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Original Article

Neonatal Septicemia: Isolation, Identification and Antibiotic Sensitivity Pattern of Bacteria in a Tertiary Hospital in Bangladesh

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Abstract:

A cross sectional descriptive study was done to find out the causative organisms and their antibiotic sensitivities in the Department of Microbiology in collaboration with the Department of Paediatrics, Rajshahi Medical College Hospital (RMCH), Rajshahi during the period of July 2014 to June 2015. A total of 116 blood samples were taken aseptically from patients who were suspected of neonatal septicemia. Blood was then inoculated in Brain heart infusion broth. Bacterial isolation, identification and antimicrobial susceptibility testing were done by standard microbiological methods. Among 116 cases, 33(28.4%) were found to be culture positive. The most commonly isolated causative agents of neonatal septicemia were *Staphylococcus aureus* 17(51.5%) followed by *Escherichia coli* 10(30.3%), *Streptococcus pneumoniae* 03(9.09%), *Klebsiella pneumoniae* 02(6.06%) and *Pseudomonas aeruginosa* 01(3.03%). In general, all the Gram positive and Gram negative isolates were highly sensitive to meropenem, amikacin, gentamicin and ciprofloxacin. Gram positive bacteria were found to be particularly sensitive to vancomycin. They were moderately sensitive to ceftazidime followed in amoxicillin but were totally resistant to ampicillin. This study revealed that *Staphylococcus aureus* and *E.coli* are predominant causative organisms in neonatal septicemia and these are highly sensitive to meropenem, amikacin, gentamicin and ciprofloxacin.

Key words: Neonatal Septicemia, Aetiological Agents, Antimicrobial Susceptibility.

Introduction:

Neonatal sepsis is an important cause of morbidity and mortality among neonates. It is responsible for 30-50% of the total neonatal death in developing countries. It is estimated that up to 20% of the neonates develop sepsis and approximately 1% die of sepsis related causes[1]. It is the second leading cause of neonatal death in Bangladesh[2]. Neonates are vulnerable to developing sepsis due to factors such as immature immune system, prematurity, low birth weight, respiratory problems and maternal infections[3].

There is difference in epidemiological data from developed and developing countries in terms of incidence, risk factors, pattern and antimicrobial sensitivity in neonatal infections[3]. In most of the developing countries Gram negative bacilli remain the major cause of neonatal septicaemia. Bacteria commonly isolated in the sample included *Klebsiella pneumoniae, Escherichia coli,* Enterobacter spp, *Pseudomonas aeruginosa, Staphylococcus aureus,* Streptococcus spp, Citrobacter spp, and coagulase negative Staphylococcus (CONS)[4,5].

In Bangladesh, a few studies have reported on causative agents of neonatal septicaemia and their antibiotic sensitivity pattern[6-8]. The spectrum of organisms that causes neonatal sepsis changes over times and varies from region to region. This is due to the changing pattern of antibiotic use and changes in lifestyle[3]. This is a life-threatening emergency and delay in diagnosis and treatment of neonatal septicemia with appropriate antibiotics may have devastating consequences. Surveillance is needed to identify common pathogens of neonatal septicemia as well as to

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find out the antibiotic sensitivity pattern of the pathogens. This study was undertaken to find out the causative organisms of neonatal septicaemia and their antibiotic susceptibility.

Materials and Methods:

This descriptive cross-sectional study was conducted in the Department of Microbiology, Rajshahi Medical College Hospital (RMCH), Rajshahi in collaboration with Department of Paediatrics of the same institution during the period from July 2014 to June 2015 after the approval of protocol by the Rajshahi Medical College Ethical Committee.

A total of 116 consecutive patients, who were suspected of neonatal septicemia and admitted in the Pediatrics Department of RMCH, were included in the study. Inclusion criteria were age up to 28 days with presence of clinical symptoms related to neonatal septicaemia such as fever, convulsion, reluctant to feed, abdominal distension and dyspnoea. Neonates with extreme low birth weight (<1kg), extreme pre-maturity (<28 weeks of gestational age), pneumonia, meningitis and those who received antibiotics prior to admission were excluded.

