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Original Research Article

Bacterial etiology and antibiotic resistance pattern of neonatal sepsis: a study in a tertiary care hospital, in Bangladesh

Mahfuza Shirin[1]*, M. Monir Hossain[1], Manifa Afrin[2], Mohammad Abdullah Al Mamun[3]

[1]Department of Neonatal Medicine and NICU, Bangladesh Institute of Child Health, Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh
[2]Department of Paediatrics, BIHS General Hospital Dhaka, Bangladesh
[3]Department of Paediatric Cardiology, Bangladesh Institute of Child Health, Dhaka Shishu (Children) Hospital Dhaka, Bangladesh

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***Correspondence:**
Dr. Mahfuza Shirin,
E-mail: mahfuzashirin@gmail.com

ABSTRACT

Background: Neonatal sepsis is a leading cause of neonatal mortality and morbidity. The objective of the study was to detect causative microorganisms of neonatal sepsis and their antimicrobial resistance patterns.
Methods: This prospective cross-sectional study was conducted from July 2017 to June 2018 in the Department of Neonatal Medicine and NICU of Dhaka Shishu (Children) Hospital (DSH). Neonates diagnosed with probable sepsis were studied. After enrollment, 1 mL blood was taken and sent to Microbiology department of DSH for culture and sensitivity. With baseline characteristics, clinical examination findings and outcome, were also recorded.
Results: Rate of isolation of single organism was 9.2% (84/913). Out of 84 isolates, gram negative bacteria were 77.4% with *Klebsiella* pneumonae being the commonest (35, 41.7%), gram positive bacteria were 11.9% with *Staphylococcus* aureus and *Streptococcus* were equal (5, 5.95% each) and the remaining (9, 10.7%) isolated organism was *Candida*. Most of the isolated gram-negative bacteria were resistant to ampicillin, gentamicin, and ceftazidime; but gram-positive bacteria preserved 20-80% sensitivity. *Klebsiella* was more resistant than *Acinetobacter* to amikacin, netilmicin, ciprofloxacin and levofloxacin. Around 45-65% of gram-negative bacteria were resistant to imipenem and meropenem but gram-positive bacteria showed lesser resistance. Among the gram-negative bacteria, *Klebsiella* and *Acinetobacter* were resistant to piperacillin as same as carbapenem group, but gram-positive bacteria were 100% sensitive to piperacillin. All the gram-negative bacteria showed more resistance to 4th generation cephalosporin, cefepime than carbapenem. Out of culture positive 84 neonates, 63 (75.0%) were cured but 21 (25.0%) died. Among the 21 expired neonates, 47.6% (10/21) were infected with *Klebsiella*.
Conclusion: This study observed that gram-negative bacteria causing neonatal sepsis predominantly, with emergence of *Candida*. All the isolated gram-positive and gram-negative organisms were mostly resistant to available antibiotics.

Key words: Antibiotic resistance pattern, Bacterial etiology, Neonatal sepsis, Sensitivity

INTRODUCTION

Sepsis is a leading cause of mortality and is responsible for nearly 25% of deaths among the neonates worldwide.[1,2] Most of these deaths occur in the developing world.[3] It also remains a significant cause of morbidity.[3,4] The clinical diagnosis of neonatal sepsis is difficult due to its non-specific presentations. So, early

diagnosis is crucial to start early and appropriate treatment for improvement of survival of neonates with sepsis. For that correct identification of causative organisms and their antibiotic sensitivity patterns are necessary. Neonatal sepsis is classified as early onset if it occurs within first 72 hours of life and as late onset if occurs after 72 hours of age until the end of the neonatal period. Early onset sepsis is conventionally regarded as maternally acquired, whereas late onset sepsis is considered environmental in origin, either hospital or community acquired.[5]

The bacteriological profile of septicemia keeps changing with the passage of time from region to region and hospital to hospital, in the same city or country. In developing countries, gram-negative organisms are the predominant causative agents of neonatal sepsis. But it is changing worldwide from predominant gram-negative to a predominant gram-positive bacteria isolation. Studies from Bangladesh over the time of two decades reported that though majority of identified organisms were gram negative bacteria but it has shifted from *E. coli*, [6] to *Klebsiella* and *Acinetobacter*.[7] Several recent studies have reported that emergence of organisms such as CONS, NFGO, and Candida spp. occurred as a cause of neonatal septicemia.[8-10] Continuous evolution of drug resistant pathogens causing neonatal sepsis is becoming a potentially devastating problem. The situation is getting worse in developing countries and reports of multi-resistant bacteria causing neonatal sepsis in developing countries are increasing.[4,11] *Klebsiella* and *Enterobacter* species are often found to be resistant.

