

Bacteriological Profile and Antibiogram of Community Acquired Neonatal Sepsis in a Tertiary Care Hospital; A Prospective Study.

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ABSTRACT

Background: To isolate the pathogenic bacteria and to know the antibiotic sensitivity in the community acquired neonatal sepsis. **Methods:** It was a prospective study undertaken on 300 neonates suspected of community acquired neonatal sepsis admitted in Pediatrics Department of Government Medical College, Amritsar over a period of one year from January 2014 to December 2014. All these cases fulfilled the inclusion criteria required for the study. Blood culture of these cases was performed by Mackie and McCartney method and antibiotic sensitivity by Kirley-Baner's disc diffusion method. **Results:** 227 (79%) cases showed positive blood culture. Gram negative isolates (N=156;65.82%) were more frequent than gram positive isolates (N=81;34.18%). Most common isolate was *Klebsiella Pneumoniae* (N=77%;32.48%) followed by *Staphylococcus Aureus* (N=66;27.84%), *E. Coli* (N=37;15.66%), *Pseudomonas Aeruginosa* (N=28;11.81%), *Acinetobacter* (N=14;5.90%), *Enterococcus* (N=8;3.37%) and *Coagulase Negative Staphylococcus Aureus* (N=7;2.99%). Both gram negative as well as gram positive isolates showed high resistance to ampicillin and gentamycin. Gram negative isolates were highly sensitive to Polymixin B and Meropenem whereas gram positive isolates were highly sensitive to Linezolid and Vancomycin. **Conclusion:** Gram negative bacteria were more frequent causes of community acquired neonatal septicemia than gram positive isolates. Both gram positive and negative isolates showed poor sensitivity towards conventional first line antibiotics, rather were mainly susceptible to higher antibiotics. So the knowledge of the pattern of bacteriological isolates and their antimicrobial susceptibility pattern can be very helpful for prompt treatment of such patients, to decrease neonatal morbidity and mortality as well as reducing the emergence of multi-drug resistant organisms.

Keywords: Antibiotic susceptibility; Bacterial isolates; Bacterial resistance; Neonatal sepsis.

INTRODUCTION

Neonatal Sepsis refers to the invasive bacterial infection occurring in the first 4 weeks of life. It encompasses various infections of the new born like septicemia, meningitis, arthritis, osteomyelitis and urinary tract infection but excludes superficial infections like conjunctivitis and oral thrush.^[1]

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It can be early onset sepsis (EOS) presenting within 72 hours of life with maternal genital tract being the main source of infection or it can be late onset sepsis (LOS) which usually presents after 72 hours

of life and the main source of infection is nosocomial (hospital acquired) or community acquired infection.^[2,3] Neonatal sepsis, the commonest cause of neonatal morbidity and mortality is responsible for 30-50% of total neonatal deaths each year in the developing countries.^[4-6] According to the World Health Organization (WHO) estimate, there are about 5 million neonatal deaths per year in the world, 98% of these occurring in the developing world. Neonatal mortality rate in developing countries from various causes is about 34 per 1000 live births, most of the deaths occurring in the first week of life. Globally, major causes of the neonatal deaths are due to prematurity (28%), sepsis (26%), and birth asphyxia (23%)^[7]. The incidence of the neonatal sepsis according to the Neonatal and Perinatal Database (NNPD) is 30 per 1000 live births. The NNPD network comprising of the 18

