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SENSITIVITY AND RESISTANCE PATTERN OF ANTIMICROBIAL AGENTS USED IN CASES OF NEONATAL SEPSIS AT A TERTIARY CARE CENTRE IN WESTERN INDIA

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ABSTRACT: Purpose: Neonatal sepsis, a major killer among neonate, necessitates urgent implementation of empirical therapy and later specific antimicrobial therapy against causative microorganism. Empirical therapy relies upon data obtained from previous studies. We conducted this study to evaluate the blood culture, sensitivity and resistance pattern of various antimicrobial agents in neonatal sepsis at Neonatal Intensive Care Unit at a tertiary care hospital in western part of India, which will show us the changing pattern of etiological organism and antimicrobials needed to curb them. Methods: We conducted a prospective cross sectional study over a period of six month duration in Neonatal Intensive Care Unit at tertiary care hospital. We collected and analyzed the blood culture reports and sensitivity pattern of antimicrobials used. Results: We enrolled 163 patients of neonatal sepsis. Paediatricians sent 163 blood samples for culture of which 62 % culture were positive. Out of 101 positive cultures 70 % were Gram negative organisms. Most frequently encountered organisms were *Klebsiella Pneumonia* followed by *Acinobacter* species while least common was *Coagulase Negative Staphylococci*. Conclusion: Our world is facing a huge issue of development of resistance to antimicrobial agents by microorganisms. Irrational prescribing habits of physicians are leading to increasing morbidity, mortality and treatment costs. To prevent antimicrobial resistance, regular educational awareness programs should be conducted in hospitals at a regular basis. Also comparative studies in the same hospital over years will help us to generate proper antimicrobial policies ultimately leading to rationale drug therapy.

INTRODUCTION: Neonatal sepsis is defined as "a clinical syndrome of bacteraemia with systemic signs and symptoms of infection in the first four weeks of life". When pathogenic bacteria gain access into the blood stream, they may cause overwhelming infection without much localization (septicaemia) or may get predominantly localized to the lung (pneumonia) or the meninges (meningitis) [1].

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Various systemic infections of the newborn such as septicaemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections are considered as part of neonatal sepsis but not superficial infection like conjunctivitis or oral thrush.

Neonatal sepsis is divided into two types based on the time of onset of the symptoms i.e. early onset sepsis (EOS) if sepsis presents within first 72 hours of life or *late onset sepsis (LOS)* if after 72 hours of life [2].

Clinically neonatal sepsis may present as hypo/hyperthermia, lethargy, poor cry, refusal of feeding, poor perfusion, hypotonia, absent neonatal reflex, respiratory distress, brady/tachycardia etc.

Sepsis is the commonest cause of neonatal mortality which is responsible for about 30-50% of the total neonatal deaths in developing countries [3, 4]. It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related causes [4]. World Health Organisation estimates that of the four million neonatal deaths all over the world every year, over 35% are due to infection in the neonatal period[5].

In India the incidence of neonatal sepsis according to the National Neonatal Perinatal Database is 30 per 1000 live births [6]. The database comprising 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. Septicemia was the commonest clinical category with an incidence of 23 per 1000 live births while the incidence of meningitis was reported to be 3 per 1000 live births. *Klebsiella Pneumoniae* was the most frequently isolated pathogen in intramural setting (32.5%) and extramural setting (27%), followed by *Staphylococcus Aureus* (in intramural-13.6% and in extramural -15%) and *Pseudomonas* in extramural setting (13%). To treat neonatal sepsis, we need immediate targeted antimicrobial treatment directed against the causative organism and based upon their sensitivity pattern. To identify causative agents, microbial culture is considered as the gold standard and antimicrobial resistance pattern is of utmost importance for a definitive and prompt cure.

However it has been observed that the etiological agents and their sensitivity towards antimicrobial agents keep on changing over a period of time, so for optimum management of neonatal sepsis we need data of all these important variables. This study will provide us important data regarding culture and sensitivity patterns of causative organisms which will help us to intensify or modify the treatment policies if necessary. Also this study was conducted in a tertiary care hospital in western India; it may help for conducting large multi-centric studies in future.

MATERIALS AND METHODS:

Study design: A prospective cross sectional observational and single centre study was conducted for 6 months in neonatal intensive care unit (NICU) at a tertiary care hospital located in western part of India. We included patients from both extramural and intramural divisions.

