

Emerging Antimicrobial Resistance in Neonatal Sepsis

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ABSTRACT

Objective: To determine the frequency and antimicrobial sensitivity pattern of microbial agents causing neonatal sepsis.

Study Design: Descriptive study.

Place and Duration of Study: Neonatal ICU, Fauji Foundation Hospital, Rawalpindi; Foundation University, Islamabad, from May 2017 to April 2019.

Methodology: Data of all neonates admitted with sepsis during study period was retrieved from computer database. Age at admission, gender, duration of hospital stay and culture reports were recorded. Culture positive patients were further analysed regarding their antibiotic sensitivity.

Results: A total of 1,070 neonates, male:female = 1.36:1, mostly newborn, were included in the study. Total mortality was 182 (17%). Blood culture was positive in 79 (7.4%). Gram positive organisms were identified in 37 (46.8%) *Staphylococci* in 29 (36.7%), *Enterococci* 7 (8.9%), *Corynebacterium* species in 1 (1.3%). Gram negative were isolated in 42 (53.2%) *Acinetobacter* Baumannii in 14 (17.7%), *Klebsiella* in 12 (15.2%), *Enterobacter* spp. in 7 (8.8%), *E.coli* in 5 (6.3%), *Pseudomonas* in 2 (2.5%) and *Proteus* in 1 (1.3%) and *Serratia* in 1 (1.3%) each. Sensitivity pattern of Gram positive organisms was: vancomycin 30/37 (81.1%), ciprofloxacin 13/37 (35.1%) and Gentamicin 12/37 (32.4%). Gram negative organisms sensitivity pattern was: meropenem 12/42 (28.6%), chloramphenicol 10/42 (23.8%), gentamicin 6/42 (14.3%), ciprofloxacin 5/42 (11.9%). Highly resistant strains of *Klebsiella* (13/14) and *Acinetobacter* (5/12) were sensitive to colomycin only.

Conclusion: Common organisms responsible for neonatal sepsis were *Staphylococci*, *Acinetobacter*, *Klebsiella* and *E.Coli*. Gram positive organisms showed sensitivity to vancomycin and gentamicin. Gram negative organisms were highly sensitive to colomycin.

Key Words: Neonatology, Neonatal sepsis, Antimicrobial sensitivity, Neonatal mortality.

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INTRODUCTION

Neonatal sepsis is defined as a syndrome of hemodynamic and clinical manifestations caused by a pathogenic infective organism.^{1,2}

Neonatal sepsis is one of the major contributors to mortality in neonatal intensive care setup. Mortality rates as high as 14.6% to 36%, higher in neonatal sepsis associated with prematurity and low birth weight.³ In a study from Ghana, neonatal sepsis was responsible for 8.3% of total deaths in a neonatal intensive care setup.⁴

Causative organisms vary depending upon the onset of sepsis. In early onset, presenting within 72 hours of life, gram positive organisms were found to occur more frequently.

In data from American Neonatology Network, main etiology for early onset neonatal sepsis was *Streptococcus agalactiae* accounting for 43% of cases while *E.coli* accounts for 29% of cases.⁵ On the other hand, in late-onset neonatal sepsis patients, i.e. those presenting beyond 72 hours of life, coagulase negative *Staphylococci* and *Staphylococcus aureus* were predominantly encountered while *E. Coli* accounted for only 7% of cases.⁶

Additional problems are presented by the fact that microbial yield of most of the culture methods is limited. In one study, only 8.98% for all blood culture specimen taken from neonates with suspected neonatal sepsis were positive for a pathological organism.⁷ This makes the rational and empiric choice of antibiotics an extremely important determinant of outcome. The problem is compounded by rapidly emerging antimicrobial resistance. In a recent study from Nepal, the organisms causing neonatal sepsis were found to be highly resistant to common antibiotics like cefotaxime, gentamicin, ciprofloxacin and chloramphenicol. Good sensitivity was observed with carbapenems and colomycin for gram negative organisms and vancomycin for Gram positive organisms.⁸

Klebsiella is among the commonest Gram negative organisms causing neonatal sepsis in some of the recent studies. The source of these organisms may include delivery rooms, operating rooms or neonatal areas.^{9,10} Microbial pattern and antimicrobial sensitivity keeps on evolving and at times, varies between different locations and healthcare setups. This makes it necessary to review the treat-

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ment guidelines in accordance with the recent data. This study was conducted to determine the frequency of different microbial agents causing neonatal sepsis and determine their antimicrobial sensitivity pattern.

