



## 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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## ABSTRACT

**Introduction:** Neonatal mortality is one of the major obstacle to achieve present days health goals of world health organization & neonatal sepsis is important preventable causes behind neonatal mortality all over the world. Analysis of etiology of neonatal sepsis and antimicrobial susceptibility pattern among isolates helps in implementation of antimicrobial policy in NICU for rational empirical antibiotics.

**Aim and Objectives:** This study was carried out to analyze etiology, the change in trends in bacteriological agents of neonatal sepsis & also studying antimicrobial susceptibility pattern in these different isolates during study period.

**Materials and Methods:** This is a retrospective observational study. In this study all the records of blood culture samples taken from suspected neonatal sepsis cases in NICU & which are received & processed in Microbiology department are analyzed, during January 2017 to August 2019.

**Results:** Total 400 blood culture reports were analyzed from 400 suspected neonatal sepsis cases, out of that 98 were pure growth indicating 24.50% culture positivity. In these cases of neonatal sepsis 57 were male & 41 were female. These newborn with sepsis had risk factors like preterm neonates (55.10%) low birth weight (53.06%) & Birth asphyxia (31.63%). Though there was Year wise variation observed in etiological agents of neonatal sepsis Overall 77(78.57%) isolates were gram negative organism & The most common etiological agent of neonatal sepsis was Pantoea Species (25 isolates) followed by Klebsiella species (24 isolates). It was observed that against antimicrobial agent's like ceftazidime, ceftazidime clavonic acid, cefoperazone, piperacillin tazobactam, ampicillin & cefoperazone sulbactam gram negative isolates showed marked resistance. While gram positive isolates showed resistance towards penicillin, ceftaxitin, azithromycin & calithromycin. High Mortality 25.51% was seen in culture proven cases of neonatal sepsis.

**Conclusion:** We observed year wise variations in etiology of neonatal sepsis during study duration which point out need of continuous monitoring and surveillance to prevent morbidity and mortality of neonatal sepsis in konkan region.

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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.<sup>1</sup> 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.<sup>2</sup>

As per data of world health organization 35% of all neonatal deaths in 2017 were due to complications

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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.<sup>3,4</sup> 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).<sup>5</sup> 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.<sup>6</sup>

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.<sup>7</sup>

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.<sup>8</sup> Neonatal sepsis is caused by a variety of Gram positive as well as Gram negative bacteria, and sometimes yeasts.<sup>9</sup> The knowledge of bacteriological profile and its antibiotic sensitivity patterns is of immense help in saving lives of neonates with septicaemia.<sup>10</sup> 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.<sup>11–13</sup>

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 reports 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.<sup>14</sup>

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	Amoxicillin clavunic acid
Ampicillin	Amikacin	Azithromycin
Amikacin	Cefoperazone	Ceferoxime
Cefoperazone sulbactam	Cefoperazone sulbactam	Cefoxitin
Cefoperazone	Cefepime	Ciprofloxacin
Gentamycin	Ceftazidime	Calirithromycin
Piperacillin tazobactam	Ceftazidime clavunic acid	Clindamycin
Ceftazidime	Ceftriaxone	Trimethoprim/Sulphamethoxazole
Ceftazidime clavunic acid	Ciprofloxacin	Imipenem
Trimethoprim/Sulphamethoxazole	Trimethoprim/Sulphamethoxazole	Linezolid
Netillin	Gentamycin	Penicillin
Ciprofloxacin	Piperacillin tazobactam	Vancomycin
Colistin	Polymyxin-B	
Tigicycline	Ticarcillin clavunic acid	
Meropenem	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
<i>Pantoea Species</i>	25
<i>Klebsiella Species</i>	24
<i>Pseudomonas species</i>	15
<i>Candida species</i>	8
<i>Staphylococcus aureus</i>	7
<i>Escherichia coli</i>	7
<i>Coagulase negative staphylococcus(CONS)</i>	5
<i>Acinetobacter species</i>	2
<i>Citerobacter species</i>	2
<i>Enterobacter aerogens</i>	1
<i>Proteus species</i>	1
<i>Streptococcus species</i>	1
<b>Total</b>	<b>98</b>