The microbiology laboratory of the hospital is accredited by NABL (National Accreditation Board for Testing and Calibration Laboratory) which is an autonomous authority under Government of India. Various microbiological cultures and testing were done as per standard guidelines.

Sample size and study population:
We included 163 neonates (zero to four weeks) admitted to NICU, who were suspected/ diagnosed cases of neonatal sepsis and in whom blood cultures were done.

Data collection:
Period of data collection:
We collected data starting from 1st April 2013 to 31st September 2013.

Inclusion criteria:

- All patients (zero to four weeks of age) of suspected/diagnosed neonatal sepsis of either sex admitted to NICU after 1st April 2013.
- Only the patients in whom blood cultures were done.
- Only the patients whose parents gave informed consent were enrolled in this study.

Exclusion criteria:

If parents of the septic neonates were not willing to participate in the study.

Informed consent:
We took informed consent from all the parents of the neonates having suspected/ diagnosed as neonatal sepsis before enrolment.

Study method:
We took prior permissions from the Head and Professor of concerned departments of the concerned hospital.

We obtained necessary ethical permission was from institutional ethics committee. We visited the NICU and Microbiology department daily to confirm that patients were selected and enrolled according to inclusion and exclusion criteria.

Blood collection method:
Two blood samples were collected aseptically from patients for routine blood culture. The vein puncture site was disinfected with 70% alcohol and 2% tincture of iodine before collecting approximately 2 ml of blood for culture.

Blood culture:
Approximately 2 ml of collected blood was added in glucose broth maintaining sample broth ratio of approximately 1:8 to 1:10. After this culture bottle was sent to microbiology laboratory as soon as possible and kept for initial incubation at 35-37 ^{0}C for 6-12 hours. Then one loop full of sample was inoculated on Mac-Conkey agar and Nutrient/blood agar each and were incubated at 35^{0}C, then observed for growth after 24 hours. If growth was seen then various tests like gram staining and microscopic examination, citrate, urease, catalase, coagulase, bile solubility etc. were done to identify and confirm the species of microorganism.

Antimicrobial resistance:
Few of the colonies were inoculated in peptone water and kept for incubation at 37^{0}C for six hours, which later was subjected to antimicrobial sensitivity tests. Modified Kirbey-Bauer method was used (Clinical and Laboratory Standards Institute). Depending upon microorganism (Gram positive and negative), various antimicrobial agents were checked for the sensitivity pattern. We collected data from indoor case papers as well as records from microbiology department and recorded on a prestructured case reporting form (CRF).

Data analysis:
We entered data in Microsoft excel sheet 2007 and tabulated later.

RESULTS:
In this study, we considered 220 patients and depending upon inclusion criteria, enrolled 163 patients having neonatal sepsis and admitted to Neonatal Intensive Care Unit (NICU), at a tertiary care hospital in western part of India. Blood cultures of these 163 patients were sent for microbial examination.

Out of total 163 microbial cultures done, we found 101 (62%) cultures to be positive which were subjected to antimicrobial sensitivity check.

In current study, out of the 101 positive results, we identified gram positive organisms in 30 isolates (30%) and gram negative organisms in 71 isolates (70 %). As shown in **Table 1**, most frequently found organism responsible for neonatal sepsis was *Klebsiella Pneumoniae* in 28 cultures (28%), followed by *Acinobacter* species in 23 cultures (23 %), while least frequently found organisms were *Pseudomonas* in 8 culture (8%) and *Coagulase Negative Staphylocci (CONS)* in 4 cultures (4%).

TABLE 1: FREQUENCY OF ORGANISMS ISOLATED BY CULTURE

Organism	No of isolates	Percentage (%)
Klebsiella Pneumoniae	28	27.7
Acinobacter	23	22.8
Staphylococcus Aureus	13	12.9
Enterococci	13	12.9
Escherischia Coli	12	11.9
Pseudomonas Aeruginosa	8	7.9
CONS	4	3.9
Total	101	100

Results displayed in **Table 2** shows that the most effective antimicrobial against *Staphylococcus aureus* were vancomycin and ceftazidime.

