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Microbiological Profile of Neonatal Septicemia in a Rural Teaching Hospital of North India

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Abstract
Objectives: *To study the microbiological profile of neonatal septicemia and to determine the anti-microbial resistance pattern of isolated bacteria.*
Study Design: *retrospective analysis of blood culture reports of neonatal septicemia cases.*
Setting: *tertiary level neonatal intensive care unit*
Patients and Methods: *case records of neonatal septicemia cases were retrieved and their culture results were analysed in terms of causative organisms and their antimicrobial sensitivity patterns. Extended spectrum beta lactamamse (ESBL) resistance and methicillin resistant Staphylococcus Aureus (MRSA) patterns were studied.*
Results: *out of 162 samples, bacterial growth was detected in 53 samples. Gram negative growth was around 66 percent and rest were gram positive. Gram negative organisms accounted for more than 75 percent in both early and late onset sepsis. Out of 12 blood cultures showing Staph Aureus, 7 (58%) were MRSA. Vancomycin was the drug to which all MRSA growths were sensitive. Out of 34 gram negative infections, 13 were ESBL in whom Klebsiella sp. was the most common followed by E. coli. All ESBL organisms were sensitive to Imipenem.*
Conclusion: *gram negative organisms predominated in the neonatal septicemia. MRSA and ESBL strains hold a large share of the bacterial growths.*

Introduction

Septicemia in neonates is the widespread bacterial infection recognized in first four weeks of life by positive blood culture [(1)]. In developing countries, factors that predispose newborns to risk of developing neonatal sepsis compared with developed countries include intrinsic factors and extrinsic factors in the antenatal, intra-partum and the neonatal period. According to World Health Organization (WHO) every year there are about 5 million neonatal deaths due to neonatal infections[(2)]. Onset of septicemia can be early (<72hrs) and late (≥72hrs – 28days). Early onset sepsis (EOS) is attained during fetal life, caused

by organisms prevalent in the maternal genital tract, infecting the neonate transplacentally or during passage through a colonized birth canal at the time of delivery or at the nursery [3]. The late onset sepsis (LOS) is acquired from environment (hospital) usually intensive care units (NICU) or the community. The infant's skin, respiratory tract, conjunctiva, gastrointestinal tract and umbilicus may become colonized from the environment and such colonization may lead to possibility of late onset septicemia from invasive microorganisms. During the past decade, extended spectrum beta lactamase (ESBL) and methicillin resistant staph aureus (MRSA) strains have repeatedly been implicated in neonatal intensive care units at tertiary care hospitals. Microorganisms develop resistance continuously by modifying or replacing the target that is penicillin binding protein (PBP) or acquiring beta-lactamases and other mechanisms.

Emergence of various β-lactamases producing multi-drug resistant strains is one of the prominent causes of septicemia, which obscures the clinical as well as therapeutic outcomes. [4]Sepsis with ESBL producing bacteria is increasingly acknowledged in Asian countries in recent years [5]. Also, Metallo-beta lactamases and Amp C beta lactamase producing GNB have emerged in neonatal septicemic case. Failure to detect various lactamases enzymes and their combination in the same organism has contributed to therapeutic failures.[6]

The organisms causing neonatal septicemia keeps changing over time and varies from place to place, due to change in lifestyle and also altering pattern of antibiotics use. Every hospital should have a policy to keep on evaluating the microbiological pattern of infection cases so that upgradation of treatment protocols can be done. This study was conducted as such an exercise to determine the microbiological profile and antibiotic susceptibility pattern in bacteria from suspected neonatal septicemia cases admitted in NICU of MMIMSR, Mullana.

Objectives

- To study the microbiological profile of neonatal septicemia and to determine the anti-microbial sensitivity pattern of isolated bacteria.
- To find out the rate of ESBL, and rate of MRSA in neonatal septicemic cases.

Material and Methods

Study Design - A retrospective study of bacterial isolates in neonatal septicemia cases

Study Population: Case records of neonatal septicemia (admitted between January 1, 2015 to December 31st 2015) were retrospectively analysed. Study was conducted on blood culture reports of all neonates in neonatal intensive care unit (NICU) with suspected septicemia during a study period of 1 year.

