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Original Research Article

Prevalence of Drug Resistance in Neonatal Sepsis- A Prospective Study

Dr. Lakshmi Rangadhol Prakash[1], Dr. Ranjini Nanjaiah[2], Dr. B. Sai Priya[3]

[1]Assistant Professor, Department of Microbiology, Basaveshwara Medical College and Hospital & Hospital, Chitradurga, Karnataka, India.
[2]Associate Professor, Department of Community Medicine, Adichunchanagiri Institute of Medical Sciences, Mandya, Karnataka, India.
[3]Associate Professor, Department of Microbiology, Basaveshwara Medical College & Hospital, Chitradurga, Karnataka, India.

Corresponding Author
Dr. Lakshmi Rangadhol Prakash, Assistant Professor, Department of Microbiology, Basaveshwara Medical College and Hospital & Hospital, Chitradurga, Karnataka, India.

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ABSTRACT

Background: Infections are the single largest cause of neonatal deaths globally**.** The emergence of Methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum ß- lactamases (ESBLs) in neonatal intensive care unit patients is increasing. This study aims to find out the bacteriological profile in neonatal sepsis and study their antimicrobial susceptibility pattern.

Method: A total of 360 suspected neonatal septicaemic cases admitted in SSIMS & RC at NICU were enrolled. Blood sample was collected for blood culture. The isolates were identified by standard biochemical tests and susceptibility performed using the disc diffusion methods as per Clinical laboratory Standard Institute (CLSI) recommendations. *Staphylococcus aureus* were tested for Methicillin resistance using Cefoxitin disc (30µg), ESBL was detected using combined disc method.

Results: Out of 360 cases 160 were bacteriologically positive, *Klebsiella* was the most common organism isolated (21%), followed by *Staphylococcus aureus* (20%) *Acinetobacter* (15%), *Pseudomonas* (13%). Maximum sensitivity was seen by Linezolid, Erythromycin for Gram positive organisms and Gram negative organisms were sensitive to Piperacillin/ Tazobactum, Imipenem, Levofloxacin, Meropenem. MRSA was detected in 53% isolates, ESBL production was majorly seen in *E.coli*, followed by *Klebsiella*.

Conclusion: *Klebsiella spp*. and *Staphylococcus aureus* were the commonest bacteria causing neonatal sepsis in this centre. Multidrug resistance among the isolates was common. Early diagnosis and institution of specific antibiotics after studying the sensitivity pattern will help in reducing neonatal morbidity and mortality and prevent emergence of drug resistant strains.

Keywords: Neonatal Septicaemia, ESBL, MRSA.

INTRODUCTION

Neonatal septicemia is an important cause of Neonatal morbidity and mortality. it is one of the indicator for measuring the health status of a nation. According to WHO estimates there are 5 million neonatal death a year with 98% occurring in developing countries. The incidence of neonatal sepsis varies from 11-24.5 /1000 live births in India. Neonatal deaths account for over a one-third of the global burden of child mortality.[1]

Neonatal sepsis can be divided into two main classes depending on the onset of symptoms namely Early-onset sepsis, which usually presents within the first 72 hours of birth and Late-onset sepsis, which usually presents 72 hours after birth.[2]

The pattern of organisms causing neonatal sepsis has been constantly changing and indiscriminate use of antibiotics has resulted in the emergence of multidrug resistant and virulent organisms. Here lies the importance of microbiological investigation and determination of antibiotic susceptibility pattern of isolate.

During the past decade, ESBL strains have frequently been implicated in neonatal intensive care units at tertiary care hospitals. Recent studies in India showed that *Klebsiella* species and *Escherichia coli* are the most common GNB causing neonatal sepsis in India

and one-third are ESBL producers in both community and hospital settings and is associated with very high mortality rate (33%) in these patients. MRSA has become one of the most common pathogens in NICU with 33% prevalence as per a recent Indian study.[3]

Therefore, this study was designed to investigate the prevalence of ESBL producing Gram negative rods in neonatal septicaemia & MRSA producing Gram positive cocci and to observe antimicrobial susceptibility pattern of ESBL and non ESBL producing gram negative isolates, which would enable formulation of appropriate antimicrobial policy for such patients.

