ORIGINAL ARTICLE

Frequency and Antimicrobial Sensitivity Spectrum of Bacterial Pathogens Responsible for Neonatal Sepsisat Sir Ganga Raam Hospital, Lahore

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ABSTRACT

Background: Neonatal sepsis is a life threatening problem in neonates which is one of the major contributor of neonatal mortality especially in developing countries.The aim of our study is to enlist the organisms predominantly responsible for neonatal sepsis in our hospital and todeterminetheirantimicrobialspectrumofsensitivity. Surveillance should be conducted shortly apart (every 3-6 months) to update the causative organisms and their sensitivity spectrum in particular localities.The administration of organism specific antibiotics will be helpful in decreasing the number of multidrug resistant strains which are spreading due to the administration of broad spectrum antibiotics, being given to patients on empirical grounds.

Aims & Objective: The aim of our study is to determine the bacterial pathogens commonly responsible for neonatal sepsis in our setup and to determine their antimicrobial spectrum of sensitivity. This will help us deciding which antibiotic should be administered empirically to the patients presenting to us with neonatal sepsis, when a culture report is awaited.

Study Design: Cross-sectional observational study.

Setting: Microbiology Laboratory of Fatima Jinnah Medical University, Lahore.

Duration of Study:1st August 2015 to 29th February 2016, (six months).

Subjects and Methods: Study was conducted on342blood culturesamples. These sampleswereculturedandafter confirmingtheoffendingagentitsantimicrobialsusceptibilitypatternwasalso determined.

Results:Outof342bloodculturesamplesreceived,117werepositiveforgrowth.Of117 positivecultures,37samples(32%)showgrowthofCitrobacterspecies,20(17%)ofStaphylococcus aureusand17(15%)ofKlebsiellaspecies.

Conclusion:Thecommonest organism responsible for sepsis in neonates in our setup is Citrobacterspecies,secondmostcommonbeingStaphylococcusaureusandthirdoneisKlebsiella species.

Key words:Neonatal Sepsis, Blood culture, Citrobacterspecies, Staphylococcus aureus, Klebsiellaspecies, Multidrugresistance.

INTRODUCTION

Neonatals epsis previously known as sepsis neonatorum is definedas "Neonatal infection occurring in the first 28 days of life".[1] It is consider edone of the major cause of mortality among neonates through out the world, especially in developing countries.[1]

Neonatal sepsis contributes to approximately 30-50% of neonatal deaths each year.[2] It has been estimated that up to 20% of neonates develop sepsis and approximately 1% of them die because of it and associated complications[2].

Neonatal sepsis refers to the presence of systemic infection in neonates including septicemia, pneumonia, meningitis, arthritis, osteomyelitis, and urinary tract infection.[2]

Neonatal sepsis is categorized according to the postnatal age of the infant at the onset of the disease. Early onset sepsis i.e. less than 72 hours and late onset sepsis i.e. greater than 72 hours.[3]

The signs and symptoms of newborns presenting with neonatal septicemia are nonspecific. These patients can present with hyperthermia or hypothermia, respiratory distress either cyanosis or apnea, difficulties in feeding,

hypotonia or lethargy, irritability or seizures, there may be increased intracranial pressure manifested as bulging fontanelle, cold extremities due to poor perfusion, bleeding problems, distended abdomen, hepatomegaly, unexplained jaundice or any other nonspecific symptom.[4]

The highest rate of neonatal sepsis has been observed in low birth weight newborns especially in those having severe birth asphyxia and maternal complications like toxemia of pregnancy, precipitous delivery, maternal Infections and intra partum hemorrhage.[4]

The current practice is to perform a complete workup of baby with the clinical suspicion of neonatal sepsis including laboratory investigations such as direct analysis and culture of blood samples taken from the patients. Of these three, cultures are considered a gold standard.[5]

Of cultures, blood culture is usually performed to isolate the causative organism responsible for neonatal sepsis as most of the blood cultures show growth confirming sepsis usually in 24-72 hours.[5]

Most frequent organisms associated with early onset neonatal sepsis are Coagulase-negative Staphylococcus, Escherichia coli, Group B Streptococcus, Listeria Monocytogensand Haemophilus influenzae[5] and those frequently responsible for late onset neonatal sepsis are Staphylococcus aureus, Coagulase negative Staphylococcus, Pseudomonas species, Enterobacter, Escherichia coli, Klebsiella species, Group B Streptococcus, Serratia species, Acinetobactersp. and Anaerobes.[5]

Day by day the rate of Neonatal sepsis is increasing alarmingly, adding up to the burden on health care service sowing to lack of proper antenatal care and follow-up. Lack of aseptic delivery practices especially those conducted at home by untrained birth attendants without following precautions and protocols such as cutting and ligation of umbilical cord under septic conditions are resulting in rapid increase in the number of cases of neonatal septicemia being admitted in hospitals.[5]

The purpose of our study is to identify pathogenic bacteria responsible for neonatal septicemia in our setup and to determine their antimicrobial susceptibility spectrum for lining up the treatment strategies for specific prevalent organisms.

