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Neonatal Sepsis; The Bacterial Causes and the Risk Factors

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Abstract

This study aimed in part to detect the bacterial types implicate in neonatal sepsis with determining the risk factors enable organisms to access neonates. The subjects of the study comprised 50 neonates who admitted to the Preterm Unit and Intensive Care Unit (ICU) in the Babylon Hospital for Pediatric and Gynecology during a period of 6 months (from October 2011 to March 2012). The neonates included both sexes, males and females whose ages ranged from 1 to 90 days. Historical data were fully recorded for each newborn. After clinically investigation and, blood samples were collected and tested for WBCs count and C-reactive protein (CRP) as preliminary indicative tests for infection. Then, a blood samples was cultured on blood agar, MacConkey agar, and nutrient agar media to identify the bacterial types causing the infection. The results indicated that Early Onset Sepsis (EOS) is more frequent than Late Onset Sepsis (LOS) and Gram negative bacteria were found to be the common causes sepsis of neonates sepsis. The results showed that abnormal WBCs count and positive CRP were significantly associated with blood culture proven septicemia.

Keywords: Neonates, sepsis, bacterial types, risk factors.

Introduction

Neonatal Sepsis can be defined as any systemic bacterial infection confirmed by a positive blood culture in the first month of life. Neonatal septicemia remains one of the main causes of mortality and morbidity despite the progress in hygiene, introduction of new and potent antimicrobial agents for treatment and advanced measures for diagnosis. Up to 10%, infants have infections in the first month of life, the matter which results in 30-50% of total neonatal deaths in developing countries[1]. These neonatal deaths are attributed principally to infection, birth asphyxia and consequences of premature birth and low birth weight[2,3]. It is well known that risk factors related to neonatal bacterial sepsis are complex; they include interaction of maternal-fetal colonization, transplacental immunity and physical and cellular defence mechanisms of the neonate[4]. The incidence of neonatal bacterial sepsis may vary from country to country as well as within the same country. In developing countries, neonatal mortality results from all expected causes of neonatal sepsis, consequently, it is about 34 per 1000 live births, occurring mainly in the first week of life, whilst it is only 5 per 1000 live births in developed countries[5]. Neonatal mortality is about 34 per 1000 live births in Asia, 42 per 1000 live births in Africa and 17 per 1000 live births in Latin America[6] versus relatively low rates being reported in the United States and Australasia which is about 6–9 per 1000 live birth and in Europe only 0.3-3 per 1000 live births[7]. Group B *Streptococcus* (GBS) has been reported to be the most frequent ethological agent of neonatal sepsis in developed countries, being responsible for high morbidity and mortality rates, although the types of microorganisms causing sepsis are different depending on the microorganism it's self and on environmental, socio-economic and hygienic policies factors[8]. Neonatal sepsis is classified as either early onset sepsis (EOS) which occurs through 1 to 7 days of the infant's age or late onset sepsis (LOS) which occurs through 8-28 days of the infant's age[9, 10]. According to Vergnano and colleagues [6], who stated that; the clinical signs for diagnosis of neonatal sepsis are any of the following signs; respiratory rate > 60 breaths/min (tacypnea), grunting, temperature >37.7oC or <35.5oC (hypothermia), lethargic or unconscious, not able to sustain sucking, tachycardia, and convulsion.

Maternal, infant and environmental factors all contribute in NNS, at a time when bacteria may invade the newborn via a number of routes such as; i. intrauterine infection which occurs due to apparent or inapparent maternal bacteremia with transplacental transmission to the fetus. *Listeria monocytogenes* septicaemia is an example of such infections[11]. Furthermore a fetus may be infected by organisms from vagina invading the amniotic fluid through the cervix with or without intact membrane[12]. The most common organisms found in the amniotic fluid and vagina are *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Group -B- beta haemolytic Streptococcus* is also occasionally present in the vaginal flora[11]. ii- Intra partum infection (Ascending Infection) which are acquired just before or during delivery with vertical transmission of the microorganisms from mother to newborn infant[13] and iii- Postpartum infection (nosocomial infection), since bacteria may be acquired from the delivery room or in the newborn nursery via the main pathways, namely the respiratory and gastrointestinal tracts. After birth, the skin and umbilical cord become an important alternative route for the entrance of bacteria into the systematic circulation. The umbilical stump is a frequent site for cutaneous infection leading to septicemia[14].