tertiary care neonatal units across India found sepsis to be the commonest cause of neonatal mortality contributing to 19% of all neonatal deaths.^[8] In India, sepsis has been reported as a cause of neonatal deaths in 20-50% of cases in the community based studies.^[9, 10] The gold standard for the diagnosis of neonatal sepsis is isolation of bacterial agents from the blood culture.^[11] Both gram negative and gram positive bacteria have been isolated from the blood and predominance of one type over the other varies from place to place and even in the same place over the time to time.^[12] In most of the developing countries, gram negative sepsis remains the major cause of the neonatal septicemia. Commonly isolated organisms include *Klebsiella Pneumonia*, *Escherichia Coli*, *Enterobacilli*, *Pseudomonas Aeruginosa*, *Staphylococcus Aureus*, *Streptococcal Species*, *Citrobacter Species* and *Coagulase Negative Staphylococcus (CONS)*.^[13,14] The bacterial susceptibility to different antibiotics varies from time to time over different geographical areas. But there is a rising concern of isolation of highly antibiotic resistant bacteria.^[15] Thus the knowledge of the bacteriological profile of neonatal septicemia and its antibiotic susceptibility pattern in geographical areas provide us the guidance to initiate empirical antibiotic treatment which is the hallmark of the management of the neonatal sepsis. So this study was undertaken with the aim to determine the bacteriological profile and its antibiotic susceptibility pattern in the community acquired neonatal sepsis. Following a rational antibiotic therapy, we can minimize the risk of severe neonatal morbidity and mortality as well as decrease the development of multidrug resistant bacteria.

MATERIALS AND METHODS

It was a hospital based prospective study conducted in the department of Pediatrics and Microbiology, Government Medical College, Amritsar, Punjab. It included 300 neonates admitted with clinically suspected community acquired sepsis during the period of one year from January 2014 to December 2014. Only those neonates were included who were less than 28 days of life, had clinically suspected septicemia and presented after 72 hours of birth. Neonates of more than 28 days of life or presenting before 72 hours of life or already on antibiotics were excluded from the study. Sepsis was suspected from the clinical history of one or more of the symptoms like refusal to feed, lethargy, fever, abdominal distension, loose stools, vomiting, features of hypoglycemia, hypothermia, tachypnea, tachycardia, grunting, chest retractions, cyanosis, apnea, pallor, shock, excessive crying, body mottling, poor cry, prolonged capillary time, bleeding from any site, neck retractions and vacant

stare. Institutional Ethics Committee clearance and informed consent from the parents of all neonates under study was obtained. With all the recommended aseptic precautions, 1ml of blood was drawn from each neonate and collected in a sterile bottle containing 1% glucose broth and inoculated at 37°C. Subcultures were made on blood agar and Mac-Conkey agar after 24 hours, 48 hours, 72 hours and 7 days which were further inoculated at 37°C for 18-24 hours. The plates were observed the next day. If no growth was observed even after 7th day, sample was reported negative for bacterial isolate. The growth of isolates were identified by colony morphology, gram staining and standard biochemical tests described in Mackie and McCartney Practical Medical Microbiology.^[16] For identification of gram positive isolates and coagulase tests were done; for gram negative organisms, Simons's citrate test, motility indole test, urea (MIU) test and triple sugar test (TST) were done. Antibiotic susceptibility test was performed on the identified isolates using commercially prepared antibiotics disks (High media Co. Mumbai, India) on Muller Hinton agar by Kirby-Baur Disk Diffusion method as recommended in the Clinical Laboratory Standard Institute (CLSI) guidelines.^[17] The antibiotic disks and their concentrations per disk (micrograms) comprised of Ampicillin (10), Amikacin (30), Gentamycin (10), Ciprofloxacin (5), Linezolid (30), Vancomycin (30), Ceftazidime (30), Cefotaxime (30), Amoxicillin (30), Cefperazone (75), Cefpodoxime (11), Piperacillin+Tazobactam (100), Imipenem (11) and Polymyxin-B (300). Multidrug resistant (MDR) isolates were phenotypically characterized into Methicillin resistant *S.aureus* (MRSA), Vancomycin resistant enterococci (VRE), Metallo β lactamase (MBL) and Extended spectrum β Lactamase (ESBL) producers.

