Int.J.Curr.Microbiol.App.Sci (2018) *7*(6): 1468-1474

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 06 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.706.174

A Study of Bacteriological Profile and Antibiogram of Early Onset Sepsis in a Tertiary Care Hospital

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Keywords

Early onset Sepsis, Neonates, Klebsiella pneumonia, Coagulase negative staphylococci, bacterial profile

Article Info

Accepted:
16 May 2018
Available Online:
10 June 2018

A B S T R A C T

Neonatal sepsis is one of the major causes of morbidity and mortality in the newborn. The most common isolates in the early onset septicemia are *Klebsiella Spp.* Bacterial isolates and their antibiotic susceptibility has been constantly changing. Thus the aim of the study was to isolate the aerobic bacteria responsible for early onset neonatal septicemia and determination of their antibiotic sensitivity pattern. The present study was conducted in department of microbiology of a tertiary care hospital. Blood sample were collected from 268 clinically suspected cases of neonatal septicemia admitted in the neonatal intensive care unit. About 1ml of blood was inoculated into 10ml of brain heart infusion (BHI) broth and incubates for 7 days. Repeated sub-culturing was done as per standard procedures. Any growth was subjected for identification by appropriate biochemical tests. Antibiotic susceptibility testing was done. Out of 83 culture positive cases, early onset septicemia was seen in 75(90.4%) cases. Gram negative organisms (63%) were predominant than Gram positive organisms (36%). *Klebsiella pneumoniae* (43%) and *Coagulase Negative Staphylococcus* (36%) were the common organisms isolated. Gram negative bacteria were resistant to routinely used antibiotics and were highly sensitive to Imipenem (100%). The Gram positive bacteria showed high resistance to Ampicilin (90%). Gram negative organisms mainly *Klebsiella pneumoniae* were most common agents causing early onset neonatal sepsis, their resistance pattern should be considered essential for deciding the empirical treatment.

Introduction

Neonatal sepsis is one of the major causes of morbidity and mortality in the newborn. Septicemia in neonates refers to generalized bacterial infection documented by a positive blood culture in the first 4 weeks of life (Rajiv Aggarwal *et al.,* 2001). Neonatal sepsis is divided into early- onset (from birth to the end of 7 days postnatal) and late onset (from 8 to 28 days postnatal) (Seyyed Mohammad Hassan Aletayeb *et al.,* 2011). Early-onset sepsis (EOS) is typically caused by organisms transmitted vertically before or at the time of birth. Late-onset sepsis (LOS) occurring after 7 days of life may be caused by pathogens acquired during delivery or during the course of hospital care (Soon Min Lee *et al.,* 2015).

Neonatal septicemia is caused by variety of Gram positive and Gram negative organisms (Bhattacharya, 2005). The spectrum of

organisms causing neonatal sepsis is quite different in developed countries in comparison with developing countries like India (Venkataseshan Sundaram *et al.,* 2009). The most common isolates in the early onset septicemia are *Klebsiella Spp* (25.8%), *Enterobacter spp* (22.4%) followed by *Escherichia coli* (14.5%). Late onset septicemia are *Enterobacter spp* (23.3%) the major pathogen followed by *Coagulase negative staphylococcus* (23.3%) (Roy *et al.,* 2002).

Bacterial isolates and their antibiotic susceptibility has been constantly changing which depends on several factors like gestational age, birth weight, maternal risk factors, place of delivery (Mathur, 1996), Thus theaim of our study was to isolate and identify the aerobic bacteria responsible for early onset neonatal septicemia and determination of their antibiotic sensitivity pattern.

Materials and Methods

The present study was conducted in department of microbiology of a tertiary care hospital. Blood sample were collected from 268 clinically suspected cases of neonatal septicemia admitted in the neonatal intensive care unit. About 1-2ml of single blood sample was collected from peripheral vein under aseptic conditions. About 1ml of blood was inoculated into 10ml of brain heart infusion (BHI) broth and incubated at 37^0C. Subcultures were done on sheep blood agar and MacCankey's agar at the earliest visual detection of turbidity or blindly on days 1, 3 and 7 if the bottles did not show turbidity. Isolates were identified by their characteristic appearance on their respective media, Gram staining and confirmed by the pattern of biochemical reaction using the standard method. Second blood culture was processed in cases where *coagulase negative staphylococcus* (CONS) was isolated. Blood culture broth which did not yield any growth following three subculture were reported negative at the end of 7 days.

The antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method for the bacterial isolates, as per Clinical and Laboratory Standard Institute guideline (CLSI). ATCC control strains were used accordingly as per standard procedures. Percentages and proportions are used for descriptive data and the level of significance for tests of significance was set at $p< 0.05$.

Results and Discussion

During the study period, a total of 268 newborns with clinical sepsis were admitted. Blood culture reports were positive in 83 cases (31%). Out of 83 culture positive cases, early onset septicemia was seen in 75(90.4%) cases and 08(9.6%) cases showed late onset septicemia. Among the 75 culture positive EOS cases, there were 38(51%) males and 37(49%) were female neonates. Among culture positive early onset sepsis cases higher percentage of culture positivity were observed among preterm neonates (60%) as compared to term and post term neonates (Table I).

Out of 75 early onset sepsis cases 21(28%), 42(56%) and 12 (16%) were very low, low and normal birth weight neonates respectively. And a higher percentage of culture positivity was observed among low birth weight neonates i.e. 42(56%). Of these culture positive early onset sepsis cases, 32(43%) were hospital inborn babies, and 39(52%) were hospital outborn babies.

Table II shows that, of the culture positive cases majority i.e. 47(63%) of isolates were Gram negative organisms, *Klebsiella pneumoniae* being the commonest isolate 32(43%) followed by *Pseudomonas*

aeruginosa 12(16%). Other organisms isolated were *Escherichia coli* 02(3%) and *Proteus vulgaris* 01(1%). Gram positive organisms were obtained in 27(36%) cases. *Coagulase negative staphylococci* 23(31%) was commonest isolate followed by *Staphylococcus aureus* 4(5%).

Table III and IV shows antibiotic sensitive pattern in Gram negative and Gram positive isolates. Best overall sensitivity among Gram negative isolates was to Imipenem and Azithromycin followed by chloramphenicol.

Variable susceptibility was seen in third generation cephalosporins, almost all isolates showed resistance to Aztrenoma, Amoxyclav, Gentamicin, and Amikacine.

Gram positive isolates had sensitivity to Azithromycin, Vancomycin, Ciprofloxacin, and Ofloxacine, they showed moderate susceptibility to third generation cephalosporins. Highly resistance was seen with Ampicillin and Methicillin.

Blood culture although considered to be gold standard in the confirmation of diagnosis of septicemia, in the present study 83/263 cases studied were culture positive, giving a culture positivity rate of 31%. It was comparable with studies conducted by Uddin Ahmed *et al.,* 35%.

Maximum culture positive cases were seen in neonates less than 1 week of age (Early onset septicemia) as compared to neonates aged more than 1 week (Late onset septicemia) in the present study. Similarly, a higher percentage of EOS was seen in the studies done by (Shashikala S. Tallur *et al.,* 2001; Roy *et al.,* 2002), this could be due to ascending infection following rupture of membranes or during the passage of baby through the infected birth canal or at the time of resuscitation in the labour room.

In this study, a higher percentage of culture positive sepsis cases were seen in preterm neonates than term and post term babies. It was also observed that septicemic cases were higher among the low birth weight neonates as compared to the very low birth weight and normal birth weight neonates. The results of present study are comparable with the study conducted by (Seyyed Mohammad Hassan Aletayeb *et al.,* 2011). Our results differ from that of (Khatua *et al.,* 1986; Shashikala S. Tallur *et al.,* 2001 and Zawar *et al.,* 2003), who reported higher proportion of cases in the very low birth weight neonates, while the study of (Maya Raghavn *et al.,* 1992) showed a higher proportion of cases among the normal birth weight neonates. According to Nelson Textbook of Pediatrics, (Richard E. Behrman *et al.,* 2004) the most important neonatal factor predisposing to infection is prematurity of LBW. Preterm LBW infants have a 3 to 10-fold higher incidence of infection than full-term normal birth weight infants. Possible explanations are premature infants have documented immune dysfunction; and premature infants often require prolonged intravenous access, endotracheal intubation or other invasive procedures that provide a portal of entry or impair barrier and clearance mechanisms.