**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	Gram negative Vs gram positive sepsis	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), Acinetobactor (n=1), E.coli (n=1), Pseudomonas aeruginosa (n=1) and Staph aureus(n=1)	12
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	13
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 Staph.aureus (15.25%)	29
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	<i>Klebsiella pneumoniae</i> (66%), Coagulase negative staphylococcus (CONS)( 12%) S. aureus others E.coli , Enterobacter , Streptococcus	30
Shri B M Patil Medical College, Bijapur.	January 2008 and December 2010 A prospective study	19.2% (131/683 )	Gram&#8209;negative bacilli (73/131, 55.7%) and Gram&#8209;positive cocci (58/131, 44.3%).	<i>Klebsiella Species</i> (30.5%),Coagulase negative staphylococci (CONS)(27.5%)	31
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.	<i>Pseudomonas aeruginosa</i> (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	32

#### 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

from NICU.

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 clavunic 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 resisto-gram of pseudomonas species was showing maximum resistance to ceftazidime (100%), cefoperazone (87%), and ticarcillin clavunic acid (80%) ceftriaxone (60%) & sensitivity in decreasing order to these antimicrobial agents like polymyxib-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%) & calirithromycin (69%) in decreasing order of resistance. (Chart No.6)

Percentage Distribution of Birth weight in culture Positive Neonatal Sepsis cases

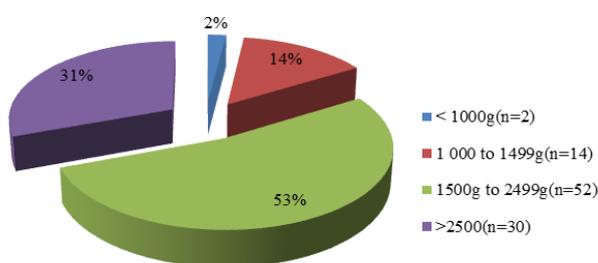


Fig. 1: Percentage Distribution of birth weight in neonatal sepsis cases

#### 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

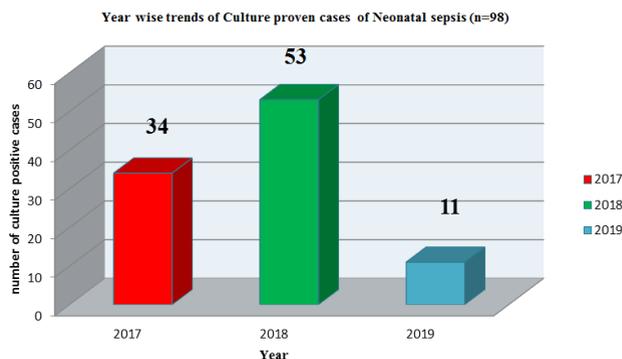


Fig. 2: Year wise trends of culture proven cases of neonatal sepsis in NICU

YEARWISE TRENDS IN BLOOD CULTURE ISOLATES AMONG CULTURE PROVEN NEONATAL SEPSIS CASES IN NICU

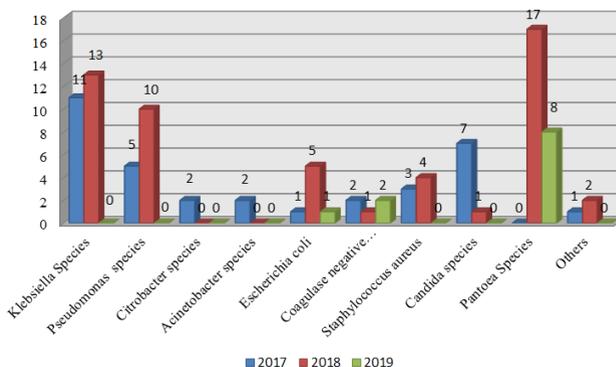


Fig. 3: Year wise Trends in Blood Culture Isolates among Culture Proven Neonatal Sepsis Cases in NICU

