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Original Research Article

Study of etiological profile, risk factors and immediate outcome of neonatal sepsis

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ABSTRACT

Background: In India, Gram negative bacteria such as CONS, *Klebsiella spp, Acinetobacter spp* and *E. coli* are the main pathogen. Due to the variation in prevalence of associated microorganism and resistant pattern, regional hospital based prospective study should be carried out to know the microorganism pattern of neonatal sepsis. Aim is to study the demographic profile of neonates with sepsis, to study maternal and neonatal risk factors for sepsis, to study pattern of causative microorganism.
Methods: The study was carried out among 226 neonates admitted in NICU at Dhiraj hospital and having sepsis. Patient's information was collected as per the prescribed proforma. Antibiotic sensitivity tests
(AST) were performed for all isolates recovered from infant cultures. Data was analysed with Epi info version 7.1.
Results: The ratio of extramural to intramural for neonatal sepsis was higher (1.9:1). Anaemia, leaking per vaginum, fetal distress, unable to cry immediate after birth, previous hospitalization were most common risk factors. Coagulase Negative Staphylococci, *S. aureus*, *Acinetobacter Baummanni*, *Klebsiella pneumonia* were commonly isolated organism. Gram positive pathogens were sensitive to vancomycin, tetracycline and gram-negative pathogens were sensitive to tigecycline and colistin.
Conclusion: Gram negative organism sepsis is still prevalent than gram positive sepsis but without major difference. The common pathogens exhibit a high degree of resistance to ampicillin, gentamicin, and third generation cephalosporins. However, most were susceptible to Vancomycin, Tetracycline, Tigecycline, Teicoplanin, and Doxycycline.

Keywords: Neonate, Sepsis, Antimicrobial susceptibility, Blood culture

INTRODUCTION

The target 3.2 of SDG3, seeks to end preventable deaths of children under 5 years of age and newborns, with all countries aiming to reduce mortality among neonates to 12 per 1,000 live births and under-5 mortality to 25 per 1,000 live births by 2030.[1] As neonatal sepsis is the most common cause of neonatal morbidity and mortality, to access the change in organism pattern of neonatal sepsis, periodic surveillance is needed. In 2018, India's neonatal mortality rate (NMR) is 22.73, that is higher than the global rate of 17.72 per 1000 live births.[2]

In most of the developing countries, gram-negative bacteria and coagulase negative *Staphylococci* (CONS) are major cause of neonatal sepsis.[3-5] On the other hand in developed countries, Group B *Streptococcus* (GBS), *Escherichia coli* and Listeria monocytogenes are leading cause of neonatal sepsis.[6,7] In India, bacteria such as CONS, Klebsiella spp, Acinetobacter spp and *E. coli* are the main pathogens.[8]

Due to the variation in prevalence, associated microorganisms and resistance and treatment patterns, regional hospital based prospective study should be

carried out to know the pattern of the risk factors, Etiology and outcome of neonatal sepsis in the neonatal unit of Dhiraj hospital, Gujarat, India.

METHODS

This hospital-based, observational, cross-sectional study was conducted in the NICU of Dhiraj Hospital, Pipariya, Waghodiya from January 2019 to July 2022. The study was pre-approved by Institutional Ethical Committee (IEC).

All NICU admitted neonates with probable or culture proven sepsis are included. Neonates with suspected sepsis (only symptoms with negative sepsis screen and culture) are excluded. The study was carried out on 226 neonates admitted in NICU at Dhiraj hospital. Each patient's parents/guardian was given information sheet with detailed explanation about this study. The subject was enrolled in this study after receiving written informed consent from parents/guardian.

Each subject's information was collected as per the prescribed proforma. All the eligible participants were subjected to investigations as per routine departmental protocol for management of neonatal sepsis. After achieving the target sample size, the details of all subjects were compiled in Microsoft Excel to make master chart and then all data were analysed statistically.

Investigations

It includes CBC (including Total Count, ANC, Platelet count), Band cell, IT ratio, CRP, RFT, Bilirubin, Serum Electrolytes, Chest Xray, CSF (where indicated), Blood Culture & sensitivity. Some other investigations according to clinical requirements of patients were also done. Blood culture was performed under strict sterile precautions. A single blood sample (2 ml) was inoculated into sterile culture bottle. The BacT alert microbial detection system was used for blood culture.