TABLE 2: SENSITIVITY PATTERNS OF *STAPHYLOCOCCUS AUREUS*

Anti-microbial Agents	Total number of isolates checked for	Sensitive strains		Resistant strains	
		Absolute numbers	Percentage (%)	Absolute numbers	Percentage (%)
Vancomycin	13	13	100	0	0
Ceftazidime	5	5	100	0	0
Levofloxacin	12	9	75	3	25
Gentamycin	13	8	61.5	5	38.5
Clindamycin	13	8	61.5	5	38.5
Ampicillin	13	7	53.9	6	46.2
Cefalothin	4	2	50	2	50
Oxacillin	13	6	46.2	7	53.9
Erythromycin	10	1	10	9	90
Cefotaxime	1	0	0	1	100

Results showed in the **Table 3** states that against *CONS* organism gentamycin, vancomycin and levofloxacin were most effective while ceftriaxone was not effective.

TABLE 3: SENSITIVITY PATTERNS OF *COAGULASE NEGATIVE STAPHYLOCOCCI*

Antimicrobial Agents	Total number of isolates checked for	Sensitive strains		Resistant strains	
		Absolute numbers	Percentage (%)	Absolute numbers	Percentage (%)
Gentamycin	4	4	100	0	0
Levofloxacin	4	4	100	0	0
Vancomycin	4	4	100	0	0
Oxacillin	4	3	75	1	25
Cefalothin	2	1	50	1	50
Erythromycin	4	1	25	3	75
Clindamycin	4	1	25	3	75
Ampicillin	4	1	25	3	75
Ceftriaxone	2	0	0	2	100

As per the results in **Table 4**, we can say that against *Klebsiella* organism meropenem, piperacillin + tazobactam combination, levofloxacin and imepenem were most effective while cefepime and cefotaxime were least effective.

TABLE 4: SENSITIVITY PATTERNS OF *KLEBSIELLA SPECIES*

Antimicrobial Agents	Total number of isolates checked for	Sensitive strains		Resistant strains	
		Absolute numbers	Percentage (%)	Absolute numbers	Percentage (%)
Meropenem	6	6	100	0	0
Piperacillin + tazobactum	10	9	90	1	10
Levofloxacin	6	5	83.3	1	16.7
Imepenem	26	20	76.9	6	23.1
Amikacin	26	15	57.7	11	42.3
Aztreonam	26	15	57.7	11	42.3
Ciprofloxacin	21	12	57.1	9	42.9
Ceftazidime	27	12	44.4	15	55.6
Piperacillin	19	6	31.6	13	68.4
Chloram-phenicol	26	7	26.9	19	73.1
Tetracycline	23	6	26.1	17	73.9
Gentamycin	9	0	0	9	100
Cefepime	3	0	0	3	100
Cefotaxime	2	0	0	2	100
Erythromycin	1	0	0	1	100

Results displayed in **Table 5** shows that against *Acinobacter* organism piperacillin + tazobactum combination, levofloxacin and meropenem were most effective.

TABLE 5: SENSITIVITY PATTERNS OF *ACINOBACTER* SPECIES

Antimicrobial Agents	Number of isolates checked	Sensitive strains		Resistant strains	
		Absolute numbers	Percentage (%)	Absolute numbers	Percentage (%)
Piperacillin +tazobacam	15	14	93.3	1	6.7
Levofloxacin	10	9	90	1	10
Meropenem	15	12	80	3	20
Imepenem	10	5	50	5	50
Ciprofloxacin	8	4	50	4	50
Gentamycin	11	5	45.45	6	54.6
Ceftazidime	12	5	41.7	7	58.3
Cefepime	12	5	41.7	7	58.3
Amikacin	23	9	39.1	14	60.9
Aztreonam	12	4	33.3	8	66.7
Cefotaxime	7	2	28.6	5	71.4
Ceftriaxone	4	1	25	3	75
Chloram-phenicol	13	3	23.1	10	76.9
Tetracycline	10	2	20	8	80
Piperacillin	18	1	5.6	17	94.4
Cefepime	1	0	0	1	100

Results have been displayed in **Table 6**, these show that against *E. coli* organism piperacillin + tazobactum combination, meropenem, aztreonam and imepenem were effective while ceftriaxon, gentamycin, cefepime and levofloxacin were not effective.

TABLE 6: SENSITIVITY PATTERNS OF *ESCHERICHIA COLI*

Antimicrobial Agents	Number of isolates checked	Sensitive strains		Resistant strains	
		Absolute numbers	Percentage (%)	Absolute numbers	Percentage (%)
Piperacillin + tazobactam	4	4	100	0	0
Meropenem	4	4	100	0	0
Aztreonam	10	8	80	2	20
Imepenem	10	8	80	2	20
Ceftazidime	9	7	77.8	2	22.2
Amikacin	12	7	58.3	5	41.7
Tetracycline	8	4	50	4	50
Ciprofloxacin	10	4	40	6	60
Chloram-phenicol	10	4	40	6	60
Pipieracillin	11	3	27.3	8	72.7
Gentamycin	3	0	0	3	100
Levofloxacin	2	0	0	2	100
Cefipime	2	0	0	2	100
Ceftriaxone	2	0	0	2	100

As per the results showed in the **Table 7,** we can say that against *Pseudomonas* organism piperacillin + tazobactum combination, amikacin, gentamycin, and meropenem, were most effective while imepenem was least effective.

