Original Research Article

A Hospital Based Prospective Study to Evaluate the Antibiotic Susceptibility of the Isolates of Neonatal Sepsis in Tertiary Care Neonatal Intensive Care Unit

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Abstract

Background: Septicemia is one of the most commonly encountered problems in neonatal nurseries and contributes considerably to the neonatal mortality and morbidity. Since indiscriminate unnecessary and excessive use of antibiotics not only replace the normal flora but also results in even emergence of multidrug resistant organisms. This method allows for microbial identification and susceptibility testing to be performed which is a critical component to managing sepsis, Periodic evaluation of organisms responsible for neonatal sepsis is essential for the appropriate management of neonates. Therefore, this study was undertaken to determine the antibiotic susceptibility from blood cultures of neonates in a tertiary care hospital in Hyderabad, India. **Materials& Methods**: A hospital based prospective study done at Tertiary Care Neonatal unit, NICE Hospital for Women, Newborns & Children, Hyderabad during June 2017 to march 2018 (10 months).The study includes all blood culture positive cases received from neonatal intensive care unit who were admitted in NICU.All negative blood cultures and contaminant growth were excluded from the study. Antibiotic susceptibility testing was done for the isolates on Muller-Hinton agar using commercially available discs (Hi-Media) by Kirby Bauer disc diffusion method, using CLSI guidelines for interpretation as resistant, intermediate sensitive and sensitive. **Results:** During the study period, a total of 521 newborns were admitted. Blood culture reports were positive in 32 cases (6.1%). Our study showed that the spectrum of antibiotic sensitivity for gram negative isolates showed all isolates to be sensitive to Tiecycline followed by Imepenem and Piperacillin-tazobactum. The isolates were least sensitive to cephalosporins **Conclusion:** It is evident from this study that Gram-negative organisms (**Klebsiella, pseudomonas**) and **S. aureus** are the leading cause of neonatal sepsis and most of them are resistant to multiple antibiotics.

Keywords: NICU, Antibiotic Susceptibility, Neonatal Sepsis, Newborn.

Introduction

Septicemia is one of the most commonly encountered problems in neonatal nurseries and contributes considerably to the neonatal mortality and morbidity.

The incidence of neonatal septicemia has been reported between 1-10/1000 live births[1]. Incidence of NNS varies from 7.1-32/1000 live birth in Asia, 6.5 to 23 /1000 live birth in Africa and from 3.3 to 8.9 / 1000 live birth in South America[2].In India the work of various workers indicates that the incidence varies between 10-20/1000 live births[3].

Low-birth weight and decreasing gestational age are associated with increased Neonatal infection rates. Neonates with a birth weight of 1500 g or less are 2.69 times more likely to acquire an infection than neonates who are born at a higher weight. Prematurity by itself is a risk factor because preterm neonates are immunecompromised and have increased susceptibility to infection due to an immature immune system, inefficient neutrophil function[4] and lack of antigen type-specific antibodies to pathogens in their environment.

Neonatal intensive care unit therapies that provide a portal of entry for pathogens include intubation and ventilation, central venous catheters and parenteral nutrition, peripheral intravenous lines, venipuncture or heel stick blood draws and indwelling urinary catheters. Of the therapeutic interventions used in the NICU, the use of central venous catheters is most frequently associated with Neonatal infection[5].

Unit design and procedures also impact the Neonatal infection rate. Overcrowded nurseries with a minimal number of sinks or lack of alternative methods of hand cleansing are at risk for increased infection rates due to the direct transmission of pathogens from the hands of the healthcare provider to the neonate[6].

The main contributory factors for these changes are the development and usage of new antibiotics, the clustering of sick neonates within relatively small areas and the prolonged survival of very low birth weight preterm babies who previously would have died**.**

Although the incidence of neonatal bacterial sepsis has remained constant during the past 50 years, the responsible pathogens have varied considerably.

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The causative organisms in neonatal infections have varied somewhat in the past 50 years and are related to the introduction of newer antibiotics and development of antibiotic resistance and changes in obstetric management[7].

