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Neonatal Infection-Focus on Bacteriological Spectrum and Drug Sensitivity in Newborn Unit of Tertiary Care Centre: A Retrospective Study

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Abstract

Context: Neonatal sepsis refers to systemic bacterial infection documented by positive blood culture in the first four weeks of life and is one of the four leading causes of neonatal mortality and morbidity in India. *Aims:* To identify the common bacterial pathogens associated with neonatal sepsis and their antibiotic susceptibility pattern. *Design:* This study was a retrospective study to identify the bacterial isolates in neonatal septicaemia and their antimicrobial susceptibility in a tertiary care hospital in KBNTGH Karnataka, from March 2017 to February 2018. *Methods:* Blood culture in newborns with clinical sign of septicaemia was retrospectively studied. Antimicrobial susceptibility testing was performed by Kirbuay -buer disc diffusion susceptibility method in accordance to clinical laboratory standard institute guidelines (CLSI). *Statistical Analysis:* Chi square test was used to check the test of proportion. *Results:* 112 cases of septicaemia could be confirmed by blood culture. Of these Gram negative was isolated in (63.3%) of cases and (36.6%) were of gram positive isolates. Kliebsiella was the predominant pathogen (45.5%) among the gram negative pathogens followed by S. Aureus (19.6%) and CONS (16.9%) were the predominant gram positive pathogen. Polymyxin and meropenem were most effective drugs against gram negative isolates were as gram positive organisms showed maximum sensitivity to vancomycin. *Conclusions:* This study highlights the growing resistance to commonly used antibiotics; also highlights the importance of kleibsiella principle organism responsible for neonatal sepsis in tertiary care settings.

Kaywords: Septicaemia; Neonate; Resistance; Antibiotics; Drugs.

Introduction

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections [1].

Neonatal septicemia remains significant but underestimated problem around the world and continues to be a problem in special care neonatal units [2]. There could be number of reasons for neonatal mortality and morbidity. The incidence is mainly higher in developing countries than developed countries [3].

The World Health Organization estimates that, there are about five million neonatal deaths a year, with approximately 98% occurring in developing countries. Despite advances in neonatal and paediatric health care, neonatal sepsis is a significant cause of neonatal morbidity and mortality [4].

The incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live births. The NNPD network comprising of 18 tertiary care neonatal units across India found sepsis to be one of the commonest causes of neonatal mortality contributing to 19% of all neonatal deaths [5].

Very little information exists on these infections and deaths due to sub optimal public health surveillance systems and lack of transportation to appropriate heath facilities where culture, other diagnostic tools, and antimicrobial susceptibility testing may be available.

While these measures are undertaken by national and international bodies there is widespread acknowledgement of a serious lack of information, which in turn limits our ability to design and implement appropriate evidence based intervention. At present, no data are available on timing of neonatal infection, types of infection (bacterial, viral, others), AMR and precise time of deaths of Indian neonates in the community setting. Due to the overlapping nature of presentation during the first days of life, multiple condition including prematurity, birth asphyxia, transient tachypnea, hypoglycaemia and other physiologic disturbances may be lumped into the "infection" category and treated with antibiotics as bacteremia. The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) global Network for Women's and Children Health. Research supported the first population – based, village level surveillance of infection conducted in the first two vulnerable months of life in Indian infants [6].

Material and Methods

Study Design and patient population

A retrospective study was conducted in NICU of Khaja banda nawaz institute of medical sciences, Kalaburagi, Karnataka. The study protocol was approved by hospital Clinical Research Ethics Committee of KBNTGH. Neonates 0-28 day with clinical sign and symptoms of sepsis at the time of admission at the time of admission or who develop sepsis during clinical stay were included in this study.

Through database searches, the information including risk factors and clinical symptoms, hematological parameters, pathogen features, major bacteria's resistant organism detection rate and antimicrobial resistant were reviewed from patient medical record on a standardized data collection form.

Blood culture were obtained from infants with clinical signs suggestive of sepsis. All blood samples were collected prior to initiation of antimicrobial therapy. Neonatal sepsis was defined as the growth of single potentially pathogenic organism from blood in patient with clinical and laboratory findings consistent with infection. Patient were divided into EOS (0-7 days) and LOS (8-28 days) groups.

