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Neonatal Bloodstream Infections

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A B S T R A C T

Neonates are immune-compromised individuals who are prone to infection. Neonatal sepsis has significant morbidity and mortality. This study is a retrospective study to assess the most important bacteria and the risk factors responsible for bloodstream in infection in Neonatal Intensive Care Unit, Mansoura University Hospital, Egypt. Gram negative bacteria were responsible for most cases of neonatal blood stream infections with *Klebsiella pneumoniae* accounting for the majority of cases (47.2 %). late-onset sepsis (61.6%) was more common than early-onset sepsis. The preterm infants represented 63% of the overall studied cases. Comparison between demographic data of cases with neonatal sepsis caused by *Klebsiella* and those caused by other bacteria revealed that there were no statistical significant differences between the two groups.

Introduction

Neonatal sepsis is a clinical syndrome characterized bacteremia and systemic signs and symptoms during the first month of life or it can be defined as a disseminated disease with a positive blood culture during the first month of life (1). Sepsis is considered the most important cause of neonatal mortality and is responsible for 30-50% of the total neonatal deaths each year in developing countries (2,3). Early onset (within first 72 hours of life) neonatal sepsis is generally acquired from pathogens of maternal genital tract, whereas late onset sepsis (after 72 hours till 28 days of life) has its environmental origin either in the community or in hospital (4).

The most common pathogens of bacterial sepsis and antibiotic sensitivity patterns vary in different parts of the world (5) and

sometimes changes from one center to another within the same country (6). Sepsis with Gram–negative microorganisms increasingly reported nowadays (7; 8). In general, gram negative bacteria are the predominant causes of neonatal sepsis and among them *Klebsiella pneumoniae* the most common pathogen, especially in developing countries (9, 10, 11). The widespread use of antimicrobial agents results in the appearance of multi-resistant strains of *Klebsiella* in hospitals (12, 13).

The aim of this study was to describe different bacterial isolates obtained from blood cultures of neonates and the predisposing risk factor for neonatal infection in the neonatal Intensive Care Unit (NICU) in Mansoura University Hospital, Egypt.

Materials and Methods

This was a retrospective review of all bacterial isolates in blood cultures obtained from the NICU of Mansoura University Hospital during period extending from August 2012 to August 2015. This neonatal unit cares for both inborn and out born neonates. A list of positive blood cultures was obtained from the Microbiology Diagnostic and Infection Control Unit (MDICU), in the department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University Computer Data Warehouse. Information obtained (were available for only 73 out of 91 cases) included identification of the isolated organisms *and* complete patient medical records (including name, gender, birth date, birth weight, age in days, Gestational age, birth location, results of physical examination, laboratory results, maternal data as mode of delivery and prenatal steroids). A neonate was defined as being ≤28 days of age. Only blood stream infections (BSIs) were included in this study as neonates with clinical signs of sepsis but having negative blood cultures were excluded. Early onset sepsis (EOS) refers to infections during the first 72 hr. whereas late onset sepsis (LOS) refers to postnatal acquisition of infections after the first 3 days of life.

Collection of blood samples performed before the start of empirical antibiotic therapy. After proper disinfection of the skin at site of venipuncture, from 0.5 to 1.5 ml of blood was withdrawn from infants and sent for blood culture together with a full blood count and C reactive protein (CRP). The CRP was repeated after 24 hours. The blood then, inoculated immediately into blood culture bottles containing either 50 ml tryptic soy broth or brain heart infusion broth. Samples were transported to laboratory and incubated at 37 ºC (14) the blood culture medium vials sent to Microbiology Diagnostic and Infection Control Unit (MDICU), in the department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University. (MDICU) laboratory for cultivation and subsequent processing.

The blood cultures were incubated aerobically at 37°C and observed daily for the first 3 days for the presence of visible microbial growth by one of the following: hemolysis, air bubbles (gas production), and coagulation of broth. At the same time, subcultures were made during three successive days on enriched and selective media including blood, chocolate, MacConkey, and mannitol salt agar plates and examined for growth after 24–48 hours of incubation. The same protocol was repeated until the 7th day before blood culture was considered to be free of microorganisms **(15).**

Data analysis

Data was collected, classified, tabulated and analyzed. Tests of significance were applied at appropriate places and interpretation was

done accordingly. To evaluate the difference between the categories, Student's t-test was used as a test of significance. A p-value of less than 0.05 was considered statistically significant (16).

