Clinico-Bacteriological Study of Neonatal Septicaemia with Special Reference to Antibiotic Resistance Pattern in Sick Newborns Admitted in a Tertiary Care Hospital, Kolkata

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ABSTRACT

Neonatal sepsis is a major healthcare burden responsible for high rates of neonatal mortality. Due to its non-specific signs and symptoms and delay in identification, it poses a great challenge to the clinician in ruling out sepsis at an early stage. The present study was undertaken to identify the recent trends in bacteriological profile associated with neonatal sepsis, their antibiotic resistance pattern and the major risk factors associated with neonatal sepsis. In this cross-sectional study, blood cultures were performed from suspected cases of neonatal sepsis. Growth was identified by standard microbiological techniques and antibiotic susceptibility testing was carried out by Kirby-Bauer disk diffusion method. MRSA (Methicillin Resistant Staphylococcus aureus) and ESBL (Extended Spectrum β-Lactamase) or AmpC β-Lactamase and carbapenemase production was detected phenotypically following CLSI (Clinical and Laboratory Standard Institute) guidelines. Out of 812 blood samples collected, 177 (21.79%) were culture positive, 85 (48.02%) isolates were Gram positive Cocci, 76 (42.93%) were Gram negative Bacilli and 16 (9.03%) were Candida spp. Staphylococcus aureus was the most common pathogen isolated followed by Klebsiella spp. & Acinetobacter spp. A high percentage of MRSA & AmpC producers were observed in present study, these were associated with clinical conditions such as respiratory distress syndrome, prolonged rupture of membrane (>12hrs) & low birth weight. Strengthening rapid detection, along with stringent infection control program and antibiotic stewardship can help to reduce neonatal sepsis and it will allow us to minimise the use of reserve drugs to battle out sepsis at an early stage.

Keywords: Neonatal septicemia, antimicrobial resistance, ESBL, AmpC, MRSA, Carbapenemase.

INTRODUCTION

Over the last two decades, prodigious progress has been made in lowering neonatal mortality rate but it continues to be a major public health care burden in many parts of the world. It is recognised as one of the major global challenges in health care. As claimed by World Health Organization (WHO), four million neonates expire every year during their neonatal period (i.e. within 4 weeks of birth) with 75% premature deaths occurring in the first week of life. Asphyxia (23%), intrapartum complication (24%), premature births (28%) and infections (35%) are the major causes responsible for global neonatal deaths. As per UNICEF data, in 2017, 2.5 million children died globally in the first month of life with 7000 neonates dying every day. However India has made significant progress in tackling the
challenge of neonatal mortality. According to the fourth edition of the National Family Health Survey conducted in 2014-15, India’s national average Infant Mortality Rate (IMR) fell sharply from 57 to 41 (NFHS 4 (2015-16), where as in 2016, India’s Neonatal Mortality Rate (NMR) is 22/1000 live births.\[5\] Neonatal sepsis is the bacterial blood stream infection in neonates with positive blood culture and is responsible for neonatal mortality. The clinical symptoms may vary from subclinical infections to severe systemic manifestations, due to immunological immaturity there is an impaired response to these pathogen. The recognised factors predisposing to neonatal sepsis are premature rupture of membrane (PROM), meconium stained liquor, prematurity, birth asphyxia and instrumental delivery. The bacterial pathogen responsible for causing neonatal sepsis varies according to geographical location.\[6,7\] In developed countries, neonatal sepsis is most commonly caused by Group B Streptococcus (GBS), Listeria monocytogenes and E.coli \[8\] where as existing literature from India reveals Klebsiella pneumoniae to be the most common pathogens for the same in this country.\[9\] Moreover increasing antibiotic resistance by the common pathogens has worsened the prognosis. Early detection of neonatal sepsis by blood culture along with antibiotic susceptibility testing of the isolates can help in reducing the morbidity and mortality from neonatal sepsis.

In the present study various maternal and child risk factors known to predispose to neonatal sepsis have been evaluated through a predesigned questionnaire to identify the major risk factors associated with this condition in our neonatal care unit. Blood cultures were performed from suspected cases of neonatal sepsis, to identify the major pathogens responsible for neonatal sepsis and to identify their antibiotic resistance patterns, with special attention to pathogens of grave concern such as MRSA and β-lactamase producing organisms. The outcome of this study will help guide current antibiotic treatment for early management of neonatal sepsis as well as help in early identification of the newborns at risk for sepsis, thus improving outcomes of this dreaded clinical complication.

