Sudan University of Science and Technology

College of Graduate Studies

Frequency of Bacterial Isolates from Neonatal Septicemia in Omdurman Maternity Hospital and Asia Specialist Hospital, Omdurman Locality

تكرار البكتيريا المعزولة من تسمم الدم لحديثي الولادة بمستشفى أم درمان للولادة ومستشفى آسيا التخصصي, محلية أم درمان

A dissertation submitted in partial fulfillment for the requirements of M.Sc. degree in Medical Laboratory Science (Microbiology)

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الآية

:قال تعالى

(اللَّهُ لَا إله إِلَّا هُوَ الْحَيُّ الْقَيُّومُ ۚ لَا تَأْخُذُهُ سِنَةٌ وَلَا نَوْمٌ ۚ لَهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الْأَرْضِ ۗ مَنْ ذَا الَّذِي يَشْفَعُ عِنْدَهُ إِلَّا بِإِذْنِهِ ۚ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ ۖ وَلَا يُحِيطُونَ بِشَيْءٍ مِنْ عِلْمِهِ إِلَّا بِمَا شَاءَ ۚ وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالْأَرْضَ ۖ وَلَا يَئُودُهُ حِفْظُهُمَا ۚ وَهُوَ الْعَلِيُّ الْعَظِيمُ).

صدق الله العظيم

سورة البقرة , الآية (255)

DEDICATION

To my beloved parents, brother, sister,

With love and respect

ACKNOWLEDGEMENTS

First of all, thanks to **ALMIGHTY ALLAH** for blessing me with good health, wellbeing, strength and patience to carry out and complete this work. I would like to express my deep thanks and appreciation to my supervisor **Dr. Wafaa Mohammed Abdalla** for her supervision, valuable advices, encouragements and continuous support throughout this study, also pleasant personality made it easy for us to do this work together. Thanks must also go to **Dr. Sami Hattra**, Asia Specifically Hospital for his help. Thanks to my colleagues for their help and support to complete this study.

ABSTRACT

This is descriptive, cross-sectional, hospital-based study aimed to determine the frequency of isolated bacteria from septicemic neonates in Omdurman Maternity Hospital and Asia Hospital during the period from August 2019 to September 2020.

A total of 150 neonates were included in this study with age ranged from 1 to 28 days with mean of age 5.51±5.52 S.D. About 2-3ml venous blood were collected aseptically and cultured in Brain Heart Infusion broth and aerobic render bottle for automated blood culture followed by subculture of isolates on blood agar, MacConkey agar, and Chocolate agar and incubated aerobically at 37ºC for 5 days up to 14 days. Identification was done by Gram's stain and biochemical tests. According to the isolates were tested for their susceptibility to antimicrobial agents using the Kirby Bauer disc diffusion method.

Out of 150 specimens, there were 80 (53.3%) yielded growth (positive blood culture) and 70 (46.7%) were showed no growth. According to onset of disease; there were 31/80 (38.8%) with early onset and 49 (61.2%) with late onset. Out of 80 growth; there were 20/80 (25%) G+ve cocci, while Gram-negative rods isolates were 60/80 (75%).

According to onset of the disease it was observed that; *Klebsiella pneumoniae* was the most common isolated bacteria (32/80 (40%)) in both onsets of neonatal sepsis, followed by *Pseudomonas aeruginosa* (21/80 (26.3%)) and *Staphylococcus aureus* (16/80 (20%)).

The antimicrobial susceptibility testing results showed that; Cefotaxime had the highest sensitivity (51(85%)) while imipenem yielded the highest resistant (41(68.3%)) among Gram-negative rods. All the isolated G+ve cocci were sensitive to vancomycin (100%) and the highest resistant to Penicillin (90%).

This study was concluded that; positive blood culture was significant from neonatal sepsis and mostly was with late onset of disease. Also Gram-negative rods pathogens took the major spectrum of isolates and *Klebsiella Pneumoniae* was the most predominant isolate and *Staphylococcus aureus* was the most frequent Gram-positive cocci. Most of the isolates were multidrug resistant and the best choice for treatment is Vancomycin and Cefotaxime for Gram-positive cocci and Gram-negative rods respectively.

