 DOI: 10.18231/2455-6807.2016.0007

Neonatal septicaemia: Its bacteriological profile and antibiogram

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Abstract

Background and Objectives: Neonatal septicaemia is a life threatening clinical emergency that demands urgent diagnosis and treatment. A wide variety of bacteria can cause neonatal septicaemia. As neonatal septicaemia is a life-threatening clinical emergency, the knowledge of epidemiological and antimicrobial susceptibility pattern of common pathogens in a given area helps to inform the choice of antibiotics, therefore a regular surveillance of important bacterial isolates and their susceptibility pattern is mandatory. So the present study was undertaken to identify the common bacterial pathogens associated with neonatal sepsis and their antibiotic susceptibility pattern.

Material and Methods: The study was carried out in the Department of Microbiology, a tertiary care hospital Jalgaon, Maharashtra during the period from December 2015 to May 2016. A total 200 clinical suspected cases of neonatal septicemia were included in the study. Blood samples were collected with all aseptic precautions and inoculated on brain heart infusion broth (BHIB).Subculture were made on blood agar and Mac-conkey agar plates. Organisms were identified as per standard recommended procedures and antibiotic susceptibility testing was carried out on isolates as per Clinical Laboratory Standard Institute (CLSI) guidelines.

Result: 60 different types of microrganisms were isolated. The incidence of neonatal septicemia was 30%.The prevalence of gram negative bacilli were 60.00% and gram positive bacilli were 40.00%.The commonest pathogen isolated was *Klebsiella pneumonia* 50.00%, followed by *Staphylococcus aureus* 30.00%. *Klebsiella pneumonia* was resistant to amikacin (86.66%) &100% resistant to ampicillin, and was 100% sensitive to pipercillin tazobactam and co-trimaxozole. The most effective antibiotics against the isolated *Staphylococcus aureus* were Vancomycin (100%), Linzeloid(100%), Ciprofloxacin (66%) ,Co-trimoxazole (66%) and Clindamycin (55%).

Conclusion: The present study reveals microbiological profile and antibiogram of neonatal septicemia. Considering the burden of mortality resulting from septicemia, better diagnostic facilities should be employed for early detection of septicemia and rational use of narrow spectrum antibiotics is recommended.

Keywords: Neonatal Septicemia, Antimicrobial Susceptibility, Narrow Spectrum Antibiotics.

Introduction

Neonatal sepsis is a significant cause of morbidity and mortality among neonates worldwide[1]. According to National Neonatal Perinatal Database (NNPD) 2002- 03 collected from 18 centers from various parts of India, Neonatal mortality rate has been reported to be 25.3 per 1000 intramural live births.[(2)] The reported incidence of nosocomial septicemia in neonates from India ranges from 1.5% to 37%.[(1,2,3)]

Neonatal sepsis is broadly categorized into early and late onset sepsis depending upon the postnatal day of presentation. Early-onset neonatal sepsis (EONS) occurs within first 72 h of life, while the late-onset neonatal sepsis (LONS) occurs between 72 h to 90 days of life.[(3)]

The bacterial agents implicated in early-onset sepsis include *Group B Streptococcus* (GBS), *Escherichia coli*, *Klebsilla Pneumoniae*, Coagulase-negative *Staphylococcus*, *Haemophilus influenza* and *Listeria monocytogenes*. The organisms commonly associated with late-onset sepsis include Coagulase-negative *staphylococci* (CONS), and *Klebsilla Pneumoniae*.[(3,4)]

Various laboratory tests are utilized to diagnose septicemia like total leucocyte count, C- reactive protein level, erythrocyte sedimentation rate, Acridine orange stained buffy coat smear examination, Nitro-bluetetrazolium test, etc. The advantages of these tests are that they are sensitive indicators of sepsis, less expensive and rapid. However, these tests never tell us anything about the aetiology of septicemia, whether the sepsis is unimicrobial or polymicrobial and their antimicrobial susceptibility pattern.[(5,6)]

The gold standard for diagnosis of septicemia is the isolation of the bacteria from a blood culture which takes at least 48 hours to confirm the diagnosis; a delay of which a neonate can ill afford for initiation of appropriate therapy. So the present study was undertaken to find out the incidence of neonatal septicemia,to determine the common bacterial agents associated with neonatal sepsis and their antibiotic susceptibility pattern in our tertiary care hospital.