**MATERIALS AND METHODS**

This study was conducted at Department of Microbiology, Medical College, Kolkata and Sick Newborn Care Unit (SNCU), Department of Paediatrics, Medical College and Hospital, Kolkata from October 2016 to March 2017, after obtaining institutional ethical clearance (Institutional Ethics Committee, Reg.No: ECR/287/Inst/WB/2013) Medical College, Kolkata.

**Data and Sample collection:** Total 812 neonates of either sex clinically suspected of septicaemia with maternal history suggestive of possible risk factors were included in the study. Clinical data (age, sex, weight, mode and place of delivery and risk factors of infection in mother like premature rupture of membrane (PROM), meconium stained liquor, asphyxia and gestational period) was collected and recorded on a predesigned proforma. Neonatal risk factors like neonatal resuscitation, respiratory distress syndrome, birth weight and other notable symptoms were also recorded. 2 ml of blood was collected from neonates and blood culture was done by BACT/ALERT 3D (biomerieux) automated blood culture systems.

**Identification of the isolates:** Blood culture bottles flagged positive by the BACT/ALERT 3D system were inoculated on MacConkey agar and Blood agar respectively (HiMedia Laboratories Pvt. Limited) and were incubated overnight at 37°C. Growth of the organisms were identified on the basis of colony morphology, gram staining and standard biochemical tests like catalase, coagulase, oxidase, urease, carbohydrate fermentation tests, indole formation, triple sugar iron test and citrate utilization test.
The turbidity of all the bacterial suspensions used in below mentioned tests were adjusted to 0.5 McFarland and all the tests were done following CLSI (Clinical and Laboratory Standards Institute: 2013) guidelines.\(^{[10]}\)

**Antimicrobial susceptibility tests (AST):** Susceptibility tests were performed using the Kirby–Bauer disk diffusion method; following antibiotic disks (Hi Media Laboratories Pvt. Ltd. Mumbai, India) were used:

For **GPC:** Amoxyclav (AMC) 30µg, Cefuroxime (CMX) 30µg, Cefoxitin (CX) 30µg, Levofloxacin (LE) 5µg, Ciprofloxacine (CIP) 5µg, Clindamycin (CD) 2µg, Erythromycin (E) 15µg, Linezolid (LZ) 30µg, Vancomycin (VA) 30 µg, Gentamicin (GEN) 10 µg, and Doxycycline (DO) 30µg.

For **GNB:** Amoxicillin (AMX) 25µg, Cefotaxime (CTX) 30µg, Cefoperazone Sulbactam (CFS) 75+30µg, Amikacin (AK) 30µg, Ciprofloxacine (CIP) 5µg, Pipercillin Tazobactam (PTZ) 36µg, Cefuroxime (CMX) 30µg, Cefepime (CPM) 30µg, Ceftazidime (CAZ) 30µg, Ertapenem (ETP) 15µg, Imipenem (IPM) 10µg, Meropenem (MRP) 10µg, Polymyxin B (PB) 50µg, Colistin (CL) 10µg, Tigecycline (TGC) 15µg. *E.coli* ATCC 25922 was used as a quality control strain for AST by disk diffusion.

**Phenotypic test for MRSA:** *Staphylococcus aureus* isolates were screened for Methicillin resistance by Cefoxitin (CX) disk diffusion test on a Muller-Hinton Agar (MHA) plate using a CX (30µg) antibiotic disk. Following overnight incubation at 35°C, a zone of inhibition halo of diameter ≤ 21 mm around the CX disk for *S.aureus* was considered as indicative of MRSA production.

For Quality Control, *S.aureus* ATCC 43300 (MRSA) and ATCC 25923 (MSSA) were taken as positive and negative QC respectively.

**Screening for ESBL β-lactamases:** Gram negative bacilli isolated were screened for ESBL β-lactamase production. Two disks, CAZ (30 µg) and CTX (30 µg) were used. An inhibition zone of ≤ 22 mm for CAZ and ≤ 27 mm for CTX indicated that the strain probably produced ESBL following CLSI guidelines.