Adherence to antibiotic policy, antimicrobial surveillance and policy updating are necessary.

المستخلص

هدفت هذه الدراسة الوصفية ,المقطعية ,مقرها المستشفى إلى تحديد تواتر البكتيريا المعزولة من تسمم الدم عند حديثي الولادة في مستشفى أم درمان للولادة ومستشفى آسيا خلال الفترة من أغسطس 2019 إلى سبتمبر 2020.

ضمنت هذه الدراسة مجموعة 150 حديثي الولادة وتتراوح أعمارهم بين يوم إلى 28 يوما بمتوسط العمر 5.51 ±5.52 انحراف معياري. تم جمع حوالي 2-3 مل من الدم الوريدي بطريقة معقمة و زرعت في زجاجات محتوية علي مرق إشراب الدماغ والقلب وزجاجة ريندر الهوائية لتذريع الدم الآلي متبوعة بإعادة تذريع المعزولات في أجار الدم وأجار الماكونكي وأجار الشكولاتة وحضنت بطريقة هوائية عند 37 درجة مئوية لمدة 5 أيام حتى 14 أيام. وقد تم التعرف بصبغة غرام والاختبارات الكيموحيوية. واستخدمت طريقة كيربي- باور باستخدام طريقة نشر القرص لمعرفة مدى تأثرها بالعوامل المضادة للميكروبات.

من بين 150 عينة، كان هناك 80 (53.3 ٪) نمو و 70 (46.7 ٪) لم يظهر أي نمو. حسب بداية المرض؛ كان هناك 80/31 (38.8٪) بداية مبكرة و 49 (61.2٪) بداية متأخرة.

من بين 80 نمو؛ كان هناك 80/20 (25٪) المكورات موجبة الجرام بينما العصي المعزولة سالبة الجرام 80/60 (75٪).

حسب بداية المرض لوحظ أن: الكلبسية الرئوية هي أكثر أنواع البكتيريا المعزولة شيوعًا(80/32(40٪)) في كلتا مجموعتي الإنتان الوليدي تليها الزائفة الزنجارية (80/21 (26.3٪) والمكورات العنقودية الذهبية (80/16 (20٪)).

أظهرت نتائج إختبارالحساسية لمضادات الميكروبات أن: السيفوتاكسيم له أعلى حساسية (51 (85٪)) بينماأعطى الإيميبينيم أعلى مقاومة (41 (68.3٪)) بين العصوية سالبة الجرام. كانت جميع المكورات موجبة الجرام المعزولة حساسة للفانكومايسين (100٪) وأعلى مقاومة للبنسلين (90٪)

خلصت هذه الدراسة إلى أن: كانت زراعة الدم الإيجابية معنوية لتسمم الدم الوليدي وكانت في الغالب مع بداية المرض المتأخرة. أيضا أخذت مسببات الأمراض سالبة الجرام الطيف الرئيسي من العزلات وكانت: الكلبسية الرئوية هي العزلة الأكثر انتشارًا وكانت المكورات العنقودية الذهبية هي أكثر موجبة الجرام شيوعًا. كانت معظم العزلات مقاومة للأدوية المتعددة وأفضل خيار للعلاج هو فانكومايسين وسيفوتاكسيم للمكورات موجبة الجرام والعصويات سالبة الجرام على التوالي.

من الضروري الإلتزام بسياسةالمضادات الحيوية وترصد مضادات الميكروبات وتحديث السياسات.

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LIST OF ABBREVIATIONS

Full name	abbreviation
American type culture collection	ATCC
Community acquire sepsis	CAS
Complete blood count	CBC
Clinical laboratory standards institute	CLSI
Cytomegalovirus	CMV
Coagulase negative staphylococcus	CONS
C- reactive protein	CRP
Cerebrospinal fluid	CSF
Ethylene diaminetera acetic acid	EDTA
Early onset	EOS
Group B *streptococcus*	GBS
Hospital acquire sepsis	HAS
Human immunodeficiency virus	HIV
Human parechovirus	HPeV
Intra partum antibiotic prophylaxis	IAP
Intensive Care Unite	ICU
Intravenous	IV
Late Onset	LAT
Muller Hinton agar	MHA
Methicillin Resistant *Staphylococcus aureus*	MRSA
Polymerase chain reaction	PCR
Pediatric intensive care unite	PICU
Respiratory syncytial virus	RSV
Statistical package for social science	SPSS
Varicella Zoster Virus	VZA
World Health Organization	WHO