Study Size and Sampling

Case records were selected in whom, blood culture showed the growth of microorganism.

Exclusion Criteria

Neonates already on antibiotics prior to admission antenatal diagnosis of intra-uterine infection, fungal growth/ mixed growth/ growth labeled as contaminant by physician

Ethics Consideration- The ethics clearance was taken from ethical committee.

Data Collection: details of clinical records were recorded in the Microsoft excel datasheet and analysed

Results

out of total 162 cases of neonatal septicemia the culture positive cases were 55 hence the culture isolation rate was (33.95%). the predominance was of bacterial isolates (95.45%) as compared to fungal isolates (4.55%).The 5 fungal isolates were found to be candida sp. distribution of bacterial isolates, showing Gram negative (64.15%) was predominant than gram positive (35.85%). Table I – Exhibits demographic characteristics of neonatal septicemic cases. Male cases 35(66.03%) were more than female cases 18(33.96%). Pre term cases were 13(24.52%), term cases were

40(75.47%). Birth weight of neonates less than 1500g were 12(22.64%), 1500-2500g were 35(66.03%), more than 2500g were 6(11.32%) Table II- illustrates that among culture positive neonates, 42 (79.24%) were having EOS and 11(20.75%) were having LOS .In EOS hospital deliveries were more (87.5%) in contrast to LOS where outborn babies were more. Table III- Illustrates In gram negative *acinetobacter* spp was 37.68% followed by *klebsiella* (28.98%), *Ecoli* (14.49%), *pseudomonas* sp(8.69%), *Citrobacter* spp (5.8%) and *Enterobacter* sp(2.89%). However, in Gram positive (35.85%) *staph aureus* (60.53%) was predominant followed by *staph epidermidis* (21.05%) and *Enterococcus* (18.42%). Table IV – Depicts distribution of isolates in early onset septicemia. In EOS, MRSA was 66.66%, among ESBL producing isolates *Klebsiellaspp* (23.80%) and *S. aureus* (21.42%) were predominent followed by *Citrobacterspp* (50%), *Enterobacterspp* (100%) and *Acinetobacterspp* (12.5%). Table V– Showing distribution of isolates in Late onset septicemia. In LOS, *Acinetobacterspp* (36.36%), *S.aureus* (27.27%), *E.coli* (18.18%), *pseudomonas spp* (9.09%), *Klebsiellaspp* (9.09%) were isolated.-. MRSA (7 out of total 12 staph aureus patients, 58.33%) were more than MSSA (41.66%). maximum susceptibility pattern of MRSA was to Vancomycin (100%) followed by Gentamicin (71.4%), linezolid (71.43%), Azithromycin (57.1%), Tetracycline (57.14%), co-trimoxazole (42.86%), Ciprofloxacin (28.57%), MRSA showed 100% resistance to Penicillin. Table VI – Shows distribution of ESBL, among gram negative isolates of neonatal septicemia. ESBL (38.23%) ESBL was exhibited by *Klebsiellaspp* 53.84%, *Ecolispp* 23%, *Citrobacterspp* 7.69%, *Enterobacterspp* 7.69% and *Acinetobacterspp* 7.69%. In case of ESBL all isolates showed 100% senstivity to imipenem, followed by amikacin (76.92%), piperacillin/tazobactam (46.15%), meropenem (46.15%), ampicillin (7.69%), chloramphenicol (23.07%), cefepime (30.76%), ciprofloxacin (30.76%), gentamicin (23.07%), tobramycin (23.07%), kanamycin (7.69%), tetracycline (7.69%).