Material and Methods

Patients: During a period of 6 months, from October 2011 to March 2012 a total of 50 neonates who admitted to the preterm unite and intensive care unit (ICU) in Babylon Hospital for children and Maternity were investigated for early onset sepsis (0-7 days of age) and late onset sepsis (>7-90 days of age). Written informed consent was obtained from their parents/guardians. Following detailed clinical examination and neonates with suspected sepsis having any one of the clinical symptoms and signs as outlined above were considered.

Blood Samples: Using aseptic conditions, by applying Povidone iodine and 70% alcohol at the site of vein puncture, 5 ml. venous blood was drawn from the antecubital or femoral vein by the attending nurse. The specimens were gently poured in sterile tubes containing anticoagulant and transported within one hour to the Core Laboratory. The blood was divided in to two equal portions; 2.5 ml. of blood were inoculated directly in brain heart infusion broth and incubated for 24 hr. for culturing assay and the remaining 2.5 ml. blood were used for white blood cell count and C-reactive protein.

Culture and Identification: All blood cultures were incubated in brain heart infusion broth at 37°C and inspected daily for 3 days for presence of visible microbial growth by observing any of one of the following; turbidity, haemolysis, air bubbles (gas production) and coagulation of broth, otherwise the results were considered as negative for microbial growth. Subcultures were made during 3 successive days on nutrient agar, blood agar, and MacConkey's agar. The inoculated plates were incubated under aerobic conditions for 24 hr. The positive blood cultures were investigated for identification of growing organisms. Gram-negative were identified according to their diagnostic characteristics as mentioned by MacFaddin[15]. Members of the family *Enterobacteriaceae* were further identified by: indole production, H2S production, citrate utilization, motility test, urease test, oxidase test and carbohydrate utilization tests. API 20E identification kits were also used to confirm the diagnosis. For diagnosis of Gram-positive bacteria; cellular and colonial morphology were examined and confirmed by: coagulase, catalase, bacitracin and optochin susceptibility tests as recommended by MacFaddin[15].

Qualitative determination of C-reactive protein: The CRP-latex is a slide agglutination test for qualitative detection of CRP in human serum. Latex particles coated with IgG are agglutinated when mixed with samples containing CRP[16].

White Blood Cell Count: Counting of WBCs in peripheral blood sample was done by auto- analyzer**.** After sampling of blood into EDTA tube approximately 50µ of blood drawn by auto-analyzer to give the result on the screen within few minutes.

Results and Discussion

The results of blood culture may take about 3 days, necessitating initial empirical treatment of suspected septicemia. Among 50 samples of blood obtained from neonates of different ages and sex, only 10 samples (20%) revealed positive culture. *Eschrichia coli* was found to be the frequent since it accounted for 30% versus *S. aureus* (20%), *Pseudomonas spp.* (20%) and *Enterobacter spp.* (20%). *Salmonella spp.* was the least, it accounted only (10%) as shown in figure 1.

