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A Prospective Study on Bacterial Isolates causing Neonatal Septicemia and their Sensitivity Pattern in a Tertiary level Hospital of Dhaka, Bangladesh

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Abstract

Now a day's high bacterial resistance is creating an alarming situation in the developing and also in the developed countries. This prospective study has been done to isolate the bacterial pathogens of neonatal septicemia and their resistance pattern to establish the best antimicrobial regimens. Here 961 suspected cases of septicemia were included who admitted to the Special Care Neonatal Unit (SCANU) of the selected tertiary care hospital from June 1, 2012 to December 31, 2013 in Dhaka, Bangladesh. Demographic and clinical data were collected. Blood culture was done in all cases. Sensitivity was tested by Kirby Bauer Disc Diffusion techniques. Clinically early onset sepsis was diagnosed in 559 and late onset sepsis in 402 cases. However, culture positivity was found only in 125 cases, among them, early onset sepsis in 73.6% and late onset sepsis in 26.4% of cases. Culture positivity showed a highly significant relationship with the age of the neonates. Blood culture was positive in more cases (74/464) of hospital delivery compared to home delivery (51/497), which was statistically significant. Here Gram negative organisms (88.0%) were predominating. Most of the drugs (Ampicillin, Gentamicin, Ceftriaxone, Cefotaxime, Ceftazidime, Amikacin) were almost 100% resistant against Acinetobacter, Pseudomonas aruginosa, Streptococcus pneumonie, Gram negative bacilli, Enterobacter Cloacae, Klebsiella pneumoniae. Ampicillin was found to be 100% resistant to Gram negative cocci. Only Imipenem (84.9%), Meropenem (92%) and Ciprofloxacin (93.6%) were found to be sensitive against most of the bacterial isolates. To overcome this situation antibiotic selection by regular monitoring is essential.

Keywords: Neonatal Sepsis, bacterial isolates, antibiotic susceptibility, antimicrobial resistance, developing countries.

Introduction

In the developing countries, neonatal sepsis remains a significant cause of neonatal deaths globally. It demands urgent diagnosis and treatment because of its clinical emergency[1]. There are about 5 million neonatal deaths in a year worldwide, 98% of this death occurring in developing countries. In Bangladesh, 57% of under-5 deaths occur within the first 28 days after birth and another 23% take place in the post natal period[2]. A study report of WHO found that below the age of 5 years perinatal deaths are responsible for most childhood mortality in the developing countries. These perinatal deaths are one of the most significant results of neonatal sepsis[1].

World Health Organization (WHO) has attributed 30% of neonatal deaths to preterm birth, 27% to sepsis or pneumonia, 23% to birth asphyxia, 6% to congenital abnormalities, 4% to tetanus, 3% to diarrhea and 7% to other causes[3]. In 2007, a global consultation convened by the Saving Newborn Lives, initiative of Save the Children, the United States Agency for International Development and the WHO concluded that there was insufficient evidence in community-based settings on infection management to make strategy for global programs, especially for young infants[4].

Neonatal septicemia can be divided into early onset in the first 7 days of life and late onset after the first 7 days of life. The most important risk factors for early onset septicemia related to the mother (early rupture of membrane and the long duration of time for the birth of the baby) and at the same time the risk factors for infection related to fetus are prematurity. Nosocomial infections are the most significant risk factor in late neonatal septicemia[5].

The findings of the study have important implications for community-based research and programs to improve maternal and neonatal health and survival not only in Bangladesh, but also in other similar developing world settings.

Material and Methods

Study design: A prospective study was conducted including 961 suspected cases of septicemia admitted to the Special Care Neonatal Unit (SCANU) of the selected tertiary care hospital from 1st June, 2012 to 31ST December, 2013 in Bangladesh. According to the American Academy of Pediatrician (AAP) classification, the SCANU of this hospital falls in level-II (A) neonatal care facility[6].