Inclusion Criteria

At birth: All newborns

(i) born to mothers with febrile illness with evidence of bacterial infection within 2 weeks prior to delivery, or prolonged rupture of membrane (>18 hours), or foul smelling or meconium stained liquor, or single unclean or > 3 sterile vaginal examination(s) during labour

(ii) Having severe prematurity necessitating active resuscitation.

After birth: All neonates with temperature instability (hypothermia/fever), lethargy, refusal to feeds, abdominal distension, respiratory distress, jaundice, seizures, vomiting, and autonomic dysfunction etc.

Exclusion Criteria

Healthy term neonates having transient tachypnea of newborn, those with gross congenital anomalies and those undergone major surgical intervention prior to sending blood culture were excluded from the study.

Patient's evaluation

The study paediatrician evaluated infants at the hospital and provide in patient care after admission. The clinical evaluation included specific questions and physical examination on the signs of sepsis.

1. Clinical variables

- Temperature instability
- Heart rate180 beats/ min or 100 beats/min
- Respiratory rate > 60 breaths/min plus grunting or desaturations
- Lethargy /altered mental status
- Glucose intolerance (plasma glucose > 10 mmol/l)

- Feeding tolerance

2. Hemodynamic variables

- Blood pressure 2SD below normal for age
- Systolic pressure < 50 mmHg (new born day 1)
- Systolic pressure < 65 mmHg (infants 1 month)

3. Tissue perfusion variables

- Capillary refill > 3s

4. Inflammatory variables

- Leukocytosis (WBC count > 34000 x 10^9/l)
- Leukopenia (WBC count <5000 x 10^9/l)
- Immature neutrophils > 10%
- Immature: Total neutrophil ratio >0.2
- Thrombocytopenia <100000 x 10^9/l
- CRP > 10mg/l or 2SD above normal value

Interpretation

- Proven Sepsis: A positive blood culture in the presence of clinical signs and symptoms of infection. For CoNS two positive blood cultures or one positive blood culture plus a positive CRP.
- Probable Sepsis: Presence of signs and symptoms of infection and at least two abnormal laboratory results when blood culture is negative.
- Possible Sepsis: Presence of clinical signs and symptoms of infection plus raised CRP when blood culture is negative [7].

Bacterial culture and identification

All centres followed standardized protocols for biospecimen collection and processing. One millilitre of blood was inoculated aseptically into Bactec-Peds Plus bottles (Becton Dickinson, India) and transported immediately to the laboratory. Blood cultures were obtained for each infant immediately after study enrolment and prior to treatment. Blood samples were inoculated in Bactec Peds plus/ F culture media and processed in a Bactec 9050 detection system (Becton Dickinson). Culture bottles were inspected visually for turbidity and Gram stain – and plated (subcultured) on Blood-agar, MacConkey agar and Chocolate-agar with 1% Iso Vitale. Bactec bottles giving positive signals or turning positive on Gram stain, but not producing growth on aerobic culture, were subcultured on BHI-agar and incubated anaerobically in an Anaerobic Jar with a Gas Pak Plus gas generating envelope and an anaerobic indicator strip at 35-37 C for 48 hrs. All bacterial isolates appearing after incubation were Gram-stained and identified by use of API tests (aerobes) or API 20A test strips (BioMerieuxInc). Bactec bottles with no signal within 5 days were considered negative.

Early onset culture proven sepsis (EOS) was defined by a positive blood culture taken during days 0-3 of life and late onset culture proven sepsis (LOS) by a positive blood culture taken during days 4-60 of life. Coagulase negative Staphylococcus, Corynebacterium, Propionibacterium, Diptheroids, Micrococcus, and Bacillus grown on blood or CSF culture were considered contaminants and the culture was not counted as positive. Clinical sepsis included negative blood culture or respiratory symptoms (Pneumonia), when the infant was treated with antibiotics for 5 or more days or died within 5 days of enrolment.

Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed by Kirby – Bauer disc diffusion susceptibility method in accordance to Clinical Laboratory Standards Institute (CLSI) guidelines.

Statistical Analysis

The data was expressed in terms of frequency and percentage. Chi square test was used to check the test of proportion. The results were analyzed using the statistical package, SPSS.

Results

Study population

Of those screened identified by the study paediatrician to have clinical sepsis were enrolled. Sepsis was not suspected by the screening physician for a majority of the babies brought to the hospital for evaluation who were not enrolled.