RESULTS

Out of 300 study cases, 166 (55.34%) were males and 134 (44.67 %) were females. Three most frequent features suggestive of septicemia were refusal to feed (n= 231; 77%), lethargy (n= 200; 66.66%) and fever (n= 147; 49 %) followed by other clinical features. 237 (79%) showed positive blood culture and 63 (21 %) showed no bacterial growth. Gram negative isolates (n =156; 65.82 %) were more frequent than gram positive growths (n=81; 34.18 %). The most common pathogen isolated was *Klebsiella pneumoniae* (n= 77; 32.48 %) followed by other organisms and CONS was the least common bacterial isolate [Table 1 and Figure 1]. The gram negative isolates showed high resistance to Ampicillin and Gentamycin but were highly sensitive to Polymyxin-B and Meropenem [Table 2]. Gram positive isolates were also quite resistance to Ampicillin and Gentamycin but were highly sensitive to Linezolid and Vancomycin [Table 3].

Antibiotic susceptibility pattern of different gram positive or gram negative organisms is depicted in [Figure 2].

Table 1: Organisms Isolated.

Organisms Isolated	Number(N=237)	Percentage
Klebsiella Pneumonia	77	32.48
Staphylococcus Aureus	66	27.84
Escherichia Coli	37	15.61
Pseudomonas	28	11.81
Acinetobacter	14	5.90
Enterococcus	8	3.37
Cons	7	2.95

Table 2: Antibiotic Susceptibility Pattern of Gram Negative Isolates.

Antibiotic	Gram Negative	Percentage
Amikacin	102	65.38
Gentamicin	65	41.66
Ceftriaxone	76	48.71
Piperacillin-Tazobactam	73	46.79
Linezolid	Nt*	Nt*
Amoxicillin-Clavulanate	Nt*	Nt*
Imipenem	154	98.71
Polymyxin-B	156	100
Ceftazidime	75	48.07
Vancomycin	Nt*	Nt*
Ciprofloxacin	105	67.30
Ampicillin	6	3.80
Cefotaxim	76	48.71
Methicillin	Nt*	Nt*
Cefoperazone	73	46.79

*NT = Not Tested

Table 2: Antibiotic Susceptibility Pattern of Gram Negative Isolates.

Antibiotic	Gram Positive	Percentage
Amikacin	75	92.59
Gentamicin	35	43.20
Ceftriaxone	43	53.08
Piperacillin-Tazobactam	Nt*	Nt*
Linezolid	81	100
Amoxicillin-Clavulanate	41	50.61
Imipenem	Nt*	Nt*
Polymixin-B	Nt*	Nt*
Ceftazidime	8	9.87
Vancomycin	74	91.35
Ciprofloxacin	51	62.96
Ampicillin	5	6.17
Cefotaxim	43	53.08
Methicillin	70	86.41
Cefoperazone	52	64.19

*NT = Not Tested

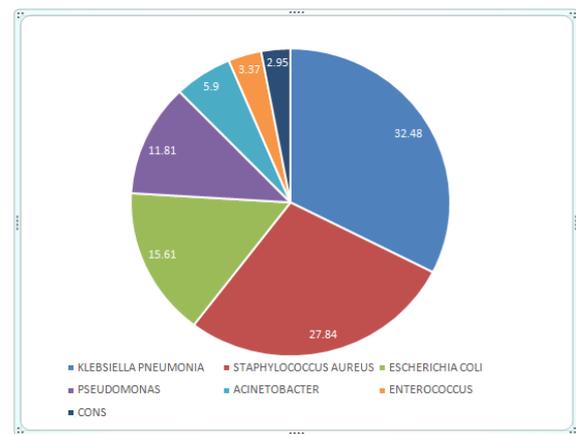


Figure 1: Organisms Isolated.

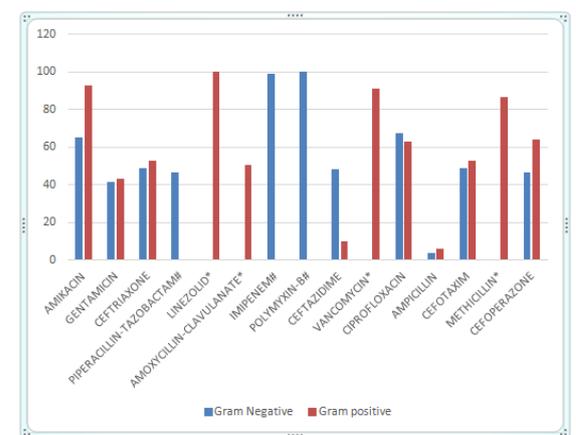


Figure 2: Percentage Susceptibility of Gram Positive and Gram Negative Isolates.