Inclusion and exclusion criteria: Septicemia was suspected

from clinical history, if one or more of the following symptoms were present: lethargy, refusal to feeds, abdominal distension, vomiting, grunting, respiratory distress, hypothermia, hyperthermia, sclerema with or without supporting evidence of risk factors such as prematurity, low birth weight, history of prolong rupture of membrane (>18 h). Neonates with extreme prematurity, respiratory distress syndrome (RDS) and gross congenital anomalies were excluded from this study.

Isolation of bacteria: Blood samples (2-3 ml) were collected from all neonates admitted to the hospital during the study period suspected to have septicemia. Blood samples were collected with all aseptic precaution before instituting antibiotic therapy in the hospital. Blood cultures were processed using the standard technique described by Cruickshank et al.[7] and were cultured on specific culture media to isolate the causative organisms. After that, isolated organism's were identified by colony characteristics, Gram staining and biochemical tests. For the identification of Gram positive organisms, Catalase and Coagulase test has done; For Gram negative organisms, Simon's Citrate test, MIU (Motility, Indole, Urea) and TSI (Triple Sugar Iron) Tests has done.

Antimicrobial Susceptibility testing: Antimicrobial susceptibility testing was performed for all blood culture isolates by Kirby–Bauer disc diffusion method as recommended in the Clinical Laboratory Standards Institute (CLSI) guidelines[8].

Antimicrobial sensitivity tests were conducted on nutrient agar and blood agar where needed. The number of selected antimicrobial agents was selected, which were commonly used for the treatment of neonatal septicemia in Bangladesh. Isolated pathogens were tested for determining their resistance to antibiotics like Gentamicin, Ampicillin, Ceftriaxone, Cefotaxime, Ceftazidime, Imipenem, Meropenem, Ciprofloxacin etc. The antibiotic sensitivity was performed by Kirby-Bauer's disc diffusion method[9].

Statistical analysis: Data on demographic characteristics of the mothers and children and the laboratory test results of blood culture were collected from recorded sheets. SPSS version 17.0 for Windows software was used for data recording and analysis. The chi-square (c^2) test was done between culture positive neonatal septicemia and sex of the child, age of the child, gestational age, and parity of mother, place and mode of delivery. A *p-value* of <0.05 was considered as statistically significant.

Results and Discussion

During the study period, a total of 961 neonates with clinical sepsis were admitted. Blood culture reports were positive in 125(13%) cases. Among the study population 559 (58.2%) were aged less than 7 days (early onset septicemia) and 402 (42.8%) were aged more than 7 days (late onset septicemia). Six hundred sixteen (64.1%) patients were male and 345 (35.9%) were female. Among the participants, 464 neonates were delivered at hospital and among this population 69.40% (322/464) were by the cesarean section and rest of other by the NVD processes.

Out of 559 early onset septicemias, 16.45% (92/559) neonates and out of 402 late onset septicemias, 8.20% (33/402) were showed the positive growth of bacterial culture. In this study, culture positivity showed a significant relationship (*p=.000*) with the age of the neonates. Table-1.

Table-1
Relationship between culture positivity and onset of septicemia

Onset	**Growth of the bacterial isolates**		***P*-value**
	No growth n(%)	**Positive growth** n(%)	
Early onset septicemia (Less than 7 days)	467 (83.5%)	92 (16.45)	.000
Late onset septicemia (More than 7 days)	369 (91.8)	33 (8.2)	

Out of 51 home delivered culture positive neonates 42 (38.2%) isolates were gram negative and 9 (60%) were gram positive organisms. Of 74 hospital delivered culture positive isolates 68 (61.8%) were gram negative and 6 (40%) were gram positive. No significant association (p=.07) was found between place of delivery and type of organisms.

