Original Research Article

Etiological divergence and antimicrobial susceptibility pattern among culture proven cases of neonatal sepsis from a tertiary care teaching institute in a konkan region of Maharashtra

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1. Introduction

Mortality and morbidity in children’s is present day concern of world, that’s why reduction of child mortality is become a part of the Millennium Development Goal formulated by United Nations.1 As per key facts of World health organization 2.5 million children died in the first month of life in 2017 approximately 7000 newborn deaths every day with about 1 million dying on the first day and close to 1 million dying within the next 6 days. On the basis of these statistics it is clear that a child’s risk of dying is highest in during the neonatal period that is the first 28 days of life.2

As per data of world health organization 35% of all neonatal deaths in 2017 were due to complications
associated with preterm birth; 24% of deaths were associated with intrapartum events, such as birth asphyxia; 14% of deaths were due to sepsis or meningitis; and 11% were associated with congenital anomalies.\(^3,4\) Despite the fact that the vast majority of these deaths in neonates are preventable there is slow decline in neonatal mortality rates all over the world since 1990. Most of the deaths due to neonatal sepsis are preventable by proper maternal care during pregnancy & care of neonates during delivery and immediately after birth.

Neonatal sepsis is a clinical presentation of underlying systemic infection during the first 28 days of life, usually classified on basis of the age at onset of the sepsis episode as early-onset (<48–72 h) and late-onset sepsis (>48–72 h).\(^5\)

No isolation of organisms from clinical samples received from cases neonatal sepsis doesn’t exclude the diagnosis of sepsis, that’s why we can label cases of sepsis depending on culture outcome as culture negative sepsis and culture positive sepsis.\(^6\)

Early onset neonatal sepsis (EOS), defined by the Centre for Disease Control and prevention (CDC) as blood or cerebrospinal fluid culture proven infection occurring in the first three days of life, is typically caused by organisms transmitted vertically from the mother to the infant before or at the time of birth.\(^7\)

Late-onset sepsis (LOS), defined as sepsis onset after 72h of life & the late-onset sepsis (LOS) is considered to originate from the community or from health-care setting. The predisposing factors for LOS mainly immaturity, low birth weight, use of prolong parental nutrition & use of invasive procedures like intubation, central line catheterization etc.

Neonatal sepsis is predominant cause of neonatal mortality and morbidity in world & greater number of neonatal sepsis cases occurs in developing countries.\(^8\)

Neonatal sepsis is caused by a variety of Gram positive as well as Gram negative bacteria, and sometimes yeasts.\(^9\)

The knowledge of bacteriological profile and its antibiotic sensitivity patterns is of immense help in saving lives of neonates with septicaemia.\(^10\) As per different studies carried out in India in different health institutes where Klebsiella species, Escherichia coli, and Staphylococcus aureus are the most common causes of infection.\(^11-13\)

Microorganisms responsible for Neonatal septicaemia have started to develop resistance to antibiotics; it results in extreme difficulty to treat cases of neonatal septicaemia. That’s why this study was under taken to evaluate bacterial profile of microorganism associated with neonatal septicaemia in Neonatal Intensive care units and analyze antimicrobial resistance pattern among isolates.

2. Materials and Methods

This is a retrospective observational study was carried out in a tertiary care rural medical college & hospital, Dervan in Konkan region of Maharashtra. In this study all the records of blood culture samples taken from suspected neonatal sepsis cases in neonatal intensive care unit(NICU) & which are received & processed in Microbiology department are analyzed, during January 2017 to August 2019.

2.1. Ethical consideration

Approval from Institutional Ethical Committee was taken & study was carried out after clearance from Institutional ethical committee.

Required Data collected from Medical records & reports were obtained from records sections of Microbiology and NICU

2.2. Inclusion criteria

All the blood samples received from NICU of clinically suspected cases of neonatal sepsis were included in study and analyzed.

2.3. Exclusion criteria

The Records of blood samples with incomplete history, age more than 28 days and repots showing growth of probable contaminants like aerobic spore bearing bacilli were excluded. All the reports fulfilling the above eligibility criteria were studied for achieving above aims and objective of this study. In these cases uniform processing pattern was followed as per departmental standard operating procedures for blood sample processing.

