Sudan University of Science and Technology

Collage of Post Graduate Studies

Detection of Bacterial Causative Agent in Preterm Neonatal Sepsis Infants in Khartoum State

الكشف عن العامل البكتيري المسبب للتسمم الدموي لدى الخدج في ولاية الخرطوم

A Dissertation Submitted for Partial Fulfilment Requirement of M.Sc. Degree in Medical Laboratory Science (Microbiology)

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(B.SC. Degree in Medical Laboratory Science (Medical Microbiology), University of Science and Technology, 2014)

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December 2020

بأسم الثالوث القدوس

(I can do all things through Christ who strengthens me”)

Philippians 4:13

DEDICATION

TO

MY TEACHERS

My HUSBAND AND MY PARENTS

MY BROTHER AND SISTER

MY FRIENDS

MY COLLEAGUES

With my faithfulness and love

ACKNOWLEDGMENT

Firstly, thanks to God who gave me health and power to do this study.

I would like to express my sincere thanks and gratitude to my supervisor **Dr.Omar Mohammed Khalil** whose stimulating suggestion, help, knowledge, experience and encouragement in all time of study.

Thanks also to my beloved parents who have been the source of encouragement through my life, they teach me to fulfil my dreams and they continuously support me.

A special thanks to my husband Tony Fathi for his vital encouragement, support, and valuable advices.

My sincere love to my brother and sister (Mark and Silvia) who have been the source of my power.

My appreciation to all staff members of Microbiology Department in Alsaaha Specialized Hospital for their help, cooperation and encouragement.

I would like to acknowledge and extend my heart full gratitude to all subjects for their participation and cooperation.

Finally, for all Sudanese people who fight for a new beginning may God bless them.

ABSTRACT

Sepsis encompasses various systemic infections of the new born such as: septicemia, meningitis, pneumonia, arthritis and osteomyelitis and urinary tract infections. Neonatal sepsis is caused by both gram-positive and gram-negative bacteria.

This is cross sectional clinical based case study aimed to detect the bacterial causative agents among preterm neonates with features of sepsis. A total of seventy blood sample (n=70) were inculcated in diphasic medium, incubated aerobically at 37C ^{O} and observed daily for the first 3 days for the presence of visible microbial growth. At the same time, subcultures were made during 3 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. Then Antimicrobial susceptibility testing of all bacterial isolates was performed and data analysis was performed by spss version 16.

The frequency of *S.epidermidis* was found 15(21.5%), *E.coli* 10(14.4%), *pseudomonas aeruginosa* 6(8.6%), *klebsiella pneumoniae* 5(7%) *and candida spp* 1(1.5%).The high rate of positive isolate was found in age group of 26-30 weeks 23(62%). C- reactive protein was tested to all study population and was found positive in all positive samples with bacterial growth. The preterms which used the nasal cannula and CPAP device had high rate of isolated bacteria 13(35%). Total white blood cell was done to all participants and was found high in the preterm with growth mean 17.9 /cumm antimicrobial susceptibility was done to all isolates, regarding gram positive bacteria *S.epidermidis* was found sensitive to Vancomycin (100%) and showed high resistance to Ampicillin and Gentamicin.

The study concluded that both Gram positive and Gram negative can cause sepsis of preterm, S.*epidermidis* was the most frequent bacteria isolated followed by *E.coli* ,*Pseudomonas aeruginosa* then *Klebsiella pneumoniae* .

المستخلص

التسمم البكتيري لللدم بالنسبه للاطفال حديثي الولاده يؤدي الي تسمم الدم واللتهاب الخلايا السحائيه والالتهاب الرئوي و التهاب المفاصل الالتهاب العظمي واللتهاب المسالك البوليه . التسمم البكتيري لللدم تسببه بكتيريا موجبة الجرام وبكتريا سالبه الجرام . كان الهدف من هذا البحث هو الكشف عن البكتيريا المسببة للتسمم البكتيري للدم عند الاطفال الخدج .تم اخذ 70 عينه من الاطفال الخدج وتمت مراقبتها في الثلاثه $37C^{O}$ وزراعتها في الوسط ذو الوجهين وتم حضنها في درجة حراره ايام الاولي للتاكد من النمو او ملاحظة اي تكتلات علي الوسط بعد النمو تم اعادة التزريع علي ثلاثه اوساط وهي وسط الشيكولاته ووسط الماكونكي ووسط المانتول الملحي .ثم تم اجراء اختبار الحساسية لمضادات الميكروبات لجميع العزلات البكتيرية عن طريق نشر قرص كيربي باور فجاء تردد العنقوديات الجلدية 15(21.5 %) ،الاشريكية القولونية 10(14.4%) ،سودوموناس ايريوجينوسا 6(8.6%) ، الكلبسيلة الرئوية 5(7.0%) والكانديدا 1(1.5%). تم العثور علي ارتفاع معدل العزلة الايجابية في الفئة العمرية 26-30 بنسبة 62% . ثم اختبار فحص الس ار بي لجميع افراد الدراسة وكان الاختبار موجب في جميع العينات الايجابية ذات النمو البكتيري. كان لدي الخدج اللذين استخدموا فنية الانف وجهاز السي باب نسبة عالية من البكتريا المعزولة 13(35%) .تم عمل فحص خلايا الدم البيضاء الكلية لجميع المشاركين ووجدت انها عالية في الخدج بمتوسط نمو 17.9/cumm لجميع العزلات لمضادات الميكروبات لاجراء الحساسية تجاة . فيما يتعلق بالبكتيريا الموجبة الجرام وجدت العنقوديات الجلدية حساسية تجاة الفانكومايسين واظهرت مقاومة عالية للامبيسيلين والجنتامسين. من ناحية اخري تم عزل البكتيريا الاشريكية القولونية ،سودوموناس ايريوجينوسا والكلبسيلة الرئوية السالبة الجرام .تم العثور علي الاشريكية القولونية حساسة لجميع المضادات الحيوية (ميروبينم ،اميبينم ،سيفترايكسون ،سيفتازيديم ،اموكلان وتيتراسيكلين بنسبة(100%) ماعدا (الاميكسين ،السيبروفلوكساسين ، والازترنام)بنسبة (0.0%). اما عن السودوموناس ايريوجينوسا فكانت حساسة بنسبة (100%) الي (ميروبينيم ،سيفتازيديم ، واموكلان)وحساسة بنسبة (50.0%) لسيبروفلوكساسين ومقاومة ل(اميكسين ،ايمبينم، ازترونام ،سيفترايكسون وتتراسايكلين).اما عن الكلبسيلة الرئوية فكانت حساسة بنسبة (100%)الي(ميروبينم ،اميكسين ،اميبينم ،سيفتازيديم و اموكلان)وحساسة بنسبة (50.0%) الي سيبروفلوكساسين ومقاومة تماما الي (سيفترايكسون وتيتراسيكلين)

LIST OF CONTENT

List of abbreviations

Abbreviation	Meaning
BPD	Bronchopulmonary dysplasia
CDC	Centers for Disease Control
CMV	Cytomegalovirus
CNS	Coagulase-negative staphylococci
CRP	C-reactive protein
HBV	Hepatitis B virus
HSV	Herpes simplex virus
LOS	late-onset sepsis
MDR	Multi drug resistance
MRSA	Methicillin resistance *S.aureus*
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
PIA	polysaccharide intercellular adhesion
PN	parenteral nutrition
RDS	Respiratory distress syndrome
ROP	retinopathy of prematurity
VLBW	very low birth weight
WHO	world health organization
WMI	white matter injury