The present study showed a higher proportion of culture positive cases among outborn / referred neonates compared to neonates who are inborn. Our results were comparable with the observations made by (Mondal *et al.,* 1991) in their study. This could be mostly because the study hospital is a referral unit and majority of the admissions are out born and referred in critically ill conditions.

In the present study majority of the isolates were Gram negative organisms accounting for 63% of the isolates with *Klebsiella pneumoniae* being the commonest isolated in 45% of the 75 culture positive cases.

Table.1 Distribution of EOS cases

DETERMINANTS	CULTURE POSITIVE	
	No	%
Gender		
Males	38	51
Females	37	49
Gestational age		
Preterm (< 37 weeks)	45	60
Term (37-41 weeks)	29	39
Post term(> 41 weeks)	01	01
Birth weight(g)		
Very low birth weight(< 1500 gms)	21	28
Low birth weight(1500-2500 gms)	42	56
Normal birth weight(>2500 gms)	12	16
Place of delivery		
Hospital		
• **Inborn**	32	43
• **Outborn**	39	52
Home	04	05

Table.2 Spectrum of bacterial isolates in Early Onset Sepsis

BACTERIAL ISOLATES	CULTURE POSITIVE	
	NUMBER	PERCENTAGE
A) GRAM POSITIVE ISOLATES		
Staphylococcus aureus	04	05
Coagulase negative staphylococci (CONS)	23	31
TOTAL	**27**	**36**
B) GRAM NEGATIVE ISOLATES		
Klebsiella pneumonia	32	43
Pseudomonas aeruginosa	12	16
Escherichia coli	02	3
Proteus vulgaris	01	1
TOTAL	**47**	**63**
C)POLYMICROBIAL ISOLATES		
Coagulase negative staphylococci and Klebsiella pneumoniae	01	1
TOTAL	**75**	

Table.3 Antibiotic sensitivity pattern among Gram positive isolates

ANTIBIOTIC	CONS (N = 23)				*Staphylococcus aureus* (N = 4)			
	SENSTIVE		RESISTANT		SENSTIVE		RESISTANT	
	No	%	No	%	No	%	No	%
Amoxycillin/ Clavlnoic acid	11	48	12	52	03	75	01	25
Azithromycin	21	91	02	09	04	100	00	00
Ampicillin	03	13	20	87	00	00	04	100
Chloramphenicol	23	100	00	00	03	75	01	25
Ciprofloxacin	20	87	03	13	03	75	01	25
Ceftazidime	08	35	15	65	02	50	02	50
Ceftriaxone	21	91	02	09	04	100	00	00
Linezolid	11	48	12	52	02	50	02	50
Ofloxacin	20	87	03	13	04	100	00	00
Vancomycin	20	87	03	13	03	75	01	25
Methicillin	02	09	21	91	01	25	03	75

Table.4 Antibiotic sensitivity pattern among Gram negative isolates

ORGANISMS	Ak	Ao	Ac	At	C	Ce	Ca	Ci	Cf	G	Pt	Tb	Of	I
Klebsiella pneumonia (32)														
Sensitive	04(12)	02(06)	01(03)	30(94)	16(50)	01(03)	01(03)	16(50)	01(03)	06(19)	04(12)	06(19)	16(50)	32(100)
Resistant	28(88)	30(94)	31(97)	02(06)	16(50)	31(97)	31(97)	16(50)	31(97)	26(81)	28(88)	26(81)	16(50)	00
Pseudomonas aeruginosa (12)														
Sensitive	07(58)	05(42)	06(50)	12(100)	07(58)	08(67)	07(58)	07(58)	08(67)	07(58)	09(75)	05(42)	07(58)	12(100)
Resistant	05(42)	07(58)	06(50)	00	05(42)	04(33)	05(42)	05(42)	04(33)	05(42)	03(25)	07(58)	05(42)	00
Escherichia coli (02)														
Sensitive	01(50)	00	01(50)	01(50)	02(100)	00	00	01(50)	01(50)	00	01(50)	00	01(50)	02(100)
Resistant	01(50)	02(100)	01(50)	01(50)	00	02(100)	02(100)	01(50)	01(50)	02(100)	01(50)	02(100)	01(50)	00
Proteus vulgaris (01)														
Sensitive	01(100)	00	00	01(100)	00	01(100)	01(100)	01(100)	00	00	01(100)	00	00	01(100)
Resistant	00	01(100)	01(100)	00	01(100)	00	00	00	01(100)	01(100)	00	01(100)	01(100)	00