The bacterial pathogens responsible for causing sepsis in neonates are acquired intrapartumeither directly from mother's blood, skin, birthcanal or from the surroundings in which delivery is being conducted. (5) Many researches have been conducted to determine the pattern and frequency of hospital acquired infections in tertiary care settings, but a limited workup has been done to find out the organisms being responsible for causing septicemia in Pakistan during the last decade.[6]

Furthermore, broad spectrum antibiotics administrated to overcome this life threatening condition is resulting in rapid emergence of multidrug resistance among the causative agents adding up to the difficulty in patient management. Unfortunately, Pakistan is ranked eighth most common country having greatest number of newborn deaths each year of which neonatal sepsis is a major contributor.[7] An important factorresponsible for this is lack of availability of microbiological diagnostic facilities. This is why most of the pediatricians and physicians have to prescribe broad spectrum antibiotics to save life of the new bornby treating them on empirical grounds.[7]

Many studies have been published about an increase in antimicrobial resistance among bacterial pathogens responsible for blood stream infection as its incidence is increasing day by day. This is because of the wide spread use of antibiotics empirically.[8] It has resulted in decreased therapeutic options available for the health team to overcome this disease.[9] An observational study conducted in Israel highlighted the fact that among the patients of neonatal sepsis, death rate was particularly higher for those who received inappropriate empirical treatment.[10]

Hence concluded that detection of pathogens responsible for blood stream infections should be considered a priority in our clinical settings. For this purpose blood culture is considered a gold standard.[11]

More than 4 million neonatal deaths have been reported each year by World Health Organization (WHO), out of which about 3 million were those of neonates.[7] Neonatal deaths caused by sepsishave been more common in developing countries like Pakistan.[12,13] Approximately 500 neonatal deaths have been reported in Pakistan every day, the neonatal mortality rate being 54/1000 live births; as described by UNICEF (2009).[7]

MATERIALS AND METHOD
Our study was across-sectional observational study, carried out on 342 blood samples received

with the clinical diagnosis of Neonatal sepsis from Neonatology ward of Pediatric units of Sir Ganga Raam Hospital during 1st of August 2015 to 29th February 2016 at the Department of Microbiology, Fatima Jinnah Medical University, Lahore.

A blood sample of 5ml is taken through venipuncture under aseptic measures by swabbing and drying the patient's skin. The sample is then added to a pre-prepared (filled with 50 ml of the medium i.e.10 times that of the sample) and sterilized 125ml blood-culture bottles. These bottles were incubated at 35–37 ºC and were inspected 2 times a day for following 3 days to look for any signs of bacterial growth. Those blood cultures in which growth was observed by any the evidence like an amorphous deposit on top of the blood layer, sub-surface or diffuse turbidity, hemolysis, coagulation of media, pellicle formation , gas formation or presence of white grain deep in the blood or on the surface were separated from those in which no signs of growth were seen. Asterile culture is the one which usually show sediment of red blood cells on the top of the culture media with an underlying pale yellow clear liquid media. Blood culture samples with visible signs of growth were observed for the presence of microorganisms by preparing a Gram-stained smear and examining it under 10X, 40X and 100X.

Positive blood samples were than cultured on appropriate sterile agar plates. The culture media we used in our study were blood agar, chocolate agar and MacConkey agar.

The bacteria isolated were further classified biochemically using Triple Sugar iron, Citrate Utilization test and Indoletest. At the end sensitivity was tested for Ceftrioxone, Ampicillin, Amoxicillin, Chloramphenicol, Tetracycline, Vancomycin, Cephradin, Penicillin, Aztreonam, Fosfomycin, Kanamycin, Imipenem, Sulphamethoxazole and Ciprofloxacin by culturing the organism on nutrient agar and using the afore mentioned antibiotic discs. After 24 hours of incubation biochemical test results were interpreted and microorganisms were identified as well as the antibiogram was reported by analyzing the sensitivity spectrum for the applied antibiotics.