Manroe's Chart and Mouzinho's Chart were used to determine Absolute Neutrophil Count in term and very low birth weight neonates respectively

Other septic screen criteria include total count <5000 or >25000 (according to age), CRP >5 mg/Dl, IT ratio >0.2, platelet count <1.0 lakh, septic screen is considered positive if TWO or more parameters are found to be abnormal.

Antibiotic sensitivity tests (AST) were performed for all isolates recovered from infant cultures following national committee for clinical laboratory standards 2001 guidelines.[9] After initiating antibiotics, babies were monitored for the response.

Depending on the blood culture report and clinical response, therapy was modified. In case of positive blood culture, antibiotics were narrowed down to target specific organism depending on the sensitivity pattern. If blood culture was negative in a septic screen positive baby, decision on antibiotics was made depending on clinical condition. The data was primarily gathered in the form of proforma and entered into Microsoft excel and analysed with Epi info version 7.1.

RESULTS

Out of total 226 subjects, 144 (63.7%) were male and 82 (36.3%) were female with M:F ratio of 1.75:1.

Out of 133/226 (58.8%) preterm babies, 51 (66.2%) and 82 (55.0%) were intramural and extramural babies, respectively. Whereas amongst 75/226 (33.2%) full term babies, 20 (26.0%) and 55 (36.9%) babies were intramural and extramural, respectively. Similarly, out of 18/226 post-term babies, 6 (7.8%) babies were intramural and 12 (8.1%) babies were extramural.

Out of total 226 subjects, 166 (73.5%) babies, 55 (24.3%) babies and 5 (2.2%) babies were AGA, SGA and LGA, respectively.

The birth weight was not known in 37/226 (16.4%) extramural neonates. So, amongst remaining 189 neonates, 39 (17.3%), 93 (41.2%), 42 (18.6%) and 15 (6.6%) neonates were belonging to NBW, LBW, VLBW and ELBW categories, respectively. Among 189 neonates, LBW: NBW ratio was 3.8:1. This ratio was higher in intramural neonates (4.5:1) as compared to extramural neonates (3.5:1).

In the present study, blood culture was performed in 201 patients. Out of them, growth was detected in 107 patients. Blood culture is still gold standard test so septic screen result was compared with blood culture. Out of 201 patients, septic screen was positive in 82 patients. Therefore, sensitivity of septic screen was 76.6% and PPV was 46.6%. However, specificity and NPV was 0.0%.

Positive septic screen with negative blood culture was considered as probable sepsis. It was reported in 119 neonates (52.7%). Culture proven sepsis was reported in 107 neonates (47.3%).

Acinetobacter baummanni (21.3%) was most common isolated in EOS followed by coagulase negative Staphylococci (16.4%) and *Klebsiella pneumoniae* (16.4%).

In LOS, most common organism was coagulase negative Staphylococci (23.9%) followed by *Klebsiella pneumoniae* (13.0%), *Staphylococcus epidermidis* (13.0%) and *Staphylococcus aureus* (10.9%).

Proportion of *Acinetobacter Baummanni* and *Staphylococcus Haemolyticus* were higher in EOS

(19.7%, 6.6% respectively) than LOS (8.7% and 2.2% respectively). However, coagulase negative Staphylococci, *Staphylococcus epidermidis* was commonly observed in LOS (23.9%, 13.0%, respectively) as compared to EOS (16.4%, 3.3% respectively).