In developing countries, wide availability of over the counter antibiotics, irrational use of antibiotics and poor hygiene are important factors of development of this condition. Also, improvement of neonatal care service especially establishment of NICU care, has led to neonates born premature or needing intensive care for more than 48 hours to be more risk prone. Spread of resistant organisms in hospitals is a recognized problem.

Most Gram-negative organism are resistant to ampicillin and cloxacillin, and many are becoming resistant to gentamicin. In many units the antibiotic policy has been changed to include third generation cephalosporin. But the organisms have reduced susceptibility to them and even quinolones. In many developing countries S. aureus is a common cause and methicillin resistant strains (MRSA) are widespread.[11]

In this respect, it has become necessary to engage in appropriate epidemiological surveillance to identify the etiological agents and their antibiotics susceptibilities so that the emergence of new pathogens and their resistance patterns can be monitored. So, this study was conducted to find out the most important organisms causing sepsis in neonates, their changing pattern and the antibiotic resistance.

METHODS

This was a prospective cross-sectional study conducted over the period of one year from July 2017 to June 2018 in the Department of Neonatal Medicine and NICU of Dhaka Shishu (Children) hospital (DSH) to identify the current pattern of organisms causing sepsis in neonates and their resistance pattern. Neonates diagnosed with probable sepsis at the time of admission were enrolled in this study after taking informed consent from the parents. Neonates who were critically ill, expired within 10 hours of admission and did not give consent were excluded from the study.

After enrollment, blood samples for culture were collected and sent to the Microbiology department of DSH. For each neonate to collect blood for culture, the skin site for venipuncture was cleaned thoroughly with diluted iodine solution followed by 2.5% chlorhexidine solution and 1 mL blood was drawn by using a butterfly needle attached to a 2 ml disposable syringe. Culture and identification of the isolates was done in Bact /Alert FAN media. Bacterial isolates were tested for susceptibility to various antimicrobial agents and categorized as sensitive or resistant. Printed reports were sent to the designated wards.

For each neonate, information including gender, gestation, age, weight, birth history, maternal perinatal history, were recorded in a questionnaire from mother or attendant. Clinical examination findings at the time of enrollment, report of blood culture with sensitivity pattern and outcome were also recorded.

Data analysis, including descriptive statistics such as frequency tabulation, mean, standard deviation, were done by using SPSS version 20.

RESULTS

A total of 1359 neonates with sepsis were admitted in DSH during this period, of them from 891 neonate were included in this study. Amongst these 891 neonates, 68.2% were male and 31.8% were female, gestational age was 36.5±2.8 weeks, of them 51.5% were preterm. At the time of admission, mean age of them was 8.9±6.6 days and out of them, 34.9% neonates were admitted within first 72 hours of age. Admission weight was 2357.7±719.9 gm and 16.2% of them were very low birth weight (<1500gm) baby (Table 1).

Regarding birth history, 69.3% were born at different types of hospital facility and more than half (59.4%) were delivered normally (Table 2). Clinically, 34.9% had early onset sepsis (Figure 1). Table 2 showed presence of maternal risk factors which might have contributed to early onset sepsis. Clinical features of sepsis are shown in Table 3.

Table 1: Baseline characteristics of neonates with sepsis (n=891).

Neonatal variables
Gender
Male: female 2.1:1
Male 608 (68.2%)
Female 283 (31.8%)
Gestational age 36.5±2.8 weeks
(23-42 weeks)
Preterm 459 (51.5%)
Term 432 (48.5%)
Admission weight 2357.7±719.9gm (800-4600)
<1500gm 144(16.2%)
1500-<2500gm 396(44.4%)
2500-4000gm 344(38.6%)
>4000gm 7 (0.8%)
Age on admission 8.9±6.6 days (14 hours - 28 days)
Age at enrollment
<3days 157 (17.6%)
3-7 days 303 (34.0%)
>7 days 431 (48.4%)

Table 2: Birth history and maternal risk factors (n=891).