Table I- Patient's characteristics of neonatal septicemia (n = 53)

Gender	Male Female	35(66.03%) 18(33.96%)
Gestation	Preterm Term	13(24.52%) 40(75.47%)
Birth weight(g)	Very low<1500 Low1500-2500 >2500	12(22.64%) 35(66.03%) 6(11.32%)

Table II: Distribution of cases according to age of onset-

Culture positive cases	EOS		LOS	
53	42(79.24%)		11(20.75)	
	Hospital Delivery	Outborn Babies	Hospital Delivery	Outborn Babies
	36(85.71%)	6(14.28%)	2(18.18%)	9(81.81%)
Fisher exact test, P Value = 0.0001 Children with EOS had a significantly higher chance of hospital deliveries where-as children with LOS had a significantly higher chance of being delivered at home				

Table III- Distribution of bacterial isolates

Total number of bacterial isolates	Gram positive isolates		Gram negative isolates	
53	Organism	N=19 (35.85%)	organism	N=34(64.15%)
	Staphylococcus aureus	12(60.53%)	Acenitobacterspp	13(37.68%)
	Staphylococcus epidermidis	4(21.05%)	Klebsiellaspp	10(28.98%)
	Enterococcus	3(18.42%)	Escherichia coli Pseudomonas spp Citrobacterspp Enterobacter Alcaligenesfaecalis	5(14.49%) 3(8.69%) 2(5.8%) 1(2.89%) 0

Table IV – Distribution of isolates in early onset septicemia (EOS)

ORGANISM	Number of isolates (N=42)	MRSA	ESBL
Klebsiellaspp	10(23.80%)		8(80%)
S.aureus	9(21.42%)	6(66.66%)	
Acinetobacterspp	8(19.04%)		1(12.5%)
CoNS	4(9.52%)		0
Ecolispp	3(7.14%)		2(66.66%)
Enterococcus spp	3(7.14%)		0
Pseudomonas spp	2(4.76%)		0
Citrobacterspp	2(4.76%)		1(50%)
Enterobacterspp	1(2.38%)		1(100%)
Alcaligenes faecalis			0

Table V – Distribution of isolates in Late onset septicemia (LOS)

ORGANISM	Number of isolates	ESBL
Acinetobacterspp	4(36.36%)	0
s.aureus	3(27.27%) MRSA 1(33.33% OF staph infections)	0
Ecolispp	2(18.18%)	1(50%)
Pseudomonas spp	1(9.09%)	0
Klebsiellaspp	1(9.09%)	0
Total	11	0

Table VI -Distribution of ESBL isolates in Gram negative bacilli:

Name of organism	ESBL
Acinetobacterspp(13)	1(7.69%)
Klebsiellaspp(10)	7(53.84%)
E.coli spp (5)	3(23%)
Pseudomonas spp(3)	0
Citrobacterspp (2)	1(7.69%)
Enterobacterspp (1)	1(7.69%)
TOTAL= 34	13

Discussion

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremia in the first month of life. An early diagnosis of neonatal septicemia is vital for proper treatment. A planned and appropriate use of antibiotics, efficient hospital policies, thorough and precise detection of resistant species, is required to check the increasing incidence of antimicrobial resistant organisms. Appropriate antibiotic policy can be made once microbial flora patterns are known along with their sensitivity analysis. The present study revealed that amongst a total of 162 clinically suspected cases of neonatal septicemia blood cultures from 55(33.85%) were positive which was in accordance with Roy II et al (47.5%) from[7]and Kayange.N et al (38.9%) [8]. This is In contrast to more developed countries with better healthcare infrastructures.[9]The range of organisms causing neonatal sepsis varies in time and place. The data of the present study showed bacterial isolates (96%) were more than fungal isolates(4%) similar to various other Indian studies[10][11][12]. This study there was the predominance of gram negative (64%) over gram positive isolates (35%). In current study GN isolates (64.49%) were more than GP isolates (35.51%) which is consistent with [13][14] . Our results are also consistent with the recent analysis at which showed similar flora pattern in similar time frame.