Timing of enrolment

Study enrolment from birth through day 3 of life for 66% of infants, while 34% were enrolled between days 4-7. The timing of enrolment was similar for infants born at home and in hospital.

Median admission weight was 2.2. A larger proportion of infants enrolled between 0-3 were preterm (51.7%) compared to those enrolled later (4-7 day)

The demographic data of these 112 cases are summarized in Table 1,2 and Figure 1.

Infant clinical signs at enrolment

Fever was the most frequently reported sign followed by poor feeding/poor sucking tachypnea, decreased activity or lethargy and chest wall retraction. Of infants enrolled between birth and 3 days, 95% presented with two or more signs compared to 75 -80% of those enrolled at later ages. Poor feeding/poor sucking decreased activity/ lethargy. Table 3 reveals the percent of infants with tachypnea varied but was highest (26.7%) among those enrolled at 0-3 days of life, as were chest wall retraction. Jaundice and hypothermia were reported more frequently (23.2%) among infants enrolled between 0-3 days. Pustules were most frequently reported among those enrolled between 4- 7 days (44.6%).

Table 1: Neonate characteristics of the neonates

Neonates characteristics	
Parameters	**Values**
No of preterm	58
IUGR	40
Birth weight	2.2
Age (Days)	2.6

Table 2: Maternal characteristics of the neonates

Maternal data	
Parameters	**Values**
No of cases of PROM > 24 HR	65
No of cases with meconium stained liquor	20
Type of delivery	
Spontaneous vaginal delivery	34
Caesarian section	38
Instrumental delivery	25
Assisted breech delivery	15

Table 3: Infant clinical signs noted by study physician at enrollment/first episode admission by age at enrollment

Clinical signs	0-3 days	4-7 days
Fever	14 (12.5)	31 (27.6)
Abdominal distension	13 (11.6)	11 (9.8)
Seizures	13 (11.6)	6 (5.3)
Tachypnea	30 (26.7)	11 (9.8)
Shock	11 (9.8)	0
Hypothermia	30 (26.7)	8 (7.1)
Jaundice	26 (23.2)	22 (19.6)
Chest wall retractions	26 (23.2)	6 (5.3)
Sclerema	4 (3.5)	6 (5.3)
Pustules	2 (1.7)	50 (44.6)

Pathogen

During the study period, there were a total of 112 cases clinically and culture positive cases of neonatal sepsis. Of these 112 neonates, 23 (20.5%) were inborn, while the other 89 (79.4%) were outborn. 87 (77.6%) were early onset, while the other 25 (22.3%) were late onset sepis.

Gram negative organisms were isolated from 71 (63.3%) out of 112 culture proven cases. Klebsiella pneumoniae was the most common causative agent of neonatal sepsis. CONS and Staphylococcus aureus were the most common gram positive agent. The etiological agents of early onset and late onset neonatal sepsis are shown in table 4. The etiological agents of sepsis in inborn and outborn neonates are compared in table 5.

Antibiotic susceptibility

Table 6 and 7, show comparative sensitivity percentage of gram positive and gram negative isolates to commonly use antimicrobial drugs.

Table 4: Etiological agents of early-onset and late-onset neonatal sepsis

Organism	Early onset	Late onset	P value
Klebsiella pneumoniae	42 (48.2)	9 (36)	0.038
Escherichia coli	16 (18.3)	2 (8)	0.017
Staphylococcus aureus	13 (14.9)	9 (36)	0.53
CONS	16 (18.3)	3 (12)	0.04
Pseudomonas	0	1 (4)	1
Proteus	0	1 (4)	1
Total	87	25	

Table 5: Etiological agents of sepsis in inborn and outborn neonates

Organism	Inborn	Outborn
Klebsiella pneumoniae	12 (52.1)	39 (43.8)
Escherichia coli	2 (8.6)	16 (17.9)
Staphylococcus aureus	4 (17.3)	18 (20.2)
CONS	4 (17.3)	15 (16.8)
Pseudomonas	0	1(1.2)
Proteus	1 (4.3)	0
Total	23	89

Table 6: Comparative sensitivity percentage of gram positive bacteria to different antimicrobial agent

Antibiotics	Staph Aureus	CONS
Amoxicillin	13 (59)	4 (21)
Gentamicin	19 (86.3)	16 (84.2)
Ceftriaxone	5 (22)	6 (31.5)
Cefuroxime	7 (31.8)	8 (42.1)
Ciprofloxacin	10 (45.4)	15 (78.9)
Pipercillin	17 (77.2)	12 (63.1)
Tigecyclin	19 (86.3)	16 (84.2)
Meropenem	13 (59)	10(52.6)
Vancomycin	19 (95.4)	19(100)