Group B *Streptococci, Escherichia coli* and *Listeria monocytogenes* have been the organisms isolated most frequently in developed countries. Gram negative organisms such as *Klebsiella spp*, and *Serratia marcescens* are more common in less developed countries.[8]

As Neonatal septicemia is associated with high mortality and morbidity, rapid diagnosis and prompt treatment with appropriate antibiotics is essential to avoid indiscriminate use of antibiotics in those neonates with sepsis and to avoid unnecessary use of antibiotics in those who do not have septicaemia. Since indiscriminate unnecessary and excessive use of antibiotics not only replace the normal flora but also results in even emergence of multidrug resistant organisms. This method allows for microbial identification and susceptibility testing to be performed which is a critical component to managing sepsis,Periodic evaluation of organisms responsible for neonatal sepsis is essential for the appropriate management of neonates. Therefore, this study was undertaken to determine the antibiotic susceptibility from blood cultures of neonates in a tertiary care hospital in Hyderabad, India.

Materials & Methods

A hospital based, prospective study done at Tertiary Care Neonatal unit, NICE Hospital for Women, Newborns & Children, Hyderabad during June 2017 to march 2018 (10 months).The study includes all blood culture positive cases received from neonatal intensive care unit who were admitted in NICU.All negative blood cultures and contaminant growth were excluded from the study.

Methodology

Sample Collection

About one ml of venous blood was drawn by venous puncture following strict aseptic precautions i.e. suitable site was cleaned with 70% ethyl alcohol v/v and 0.5% chlorhexidine gluconate w/v rubbing vigorously and allowed to dry. Starting in the centre of the circle the antiseptic solution was applied in widening circles until the entire circle has been saturated and allowed to dry on the skin for at least one min[8].

Blood Culture

About one ml of blood was drawn aseptically and inoculated into a blood culture bottle infusion broth, thus making dilution of 1 in 10 to nullify the natural bacteriostatic or bactericidal activity of blood. Brain Heart infusion broth was prepared using the commercially available ready to use powder supplied by Hi-Media Laboratories Pvt. Limited, Mumbai, India. The broth was distributed into 10 ml quantity in McCartney bottles and sterilized by autoclaving at 121℃ for 15 minutes.

After inoculation, the blood culture bottles were incubated at 37℃ under aerobic conditions in the incubator for 7 days. The first subculture was done after 24 hours of incubation the second on the 3 rd day and a final on the seventh day. Subcultures were done onto chocolate agar, 5% sheep blood agar and Mac-conkey agar plates. The inoculated plates were incubated aerobically in the incubator at 37℃ for 24 hours and the plates were observed for growth. The growth was identified by colonial characteristics, Gram's stain and standard biochemical tests, described in Mackie and McCartney, Practical Medical Microbiology and Bailey and Scott's Diagnostic Microbiology.

Culture which did not yield any growth following three subcultures was reported negative at the end of 7th day.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was done for the isolates on Muller-Hinton agar using commercially available discs (Hi-Media) by Kirby Bauer disc diffusion method, using CLSI guidelines for interpretation as resistant, intermediate sensitive and sensitive. Control strains of Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 25923 were used.

Results

During the study period, a total of 521 newborns were admitted. Blood culture reports were positive in 32 cases (6.1%). Our study showed that the spectrum of antibiotic sensitivity for gram negative isolates showed all isolates to be sensitive to Tiecycline followed by Imepenem and Piperacillin-tazobactum. The isolates were least sensitive to cephalosporins (table 1).

The spectrum of antibiotic sensitivity for klebsiella pneumoniae showed all isolates to be sensitive to Tigecycline and Imepenem followed by Piperacillin-tazobactum (figure 1).

The antibiotic sensitivity pattern of the grampositive blood culture isolates indicates that all isolates were sensitive to Vancomycin and linezolid, whereas all isolates were resistant to Amoxicillin clavulanic acid (table 2).