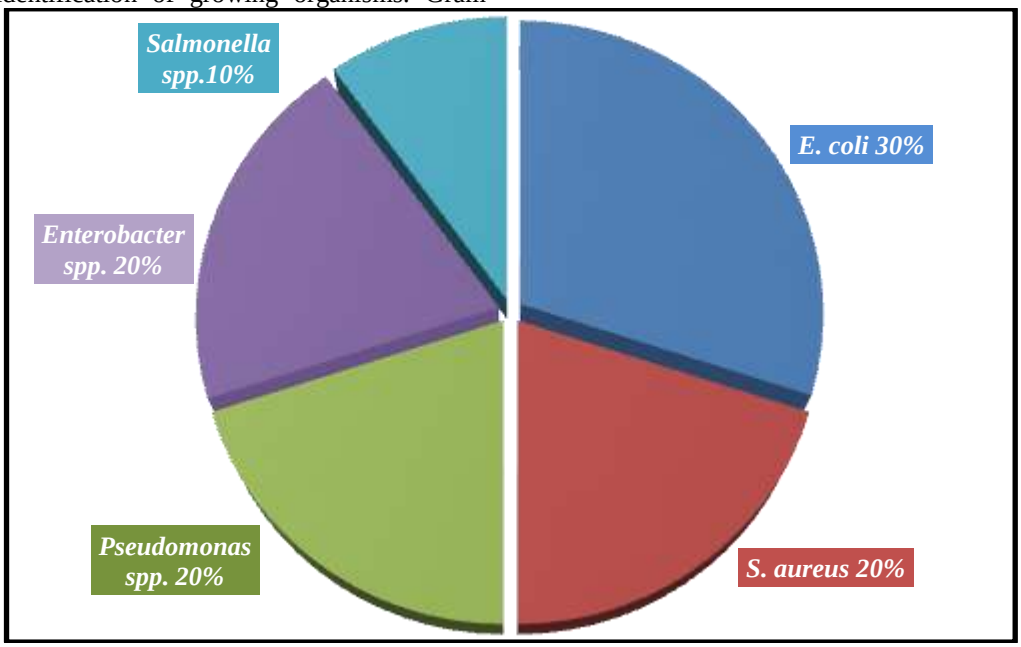

Figure-1
Frequency of bacterial types detected in blood of neonates

Age and Sex in Septicemia: Distributions of investigated neonates for bacterial sepsis according to the age, sex and type of septicemia are shown in table 1 below.

Table-1
Distribution of 50 neonates with suspected sepsis according to the sex, age and type of sepsis

Category	Neonates with EOS*, n. (%)	Neonates with LOS**, n. (%)	Total, n. (%)
Males	21 (42%)	11 (22%)	32 (64%)
Females	8 (16%)	10 (20%)	18 (36%)
Total	29 (58%)	21 (42%)	50

.*age 0 to7 days, **age > 7 to 90 days

Out of the 50 septic neonates, 32 (64 %) were males versus 18 (36%) were females resulting in an overall females to males ratio of 1: 1.8. A total of 29 (58%) neonates presented with EOS and 21 (42 %) neonates presented with LOS as shown in table 1. The results indicated that the incidences of EOS were common compared with LOS, which is in consistence with the reports from other developing countries e.g. in Iran (77.5% vs. 22.5%)[2] and Bangladesh (70.7% vs. 29.3%)[17]. However, an increased LOS compared with EOS has been reported in Saudi Arabia (39% vs. 61%)[18], Pakistan (42% vs.58%)[19] and Libya (31% vs. 69%)[20]. The possible explanation for low frequency of LOS in this study might be attributed to the early discharge policy in the Iraqi hospitals since the newly delivered mother is discharged from hospital within 3 hrs. for normal vaginal delivery and after 24 hr. for cesarean section. Data being recorded about each neonate indicated that out of the 50 neonates included in this study; 31(62%) were preterm (gestational age less than 37 weeks), 17(34%) were born in the midwife and 34(68%) were delivered by normal vaginal delivery. Approximately, 33(66%) neonates with sepsis had low birth weight (<2500 g) and 7(14%) neonates had very low birth weight (< 1500 g).