**Phenotypic confirmatory test for ESBL production:** Here two of the cephalosporin disks (CTX/CAZ) were tested individually as using more than one antimicrobial agent increases the sensitivity of ESBL detection. A ≥ 5mm increase in the zone diameter of Cefotaxime-Clavulanate (30µg + 10µg) / Ceftazidime-Clavulanate (30µg + 10µg) vs. the zone diameter of CTX/CAZ alone indicated the test strain produced ESBL. The distance between the disks was critical and 20 mm centre-to-centre has been found to be optimal for cephalosporin (30µg) disk as per CLSI guidelines.

For standard Quality Control, *K. pneumoniae* ATCC 700603 and *E.coli* ATCC 25922 were used as positive and negative QC respectively.

**Screening for AmpC β-lactamases:** Test strains producing AmpC β-lactamase were screened using cefoxitin disk (30µg), after overnight incubation, isolates producing zone diameter ≤ 18mm \(^{[11]}\) were subjected to cefoxitin-cloxacillin double disk synergy (CC-DDS) test for confirmation of AmpC production.

**Phenotypic confirmatory test for AmpC β-lactamases production:** The cefoxitin-cloxacillin double disk synergy test (CC-DDS) was performed. This test is based on the inhibitory effect of cloxacillin on AmpC β-lactamase. The CC-DDS was performed using CX (30µg) and CX + Cloxacillin (30µg + 200µg). A difference of ≥ 4mm in the inhibition zone diameter of cefoxitin-cloxacillin as compared to that of cefoxitin zone alone was considered as indicative of AmpC β-lactamase production.

**Screening test for carbapenemase production:** As per CLSI guidelines, a zone diameter of ≤ 19mm for meropenem (10µg) and ≤ 18mm for ertapenem (10µg) were used for screening carbapenem resistant strain.

**Phenotypic confirmatory tests for Carbapenemase production:**
Combined Disk Synergy Test (CDST) was performed with IPM and IPM-EDTA using (10µg) of IPM disk and 5µL of EDTA (EDTA solution was prepared by adding 1.86gm of EDTA powder in 10ml of distilled water, pH was adjusted to 8) solution, which was added to a blank disk of 6mm. The disk containing EDTA soln. was placed beside IPM disk. IPM disk was kept 10 mm edge – to – edge apart from the IPM-EDTA disk. After overnight incubation of the plate at 37ºC, A ≥ 5mm increase in the zone around IPM-EDTA disk as compared to IPM disk alone was interpreted as positive for carbapenemase producing clinical isolates.

The Modified Hodge Test (MHT) was done to detect carbapenemase production, an overnight culture suspension of Escherichia coli ATCC 25922 adjusted to 0.5 Mac Farland standard, was inoculated on the surface of MHA. After drying for 5-10 minutes, 10µg MRP disk was placed at the centre of the plate and the three strains (Positive Control: K.pneumoniae ATCC BAA 1705, Negative Control: K.pneumoniae ATCC BAA 1706 and the test strain) were streaked from the edge of the disk to the periphery of the plate in three different directions. The plate was incubated overnight at 37ºC for 24 hours; plate was then examined for clover leaf pattern indentations at the intersection of the test organisms and E.coli 25922, within the zone of inhibition of the carbapenem susceptibility disk. MHT positive test shows clover leaf pattern indentations of the E.coli 25922 growing along the test organism growth streak within the disk diffusion zone. Positive test indicate Carbapenemase production by the test organisms.

RESULTS
Out of 812 clinically suspected cases of neonatal septicaemia blood culture were positive in 177 (21.79%) neonates, among which 96 (54.23%) were female and 81 (45.76%) were male with female to male ratio 2:1.8. Early onset septicaemia (EOS) was found in 99 (55.93%) neonates while late onset septicaemia (LOS) was found in 78 (44.07%) neonates. 17 (9.60%) babies who had positive blood cultures expired during the course of this study.

Distribution of isolates from blood culture
Out of the 177 culture positive cases, 85 (48.03%) were Gram-positive cocci, 76 (42.94%) were Gram-negative bacilli and 16 (9.03%) isolates were of Candida species. Two (1.129%) samples showed poly microbial growth. Amongst Gram-negative bacilli the most commonly isolated organism was Klebsiella spp. followed by Acinetobacter spp. and Pseudomonas spp., whereas among Gram-positive Cocci Staphylococcus aureus was predominant followed by Coagulase Negative Staphylococcus spp. (CONS) and Enterococcus spp. (Figure.1)