All blood culture samples received from NICU were processed as per standard norms with automated Bactec 9050 blood culture system and positive blood culture samples were cultured on Blood Agar, Mac- Conkey Agar & Chocolate agar respectively.

These plates were incubated overnight at appropriate temperature (35°C to 37°C) aerobically & presence of growth observed on next day, pure growth without any contaminations processed as per colony morphology and gram nature, identification was performed by Vitek 2 (bioMérieux, Marcy-l’Etoile, France) with help of manufacturers standard operating procedures(SOP) using Identification cards (ID cards) and Antimicrobial susceptibility cards. Whenever required the manual antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method and conventional identification methods based on bio-chemical reactions were also used. Selection of antimicrobial agents as the Clinical & Laboratory standards Institute guidelines based departmental list of antimicrobial agents used for antimicrobial susceptibility testing.\(^14\)

Antimicrobial susceptibility against following antimicrobial agents evaluated in study.
**Table 1:**

| Antimicrobial agents evaluated against Gram Negative organism | Antimicrobial agents evaluated against Gram Positive organism |
|--------------------------------------------------------------|-------------------------------------------------------------|
| All other gram negative bacilli                             | Pseudomonas species                                          |
| Ampicillin                                                   | Amoxicillin clavunic acid                                   |
| Amikacin                                                     | Azithromycin                                                |
| Cefoperazone sulbactum                                      | Cefoxitin                                                   |
| Cefoperazone                                                 | Ciprofloxacin                                               |
| Gentamycin                                                   | Calirithromycin                                             |
| Piperacillin tazobactum                                     | Clindamycin                                                 |
| Cefazidime                                                   | Trimethoprim/Sulphamethoxazole                              |
| Cefazidime clavunic acid                                    | Imipenem                                                    |
| Trimethoprim/Sulphamethoxazole                              | Linezolid                                                   |
| Gentamycin                                                   | Penicillin                                                  |
| Ciprofloxacin                                                | Vancomycin                                                  |
| Colistin                                                     | Meropenem                                                   |
| Tigicycline                                                  | Meropenem                                                   |

**Table 2:** Different variables observed in study

| Variables | Number |
|-----------|--------|
| Total blood culture sample received from NICU | 400    |
| Microorganism isolated (culture positive sepsis) | 98(24.50%) |
| Gram negative organisms isolated | 77(78.57%) |
| Gram Positive Organisms isolated | 13(13.26%) |
| Fugal isolates | 8(8.16%) |
| Mortality among culture positive cases | 25(25.51%) |
| Pre Term babies amongst culture positive cases | 54(55.10%) |
| low birth weight babies (less than 2.5kg birth weight) | 52(53.06%) |
| Birth Asphyxia | 31(31.63%) |
| Sex predominance | 57(58.16% male) |
|                          | 41(41.83% female) |

**Table 3:** Different etiological agent isolated in Blood culture samples of Suspected Neonatal Sepsis received from NICU

| Organism isolated | No. of isolates |
|-------------------|-----------------|
| Pantoea Species   | 25              |
| Klebsiella Species | 24             |
| Pseudomonas species | 15             |
| Candida species   | 8               |
| Staphylococcus aureus | 7             |
| Escherichia coli  | 7               |
| Coagulase negative staphylococcus(CONS) | 5 |
| Acinetobacter species | 2 |
| Citrobacter species | 2             |
| Enterobacter aerogens | 1             |
| Proteus species   | 1               |
| Streptococcus species | 1            |
| **Total**         | **98**          |
Table 4: Brief analysis of different studies of neonatal sepsis carried out in different part of India