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Chapter one

Introduction

Introduction

1.1. Background:

Neonatal sepsis is a systemic infection occurring in infants within 28 days of life and is a major cause of morbidity and mortality in newborns (Simonsen *et al*,2014). According to the international pediatric consensus conference of 2001, neonatal sepsis was defined as systemic inflammatory response syndrome in the presence of or as a result of suspected or proven infection with or without accompanying bacteremia, documented by a positive blood culture in the first 28 days of life (Goldstein *et al*, 2015). Sepsis encompasses various systemic infections of the new born such as: septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infections (Woldu, 2014). Neonatal sepsis is caused by both gram-positive and gram-negative bacteria's (Milka, 2013). Neonatal sepsis is classified into two major categories based on the time of onset: early-onset neonatal sepsis (EONS) and late onset neonatal sepsis (LONS). Early-onset neonatal sepsis appears within the first seven days of life and most cases appear within 24 h of birth. While late onset neonatal sepsis occurs after 8 days of infant's life and is mostly acquired after delivery (El-Din *et al*,2015). Sepsis is diagnosed a complete white blood cell count with differential, blood culture, urine cultures, and a lumbar puncture for cell count and culture. To clear the diagnosis of early onset sepsis factors that predispose the neonate for sepsis such as maternal infection and prolonged rupture of membranes, and prematurity are also considered (El-Din *et al*, 2015). Signs and symptoms of infection in neonates are subtle and non-specific, may present with one or more of the following: hypothermia or fever, lethargy, poor cry, refusal to suck, poor perfusion, prolonged capillary refill time, hypotonia, absent neonatal reflexes, bulging fontanel, brady/tachycardia, respiratory distress, apnea and gasping respiration, hypo/hyperglycemia, and metabolic

acidosis (Hoogen*et al*, 2009). Risk factors for early onset of sepsis includes premature rupture of membrane (PROM), fever, chorioamnionitis, repeated vaginal examination, meconium stained amniotic fluid, dietary intake of contaminated foods, cervical cerclage, place of birth, prematurity, low birth weight, complicated or instrument-assisted delivery, and low appearance pulse grimace activity respiration (APGAR) scores. Late onset of sepsis acquiring nosocomial infections and invasive procedures during hospital admission (Setiawan *et al*, 2004). Antimicrobials used to treat sepsis are combinations and in most units are penicillin (Benzyl penicillin, Ampicillin, or Cloxacillin) together with an aminoglycoside, most commonly Gentamicin and is largely preventable by timely recognition, rational antimicrobial therapy and aggressive supportive care (Shah and Padbury,2014). Globally, sepsis is one of the major causes of morbidity and mortality among neonates, according to WHO sepsis caused approximately 12% of the 2.9 million neonatal deaths in 2012 out these deaths 99% occur in developing countries (WHO, 2012). In Africa sepsis accounts 28% neonatal deaths (Aggarwal*et al*, 2014) and infectious cause's accounts 68 deaths per 1000 live births. In Ethiopia from prenatal mortalities sepsis covers 5%. In Debrezeyt, Ethiopia the overall poor outcomes of NS were 26% including deaths. (Lawn andMongi, 2012).

1.2. Rationale:

Globally, sepsis is still one of the major causes of morbidity and mortality in neonates. Neonatal sepsis is a systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections (Shaha *et al,*2012).More than 40% of under-five deaths globally occur in the neonatal period, resulting in 3.1 million new-born deaths each year. The majority of these deaths usually occur in low-income countries and almost 1 million of these deaths are attributed to infectious causes including neonatal sepsis, meningitis, and pneumonia (World Health Organization, 2012). On the other hand, the survivors of neonatal sepsis are vulnerable to short and long-term neurodevelopmental morbidity. This study was aimed to identify the bacterial causative agent of preterm sepsis.

1.3. OBJECTIVES

1.3.1. General objective:

To detect the bacterial causative agents among preterm neonates with features of sepsis.

1.3.2. Specific objectives:

1. To isolate causative agents from pediatric blood culture aerobic (Brain heart infusion) and anaerobic (Thioglycolate broth).
2. To correlate between the infection and relative risk (age, weight, gender and medical device used).
3. To make association between sepsis and clinical remark.
4. To correlate the infection with some hematological indices (e.g. Number of white blood cells, neutrophils and platelets) and biochemical markers of inflammation such as C-reactive protein.
5. To determine antibiotic sensitivity for this isolates using disk diffusion method.

Chapter Two

LITERATURE REVIEW

LITERATURE REVIEW

2.1. Epidemiology and Public Health Issues

Neonatal mortality is increasingly recognized as an important global public health challenge that must be addressed if we wish to reduce child death disparities between rich and poor countries. Most of the estimated (Qazi*et al*, 2009) million neonatal deaths per year occur in low and middle income countries. More than one-third of neonatal deaths are estimated to be due to severe infections, and a quarter is due to the clinical syndrome of neonatal sepsis/pneumonia (Qazi*et al*, 2009).

The reported incidence of neonatal sepsis varies from 7.1 to 38 per 1000 live births in Asia, from 6.5 to 23 per 1000 live births in Africa, and from 3.5 to 8.9 per 1000 live births in South America and the Caribbean. By comparison, rates reported in the United States and Australia range from 1.5 to 3.5 per 1000 for early onset sepsis and up to 6 per 1000 live births for late onset sepsis, a total of 6 – 9 per 1000 for neonatalSepsis (Vergnano*et al*, 2005).

Currently, neonatal mortality rate in Bangladesh is 32 per 1000 live births which accounts for 60% of all under five deaths (Bangladesh Demographic and Health Survey, 2011). In a study assessing causes of neonatal deaths in rural Bangladesh it is shown that sepsis/ meningitis constituted 12% of direct causes of neonatal deaths (Chowdhury, 2010). In another study conducted in Dhaka slums showed sepsis as a direct cause of neonatal deaths in 20% cases (Khatun*et al*, 2012). In another study in Bangladesh, estimated causes of mortality around the year 2010 for 102,000 neonatal deaths showed that severe infections (sepsis, meningitis, pneumonia and tetanus) contributed 20% of neonatal deaths(Liu L *et al* ,2012) ,(Rubayet*et al*,2012).

2.2. Definition

Systemic illness caused by microbial invasion of normally sterile parts of the body is referred to as ‘sepsis’. This is a term that specifically serves to differentiate an illness of microbial origin from an identical clinical syndrome that can arise in several non-microbial conditions. When accompanied by evidence of hypoperfusion or dysfunction of at least one organ system, this becomes ‘severe sepsis’. Finally, where severe sepsis is accompanied by hypotension or need for vasopressors, despite adequate fluid resuscitation, the term ‘septic shock’ applies. Within this terminology, the archaic term ‘septicemia’, which persists in the language of the non-specialist and layman, straddles the definitions of sepsis, severe sepsis, and septic shock (Lever *et al*, 2007).

Neonatal sepsis is a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first month of life (Tricia *et al*, 2009). The condition may be defined both clinically and/or microbiologically, by positive blood and/or cerebrospinal fluid cultures (Vergnano*et al*, 2005).

2.3. Classification

Neonatal sepsis may be classified according to the time of onset of the disease: early onset (EOS) and late onset (LOS). The distinction has clinical relevance, as EOS disease is mainly due to bacteria acquired before and during delivery, and LOS disease to bacteria acquired after delivery (nosocomial or community sources). In the literature, however, there is little consensus as to what age limits apply. A few papers distinguish between very early onset (within 24 hours), EOS (24 hours to six days), and LOS (more than six days) sepsis (Vergnano*et al*, 2005). Very late onset sepsis is demarcated by onset at >30 days of age.