In gram positive isolates majority was *staph. aureus 60*.53% followed by *staph. epidermidis* 21.05% , *Enterococcus*18.42%. in gram negative bacteria, leading organism was *Acenitobactersp* (37.68%) followed by *Klebsiella pneumonia* (28.98%), *Escherichia coli* (14.49%), *Citrobactersp* (10.14%), *Pseudomonas sp*(8.69%) and *Enterobacter* (2.89%). *S.aureus* was most usual Gram-positive organism and among all isolates the second most common organism. A substantial percentage (21.05%) was Coagulase negative *staph aureus* (CONS). In case of Gram negative bacilli the predominant organism was *Acinetobacter* species (37.68%) followed by *Klebsiella 30744neumonia* (28.98%) and *E coli*(14.49%) in present study which is in accordance to Marwah.P et al[15]*Acinetobacter* (14.9%) and *Klebsiella pneumonia* (14.9%), Mishra. A[16] *Acinetobactersp* (31.5%), *E.coli* (26.3%), *Klebsiella pneumonia* (10.7%). Non-fermenter, *Acinetobacter* appeared as significant nosocomial pathogen in neonates. *Klebsiella* was the predominant organism in a similar study[17] (*Klebsiellasp* 22.53%, *Pseudomonas sp* 21.12%, *Acinetobacter sp* 15.49%)

The upward moving graph of prevalence of MRSA in NICUs reflected by the recent data and the consequent evolution of its various strain within the hospital and community settings suggest its alarming trendas a pathogen. In present study, 7(58%) were MRSA which were more than MSSA similar to Chelliah. A et al [18](MRSA 56%) in contrast Kaistha.N et al [19] (11.11%).The higher rate of MRSA could, to a great extent be attributed to the fact that these organisms are now both hospital acquired and community flora. In present study all MRSA were 100% sensitive to Vancomycin. Present study demonstrates 100 % sensitivity to vancomycin followed by Gentamicin (71%), linezolid (71.43%), azithromycin (57%), tetracycline (57.14%), co-trimoxazole (42.86%), ciprofloxacin (28.57%), Erythromycin (28%) and clindamycin , levofloxacin (14.29%) and analogous with Pandey.S et al[20]with Vancomycin (100%), Gentamicin (79.32%) but in contrast to Saravanan. M et al[21] with oxacillin (100%), Vancomycin (75%).The antibiotic susceptibility pattern varies in space and time and within localized communities as well.

ESBLs are now a problem in hospitalized patients throughout the world. The study revealed ESBL production (38.2%) majority were *klebsiella* (53%) and *Ecoli* strains (23%) this is closer to study by Chellaih. A et al [18] (ESBL 67.3%, *Klebsiella* 70.9%, *Ecoli* 57.14%), Kamble.R and Ovhal.R [17] (*Klebsiella* 62.5%, *Ecoli* 20%), Bhattacharjee. A et al [10](*Klebsiella* 62.7%, *Ecoli* 46.5%).The extensive use of broad spectrum β-lactam antibiotics has led to marked increase in the incidence of Amp C in Gram negative organisms particularly family Enterobacteriaceae. In current study, 1(50%) *Klebsiella i*solates in which both ESBL and Amp C are co-expressed in the same isolate and 1 (50 %) *Escherichia coli* isolates were AmpCβ-lactamase producers similar to Subitha.B et al [22] with *Klebsiellasp* (25%) and *Ecoli* (11%).All ESBL isolates were sensitive to imipenem (100%) followed by amikacin(77%), piperacillin/tazobactam (46%), meropenem (46), ampicillin (7.7%), chloramphenicol(23%), cefepime (26.92%), ciprofloxacin (23.08%), gentamicin (19.23%), tobramycin (11.54%), kanamycin (7.69%), tetracycline (7.6%) (Table VII) similar to Kulkarni.R et al imipenem (100%), amikacin (70.4%), Chelliah. A et al[18]imipenem (100%) in contrast to Singh.et al[23]Imipenem (65.62%), Piperacillin/tazobactam (56.25%) and Amikacin (34.36%). The 100% sensitivityto imipenem signifies the absence of selective pressure thus can be dispensed only after antibiotic susceptibility testing