Results and Discussion

Gram negative bacteria were responsible for most cases of neonatal blood stream infections in Mansoura University Hospital during the period of this study and *Klebsiella pneumoniae* (47.2 %) was the most frequent isolated pathogen as shown in Table (1)

Over the period of the study, 91 cases of neonates admitted in NICU of Mansoura University Hospitals and had a culture proven sepsis. Complete patient medical records (including name, gender, birth date, birth weight, age in days, Gestational age, birth location, results of physical examination, laboratory results, maternal data as mode of delivery and prenatal steroids) were available for only 73 out of 91 (80%) cases.

Organism isolated in early-onset (EOS) and late-onset (LOS) septicemia

Early onset sepsis (EOS) refers to infections during the first 72 hr. whereas late onset sepsis (LOS) refers to postnatal acquisition of infections after the first 3 days of life (Bizzarro *et al.*, 2008). As shown in Table 2, late-onset sepsis was more common than early-onset sepsis as, early onset sepsis detected in 28 (38.4%) cases and late onset sepsis in 45 (61.6%) cases.

Maternal and neonatal data of neonates investigated for sepsis

Both Maternal and neonatal data of neonates investigated for sepsis shown in Table 3 and Table 4. Of the 73 studied neonates, 63% were males and the rest 37% were females. The preterm infants represented 63% of the overall studied cases. Most of the studied cases (98.7%) were born in the health care facilities (hospitals/ private clinics), while only one case was born in home. Caesarean section represented the major delivery mode (80.8%). The majority of the studied neonates (63%) had either low birth weight (LBW) or very low birth weight (VLBW).

Table 5 summarized the clinical presentations and signs among the studied cases with sepsis. As shown from this table, prematurity and respiratory distress syndrome were the most common clinical diagnosis among the studied cases.

Comparison between demographic and clinical data of neonates infected by *Klebsiella* and those infected with other bacteria

Demographic data of cases with neonatal sepsis caused by *Klebsiella* were compared with those caused by other bacteria. Table 6 shows no statistical significant differences in the mean of age, gestational age, and birth weight of *Klebsiella* Infected neonates and other neonates.

Neonatal sepsis represents a very important health problem all over the world (17). Pathogens encountered in neonatal sepsis vary worldwide (even vary from hospital to hospital in the same country).These differences in bacterial etiology of neonatal sepsis attributed to many factors such as environmental differences, patterns of antibiotics used, nursery practices, differences in the supportive care and infection control practices (18).

In this study, Gram-negative bacteria represent the major cause of bloodstream infections in neonates in Mansoura University Hospital and responsible for about 66% of the

overall cases. Neonatal sepsis caused by Gram-negative bacteria is more frequent in developing countries (19). A comparable results to our study were recorded by Kamath *et al.*, (2010) who reported that 71.8% of BSIs in India were caused by Gram-negative bacteria, with *Klebsiella* species accounting for 16.4%, *Pseudomonas* spp. 13.6%, *E. coli* 11.8%, *Enterobacter* spp. 11.4% and *Acinetobacter* spp. 10% (19).

Table.1 Microbiological profile found in positive blood cultures from neonates

Isolated microorganism	No of isolates	%
Gram – negative bacteria		
Klebsiella pneumonia	43	**47.2 %**
Escherichia coli	10	**11%**
Acinetobacter species	1	**1.1%**
Pseudomonas species	5	**5.5%**
Proteus mirabilis	1	**1.1%**
Gram – positive bacteria		
a hemolytic streptococci	3	**3.3%**
Staphylococcus aureus	**18**	**19. 8%**
MRSA*	1	**1.1%**
Fungi		
Candida species	9	**9.9%**
Total	91	**100%**