| Place                                      | Duration of study & Type of study | Positive blood cultures | Common organisms                                                                 |
|--------------------------------------------|----------------------------------|-------------------------|----------------------------------------------------------------------------------|
| Stanley medical college, in North Chennai region, India | July 1, 2015 to December 31, 2015 Retrospective study | 10% (10/120) Clinically suspected cases are more. In culture positive 50% gram negatives sepsis | Coagulase negative staphylococcus (n=3) Klebsiella pneumonia (n=2) Non candida albican (n=2), Acinetobacter (n=1), E.coli (n=1), Pseudomonas aeruginosa (n=1) and Stap aureus (n=1) |
| School of Tropical Medicine, Kolkata       | 1 year Institution based observational, epidemiological study | 55.43% (51/92) Gram negatives sepsis common but fungal sepsis also contribute 50% cases | Klebsiella pneumoniae (48.18%), E..coli (14.81%), Staph.epidermidis (18.51%) fungal pathogens for 47.5% cases |
| Microbiology Department of Pravara Rural Hospital, Loni Maharashtra | Two years A retrospective observational Study | 23.31% (259/1111) Gram Negative sepsis (59.32%) | Klebsiella species (28.81%), Coagulase negative staphylococcus CONS (17.96%), Pseudomonas species (15.93%) and Stapheureus (15.25%) |
| Jawaharlal Institute of Post graduate, Medical Education and Research (JIPMER),Puducherry | August 2004 to July2006 A prospective observational cohort study | 41.6% (50 / 120) 70% cases belong to gram negatives sepsis | Klebsiella pneumoniae(66%), Coagulase negative staphylococccus (CONS) (12%) S. aureus others E.coli , Enterobacter , Streptococcus |
| Shri B M Patil Medical College, Bijapur.   | January 2008 and December 2010 A prospective study | 19.2% (131/683) Gram&#8209;bacilli (73/131, 55.7%) and Gram&#8209;positive cocci (58/131, 44.3%). | Klebsiella Species (30.5%),Coagulase negative staphylococci (CONS)(27.5%) |
| PGIMS, Rohtak, Haryana                    | July 2010 to September 2013 A retrospective review | 11.62% (336 /28,927) Gram negative organisms were isolated in 81.18% cases whereas 18.82% cases were with gram positive organisms. | Pseudomonas aeruginosa (43.82%),E.coli (23.03%), Acinetobacter(9.55%) in early onset sepsis, pseudomonas aeruginosa (51.35%), Acinetobacter (21.15%), S.aureus (17.41%) in late onset sepsis |

2.4. Statistical analysis

Data was tabulated and analyzed by using the Microsoft excel

3. Results and Observations

Out of 400 blood culture reports examined from 400 suspected neonatal sepsis cases, 98(24.50%) blood culture specimens showed pure growth of organisms. All of these neonates showed symptoms related to Systemic inflammatory response syndrome (SIRS) & some of them had risk to develop neonatal sepsis as per clinical records. Among these culture positive cases 57 were male and 41 are female newborns. Overall, 55.10 % of cases of neonatal sepsis were seen in preterm neonates, 53.06% cases seen in babies with low birth weight & birth asphyxia in 31.63% newborns (Table 2)

Among the culture positive cases the birth weight ranged from 820 grams to 3800 grams with a mean of 2089grams. Maximum no of the patients belonged to the 1500 to 2499 g rams group (Figure 1).

Among isolated microorganisms from blood culture samples, 77(78.57%) isolates were gram negative organisms (Table 2). The most common etiological agent in culture proven cases of neonatal sepsis was Pantoea Species (25 isolates) followed by Klebsiella species (24 isolates). Table 3 shows the distribution of different etiological agents of neonatal sepsis isolated from clinically suspected cases & those having risk factors to develop neonatal sepsis.

There were year wise variations in appearance of different etiological agents associated with neonatal sepsis yearly (Figures 2 and 3). In 2017 Klebsiella species (n=11), Pseudomonas species (n=5) & Candida species (n=7) were most common isolates in blood culture received.
But 2018 trends of appearance and association of these organisms changed dramatically. *Pantoea species* (n=17) emerged as leading cause of neonatal sepsis & *Klebsiella species* (n=13), *Pseudomonas species* (n=10) & *Escherichia coli* (n=5) were other common organisms associated with neonatal sepsis. While in 2019 it was found that *Pantoea species* (n=8) still remain on top and it’s was responsible for near about 80% culture proven neonatal sepsis cases in this year.