Variables		N (%)
Mode of delivery	Normal vaginal	529 (59.4)
	LUCS	362 (40.6)
Place of delivery	Home	273 (30.7)
	Clinic	461 (51.8)
	Hospital	157 (17.5)
Maternal risk factors*	Maternal fever with last 7 days of delivery	4 (0.4)
	PROM	30 (3.4)
	Foul smelled PV discharge	55 (6.2)
	Foul smelled liquor	202 (22.7)
	PV examination without gloves	95 (10.7)

*Multiple responses

From all (891, 100%) neonates blood culture was sent for 2nd time. So, total 913 samples were sent for blood culture, out of them only 9.2% (84/913) culture specimens had growth of single organism. Out of 84 positive cultures, 76 were yielded from first time blood culture, 8 were isolated from 2nd time blood culture and 3 were isolated from both the time. Out of 84 isolates, gram negative bacteria were found in 77.4% with *Klebsiella* being the commonest (35, 41.7%), gram positive bacteria in 11.9% with *Staphylococcus aureus* and *Streptococcus* were equal (5, 5.95% each) and the remaining (9, 10.7%) isolated organism was *Candida* (Figure 2).

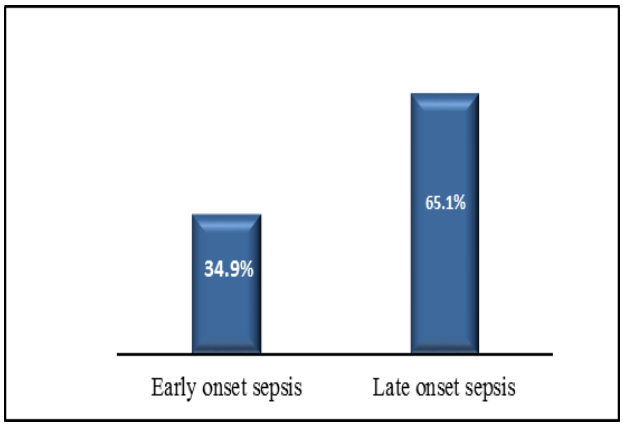

Figure 1: Type of sepsis of the study participants (n=891).

Table 3: Clinical findings at the time of enrollment (n=891).

Clinical findings*	N (%)
Lethargy	103 (11.6)
Abdominal distension	35 (3.9)
Vomiting	160 (18.0)
Jaundice	203 (22.8)
Tachypnoea	40 (4.5)
Apnoea	109 (12.2)
Convulsion	108 (12.1)
Prolonged CRT	527 (59.2)
Hypothermia	397 (44.6)
Fever	129(14.5)
Hypoglycemia	12 (1.3)

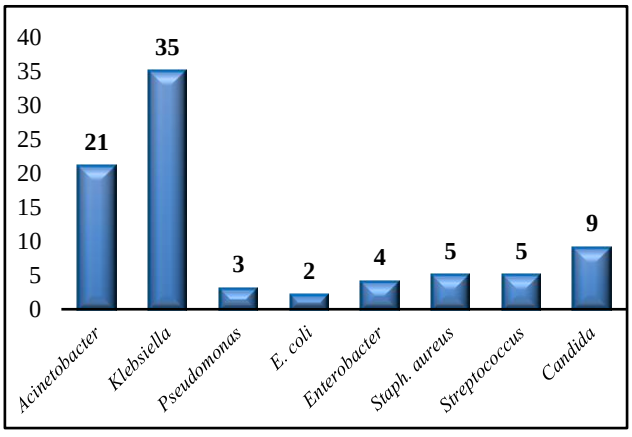

Figure 2: Distribution of isolated organisms in blood culture (n=84).

Susceptibility pattern of isolated bacteria is shown in Table IV. Most of the gram-negative bacteria were 90%-100% resistant to ampicillin, gentamicin, and ceftazidime; but gram-positive bacteria *Streptococcus*

and *Staphylococcus* preserved 20-80% sensitivity, except *staphylococcus* was 100% resistant to ampicillin and *Streptococcus* was 100% resistant to gentamicin. *Klebsiella* was more resistant than *Acinetobacter* to amikacin (89% vs 86%), netilmicin (91 vs 52%), ciprofloxacin (86% vs 62%), levofloxacin (85% vs 67%) and cotrimoxazole (77% vs 52%), but *Acinetobacter* was more resistant to chloramphenicol (86% vs 43%). Around 45-65% of gram-negative organisms were resistant to imipenem and meropenem but gram-positive bacteria showed lesser resistance (0-20%). Among the gram-negative bacteria, *Klebsiella* and *Acinetobacter* were resistant to broad spectrum β-lactam antibiotic, piperacillin as same as carbapenem group, but gram-positive bacteria were 100% sensitive to piperacillin. All the gram-negative bacteria showed more resistance (86%-100%) to 4th generation cephalosporin, cefepime than carbapenem.