Informed written consent from legal guardians were taken. About 2-3ml of blood was withdrawn under strict aseptic precautions from each patient from antecubital vein or dorsal vein using disposable syringe. The local site (antecubital vein or dorsal vein) was cleansed with 70% alcohol and povidone iodine (1%), followed by 70% alcohol again. Blood was inoculated in a brain heart infusion broth and incubated at 37^0C for up to 7 days and subcultured on blood agar and MacConkey's agar media. Depending upon the observation of Gram's staining, specific biochemical tests were put up for the final identification.

The antibiotic susceptibility testing of the isolates was done by Kirby-Bauer disk diffusion method on Mueller Hinton agar as per Clinical Laboratory Standards Institute (CLSI) guidelines[9]. Antibiotic discs (Himedia Com. Ltd, India) used for Gram negative bacilli were ampicillin, amoxicillin, ceftazidime, amikacin, ciprofloxacin, gentamicin and meropenem; and for Gram positive cocci vancomycin was used in addition to the disc used for Gram negative bacilli.

Results:

Among 116 blood samples of patients with clinical diagnosis of neonatal septicaemia, 33(28.4%) yielded growth; of which Gram positive cocci was 20(60.6%) and Gram negative bacilli was 13(39.4%) and rest 83(71.6%) showed no growth (Table-I).

Table-I: Table showing the results of blood culture (n=116)

Growth		No growth	Total
Gram positive cocci	20(60.6%)	83(71.6%)	103
Gram negative bacilli	13(39.4%)		13
Total	33(28.4%)	83(71.6%)	116

Among 20(60.6%) Gram positive cocci isolates; *Staphylococcus aureus* was 17(51.5%) and *Streptococcus pneumoniae* was 03(9.09%). Number of Gram negative bacilli isolates was 13(39.4%) of them 10(30.3%) were *Escherichia coli*, 02(6.06%) were *Klebsiella pneumoniae* and 01(3.03%) was *Pseudomonas aeruginosa* (Table-II).

Table-II: Table showing the types of organisms in culture isolates (n=33)

Isolates		Number (%)	Total (%)
Gram positive cocci	*Staphylococcus aureus*	17 (51.5)	20 (60.6)
	Streptococcus pneumonie	03 (9.09)	
Gram negative bacilli	*Escherichia coli*	10 (30.3)	13 (39.4)
	Klebsiella pneumonia	02(6.06)	
	Pseudomonas aeruginosa	01(3.03)	

Staphylococcus aureus was sensitive to amoxicillin (23.5%), amikacin (94.1%), gentamicin (94.1%), vancomycin (94.1%), ciprofloxacin (82.3%), ceftazidime (58.8%), meropenem (100%); but was resistant to ampicillin. While *Streptococcus pneumoniae* was 100% sensitive to amikacin, gentamicin, vancomycin and meropenem; 66.6% to ciprofloxacin and 33.3% to ceftazidime but all are resistant to ampicillin and amoxicillin (Table-III).

Escherichia coli was sensitive to meropenem (100.0%), gentamicin (90.0%), amikacin (90.0%), ciprofloxacin (90.0%), ceftazidime (60.0%) and amoxicillin (10.0%); but was resistant to ampicillin (Table-III).

Klebsiella penumoniae was 100% sensitive to amikacin, gentamicin and meropenem; while 50% sensitivity to ciprofloxacin and ceftazidime but was resistant to ampicillin and amoxicillin (Table-III).

Table-III: Antibiotic sensitivity pattern of bacterial isolates in neonatal septicaemia

Antibiotics	S. aureus (n=17)	S. pneumonie (n=3)	*E. coli* (n=10)	*K. pneumoniae* (n=2)	*P. aeruginosa* (n=1)
Ampicillin	0	0	0	0	0
Amoxicillin	4(23.5%)	0	1(10%)	0	0
Amikacin	16(94.1%)	3(100%)	9(90%)	2(100%)	1(100%)
Gentamicin	16(94.1%)	3(100%)	9(90%)	2(100%)	1(100%)
Ciprofloxacin	14(82.3%)	2(66.6%)	9(90%)	1(50%)	1(100%)
Vancomycin	16(94.1%)	3(100%)	-	-	-
Ceftazidime	10(58.8%)	1(33.3%)	6(60%)	1(50%)	0
Meropenem	17(100%)	3(100%)	10(100%)	2(100%)	1(100%)

Pseudomonas aeruginosa was 100% sensitive to amikacin, gentamicin, ciprofloxacin and meropenem but 100% resistant to amoxicillin, ampicillin, and ceftazidime (Table-III).