2.4. Risk factors

Several obstetric and neonatal factors have been identified that may be associated with an increased risk of neonatal infection. The presence of any

of these factors alone is not an indication for a complete sepsis (Vergnanoet*al*, 2005) work-up and antibiotic therapy; however, combinations of risk factors are clearly additive and should greatly enhance the suspicion of sepsis (Mary*et al*, 2007).

• Prematurity and low birthweight

• Premature or prolonged rupture of membranes (>18 h)

• Maternal peripartum fever (>100.40F) or infection

• Resuscitation at birth

• Multiple gestations

• Invasive procedures

• Infants with galactosemia (predisposition to *E. Coli*sepsis), immune defects, or asplenia.

• Other factors: Male sex (four times more affected than females), bottle-feeding (as opposed to breast-feeding), low socio-economic status, improper hand washing practice of NICU staff and family members etc.

2.5. Bacteria causing neonatal sepsis

Bacteria, such as *Staphylococcus* , *Streptococcus*, *L. monocytogenes, E.faecalis,E. faecium*, group D Streptococci, α-hemolytic Streptococci and Staphylococci,*S.pneumoniae,H. Influenza* type B, are recognized as the principal cause of earlyneonatal sepsis. Less commonly, *N. meningitidis* and *N. gonorrhoeae* have been also reported as a cause of neonatal septicemia and Gram-negative enteric organisms

Predominantly *E. coli, Klebsiella* species are included (Simonsen*et al*, 2014). On the other hand, late-onset sepsis is predominantly caused by Staphylococci species and E. coli and most frequently related with low birth weight of infants and use of intravascular catheters, endo-tracheal intubation, assisted ventilation, surgery, contact with hand of colonized personnel and contact with contaminated equipment as the main risk factors for late onset of neonatal sepsis. Sometimes *E. cloacae* or *Citrobactor* from

blood or CSF may be due to contaminated feedings. Contaminated respiratory equipment is suspected in outbreaks of hospital-acquired *P. aeroginosa* pneumonia or sepsis (Mohammad *et al*, 2011).

Staphylococci are Gram-positive cocci, which often stick together in grape-like clusters. They belong to the family *Micrococcacea.* There are 45 species and 24 subspecies of the genus *Staphylococcus*. With a few exceptions, all species are catalase-positive, and they are all facultative anaerobe. The genus can be separated into two groups based on the ability to produce coagulase, an enzyme that causes clotting of blood plasma: The coagulase-positive *staphylococci* (*Staphylococcus aureus* and a few others) and the coagulase-negative staphylococci (CONS) (Shaw *et al*, 2015)

2.6. Pathogenesis

The pathophysiology of sepsis arises largely from the response of the host's innate immune system under the influence of genetic factors. Sepsis originates from a breach of integrity of the host barrier, either physical or immunological, and direct penetration of the pathogen into the bloodstream, creating the septic

state. (Lever *et al*, 2007).

2.7. Clinical manifestations

The signs and symptoms of sepsis are influenced by the virulence of the pathogen, the portal of entry, the susceptibility and response of the host, and the temporal evolution of the condition. (Lever *et al*, 2007).

Initial signs and symptoms of infection in newborn infants:(Barbara *et al*, 2011*)*

• General: Fever, temperature instability, "not doing well", poor feeding, edema

• Gastrointestinal system: Abdominal distension, vomiting, diarrhea, hepatomegaly

• Respiratory system: Apnea, dyspnea, tachypnea, retractions, flaring, grunting, cyanosis

• Renal system: Oliguria

• Cardiovascular system: Pallor, mottling; cold, clammy skin, tachycardia, hypotension, bradycardia

• Central nervous system: Irritability, lethargy, tremors, seizures, hyporeflexia, hypotonia, abnormal Moro reflex, irregular respirations, full fontanel, high-pitched cry

• Hematologic system: Jaundice, splenomegaly, pallor, petechiae, purpura, bleeding

Early-onset sepsis is usually a multisystem illness with prominent respiratory symptoms. It is characterized by a sudden onset and fulminant course that can progress rapidly to septic shock and death. Late-onset sepsis is usually more insidious but it can be fulminant at times. In addition to bacteremia, these infants may have an identifiable focus, most often meningitis in addition to sepsis. (Tricia *et al*, 2009)

2.8. *S. epidermidis* - clinical significance

Ubiquitous colonization of *S. epidermidis* on the iucus membranes gives them the opportunity to cause infections under special circumstances. However, in general *S.epidermidis*are low virulent bacteria with few virulence factors.(Otto,2007)*epidermidis*has emerged as an important opportunistic human pathogen, reflecting the increased use of indwelling medical devices and an increasing number of patients with impaired immune systems,e.g. patients receiving immune-suppressive therapy, preterm infants, AIDS patients, and drug abusers. *S. epidermidis* is now considered one the most frequent causes of nosocomial infections

2.9. S. *epidermidis* infections in neonates

*S. epidermidis*may cause a wide spectrum of infections in neonates. Isolation of *S. epidermidis*has been associated with wound abscesses, pneumonia, urinary tract infections, necrotizing enterocollitis (NEC), endocarditis, omphalitis and meningitis. However, clearly the most important and prevalent *S.epidermidis* infection in neonates is sepsis with or without the association to indwelling catheters.Neonatal sepsis is an important cause of morbidity in neonatal intensive care units. Over the past twenty years there has been a substantial shift in pathogen patterns for late-onset sepsis in neonates (305), where the nosocomial pathogens have become more important. CONS are now the most prevalent pathogen causing late-onset sepsis, accounting for more than 50% of the episodes. (Van den Hoogen*et al,*2010)These infections are associated with low birth weight, low gestational age, need of mechanical ventilation, parenteral nutrition (PN) and a history of intravascular catheterization. A large proportion of systemic infections due to *S. epidermidis* in the neonatal period are associated with indwelling catheters or other devices that causes a break in the skin. Many neonatal infections are caused by bacteria that colonize the patient's own skin, indicating that invasive *S. epidermidis* infections often are derived from the skin and that indwelling vascular lines may be a major source of infection. Late onset-sepsis caused by *S. epidermidis* is seldom fatal, but they cause significant morbidity with longer in-hospital time, and a significant increase in total hospital costs (Blot *et al,* 2005).The diagnosis of *S. epidermidis* late onset-sepsis in neonates is difficult. The clinical signs of infection in neonates, and especially in premature neonates, are subtle and non-specific and the laboratory tests including the "gold standard" blood culture are not always reliable. (Blot*et al,*2005).To minimize the amount of blood drawn and puncture of the skin of the neonates, usual practice in many neonatal

intensive care units (NICUs) are to obtain only a single blood culture. The most used definition for *S. epidermidis* sepsis in neonates is: One positive blood culture and the addition of clinical signs of sepsis such as apnea, tachypnea, need for increased respiratory support, bradycardia, hypotonia, feeding intolerance, abdominal distentionor in the early phase only "the baby is just not right .*S. epidermidis*infections may have a significant impact on the innate immune response of the neonate. The preterm neonates are especially vulnerable because of an immature functioning immune system. *S.epidermidis* infections induce significant secretion of both pro- and anti-inflammatory cytokines, but the secretion of pro-inflammatory cytokines seems to be gestational age dependent. It has been reported that glucose and especially intravenous lipids may modulate host defense and increase the risk of infections in neonates. The use of total parenteral nutrition (TPN) may also reduce the function of neutrophils. Recently it was shown that the pro-inflammatory cytokine response to *S. epidermidis in vitro* was affected by both lipids and glucose. However, further studies are needed to investigate whetherthese findings are applicable to clinical settings and to evaluate the role of cytokine monitoring in infants receiving long-term parenteral nutrition.(Haase *et al,* 2011) *S. epidermidis* biofilms also activate leukocytes, but their ability to up-regulate oxidative burst, induce opsonophagocytosis and bacterial killing is impaired in infants compared to adults. This is probably due to the immaturity of their immune system, with a significant hypo gamma globulinemia and reduced complement activity both in the classical and alternative pathway. Also, the inflammatory response in neonates, assessed by CRP, is compromised when challenged with *S. epidermidis* biofilm producing strains. Deficiency of complement factor C3 and IgG have been related to greater risk for CONS associated infections in neonates. In conclusion, defects in the

neonatal immune response, may partly explain why this otherwise low virulent pathogen, causes such serious infections among these patients.