MATERIALS & METHODS

This study was conducted in S. S. Institute of Medial Science and Research Centre, Davangere. Neonates admitted with the clinical diagnosis of neonatal septicaemia are included in the study as per the criteria by Vergnano.[4]

Exclusion Criteria

Neonates already on antibiotics and with diagnosis of intra-uterine infection and congenital anomalies were excluded from the study.

The study group comprised of 360 suspected cases of neonatal septicaemia in the neonatal intensive care unit,160 were positive for blood culture. Thus prevalence of neonatal septicemia in the present study is 44.4%

Specimen Processing

Blood samples were collected from fresh veni-puncture site after preparing the area with aseptic precaution. They were subjected to aerobic bacterial culture by conventional method using brain heart infusion broth with sodium polyethanol sulphonate (Himedia Labs, India). Bacteria isolated by standard microbiological procedures were subjected to antimicrobial susceptibility testing by Kirby Bauer's disc diffusion method. Suspected multidrug resistant strains were confirmed by phenotypic methods. Relevant ATCC strains were used as controls wherever necessary.

Phenotypic Detection of MRSA

- Detection of Methicillin resistance among *Staphylococcus aureus* isolates was done using Cefoxitin disc (30 ug) on Mueller Hinton agar and results interpreted according to CLSI guidelines.[5]
- With zone of inhibition <21mm considered as positive
- With zone of inhibition >22mm is considered negative

Phenotypic Detection of ESBL Production

The screening for ESBL production was done as per Clinical and Laboratory Standards Institute (CLSI) recommended method. Isolate showing inhibition zone of ≤22 mm for Ceftazidime (30 µg), 27 mm for Cefotaxime (30 µg), was taken for ESBL confirmation.

The confirmation was done by CLSI phenotypic disk confirmatory test using disks of Ceftazidime (30 µg) and Ceftazidime-Clavulanic acid (30 µg/10 µg). Both the disks were placed at least 20 mm apart, centre to centre on Mueller-Hinton agar plate and incubated over night at 37°C. A zone difference of ≥5 mm around Ceftazidime and Ceftazidime-clavulanic acid was taken as ESBL positive. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative control respectively.

RESULTS

Totally 360 neonates who were suspected to have septicemia on clinical basis were included in the study. Among these 160 neonates had definitive septicemia as they were bacteriologically positive. Among these 100 cases (62.5%) were Gram-negative organisms & 60 cases (37.5%) were Gram-positive organisms.

Bacterial isolates	No. of isolates
Klebsiella	34
Acinetobacter	23
Pseudomonas	22
E.coli	12
Citrobacter	5
Others	4
Staphylococcus aureus	32
CONS	20
Enterococcus Spp	8

Table 1. Microbial Profile of Neonatal Septicemia

In our study 32 Staphylococcus were isolated, out of which 14 were Methicillin resistant and ESBL was detected in 58% of Klebsiella pneumoniae isolates and 66% of Escherichia coli isolates.

Bacterial isolates and number	MRSA detected by using cefoxitin disc
Staphylococcus aureus (32)	14(43%)

Table 2: Detection of MRSA

Bacterial isolates	No. of isolates	ESBL detection
Klebsiella	34	20
Acinetobacter	23	14
Pseudomonas	22	6
E.coli	12	8
Citrobacter,	5	2

Table 3: Detection of ESBL in Gram negative organisms

All the Gram positive isolates were 100% sensitive to Vancomycin,96% to Linezolid,80% to Gentamicin, Gram negative organisms isolated were more sensitive to Amikacin, Levofloxacin, Imepenem, Pipercillin tazobactum, Meropenem.

DISCUSSION

Neonatal septicemia is much higher in developing countries than in developed nations. Neonates are particularly vulnerable to infections because of weak immune barrier. Moreover, several risk factors have been identified both in the neonates and in the mothers making them susceptible to infections. Blood stream infections have been quoted as the most common infections in this neonatal age group.[6,7]

In our study most of the babies had clinical presentation of breathlessness, poor cry and poor activity. Other manifestations included poor feeding, convulsions, fever, sclerema, jaundice, tachypnoea, grunt, lethargy, irritability, diarrhea and abdominal distension.