Table-2
Relationship between culture positivity with the place of delivery

Place of delivery	**No. of Organisms (%)**		***P* – value**
	Gram positive N (%)	**Gram negative** n(%)	
Home	9 (60)	42 (38.2)	.07
Hospital	6 (40)	68 (61.8)	
Total	15 (100)	110 (100)	

Culture positivity has been shown for home delivery 10.35% (51/497) and for hospital delivery 15.9% (74/464). Interestingly culture positivity rate was significantly high (p=.009) among hospital delivered neonates in this study. So, place of delivery plays an important role with bacterial culture positivity. Again, culture positivity rate is high in mature neonates (13.7%, 81/593) compared to premature neonates (11.9%, 44/368). But there was no significant relationship (p=0.446) found. Neonate by normal delivery showed 10.38% (54/520) and by cesarean section 16.09% (71/441) culture positivity. From this study, it has been observed that type of delivery is significantly (*p=0.009*) associated with culture positivity.

Table-3
Significance of culture positivity with place of delivery, maturity and type of delivery

Variables	**No Growth of Bacterial isolates** N (%)	**Culture Positive Growth** N (%)	***p*-value**
Place of delivery			
Home	446 (89.7)	51 (10.3)	0.009
Hospital	390 (84.1)	74 (15.9)	
Maturity			
Mature	512 (86.3)	81 (13.7)	0.446
Premature	324 (88.0)	44 (12.0)	
Type of delivery			
NVD	575 (90.0)	64 (10.0)	0.009
LUCS	261 (81.1)	61 (18.9)	

Out of 125 bacterial pathogens, Gram negative organisms (88.0%) predominated over gram positive organisms (12.0%). The common isolates were *Pseudomonas aruginosa* 67(53.6%), *Klebsiella pneumonia* 17(13.6%), *Acinetobacter* 10(8.0%), *Streptococcus pneumonia* 7(5.6%), Gram negative bacilli 5(4.0%), *Enterobacter cloacae* 5(4.0%), *Staphylococcus aureus* 8(6.4%) and Gram negative cocci 6(4.8%).

Table-5 shows the antimicrobial resistance pattern of the isolated organisms. Most of the drugs that is Ampicillin, Gentamycin, Ceftriaxone, Cefotaxime, Ceftazidime, Amikacin was 100% resistant against *Acinatobacter*, *Pseudomonas aruginosa, Streptococcus pneumonia*, Gram negative bacilli, *Enterobacter cloacae*, *Klebsiella pneumonia*. All the drugs except Imipenem and Amikacin was found 100% resistant to *Staphylococcus aureus* and only Ampicillin was found to be 100% resistant to Gram negative cocci.

Discussion: Neonatal Infection is one of the major problems in developing countries, including Bangladesh[10]. The mortality rate was estimated as high as 50% for untreated neonates[11]. In this study, culture positivity was found in 13% (125/961) cases, among them Gram negative isolates were 88% and Gram positive were 12%. The most common bacteria was *Pseudomonas aruginosa* (53.6%) followed by *Klebsiella pneumonia* (13.6%). In a previous study of Monjur et al., 2012, culture positivity was found in 19.4% cases. Among them *Pseudomonas aruginosa* was 31.4% followed by *Klebsiella pneumonia* (23.2%)[12]. Santosh Kumar Mondol et al. (2012) showed that Gram-negative organisms were more common (68.4%) than gram-positive organisms (31.6%). In their study, *Klebsiella pneumonia* was the communal bacteria (52%), followed by *Staphylococcus aureus* (26%)[1]. A report from NNPD (2007), which revealed *Klebsiella pneumonia* and *Staphylococcus aureus* to be the most frequent causative organism in India 4. However, some of the previous workers reported *Pseudomonas aruginosa* and *Klebsiella pneumonia* are the common isolates in early onset sepsis[4]. Misra R N et al. (2012) found that out of 75 culture positive cases, 39 were gram negative bacilli, 36 were gram positive cocci. However, they found *Staphylococcus aureus* (32%) to be more common followed by *Klebsiella pneumonia* (16%)[13]. Shah A Jand her co-workers (2012) found that Gram negative isolates *Escherichia coli* (20%) was the commonest organism followed by *Klebsiella pneumonia* (12%)[14]. The reasons for these discrepancies remain unclear. It is possible that other factors like place and type of delivery, diagnosis of the causative organisms, treatment facilities, etc. may have played some role in these cases.