Table 7: Comparative sensitivity percentage of gram negative bacteria to different antimicrobial agent

Antibiotics	Klebsiellapneumoniae	Escherichia coli	Pseudomonas	Proteus
Gentamicin	34 (66.6)	9 (50)	0	0
Cefotaxim	38 (74.5)	10 (55.5)	0	0
Ceftazidine	38 (74.5)	12 (66.6)	1 (100)	1 (100)
Cefaperazonesulbactum	44 (86.2)	18 (100)	1 (100)	1 (100)
Ciprofloxacin	41 (80.3)	18 (100)	1 (100)	1 (100)
Meropenem	51 (100)	18 (100)	1 (100)	1 (100)
Polymixin	50 (98)	15 (83.3)	1 (100)	1 (100)

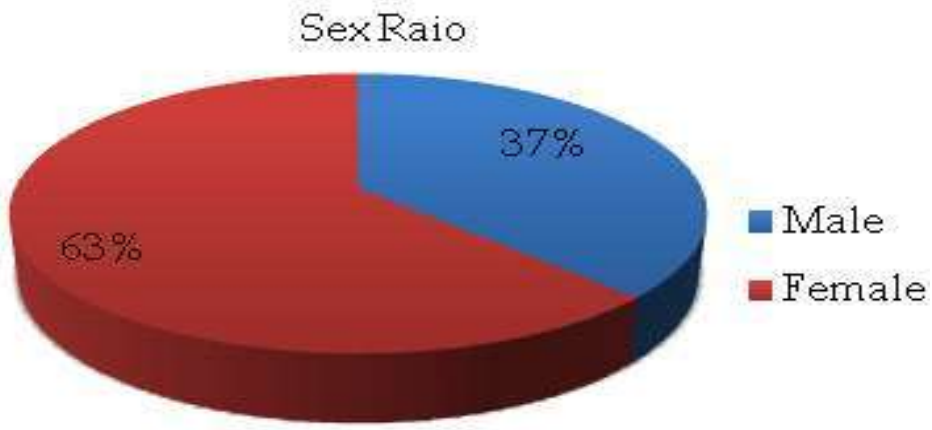

Fig. 1: Sex ratio of neonates

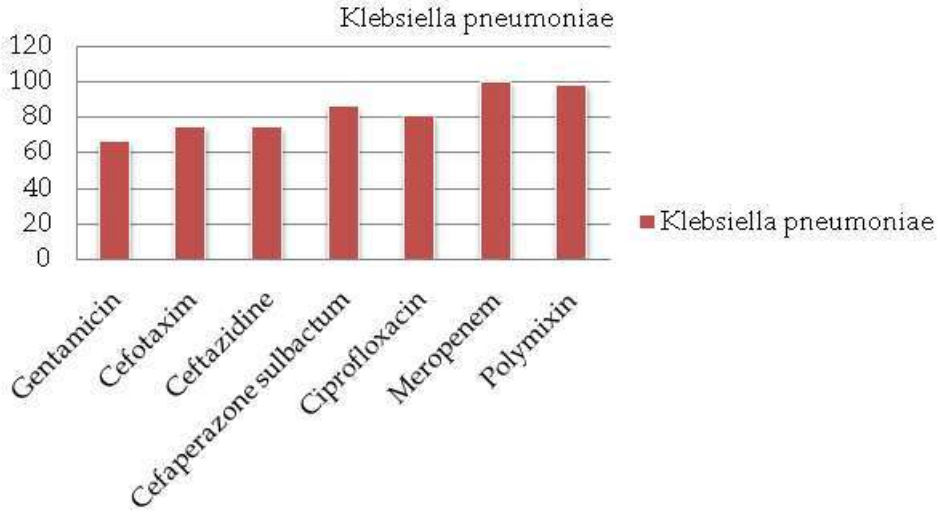

Fig. 2: shows the sensitivity pattern of most common gram negative isolates klebsiella pneumoniae.

Among sensitivity pattern in gram negative isolates (Figure 2) there was a high degree of sensitivity to drugs like Meropenem and Polymixin and among gram positive to Vancomycin.