Table 1: Sensitivity pattern of gram-negative organisms in positive blood culture

Antimicrobial Agent	Sensitivity Pattern		
	Sensitive	Intermediate	Resistant
Imepenem	25(96%)	00	01
Ciprofloxacin	16(62%)	03	07
Cefotaxime	08(30%)	08	10
Cefepime	11(42%)	04	11
Ceftriaxone	10(38%)	06	10
Piptaz	23(88%)	03	00
Colistin	17(65%)	08	01
Aztreonam	20(77%)	04	02
Tigecycline	26(100%)	00	0
Amikacin	17(65%)	03	06
Amoxyclav	00(00%)	01	25

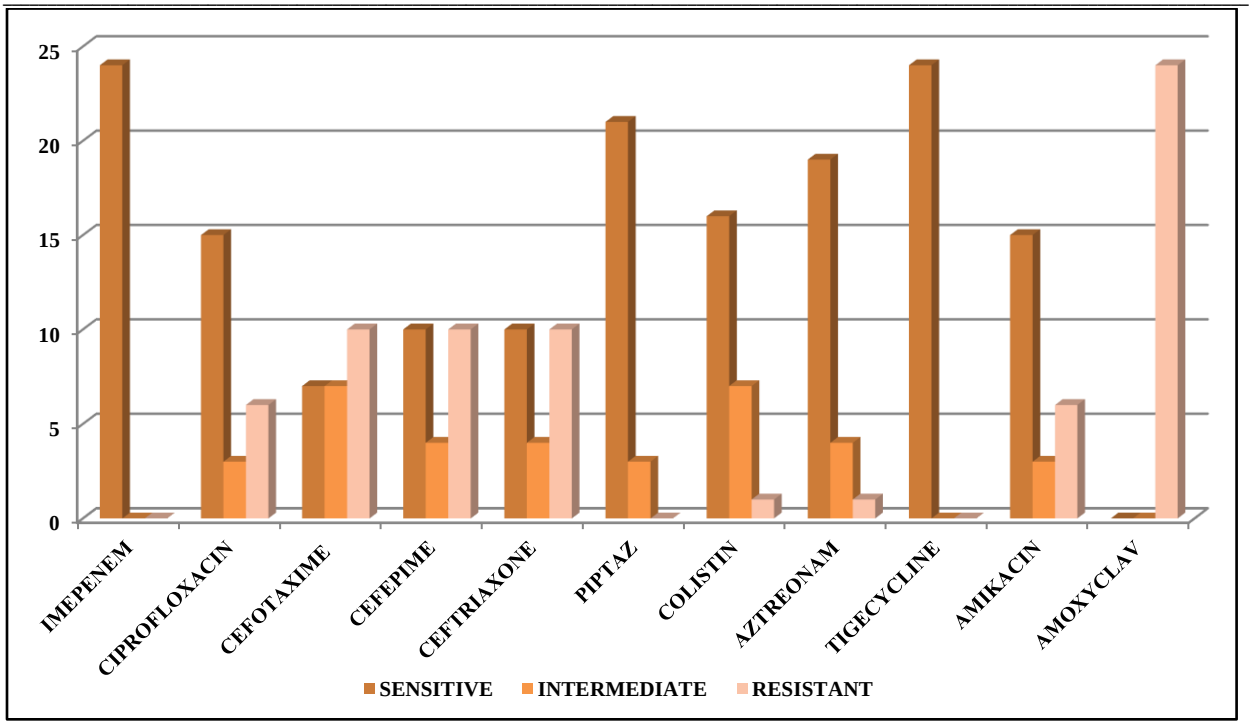

Figure 1: Sensitivity Pattern of Klebsiella Pneumoniae

Table 2: Sensitivity Pattern ofGram-Positive Organisms in Positive Blood Culture

Antimicrobial Agent	OrganismStaphylococcus Aureus		
	Sensitive	Intermediate	Resistant
Ciprofloxacin	04(66%)	01	01
Ceftriaxone	03(50%)	00	03
Amikacin	04(66%)	00	02
Amoxyclav	00(00%)	01	05
Cefoxitin	04(66%)	01	01
Cefalaxin	03(50%)	01	02
Azithromycin	04(66%)	01	01
Linezolid	05(84%)	01	00
Vancomycin	05(84%)	01	00