2.10. Virulence factors –general.

Virulence has been defined in several different ways, such as:"Harmfulness, and describes the ability of a pathogen to reduce host fitness",(Massey *et al*, 2006) or "The ability of a microorganism to establish an infection and cause disease in a host". Factors important for the pathogens virulence generally contributes to either i) immune evasion, ii) immune stimulation, iii) colonization, or iv) factors that cause damage to the host. In general, *S.epidermidis*has few virulence factors which directly cause damage to the host, compared to its more virulent relative, *S. aureus, S. epidermidis* therefore have to rely on factors modulating the immune system of the host in order to maintain a persistent infection.

2.11. Biofilm formation in *S. epidermidis*

Biofilm formation is the most important virulence factor of *S. epidermidis*. The adaptation to environmental factors and the metabolic shift contributes to *S. epidermidis* success in colonization of host tissue and medical devices, and protects the bacteria against the host's immune system (Foster, 2005) and attempts of antibiotic treatments. It is now generally accepted that *S. epidermidis* infections are dependent on the species ability to adhere to artificial surfaces and to assemble biofilm consortia. (Mack *et al*, 2006)

Biofilm formation is commonly described as two-step process with initial attachment to surfaces with ii) a subsequent aggregation and maturation into multicellular structures (Horstkotte *et al*, 2006). A final detachment phase after steady-state has been acquired then follows. The detachment phase involves the detachment of single cells or cell cluster by various mechanisms and is believed to be crucial for the dissemination of the bacteria.

2.12. Premature rupture of membranes (PROM)

Mother and respiratory distress syndrome (RDS) symptoms related to preterm sepsis PROM may occur in response to an untreated urinary tract infection (UTI) or birth canal infection. Other risk factors are previous preterm delivery, uterine bleeding in pregnancy and heavy cigarette smoking during pregnancy. Rupture of membranes without other complications for more than 24 hours before delivery is associated with a 1% increase in the incidence of neonatal sepsis; however, when chorioamnionitis accompanies the rupture of membranes, the incidence of neonatal infection is quadrupled. When membranes have ruptured prematurely before 37 weeks' gestation, a longer latent period precedes vaginal delivery, increasing the likelihood that the infant will be infected. The duration of membrane rupture before delivery and the likelihood of neonatal infection are inversely related to gestational age. Thus, the more premature an infant is, the longer the delay between rupture of membranes and delivery and the higher the likelihood of neonatal sepsis.

2.13. Prematurity

In addition to the relation between preterm PROM and neonatal sepsis, there are other associations between prematurity and neonatal sepsis that increase the risk for premature infants. Preterm infants are more likely to require invasive procedures, such as umbilical catheterization and intubation. Prematurity is associated with infection from cytomegalovirus (CMV), herpes simplex virus (HSV), hepatitis B virus (HBV), Toxoplasma, Mycobacterium tuberculosis, Campylobacter fetus, and Listeria species. Intrauterine growth retardation and low birth weight are also observed in CMV infection and toxoplasmosis. Premature infants have less immunologic ability to resist and combat infection. Consequently, they are more susceptible to infection caused by common organisms such as

coagulase-negative *Staphylococcus*-an organism usually not associated with severe sepsis.

2.14. Physical Examination

The clinical signs of neonatal sepsis are nonspecific and are associated with the characteristics of the causative organism and the body's response to the invasion. These nonspecific clinical signs of early sepsis are also associated with other neonatal diseases, such as respiratory distress syndrome (RDS), metabolic disorders, intracranial hemorrhage, and a traumatic delivery. In view of the non-specificity of these signs, it is prudent to provide treatment for suspected neonatal sepsis while excluding other disease processes. To obtain the most information from the examination, systematic physical assessment of the infant is best performed in a series that should include observation, auscultation, and palpation, in that order. Changes in findings from one examination to the next provide important information about the presence and evolution of sepsis.

2.15. Practices related to late onset sepsis in very low birth weight preterm infants.

Late-onset sepsis (LOS) is a major cause of neonatal morbidity and mortality. The World Health Organization (WHO) estimates that of the four million neonatal deaths worldwide per year, more than one-third are caused by severe infections and one-quarter are due to neonatal sepsis/pneumonia. (Qazi SA *et al*, 2009)1 and 2 In Brazil, the neonatal mortality rate represents 60% of infant mortality, and sepsis is a major cause of neonatal deaths. (Brasi*et al*, 2007)

Research networks worldwide are dedicated to the study of neonatal sepsis. The National Institute of Child Health and Human Development (NICHD Neonatal Research Network) documented a 21% incidence of LOS confirmed in preterm infants weighing < 1,500 g, ranging from 10-38%

among centers. In the Brazilian Neonatal Research Network (BNRN), consisting of 16 reference centers in the maternal-child health area, the LOS study group showed, in the years 2009-2010, 50% incidence of LOS (range 29-72% among centers) in preterm infants weighing < 1,500 g, with 27.5% confirmed LOS (unpublished data). LOS is related to the postnatal environment, characteristics of the newborn, and care practices. Among these practices, the use of vascular catheters and parenteral nutrition are important risk factors for sepsis. (Richtmann*et al*, 2011). Hand hygiene is another practice that deserves attention, as the transmission of microorganisms through the caregivers' hands is a constant concern. The Centers for Disease Control and Prevention (CDC), the WHO, (WHO , 2012)and the Brazilian National Health Surveillance Agency (ANVISA) periodically publish guides on hand sanitizing, but warn that the adherence of health professionals is low, even in campaign periods.(Brasil,2012). The percentage of professionals' adherence to hand washing varies from 28% to 62%, and greater adherence to hand hygiene practices is associated with reduced rates of hospital infection.(Ferraz and Higienização das mãos,2011) Another relevant aspect is the difficulty in the diagnosis of sepsis, as clinical data and laboratory tests are nonspecific and blood culture, considered the gold standard, has low positivity. The difficulty in diagnosis can lead to treatment delay or excessive use of antibiotics, allowing the selection of flora and development of bacterial resistance, another frequent problem in the neonatal intensive care unit (NICU).

2.16. *S.epidermidis* major cause of preterm sepsis.