National Neonatal Perinatal database (NNPD) 2003 categorizes sepsis as EOS (presenting within 72 hours of birth) and LOS (presenting 72 hours after birth).In the present study, out of 53 culture positive (bacterial) cases , EOS (79%) was more frequent than LOS (21%) which is in agreement with Samaga.M.P et al[14] (53.6%, 46.4%), Mustafa.M et al[13] (58%,42%), Premalatha. D.E et al (78.5%,21.5%) [24] On the contrary, Kayange . N et al[8](EOS-47%, LOS-51.4%) , Thakur.S et al (LOS 51%,EOS 49%)[25] LOS was predominant

In present study, *Klebsiella sp*(24%) and *s.aureus* (21%) were predominently isolated from EOS which is similar to Mane.A.K et al [26]*klebsiella* (20.83%) , *S.aureus* (20.83%) but in contrast to Gandhi.S et al [27] with *Ecoli* (26.08%), *s.aureus* (23.91%) and Shehab El-din.E.M.R et al [28]shows predominance of CoNS, *Acinetobacter*. Majority cases of MRSA, ESBL, MBL were reported in EOS as compared to LOS. Further in EOS, MRSA was shown by (66%). The present study depicted predominance of *Staph aureus* in EOSas compared to LOS. EOS is considered to pass from mother to child at time of labour and during delivery. *S.aureus* often colonises the human skin, mucous membranes. Out of 3 *E.colisp* 2 produced ESBL (66%) followed by *Klebsiella sp* (80%), *Citrobacter sp* (50%), *Enterobacter sp* (50%), *Acinetobacter sp* (5.56%). Predomience of *E.coli* could be explained by the fact that *E.coli* is normal vaginal flora but irrational use of antibiotics can cause change in E.coli producing ESBL which gets transmitted to neonates during

delivery. In present study, predominent organisms isolated in LOS was *acinetobacter sp* (36%) followed by *S.aureus* (27%). This is similar to Shrestha .N.J et al[29] *E.coli* (52.3%), *s.aureus* (46.7%) as predominent organisms But in contrast with Gandhi.S et al [27] CoNS (19.44%) and *klebsiella pneumoniae* (16.67%). MRSA was shown by 2 out of 3 S.aureus isolates (67%). Similarly, out of 2 *E.coli sp* 1(50%) showed ESBL,

LWB and prematurity have been mentioned to be significant risk factors for neonatal sepsis In the current study, neonatal septicemic cases in males (67%) were reported more than females (33%) which is similar to Khinchi.Y.R et al [30] with male (65.1%), female (34.9%) and Shehab El- din. E.M.R et al[28] male (61.2%), female (38.8%). Perhaps the synthesis of γ-globulins was regulated by X–linked immune regulatory genes and males with one X chromosome are more susceptible for neonatal septicemia than females. In present study, preterm neonates were 25% and term neonates were 75% this is in accordance with Shrestha.P et al [31] with preterm cases (24.27%), term (72.81%). Also, in present study, neonates with birth weight <1500g were 21.82%, 1500-2500g were 67.27% and >2500g were 10.91% which was in accordance to Shehab El-din et al (28) VLBW (6.9%), LBW (62.8%), >2500g (30.3%).

Conclusion

In this study, out of 162 cases, the culture positive cases were 55 (34%),

Predominance was of bacterial isolates (96%) as compared to fungal isolates (4%)

MRSA (58%) was more than MSSA (42%). MRSA showed 100% sensitivity to Vancomycin.

Among GNB, ESBL production was seen in 38% isolates

Antibiotic susceptibility pattern of ESBL, showed 100% senstivity to imipenem by all isolates and lowest was to tetracycline (7%).

To overcome the problem of community acquired drug resistance and MDR which is a domain of hospital, a combined effort between the microbiologists, and physicians is required. We endorse the periodic appraisal in antibiotic policy in the hospital according to available data of antibiotic sensitivity and microorganism profile from time to time. Proper antibiotic policy to restrict the indiscreminate use of antibiotics particularly cephalosporins and carbapenems should be taken to minimize the emergence of beta lactamase producing pathogens.

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