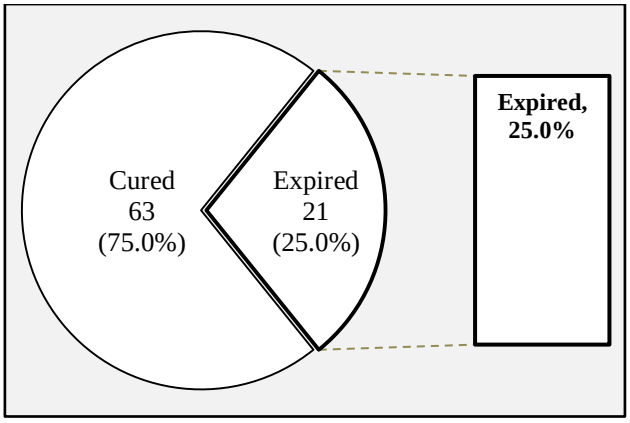

Figure 3: Outcome of neonates with positive blood culture (n=84).

Table 4: Sensitivity pattern of isolated organisms (n=75).

Antibiotic	Sensitive/ Resis*	Isolated bacteria						
		Klebsiella **35** **n (%)**	*Acinetobacter* **21** **n (%)**	*Pseudomonas* **3** **n (%)**	*E. coli* **2** **n (%)**	*Enterobacter* **4** **n (%)**	*Streptococcus* **5** **n (%)**	*Staph. aureus* **5** **n (%)**
Ampicillin	S	2 (5.7)	0	0	0	0	2(40)	0
	R	33(94.3)	21(100)	3(100)	2 (100)	4(100)	3(60)	5(100)
Gentamicin	S	3(8.6)	3(14.3)	0	0	0	0	3(60)
	R	32(91.4)	18(85.7)	3(100)	2(100)	4(100)	5(100)	2(40)
Amikacin	S	4(11.4)	3(14.3)	0	1(50)	2(50)	2(40)	4(80)
	R	31(88.6)	18(85.7)	3(100)	1(50)	2(50)	3(60)	1(20)
Netilmicin	S	3(8.6)	10(47.6)	0	1(50)	2(50)	2(40)	3(60)
	R	32(91.4)	11(52.4)	3(100)	1(50)	2(50)	3(60)	2(40)
Ceftazidime	S	2 (5.7)	3(14.3)	1(33.3)	1(50)	0	2(40)	3(60)
	R	33(94.3)	18(85.7)	2(66.7)	1(50)	4(100)	3(60)	2(40)
Ciprofloxacin	S	5(14.3)	8(38.1)	1(33.3)	0	4(100)	1(20)	3(60)
	R	30(85.7)	13(61.9)	2(66.7)	2(100)	0	4(80)	1(40)
Levofloxacin	S	11(31.4)	7(33.3)	1(33.3)	0	4(100)	2(40)	3(60)
	R	24 (84.6)	14(66.7)	2(66.7)	2(100)	0	3(60)	2(40)
Cotrimoxazole	S	8(22.9)	10(47.6)	1(33.3)	1(50)	2(50)	0	4(80)
	R	27(77.1)	11(52.4)	2(66.7)	1(50)	2(50)	5(100)	1(20)
Piperacillin	S	14(40.0)	6(28.6)	3(100)	2(100)	4(100)	5(100)	5(100)
	R	21(60.0)	15(71.4)	0	0	0	0	0
Imipenem	S	15(42.9)	8(38.1)	2(66.7)	1(50)	4(100)	3(60)	4(80)
	R	20(57.1)	13(61.9)	1(33.3)	1(50)	0	2(40)	1(20)
Meropenem	S	16(45.7)	7(33.3)	2(66.7)	1(50)	4(100)	3(60)	5(100)
	R	19(45.3)	14(64.7)	1(33.3)	1(50)	0	2(40)	0
Chloramphenicol	S	20(57.1)	3(14.3)	2(66.7)	1(50)	0	2(40)	4(80)
	R	15(42.9)	18(85.7)	1(33.3)	1(50)	4(100)	3(60)	1(20)
Cefepime	S	5(14.3)	2(09.5)	1(33.3)	0	2(50)	2(40)	4(80)
	R	30(85.7)	19(90.5)	2(66.7)	2(100)	2(50)	3(60)	1(20)

*S=Sensitive; R=Resistant

Out of 84 neonates with positive blood culture, 63 (75.0%) were cured but 21 (25.0%) died. Out of the 21 expired neonates, 47.6% (10/21) were infected with *Klebsiella* (Figure 3).

