Original Research Article

Five year trend of aerobic bacteria isolated from patients with neonatal sepsis in a tertiary care centre, North Kerala, India

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ABSTRACT

Background: Antimicrobial resistance has reached to a significant proportion globally. This antimicrobial resistance increases the cost of health care in addition to the existing burden of the prevalence of infectious disease in developing countries. We need to have institutional protocols based on the standard guidelines. It is important for the clinician to use antibiotics only when it is necessary. Thus, the present study was undertaken to know the bacteriological profile and antibiogram of aerobic pathogens isolated from blood samples of patients in NICU during 2012 -2016.
Methods: This retrospective study was conducted at Clinical Microbiology and Paediatric department, MES Medical College, Perinthalmanna, between January 2012 to December 2016. All blood samples from suspected Neonatal sepsis were included in the study. The positive samples were cultured as per standard microbiological procedure and antimicrobial susceptibility was done as per CLSI guidelines.
Results: During the study period of 5 years, out of 2022 blood samples received from NICU 251 (12.41%) were positive. During the study period, male patients (146, 58.17%) were more than the female. The Gram-positive isolates accounted for 135 (53.78%) isolates compared to Gram negative isolates 115 (45.81%). Authors had a single isolate of Candida species in 2014. Among the Gram positive isolates, *Staphylococcus aureus* (77/135) was the commonest and *Klebsiella pneumoniae* (51/115) among the Gram negative isolates. There was a steady rise in MRSA isolation rate in five years. The commonly used antibiotic in neonatal sepsis i.e., Ampicillin and Gentamicin, was observed to have high level of resistance. No resistance was observed against Vancomycin, Teicoplanin, Linezolid among Gram positive and against Carbapenems among Gram negative pathogens.
Conclusions: As Gram positive organisms were the most common pathogen isolated in patients with neonatal septicaemia in our study population. The *Staphylococcus aureus* and *Klebsiella pneumoniae* were the common isolates. Following this study, Piperacillin tazobactum is considered as the drug for empirical therapy. Vancomycin and carbapenems are considered as reserve drug and escalated only following the report from microbiology report.

Keywords: Antibiotic, Neonatal sepsis, Resistance, Trend

INTRODUCTION

Septicemia is a significant cause of morbidity and mortality in neonates especially in developing countries. The frequent emergence of resistant bacteria makes the problem more difficult, so early detection and identification of pathogen is very important. Thus, the present study was undertaken to know the bacteriological profile and antibiogram of aerobic pathogens isolated from blood samples of patients in NICU over a period of 2012-2016. Antibiotic is one of the common drugs prescribed for the patients especially in inpatients. The antimicrobials have saved many lives from dreadful infectious diseases. It is a double-edged sword as its

injudicious use will give rise to antimicrobial resistance that can endanger many patients life. The first antibacterial resistance was known in 1948. In the present 21st century every known pathogen is resistant to one or more antimicrobials.[1] Antibiotic medications are over used in both developed and developing countries. Antimicrobial resistance has reached to a significant proportion globally. Indiscriminate use of drugs is an important global threat.[2] Authors need to develop new antimicrobial and at the same time antibiotics has to be used judiciously. The data available on profile of antibiotic usage is less and its usage among pediatric patients is very meager. Multidisciplinary approach is required to overcome the current trends of antimicrobial resistance across the globe.[3] This study was undertaken to study the flora causing neonatal sepsis and their antibiogram in the five years.

METHODS

This retrospective study was conducted at department of Clinical Microbiology and Paediatric department, MES Medical College Hospital, Perinthalmanna after approval from Institutional Ethics Committee. The blood samples of patients suspected of neonatal sepsis during the study period of January 2012 to December 2016 were included in the study. The paediatric patients above the age of 28 days were excluded from the study.