Maternal, perinatal and neonatal events put the baby at increased risk of infection. These factors play a major role in the incidence and the pattern of organisms in neonatal septicemia.The most significant factors in septicemia are low birth weight babies and prematurity.[8] In India, the problem is magnified as a majority of admissions to the neonatal ICU comprises of septicemia. Some times septicemia may be the result of intensive life support measures used to survive pre-term babies or sick neonates.

In our study *Klebsiella* (21%) is the commonest organism isolated from cases of neonatal septicaemia followed by *Staphylococcus aureus* (20%) and *Acinetobacter* (15%).

This is similar to study by Zakariya et al[7] and Chandel et al,[9] NNPD data 2002- 2003 shows that *Klebsiella pneumoniae* is the commonest pathogen isolated in neonatal sepsis both in babies born within the institution (intramural) and in babies referred from community and other hospitals (extramural).[6]

One of the reasons expounded for the predominance of an organism in causing septicemia in the nursery is the selective pressure of antimicrobial agents, so that the resistant organisms tend to colonize and proliferates in the nurseries.[10]

In our study,as per [Table/Fig-2], there were a total of 32 *Staphylococcus aureus* isolated, out of which 14 were Methicillin resistant. All the MRSA strains were sensitive to Vancomycin. *Enterococcus faecalis* and *Coagulase negative Staphylococci* were also 100% sensitive to Vancomycin similar to a study by Amutha Chelliah et al.[10] So screening for MRSA in every *Staphylococcus aureus* isolated will be of immense value for providing efficient patient care.[11]

K. pneumoniae and *E. coli,* were found to be highly resistant to Cephalosporins and completely sensitive to Imipenem this is a typical characteristic of Extended-spectrum beta-lactamases (ESBLs).[12]

ESBL was detected in 58% of *Klebsiella pneumoniae* isolates and 66% of *Escherichia coli* isolates. This is closer to study by Bhattacharjee et al[1], in which ESBL was observed in 62.7% of Klebsiella pneumonia and 46.5% of Escherichia coli and Jain et al.[13] in which ESBL was in 86.6% of Klebsiella pneumoniae and 63.6% of Escherichia coli.

In most centers β-lactamase production is not routinely tested which ultimately results in the dissemination of β-lactamase producing strains in hospitals, and it remains undetected for longer periods. Inappropriate usage of antimicrobials leads to escalating percentage of β-lactamase production. Therefore, preventive antibiotics should be used as little as possible and specific therapeutic antibiotics should be used for short period as suggested by Singh *et al*.[14] In conditions, wherein the use of antibiotics is necessary, rotation of antibiotic regimens is suggested

The difference in percentage of isolation of ESBL compared to few other studies could be due to regional variations. Distinct regional variations have been detected in the incidence of ESBL producing isolates.[15] Recent study demonstrated that restricted use of cephalosporins significantly reduced the incidence of ESBL producing, cefotaxime resistant and ciprofloxacin resistant gram negatives.[16]

Though molecular techniques are the gold standard, it is difficult for most of the diagnostic laboratories to do on a routine basis. On the other hand, although phenotypic methods give little imprecise results, they are widely being used because of their simplicity and cost-effectiveness. ESBL detection by only CLSI method and lack of molecular data is the limitation of the study.

Simple hygienic measures, such as hand-washing practices, the use of sterile equipment, patient cohorting (i.e., grouping patients with similar infections in the same location) and screening of attending staff and mothers for MRSA and ESBL help to prevent the further spread of these resistant strains. This study stresses that antimicrobial resistance is a global problem and emphasizes the need for surveillance and promotion of correct and restrictive antibiotic policies including specific antibiotic therapy after studying sensitivity pattern.

CONLUSION

Along with blood culture other septic markers can be used in rapid diagnosis and early management of septicemia. Reviewing antimicrobial prophylaxis and treatment regimens based on local antibiotic resistance patterns can reduce morbidity and mortality in the neonates.

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