Antibiotic resistance pattern of isolates recovered from positive blood culture
Most of the Gram-positive isolates were resistant to amoxicillin (50.02%) and amoxicillin-clavulanate (48.11%) whereas Linezolid showed high efficacy against Gram-positive cocci with 81.39% sensitivity. Among the Gram-negative organisms, nearly all the isolates were resistant to Amoxicillin-clavulanate (82.43%) and most of the isolates were resistant to cefuroxime (33.39%) and cefotaxime (67.09%) while imipenem and meropenem were found to be most effective antibiotics showing 77.27% and 76.74%
sensitivity against Gram-negative bacilli. (Figure. 2, 3, 4)
Among Gram negative organisms isolated in this study, 30.78% were ESBL producers, 35.71% were carbapenemase producers & 55.76% were AmpC producers whereas among Gram-positive organisms, Methicillin resistance S.aureus was seen as high as 45.94%.

Analysis of Risk factors in cases of culture-proven sepsis

Assessment of risk factors associated with neonatal sepsis was carried out through a predesigned proforma. Analysis of the results showed that preterm babies, babies with low birth weight and babies born out of vaginal delivery were significantly at higher risk for contracting sepsis. (Table 1)

Premature neonates are more vulnerable to infection due to their immature immune system and along with invasive life support apparatus, makes them highly susceptible to nosocomial infection.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>CULTURE POSITIVE CASES (177)</th>
<th>P-VALUE</th>
</tr>
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<tbody>
<tr>
<td>GESTATION WEEKS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤36</td>
<td>149 (84.18%)</td>
<td>0.01</td>
</tr>
<tr>
<td>&gt;36</td>
<td>28 (15.82%)</td>
<td></td>
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<tr>
<td>BIRTH WEIGHT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2.5KG</td>
<td>140 (79.09%)</td>
<td>0.01</td>
</tr>
<tr>
<td>&gt;2.5KG</td>
<td>37 (20.91%)</td>
<td></td>
</tr>
<tr>
<td>MODE OF DELIVERY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAGINAL</td>
<td>162 (91.52%)</td>
<td>0.01</td>
</tr>
<tr>
<td>CAESAREAN</td>
<td>15 (8.48%)</td>
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<tr>
<td>PLACE OF DELIVERY</td>
<td></td>
<td></td>
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<tr>
<td>INBORN</td>
<td>104 (58.75%)</td>
<td>0.02</td>
</tr>
<tr>
<td>OUTBORN</td>
<td>73 (41.25%)</td>
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<tr>
<td>POOR FEEDING</td>
<td>100%</td>
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<tr>
<td>ALTERATION OF CRY</td>
<td>57.62%</td>
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<tr>
<td>RESPIRATORY SYMPTOMS</td>
<td>43.50%</td>
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<td>GASTROINTESTINAL SYMPTOMS</td>
<td>23.72%</td>
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<tr>
<td>CONVULSIONS</td>
<td>14.68%</td>
<td></td>
</tr>
<tr>
<td>LETHARGY</td>
<td>84.74%</td>
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</tbody>
</table>

DISCUSSIONS

With several advances in the prevention and management of sepsis, the rate of neonatal sepsis has decreased considerably, yet it remains a significant cause of neonatal mortality worldwide. Blood culture is the most conclusive investigation for the diagnosis of sepsis. The present study revealed blood culture positivity rate of 21.79%, which is lower than most other similar studies, Sharma et al 2013 (37.63%), Shah AJ et al 2012 (31.75%). Some studies have also reported lower rates of Culture positivity, Reddy et al 2016 (12.14%). However, negative blood culture does not exclude sepsis as about 25.9% of all neonatal sepsis could be due to anaerobes.

In this study Gram-positive cocci (48.02%) were predominant over Gram-
negative bacilli (42.93%), this scenario is comparable to studies where Gram-positive cocci were more prevalent like Karnataka, India (80%) Kerala, India (51.92%) and Nepal (63.8%) Rajana et al 2018. The most predominant organisms isolated in this study was *S. aureus* accounting for about (29.92%) followed by *Klebsiella spp.* (15.81%) and *Acinetobacter spp.* (11.86%). Our results are in agreement with other studies that reported *S. aureus* as predominant organism, Sharma et al 2013 (37.22%), Shaw et al 2007 (42.75%) and Karthikeyan et al 2001 (61.5%). Among Gram-negative isolates, *Klebsiella spp.* (18%) is the most commonly encountered organisms in our study and is second most predominant organisms after *S. aureus* which is in accordance with other studies by Sharma et al 2013 (27.01%) but this finding is different from many other studies which reported *Klebsiella spp.* to be most frequently isolated organism, Reddy et al 2016 and Agrawal et al 2018.