The venous blood (0.5-1 ml) of the suspected neonatal sepsis was collected and inoculated in automated blood culture (BACTEC 9050, Becton Dickinson) system. The positive samples were subcultured on 5% sheep blood agar, MacConkey's agar and incubated aerobially at 370C for 18-24 hours. The bacterial colonies grown were identified using Gram's staining and standard operating procedures. Antibiotic sensitivity testing was done by Kirby Bauer's Disc diffusion method on Mueller Hinton agar as per CLSI guidelines.[4]

The antimicrobial agents tested were Ampicillin (10µg), Ceftriaxone (30µg), Cotrimoxazole (25µg), Gentamicin (10µg), Ciprofloxacin (5µg), Levofloxacin (5µg). In addition to these antimicrobial agents, Cephalexin (30µg), Erythromycin (15µg) Clindamycin (2µg), Vancomycin (30µg), Linezolid (30µg), Teicoplanin (30 µg) were tested for Gram positive pathogens and Piperacillin/ Tazobactum (100/10µg) and Meropenem (10µg) were tested for Gram negative pathogens. The quality control was done using *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923).

The demographic detail of the neonates, the pathogens causing neonatal sepsis and their antibiogram was noted.

The antibiogram of most common Gram positive isolate i.e., *Staphylococcus aureus* and Gram negative pathogens i.e., *Klebsiella pneumoniae* were analysed for changing trend.

The collected was entered in Microsoft Excel 2007 and analysed by percentage.

RESULTS

During the study period of 5 years, out of 2022 blood samples received from NICU 251 (12.41%) were positive. During the study period, male patients (146, 58.17%) were more than the female with male: female ratio being 1.39:1 (Table 1).

Table 1: Gender wise distribution of the Neonatal sepsis patients studied.

	Male	Female	Total
No of isolates/ year	146 (58.17%)	105 (41.83%)	251
2012	35	24	59
2013	34	20	54
2014	15	14	29
2015	33	21	54
2016	29	26	55

The Gram-positive isolates accounted for 135 (53.78%) isolates compared to Gram negative isolates 115 (45.81%). Authors had a single isolated of *Candida* species in 2014. Among the Gram positive isolates, *Staphylococcus aureus* (77/135) was the most common isolate, followed by Coagulase Negative *Staphylococcus* Species (39/135). *Klebsiella pneumoniae* (51/115) was commonest followed by *Citrobacter* species (24/115) among the Gram negative pathogens (Table 2).

The antimicrobial resistance pattern was studied according to the Gram reaction to the organism. Among the Gram negative organism, resistance was observed against Ampicillin, 3rd generation cephalosporins, Cotrimoxazole, Gentamicin throughout the study period. But, emergence of resistance was observed against amikacin, levofloxacin, Piperacillin/ Tazobactum. No resistance was observed against Carbapenems (Figure 1). Similarly, among Gram positive isolates, resistance was observed against Ampicillin, 3rd generation cephalosporins, Cotrimoxazole, Ciprofloxacin and erythromycin throughout the study period. There was emergence of resistance to amikacin. No resistance was observed against high end antimicrobials against Gram positive pathogens, i.e., Vancomycin, Linezolid, Tiecoplanin (Figure 2). Authors studied the trend among the most common pathogen among the Gram positive and Gram negative, i.e, *Staphylococcus aureus* and *Klebsiella pneumoniae*, respectively. Among the *Staphylococcus aureus* isolates, there was steady rise in resistance to Ampicillin, 3rd generation cephalosporins, Cotrimoxazole, Ciprofloxacin and erythromycin from 2012-16 (Table 3).