In our study when antimicrobial resistance pattern was analyzed in gram negative isolates, it was found that against many routinely used antimicrobial agent’s like ceftazidime (74%), ceftazidime clavunatic acid (64%), cefoperazone (58%), piperacillin tazobactum (52%), ampicillin (48%) & cefoperazone sulbactum (47%), gram negative micro-organism started showing marked resistance. (Figure 4)

The analysis of resistogram of pseudomonas species was showing maximum resistance to ceftazidime (100%), cefoperazone (87%), and ticarcillin clavunatic acid (80%) ceftriaxone (60%) & sensitivity in decreasing order to these antimicrobial agents like polymyxin-B (100%), gentamycin & amikacin (73%) meropenem (73%), piperacillin tazobactum (60%). (Figure 5)

The resistance pattern amongst antimicrobial agents used against gram positive isolates was penicillin (92%) cefoxitin (77%), azithromycin (69%) & cilmithromycin (69%) in decreasing order of resistance. (Chart No.6)

**Fig. 1:** Percentage Distribution of birth weight in culture positive neonatal sepsis cases

**Fig. 2:** Year wise trends of culture proven cases of neonatal sepsis in NICU

**Fig. 3:** Year wise Trends in Blood Culture Isolates among Culture Proven Neonatal Sepsis Cases in NICU

**Fig. 4:** Resistance pattern of Gram negative blood culture isolates (excluding *Pseudomonas species*) in NICU

4. **Discussion**

Neonatal sepsis is present day concern of developing world & major obstacle in their efforts to achieving different health goals put forth by world health organization. Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteraemia in the first month of life. Even tough bacteria’s are most common etiological agents behind neonatal sepsis cases; other organisms like Viruses and...
fungi are also responsible for significant proportion of neonatal septicemia. due to this reason not all blood culture sample received from clinically suspected cases will turn positive for pathogenic organisms. In spite of this timing of collection of blood sample also play important role; blood culture should be send before starting empirical antimicrobial therapy. 

So many studies put forth concept of clinical sepsis, When in the infant clinical manifestations & signs of infection present but blood and other sterile site cultures are negative, such cases are considered to have “clinical ” sepsis or culture negative sepsis. 

For adequate and effective management of neonatal sepsis knowledge of profile of causative organism and their sensitivity pattern plays a significant role. The emergence of resistant bacteria in NICU settings is one of the important reasons behind failure in the treatment of neonatal septicemia and increase neonatal mortality. 

In this study, out of 400 blood specimens examined from 400 clinically suspected cases of neonatal sepsis, (24.50%) blood culture specimens showed pure growth of organism’s indicating Culture proven cases of neonatal sepsis. Thus prevalence of culture positive neonatal sepsis in NICU was 24.50%. This culture positivity fall between the ranges from 10% to 60%, observed in different studies conducted by various authors. (Table 4).

In Our study 78.57 % isolates were gram negative organisms this is similar to other studies carried out in different part of India. In present study 55.10% of neonatal sepsis cases were observed in the preterm neonates & overall 25.51%(25 death out of 98 cases) mortality seen in culture proven cases of neonatal sepsis. In present study mortality was less compare to another study (46.7% in 2014) carried out in other part of India. 

After analyzing birth weight of culture proven cases of neonatal sepsis, it was found that near about 53% belong to Low birth weight and 31% in very low birth weight group while in 2 cases birth weight was less than 1000gram. This suggest association low birth weight with neonatal sepsis, which was also observed other studies carried out by Jajoo M et al. & Seth Riti J et al.

WHO has defined low birth weight neonate as any neonate weighing less than 2500 g at birth. The incidence of low birth weight neonates in India is 16% to 30% taking 2500g. LBW is associated with immaturity of vital organs and lack of immunological response. This makes them to susceptible to infections. This study was carried out to find out the trends and transient changes in association of different microorganism with culture proven cases of neonatal sepsis it was found that association of organism with septicaemia varies yearly i.e in 2017 Kelbsella species & pseudomonas species are most common causes of neonatal sepsis in our NICU. This association was similar to retrospective hospital based studies carried out in India (Table 4).

