ORIGINAL ARTICLE

Neonatal Sepsis; Incidence and Microbiological Profile along with Antibiotic Sensitivity of Causative Microorganisms

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ABSTRACT

Objective: To find the incidence of neonatal sepsis, microorganisms involved and their antibiotic sensitivity among neonates admitted with neonatal sepsis during the study period.

Study Design: Prospective observational study.

Place and Duration of Study: The study was carried out at neonatal intensive care unit (NICU) of combined military Hospital (CMH), Kharian, Pakistan from January 2019 to December 2020.

Materials and Methods: After getting permission from Ethical Review Board of hospital, all the neonates who were admitted in neonatal intensive care unit (NICU) of hospital with neonatal sepsis were included in subject study. All the neonates with fever, irritability, difficulty in feeding, respiratory distress, fits, bulging fontanels, jaundice, bleeding etc. were suspected as case of "Neonatal sepsis". Venous blood sample of neonates was drawn under aseptic technique and collected in sterile BacT/ALERT (PF) blood collecting culture bottles and sent to laboratory immediately for culture, sensitivity procedure and reporting.

Results: A total of 4892 neonates were admitted in neonatal intensive care unit from January 2019 to December 2020. Out of these, 342 neonates were clinically diagnosed as cases of neonatal sepsis. Incidence of neonatal sepsis was 6.99%, comprising 185 males (54.1%) and 157 females (45.9%). The age range was from 2 days to 88 days with a mean age 35.47+23.40 days. *Staphylococcus aureus* was found to be the most commonly infectious microorganism (33.9%) followed by Candida species (18.5%), *Klebsiella* pneumoniae (14.5%) and Coagulase negative Staphylococci (12.3%). Non-fermenting Gram negative bacteria were found to be infecting 26 (11.5%) out of the total positive cases (*Acinetobacter baumannii* complex 8.4%, *Stenotrophomonas maltophilia* 3.1% and *Pseudomonas aeruginosa* 2.6%).

Conclusion: *Staphylococcus aureus* was the most prevalent organism found to be responsible for neonatal sepsis with majority being resistant to Methicillin. Methicillin-resistant Staphylococcus aureus (MRSA) is a 100% percent sensitive to vancomycin and Teicoplanin. On the other hand, Gram Negative organisms have shown promising sensitivity to Colistin.

Keywords: *Antibiotics, Microorganisms, Neonatal Sepsis, Sensitivity.*

How to cite this: *Hussain MA, Akram S, Khan MA, Nawaz S, Ali S, Amir M. Neonatal Sepsis; Incidence and Microbiological Profile along with Antibiotic Sensitivity of Causative Microorganisms. Life and Science. 2023; 4(1): 3-8. doi: http://doi.org/10.37185/LnS.1.1.248*

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Introduction

Sepsis is defined as immunological response of body to infection that can result in organ dysfunction and death.[1] Sepsis is associated with significant mortality and morbidity in children globally. It affects over 25 million children per year with approximately 3 million deaths in children, adolescents, and neonates worldwide.[2] Neonatal deaths comprises of 41% deaths in children less than 05 years old.[2] Neonatal sepsis is categorized into early onset and late onset neonatal sepsis. Early onset neonatal sepsis occurs within 72 hours of birth and late onset

Funding Source: NIL; Conflict of Interest: NIL
Received: Mar 13, 2022; Revised: Jun 15, 2022
Accepted: Dec 08, 2022

neonatal sepsis occurs after 72 hours of birth till 03 months of age.[3] The causative pathogen/micro-organism can be acquired via intrauterine, maternal flora, hospital, or community.[3] In early onset neonatal sepsis, maternal genital tract is the source of acquiring infection and premature rupture of membranes, prolonged rupture of membranes (>18 hours), urinary tract infection and caesarean sections are associated with increased risk.[4] Important risk factors for late onset neonatal sepsis include low birth weight, prematurity, prolonged rupture of membranes, neonatal resuscitations, lack of breast-feeding, invasive procedures (mechanical ventilation, intravascular catheterization), surgery, any cardio-vascular/respiratory disease and long hospital stay etc.[4] Microorganisms responsible for early onset neonatal sepsis are transmitted vertically in the majority of cases and those responsible for late onset are mostly acquired from the surrounding atmosphere.[5] The pathogenic microorganisms involved in neonatal sepsis vary from country to country.[6] From the reported literature it is evident various Gram Negative and Positive strains such as *Klebsiella* spp, *Pseudomonas aeruginosa*, *Staphylococcus* spp. including Methicillinresistant *Staphylococcus aureus* and *E. coli* predominates in developing countries.[7]

