Original Article

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Microbiology Section

Staphylococcus Aureus- The Predominant Pathogen in the Neonatal ICU of a Tertiary Care Hospital in Amritsar, India

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ABSTRACT

Background: An early treatment and the appropriate and the rational use of antibiotics would minimize the risk of severe morbidity and mortality in neonatal sepsis, and reduce the emergence of multi-drug resistant organisms in intensive care units. For the success of an early empiric treatment, a periodic review of the cases to assess any changing trends in the infecting organisms and their antimicrobial susceptibility is important.

AIM: To study the most commonly encountered bacterial pathogens which caused neonatal sepsis and their sensitivity patterns, so that guidelines could be prepared for a rational antibiotic therapy.

Setting and Design: This was a retrospective study which was conducted in the Department of Microbiology and the Neonatal Intensive Care Unit (NICU) at SGRDIMSAR, Amritsar, during June 2011 to June 2012.

Methods and Materials: Blood specimens for culture were drawn from 311 newborns who were admitted in an NICU with sepsis. The specimens were inoculated into brain heart infusion broth. Subcultures were performed on days 1, 2, 3, 5, 7 and 10. The isolates were identified by doing standard biochemical tests. The antibiotic resistance patterns of the isolates were studied by the Kirby Bauer disc diffusion technique.

Results: A total of 131 organisms were isolated from the 311 blood cultures. These included Staphylococcus aureus (n=68), Coagulase Negative Staphylococcus (CoNS) (n=30), Klebsiella pneumoniae (n=10), Acinetobacter baumannii (n=9), Escherichia coli (n=05), Enterobacter cloacae (n=04), Citrobacter diversus (n=02), Pseudomonas aeruginosa (n=02) and Candida (n=01). Staphylococcus aureus was the main pathogen in both early and late-onset sepsis. On antibiotic sensitivity testing, 57.35% of the Staphylococcus aureus isolates were found to be methicillin resistant. More than 90% gram negative rods were resistant to amikacin. The resistance to the third generation cephalosporins varied between 50-55%. The resistance to ciprofloxacin was quite high; however, most of the isolates were susceptible to levofloxacin. A majority of the isolates were susceptible to piperacillin-tazobactum and imipenem.

Conclusion: The present study emphasized the importance of periodic surveys on the microbial flora which was encountered in particular neonatal settings to recognize the trend.

Key Words: Septicaemia, Drug resistance, Antimicrobial sensitivity tests, S.aureus neonate, India

INTRODUCTION

Septicaemia in neonates is one of the major causes of morbidity and mortality among the newborns in the developing world [1]. It can be defined as “a clinical syndrome which is characterized by systemic signs and symptoms and bacteraemia during the first month of life”. It is labelled as “early onset” disease, if it presents during the first 5-7 days of life and as “late onset” if it occurs after the first week of life. The factors which are associated with sepsis in newborns include: low birth weight, foetal distress, a low Apgar score, the requirement of mechanical ventilation, umbilical catheterization and a history of preeclampsia in the mothers [2]. The incidence of neonatal sepsis which has been reported in the literature varies from 1-506/1000 live births [2-5]. In India, according to the National Neonatal Perinatal Database (NNPD) 2002-03, the incidence of neonatal septicemia has been reported to be 30/1000 live births. To initiate the appropriate antibiotic treatment, it is extremely important to diagnose the cases in time. The uncertainty which surrounds the clinical approach to the treatment of neonatal septicaemia can be minimized by undertaking periodic epidemiological surveys on the aetiological agents and their antibiotic sensitivity patterns, which lead to the recognition of the most frequently-encountered pathogens in a particular neonatal setting.

The same was the aim of this study; to find out the most commonly encountered pathogens and to formulate guidelines which could be provided to the paediatricians regarding the appropriate empirical antibiotic treatment of the cases.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, during June 2011 to June 2012. A total of 311 neonates (0-28 days) with the clinical signs and symptoms of sepsis at the time of admission or who developed sepsis during their hospital stay were included in this study. The neonates with congenital malformations or dysmorphic features were excluded. The neonatal septicaemia was categorized according to its time of onset as early -onset sepsis (0-7 days) and late-onset sepsis (8-28 days). An informed consent was taken from the parents of the neonates before the

performance of this study.