Re-inoculation was done for slow growers after 10 days. Antibioticsusceptibity of the isolates was then assessed and reported to guide the clinician regarding the choice of antibiotics for a particular case.

RESULTS

A total of 342 samples with the clinical suspicion of neonatal sepsis were submitted to Microbiology Department. Out of 342 samples 117 (34%) were positive for growth whereas 225 (66%) samples did not show any growth.**(Figure: 1)**
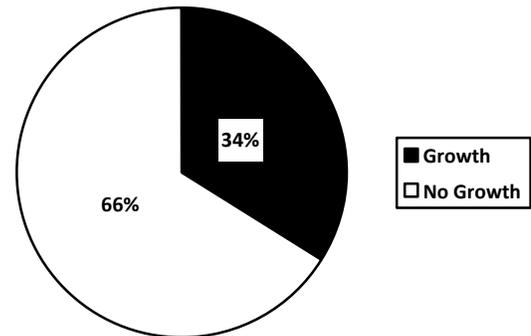
Figure 1: Proportion of Blood cultures positive for bacterial growth.

Out of 117 culture positive isolates, 37 samples (32%) showed growth of Citrobacter species, 20 (17%) of Staphylococcusaureusand 17 (15%) of Klebsiella species. Hence in our study the most common bacterial pathogen being responsible for neonatal sepsis was Citrobacter species, second most common being Staphylococcus aureus and third one Klebsiella species.**(Figure: 2)**
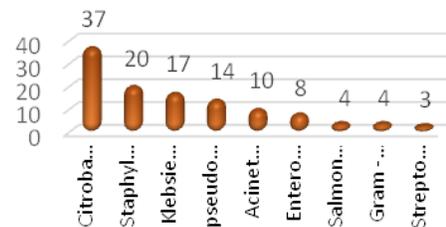
Figure 2: Frequency of bacterial pathogens responsible for neonatal septicemia.

Regarding antimicrobial spectrum of sensitivity Citrobacter species, showed 76% sensitivity to chloramphenicol, Staphylococcusaureus showed 60% sensitivity to chloramphenicol and Klebsiellaspecies showed 47% sensitivity aztreonam.(Table 1).

Bacterial Pathogens	Antibiotics													
	CTX	AMP	AMX	C	TE	VA	CE	P	AT	F	K	IME	STX	CIP
Citrobacter species.	2.7%	5.4%	0%	75.6%	21.6%	13.5%	0%	0%	56.7%	16.2%	8.1%	24.3%	10.8%	0%
Staphylococcus aureus.	0%	30%	35%	60%	15%	50%	20%	15%	20%	10%	25%	5%	0%	0%
Klebsiella species.	17.6%	29.4%	29.4%	41.1%	23.5%	11.7%	29.4%	0%	47%	5.8%	29.4%	11.7%	5.8%	23.5%
Pseudomonas aureginosa	7.1%	0%	0%	14.2%	0%	0%	0%	0%	35.7%	0%	14.2%	28.5%	0%	7.1%
Acinetobacter sp.	0%	0%	10%	50%	20%	0%	10%	0%	40%	10%	10%	10%	10%	10%
Enterobacter sp.	12.5%	0%	0%	0%	25%	12.5%	0%	0%	25%	12.5%	12.5%	25%	0%	0%
Salmonella sp.	0%	25%	25%	50%	0%	0%	25%	0%	25%	0%	0%	25%	0%	0%
Gram –ive cocci	0%	25%	50%	100%	50%	75%	50%	0%	50%	0%	75%	50%	0%	0%
Streptococcus sp.	0%	0%	0%	0%	0%	0%	0%	0%	33.3%	0%	0%	0%	0%	0%

Table 1: Antibiotic spectrum of sensitivity of Bacterial Isolates.

CTX	Ceftrioxone (30µg)
AMP	Amplicillin (10µg)
AMX	Amoxicillin (25µg)
C	Chloramphenicol (30µg)
TE	Tetracyclin (30µg)
VA	Vancomycin (30µg)
CE	Cephradin (30µg)
P	Penicillin 10 U
AT	Aztreonam (30µg)
F	Fosfomycin (50µg)
K	Kanamycin (30µg)
IME	Imepnam (10µg)
SXT	Trimethoprim+ Sulphamethoxazole (1.25 + 23.75µg)
NA	Ciprofloxacin(50µg)

DISSCUSSION

Neonatal Sepsis is a life threatening disease of neonates all over the world.[14] It is one of the medical emergencies which if not treated properly on time can have serious consequences.[15] Thus its empirical treatment must comprise of a combination of drugs which can cover the bacterial pathogens most commonly responsible for neonatal sepsis in a particular location.[15] Time to time surveillance is required to identify the common pathogens being responsible for it as well as their antimicrobial sensitivity spectrum to overcome this problem.