TABLE 7: SENSITIVITY PATTERNS OF *PSEUDOMONAS*

Antimicrobial Agents	Total number of isolates checked for	Sensitive strains		Resistant strains	
		Absolute numbers	Percentage (%)	Absolute numbers	Percentage (%)
Amikacin	8	8	100	0	0
Piperacillin + tazobactum	6	6	100	0	0
Gentamycin	4	4	100	0	0
Tetracycline	3	3	100	0	0
Chloram-phenicol	3	3	100	0	0
Piperacillin	2	2	100	0	0
Meropenem	6	5	83.3	1	16.7
Ceftazidime	5	4	80	1	20
Ciprofloxacin	8	6	75	2	25
Aztreonam	6	4	66.7	2	33.3
Imepenem	6	1	16.7	5	83.3

The results displaying sensitivity pattern of *Enterococci* have been shown in **Table 8**. Results showed that against *Enterococci* organism linezolide and vancomycin were most effective while gentamycin, erythromycin, ciprofloxacin and ceftazidime were not effective.

TABLE 8: SENSITIVITY PATTERNS OF *ENTEROCOCCI*

Antimicrobial Agents	Total number of isolates checked for	Sensitive strains		Resistant strains	
		Absolute numbers	Percentage (%)	Absolute numbers	Percentage (%)
Linezolide	11	11	100	0	0
Vancomycin	10	10	100	0	0
Piperacillin	3	2	66.7	1	33.3
Levofloxacin	9	5	55.6	4	44.4
Ampicillin	13	4	30.8	9	69.2
Penicillin	12	3	25	9	75
Doxycycline	12	2	16.7	10	83.3
Erythromycin	11	0	0	11	100
Gentamycin	4	0	0	4	100
Ciprofloxacin	3	0	0	3	100
Ceftazidime	2	0	0	2	100
Tetracycline	1	0	0	1	100

DISCUSSION: Septicaemia is one the major causes of morbidity and mortality in the neonatal period, and it often has a rapid and fulminant course. The database comprising 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 [6].

In neonatal sepsis, culture and sensitivity reports are of utmost importance. We observed that in our study total 163 microbial culture and sensitivity tests were done, of which 62 % culture were found to be positive. This is higher than that reported by Shreshtha S et al and Shahian et al in which the blood culture positivity rate was 44% and 43% respectively [7-8]. In our hospital culture and sensitivity reports are done on the day of admission and empirical antimicrobial therapy is started with Cefotaxime and Amikacin combination. Culture is repeated later, if clinical improvement is not seen despite of empirical antimicrobial therapy. So many times undue culture tests are avoided, yielding higher rate of positive culture observed in our study.

In this study gram positive organisms were identified in 30 % cultures and gram negative organisms were seen in 70 % of cultures. Study conducted by Rahman showed that in their study 70 % cases of neonatal sepsis were caused by gram negative organisms [10]. The results in our study reflects what have been earlier established that in developing countries gram negative organisms are causative organisms in majority of cases of neonatal sepsis [4].

We observed that most frequently found organism was *Klebsiella Pneumoniae*, followed by

Acinobacter species and *Staphylococcus Aureus*. Least frequently found organisms were *Pseudomonas* and *Coagulase Negative Staphylocci (CONS).*

Aletayeb et al reported *Klebsiella Pneumoniae*, *E.coli* and *Acinobacter* as the major organisms, which is in accordance with our study [11]. *Klebsiella Pneumoniae*, *Staphylococcus Aureus* and *CONS* were the predominant organisms in the study done by Shrestha S et al and Jyothi P et al [7, 12]. *Pseudomonas Aeroginosa* was the predominant organism for neonatal sepsis followed by *Klebsiella* and *Acinobacter* in the study done by Bhat R et al [13]. Shahian et al reported *CONS*, *E. coli* and *Staphylococcous* as the major organisms which is in contrast to observations in our study [9].