All the blood cultures were collected from the peripheral veins by following proper aseptic precautions before any antibiotic therapy was started with. Approximately, 2- 3 ml of blood was inoculated into brain-heart infusion broth. The inoculated broth bottles were transported immediately to the Department of Microbiology and they were incubated at 37°C. Subcultures were done on blood and MacConkey's agar plates on days 1,2,3,5,7 and 10. The colonies which were isolated were identified on the basis of their colony morphology, their gram staining patterns, and their standard biochemical tests [6]. The antibiotic sensitivity patterns of the isolates were studied by using the Kirby Bauer disc diffusion technique [7]. Staphylococcus aureus ATCC 27853 was included as the control strain. Staphylococcus aureus was further screened for methicillin resistance by the Kirby-Bauer method by using cefoxitin (30 mcg) discs according to the CLSI guidelines [8]. The antibiotics which were tested for S.aureus included amoxycillin, oxacillin, gentamicin, amikacin, ciprofloxacin, levofloxacin, erythromycin, clindamycin, cefoxitin and vancomycin. When an isolate was erythromycin resistant and clindamycin susceptible, the inducible Macrolide-Lincosamide-Streptogramin B (MLSB) resistance was excluded by the disk diffusion and the D-zone test methods [7]. The antibiotics which were tested for the gram-negative isolates were gentamicin, amikacin, ciprofloxacin, levofloxacin, cefotaxime, imipenem and piperacillin+tazobactam. Quinolones (ciprofloxacin) are not recommended for use in young children but they may be used in culture proven sepsis with bacteria not sensitive to other antibiotics.

Organism isolated on	Number and percentage of positive blood cultures
First subculture	94 (71.75%)
Second subculture	17 (12.97%)
Third and subsequent subcultures	20 (15.27%)
Total positive cultures	131 (100%)

[Table/Fig-1]: Organisms isolated on subcultures

Organism	Number and percentage of organisms		Total no. of each organism isolated
	Early onset sepsis	Late onset sepsis	
Staphylococcus aureus	31 (63.35%)	37 (77.08%)	68
Coagulase negative staphylococcus	20 (15.26%)	10 (12.05%)	30
Klebsiella pneumoniae	10 (12.05%)	0	10
Acinetobacter baumannii	09 (10.84%)	0	09
Escherichia coli	05 (6.02%)	0	05
Enterobacter cloacae	04 (4.82%)	0	04
Citrobacter diversus	02 (2.41%)	0	02
Pseudomonas aeruginosa	02 (2.41%)	0	02
Candida	0	1 (1.20%)	01
Total no. of isolates	83 (63.35%)	48 (36.64%)	131

[Table/Fig-2]: Number and percentage of organisms isolated from cases of Early and Late onset sepsis

RESULTS

1. One hundred and thirty one cases (42.12%) out of the 311 which were included in the study were confirmed as having neonatal sepsis, as their blood cultures yielded the growth of some bacterial pathogens.

2. [Table/Fig-1] depicts the yield of the organisms on subcultures.

3. 61.07% neonates who were confirmed to be cases of neonatal sepsis were males (n=80).

4. The organisms which were isolated from the cases of early onset sepsis were, n=83 and those which were isolated from the cases of late onset sepsis were, n= 48. [Table/Fig-2] shows the numbers of various organisms which were isolated from the cases of early and late onset sepsis.

5. Staphylococcus aureus was the predominant pathogen which was isolated from both the early and the late-onset septicaemia cases. The results of the antibiotic resistance patterns of Staphylococcus aureus and other isolates are shown in [Table/Fig-3].

6. The NICU patients with positive cultures for Staphylococcus aureus were 68 (51.9%). Out of these 68 Staphylococcus aureus isolates, 39 were methicillin resistant Staphylococcus aureus (MRSA)

Antibiotics	S.aureus (n=68)	CoNS (n=30)	K.pneumoniae (n=10)	Acinetobacter (n=9)	E.coli (n=5)	Enterobacter (n=4)	Citrobacter (n=2)	Pseudomonas aeruginosa(n=2)
Amoxycillin	29(42.6%)	12 (40%)	-	-	-	-	-	-
Oxacillin	25(36.7%)	24(80%)	-	-	-	-	-	-
Erythromycin	32(47%)	17(56.6%)	-	-	-	-	-	-
Gentamicin	40(58.8%)	19(63.3%)	0(0%)	4(44.4%)	1(20%)	4(100%)	2(100%)	1(50%)
Amikacin	50(73.5%)	21(70%)	2(20%)	4(44.4%)	3(60%)	1(25%)	2(100%)	1(50%)
Cefoxitin	29(42.6%)	27(90%)	-	-	-	-	-	-
Cefotaxime	23(34%)	12(40%)	5(50%)	5(55.55%)	0(0%)	2(50%)	2(100%)	1(50%)
Ciprofloxacin	34(50%)	16(53.3%)	3(30%)	4(44.4%)	0(0%)	1(25%)	2(100%)	1(50%)
Levofloxacin	41(60.3%)	25(83.3%)	3(30%)	7(77.7%)	2(40%)	4(100%)	2(100%)	1(50%)
Imipenem	-	-	6(60%)	7(77.7%)	5(100%)	4(100%)	2(100%)	2(100%)
Vancomycin	68(100%)	30(100%)	-	-	-	-	-	-
Piperacillin-Tazobactum	40(58.8%)	24(80%)	2(20%)	7(77.7%)	3(60%)	2(50%)	2(100%)	2(100%)
Clindamycin	42(61.7%)	21(70%)	-	-	-	-	-	-

[Table/Fig-3]: Showing Pattern of sensitivity of Gram-positive and Gram-negative organisms to antibiotics

and 29 were methicillin sensitive. A majority of the MRSA infections were late onset (occurring at >72 hrs of age). From the antibiotic sensitivity patterns, it was clear that the most effective antibiotic against the Staphylococcal isolates was vancomycin (100%) followed by clindamycin (61.7%).