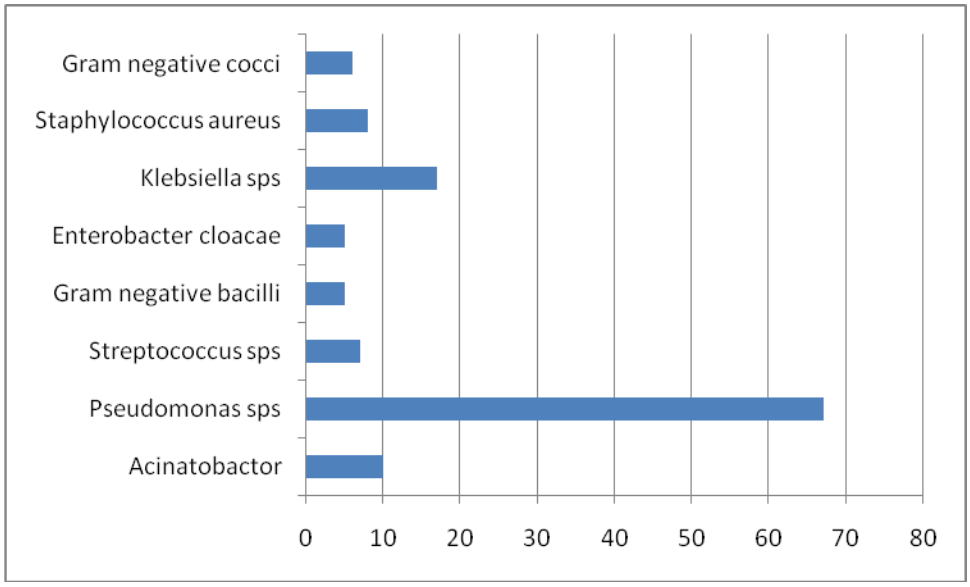

Figure-1
Isolated organisms and their numbers

Table-4
Relation between maturity and onset of sepsis with causative organisms

Organisms	Frequency in (%)	Less than 7 days in (%)	More than 7 days in (%)	Mature in (%)	Premature in (%)
Acinatobacter	10 (8)	10(100.00)	0 (0)	8 (80.0)	2(20)
Pseudomonas sps	67 (53.6)	48 (71.6)	19 (28.4)	43 (64.2)	24 (35.8)
Streptococcus sps	7 (5.6)	7(100)	0(0)	4(57.1)	3(42.9)
Gram negative bacilli	5 (4.0)	0(0)	5(100)	3(60)	2(40)
Enterobacter cloacae	5 (4.0)	0(0)	5(100)	3(60)	2(40)
Klebsiella sps	17 (13.6)	13(76.5)	4(23.5)	10(58.8)	7(41.2)
Staphylococcus aureus	8 (6.4)	8(100)	0(0)	6(75.0)	2(25.0)
Gram negative cocci	6 (4.8)	6(100)	0(0)	4(66.7)	2(33.3)
Total	125 (100%)	92(73.6)	33(26.4)	81(64.8)	44(35.2)

Table-5
Antimicrobial Resistance pattern of the isolated organisms

Organisms	CRO	CN	AMP	CTX	CAZ	CIP	IPM	MEM	AK
Acinatobacter	50% (4/8)	100% (8/8)	100% (8/8)	0 % (0/8)	0% (0/8)	0% (0/8)	0% (0/8)	0% (0/8)	------
Pseudomonas sps	39.3% (22/56)	80% (48/60)	85.7 % (48/56)	50 % (28/56)	21.4 % (12/56)	0 % (0/60)	21.4% (12/56)	0 % (0/52)	100 % (8/8)
Streptococcus sps	100% (6/6)	0% (0/6)	100 % (6/6)	100% (6/6)	100 % (6/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)
Gram negative bacilli	0% (0/4)	100 % (4/4)	100% (4/4)	------	0% (0/4)	0% (0/4)	0% (0/4)	----	100% (4/4)
Enterobacter cloacae	100% (4/4)	100% (4/4)	100% (4/4)	100% (4/4)	100% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)
Klebsiella sps	75% (12/16)	25% (4/16)	100% (16/16)	56.3% (9/16)	75% (12/16)	0% (0/16)	25% (4/16)	0% (0/12)	0% (0/8)
Staphylococcus aureus	100% (7/7)	100% (7/7)	100% (7/7)	100% (7/7)	100% (7/7)	100% (7/7)	0% (0/7)	100% (7/7)	0% (0/7)
Gram negative cocci	0% (0/5)	0% (0/5)	100% (5/5)	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/5)	-------