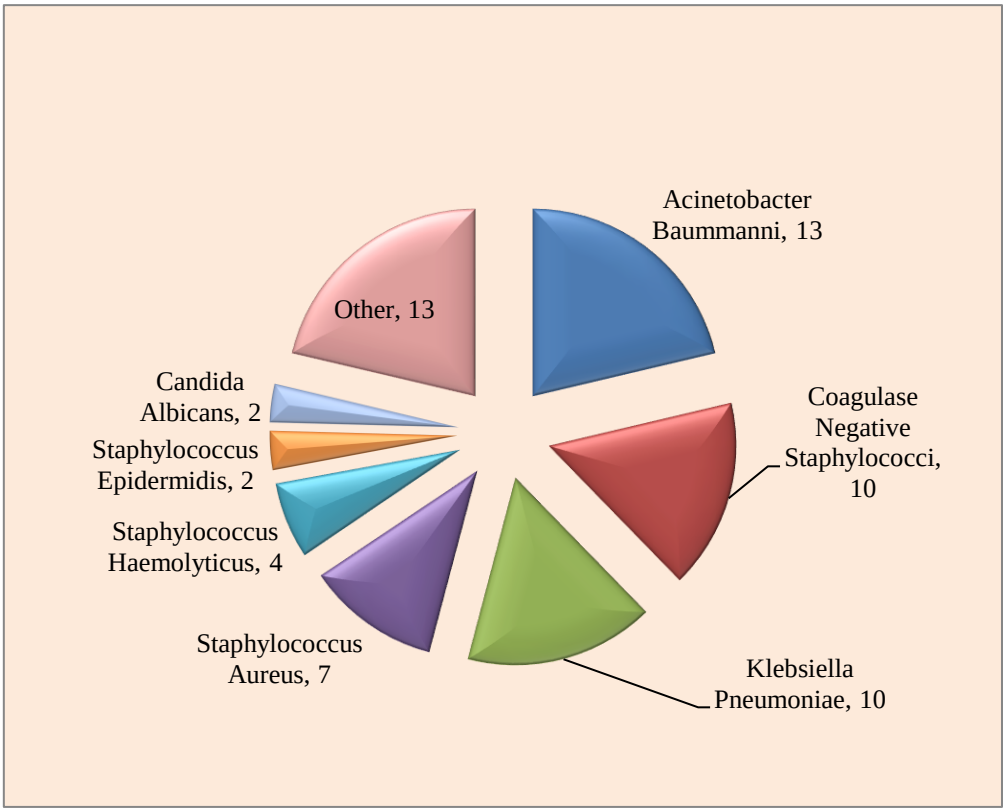

Figure 1: Organism pattern in EOS.

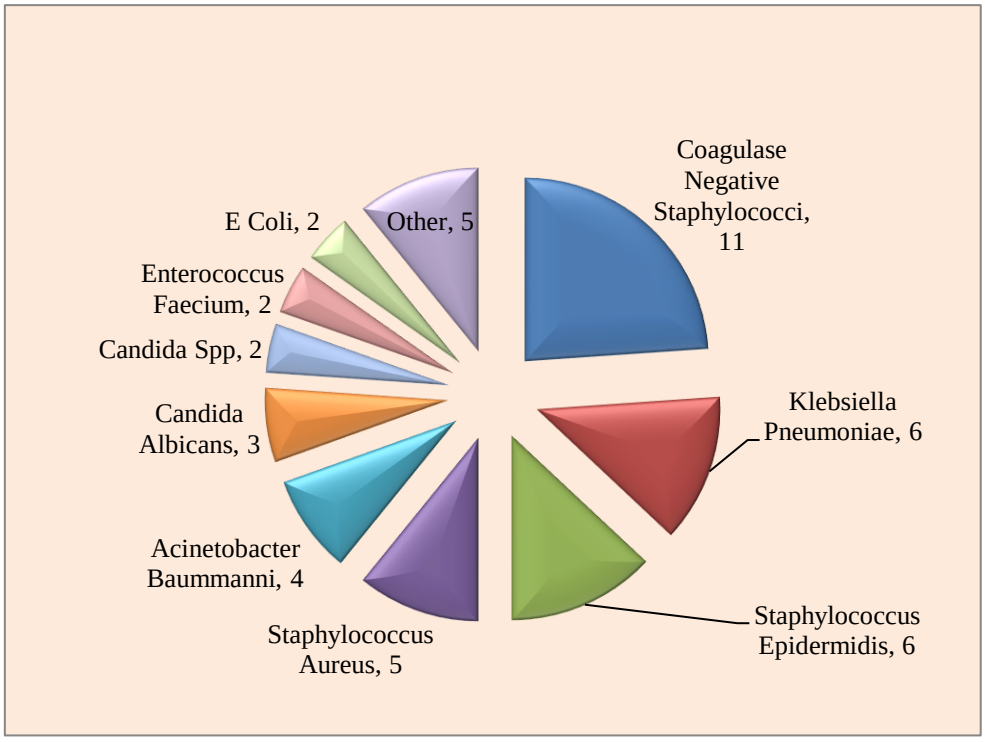

Figure 2: Organism pattern in LOS.