In our study, GPC showed high rate of resistance to penicillin (AMX/AMC) 49.07% followed by fluoroquinolones (LE/CIP) 46.63% and cephalosporins (CXM/CX) 41.9%, whereas GPC were highly susceptible to vancomycin 100% and linezolid 81.39%. A similar type of antibiotic resistance pattern in the commonly used antibiotic has been noted by Aurangzeb et al 2003, Gandhi et al 2013, Sharma et al 2013 and Rajana et al 2018. MRSA among GPC observed in this study was 45.94% which corroborates with findings of Gandhi et al 2013 (31.25%) and Patel et al 2014 (33%).

GNB in our study showed high degree of resistance to Gentamicin 64.09% followed by penicillin (AMC/PTZ) 56.81% and cephalosporins (CXM/CTX/CAZ/CFS/CPM) 52.49% whereas GNB were highly susceptible to polymyxin 100% and moderately susceptible to carbapenems (IPM/MRP) 77.01%. Lower rate of sensitivity to commonly used antibiotics has been observed in many similar studies, Shah et al 2012, Gandhi et al 2013, Patel et al 2014 and Samaga et al 2016. The prevalence of ESBL-producing organisms in neonatal sepsis varies in different geographical areas, 37% in Latin America Vijayankanti et al 2013, 7% in United States Sader et al 1998, 5-56% in Asian countries Kaftandzhieva et al 2009, Bell et al 2002. The incidence of ESBL producing organisms in this study is 30.78% in which ESBL producing *Klebsiella spp.* and *E.coli* is 25% and 18.75% respectively which is comparable with study from India that reported ESBL producing *Klebsiella spp.* 28.6% and ESBL producing *E.coli* 36.5%. This study reported 55.76% AmpC β-lactamase producing GNB among which AmpC producing *Acinetobacter spp.* and *Klebsiella spp.* reported 48.27% and 20.68% respectively which is higher than similar studies showing 22% Salamat et al 2010, 20.7% by Machanda et al 2003 AmpC producing GNB. The incidence of carbapenemase producing GNB in this study is 35.71% in which 33.34% for *Klebsiella spp.* and 26.66% for *Acinetobacter spp.* which is similar to study by Mushi et al 2013 (35.24%).

Various birth related parameters such as prematurity, low birth weight, neonatal asphyxia etc puts the infant at higher risk for the development of sepsis. We noted 84.18% neonates with positive blood culture had gestational period ≤ 36 weeks (p value = 0.01), this is due to immature immune system of the preterm babies and moreover they lack adequate humoral immunity as 50% of maternal IgG is transported during later stage of pregnancy, Gonzalez et al 2015, Malek et al 2003 and Rajana et al 2018. In this study, 79.09% neonates born with positive blood culture were of low birth weight (≤2.5kgs), similar results has been reported by Iyer et al 2017, Rajana et al 2018, Agrawal et al 2018 (86%) and Verma et al (60.94%) 2015. 91.52% babies born out of vaginal delivery were associated with positive blood culture, which means neonates are at a higher risk of...
contracting sepsis because the endogenous bacteria present on maternal vaginal tract can cause disease in the neonates, Ayengar et al 1991, [36] Al-Adnani et al 2007 [37] and Grace et al 2013. [38]

The various predisposing factors responsible for sepsis were RDS, GI symptoms (loose stools, abdominal distension, vomiting etc), lethargy and poor feeding. Similar predisposing factors have also been noted by Karthikeyan et al 2001, [18] Samaga et al 2016, [23] and Chandrakala et al 2017. [34]

CONCLUSIONS

Neonatal Septicaemia is most frequent and severe condition which threatens survival of neonates during first few weeks of life. In this cohort study, the burden of sepsis was nearly similar in EOS and LOS; moreover, we observed that mode and place of delivery plays a significant role in contracting sepsis which indicates proper hand sanitization, maintaining cleanliness and monitoring maternal vaginal colonization during vaginal delivery is of utmost importance. With increase in rate of sepsis by XDR organisms antibiotic stewardship is absolutely paramount to ensure right choice of antibiotic. Key points to reduce the rates of acquiring nosocomial infections and development of bacterial resistance are adherence to infection-control programme, regular monitoring of antibiotic susceptibility and evaluation and encouragement of rational antibiotic use.

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