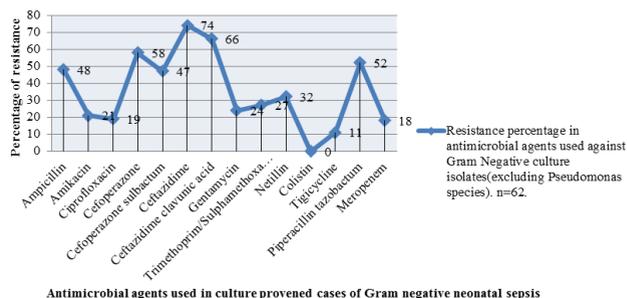
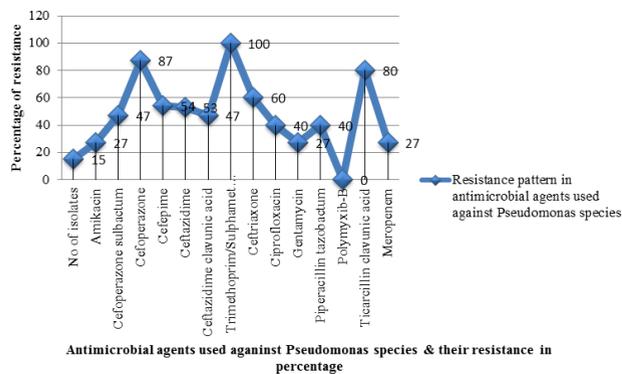
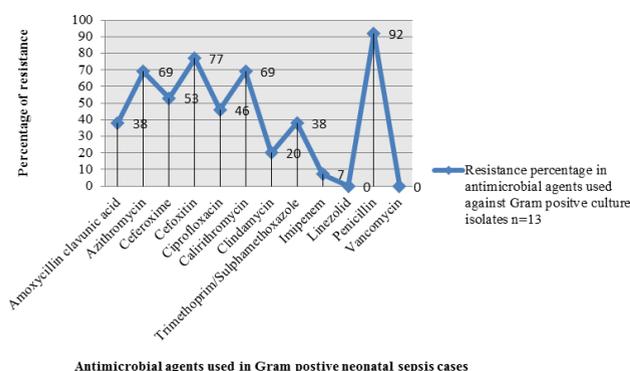


Fig. 4: Resistance pattern of Gram negative blood culture isolates (excluding Pseudomonas species) in NICU



**Fig. 5:** Resistance pattern in antimicrobial agents used against Pseudomonas species



**Fig. 6:** Resistogram of gram positive culture isolates

fungi are also responsible for significant proportion of neonatal septicaemia. 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.<sup>15</sup>

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.<sup>16</sup>

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 septicaemia and increase neonatal mortality.<sup>17</sup>

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.<sup>18,19</sup>

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.<sup>20,21</sup>

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 Kelbseilla 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.<sup>22</sup> There were only few cases reports of Neonatal sepsis due to Pantoea species in India.<sup>23,24</sup>

Pantoea species is an opportunistic pathogen not normally associated with neonatal infection

*P.agglomerans* 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.<sup>25</sup> 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.<sup>26</sup>

In present study, we found that most of the gram negative isolates showed resistance against third generation cephalosporins like ceftazidime, ceftazidime clavulanic acid, cefoperazone & cefoperazone sulbactam they also demonstrate resistance against commonly used broad spectrum antibiotics like piperacillin tazobactam & 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.<sup>27</sup>

In our study, analysis of resistogram of pseudomonas species was showing resistance in decreasing order to trimethoprim/sulphamethoxazole (100%), cefoperazone (87%), ticarcillin clavulanic 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%) ceftazidime (77%), azithromycin (69%) & clarithromycin (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 concluded 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.'<sup>28</sup>

## 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 clavulanic acid, cefoperazone, cefoperazone sulbactam 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 antimicrobial 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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### Author biography

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