Discussion

In the present study, Klebsiella pneumoniae 24 (75%) was the predominant isolate, followed by staphylococcus aureus 10 (18.7%). Gram negative organisms formed the majority of the isolates as compared to Gram positive organisms (81.2% v/s 18.8%). Similarly, a higher proportion of Klebsiella pneumoniae were isolated by Tallur et al[9] (53.50%), Roy et al[10] (24.60%). Studies conducted by G. Karthikeyan et al[11](61.50%), showed Staphylococcus aureus as the commonest isolate, while Pseudomonas aeruginosa was the commonest pathogen isolated by Betty Chacko et al[12] (60%) and A. H. Movahedian et al[13] (36%), in their study. In the present study majority of the isolates were Gram negative organisms accounting for 81.2% of the isolates with Klebsiella pneumonia being the commonest isolated in 24(75%) of the 32 culture positive cases. Among the Klebsiella pneumonia all isolates are susceptible to tigecycline and 96% of the isolates were susceptible to Imipenem followed by Piptaz (88%), Colistin have 65% while only around 65% susceptibility was noted to Amikacin and Ciprofloxacin. Very less sensitivity to Ceftriaxone and Cefotaxime. Resistance to Amoxyclav was 100%. A varied resistance pattern was seen to Aztreonam. It is evident from the present study that maximum susceptibility was noted to Tigecycline, Imipenem and Piptaz. Majority of the isolates were less susceptible to third generation cephalosporin. In a study done by Tallur et al[9] 2000, Klebsiella pneumonia was the commonest pathogen found which were more susceptible to Gentamicin, Amikacin and third generation cephalosporin.

In a study done by Tallur et al[9] 2000, Klebsiella pneumonia was the commonest pathogen found which were more susceptible to Gentamicin, Amikacin and third generation cephalosporin.

Gram negative organisms continue to be a menace to the sick, fragile and debilitated newborns. Multidrug resistance of the causative organisms of septicemia is a rapidly emerging, potentially disastrous

problem. Our data is no exception to the worldwide antimicrobial emergency. Among these, Klebsiella septicemia continues to be a challenge to the neonatologist and microbiologists. One of the reasons for the predominance of an organism in causing septicemia in the neonatal units is the selective pressure of antimicrobial agents, so that resistant microorganisms tend to colonize and proliferate in the neonatal units. Cross contamination and nosocomial transmission may play significant role in the etiology of Klebsiella septicemia[9]. Also in the present study, 1 (3.1%) isolate was Pseudomonas aeruginosa which was 100% susceptible to Tigecycline and imepenem and 100% resistance to Amoxicillin- clavulanic acid.

Study of AH Movahedian et al[13] 2006 observed 70% of susceptibility of Pseudomonas aeruginosa to Amikacin followed by Gentamicin 53% and lower susceptibility to Ampicillin, Cephalexin and Ceftriaxone. Overall the Gram negative organisms were susceptible to Tigecycline,Imipenem and piptaz. Almost all isolates showed resistance to Amoxicillin and clavulanic acid.

In the present study staphylococcus aureus was the only Gram-positive organism isolated constituting 18.7%. Most of the gram-positive isolates were susceptible to Vancomycin and Linezolid. Around 50% resistance to cefalexin and ceftriaxone and 100% resistance to amoxiclav.

Tallur et al[9] noted a high incidence of Staphylococcus spec. in their study, pointing towards its nosocomial origin. All these isolates were 100% susceptible to Vancomycin. In the study by G. Karthikeyan et al[11] 2001 major isolate was Staphylococcus aureus and most of these isolates belonged to early onset septicemia group; 24/59 (40.7%). The significant proportions of these cases were presumed to occur as a result of vertical transmission thereby implying maternal colonization. Multidrug resistant Staphylococcus aureus has been accepted as an indication for the use of vancomycin, which is the drug of choice in these situations. Similar observations has been made in the study by Roy et al[10] who found Staphylococcus aureus isolates to be resistant to Penicillin, Erythromycin, Gentamicin and Ciprofloxacin.

Coagulase negative Staphylococci are emerging as causative organisms of neonatal septicemia and recovery of these pathogens from the blood culture of newborn infants should no longer be consider as a contaminant especially in the preterm and low birth weight neonates with relatively longer hospital stay and excessive exposure to diagnostic and supportive procedures.

Conclusion

It is evident from this study that Gram-negative organisms **(**Klebsiella, pseudomonas) and S. aureus are the leading cause of neonatal sepsis and most of them are resistant to multiple antibiotics.Furthermore, we advise that health education be provided to the public on the dangers of indiscriminate use of antibiotics, which is currently considered to be a menace in our society and which has been responsible for the ineffectiveness of most commonly used antibiotics such as amoxicillin and cephalosporins as observed in our study.

Conflict of Interest: Nil
Source of support:Nil

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