Table.2 Organism isolated in early-onset (EOS) and late-onset (LOS) septicemia

Isolated microorganism	Early-onset ≤72 hr.	Late- onset >72 hr.	Total no. of organism
Gram – negative bacteria			
Klebsiella pneumonia	11	20	**31**
Escherichia coli	3	6	**9**
Acinetobacter species	-	1	**1**
Pseudomonas species	2	2	**4**
Proteus mirabilis	1	-	**1**
Gram – positive bacteria			
a hemolytic streptococci	3	-	**3**
Staphylococcus aureus	**7**	9	**16**
MRSA	-	1	**1**
Fungi			
Candida species	1	6	**7**
Total	**28 (38.4%)**	**45 (61.6%)**	**73**

Table.3 Neonatal data of the neonates investigated for sepsis

Features	Total (n=73) number %
Sex: Male Female	 **46 (63 %)** **27(37 %)**
Birth weight: ≤ 1500 g (VLBL) **1501**-2500 (LBW) >2500	 **30 (41%)** **16 (21.9%)** **27 (36.9%)**

Table.4 Maternal data of neonates investigated for sepsis

Characteristics	Total (n=73) number %
Gestational age ≤ 33 weeks (preterm) 34-36 (late preterm) ≥ 37 weeks (term)	 31(42.5%) 15 (20.5%) 27 (37%)
Birth location Hospital Private clinic Home	 58 (79.5%) 14 (19.17%) 1 (1.36%)
Delivery Mode Vaginal/Spontaneous Caesarean section	 14 (19.17%) 59 (80.82%)
Prenatal steroids	21 (28.76%)

Table.5 Clinical presentations and signs accompanied the diagnosis among studied cases with suspected sepsis

Clinical signs/ accompanied diagnosis	Number (%)
Respiratory distress syndrome	18 (24.6%)
Apnea	2(2.7%)
Hypothermia	2(2.7%)
Jaundice	4(5.5%)
Prematurity	46 (63%)
Convulsions and neurologic alterations	8 (10.9%)
Poor feeding	6(8.2%)
Hypoxic ischemia	2(2.7%)
Neonatal hypoglycemia	2(2.7%)
Neonatal pneumonia	2(2.7%)
Bleeding tendency or hemorrhage	1(1.4%)

Table.6 Comparison between demographic data of neonates infected by *Klebsiella* and those infected with other bacteria

Variables	*Klebsiella* N=31	Other bacteria N=42	p. value
Gestational age (weeks)(mean±SE)	34.5 ± 0.8	34.2 ± 0.7	0.774
Age (day)(mean ± SE)	8.3 ± 1.3	9.4 ± 1.3	0.541
Weight(g) (mean ± SE)	2071.1± 143.4	2156.6± 169.8	0.702

Table.7 Results of white blood count (WBC), PLT and C - reactive protein (CRP) of studied neonates

Clinical manifestations	*Klebsiella* N=31	Other bacteria N=42	p. value
WBC Range Mean +SE	 5.3-87.9 21.4±3.5	 6.7-69.4 18.9±2.4	0.575
PLT Range Mean +SE	 22-678 173.9±30.5	 28-679 169.9±30.9	0.927
CRP Range Mean +SE	 12-180 59.1±9.55	 11-260 56.43±8.8	0.839

Our results demonstrated that, *Klebsiella pneumonia* is the main causative agents for neonatal bloodstream infections and accounts for 47.2 % of all cases. Other studies from Egypt reported that *Klebsiella pneumoniae* is the main organism associated with neonatal sepsis (20 & 21).

Our results are somewhat higher to what reported by Mohammed and El Seifi, 2014 in Zagazig University Hospital in Egypt, who found that *Klebsiella* was the most frequently isolated bacteria in neonatal intensive care unit and represent 34.2% of the recovered microorganisms (22).

By contrast, In Europe, Gram-positive bacteria, caused most of nosocomial neonatal infections (76.4%) with CoNS being the most frequently occurring pathogens (23).

In this study, *E.coli* represented the second cause of neonatal Gram-negative blood stream infections and accounts for 11% of all the cases, followed by *Pseudomonas species* (5.5%). Ghotaslou *et al.*, 2007, reported similar results that *K. pneumoniae* was the most common Gram-negative causing neonatal sepsis and *E.coli* is the second most common among Gram-negative isolates (24). On the other hand, other Gram-negative bacteria such as *P. aeruginosa* and *Enterobacter* spp were identified as the most common Gram-negative isolates associated with neonatal sepsis in other studies (25&26).