DISCUSSION

This study was conducted to find out the causative organisms, their changing pattern and the antibiotic resistance in neonatal sepsis. Authors found that the rate of isolation of single organism was about 9.2%. In a previous study reported from Bangladesh, the culture positivity rate was 36%.[7] In studies done in India, it has ranged from 16%-54%.[4,12,13] One study from Africa also reported similar rate of culture positivity, approximately 44-47%.[14,15] Rate of culture positivity in this study might be lower than that of actual, because DSH is solely a children hospital, all of the enrolled neonates were out born, no options to get inborn babies and many of them got antibiotics beforehand. Most of the neonatal septicemia cases now are either LBW or preterm.[16] In this study, 60% were LBW and 51.5% were preterm neonates. Authors found foul smelled liquor (22.7%) and PV examination without gloves (10.7%) among the maternal risk factors for occurrence of EOS. In this study LOS (51%) was more than EOS (34.9%) but percentage of EOS was more than that of other studies.[17]

The bacteriological profile has changed worldwide from predominant gram-negative to a predominant gram-positive bacteria isolation.[18-21] But among the positive cultures Authors found more gram-negative bacteria (77.4%) contrasted with gram-positive bacteria (11.9%) and a good number of *Candida spp.* (10.7%). Our finding was similar to that of previous studies reported from Bangladesh.[6,7] Several recent studies also have reported that emergence of organisms such as CONS, NFGO, and *Candida spp.* occurred as a cause of neonatal septicemia.[8-10]

Regarding changes in distribution of organism it was clearly evident from this study that about 11% *Candida* was isolated, which should be taken into account.

The pattern of bacterial organisms is constantly changing with time and place. Previously sensitive organisms are rapidly becoming resistant to commonly used antibiotic due to indiscriminate use thus making the treatment difficult and costly.[22] Reports showed that almost all isolated *Enterobacteriaceae* were either completely resistant to early beta-lactam antibiotics including ampicillin, amoxicillin, carbenicillin alone or in combination with beta-lactam inhibitor clavulanic acid along with resistant to second generation cephalosporin and to some extent to third generation drugs such as ceftriaxone.[8,23] Authors found most of the isolated gram-negative bacteria were resistant to ampicillin, gentamicin, and ceftazidime; but gram-positive bacteria *streptococcus* and staphylococcus preserved 20-80% sensitivity to those antibiotics. But Authors found that staphylococcus was 100% resistant to ampicillin and *Streptococcus* was 100% resistant to gentamicin. Similar findings of high resistance to ampicillin (71%) was reported by Bhat et al.[9] It was also reported by Bhat et al [9] that *Klebsiella pneumoniae* showed resistance to all antibiotics tested except imipenem. Authors found that *Klebsiella* was more resistant than *Acinetobacter* to amikacin, netilmicin, ciprofloxacin, levofloxacin, cotrimoxazole but sensitivity was still present. This high resistance pattern could be attributed to easy availability and widespread use of broad-spectrum antibiotics in the presumptive treatment of infections prevailing in our country. Blood culture facilities are often not available in most of the settings in Bangladesh. In such scenarios, clinicians have to depend on empirical antibiotic regimens. The high prevalence of resistance to ampicillin makes it out of use in neonatal sepsis in our hospital. The increasing resistance of Gram-negative organisms to extended spectrum cephalosporins and carbapenems makes the choice of antibiotics difficult.

This was a prospective cross-sectional study in a single center. So, the study results might not be reflected throughout the whole country.

CONCLUSION

This study concluded that gram-negative bacteria causing neonatal sepsis predominantly with emergence of *Candida*. It was also evident that all isolated gram-negative and gram-positive organisms were mostly resistant to available antibiotics. So, Authors recommend to take strict policy to prevent sepsis, and use available antibiotics rationally, to reduce the risk of death from neonatal sepsis.

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