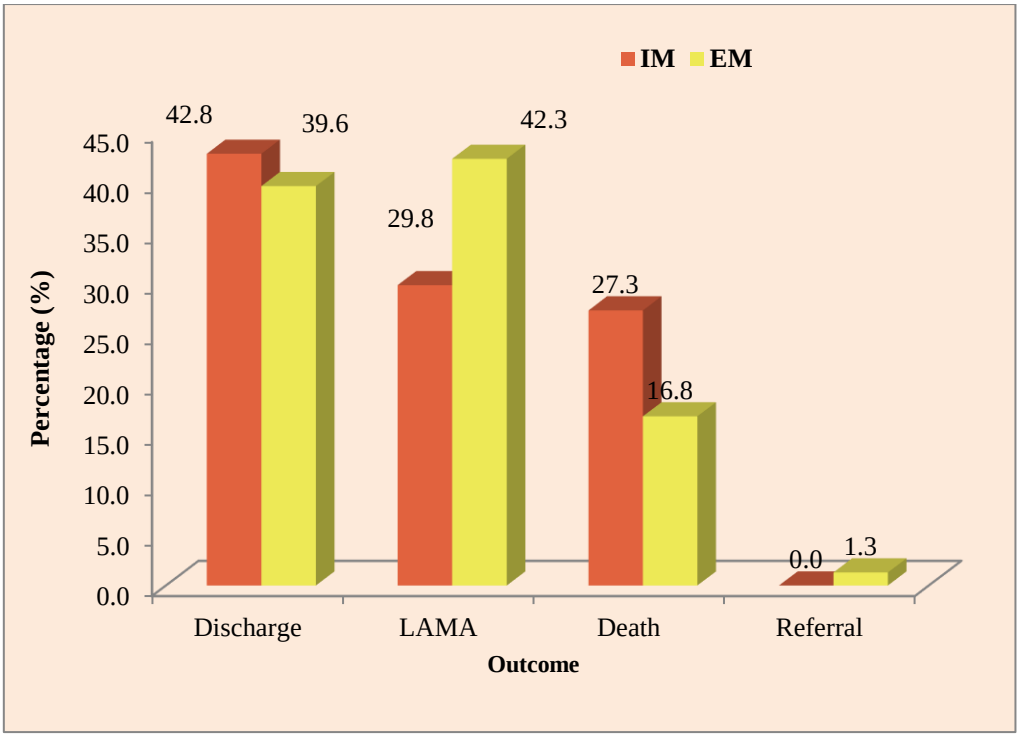

Figure 3: Immediate outcome of neonates with sepsis.

Most of the gram-positive organism was sensitive against daptomycin (100.0%), nitrofurantoin (100.0%), vancomycin (91.5%), tigecycline (96.7%), linezolid (80.9%), doxycycline (76.2%), tetracycline (76.2%) and teicoplanin (74.1%). resistance was observed for penicillin (90.9%), oxacillin (89.5%), cefoxitin (88.0%), erythromycin (84.1%), ciprofloxacin (79.2%), levofloxacin (77.3%), and clindamycin (70.4%). Gram negative organism was resistant against majority of medicine such as cefuroxime (95.7%), piperacillin+ tazobactam (93.1%), ceftriaxone (88.2%), levofloxacin (85.7%), imipenem (85.7%), minocycline (83.3%), gentamycin (82.1%), ciprofloxacin (80.0%) etc. Sensitive was reported against tigecycline (86.1%), colistin (97.4%). In present study, amongst 226 enrolled subjects' overall rate of discharge, LAMA, death and referral were 92 (40.7%), 86 (38.0%), 46 (20.4%) and 2 (0.9%) accordingly

Table 1: Observed risk factors for sepsis.