Blood infection (sepsis) causes millions of deaths worldwide and is the most frequent cause of death in hospitalized patients. Sepsis is most commonly due to bacteria. (Stearns and Kurosawa et *al*, 2011). In addition to symptoms related to the specific bacterial infection, symptoms of sepsis include fever, tachycardia and tachypnea. The definition of sepsis is clinical, with the sepsis-related organ failure assessment score, a combination of scores for the respiratory, cardiovascular, hepatic, coagulation, renal and neurological conditions, giving an understanding of the severity of sepsis in terms of the prediction for a fatal outcome. Sepsis develops from bacteria being present in the blood (bacteremia) when the immune system launches an overwhelming responsive defense. (Stearns and Kurosawa*et al*, 2011)While sepsis is well defined clinically, the molecular immunological mechanisms that underlie the development of sepsis are poorly understood. Long believed to be predominantly due to Gram-negative bacteria, during the second half of the 20th century, Gram-positive bacteria, in particular *Staphylococcus aureus*, have become leading causes of sepsis and sepsis-related deaths. Coagulase-negative staphylococci (CNS), with the major species *S. epidermidis*, are also often reported as frequent causes of sepsis, particularly among neonates (Qin *et al*, 2016). However, the fact that they are ubiquitous commensals on the human skin makes the microbiological diagnosis of a true CNS blood infection difficult, as the detection of CNS in blood samples is often due to contamination rather than a true infection (Kleinschmidt*et al*, 2015). The precise percentage of how many positive blood cultures are due to contamination varies considerably among the many studies that have attempted to estimate that number. Nevertheless, there is agreement that *S. epidermidis* is among the most frequent bacterial sources underlying

bacteremia and sepsis. (Wang et *al,* 2010). In theory, bacterial factors can influence the development of sepsis in at least two different ways. First, the reaction of the immune system differs depending on the bacterial triggers or pathogen-associated molecular patterns. In Gram-negative bacteria, the most important proinflammatory trigger is lipopolysaccharide. Structural modifications of this surface structure, which is ubiquitous in Gram-negative bacteria, can have a significant impact on the strength of the immune reaction. In Gram-positive bacteria, lipoteichoic acid is often stated to play a similar role (Spaan*et al,* 2017). In addition to those invariant structures on the Gram-negative or Gram-positive cell envelope, some particularly aggressive bacteria such as *S. aureus* produce toxins that may further significantly exacerbate the immune reaction. Sepsis often arises from the constant seeding of bacteria from contaminated indwelling medical devices. For CNS, this is by far the most common cause of bacteremia. CNS are among the most frequent causes of bloodstream infection originating from indwelling medical device contamination. (Qin *et al*, 2016). The most frequent types of such device-associated infections are central line-associated bloodstream infection and catheter-related bloodstream infection. Their severity is ultimately dependent on the extent of bacterial colonization. Thus, the most important bacterial factors underlying the pathogenesis of such infections have always been considered those that increase the propensity of the bacteria to form biofilms on the devices, which significantly decreases the capacity of phagocytic cells and other innate immune mechanisms to remove the bacteria. For *S. epidermidis*, these factors include the biofilm exopolysaccharide polysaccharide intercellular adhesin (PIA), in addition to a series of proteins and other matrix components such as teichoic acids.(Qin *et al,*2008). In contrast to *S. aureus, S. epidermidis* as well as other CNS have not been known to produce aggressive proinflammatory or even cytolytic toxins.

(Qin *et al*, 2016) Reports that claim that α-toxin or superantigenic toxins are widespread in *S. epidermidis* or other CNS have to be regarded as incorrect due to the failure to appropriately identify isolates. The notion that CNS do not produce toxins, however, had to be corrected when it was found that *S. epidermidis* produces PSMs. PSMs are amphipathic, α-helical peptides with surfactant character. Many PSMs have strong proinflammatory and broadly cytolytic properties. At nanomolar concentrations, in predominantly formyl peptide receptor-2 mediated fashion, (Kretschmer*et al*, 2010) they elicit leukocyte chemotaxis, activation and cytokine release. In contrast, their cytolyticproperties, which are observed at micromolar concentrations and have been described for neutrophils, monocytes, erythrocytes and osteoblasts are receptor-independent. Notably, PSMs are produced in high amounts in a quorum-sensing controlled fashion, with stationary phase bacterial cultures reaching far more than the concentrations needed for cytolysis. In *S. aureus*, PSMs have been shown to contribute significantly to pathogenesis.(Queck*et al, 2008*). PSMs have been grouped according to length. The α-type PSMs are 20–25 and the β-type PSMs are approximately 44–45 amino acids long. All PSMs are secreted by a dedicated transporter without a signal peptide and thus have an N-terminal formyl-methionine. (Chatterjee *et al, 2013*). While virtually every staphylococcal species produces PSMs, the pattern is different and characteristic for every species. After the initial description of three PSMs in *S. epidermidis* (PSMα, PSMβ and PSMγ, which is equal to δ-toxin), the entire *S.epidermidis* PSM pattern was determined. One particular *S.epidermidis* PSM, PSMδ, has astounding cytolytic character at the level of the most strongly cytolytic *S. aureus* PSM (PSMα3).(Qin *et al*, 2016)

2.17. Previous studies:

Coagulase-negative staphylococcus especially *S. epidermidis* have emerged as the predominant pathogen of late-onset sepsis (LOS) In very low birth weight (VLBW) infants. Accounting for up to 77.90, of Neonatal in industrialized countries and 46.5 % in some developing countries. VLBW neonates with indwelling medical devices are most susceptible for *S.epidermidis* sepsis, the incidence rate of which is approximately 25% .Four Studies in preterm sepsis In Sudan. The objectives of the study were to determine the causes, clinical presentation, management and outcome of neonatal sepsis in Gadarif Teaching Hospital, Sudan, inmethods:The study was descriptive conducted in the department of neonatology in Gedarif Teaching Hospital, Sudan. All the 240 records of patients attended the hospital during 2008-2010, and diagnosed as neonatal sepsis were considered in the study. Data were collected by a check list and analyzes by the computer using SPSS version 16, inresults. The causes of neonatal sepsis were low birth weight, premature rupture of the membrane, mother infection and meningocele constituted 39.6%, 31.3%, 20.8% and 2.5% respectively, other causes constituted 5.8%. Most ofthe patients.Were presented with poor sucking (59.2%). Other presentations were fever, pallor, respiratory distress, vomiting, diarrhea, and jaundice constituted 43.8%, 43.8%, 39.6%, 35.4%, 27.1%, and 27.1% respectively. Other causes constituted 16.3%. Death ratio for neonatal sepsis was 12.5%, in conclusion:The study concluded that neonatal sepsis is more common among males. Neonatal sepsis is related to Low birth weight, premature rupture of the membrane, mother infection before delivery and meningocele. Most of the patients with neonatal sepsis present with poor sucking, fever, pallor and respiratory distress. Death ratio from neonatal sepsis is 12.5%.(Elsadig*et al*,2018). In Sudan prevalence and Causes of