Staphylococcus Aureus was the predominant organism for neonatal sepsis followed by *Klebsiella* and *Escherischia Coli* in the study done by Mhada TV(2012) et al [8]. Shahian et al and Dias E et al reported *CONS* as the major organisms for neonatal sepsis[9 and 14]. The similar or contradictory findings found in above mentioned studies reflect differential patterns of causative organisms in neonatal sepsis at different geographical areas.

The results in our study shows that for *Staphylococcus Aureus* infection most effective antimicrobial given in the mentioned study were vancomycin and ceftazidime as all isolates checked were found to sensitive to them. This was in accordance with the study by Shrestha, which showed that vancomycin was effective in 100 % isolates[7]. However, in study conducted by Kayagne vancomycin was effective against 14 % strains only while study by Shahian showed that ceftazidime was effective only in 22% [15, 9].

In our study according to sensitivity results we noted showed that against *CONS* organism gentamycin, vancomycin and levofloxacin were most effective in all cultures while ceftriaxone was not effective in any. These results were comparable with study done by Shrestha [7]. In some other studies done by Shahian [9] and Dias [14], results showed that strains were sensitive to gentamycin only in 50 % and 63 % cultures respectively [9, 14].

Our study also showed that *Klebsiella* organism were sensitive to meropenem in 100 % cultures, levofloxacin in 83 % and imepenem in 77 % of cultures while cefepime and cefotaxime were not effective at all. Similar results were observed in studies done by Aletayeb and Shrestha who showed 90 % and 100 % sensitivity towards imepenam, respectively [11, 7]. In contrast to our study, Kayagne showed only 2 % sensitivity to meropenem and Shahian showed only 33 % sensitivity to imepenem[15, 9].

As per our study *Acinobacter* organism were most sensitive to piperacillin + tazobactum combination in 93 % of cultures, levofloxacin in 90 % of cultures and meropenem in 80 % of cultures. These results were comparable to study done by Shrestha showing 100% sensitivity to meropenem[7].

In our study results showed that against *E. coli* organism piperacillin + tazobactum combination, meropenem, aztreonam and imepenem were effective in 100 %, 100 %, 80 % and 80 % of positive cultures respectively while ceftriaxon, gentamycin, cefepime and levofloxacin were not effective. Other studies to be compared are, by Shrestha showing 100 % sensitivity to imepenem but 100 % resistance aztreonam, Shahian showing 67 % and 33 % sensitivity to imepenem and gentamycin respectively, Mhada showing 43 % sensitivity to gentamycin, Kayagne showing 0% and 68 % sensitivity to meropenem and gentamycin[7, 9, 8, 15].

We observed that against *Pseudomonas* organism piperacillin + tazobactum combination, amikacin, gentamycin, and meropenem, were found to be effective in 100 %, 100 %, 100 % and 83 % of cultures respectively while imepenem was effective only in 17 % of cultures. Study by Rehman showed gentamycin was effective only against 21 % strains [10]. Shrestha showed 100 % sensitivity to imepenem but only 11 % and 0 % sensitivity to gentamycin and amikacin respectively[7]. Mhada showed that amikacin and gentamycin were not effective [8].

This study showed that against *Enterococci* organism linezolide and vancomycin were effective in all cultures while gentamycin, erythromycin,

ciprofloxacin and ceftazidime were not effective. Similar results were shown by Shahian and Shrestha [9, 7].

The differences found in sensitivity patterns in neonatal sepsis mentioned in above studies shows that susceptibility of various microorganisms differs at different places and different time period. Limitations of current study were shorter duration of study and study was conducted in single centre only. Ideally blood samples for culture should be repeated after 3-4 days and antimicrobial therapy should be changed as per the report, however as the hospital is a limited resource hospital and to minimize the cost of the treatment, blood cultures were repeated only in case the empirical antimicrobial therapy was not effective.

CONCLUSION: Various studies indicate gradual increase in the emergence of antibiotics resistance organisms. Factors which play role in the development of resistance include no uniformity in the usage of antibiotics, indiscriminate use, availability of antibiotics etc. Also microbial pattern and their sensitivity towards antimicrobials vary depending on the study group and the hospital setup. So trend nowadays is towards comparative studies in the same hospital over years, which will help us to generate proper antimicrobial policies for various infective conditions. This may ultimately lead us towards rationale drug therapy.

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CONFLICT OF INTEREST: We, the authors of this article declare that we have no conflict of interest of any means.

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