of GNBs more predominantly that was reported in 67.2% cases in their study. (Kaistha *et al.,* 2009) recorded gram negative septicemia in 80.4% of neonates.

(Nwadioha *et al.,* 2010) also documented high incidence of gram negative bacteremia seen in 69.3% of cases and stated that predominance of either GPCs or GNBs is influenced by geographical location and changes taking place on time.

As presented in table 5, in our study, out of 47 bacterial isolates, *K.aerogenes* was the principal etiological agent seen in 29.78% neonates that was similar to the reports of those of (Tallur *et al.,* 2000) who also reported Klebsiella species (*K.pneumoniae, K.aerogenes, K.oxytoca*) as the most common bacterial pathogen that was detected in 84(33.76%) cases, (Kaistha *et al.,* 2009) who also recorded Klebsiella species in 84(28.3%), and (Mustafa and Ahmed, 2014) who also noted in their study 35% *K. pneumoniae* isolates as most commonest bacterium.

In the present study, EOS was predominantly seen compared to LOS. Most common organism isolated was *K. aerogenes*.

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