Neonatal Sepsis in Soba University Hospital, Sudan Wafa Babiker1 ,Amany Ahmed1 , Taiser Babiker1 , Elamin Mohamed Ibrahim1 and BabikerSaad Almugadam2 Abstract objective,this study aimed to determine the isolated organisms and, the most common causes of neonatal sepsis; and to evaluate the susceptibility pattern of isolates to different antibiotics. Methods: A total of 119 blood samples were collected and inoculated in brain heart infusion broth, then incubated up to 7days at 37°C. All isolates were identified based on culture charters, Gram stain, and standard biochemical test. Antimicrobial susceptibility tests were done according to CLSI guidelines 2011. Results: Out of 119 blood samples investigated only 37.8% (45/119) were found to be positive for neonatal septicemia and all cases was early onset sepsis. The frequency of Gram-positive and Gram negative bacteria is 57.8% and 42.2% respectively. MRSA and *K. pneumoniae* are the most common isolated organisms. All Gram–ve isolates were resistant to ceftriaxone, cephalexin, and cotrimoxazol and sensitive to imipenem (100%). While most isolates were sensitive to Vancomycin, and resistant to Ciprofloxacin, Amoxyclav, Erythromycin, and Oxacillin. Conclusion: Neonatal sepsis is a major health problem worldwide, and the emergence of MDR isolates can limit the therapeutic options. Proper antibiotic discretion and regular updating of antibiotics susceptibility through continuous surveillance is essential to maintain a good infection control program and it can play a key role in avoiding and limiting the extending of this problem. Keywords Neonatal sepsis; Antimicrobial; Gram -ve; Gram +ve; Sudan (WafaBabiker andAmany Ahmed, 2018). In South Sudan by (NorthanHurtado*etal*, 2015). neonatal deaths comprise a growing proportion of global under-five mortality. However, data from the highest-burden areas is sparse. This descriptive retrospective study analyses the outcomes of all infants exiting the Médecins sans Frontières-managed neonatal unit in Aweil Hospital, rural South Sudan from 2011 to 2014. A

total of 4268 patients were treated over 4 years, with annual admissions increasing from 687 to 1494. Overall mortality was 13.5% (n = 576), declining from 18.7% to 11.1% (p for trend <0.001). Newborns weighing <2500 g were at significantly increased mortality risk compared with babies ≥2500 g (odds ratio = 2.27, 95% confidence interval = 1.9–2.71, $p < 0.001$). Leading causes of death included sepsis (49.7%), tetanus (15.8%), respiratory distress (12.8%) and asphyxia (9.2%). Tetanus had the highest case fatality rate (49.7%), followed by perinatal asphyxia (26.5%), respiratory distress (20.4%) and neonatal sepsis (10.5%). Despite increasing admissions, overall mortality declined, indicating that survival of these especially vulnerable infants can be improved even in a basic-level district hospital programme. Delair, H. done in ,Late-onset sepsis (occurring after 3 days of age) is an important problem in very low birth weight (VLBW) infants. To determine the current incidence of late-onset sepsis, risk factors for disease, and the impact of late-onset sepsis on subsequent hospital course, we evaluated a cohort of 6956 VLBW (401–1500 g) neonates admitted to the clinical centers of the National Institute of Child Health and Human Development Neonatal Research Network over a 2-year period (1998–2000), in *methods.* The National Institute of Child Health and Human Development Neonatal Research Network maintains a prospective registry of all VLBW neonates admitted to participating centers within 14 days of birth. Expanded infection surveillance was added in 1998, r*esults.* Of 6215 infants who survived beyond 3 days, 1313 (21%) had 1 or more episodes of blood culture-proven late-onset sepsis. The vast majority of infections (70%) were caused by Gram-positive organisms, with coagulase-negative staphylococci accounting for 48% of infections. Rate of infection was inversely related to birth weight and gestational age. Complications of prematurity associated with an increased rate of late-onset sepsis included patent ductusarteriosus, prolonged ventilation, prolonged

intravascular access, bronchopulmonary dysplasia, and necrotizing enterocolitis. Infants who developed late-onset sepsis had a significantly prolonged hospital stay (mean length of stay: 79 vs 60 days). They were significantly more likely to die than those who were uninfected (18% vs 7%), especially if they were infected with Gram-negative organisms (36%) or fungi (32%),. Late-onset sepsis remains an important risk factor for death among VLBW preterm infants and for prolonged hospital stay among VLBW survivors. Strategies to reduce late-onset sepsis and its medical, social, and economic toll need to be addressed urgently.*Staphylococcus epidermidis* accounts for the majority of cases of neonatal sepsis. Moreover, it has been demonstrated to be associated with neonatal morbidities, such as bronchopulmonary dysplasia (BPD), white matter injury (WMI), necrotizing enterocolitis (NEC) and retinopathy of prematurity (ROP), which affect short-term and long-term neonatal outcome. Imbalanced inflammation has been considered to be a major underlying mechanism of each entity. Conventionally regarded as a harmless commensal on human skin, *S.epidermidis* has received less attention than its more virulent relative *Staphylococcus aureus*. Particularities of neonatal innate immunity and nosocomial environmental factors, however, may contribute to the emergence of *S. epidermidis* as a significant nosocomial pathogen. Neonatal host response to *S.epidermidis* sepsis has not been fully elucidated. Evidence is emerging regarding the implication of *S.epidermidis* sepsis in the pathogenesis of neonatal inflammatory diseases. This review focuses on the interplay among *S.epidermidis*, neonatal innate immunity and inflammation-driven organ injury. (Ying Dong*et al*, 2018).

Chapter Three

Material and method

Material and method

3.1. Study design:

Clinical base, case study.

3.2. Study area:

Study was conducted in ALSAAHA Specialized Hospital south Khartoum State Sudan, in period between June 2018-Febuary 2019.

3.3. Study population:

Preterm neonates with signs of sepsis

3.4. Inclusion Criteria:

Preterm neonates under 35 weeks of gestational age with clinical sign of sepsis

3.5. Exclusion criteria:

Preterm neonates with cancer and HIV

Preterm neonates on antibiotics before the blood culture sample

3.6. Ethical consideration:

This study was approved by the college of MedicalLaboratory Science Ethical Committee SUST.

3.7. Sample Size:

Following equation was used to determine the samplesize:

$$n = \frac{zpq\ 2}{d^2}$$

n:samplesize

z: stander deviation when significant level is 95%

p:previousprevalence

q:1-p

d^2:desiredmargin of error

The 70 blood samples were collected to carry the study.

3.8. Data collection tools:

Data was collected by questionnaire sheet (appendix)1.

3.9.Method:

3.9.1. Sample collection

Five ml of blood were drawn from each preterm with clinical sign of sepsis by Aseptic technique. 3 ml of blood were inoculated into two blood culture bottle one incubated aerobic and the other anaerobic, all the procedures were done under Aseptic technique, and the other 2 ml of blood drown into EDTA container and was used for the hematological indices and biochemical marker of inflammation such as C - reactive protein

3.9.2. Processing of Specimens:

The blood cultures were incubated aerobically at 37C ^{O} and observed daily for the first 3 days for the presence of visible microbial growth by one of the following: haemolysis, air bubbles (gas production), and coagulation of broth. At the same time, subcultures were made during 3 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. Isolates obtained were identified by standard microbiological techniques, namely, Gram staining, colony characteristics, and biochemical properties including catalase, coagulase (free and bound), DNase production, growth on mannitol salt agar, and hemolytic activity on blood agar plates for Gram-positive isolates, and triple sugar iron (TSI), motility, indole, citrate utilization, urease, oxidase and hydrogen sulphide production (B. Murray and T. Pfaller,1999). Candida isolate was confirmed by growth on Sabouraud media.

3.9.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of all bacterial isolates was performed by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Oxoid) according to the recommendations of the CLSI (2010).