CHAPTER I

INTRODUCTION AND OBJECTIVE

CHAPTER I

1. INTRODUCTION

1.1.Introduction

Neonatal sepsis refers to an infection involving bloodstream in newborn infants less than 28 days old. It continues to remain aleading cause of morbidity and mortality among infants, especially in middle and lower-income countries (Wynn, 2016).It may be categorized as early or late onset. Eighty-five percent of newborns with early-onset infection present within 24 hours and onset is most rapid in premature neonates (Mohamed *et al*., 2015).

Despite the decrease in neonatal deaths, research shows that neonatal sepsis remains a remarkable hindrance to the progress in the decline of cause specific mortality rates in the world, particularly Africa (Liu*et al*., 2015).Every year sepsis affects 30 million people worldwide, 3 million newborns, 1.2 million children and can kill 6 million people (WHO,2018)

Neonatal sepsis is a major cause of mortality during the first month of life, contributing to 13% - 15% of all neonate death. Low birth weight and Gram-negative infection are associated with worse outcomes (Gollehon and Anderson *et al*., 2019).

In Sudan, the prevalence of neonatal sepsis is 17.5% and the mortality is 14.5% (Babiker *et al*., 2018).

Bacterial sepsis acquired in the hospital, especially when a patient is already in the ICU, tends to follow a much more severe course than sepsis for which a patient is admitted to the hospital. Hospital acquire sepsis (HAS) is thus approximately five times more expensive than community acquire sepsis (CAS), and the mortality rate of HAS is approximately twice that ofCAS, with calculated in hospital mortality rate of 19.2% as opposed to 8.6% (Page *et al*., 2015; López-Mestanza *et al*., 2018; Meyer *et al*., 2018).

The organisms responsible for HAS also differ from CAS; they are often opportunistic and resistant to some or all of the first line antibiotics used to treat them. Whereas pathogenic Gram-negative bacteria are at least partially responsible for the majority of bacterial sepsis cases, a heterogeneous mixture predominates in HAS. A large number of cases, for example, can be attributed to drug resistant *Pseudomonas* species **(**Palavutitotai *et al*., 2018).

The majority of Gram positive HAS, on the other hand, can be attributed to *Staphylococcus* species, especially methicillin resistant *Staphylococcus aureus* (MRSA), a bacterium that is a growing concern among the lay and medical populations alike (Thaden *et al.*, 2017).

The diagnosis of infection in neonates is difficult, because of the non-specific clinical presentation and the lack of reliable diagnostic tests. As a result of this uncertainty, antimicrobial chemotherapy is often commenced on the slightest clinical suspicion of infection (Lam and Ng, 2008).

1.2. Rationale

Due to the non-specific neonatal presentation for sepsis and the high risk of mortality and morbidity without treatment, many asymptomatic neonates undergo a sepsis workup, if concerning factors are present. Although approximately 7% to 13% of all neonates are worked up for sepsis, only 3% to 8% develop positive cultures neonatal sepsis (Singh and Gray, 2019).

This study was conducted to provide epidemiological data about the frequency of neonatal sepsis in Khartoum State, whichwill assess sepsis threatens the health of neonates andto evaluate the burden of infection that may needmedical intervention.

Besides tochoose the suitable antimicrobial agents correctly which will eliminate the causative agent successfully.

1.3. Objectives

1.3.1 General objective

To detect the frequency of isolated bacteria from neonatal septicemia in selected hospitals in Omdurman Locality.

1.3.2. Specific objectives

1.To isolate and identify bacteria from blood specimens of neonates with sepsis.

2. To determine the frequency of isolates according to onset of disease.

3. To assess the antimicrobial activity of different antimicrobial agents against isolated bacteria.

CHAPTER II

LITERATURE REVIEW

CHAPTER II

2. LITERATURE REVIEW

2.1. Septicemia

2.1.1. Definition

Is a bacterial infection that spreads into the bloodstream and the term sepsis is the body's response to that infection (Torrey, 2020).