Observed risk factor	IM	EM	Total
Previous hospitalisation	2 (2.6%)	112 (75.2%)	114 (50.4%)
Fetal distress	27 (35.1%)	38 (25.5%)	65 (28.8%)
Did not cry immediately after birth	30 (39.0%)	30 (20.1%)	60 (26.5%)
Leaking per vagina	18 (23.4%)	30 (20.1%)	48 (21.2%)
Need for intubation	27 (35.1%)	12 (8.1%)	39 (17.3%)
Meconium-stained liquor	20 (26.0%)	16 (10.7%)	36 (15.9%)
Unhygienic feeding practice	1 (1.3%)	32 (21.5%)	33 (14.6%)
Unsterile cord cutting	0 (0.0%)	12 (8.1%)	12 (5.3%)
Foul smelling liquor	0 (0.0%)	8 (5.4%)	8 (3.5%)
Multiple per vaginal examination	4 (5.2%)	4 (2.7%)	8 (3.5%)
Maternal infection	1 (1.3%)	0 (0.0%)	1 (0.4%)

Table 2: Relation between septic screen and blood culture.

		Blood culture		Total
		Positive	Negative	
Septic screen	**Positive**	82	94	176
	Negative	25	0	25
Total		107	94	201

Table 3: Sensitivity pattern of various organisms.

Antibiotics	Resistant	Sensitive
Antibiotics against gram positive organisms		
Nitrofurantoin	0 (0.0%)	8 (100%)
Daptomycin	0 (0.0%)	22 (100%)
Tigecycline	1 (3.3%)	29 (96.7%)
Vancomycin	4 (8.5%)	43 (91.5%)
Linezolid	9 (19.1%)	38 (80.9%)
Doxycycline	5 (23.8%)	16 (76.2%)
Teicoplanin	7 (25.9%)	20 (74.1%)
Trimethoprim+sulbactam	4 (44.4%)	5 (55.6%)
Cotrimoxazole	18 (45.0%)	22 (55.0%)
Ceftriaxone	9 (47.4%)	10 (52.6%)
Gentamicin	22 (50%)	22 (50%)
Clindamycin	19 (70.4%)	8 (29.6%)
Levofloxacin	34 (77.3%)	10 (22.7%)
Ciprofloxacin	19 (79.2%)	5 (20.8%)
Penicillin	40 (90.9%)	4 (9.1%)
Antibiotics against gram negative organisms		
Aztreonam	0 (0.0%)	2 (100%)
Clindamycin	0 (0.0%)	2 (100%)
Tetracycline	0 (0.0%)	1 (100%)
Colistin	1 (2.6%)	38 (97.4%)
Tigecycline	5 (13.9%)	31 (86.1%)
Trimethoprim+sulbactam	5 (50.0%)	5 (50.0%)
Piperacillin	1 (50.0%)	1 (50.0%)
Nitrofurantoin	2 (50.0%)	2 (50%)
Amikacin	23 (71.9%)	9 (28.1%)
Cefepime	34 (81.0%)	8 (19.0%)
Gentamycin	32 (82.1%)	7 (17.9%)
Minocycline	10 (83.3%)	2 (16.7%)
Levofloxacin	12 (85.7%)	2 (14.3%)
Imipenem	36 (85.7%)	6 (14.3%)
Meropenem	30 (85.7%)	5 (14.3%)
Ceftriaxone	15 (88.2%)	2 (11.8%)
Piperacillin + tazobactam	27 (93.1%)	2 (6.9%)
Cefuroxime	22 (95.7%)	1 (4.3%)

DISCUSSION

In this study, the ratio of extramural (EM) to intramural (IM) admission rates was 1.9:1 while it was 1.5: 1 in the study conducted by Pandya et al and 1.7:1 in study of Chhabra et al. This is because of the fact that intramural deliveries in the study institution are conducted with preventive aspects with adequate perinatal care whereas in extramural including home deliveries there may be various predisposing and risk factors including unsafe or unclean environment, limited skilled manpower and inadequate facilities etc.[10,11]