In our country, neonatal sepsis continues to be the most common cause of newborn morbidity and mortality. The data of incidence of neonatal sepsis is lacking from many countries.[8] In our country, incidence of neonatal sepsis has been reported to vary from 1.1 to 3.8 per 1000 live births.[9] In underdeveloped nations, it is the most frequent reason for neonatal hospitalization's to intensive care units.[10] It is for this reason we have carried out this study to find incidence and microorganisms responsible in neonatal sepsis in the neonatal intensive care unit of CMH, Kharian.

Materials and Methods

The study was carried out at neonatal intensive care unit (NICU) of CMH, Kharian, Pakistan from January 2019 to December 2020. This was a prospective observational study. After getting permission from the Ethical Review Board of the hospital, all the neonates who were admitted in neonatal intensive care unit (NICU) of hospital with neonatal sepsis were included in the study. Proper consent was taken from the parents for the subject purpose.

All the neonates with fever, irritability, difficulty in feeding, respiratory distress, fits, bulging fontanels, jaundice, bleeding etc. were suspected as case of "Neonatal sepsis". They were admitted to NICU.

Sterile BacT/ALERT (PF Plus) blood culture bottles were used for the purpose of culturing blood specimen from study population. 80% ethyl alcohol was applied to the rubber stoppers of the culture bottles and allowed to dry for 1 minute before inoculation of the specimen. A venous blood sample of neonates was drawn under aseptic technique using successive application of 80% ethyl alcohol and tincture of iodine over the veni-puncture site before blood collection. 1-3 ml of venous blood was drawn for each culture bottle and delivery to microbiology laboratory was ensured within 1 hour of collection for timely incubation. Blood culture bottles were loaded in BacT/ALERT 3D automated blood culture system for incubation and growth detection as per the manufacturer's instructions for a maximum incubation duration of 7 days after which all culture bottles which showed no growth were marked negative and were not processed further. Culture bottles exhibiting positive microbial growth during incubation were further sub-cultured on Blood, MacConkey and Chocolate agar plates. Gram staining was used to presumably identify the isolates. Further biochemical testing for Catalase, coagulase or oxidase production, API 10S profile, bile aesculin agar growth characteristic etc. were used depending on colony morphology and staining results to precisely identify the isolate. CHROMagar Candida and API 20C aux were used for yeast identification. Sensitivity testing was performed following the Disk Diffusion technique on Mueller Hinton agar as described in CLSI 2018-20 M100 document. Each isolate received a 0.5 McFarland standard colony suspension for disc diffusion testing on Mueller Hinton Agar with a selective antimicrobials. 90mm disposable Agar plates were used for testing antimicrobial sensitivity and a maximum of 6 antibiotic disks were applied on each agar plate maintaining a distance of 24mm between the centers of neighboring disks. Culture plates were

incubated for 18-24 hours at 35 ^{0}C. Since possibility of multi-resistant bacterial isolates from several blood specimens was anticipated so, both primary testing panel of antibiotics as well as supplemental antimicrobial agents as stated in Table 1A of CLSI document M-100 for the respective year (2018-20) for each particular isolate were tested.[1] Sensitivity of each of the isolate for the tested antibiotic was determined through measurement of the diameter of zone of inhibition as per CLSI guidelines and assigned a sensitive, intermediate or resistant category according to the reference criteria given in CLSI document M-100 for the respective bacterial group:

Bacterial Group Details	
Bacterial Group	**Sensitivity interpretation reference**
Enterobacterales	Table 2A CLSI document M-100
Pseudomonas aeruginosa	Table 2B -1 CLSI document M - 100
Acinetobacter species	Table 2B -2 CLSI document M - 100
Stenotrophomonas maltophilia	Table 2B -4 CLSI document M - 100
Staphylococcus species	Table 2C CLSI document M-100
Enterococcus species	Table 2D CLSI document M-100

Sensitivity of Gram Negative bacterial strains against Colistin (Polymyxin E) were tested using CHROMagar COL-APSE. FDA approved interpretive criteria were followed for assigning sensitivity of the bacteria against Tigecycline. Sensitivity results of bacteria against Doxycycline, Tigecycline and Minocycline were withheld at the lab since these agents are unsafe for use in the tested population (neonates) and mostly contributed to the epidemiological data. MICs (minimum inhibitory concentration) were performed using E-test strips for determining the sensitivity of Staphylococci against Vancomycin and Teicoplanin.

The data including age, gender, microorganism isolated from blood culture and their antibiotic sensitivity were entered in statistical package for social sciences (SPSS) 21 and analyzed. Percentages were used to express frequencies.

Results

A total of 4892 neonates were admitted in neonatal intensive care unit from January 2019 to December 2020. Out of these, 342 neonates were clinically diagnosed as cases of neonatal sepsis. The incidence of neonatal sepsis was 6.9% of the total admitted cases. There were 185 males (54.1%) and 157 females (45.9%). The age range was from 2 days to 88 days with a mean age 35.47+23.40 days. Upon blood culture, 227 (66.4%) patients exhibited positive growth. Based on positive blood culture results, bacteria were found to be the cause in 185 (81.5%) of the sepsis cases, while the rest 42 (18.5%) were caused by various Candida species. Gram positive constituted 59.5% of the isolated bacteria whereas 40.5% were caused by Gram negative (Fig 1).

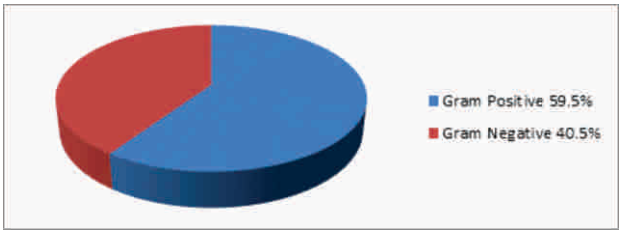

Fig 1: Gram Positive and Negative Percentage (%)

Staphylococcus aureus was found to be the most common bacterial strain (33.9%) followed by Candida species (18.5%), *Klebsiella pneumonia* (14.5%) and Coagulase negative Staphylococci (12.3%). Non-fermenting Gram negative bacteria were found to be infecting 26 (11.5%) out of the total positive cases (*Acinetobacter baumannii* complex 8.4%, *Stenotrophomonas maltophilia* 3.1% and *Pseudomonas aeruginosa* 2.6%) as shown in Table 1.

Table 1. Frequency of microorganisms involved in Neonatal Sepsis

S.No	Microorganism	N	Percentage
1	*Staphylococcus aureus*	77	33.9%
	1. *MRSA (Methicilin resistant staph aureus)*	48	
	2. *MSSA (Methicillin sensitive staph aureus)*	29	
2	Candida species	42	18.5%
3	*Klebsiella pneumoniae*	33	14.5%
4	Coagulase negative Staphylococci	28	12.3%
5	*Acinetobacter baumannii*	19	8.4%
6	*Stenotrophomonas maltophilia*	7	3.1%
7	*Pseudomonasaeruginosa*	6	2.6%
8	*Enterococcus faecalis*	5	2.2%
9	*Enterobacter cloacae*	4	1.8%
10	*Klebsiella oxytoca*	3	1.3%
11	*Serratia marcescens*	1	0.4%
12	*Escherichia coli*	1	0.4%
13	*Citrobacter freundii*	1	0.4%
	Total	227	100%

There was one culture each of *Serratia marcescens*, *Escherichia coli* and *Citrobacter freundii*. Out of 77 isolates of *Staphylococcus aureus* (62.3%) 48were methicillin resistant (MRSA). Methicillin resistance was observed in 46.4% of the isolated strains of Coagulase Negative Staphylococci.