Neonatal septicemia is a clinical ailment which is characterized by signs of circulatory compromise such as poor peripheral perfusionusually

characterized by pale or cyanosed skin, hypotonic and poorly responsive lethargic baby.[5] Before the development and establishment of antibiotics administration protocols, neonatal sepsis was life threatening. Even now mortality rates among infants who are treated with antibiotics are 5% to 60%, having disproportionate distribution worldwide being the highest in developing countries.[5]

According to a statistical data provided by World Health Organization (WHO) about 1 million neonates die each year because of neonatal sepsis, of which 42% die in their first week of life.[5] A major factor responsible for a marked difference between the rates of neonatal sepsis in developing and developed countries is disparities in neonatal care, health facility infections and home conducted deliveries in septic environment.[16] Premature infants are at greater risk. One of the main reason being reported especially in developed countries is increased admissions of premature babies who are at a greater risk of acquiring hospital infections most of which being caused by multi drug resistant bacterial pathogens[16].

Our study results are somewhat similar (regarding second an third most prevalent organism) to a study conducted in a private hospital in Festac town, Nigeria, which showed that Klebsiellaspecies (34.3%) is the most common Gram-negative bacteria while Staphylococcus aureus(28.1%) is the commonest Gram-positive bacteria associated with neonatal septicemia. But it differs regarding the most common organism which in our study is Citrobacter sp. The sensitivity spectrum results are also different as in this study Klebsiellaspecieswas found to beresistant to chloramphenicol.[17]

A studied conducted in Rawalpindi showed that the most common Gram positive pathogen responsible for sepsis in neonates is Staphylococcus species (47.7%) which is similar to our results, while Acinetobacter, Pseudomonas sp. (43), E. coliand Salmonella sp. (30) were the most common Gram negative isolates[11], which is in contrast with our results.

A research conducted in Peshawar showed that Escherichia coli was the most common organism (36.6%), Staphylococcus aureus (29.5%) being the second most common followed by Pseudomonas species (22.4%), Klebsiellaspecies (7.6%), and Proteus (3.8%).[18] The results differs from ours regarding the prevalence of E.coli but show similarity regarding the prevalence of

Staphylococcus aureus. In their study Pseudomonas sp. and E.coli showed a great degree of resistance to commonly used antibiotics (augmentin, gentamicin andampicillin) similar to our study in which pseudomonassp. showed high resistance to cephalosporins and sensitivity to aztreonam and imipenem. In their study Staphylococcus aureus showed a low resistance to all of the three antibiotic groups[18] which is in contrast to our results in which Staphylococcus showed high resistance to cephalosporins and sensitivity to chloramphenicol and vancomycin. (18)

A study carried out in Department of Pediatrics, Holy Family Hospital, Rawalpindi Medical College included 50 newborns with diagnosis of neonatal sepsis. The predominant microorganisms isolated were gram negative (84%), with Enterobactersp. as the most common organism (48%) followed by E. coli (16%), Klebsiellasp. (14%) and Pseudomonassp.(6%). Among gram positive organisms the most common organism was Staphylococcusaureus(10%) and the second one is Streptococcuspneumoniae (6%). (19) These results are partially similar to ours in which Klebsiella sp. were isolated in 17% cases and were also the third most common organism. In this study Staphylococcsaureus causes only 10% cases of neonatal sepsis.[19]

A study carried out at the Department of Pediatrics, Post Graduate Medical Institute, HayatabadMedicalComplex, Peshawar, showed that among 140 cases of culture proven sepsis, gram-negative organisms were more common in neonatalsepsis (75%) which is in contrast with our results. Escherichia coli (44.3%) was the commonest followed byStaphylococcusaureus (26.3%), Klebsiella sp. (18.6%) and Pseudomonassp. (12.1%).Escherichia coliwas sensitive to Ciprofloxacin (93.5%), Cefipime (83.9%) and Amikacin (74.2%). Staphylococcus aureusalso show sensitivity toCiprofloxacin (81.8%), Cefipime (75.8%) and Amikacin (66.7%). The sensitivity spectrum also differs from ours in which most of the gram positive and negative organisms were sensitive to chloramphenicol, Aztreonam and Vancomycin.[20]