Materials and Methods

Study Design: The present study after the permission from ethical committee was conducted in the Department of Microbiology at tertiary care hospital, Jalgaon Maharashtra from December 2015 to May 2016. During this period blood samples will be collected from new-borns admitted to Department of Paediatrics and Neonatal Intensive Care Unit (NICU).

Selection of subject: Neonates of normal and low birth weight of both sexes were included in this study. Blood cultures was done for all suspected septicaemic neonates referred by pediatricians. Neonates presenting with signs and symptoms such as refusal of feed, lethargy, fever,

hypothermia, vomiting, diarrhoea, abdominal distension, jaundice, respiratory distress, seizures, irritability, cyanosis, bulging of anterior fontanels, pustular skin, apnoea, conjunctival discharge etc. were taken up for study. History and clinical findings of suspected neonates was recorded. Blood samples from referred cases were collected from peripheral vein with proper aseptic precautions preferably before starting any antibiotic therapy.

Exclusion criteria: Neonates who had undergone surgery because of risk of wound infection, congenital anomalies, birth weight less than 1000 gms and age more than 28 days at the time of diagnosis was excluded from the study.

Sample collection: 2 ml of blood for culture was drawn in sterile syringe after skin preparation by a two step process with 70% alcohol and povidone iodine application and then dried for 1 min. Blood was collected aseptically and incubated into blood culture bottle containing 10 ml of Brain Heart Infusion Broth (BHIB). These bottles were incubated at 37° C temperature under aerobic conditions in the incubator maximum for 7 days. Subculture were made on Blood agar and Mac-conkey's agar daily from 1st to 7th day. However if the growth was observed further sub cultures were not done. Growth if any was processed according to standard microbiological techniques which includes Gram staining, colony characteristics and biochemical properties described in Mackie and McCartney Practical Medical Microbiology and Bailey and Scott's Diagnostic Microbiology.[7,8] Blood culture broth which showed no microbial growth after 7 days were reported as culture negative. Criteria for antimicrobial sensitivity testing used were as per Clinical Laboratory Standard Institute guidelines (CLSI 2015).[9]

Antimicrobial sensitivity testing was done on Muller Hinton Agar (MHA) by Kirby Bauer disc diffusion method. Commercially available discs (Hi-media) were used. Concentration of discs used were Erythromycin (15 mcg), Co-trimoxazole (25mcg), Ciprofloxacin (5mcg), Ampicillin (30mcg), Piperacillin+Tazobactam (100/10mcg), Ceftazidime (30 mcg), Amikacin (30 mcg), Cefoperozone(30mcg), Ofloxacin (5mcg), Vancomycin(30mcg), Linzeloid (30mcg) and Imipenem (10mcg).

Staphylococcus aureus (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruoginosa* (ATCC 27853) was used as quality control throughout the study for culture and antimicrobial susceptibility testing.

Statistical analysis: The results were expressed as percentages for analysis of various epidemiological details and for analysing the distribution of different bacterial isolates and their sensitivity pattern. Microsoft excel was used for the interpretation of these results.

Results

This study was conducted in the Department of Microbiology at tertiary care hospital, Jalgaon Maharashtra during the period of 6 months. Total 60 organisms were isolated out of 200 suspected neonatal cases, incidence of neonatal septicaemia was 30%.

Table 1: Results of blood culture

Blood culture	Cases	
	Number (n)	Percentage (%)
Positive	60	30
Negative	140	70
Total	200	100

Table 2: Organisms isolated by blood culture

Organism	Isolates	
	Number (n)	Percentage (%)
Klebsiella pneumonia	30	50.00
Staphylococcus aureus	18	30.00
Acinetobacter	6	10.00
CONS	4	6.66
Candida albicans	2	3.33
Total	60	100

The microbial profile of neonatal septicaemia is depicted in Table 2. *Klebsiella pneumonia* 30 (50%), *Staphylococcus aureus*18 (30%) were major isolates. Other isolates include *Acinetobacter species* 6 (10%), *Coagulase Negative Staphylococcus* (CONS) 4 (6.66%) and *Candida albicans*2 (3.33%).