Discussion:

The study of bacteriological profile along with the antimicrobial sensitivity pattern plays a very important role for effective management of neonatal septicaemia[10-12]. In this study, out of 116 blood samples, 33(28.4%) had yielded growth of both Gram positive and Gram negative bacteria which was nearly similar with the study of Shrestha et al[13] and Jain et al[14] in Nepal, Desai et al[15] in South India, Tsering et al[16] in Sikkim where positive culture rates were 30.8%, 28.3%, 32%, 23% respectively. However higher rate of isolation of pathogenic bacteria were reported by Sharma et al[17] in Amritsar of India, Mustafa et al[18] in Andhra prodesh of India where isolation rates were 42%, 44.2% respectively.

In this study Gram positive bacteria were more frequent isolates (60.6%) than the Gram negative bacteria (39.4%) which is consistent with the study of Shrestha et al[13] in Nepal where Gram positive bacterial isolates were 56.8% and Gram negative was 44.2%. In another study done by Nahar et al[7] in Bangladesh show the dominance of Gram negative bacteria was 78% in neonatal sepsis and Gram positive bacterial isolates was 22%.

Staphylococcus aureus was the predominant (51.5%) bacterial isolate in this study. This was supported by the study of Sharma et al[17] in Amritsar India, where *Staphylococcus aureus* was 51.9%. On the contrary, our finding differed with that of Mohamadi et al[19] in Iran which showed that isolation rate of *Staphylococcus aureus* 11.1%. Isolation rate of *Streptococcus pneumoniae* was 9.09% in this study which was consistent with other studies[19,20].

Isolation of *E.coli* was the 2nd most common organism (30.3%) in our study which is comparable to the study of Ahmed et al[21] in Bangladesh and Tamboli et al[22] in Maharashtra, India which showed 30% and 31.57% isolates of *E.coli*, respectively. A lower isolation rate was also reported by Hiral et al[23] in Gujrat, India where they found 9.92% *E.coli*. Isolation rate of *Klebsiella pneumoniae* in the present study was 6.06%, which was comparable to some other studies[7,17,19,24]. A higher isolation rates was also observed in other studies[6,19,25]. *Pseudomonas aeruginosa* was isolated at the rate of 3.03% which was comparable to other studies[6,24-26]. A higher isolation rates was also observed by others[7,22]. This extreme rate of variation may be due to geographical variations as well as variation in gestational age, birth weight, child health care facilities, maternal nutrition and maternal vaginal flora, perinatal care and hygienic conditions of mother.

In this study the pattern of antibiotic sensitivity of *Staphylococcus aureus* and *E.coli* were consistent with the study of Khan et al[27] in Pakistan where *Staphylococcus aureus* sensitivity to meropenem (92.85%), vancomycin (100%), ceftazidime (57.14%) & *E.coli* sensitivity to amikacin (80.55%), meropenem (97.2%), ciprofloxacin (77.7%), ceftazidime (61.1%) were found. But the current study disagrees with the study of Rizwan et al[20] in Dhaka, Bangladesh who reported 100% resistant to ciprofloxacin, gentamicin, ceftazidime, Meropenem against *Staphylococcus aureus*. Different rate of sensitivity of *E. coli* was reported by Hannan et al[28] in Pakistan and their observation was cent percent resistant to ciprofloxacin, amikacin and ceftazidime.

The pattern of antibiotic sensitivity to *Streptococcus pneumoniae* in this study was found in line with some other studies[17,20]. The pattern of antibiotic sensitivity to *Klebsiella pneumoniae* in the current study was in agreement with some other studies[13,18,27]. The pattern of

antibiotic sensitivity to Pseudomonas aeruginosa in this study was concordant with some studies[13,27]. But dissimilarity was observed in other studies[17,25]. Above mentioned all dissimilarity about antibiotic sensitivity pattern of Gram positive and Gram negative bacteria may be due to geographical variation and some other factors such as frequent use of antibiotics for both prophylaxis and treatment of neonates in hospital which is responsible to emergence of resistant strains[29-32].

Conclusion:

According to this study, the antibiotic susceptibility profile suggested that meropenem, amikacin, gentamicin, ciprofloxacin, vancomycin (particularly for Gram positive bacteria) may be the drug of choice in case of neonatal septicemia. Culture of anaerobic bacteria could not be performed due to limitations of lab facilities. So, further study should be undertaken with more samples and utilizing more advanced technology.

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