The sensitivity of Gram-positivestrainsto various antibiotics have been shown in Table 2. Methicillin Resistant *Staphylococcus aureus* has 100 percent sensitivity to *Vancomycin* and Teicoplanin. Methicillin Sensitive *Staphylococcus aureus* has promising sensitivity to many antibiotics including Amikacin, Amoxacillin/clavulanic acid, cefepime and ciftriaxone etc. Table 2 also comprises of sensitivity patterns of all other strains.

Similarly, Table 3 represents sensitivity of Gram-negative strains to various antibiotics. They had excellent sensitivity to Colistin. Sensitivity to other antibiotics varied from strain to strain.

Table 2 : Sensitivity pattern of Gram Positive microorganisms implicated in Neonatal sepsis

	Methicillin Resistant *Staphylococcus aureus*	**Methicillin Sensitive *Staphylococcus aureus***	**Coagulase *Negative Staphylococcus spp***	***Enterococcus faecalis***
Amikacin	83.3%	100%	92.8%	-
Amoxicillin/Clavulanic acid	0%)	100%	46.4%	-
Ampicillin	0% (IR)	3.4%	7.1%	80%
Cefepime	0% (IR)	100%	46.4%	-
Ceftriaxone	0% (IR)	100%	46.4%	-
Ciprofloxacin	10.4%	31.03%	50%	-
Co-trimoxazole	43.7%	55.2%	71.4%	-
Doxycycline	75%	58.6%	64.28%	25%
Gentamicin	39.6%	68.9%	82.1%	-
Imipenem	0% (IR)	100%	46.4%	50%
Levofloxacin	-	-	-	60%
Meropenem	0% (IR)	100%	46.4%	-
Minocycline	91.6%	93.1%	100%	-
Moxifloxacin	32.2%	100%	72.7%	-
Sulbactam-Cefoperazone	0% (IR)	100%	46.4%	-
Tazobactam -piperacillin	0% (IR)	100%	46.4%	-
Tigecycline[2]	100%	-	81.8%	100%
Cloxacillin	0% (IR)	100%	46.4%	-
Cephradine	0% (IR)	100%	46.4%	-
Clindamycin	45.8%	79.3%	71.4%	-
Fusidic Acid	23.5%	55.2%	46.4%	-
Erythromycin	14.6%	68.9%	39.3%	-
Linezolid	97.9%	96.5%	90.9%	100%
Rifampicin	58.8%	100%	57.1%	-
Teicoplanin	100%	100%	72.7%	100%
Vancomycin	100%	100%	100%	100%
Chloramphenicol	93.5%	-	-	80%

Discussion

Neonatal sepsis is a life-threatening condition, warranting prompt diagnosis and treatment. Delay in treatment can cost lives. Empirical anti-microbial should be started without delay to halt progression of sepsis. Empirical treatment is given, keeping in view the prevalent strains in the region/hospital. Spectrum of strains involved in neonatal sepsis varies from region to region and over the period.[10]

In this study, we have reported 6.9% incidence of neonatal sepsis of all the neonatal ICU admissions in CMH kkharian hospital. This incidence was almost similar to neonatal sepsis in an Iranian tertiary care hospital where 6.4% incidence have been reported.[10] On the other hand, it is quite less as compared to Getabele*et al,* reported incidence of neonatal sepsis 77.9% (65% for early onset and 35% for late onset neonatal sepsis) in Ethopia.[11] Fleischmann C and colleagues carried out systemic review and meta-analysis of 26 researches regarding incidence of neonatal sepsis and reported 2824 cases of sepsis

Table 3: Sensitivity pattern of Gram -Negative microorganisms implicated in Neonatal sepsis