Staphylococcus aureus remain a very important neonatal pathogen. In this study, *Staphylococcus aureus* represented 19.8% of the overall blood stream infections among the studied cases. This was in accordance with what was mentioned by Zaidi *et al.,* (2005) who reported that, *Staphylococcus aureus* in developing countries, responsible for 8-22% of neonatal blood-stream infections (27)

Candida spp. was isolated from 9.9% of the studied cases. Lower incidence was reported in Kenya by Kohli-Kochhar *et al.,* 2011 and in India by Gandhi *et al.,* 2013 as they reported incidence of (2.41%) and (2.63%) respectively (28, 29).

Early onset sepsis usually presents within the first 72 hours of life and is associated with acquisition of microorganisms from the mother either an ascending infection from the cervix (may be caused by organisms that colonize in the mother's genitourinary tract) or Trans placental infection. While, late onset sepsis usually presents after 72 hours of life and is acquired from the care-giving environment. (30).

In the current study, early-onset sepsis (EOS) was less common than late-onset sepsis (LOS) (38.4% versus 61.6%). This finding was in accordance with previous study performed by Shehab El-Din *et al.,* 2015 (between March 2011 and August 2012) who reported an incidence of 55.8% for LOS and 44.2% for EOS (15). In addition, other studies by Kayange *et al.,* 2010 and Ballot *et al.,* 2012 reported a higher incidence for LOS than EOS (31,32).

The male neonate is predominant in almost all studies of neonatal sepsis. In this study, the percentage of male neonates is 63.0%. The explanation of this phenomenon is not clear but this may be due to a sex-linked factor related to host susceptibility (33 & 15). Moreover, the ESBL phenotype of *K. pneumonia* has been reported more frequently in males (34).

Neonates, especially preterm and with very low birth weight (VLBW) are more susceptible to acquire nosocomial infections because of immaturity of their immune system, receiving total parenteral nutrition in the NICU, prolonged hospital stay and exposure to invasive procedures (35).

Generally, birth weight is inversely proportional to incidence of neonatal sepsis. Many studies confirm this correlation, Kaufman and Fairchild, 2004 reported that a serious systemic infections occurred in 20% of very-low-birth-weight (<1500 g) preterm infants (36). In this work, very low birth weight neonates (VLBW) and low birth weight neonates (LBW) represent 41 % and 22% respectively of the overall cases. The same results were reported by Mutlu *et al.,* 2011 who recorded forty-one percent for VLBW neonates with sepsis (37). Premature neonates are more susceptible to nosocomial infections (38). Most of the neonates in this study were premature. Both preterm neonates (≤ 33 weeks) and late preterm (34-36 weeks) represented together 63% of the studied cases. A comparable result was reported by Mutlu *et al.,* 2011 who reported that 66 % of the neonates having sepsis were premature (37).

Generally, prematurity and low birth weight considered as the two most important factors exposing the neonates to infections and the premature infants with low birth weight have a 3 to 10 fold higher incidence of infection and sepsis than do full-term normal birth weight infants (31).

In this study, most of neonates having sepsis was born via Caesarean section (80.8%).This is similar to what was reported by previous studies (26 & 39). While Wrener *et al.,* 2012

reported that there was no significant difference in intra-ventricular hemorrhage, subdural hemorrhage, seizure, or sepsis between the cesarean delivery and vaginal delivery groups (40). The Cesarean delivery when compared with vaginal delivery, it was associated with increased odds of respiratory distress syndrome.

In this study, prenatal steroids were given in 28.7% of the studied cases. Some studies found that, the incidence of neonatal infections were not affected significantly with prenatal steroids (41). In contrast, several studies suggested that steroid treatment was considered as an independent risk factor for early onset neonatal sepsis (EOS) (42& 43). Indeed, the multiple courses of steroids may have a significant effect over the appearance of neonatal sepsis. Single course of steroids is recommended than multiple courses and have the same effect (42).

In this work, demographic data of cases with neonatal sepsis caused by *Klebsiella* were compared with those caused by other bacteria and there is no statistical significant differences in the mean of age, gestational age, birth weight, WBC, PLT and CRP of *Klebsiella* Infected neonates and neonates infected by other bacteria.

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