Table 3: Antibiotic sensitivity pattern of isolated gram negative organisms

Drug		*Klebsiella pneumoniae* (n=30)	*Acinetobacter species* (n=6)
Ampicillin	R	30(100%)	2(33.33%)
	S	0(00%)	4(66.66%)
Piperacillin + Tazobactam	R	0(00%)	0(00%)
	S	30(100%)	6(100%)
Cetazidime	R	2(6.66%)	6(100%)
	S	28(93.33%)	0(00%)
Cefoperozone	R	2(6.66%)	6(100%)
	S	28(93.33%)	0(00%)
Amikacin	R	26(86.66%)	6(100%)
	S	4(13.33%)	0(00%)
Ofloxacin	R	0(00%)	0(00%)
	S	30(100%)	6(100%)
Imipenem	R	0(00%)	2(33.33%)
	S	30(100%)	4(66.66%)
Co-trimoxazole	R	0(00%)	6(100%)
	S	30(100%)	0(00%)

In isolated gram negative bacteria *Klebsiella pneumoniae* was 100% sensitive to Piperacillin+ Tazobactam, Ofloxacin, Imipenem and Co-trimaxazole, 93.33% were sensitive to Ceftazidime and Cefoparozone, 13.33% were sensitive to Amikacin and 100% resistant to Ampicillin. Regarding *Acinetobacte*r species 100% sensitivity was seen to Piperacillin +Tazobactam and ofloxacin, 66.66% sensitivity was seen to Ampicillin and 100% resistance to Ceftazidime, Cefoparozone, Co-trimaxazole and Amikacin. Both

isolated gram negative organisms were 100% sensitive to Piperacillin+ Tazobactam and Ofloxacin.

Table 4: Antibiotic sensitivity pattern of isolated gram positive organisms

Drug	*Staphylococcus. aureus* (n=18)		*CONS* (n=4)
Penicillin	R	16(88.88%)	2(50%)
	S	2(11.11%)	2(50%)
Erythromycin	R	14(77.77%)	2(50%)
	S	4(22.22%)	2(50%)
Clindamycin	R	8(44.44%)	0(00%)
	S	10(55.55%)	4(100%)
Co-trimoxazole	R	6(33.33%)	0(00%)
	S	12(66.66%)	4(100%)
Ciprofloxacin	R	6(33.33%)	0(00%)
	S	12(66.66%)	4(100%)
Vancomycin	R	0(00%)	0(00%)
	S	18(100%)	18(100%)
Linzeloid	R	0(00%)	0(00%)
	S	18(100%)	18(100%)

Isolated *Staphylococcus aureus* strains were 100% sensitive to Vancomycin and Linzeloid ,66% sensitive to Co-trimaxazole and Ciprofloxacin, 55% sensitive to Clindamycin, 22% sensitive to Erythromycin and 11.4% sensitive to Penicillin. Regarding Coagulase Negative Staphylococcus, it showed 100% sensitivity to Vancomycin, Linzeloid Clindamycin, Co-trimoxazole, and Ciprofloxacin, 50% sensitive to Erythromycin and Penicillin.

Table 5: Day on which the microorganisms were isolated from blood cultures

Organisms	Number of blood culture yielding organisms on day							
	0	1	2	3	4	5	6	7
Klebsiella pneumonia	0	0	6	6	18	0	0	0
Staphylococcus aureus	0	0	4	2	12	0	0	0
Acinetobacter spp	0	0	0	2	4	0	0	0
CONS	0	0	2	2	0	0	0	0
Candida albicans	0	0	0	2	0	0	0	0
Total (n)	0	0	12	14	34	0	0	0
Total percentage (%)	0	0	20.00	23.33	56.66	0	0	0

Day on which the microorganisms were isolated from blood cultures are shown in Table 5. Organisms isolated on day 2 were 12 (20.00%), on day 3 were 14 (23.33%) and on day 4 were 34 (56.66%). The maximum yield was seen on day 4 of subculture whereas on day 0, 1, 5, 6 and 7 no organisms were isolated.