Among the *Klebsiella pneumoniae* isolates, there was resistance observed against 3rd generation cephalosporins,

Cotrimoxazole, gentamicin, Ciprofloxacin. Resistance against Piperacillin Tazobactum was observed to rising to a significant level among *Klebsiella* species isolates from the neonates during the study period (Table 4)

Table 2: Distribution of pathogens isolated during the study period.
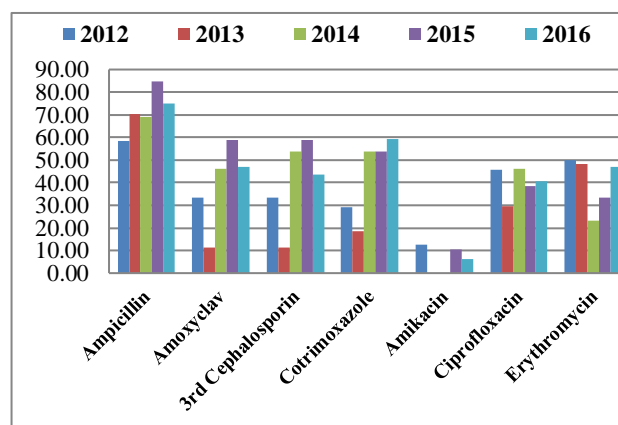
	2012	2013	2014	2015	2016	
Gram Negative	**35**	**27**	**15**	**15**	**23**	**115**
Klebsiella sp	22	9	5	8	7	51
Citrobacter sp	6	10	3	1	4	24
Pseudomonas sp	7	2	3	-	6	18
Acinetobacter sp	-	4	4	5	4	17
Escherichia coli	-	2	-	1	2	5
Gram Positive	**24**	**27**	**13**	**39**	**32**	**135**
Staphylococcus aureus	20	20	6	15	16	77
CoNS	2	7	3	19	8	39
Enterococcus sp.	2	-	4	1	5	12
Pneumococcus	-	-	-	4	3	7
Fungus						
Candida sp.	-	-	1	-	-	1
Total	59	54	29	54	55	251
MRSA	6	3	2	10	12	33
MRSA isolation rate (% of MRSA/ SA)	30.0	15.0	33.3	66.7	75.0	42.9

Table 3: Antibiotic resistance trend in *Staphylococcus aureus* (in Percentage).

Year	2012	2013	2014	2015	2016
No of Isolates	**20**	**20**	**6**	**15**	**16**
Ampicillin	50.00	65.00	83.33	93.33	100.00
Cephalexin	45.00	25.00	33.33	73.33	75.00
3rd Generation cephalosporin	30.00	15.00	33.33	66.67	75.00
Cotrimoxazole	25.00	5.00	50.00	80.00	81.25
Gentamicin	35.00	25.00	33.33	33.33	31.25
Ciprofloxacin	45.00	25.00	33.33	46.67	68.75
Levofloxacin	0.00	0.00	0.00	13.33	0.00
Erythromycin	55.00	45.00	33.33	40.00	75.00
Clindamycin	15.00	0.00	33.33	6.67	0.00
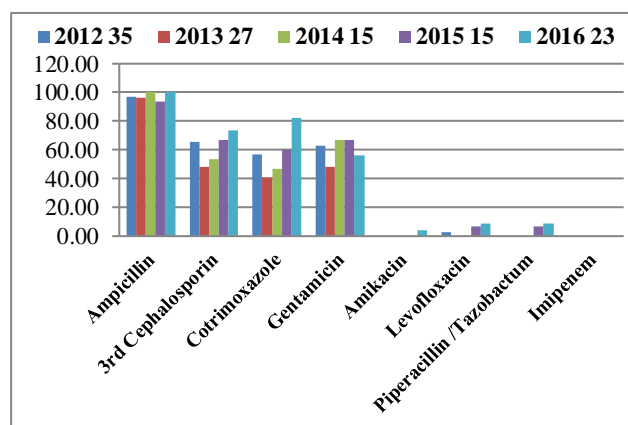
Figure 1: Antimicrobial resistance trend among Gram negative isolates.

Figure 2: Antimicrobial resistance trend among Gram positive isolates.

Table 4: Antibiotic resistance trend in *Klebsiella pneumoniae* (in Percentage).