Table 4.1: Antimicrobial Agents used for Gram Positive bacteria:

NO	Antimicrobial agent	Code	Resistance mm	Intermediate mm	Sensitive mm
1.	**Vancomycin**	(VA)	--	--	15
2.	**Clindamycin**	(DA)	14	15-20	21
3.	**Co-trimoxazole**	(COT)	10	11-15	16
4.	**Tetracycline**	(TE)	14	15-18	19
5.	**Ampicillin**	(AM)	28	--	29
6.	**Penicillin**	(P)	28	--	29
7.	**Erythromycin**	(E)	13	14-22	23
8.	**Amoxicillin+ Calvulanic acid**	(AMC)	19	--	20
9.	**Gentamicin**	(CN)	12	13-14	15
10.	**Ceftazidime**	(CAZ)	14	15-17	18

Table 4.2:Antimicrobial Agents used for Gram Negative bacteria:

NO	Antibiotic	Code	Resistance mm	Intermediate mm	Sensitive mm
1.	**Meropenem**	(MEM)	19	20-22	23
2.	**Amikacin**	(AK)	14	15-16	17
3.	**Imipenem**	(IPM)	19	20-22	23
4.	**Ciprofloxacin**	(CIP)	15	16-20	21
5.	**Ceftriaxone**	(CRO)	19	20-22	23
6.	**Ceftazidime**	(CAZ)	14	15-17	18
7.	**Aztreonam**	(ATM)	17	18-20	21
8.	**Amoxicillin+ Calvulanic acid**	(AMC)	13	14-17	18
9.	**Tetracycline**	(TE)	14	15-18	19

3.9.4. C-reactive protein detection

Three ml of venous blood from preterm neonates were taken for in vitro qualitative determination of CRP. Neonate CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The aggregates were determined turbidimetrically by Cobas C311 machine.

3.9.5. Hematology analyzer

EDTA blood samples were analyzed using Sysmex for hematological indices.

3.10. Data Analysis:

Data collected was analyzed by SPSS program version 16.

Chapter Four

REULST

REULST

Total of 70 (100%) samples were collected to detect preterm sepsis, the isolates detected were as follows: *S.epidermidis*15(21.5%), *E.coli*10(14.4%),*Psuedomonasaeruginosa*6(8.6%) , *Klebiella pneumonia* 5(7%)*and Candida species* 1(1.5%). while no growth was found in 33 blood culture (47%) **Table: (4.1)**

The study population were distributed according to the age interval , high rate of frequency isolates were found in age group of 26-30 week with positive isolate of 23(62%) while those with no growth were found to be 2(6%) among this group, followed by age group of 35-38 week with positive isolate of 9(24.5 %) and no growth in this group was found 18(54.5%), lastly age group of 31-34 week the positive isolate was found 5(13.5%) and no growth was found 13(39.5%). It was significant value *p.value(0.03)***Table: (4.2)**

The study population was distributed according to weight, the high rate of isolate was found in weight of (1.1 - 2.0) kg the isolate detected 26(70.3 %) and no growth was found 15(45.5%), then weight of (2.1 - 3.0) kg 11 (29.7%) and no growth was found 18(52.5%).it was significant *value (0.01)***Table: (4.3)**

The gender was found insignificant in this study *p.value(0.3)*.male had high rate of growth 24(65%) no growth found 13(39.5%) compared with female growth 13(35%) no growth in this group was found 20(60.5%)**Table: (4.4)**

The contamination of the medical device was taken as probable source of infection ,the high rate of growth was found in nasal cannula8(21.5%) and CPAP 6(16.5%) no growth in this group was found 4(12%) followed by Nasal cannula and the growth found 13(35%) no growth in this group was found 6(18.2%) then CPAP device the growth found 6(16.5%) no growth in

this group was found 4(12%) lastly UVC device the growth found 3(8%) no growth in this group was found 1(3%). On the other hand in patients with no device the growth was found 4(21.1%) no growth in this group was found 9(42.9%) this was appear to be in significant*p.value(0.4)***Table: (4.5)**

The frequency of growth for the following clinical remarks was found as follow Distressed(RDS) 53 (43%), Coffee ground secretion 6(16%), Tachycardia 18 (48.7%), Cannot tolerate feeding 23(62%), Apnea 23(62%) and Convolution 2(5.4%) look ills 13(35%)**Table: (6), Table: (4.7)**

The white blood cells and platelet were estimated to the study population, the mean of lymphocyte was found in positive growth 17.9 and in the no growth 13.5 which is significant *p.value(0.02)* , Neutrophil mean found 59.6 in growth and 37.4 in the no growth this is insignificant *p.value(0.4)* and plat let mean was detected 188.7 and in the no growth the platelet mean was found 270 *p.value(0.01)***Table: (4.8)**

c- reactive protein was identify to study population and was found positive in 37(100%) in the group of isolated microorganism, while the group of no growth was detected positive in 4(12.0%) and negative in 29(88.0%) significan*p.value(0.00)***Table: (4.9)**

the antimicrobial susceptibility was done to all isolate of *S.epidermidis* and found as follow Vancomycin (100%), DA(60%) , Cotrimethaxazol (33%), TE(40%) ,penicillin (13%),erythromycin (27%), and resistance (0%) for AMC, CN, CAZ, AM**Table: (4.10)**

Antibiotic susceptibility was done against *E.coli, pseudomonas* and *klebiellaspp.E.coli*was found sensitive to all antibiotic used (MEM, IPM, CEFO, CAZ, AMC and TE)(100%) except AK ,CIP and ATM(0.0%) .*Psuedomonas*was found sensitive (100.0%) to (MEM, CAZ and AMC) and resistance (0.0%) to (AK, IPM, CIP, CRO, ATM and TE) and (50.0%) of

the *Psuedomonas* appear sensitive to (CIP). Regarding *klebsiella*spp it was found sensitive (100.0%) to (MEM, AK, IPM, CAZ and AMC**)** resistance (0.0%) to (**CRO** and TE) and (50.0%) of them appears sensitive to (CIP) **Table (4.11)**

Table (4.3) Frequency of isolated microorganism

No	**Isolation**	**Frequency (%)**
1.	***S.epidermidis***	15(21.5%)
2.	***E.coli***	10(14.4%)
3.	***Psuedomonas aeruginosa***	6(8.6%)
4.	***Klebiella pneumoniae***	5(7%)
5.	***Candida spp***	1(1.5%)
6.	***No growth***	33 (47%)
	Total	70(100%)

Table (4.4) distribution of isolated bacteria according to age

age per week		**Isolation growth**		***p.value***
		growth	**no growth**	
26-30 week	**Count**	23	2	0.03
	%	62 %	6 %	
31-34 week	**Count**	5	13	
	%	13.5%	39.5%	
35-38 week	**Count**	9	18	
	%	24.5%	54.5%	

Table (4.5) distribution of isolated bacteria according to Weight

Weight		Isolation growth		*p.value*
		Growth	No growth	
(1.1 - 2.0) kg	Count	26	15	0.01
	%	70.3%	45.5%	
(2.1 - 3.0) kg	Count	11	18	
	%	29.7%	54.5%	

Table (4.6) distribution of isolated bacteria according to Gender

Gender		Isolation growth		*p.value*
		Growth	No growth	
Male	Count	24	13	0.3
	%	65%	39.5%	
Female	Count	13	20	
	%	35%	60.5%	

Table (4.7) Distribution of isolate among medical device

Medical devices		Isolation growth		p.value
		Growth	No growth	
Nasal cannula	Count	8	8	0.4
	%	21.5%	24.3%	
UVC	Count	3	1	
	%	8 %	3 %	
CPAP	Count	6	4	
	%	16.5%	12%	
nasal cannula and CPAP	Count	13	6	
	%	35%	18.2%	
NON	Count	7	14	
	%	19%	42.5%	