METHODOLOGY

This descriptive study was conducted in NICU of Fauji Foundation Hospital, Foundation University, Islamabad from May 2017 to April 2019. After obtaining ethical approval from the Institution Review Board, data of all neonates admitted in NICU during the study period was retrieved from computer database by consecutive non-probability sampling. Age at admission, gender, duration of hospital stay and culture reports were recorded. Culture positive patients were further analysed regarding their antibiotic sensitivity patterns. Antibiotic sensitivity was tested in a stepwise manner; sensitivity to common antibiotics was tested first and only highly resistant organisms were tested for sensitivity to antibiotics like colomycin.

All data was recorded and analysed using SPSS version 20. Descriptive variables including culture positivity, gram staining, gender, onset of neonatal sepsis, outcome and organism identified were

described as frequencies and percentages, while continuous variables including age and hospital stay were subjected to normality testing. As both variables were non-parametric, they were described as median and interquartile range. Data was divided into groups based upon the onset of sepsis (early onset within 3 days of life and late onset beyond 3 days of life), and culture positivity. Comparison between the groups was done using Mann Whitney-U test and Chi-square test for quantitative and qualitative variables respectively. P-value of less than 0.05 was considered significant.

RESULTS

A total of 1,070 neonates, males 617 (57.7%) and females 453 (42.3%), were entered into study after removing duplicates. Median age at time of admission was 0 days (newborn) with an interquartile range of 0 - 1.9 days. Blood culture was positive in 79 (7.4%) patients. Median duration of admission was 5.5 days with an interquartile range of 2.9 - 9.7 days. Total number of expired neonates was 182 giving an expiry rate of 17%.

Among all, 886 (82.8%) neonates presented with early onset neonatal sepsis (within 3 days of life) while 184 (17.2%) presented with late onset of neonatal sepsis (beyond 3 days of life).

Table I: Demographic Features of Study Population.

		Early presentation (<72 hours) (n=886)	Late presentation (>72 hours) (N=184)	p-value
Gender	Male	506 (57.11%)	111 (60.33%)	0.422*
	Female	380 (42.89%)	73 (39.67%)	
Organism identified	Gram +ive	25 (2.82%)	12 (6.52%)	<0.001*
	Gram -ive	16 (1.80%)	26 (14.13%)	
Expiry rate		141 (15.91%)	41 (22.28%)	0.036*
Duration of admission (days)	Median	4.7	10.4	<0.001 [#]
	IQR (Q1 - Q3)	2.6 - 8.3	5.8 - 17.6	

Chi-square test; [#]Mann-Whitney U-Test.

Table II: Gram positive organisms; Antibiotic Sensitivity (n=37).

Organism	No	Doxycycline	Chloramphenical	Vancomycin	Linezolid	Ciprofloxacin
MRSA	19	13 (68.42%)	14 (73.68%)	19 (100%)	16 (84.21%)	5 (26.31%)
MRSE	1	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0 (0%)
MSSA	8	6 (75%)	5 (62.5%)	6 (75%)	8 (100%)	5 (62.50%)
Coagulase negative <i>staphylococcus</i>	1	1 (100%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)
<i>Enterococcus spp</i>	7	2 (28.57%)	2 (28.57%)	3 (42.86%)	3 (42.86%)	1 (14.29%)
<i>Corynebacterium spp</i>	1	1 (100%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)
Total	37	24 (64.86%)	22 (59.46%)	31 (83.78%)	30 (81.08%)	13 (35.13%)

MRSA: Methicillin Resistant *Staphylococcus Aureus*; MRSE: Methicillin Resistant *Staphylococcus Epidermidis*; MSSA: Methicillin Sensitive *Staphylococcus Aureus*.

