Neonatal Bacteriemia Isolates and Their Antibiotic Resistance Pattern in Neonatal Insensitive Care Unit (NICU) at Beasat Hospital, Sanandaj, Iran

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Abstract- Bacteremia continues to result in significant morbidity and mortality, particularly among neonates. There is scarce data on neonatal bacteremia in among Iranian neonates. In this study, we determined neonatal bacteremia isolates and their antibiotic resistance pattern in neonatal insensitive care unit at Beasat hospital, Sanandaj, Iran. During one year, all neonates admitted to the NICU were evaluated. Staphylococcal isolates were subjected to determine the prevalence of MRS and mecA gene. A total of 355 blood cultures from suspected cases of sepsis were processed, of which 27 (7.6%) were positive for bacterial growth. Of the 27 isolates, 20 (74%) were *Staphylococcus spp* as the leading cause of bacteremia. The incidence of Gram negative bacteria was 04 (14.8%). The isolated bacteria were resistant to commonly used antibiotics. Maximum resistance among *Staphylococcus spp* was against Penicillin, and Ampicillin. In our study, the isolated bacteria were 7.5 % Vancomycin and Ciprofloxacin sensitive. Oxacillin disk diffusion and PCR screened 35% and 30% mec a positive *Staphylococcus spp*. The spectrum of neonatal bacteremia as seen in NICU at Beasat hospital confirmed the importance of pathogens such as *Staphylococcus spp*. Penicillin, Ampicillin and Cotrimoxazol resistance was high in theses isolates with high mecA gene carriage, probably due to antibiotic selection.

Introduction

Bacterial infections such as bacteremia pose a significant threat to neonate's mortality and morbidity (1-2). Many microorganisms are responsible for bacteremia, commonest being Gram-positive cocci particularly *Staphylococci spp*. These are commensal on the human body surfaces and colonize intravenous devices, which become a focus of infection in hospitalized patients more so in neonates and paediatric patients. Rapid detection and identification of bacteria in blood is crucial in patient management because the mortality rate associated with blood stream infections is very high (3-4).

In addition, the emergence of antibiotic resistant among bacteria is considered a major problem in most hospitals all over the world particularly in the developing countries (5-7). Moreover, Centre for Disease Control and Prevention reported that many bacteria are resistant to two or more classes of antibiotics in both large and small hospitals (8).

Staphylococcus spp has showed up as a significant nosocomial pathogen in the neonatal intensive care unit (NICU). Recent reports on *Staphylococcus spp*. infections in neonates have focused especially on the molecular epidemiology in the NICU. However, we have very scant information about the current status of this infection among neonates and the most suitable measures to interrupt the transmission of *Staphylococcus*

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strains. Moreover, with the increased use of prophylactic antibiotics to mothers, careful surveillance of the changing trend of bacterial organisms and their antibiotic resistance pattern among neonates is warranted.

Therefore, early diagnosis and proper management of neonatal bacteriemia could bring down the morbidity and mortality substantially. To the horizon of our knowledge there has been very few studies done in this field in the Province of Kurdistan, Iran. (9).

The present study was undertaken to study the isolates in neonatal bacteremia and their antibiotic resistance pattern in a NICU in this Province.

Materials and Methods

Setting

This study was carried out at the NICU 22 bedded in Beasat hospital which is a teaching hospital affiliated to Kurdistan University of Medical Sciences. Beasat hospital is a 350-bed hospital, serving a population of about 1.6 million, Kurdistan Province, in the west part of Iran. This hospital is the only referral center in Kurdistan Province. In total; 1,650 neonates are admitted in 2010, of which 436 patients were followed in the NICU.

The study included consecutive cases of clinically suspected septicemia episodes in children aged 1 – 30 days in the NICU. Blood samples were collected with all aseptic precautions for culture and sensitivity studies following universal precautions. Blood cultures were processed using the standard technique (10).

Sample collection

Samples were inoculated into brain heart infusion broth (HiMedia, India). The broth was incubated aerobically at 37°C. A subculture was done after over night; if no growth was obtained, the bottles were tested for seven consecutive days. Any sign of growth was followed-up by subcultures. Isolates were identified using standard biochemical tests. Repeated isolation of the same organism from the same patient was considered as one isolate.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing for all the bacterial strains to various antimicrobial agents were determined according to the Clinical Laboratory Standards Institute guideline. Staphylococcal isolates from those neonates whose blood culture was positive for staphylococci were subjected to determine the

prevalence of MRS according to CLSI guideline (11). Similarly, Staphylococcal isolates from those neonates whose blood culture was positive for staphylococci were subjected to determine the prevalence of MRS mecA gene according to the procedure described by Cekovska et al (12).

Results

During the study period, 355 blood samples were taken from suspected cases of sepsis and processed, of which 27 (7.6%) were positive for bacterial growth. In total, 194 (54.6) of the neonates were male. The mean age of neonates was 4.9±6.0 days and the mean weight of the patients was 2842±684 grams (range 1100 – 4500 grams). The mean time of admitted in hospitals was 5.67±4.34 days (range 0-30 days).

Coagulase-negative *Staphylococcus* were found the most frequent causative agents as 17 isolates (63.0). The following causative bacteria were found S. *aureus* in three isolates (11.1%), *Streptococcus pneumoniae* three isolates (11.1%), *Klebsiella pneumoniae* two isolates (7.4%) and *Enterobacter spp* two isolates (7.4%). There was no polymicrobial bacteremia. Of the 27 isolates, 20 (74.1%) were *Staphylococcus spp* as the leading cause of bacteremia.