Antibiotic disks (Himedia) and their concentrations per disk comprised : Azithromycin-At (15µg), Amoxyclav-Ac (30µg), Amikicin-Ak (30µg), Aztreonam-Ao (30µg), Chloramphenicol-C (30µg), Cephotaxime-Ce (30µg), Ceftazidime-Ca (30µg), Ceftriaxone-Ci (30µg), Ciprofloxacin-Cf (5µg), Ofloxacin-Of (5µg), Gentamicin-G (10µg), Piperacillin/Tazobactam-Pt (10µg), Tobramycin-Tb (5µg), Imipenem- I (10µg).

Among the *Klebsiella pneumonia* maximum isolates i.e. 30(94%) were susceptible to Azithromycin followed by Chloramphenicol 16(50%). 94% resistance was seen to Amoxyclav. A varied resistance pattern was seen to Ceftazidime, Aztreonam (94%) and Ciprofloxacin (97%). It is evident from the present study that maximum susceptibility was noted to Chloramphenicol and newer macrolides like Azithromycin. Majority of the isolates were susceptible to third generation cephalosporin. In a study done by (Shashikala S. Tallur *et al.*, 2000), *Klebsiella pneumonia* was the commonest pathogen found which were more susceptible to Gentamicin, Amikicin and third generation cephalosporin. The study of (Joshi *et al.*, 2000) shows a predominance of Gram negative bacteremia (67.2%) in their study, which had 25-75% resistance to Cephalosporin, 68-78% resistance to piperacillin and 23-69% resistance to Gentamicin.

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. Present study 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 (Shashikala S. Tallur *et al.,* 2000)

Also in the present study, 12(14.5%) isolates were *pseudomonas aeruginosa* which was 100% susceptible to Azithromycin, Study of (Movahedian *et al.,* 2006) observed 70% of susceptibility of *Pseudomonas aeruginosa* to Amikicin followed by Gentamicin 53% and lower susceptibility to Ampicillin, Cephalexin, Ceftriaxone and cefotaxime.

Overall the Gram negative organisms were susceptible to Azithromycin. Except *Proteus vulgaris* all other Gram negative organisms were susceptible to Chloramphenicol. A variable susceptibility was seen to third generation cephalosporin. Almost all isolates showed resistance to Aztreonam. The sensitivity to Azithromycin may be due to its nonuse among neonates by intravenous route.

In the present study *Coagulase negative staphylococcus* was the major Gram positive organism isolated constituting 26(31%) of the isolates and *Staphylococcus aureus* being 4(5%). All the gram positive isolates were 100 % susceptible to Azithromycin and Ceftriaxone. Of about 80% susceptibility was seen to Vancomycin and Ofloxacin. (Shashikala S. Tallur *et al.,* 2000) noted a high incidence of *Staphylococcus spp* in their study, pointing towards its nosocomial origin. All these isolates were 100% susceptible to Vancomycin. In the study by (Karthikeyan and Premkumar, 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.

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 (Anand *et al.,* 1991).

This research study identified *CONS, S. aureus, and Klebsiella pneumoniae* as the predominant etiological agents of bloodstream infection during early neonatal period. Effective prophylactic measures, prompt and accurate diagnoses and subsequent administration of targeted therapy are vital to curb the excessive burden of the disease. An alarmingly high degree of antibiotic resistance observed calls for an urgent evaluation and development of antibiotic policies and protocols for neonatal sepsis. Future epidemiological and clinical studies are also needed to monitor changes in the microorganisms causing early onset neonatal sepsis.

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How to cite this article:

Arati Bhurle, Shivakumar Solabannavar and Anushka V. Devnikar. 2018. A Study of Bacteriological Profile and Antibiogram of Early Onset Sepsis in a Tertiary Care Hospital. *Int.J.Curr.Microbiol.App.Sci.* 7(06): 1468-1474. doi: https://doi.org/10.20546/ijcmas.2018.706.174