Table (4.8) Frequency of clinical remarks among study population

No	Clinical remarks	Frequency (%)
1.	Distressed (RDS)	53 (43%)
2.	look ills	18(45%)
3.	Cannot tolerate feeding	3(8%)
4.	Apnea	3(8%)
5.	Coffee ground secretion	7(18%)
6.	Convolution	1(3%)
7.	Tachycardia	4(10%)

Table (4.9) Distribution of isolation according to clinical remarks

Clinical remarks		Isolation growth		*p.value*
		Growth	No growth	
Distressed RDS	**Count**	26	11	0.3
	%	70.0%	30.0%	
look ills	**Count**	13	24	0.00
	%	35%	65%	
Cannot tolerate feeding	**Count**	23	14	0.4
	%	62%	38%	
Apnea	**Count**	23	14	0.4
	%	62%	38%	
Coffee ground secretion	**Count**	6	31	0.5
	%	16%	84%	
Convolution	**Count**	2	35	0.2
	%	5.4%	94.6%	
Tachycardia	**Count**	18	19	0.9
	%	48.7%	51.3%	

Table: (4.10) distribution of isolation according to white blood cells

Growth		Mean	*p.value*
TWBC	**Growth**	17.9	0.02
	No growth	13.5	
L	**Growth**	33.3	0.4
	No growth	37.4	
N	**Growth**	59.6	0.3
	No growth	54.7	
PLT	**Growth**	188.7	0.01
	No growth	270.0	

Table: (4.11) identification of CRP among study population

Growth		CRP		*p.value*
		positive	**negative**	
Growth	**Count**	37	0	0.00
	%	100.0%	0.0%	
No growth	**Count**	4	29	
	%	12.0%	88.0%	

Table: (4.12) Antibiotic susceptibility against *S.epidermidis*

Antibiotic	sensitive to *S.epidermidis*
VA	100%
DA	60%
COT	33%
TE	40%
AM	0%
P	13%
E	27%
AMC	0%
CN	0%
CAZ	0%

VA=vancomycin , DA= Clindamycin , COT=Co-trimoxazole , TE= tetracycline, AM=Ampicillin , P= penicillin , E=erythromycin, AMC=Amoxicillin+Calvulanic acid , CN= Gentamicin , CAZ=Ceftazidim

Table: (4.13) Antibiotic susceptibility against *E.coli,* *Pseudomonasaeruginosa*and *Klebsiella pneumoniae*

Sensitive of antibiotic	Isolation		
	E.coli	***P.aeruginosa***	***K.pneumoniae***
MEM	100.0%	100.0%	100.0%
AK	0.0%	0.0%	100.0%
IPM	100.0%	0.0%	100.0%
CIP	0.0%	50.0%	50.0%
CRO	100.0%	0.0%	0.0%
CAZ	100.0%	100.0%	100.0%
ATM	0.0%	0.0%	50.0%
AMC	100.0%	100.0%	100.0%
TE	100.0%	0.0%	0.0%

MEM=Meropenem, IPM=Imipenem, CRO=ceftrixone,CAZ =Ceftazidim, AMC =Amoxicillin+Calvulanic acid ,TE=tetracycline,AK=amkacin ,CIP = ciprofloxacin, ATM=Aztreonam

Chapter Five

Discussion

Discussion

5.1. Discussion

The clinical signs and symptoms of neonatal sepsis are subtle and non-specific, making its early diagnosis difficult, and it can interfere with other life-threatening diseases, such as necrotizing enterocolitis and perinatal asphyxia . Blood culture is still the gold standard for definitive diagnosis of neonatal sepsis, in spite of some drawbacks of blood cultures as being time consuming, low sensitivity, and possible contamination especially with commensal that could be produced.

In our study, the prevalence rate of suspected neonatal sepsis during the study period was 21 (52.5%) which is Similar in high rates were previously reported in Egypt (Moore *et al,* 2005) and other developing countries such as Tanzania 39% (N. Kayange*et al* ,2010) and Cameroon 34.7% (Chiabi *et al,* 2011). In contrast, very low rates were reported in the developed countries (*Li et al* ,2013), which can be explained by the high quality of life and high standard measures of health care and hospital services in these countries.

In our study, the incidence of neonatal sepsis was predominantly associated with Gram positive cocci, specifically *S. epidermidis* compared to Gram-negative and *Candida* spp. Similar findings were obtained in other studies in Egypt (Shokry *et al* ,2007) and other different countries (including China,Mexico, South Africa, and Kenya) (Li *et al* ,2013) (Ballot,2012) (Leal ,2012).High rates of infections were reported in the Middle East, Southeast Asia, and Latin America .On the contrary, Gram-negative neonatal sepsis was predominant in other studies (Chiabi *et al* ,2011) (Afsharpaiman ,2012) (Shah,2012).

The extensive use of invasive devices for caring for the immunologically immature neonates especially preterm is the main cause of bacteraemia. This finding is supported by the study of (Afsharpaiman, 2012), as, in more

than 50% of the cases of bacteremia in NICU, the infection could be correlated with the use of venous catheters. Despite the importance and role of CONS as etiological agents of neonatal sepsis as proved in many studies, determination of the identity of coagulase negative isolates whether being true pathogens or contaminants is still problematic.

In ourstudy, *S. epidermidis*15 (37.5%) was the most frequently recovered isolate in blood cultures, followed by*E.coli 10 (14.4) then Psuedomonas aeruginosa* 6 (8.6%) *and Klebiella pneumoniae*5(7%), *Candida spp*1(1.5%)., respectively. Similar findings were reported in other previous studies (Li*et al*, 2013) (F. Koksal*et al*, 2009) (Piette and Verschraegen, 2009)

In our study, all isolates showed high resistance to ampicillin and gentamicin Interestingly, all staphylococcal isolates were sensitive to vancomycin as previously found in other reports (A. Chiabi*et al* ,2011), (Kari *et al* ,2007), but it's over prescription may result in the development of vancomycin-resistant strains such as enterococci.

5. 2. Conclusion:

- Both Gram positive and Gram negative bacteria can cause sepsis of preterm.
- *S.epidermidis* was the most frequent bacteria isolated followed by *E.coli, Pseudomonas aeruginosa* then *Klebsiella pneumoniae.*
- VLBW and gestational age of the preterm was found to be a high risk factor for preterm sepsis.
- Preterm gender had no effect on the preterm septic status.
- CRP test came out as a good indicator for the preterm septic status.
- RDS was the most clinical remark that showed septicaemia.
- Medical devices were found to be as source of infection.

5.3. Recommendations:

1. Follow hand hygiene protocol (your 5min for hand hygiene) when handling with the Preterm.
2. Good and frequent care of the preterm by the nurses can prevent preterm sepsis.
3. Appropriate identification of the sepsis source.
4. Prompt antibiotic prescription and aggressive management can effectively prevent adverse events following neonatal sepsis.
5. Determination of the neonatal sepsis incidence, causative pathogens, and the patterns and rates of antibiotic resistance among all the neonate and infant populations are necessary to prevent complications.
6. More studies using molecular to identify the resistance gene
7. Minimise the use of clinical device and asses daily the need of them.

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Appendix 1

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Detection of Bacterial Causative Agent in Preterm Neonatal sepsis Infants in Khartoum state

Questionnaire

Patient name: ……..

Patient gender:…….

Patient age:……..

Patient weight:…….

Currently antibiotics used……...

Medical devices attached to the patient: ……

Month of delivery or section operation:……

Name of hospital admitted to:……...

Clinical remarks of sepsis:……

Blood culture result: …….

Antibiotic sensitivity test ……..

CRP result: ……

Hematological indices result: …….....