Higher male: female ratio (1.8:1) was reported in this study. This could be due to increased emphasis on health intervention for male child versus female child. Male predominance has been observed in studies of developing countries: as male: female ratio in various studies is 1.4:1 in India by Pandya et al, 1.7:1 in Pakistan by Ali et al.[10,12] The LBW: NBW ratio for sepsis was 3.8:1. The study incidence of LBW neonates was 66.37% (150/226) which is similar to another study conducted by Pandya et al in Gujarat, 62.4%. It was higher than average incidence of LBW (30%) in India. This can be explained by higher number of preterm deliveries 59%, institute being a tertiary care centre and catering to high-risk deliveries. Low socio-economic strata, anaemia, under nutrition and maternal illness are the main contributors to low birth weight. In the present study, EOS was more common (60.2%) than LOS (39.8%). Similar result was observed in the study of Nayak et al (EOS 84.1% and LOS 25.9%). EOS in general is more common because of various high risk perinatal factors for sepsis that operate during this period. In the present study, higher proportion of LOS

was reported in extramural babies (49.0%) as compared to intramural babies (22.1%). Infection may be acquired during transportation. This is in consonance with study of Khinchi et al, as higher LOS accounted for 44% in the extramural babies and 20% in intramural babies.[13]

There has been a wide variation in the growth positivity in India; a higher isolation rate of 52.6% and 46.6% were reported by Movahedian et al, and Nayak et al In present study, similar blood culture yield was observed 53.2%. which is much higher, than study by Sharma et al (37.6%), Shah et al (31.7%).[14-17] These indicated that difficulty in obtaining an adequate volume of blood and the low levels of bacteraemia, blood cultures tend to be sterile in many neonates' various hospitals. High growth positivity in our study revealed trained nurses, phlebotomist and microbiologist in hospital. Similar blood culture yield was observed in EOS (50.4%) and LOS (57.5%). In the present study, positive septic screen was reported in 52.7% neonates. Sensitivity of septic screen was 76.6% and PPV was 46.6.

Growth was detected in 53.2% (107/201) out of 201 blood culture. Of 107 positive blood culture, about 49.5% (53/107) were gram positive bacteria, 41.9% (44/107) were gram negative bacteria and 9.3% (10/107) were fungus.

Nayak et al, reported gram negative bacteria as principal pathogens (61.3%), followed by gram positive bacteria (28.0%) and fungus (10.6%). Similar preponderance of the gram-negative bacteria was reported in other studies conducted Roy et al and Jain et al.[16,18,19]

The most commonly identified bacteria in the present study are coagulase negative staphylococci (CONS), *Acinetobacter baummanni*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* etc. Compare to 19% CONS found in present study, 11.1% in Nepal by Thapa et al. *Klebsiella pneumoniae* was present 15% in the current study. However, studies conducted in southern part of India shows 37% by Chandrakala et al, Thapa S et al shows 7.1% in Nepal.[20,21]

In the present study, *Acinetobacter baummanni* and *Staphylococcus haemolyticus* were more commonly isolated in EOS. However, coagulase Negative Staphylococci, *Staphylococcus epidermidis*, were commonly reported in LOS. Other pathogen profile was similar between EOS and LOS. This challenges the assumption of attributing early onset sepsis to vertical transmission from the mothers. The source of infection in early onset sepsis may be the unhygienic practices in the labour rooms and neonatal intensive care units. Identifying the source and transmission pathways of common pathogens of early onset sepsis is essential to determine the appropriate steps to prevent infection that will help to reduce the high burden of mortality associated with early onset sepsis in the region. In the present study, most of the gram-positive organism was sensitive against daptomycin (100.0%), nitrofurantoin (100.0%), vancomycin (91.5%), tigecycline (96.7%), linezolid (80.9%), doxycycline (76.2%). resistance was observed for penicillin (90.9%), oxacillin (89.5%), cefoxitin (88.0%), erythromycin (84.1%), ciprofloxacin (79.2%). gram negative organism was resistant against majority of medicine such as cefuroxime (95.7%), piperacillin + tazobactam (93.1%), ceftriaxone (88.2%), levofloxacin (85.7%) etc. sensitive was reported against Tigecycline (86.1%), Colistin (97.4%).