Table 6: Neonatal risk factors (n=30)

Culture	Sex		Birth weight		Gestational age	
	Male	Female	Low	Normal	Preterm	Term
Culture positive	36 (60%)	24 (40%)	34 (56.6%)	26 (46.6%)	32 (53.3%)	28 (46.6%)
Culture negative	84 (60%)	56 (40%)	78 (55.71%)	62 (44.28%)	80 (57.14%)	60 (42.85%)
Total	120	80	112	88	112	88

Culture positivity was seen more in male babies. Out of 120 male babies 36 were culture positive and out of 480 female babies 24 were culture positive. Preterm babies were significantly more susceptible to infection than term babies. Culture positivity was more in low birth weight babies than in normal birth weight babies.

Discussion

This study was conducted to determine the predominant organisms responsible for neonatal septicaemia and their antibiotic sensitivity pattern in our NICU.In the present study 30% cases are blood culture positive, where as in 70% cases there is no growth. This result is in accordance with earlier study done by Jain N.K. *et al*[10] they have also reported 28.30% culture positivity in their study. In the present study Gram negative organisms (60%) constitutes the major group of isolates in neonatal septicaemic cases. This result is in accordance with the earlier study done by other researchers.[11-15] Amongst the gram negative group dominant pathogen isolated was *Klebsiella pneumonia* 50% Similar results have been reported by some other workers[16,17] followed by *Acinetobacter species* in 10% samples and nearly same findings were reported by Ramesh Bhat *et al* and Vinodkumar*et al*.[18,1]

Low incidence of gram positive organisms (40%) were isolated in comparison with gram negative organisms (60%) in our study, same observations were made by Ghyamshyam D. Kumhar*et al*.[12] In our study *Staphylococcus aureus* (30%) is the predominant isolate, followed by *Coagulase Negative Staphylococcus* (6.66%) and *Candida species* (3.33%). Our findings correlates well with the various studies done by other workers.[13,14,15]

All isolated *Klebsiella pneumonia* were 100% susceptible to Piperacillin + Tazobactam, Ofloxacin, Imipenem, Co-trimoxazole, while 93% susceptible was seen with Ceftazidime and Cefoperozone, similar pattern of susceptibility were seen in other studies.[11,12] In our study *Klebsiella pneumoniae* showed 86.66% resistance to Amikacin and 100% resistant to Ampicillin which was on higher side as compared to other researchers.[10,11] *Acinetobacter species* strains were 100% sensitive to Piperacillin + Tazobactam and Ofloxacin; it closely correlates with the findings of Vinod Kumar *et al*[1] High resistance to Ceftazidime, Cefoperozone, Amikacin and Co-trimoxazole is seen in our study. Mane *et al*[19] also reported low sensitivity to Ceftazidime(20%) and Amikacin(20%). Whereas Ramesh Bhat *etal*[18] observed high susceptibility to Amikacin.

Antibiotic sensitivity pattern of Gram positive organisms, shows that 89% of *Staphylococcus aureus* isolates were resistant to penicillin. Similar high penicillin (70%) resistance was reported by Dhechen C Tsering *et al*.[20] The most effective antibiotics against the isolated *Staphylococcus aureus* are Vancomycin (100%), Linzeloid (100%), followed by Clindamycin (55%), Ciprofloxacin (66%) and Co-trimoxazole (66%) our findings correlates with the findings of Vinodkumar*et al*,[1] Mane AK *et al*[19] while coagulase negative *Staphylococcus* shown 100% sensitivity to Vancomycin, Linzeloid, Clindamycin, Co-trimoxazole, Ciprofloxacin and 50% sensitivity to Penicillin and Erythromycin in our study. Nearly similar sensitivity pattern to penicillin was reported by Jeeva Shanker *et al*[3] and vinodkumar *et al*.[1] Dhechen C tsering*et al*[20] reported Vancomycin, Linzeliod followed by Clindamycin was the most effective drug in their study as that of ours.

Conclusion

Blood culture has got an important value in diagnosing Neonatal septicaemia. Early identification and finding out antibiotic sensitivity pattern could be life saving. The blood culture positivity rate was 30%. *Klebsiella pneumoniae* and *Staphylococcus aureus* were the commonest isolates from cases of neonatal septicemia and recommends cotrimaxozole and third generation cephalosporins as empirical treatment for gram negative isolates and cotrimaxozole and clindamycin for gram positive isolates. Our study also highlights alarming resistance to amikacin and ampicillin. Preterm babies and low birth weight babies are significant risk factors for development of neonatal septicemia.

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