Table III: Gram negative Organisms; Antibiotic Sensitivity (n=42).

Organism	No.	Ciprofloxacin	Gentimicin	Meropenem	Colomycin	Chloramphenical
<i>Klebsiella</i>	12	2 (16.67%)	2 (16.67%)	3 (25%)	11 (91.67%)	2 (16.67%)
<i>Acinetobacter Baumannii</i>	14	0 (0%)	0 (0%)	0 (0%)	14 (100%)	1 (7.14%)
<i>E.coli</i>	5	4 (80%)	3 (60%)	4 (80%)	Not Tested	2 (40%)
<i>Enterobacter spp.</i>	7	0 (0%)	0 (0%)	1 (14.29%)	3/3 (100%)	5 (71.43%)
<i>Proteus</i>	1	0 (0%)	0 (0%)	1 (100%)	Not Tested	0 (0%)
<i>Pseudomonas</i>	2	1 (50%)	0 (0%)	2 (100%)	Not Tested	0 (0%)
<i>Serratia</i>	1	1 (100%)	0 (0%)	1 (100%)	Not Tested	0 (0%)
Total	42	8 (19.0%)	5 (11.9%)	12 (28.6%)	28/29 (96.55%)	8 (19.0%)

Age at onset was the most significant determinant of microbiological spectrum and outcome. Culture proven sepsis was much more frequent in neonates with late onset sepsis and they had significantly higher frequency of gram negative organisms and higher expiry rate. However, there was no difference of gender among late onset and early onset sepsis (Table I).

Blood culture was positive in 79 (7.4%). Gram positive organisms were identified in 37 (46.8%) out of 79 patients; *Staphylococci* in 29 (36.7%), *Enterococci* 7 (8.9%), *Corynebacterium* species in 1 (1.3%). Gram negative organisms were isolated in 42 (53.2%) patients; *Acinetobacter baumannii* in 14 (17.7%), *Klebsiella* in 12 (15.2%), *Enterobacter* spp. In 7 (8.9%), *E.coli* in 5 (6.3%), *Pseudomonas* in 2(2.5%), *Proteus* in 1 (1.3%) and *Serratia* in 1 (1.3%).

Penicillin, ampicillin, cephradine, cefotaxime, ceftazidime, cefoperazone/salbactam and aztreonam showed very high resistance pattern with 5% or less organism isolated having sensitivity to these antibiotics. Sensitivity pattern of Gram positive organisms was: vancomycin 30/37 (81.1%), chloramphenical 26/37 (70.3%), ciprofloxacin 13/37 (35.1%) and gentamicin 12/37 (32.4%) (Table II). Gram negative organisms showed meropenem 12/42 (28.6%), chloramphenical 10/42 (23.8%), gentamicin 6/42 (14.3%) ciprofloxacin 5/42 (11.9%). Highly resistant strains of *Klebsiella* and *Acinetobacter* showed a high sensitivity (100%) to colomycin, but were resistant to all other antibiotics tested. Out of total 14 strains of *Acinetobacter* isolated; 13(92.9%) were sensitive only to colomycin while among 12 strains of *Klebsiella* isolated 5 (41.7%) were sensitive to colomycin only (Table III). No organism showed complete resistant.

Overall mortality 182 (17%). There was no significant difference of expiry rate between culture-positive and culture-negative cases ($p = 0.89$). Among culture-positive sepsis patient, there was no significant difference of mortality between early onset and late onset sepsis ($p = 0.090$). Median hospital stay in culture-negative neonates was 5.3 days with interquartile range of 2.9 -9.1 days while culture-positive patients had a median hospital stay of 9.6 days with interquartile range of 4.0 - 17.6. The hospital stay was significantly longer in culture-positive patients ($p = <0.001$).