A study carried out at a Tertiary care Hospital of Nepal showed that neonatal sepsis was 20.3% prevalent in their hospital.[21] The predominant isolates in their study were Gram positive cocci(88.40%) which is in contrast to our study. In their study the most common gram positive

organism isolated was Staphylococcus epidermidis(72.46%) the second one being Staphylococcus aureus (7.24%), third one Staphylococcus saprophyticus (4.34%) and Enterococcus fecalis as the fourth most common isolate (4.34%).[21] This is also in contrast to our study in which the most predominant gram positive species was Staphlococcusaureus. Approximately 11.60% positive culture samples showed growth of Gram negative bacilli especially E.coli10.14% and Klebsiella species1.44%.[21] It also differs from our study. Sensitivity of their isolates was highest for Amikacin. In their study Vancomycinwas the drug to which most of the gram positive pathogens were sensitive which resemble our results for positive isolates.

Regarding protection against this deadly disease early breast feeding is of prime importance. The initiation of breast feeding within first twenty four hours of birth is known as early breast feeding. Its benefits have been known for years which are now confirmed.[22]

Analysis of maternal risk factors (especially intrapartum) revealed a considerable association between maternal factors and neonatal sepsis. Some of these factors are maternal urinary tract infection, pyrexia and vaginal infections. Newborns of these mothers had significantly lowAPGAR scores and most of them need to be intubated immediately after birth. Analytical data suggests the possibility of vertical transmission as well as horizontal transmission of infection postnatally.[23]

Today a major concern in treating neonatal sepsis is an increased incidence of antibiotic resistance among bacterial pathogens which is mainly effecting the treatment of ill neonates empirically. It highlights the need for continuous microbiological surveillance in all clinical and hospital setups.[11]

CONCLUSIONS & RECOMMENDATIONS

As in the past, neonatal sepsis is stilla leading cause of neonatal hospital admissions, ailments and deaths in developing countries.[18] The reasons for such a persistence in the incidence of neonatal sepsis is home deliveries conducted in septic conditions, lack of breast feeding, nosocomial resistant infections, multidrug resistant bacterial pathogens and lack of proper treatment strategies regarding general as well as specific empirical treatment approaches to that particular environment, based on continuous surveillance.[18]

Developing countries contributes to 99% of 4million neonatal deaths worldwide each year.[10] Infections such as sepsis, pneumonia, diarrhea and tetanus are the major ailments which are responsible for approximately 34 neonatal deaths per 1000 live births (10) in contrast to the proportion in developed countries where neonatal mortality caused by sepsis is around 5/1,000 livebirths.[24]

Citrobacter species(37%) is the most common bacterial pathogen responsible for neonatal septicemia in our setup followed by Staphylococcus aureus(20%) and Klebsiellapneumoniae (17%).In our study most of the bacterial pathogens showed sensitivity to Chloramphenicol, Aztreonam, Vancomycin and Imipenem.

For the diagnosis and treatment of neonatal sepsis blood culture is considered a gold standard.(25) Currently, the greatest challenge being faced in the treatment of neonatal septicemia is multidrug resistance among the causative organisms which is adding up to the difficulty in controlling the morbidity and mortality rates attributed to neonatal septicemia.[26] The disproportionate pace of the development of multidrug resistance and the development of new antibiotic drugs are major points to ponder.[26] It is the need of the era to use antibiotics wisely and to limit the administration of broad spectrum empirical antibiotics, otherwise, after a short time span there will be no drugs available for the treatment of Neonatal sepsis.[26]

Apart from focusing on the treatment strategies, preventive measures should be considered especially to recognize infants who are at a greater risk, consideration of proper aseptic measures during labor and increasing community awareness regarding exclusive and early Breast feeding.[23] Indeed, new strategies need to be develop which can be helpful in early diagnosis and prompt treatment of neonates.[11]

The aim of our study is to list the organisms predominantly responsible for neonatal sepsis in our hospital. It is recommended that surveillance should be conducted shortly apart (every 3-6 months) to update the causative organisms and their sensitivity spectrum in particular localities.[26] Each hospital must maintain its own specific antibiogramconsidering all the possible empirical treatment options[26]. Efforts must be directed to keep this life threatening disease under control by administration of an effective empirical treatment to

the baby keeping in mind the prevalent organisms to save the life of the patient, when the blood culture and sensitivity report is awaited.[26]

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