Year	2012	2013	2014	2015	2016
No of Isolates	22	9	5	8	7
3rd Generation Cephalosporin	77.27	44.44	60.00	62.50	85.71
Cotrimoxazole	63.64	88.89	40.00	50.00	85.71
Gentamicin	77.27	44.44	40.00	75.00	71.43
Ciprofloxacin	68.18	33.33	20.00	75.00	71.43
Levofloxacin	4.55	0.00	0.00	12.50	14.29
Piperacillin-Tazobactum	22.73	11.11	0.00	12.50	28.57

DISCUSSION

As per the W.H.O recommendations, surveillance system is needed in every country to assess the antibiotic use by experts in clinical practice at local as well as at national level. Antibiotics overuse is well documented in developed countries. This study authors have observed the change in the antimicrobial drug resistance pattern over a period of five years in a neonatal ICU in a tertiary care hospital.[3] In our study, male neonates were more than the female with the ratio 1.39:1, this is observed in various other studies worldwide.[5,6] Authors have observed predominance of Gram positive pathogens in this NICU. Authors have observed *Staphylococcus aureus* is the commones, similar to few other studies.[7-9] In contrary, few of the Indian studies have reported, Gram negative bacteria as the commonest pathogen causing Neonatal sepsis.[5,10,11] This emphasis the need for study the flora causing neonatal sepsis in a particular Neonatal Intensive unit, as it can vary among the various geographical area and hospital, profile of the neonates treated. Coagulase Negative *Staphylococcus* species were included in the study as these were isolated from clinically suspected neonatal sepsis, even though studies have considered them as contaminant.[12]

There are even studies in which CoNS accounted for about two third of the neonatal sepsis.[13] Among the gram-negative bacteria, *Klebsiella* and *Citrobacter* species were isolated from this neonatal population. *Klebsiella pneumoniae* has been isolated among the neonates in sepsis in various population.[6,9,14]

When the antimicrobial resistance was studied among Gram positive pathogens, it showed high resistance to Ampicillin, Cotrimoxazole, Ciprofloxacin and Erthromycin, similar to other studies.[5,9] Anti-MRSA drugs like Vancomycin, linezolid ,Teicoplanin were sensitive among all Gram positive pathogens.[10,15] Among gram negative pathogens revealed high level of resistance to Ampicillin, 3rd generation Cephalosporins, and gentamicin and emergence of resistance to levofloxacin, piperacillin/ tazobactum. High level resistance to cephalosporins have been reported in various studies.[5,6,14] Especially when the isolates are ESBL producers.

Whereas amikacin, levofloxacin were more effective in these pathogens.

Staphylococcus aureus isolates showed resistance to Ampcillin, from 50%-100%. Similarly, rise in resistance was observed for cephalexin, 3GC, Cotrimoxazole and erythromycin. Antimicrobials like fluoroquinoles, clindamycin were observed to have low resistance.[9] Among the *Klebsiella* isolates, there was rise in the resistance to 3GC, Aminoglycosides and Fluoroquinolones, no strain resistant to carbapenem were isolated.[10,15]

This study revealed the change in the flora causing neonatal sepsis in this NICU. The emergence of resistance to the first line antimicrobial drugs was very much observed over the five years. Hence, the drug of choice for the empirical therapy needs to change. From this study, beta lactam- beta lactamase inhibitor combination like Piperacillin tazobactum was considered for empirical therapy. Vancomycin and Amikacin/ Meropenem can be considered in case of MRSA or MDR-GNB were suspected, respectively. But as it was a retrospective study, the clinical details, antibacterial therapy administered, duration and outcome was not able to be studied, which is the major drawback of the study.

CONCLUSION

Gram positive organisms, *Staphylococcus aureus* was the most common pathogen isolated in patients with neonatal septicaemia, followed by *Klebsiella sp.* among the Gram negative. There was rise in antimicrobial resistance to high level antimicrobials like 3GC, fluoroquinoles. This has helped us to know the flora causing neonatal sepsis and start appropriate empirical antimicrobial therapy.

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