Gladstone et al also reported that gram-negative bacteria showed high resistance to multiple drugs while imipenem was still the best for infections with multidrug-resistant gram-negative organism.[23]

In the present study, 84.0% CONS were sensitive for vancomycin, 83.3% for tetracycline and tigecycline, 80.0% for teicoplanin, 75.0% for doxycycline and 70.0% for linezolid. All CONS were resistant for penicillin, 93.8% for cefoxitin and 73.7% for erythromycin, about 90.0% *Staph aureus* were sensitive for vancomycin, 83.3% for linezolid, 75% for cotrimoxazole, 71.4% for doxycycline and 71.4% for ceftriaxone all *Staph aureus* were resistant for erythromycin, 77.8% for rifampicin, 71.4% for penicillin, cefoxitin, and levofloxacin Nayak et al observed 93%, 40%, 47%, and 73%.[15]

Acinetobacter Baummanni were resistant to commonly used antibiotic (100% for levofloxacin, gentamycin, ceftriaxone, ciprofloxacin, cefuroxime, minocycline, amikacin, cefepime, imipenem, piperacillin, piperacillin + tazobactam etc.). it was sensitive for tigecycline (100.0%) and colistin (100.0%), cotrimoxazole (83.3%).

Klebsiella pneumonia was resistant against most of the medicine. (100% for piperacillin+tazobactam, ampicillin. amoxycillin, cefotaxime, oxacillin) sensitivity was observed against tigecycline (93.8%), and nalidixic acid (73.3%). klebsiella pneumoniae was resistant to ceftazidime, ceftriaxone, and cefepime, gentamicin (87.0% each); ampicillin (100%) and ciprofloxacin, amikacin (82.6%).

Sharma et al reported that all penicillin. Ampicillin, gentamicin & ciprofloxacin had lowest sensitivity to all bacterial isolates.[16] Highest sensitivity was recorded with meropenem and vancomycin followed by amikacin and cefepime. As far as cephalosporins are concerned, moderate sensitivity was observed for third generation cephalosporins i.e., cefotaxime while higher sensitivity was documented for fourth generation cephalosporins i.e. cefepime.

The high antimicrobial resistance brings into focus the overuse of antibiotics in neonates with culture negative sepsis. Low sensitivity of commonly used antibiotics and fair sensitivity to Amikacin was also observed by other authors Tallur et al, concur with us that most isolates

were resistant to ampicillin, gentamicin and cotrimoxazole. Almost all the isolates in their study were sensitive to either cefotaxime or amikacin.[23-25]

More reliable and accurate point-of-care diagnostic method(s) are needed to rule out sepsis, thereby preventing indiscriminate use of antibiotics in neonatal intensive care units. Present study shows death in 20% of neonatal sepsis patients while 41% cases were discharged and survived. Cases leave against the medical advice (LAMA) or completing proper treatment were 38% and only 1% cases were referred to higher facilities for further management.

Comparing to present study, a higher discharge rate has found in other studies, such as 66% in Central India by Sharma et al, 83% in South India by Chandrakala et al, 75% in Gujarat by Pandya et al.[10,16,21] Likewise refer cases were also noted in higher proportions in other studies, such as 2% in Chandrakala et al. Lastly, a mixed response has been observed in case of deaths. A lower result has been depicted in studies conducted in Gujarat, 19% by Pandya et al and in South India, 4% by Chandrakala et al while a higher death rate has seen in studies of central India by Sharma AK et al.[10,16,20,21]

CONCLUSION

Risk factors like history of leaking per vagina, LBW, fatal distress, unable to cry immediate after birth, previous hospitalisation, and need for PPV are still predominant factors causing neonatal sepsis. It means need of improvised maternal and neonatal care is very crucial in reducing burden of neonatal sepsis. The group of microorganisms causing neonatal sepsis as well as antibiotics sensitivity pattern vary widely not only across the globe but also from centre to centre. Therefore, each NICU centre should analyse relevant data periodically and update their own neonatal sepsis management protocol.

Gram negative organism sepsis is still prevalent than gram positive sepsis but without major difference in proportion. This finding provokes thought to conduct further study to assess microorganism growth pattern from different part of world. The common pathogens exhibit a high degree of resistance to first line drugs recommended by the World health organization-namely, ampicillin, gentamicin, and third generation cephalosporins such as cefotaxime. However, most were susceptible to WHO classified “watch group” and “reserved group” antibiotics, such as vancomycin, tetracycline, tigecycline, teicoplanin, and doxycycline. It suggests the necessity to analyse antibiotic sensitivity pattern all over nation which may be helpful to update available national management guideline for neonatal sepsis.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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