7. The infants with lower gestational ages and birth weights had a higher incidence of the MRSA infection. The mean birth weight was 1204 g (range, 542–2800 g); 7 had a very low birth weight (<1500 g).

8. Most of the gram-negative bacteria were resistant to ciprofloxacin, except the isolates of Citrobacter. Levofloxacin showed 100% sensitivity in Enterobacter sp. and Citrobacter sp. However, a 30-77.7% resistance was seen in other isolates. All the Citrobacter isolates were sensitive to all the antibiotics. The Klebsiella species were resistant to most of the antibiotics. The gram negative isolates showed 50-100% sensitivity to cefotaxime; however, 20-60% of the isolates were sensitive to amikacin. The maximum no. of gram-negative isolates was sensitive to imipenem and piperacillin-tazobactum.

DISCUSSION

S. aureus was the predominantly isolated pathogen in this study; similar findings were seen in several studies [9,10]. Group B Streptococcus, which is a common cause of neonatal sepsis in the west, is infrequent in India and in other tropical countries [11]. The culture-positivity of the aerobic organisms in the neonates in our study was 42.12%, whereas in a study which was done by Shaw CK et al., [12], it was 54.64%, Bhattacharjee et al., [13]. found it to be 48% and Dias et al., [14] found it to be 32%.. Repeated subcultures increase the chances of isolation of the organisms from blood cultures, as was seen in this study. We might have missed many anaerobes in the present study. Zaidi et al., reported that anaerobic blood cultures are rarely helpful in the majority of paediatric patients and that they usually show positive results only in the clinical settings which are associated with anaerobic infections [15].

The horizontal transmission of S. aureus from colonized visitors or health care workers to the infants in the NICU has previously been documented [16-18] and it could have been a mode of transmission in some of our patients. In a similar study which was done by Sundaram V et al., [19], they reported an increase in the incidence of neonatal sepsis which was caused by S. aureus and a decrease in the incidence of neonatal sepsis which was caused by gram-negative bacilli. Similar findings were obtained in our study. S. aureus is the second most common pathogen which causes late-onset septicaemia in NICU infants with very low birth weights [20]. Poorly developed host defense mechanisms, the necessity of central venous catheters, endotracheal and upper gastrointestinal tract tube placement, procedures which cause an interruption in the skin integrity, a prolonged total parenteral nutrition, and the use of steroids or antimicrobial agents, all increase the risk of the Staphylococcal infection in premature infants. S. aureus bacteraemia in the neonates is historically associated with septic shock, which can be rapidly fatal [20-22]. These high fatality and morbidity rates which occurred despite the prompt initiation of the appropriate antimicrobial agents and the intensive care support, highlighted the fact that in geographic regions where the community MRSA was prevalent, eliminating vancomycin from the empirical therapy for presumed late-onset neonatal septicaemia potentially could be harmful. The community MRSA strains demonstrate less in vitro antimicrobial resistance than do the traditional health care-associated isolates. However, many agents with an in vitro activity, such as clindamycin , are inappropriate for the treatment of invasive infections in the NICU patients, maybe because of their bacteriostatic rather than bactericidal activities (clindamycin). MRSA causes a significant proportion of S. aureus infections in the NICU, both in our NICU and in those at other centres [20], resulting in substantial morbidity and mortality, whether the infecting strain is health care associated or whether it has the genetic characteristics of the community strains. Although we support the efforts which are being made to restrict the use of vancomycin to prevent the emergence of drug-resistant Staphylococci [23], we believe that eliminating vancomycin from the initial therapy for late-onset neonatal sepsis is imprudent in the areas where MRSA is prevalent in the community.

CONCLUSION

The present study emphasizes the importance of periodic surveys on the microbial flora which is encountered in particular neonatal settings, to recognize the trend. Vancomycin should be used when the patient does not respond to the first line treatment or the combination of drugs; however, in view of the isolation of the highly antibiotic resistant organisms, vancomycin, in combination with the third generation cephalosporins or a carbapenem, was the drug of choice for empirically treating the late-onset neonatal sepsis in our institute. In order to prevent the horizontal transmission of infections in neonates, it is important for the health care workers to adopt strict universal precautions and there should be a restricted entry of visitors in the NICU.

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