The highest resistance rates among *Staphylococcus* were found against Penicillin, Ampicillin and Cotrimoxazol. In total 18 (90%) *Staphylococcus* strains were found sensitive against Vancomycin and Ciprofloxacin (Table 1). Seven (35%) of the *Staphylococcus* strains were found resistant against Oxacillin and PCR screened six (30%) *mec* A positive *Staphylococcus spp* (Figure 1).

Table 1. Antibiotic resistance pattern of isolated *Staphylococcus* strains from positive blood culture in hospitalized neonates

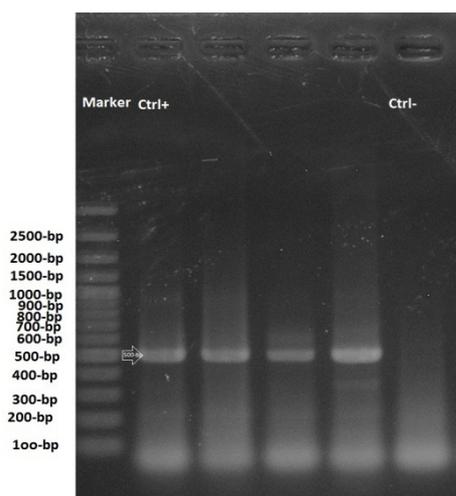| Antibiotic	Resistance	Intermediate	Sensitive
	Number (%)		
Ciprofloxacin	02 (10)	01 (05)	17 (85)
Clindamycin	06 (30)	03(15)	11 (55)
Gentamicin	03 (15)	02(10)	15 (75)
Erythromycin	06 (30)	04 (20)	10 (50)
Vancomycin	02 (10)	0.0 (0.0)	18 (90)
Ampicillin	15 (75)	04 (20)	1.0 (05)
Cotrimoxazol	14 (70)	04 (20)	2.0 (10)
Penicillin	16 (80)	04 (20)	0.0 (0.0)
Oxacillin	07 (35)	01(05)	12 (60)

Figure 1. PCR analysis for the *mec*A gene among *Staphylococci spp* isolated from positive blood culture in hospitalized neonates Lane 1, DNA marker; lane 2, 3, 4 and 5 have *mec*A gene; Lane 6 negative control, lacking *mec*A gene

Discussion

Each year in Kurdistan Province, Iran, many neonates die before reaching the age of five years, and the vast majority of these deaths are not fully investigated and, therefore, the data on bacterial infections are very limited in this area. In addition, there are almost no estimates of incidence, mortality, or hospital burden for bacterial infections. Thus, the impact of childhood bacteremia is largely unknown.

Timely and accurate detection and reporting of bloodstream infection are, therefore, some of the most important functions of a clinical laboratory (13-14). According to global surveillance reports, bloodstream isolates are the best candidates for the study of antimicrobial susceptibility of human bacterial pathogens (15-16). Unfortunately, data regarding the neonatal bacteremia isolates and their antibiotic resistance pattern in neonatal insensitive care unit in Kurdistan province are scarce.

In this study, the commonest bacterial isolates were Staphylococci *spp* (74%), and this is consistent with the general increase in Staphylococci infections worldwide17-18. However, in a previous study from our own university, Bahmani (9) had reported a 30% neonatal bacteriemia.

For the effective management of neonatal bacteremia cases, study of the bacteriological profile with their antibiotic pattern plays a significant role. In addition, the growing problem of bacteremia is magnified by reports of clinical failure and resistance to various antibiotics particularly methicillin (19-20). In this study, the results of antibiotic sensitivity pattern revealed that the majority

of isolates were resistant to commonly used antibiotics like Penicillin, Ampicillin and Cotrimoxazol. However, it has been shown that Vancomycin and Ciprofloxacin were the two most effective antibiotics against all the bacterial isolates which is a correlate with the findings of Shahsanam *et al.* (21). Ciprofloxacin is not useful alternative in childhood because of side effects.

In addition, our data suggesting the best choice of antibiotics is important for the local physicians when treatment of the neonatal bacteremia has to be initiated before the result of the blood culture is known. The authors suggest that a local dynamic database has to be established. This should contain relevant data on the positive blood cultures.

There was a significant difference in the frequency of neonatal septicemia caused by *Staphylococcus spp* with the previous studies from Iran. Prevention of neonatal bacteremia caused by *Staphylococcus spp* remains a challenge. Of note, *Staphylococcus spp* from this study had almost a twofold lower incidence of MRS than that of Setareh *et al.* (17). Two additional studies revealed a high frequency of MRSA infections during 200822 and during 2010 (23). A recent study in Iran carried out by Saeid et.al (24). Confirmed resistance to methicillin by the detection of the mecA gene using PCR and reported prevalence of 89%, which is far higher to the prevalence in this study.

In conclusion, the spectrum of neonatal bacteremia as seen in NICU at Beasat Hospital, confirmed the importance of pathogens such as *Staphylococcus spp*. The resistance against Penicillin, Ampicillin and Cotrimoxazol were high in these isolates with a 30% mecA gene carriage.

Acknowledgment

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