*Not Tested For Gram Negative Isolates

Not Tested For Gram Positive Isolates

DISCUSSION

Neonatal Sepsis is a life threatening emergency and any delay in its treatment may lead to mortality.^[4,7] Bacteriological profile of community acquired neonatal sepsis changes from region to region and time to time. In addition, there is increasing multidrug resistance over the last few years. Therefore knowledge of pattern of the bacteriological profile and its antimicrobial susceptibility pattern can be very helpful for prompt empirical treatment of neonatal sepsis. This study was undertaken keeping these objectives in mind. Out of 300 study cases; male to female ratio 1.2:1 which is comparable to other studies like by Begums et al.^[18] Reason for male preponderance may be sex dependent factors as X-linked immunoregulatory genes may play some protective roles in females.^[19] Positive blood culture was observed in 79% cases in the present study which is comparable to study by Premlata et al.^[20] showing blood culture positivity in 82% cases although the results are higher as compared to Karthikeyan et al.^[21] showing blood culture positivity in 51% cases. The difference may be due to variable incidence of neonatal sepsis from place to place and due to many other factors like perinatal care, birth weight etc. Gram negative isolates (65.82%) were more frequent than gram positive (34.18%). These results were consistent with the NNPD data. But our results are in contrast to studies from developed countries showing gram positive isolates more frequently.^[22] Out of all culture positive cases, Klebsiella pneumonia was most frequent (32.48%) gram negative isolate and Staphylococcus Aureus was the most common (23.84%) gram positive isolate. These results are similar to many other Indian studies. Most of the gram negative isolates in the present study were resistant to the routinely used first line empirical antibiotics with 96.15% resistant to Ampicillin and 58.34% to Gentamicin. But all the negative organisms showed 100% sensitivity to Polymixin B, 98.71% to Imipenem, 67.30% to Ciprofloxacin and 65.38% to Amikacin. Our findings correlate well to those of Mustafa et al and many other studies.^[23] Out of gram positive isolates, again 93.82% were resistant to routinely used first line antimicrobial Ampicillin and 56.80% were resistant to Gentamicin whereas 100% gram positive isolates were sensitive to Linezolid, 92.59% were sensitive to Amikacin and 91.35% to Vancomycin. Our findings again correlate well with those reported by Mustafa et al and Kaistha et al.^[23,24] Increasing resistance by both gram positive and gram negative isolates to routinely used antimicrobials may be due to inappropriate use of antibiotics

CONCLUSION

Gram negative organisms were more frequent causes of community acquired (CA) neonatal septicemia than gram positive isolates. Klebsiella pneumoniae was the most common, Staphylococcus aureus was the second most common and CONS was the least common organisms isolated. Both gram negative and gram positive showed poor sensitivity towards routinely used first line antimicrobials like Ampicillin and Gentamicin. Gram negative organisms were highly sensitive to Polymixin-B, Imipenem, Ciprofloxacin, Amikacin and third generation Cephalosporins in the descending order. Gram-positive isolates were mainly sensitive to Linezolid, Amikacin, Vancomycin, Methicillin, Ciprofloxacin and third generation Cephalosporins in descending order. Therefore, the bacteriological profile and the sensitivity pattern of Community acquired neonatal septicemia in a particular geographical area must be considered before deciding the empirical antibiotic treatment of community acquired neonatal septicemia. The higher antibiotics like the Polymixin B, Linezolid and Vancomycin should be kept reserved for multidrug resistance bacteria. Judicious or rational use of antibiotic can serve the dual purpose of not only reducing the neonatal morbidity and mortality but also reducing the multi-drug resistance rising to the dangerous level.

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