	Klebsiella pneumoniae	*Acinetobacter baumannii*	*Stenotrophomonas maltophilia*	*Pseudomonasaeruginosa*	*Enterobacter cloacae*	*Klebsiella oxytoca*
Amikacin	15.1%	10.5%	0% (IR)	33.3%	75%	100%
Amoxicillin-Clavulanic acid	11.1%	0% (IR)	0% (IR)	-	0% (IR)	0% (IR)
Ampicillin	0% (IR)	0% (IR)	-	-	0% (IR)	0% (IR)
Aztreonam	12.1%	0% (IR)	-	16.7%	100%	100%
Cefepime	12.1%	10.5%	-	50%		100%
Ceftazidime	6.1%	5.3%	-	33.3%	50%	100%
Ceftriaxone	6.1%	10.5%	-		25%	100%
Ciprofloxacin	15.1%	10.5%	-	66.7%	50%	100%
Colistin[1]	81.8%	94.7%	-	100%	100%	100%
Co-Trimoxazole	33.3%	31.6%	85.7%		50%	100%
Doxycycline	18.2%	47.3%	-		25%	100%
Gentamicin	6.1%	15.8%	0% (IR)	33.3%	75%	100%
Imipenem	12.1%	15.8%	0% (IR)	33.3%	100%	100%
Levofloxacin	30.3%	26.3%	85.7%	66.7%	100%	100%
Meropenem	18.2%	10.5%	0% (IR)	33.3%	100%	100%
Minocycline	15.1%	58%	71.4%		-	-
Moxifloxacin	24.2%	-	-	33.3%	-	
Sulbactam-Cefoperazone	9.1%	52.9%	-	66.7%	75%	100%
Tazobactam - Piperacillin	12.1%	23.5%	0% (IR)	66.7%	100%	100%
Tigecycline[2]	48.5%	29.4%	-		50%	100%
Tobramycin	-	-	-	33.3%		

1. Using CHROMagar COL-APSE, 2. FDA-Identified Interpretive criterion, IR = Intrinsic resistance, the cells left blank show the organisms were not tested against these antibiotics

per 100000 live births.[12] Nyma *et al.* reported incidence of neonatal sepsis 69.35% in Dhaka, Bangladesh.[13] The incidence of neonatal sepsis in Rawalpindi (Pakistan) has been reported 29.5%.[14] The incidence of neonatal sepsis in our study is less as compared to the aforementioned reported data.

In our research, we found *Staphylococcus aureus* to be the most common strains (33.9%)responsible for in neonatal sepsis followed by Candida species (18.5%), *Klebsiella pneumonie* (14.5%) and Coagulase negative Staphylococci (12.3%). Ahmed *etal*, in their study, found that Gram negative bacilli (Escherichia coli) is the most common strain cultured in 42% of neonates followed by Gram negative rod (Klebsiella pneumoniae).[14]*Staphylococcus aureus* was also reported as the most common strain by Braima et al.[15]According to El-Jadba *et. al*. most frequent isolate cultured from neonates with sepsis wereGram positive (Coagulase negative Staph) in 57.3% of cases, followed by *E. coli* (10.4% cases), Klebsiella spp (8% cases), Pseudomoanasaeeruginosa (5.8% cases) and streptococcus viridians in 3.4% cases.[16]

High prevalence of Methicillin resistance among Staphylococcus aureus (62.3%) and coagulase negative Staphylococcus (46.4%) was observed in our study which is in agreement with the reported literature.[15,17]Bassetti et al have also reported a remarkably high frequency of MRSA (94%) in two Italian University hospitals.[18] The reasons/risk factors for high Methicillin resistance have been related to low birth weight, asphyxia at birth, prematurity, umbilical catheterization, mechanical ventilation, and many other yet unknown factors.[15] Few researchers also have attributed the high prevalence of *Staphylococcus aureus* among neonates to vertical transmission from infected mothers.[19]

Conclusion

The study has shown various bacterial strainscausing neonatal sepsis and their sensitivity to antibiotics. *Staphylococcus aureus* is the most commonstrainsobserved in neonatal sepsis with majority being resistant to Methicillin. MRSA is 100% percent sensitive to vancomycin and Teicoplanin. On the other hand,Gram negative strainshave shown promising sensitivity to Colistin. To counter the grave problem of antibiotic resistance, we propose strict measures to control infections, use above aforementioned antibiotic according to suspected strainsand carry out culture sensitivity investigation without hesitation in sick neonates.

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