C - reactive protein (CRP): The results of C-reactive protein were positive in 33(66%) and the remaining 17(34%) were negative as indicated in figure 2. Those neonates with positive CRP revealed positive bacterial culture in 10 cases. This mean that (30.30%) of positive CRP had positive bacterial culture. Measurement of the acute phase response is a helpful indicator of the presence and extent of inflammation or tissue damage and response to treatment. It has been suggested that the combination of IL-8 and CRP appeared to provide a more reliable method in the early diagnosis of neonatal sepsis. CRP assay is sensitive and fairly precise small rises in serum level and can often be detected before any clinical feature becomes apparent. Whilst as a tissue damaging process resolves the serum level rapidly decreases toward the normal rang. The outcome of neonates with infections is strongly related to their appropriate diagnosis and management. Diagnosing of neonatal infection, however, is a challenge, since clinical signs and symptoms are often nonspecific for a particular infection. As a consequence, deciding whether to treat or not, balancing optimal patients care with aspects such as possible adverse events or antibiotic resistance, may be difficult. In line with this idea, the recognition of the risk factors for neonatal infections is extremely relevant in the clinical setting, since it contributes to the diagnostic reasoning and supports clinical decisions.

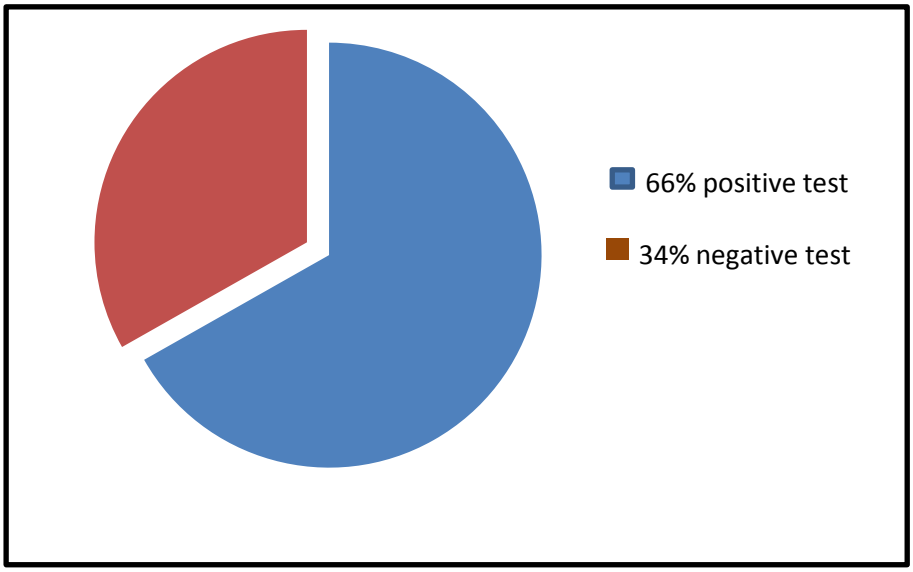

Figure-2
Percentage of positive to negative CRP

White Blood Cells Count (WBCs count): Results of this test indicated that 28 (56%) of septic neonates had normal white blood cells (5000 20,000/mm^3), 8 of them (16%) had high WBCs count (>20,000/mm^3) and 14 neonates (28%) had low WBCs count (<5000/mm^3) table 2.

Table-2
White blood cell counts in neonates admitted with suspected cases of sepsis

WBC count/mm.3	No. (%)
5000 - 20.000 (normal)	28 (56%)
>20.000 (high)	8 (16%)
<5000 (low)	14 (28%)
Total	50

The WBCs and differential count are useful for assessing a neonate who may have sepsis and for evaluating a neonate being treated for proven sepsis. The bone marrow reserves of leukocytes in a newborn are relatively small compared to those of older children and adults and leukopenia (low WBC count) occurs more frequently as sign of overwhelming infection.

Conclusion

Early onset sepsis (EOS) of neonates is more common compared with late onset sepsis (LOS) in Babylon population. Prematurity, low birth weight, abnormal WBCs count and C-reactive protein are indicative parameters associated with neonatal sepsis. *Enterobacter* spp. and *Klebsiella* spp.are the common organisms causing neonatal sepsis and the multi-drugs resistance character was detected in *Staphylococcus aureus* and *Pseudomonas* spp.

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