Here, CRO = Ceftriaxone, CIP= Ciprofloxacin, AMP= Ampicillin, CN= Gentamicin, CTX= Cefotaxime,CAZ= Ceftazidime, IPM= Imipenem, MEM= Meropenem, AK= Amikacin.

In this study, early onset sepsis cases were found to be higher than late onset sepsis. Out of 125 culture positive cases, 92 had early onset and 33 had late onset septicemia. From the study of Shah A J et al., 2012, early onset was noted in 95%, while late onset only 5% [14]. From another study, isolated pathogen in Al-Nasser Hospital with early and late onset of sepsis was 47.2 % and 52.8%, respectively[15]. Whereas the early and late onset sepsis in Al- Shifa Hospital was 81.2% (91) and 18.8% (21)[11]. Boia et al., showed in their study that out of 34 premature neonates, early onset septicemia occurred to 16 infants (47.06%) and late onset septicemia occurred in 18 cases (52.94%)[5].

Prematurity represents one of the most important risk factor for infections. The risk of developing complications increases with decreasing gestational age. Preterm infants have a 3- to 10-fold higher incidence of infection than full-term normal infants. However, in our study we found that 12% premature babies had positive blood culture and 13.7% mature babies showed culture positivity.

It is difficult to compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is extremely variable[15]. Hospital data from developing countries suggest that the pathogens causing neonatal infections (in the first 28 days of life) are common, 71% of isolates of *Klebsiella pneumonia* and 50% of *Escherichia coli* is resistant to gentamicin[16]. Resistance to Ampicillin (92.5%), Gentamicin (68.2%), Ceftriaxone (51.9%), Ciprofloxacin (6.4%), Ceftazidime (38.7%), Imipenem (15.1%), Meropenem (8.0%), Cefotaxime (52.9%), Amikacin (32.4%) has been observed in our study. Aurangzeb et al. reported considerable resistance to commonly used antibiotics such as ampicillin, amoxicillin, ceftazidime, cefotaxime and comparatively low resistance to gentamicin, impenem, ofloxacin and ciprofloxacin[17].

Multiple antibiotic resistances among neonatal sepsis are

currently one of the greatest challenges to the effective management of infections. Nalini Agnihotri et al., 2004 suggested that Aminoglycoside, third generation cephalosporin and quinolones are the drug of choice for septicemia[18]. Our study also suggests that majority of the commonly used antibiotics such as Ampicillin, Gentamicin, Cefotaxime and Ceftriaxone are resistant against the organisms. Ceftazidime, Amikacin are moderately sensitive. Only Imipenem, Meropenem and Ciprofloxacin are highly sensitive in these cases.

Conclusion

Antibiotic resistance is today a global problem. Neonatal septicemia is a life-threatening emergency, and rapid treatment with antibiotics is essential for a favorable outcome[11]. In the present study, high bacterial resistance among the pathogens suspected to cause neonatal septicemia is demonstrated which can be controlled by prudent use of available antibiotics. This study suggests regular monitoring of the antimicrobial sensitivity of the causative organisms in a particular setting is very important. It also emphasizes the need to implement infection control policies at the national level for effective management of such infections. Countries that have implemented comprehensive national strategies have been the most successful in controlling resistance[17]. It has been noticed that a vulnerable situation is waiting for the people of low income and middle income countries, because the use of antibiotic is increasing with the rising of their incomes. At the same time, high rates of hospitalization and high prevalence of hospital infections are also increased[18].

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