DISCUSSION

Neonatal sepsis is a very important cause of neonatal mortality that importantly is potentially treatable. Most of the patients in the study presented within 72 hours of life and had early onset sepsis with slight male predominance.

Culture positivity rate in the current study was 7.4%. The rate is low because all neonates were included in the study as blood cultures we sent for every neonate irrespective of clinical features suggestive of sepsis. Culture positivity rates reported in different studies are variable ranging from 19.56% to 48%.^{11,12} Gram-positive and Gram-negative organisms were encoun-

tered in 37 (46.8%) and 42 (53.2%) of cases. Most common organisms were *Staphylococcus aureus*, *Acinetobacter* and *Klebsiella* in descending order. Group B *Streptococcus* was not isolated in any case and *E. coli* was encountered in only 6.3% of culture positive cases. Similar bacteriological pattern was documented in the study done. in Nepal where *Klebsiella*, *Enterobacter* and *Acinetobacter* were the predominant Gram negative organisms and *Staphylococci* were the predominant gram positive organisms. Other studies have also reported similar results. In a meta-analysis of neonatal sepsis data from Pakistan, *Klebsiella* and *E. coli* were the predominant causative organisms.^{8,13,14}

Gram positive organisms showed a high sensitivity towards vancomycin and linezolid while gram negative organisms showed a high sensitivity towards colomycin. High resistance was observed towards most of the common antibiotics. These ominous findings are endorsed by the results of numerous contemporary studies.^{15,16} Duration of hospital stay was significantly higher in culture-positive patients which is self-explanatory.

Overall mortality was 17% and rate was significantly higher in neonates with late-onset sepsis. However, among culture positive sepsis patients, there was no significant difference of expiry rate in early-onset and late-onset sepsis. This outcome is comparable with studies from India, Nepal and Egypt.^{16,17}

Multiple local studies demonstrate the evolving etiological and antimicrobial sensitivity pattern of neonatal sepsis. A study published in 2012 shows a predominance of *Staphylococci* and *E.coli* with intermediate sensitivity to penicillins and third generation cephalosporins while aminoglycosides, fluoroquinolones, carbapenems and vancomycin showed high level of sensitivity and low resistance.¹⁸ A study from Peshawar published in 2016, showed a predominance of *E.coli* and *staphalococcusaureus* with high sensitivity towards carbapenems and fluoroquinolones.¹⁹ Both these studies showed a low frequency of *Klebsiella* and *Acinetobacter* was not reported in either study. A more recent study from Karachi has reported a high frequency of highly resistant gram negative bacteria responsive only to colomycin.²⁰

Bacteriological and antimicrobial sensitivity pattern is variable and rapidly changing. Colomycin resistance is also starting to emerge gradually as one organism reported in current study. There is a need for careful and continuous antimicrobial sensitivity surveillance and update of treatment guidelines accordingly. Development of newer antimicrobial agents is also need of the day.

CONCLUSION

Common organisms identified in our study were *Styphylococci*, *Acinetobacter*, *Klebsiella* and *E.coli*. Gram positive organisms showed sensitivity to vancomycin, chlormphenical and gentamicin. Gram negative organisms were highly sensitive to

colomycin, but only sparsely sensitive to meropenem, chloramphenicol and gentamicin. *Klebsiella pneumoniae* and *Acinetobacter* isolated were highly resistant to most of the antibiotics tested.

ETHICAL APPROVAL:

Ethical approval was obtained from the Institutional Review Board of Fauji Foundation Hospital Rawalpindi, before starting data collection.

PATIENTS' CONSENT:

As data was collected from the computer database, consent was not taken from the parents / guardians.

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

MAH: Data acquisition, data analysis, writeup.

MAL: Critical review.

KMT: Writeup.

AT, HSK, RA: Drafting, critical revision.

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