While during 2018 and 2019 Pantoea species emerged as leading cause of neonatal sepsis in culture positive cases. The Pantoea species is an environmental Gram-negative bacterium, motile organism. Pantoea is separate genus belong to the Enterobacteriaceae family containing strains which was previously known as Enterobacter agglomerans,Erwinia herbicola & Erwinia milletiae. One species pantoea agglomerans associated with out breaks of septicaemia in North America. There were only few cases reports of Neonatal sepsis due to Pantoea species in India. Pantoea species is an opportunistic pathogen not normally associated with neonatal infection 

Paggglomerans is reported to cause osteomyelitis, septic arthritis; urinary tract infection, blood infections, and abscess like infections in children and infants Infections caused by pantoea species have been reported in samples obtained from environmental samples like cotton swabs , intravenous catheters etc. Though spontaneous infection from pantoea species is rare, In our health set up as instruction of hospital infection control committee, different environmental samples like Intravenous catheters,
intravenous drugs and fluids, aero-humidifiers, ventilator masks, disinfectant solutions, sinks & door handles in NICU with help of infection control nurse were obtained and screened for growth of *Pantoea species*, with all of the environmental samples being negative. Synonymous association neonatal sepsis & *pantoea* species without known exogenous source was reported in a teaching hospital from Sri Lanka.\(^{26}\)

In present study, we found that most of the gram negative isolates showed resistance against third generation cephalosporins like ceftazidime, ceftazidime calvunic acid, cefoperazone & cefoperazone sulbactum they also demonstrate resistance against commonly used b road spectrum antibiotics like piperacillin tazobactum & ampicillin. But most of the gram isolates were sensitive to colistin & tigecycline. Similar type of multi-drug resistance in culture proven cases of neonatal sepsis was observed in study conducted by Bandyopadhyay T et al.\(^{27}\)

In our study, analysis of resistogram of pseudomonas species was showing resistance in decreasing order to trimethoprim/sulphamethoxazole (100%), cefoperazone (87%), ticarcillin clavunic acid (80%) ceftriaxone (60%) & all isolates showed 100% sensitivity to polymyxin B.

The resistance pattern amongst antimicrobial agents used against gram positive isolates was penicillin (92%) cefoxitin (77%), azithromycin (69%) & calirithromycin (69%) in decreasing order of resistance. The resistance pattern in isolates of this study is similar to observations of one study carried out in South East Asia. That study conclude that ‘ Antimicrobial resistance has worsened in recent days, result of this most antibiotics became obsolete. & microorganisms become resistance to even “reserve” antibiotics. About 50-70% of the common Gram negative isolates are now multidrug resistant.\(^{28}\)

### 5. Conclusion

There is not much published literature on neonatal sepsis available for the Konkan region of Maharashtra, western coastline of India. As our study was carried out in tertiary care Rural Hospital & medical college at Dervan, Ratnagiri, and western coastline of India which is only single charitable teaching institute & hospital serving in such remote, hilly area. This study was little efforts towards finding aetiology of neonatal sepsis & divergence in aetiology of neonatal sepsis in this remote & hilly area. This study showed that Gram-negative organisms (*Pantoea species*, *Klebsiella species*, and *Pseudomonas species*), Candida species and *S. Aureus* are the leading cause of neonatal sepsis and most of them are resistant to multiple antibiotics. The *Pantoea species* are emerged as leading causes of neonatal sepsis in recent years since 2018. Emergence of such newer microorganism like *Pantoea species* may be due to underlying risk factor present in new born like low birth weight, Birth asphyxia & Preterm delivery. These risk factors are responsible for reduced immune response against invading opportunistic pathogens like *Pantoea species*. Most of the cases of neonatal sepsis due to *Pantoea* species are seen in referred cases from surrounding different health care facilities. We are not able to find source of infection in our neonatal intensive care unit even after proper efforts taken to find out localised source as Infection control policy of our institute. Therefore we suggest continuous monitoring & surveillance to find out transient changes in appearance of organisms associated with neonatal sepsis and their sensitivity in surrounding health care setups.

A low susceptibility observed in this study to commonly used antibiotics like ampicillin,penicillin, cefotaxime, ceftazidime calvunic acid, cefoperazone, cefoperazone sulbactum is a matter of concern. As tertiary care centre in remote & hilly area of konkan most of the cases were referred from general practitioners, and other health setups. So we also advise that information to be provided to all general practitioners, and other government and non government health facilities regarding dangers of indiscriminate use of antibiotics.

This study helps us to modify our antimiicrobial policy in NICU and help us to use appropriate antimicrobial agents as per changing trends in susceptibility pattern among isolates.

### 6. Source of Funding

None.

### 7. Conflict of Interest

None.

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