Discussion

Neonatal sepsis is a clinical syndrome characterized bysigns and symptoms of infection with or without accompanying bacteremia in the first month of life [8]. Although bacteria are the most common agents implicated in neonatal sepsis, neonatal sepsis syndrome can also be caused byorganisms other than bacteria like adenovirus, enterovirus, coxsackievirus, rubellavirus, Toxoplasma species andCandida species [9]. Therefore, only a proportion of theblood cultures

from cases with clinical sepsis will bepositive for pathogenic organisms.

Sneha S et al. conducted a study of 200 cases, 92 [46%} were blood culture positive which included 49 [53.26%] males, 62 [67.39%] were preterm and 53 [57.60%] were very low birth weight, 68 [73.91%] were spontaneous vaginal delivery and 59 [64.13%] were hospital out born neonates. Early onset septicaemia was more common, seen in 66.33% of cases than late onset septicaemia in 33.69% cases [10].

The etiological agents of neonatal sepsis vary between developed and developing countries. Klebsiella pneumonia and other Gram-negative organisms were the common causes of sepsis in the present study as well other studies from India and Nigeria. However, in the developed countries Group B Streptococcus and coagulase negative staphylococci (CONS) are the predominant causes of sepsis [11].

Klebsiella pneumonia was the commonest agent causing both earlyonsetand late-onset sepsis. The authors also documented an outbreak of ESBL producing Klebsiella pneumoniae.

Klebsiella pneumonae was the most common organism isolated in our study. This finding are consistent with the studies carried out by Dr. Desai KJ et al., Anwer BK et al., Nazeer S et al. where *Klebsiella pneumonae* was the most commonly isolated microorganism. *Pseudomonas aeruginosa* was the most common organism by (36%) Movahedian AH et al study and *E. coli* was common organism for Moncef et al. study. In our study *Pseudomanas* is 1.1% and *E. Coli* is 16 % cases [12].

Antibiotic resistance is today a global problem Reports of multi-resistant bacteria causing neonatal sepsis in developing countries are increasing. The wide availability of the over the counter antibiotics and the in appropriate use of broad - spectrum antibiotics in the community may explain this situation. It is difficult to compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is extremely variable [13].

The present study shows high degree of resistance to first time of antibiotics, gram negative *Klebsiella pneumonae* showed resistance to Ampicillin, Gentamicin, and cefotaxime.

Similar observations were by Nazeer S et al. that ampicillin (76.82), ceftazedime (61.6%), amoxiclavulnic acid (57.7%), ceftrioxone (42.3%) [14]. Movahedian et al. in the study reported the observation that *Klebsiella Pneumonae* showed a high degree of resistance to commonly used antibiotics (ampicillin), as well as third generation cephalosporin. Similar observations were in Mahmood A et al. study that resistance to gentamycin was as high as 90.4% in cases with *Klebsiella Pneumonae* septicaemia [15].

This increase in resistance in aminoglycosides and third generation cephalosporius are alarming as they are being used in our set up as empirical therapy. Imipenem, levofloxacin, piperacillin+tazobactum are having higher sensitivity for *Klebsiella pneumonae* and timely use of these antibiotics will definitely reduce mortality and morbidity in NICU. Hence, the changing antibiotic susceptibilities need continuous monitoring and reevaluation for putting forth the guidelines for empirical treatment in NICU [12].

In many of these deaths, we cannot rule out early onset sepsis. Infection vertically transmitted from the mother or acquired during delivery and perinatally in the home environment or in the hospitals can theoretically cause such early sepsis in the newborns. However, any such infection would typically take about 48 hours to manifest and will not produce frank sepsis and death in less than 24 hours unless there is history of extremely prolonged rupture of membranes. Only 5% of our babies were delivered more than 24 hours after rupture of membranes. Although many non-sepsis related indications were recorded in the system pointing towards birth asphyxia (e.g. baby did not cry at birth), there is a need to improve case detection in the first 12–24 hours of neonatal life. As we strive to get answers to these fundamental questions, many other health system related changes have taken place in India. For example, introduction of a new cadre of village level lady workers called ASHA (Accredited Self Help Activist), and promotion of hospital delivery with financial incentive to the mother has resulted in a sharp drop in home deliveries [6].

Conclusions

This study highlights the growing resistance to commonly used antibiotics; also highlights the importance of kleibsiella principle organism responsible for neonatal sepsis in tertiary care settings.

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