Sepsis is a complex condition characterized by the simultaneous activation of inflammation and coagulation in response to microbial insult. These events manifest as systemic inflammatory response syndrome or sepsis symptoms through the release of proinflammatory cytokines, procoagulants, and adhesion molecules from immune cells and/or damaged endothelium (Polat*et al*., 2017).

2.1.2. Causative agents

2.1.2.1. Bacterial Causative agents

Pathogenic sepsis is not a monolithic condition and there are three major types of sepsis exist within this category: bacterial, viral, and fungal; each with its own mechanism of action. While similar in symptoms, the etiologies and immune mechanisms of these types differ enough that a discrete patient base can be recognized for each one(Dolin, 2019).

Bacteria are the most common cause of sepsis, with 62.2% of patients with positive blood cultures harboring Gram-negative bacteria and 46.8% infected with Gram-positive bacteria (Mayr *et al*., 2014).

Escherichia coli can be found in approximately 1 in 6 culture positive patients and Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) have made up an increasing percentage of sepsis with the advent of excessive antibiotic treatment (Saner, 2018).Other common Gram negative species include *Klebsiella* and *Enterobacter* (Thaden *et al.*, 2017).

2.1.2.2. fungal Causative agents

Fungal sepsis shares common mechanisms with bacterial sepsis, but in contrast, it is a fast growing and often lethal subtype (Spec *et al*., 2016; Bassetti *et al*., 2017). Approximately 17% of sepsis can be attributed to *Candida* species, with 2% to 3% more caused by *Aspergillus* and others and in an invasive situation, however, fungal sepsis can kill at a rate of 40% to 60% (Upperman *et al*., 2003; Delaloye and Calandra, 2014).

This is far higher than the approximately 30% average case fatality rate of bacterial or viral sepsis, and approaches or exceeds the upward of 45% case fatality rate of antibiotic resistant HAS (Mayr *et al*., 2014; Page *et al*., 2015).

2.1.2. 3. Viral Causative agents

A recent study of adult patients with sepsis showed that viral respiratory pathogens, namely influenza A virus, human metapneumovirus, coronavirus, and respiratory syncytial virus (RSV), were overlooked in 70% of patients (Ljungstrom*et al*., 2017).In the pediatric intensive care unit (PICU), influenza virus is a leading cause of viral sepsis and caries an especially high mortality rate. RSV has also been found to cause severe bronchiolitis and may present with sepsis, especially in children with history of premature birth, chronic lung disease, congenital heart disease or primary immunodeficiency (Caballero and Polack, 2018).

Sepsis has also been observed in neonates with HSV, human parechovirus (HPeV) and enteroviral infection (Gupta *et al*., 2018).

Patients with immunodeficiency due to human immunodeficiency virus (HIV) infection are highly susceptible to viral sepsis depending on the stage of disease and access and response to the treatment. In these patients, common viral infections observed to cause sepsis include RSV, influenza, parainfluenza, adenovirus, CMV, EBV, and VZV (Hatherill*et al*.,2005).

2.2. Neonatal sepsis

2.2.1. Definition

The term neonatal sepsis is used to describe a generalized bloodstream infection of bacterial, viral, or fungal origin which is associated with hemodynamic changes and other clinical symptoms and signs, however, there is no unified definition(Mach *et al*.,2019).

2.2.2.Types on neonatal septicemia

It is divided into 2 groups based on the time of presentation after birth; early onset sepsis (EOS) and late onset sepsis (LOS). EOS refers to sepsis in neonates at or before 72 hours of life and LOS is defined as sepsis occurring at or after 72 hours of life. Although, some experts use 7 days as the cutoff date (Simonsen*et al*., 2014).

2.2.3. Risk factors

The predictive factors for neonatal sepsis are associated to gestational age, premature rupture of amniotic membranes and maternal infection. Birth conditions, low weight and prematurity are strong evidence of sepsis. The factors related to the environment

of the neonatal intensive care unit are potential contributing factors associated to late onset sepsis (Cortese *et al*., 2016).Premature rupture of fetal membranes is a major risk factor for prematurity and early onset sepsis and a leading cause of neonatal mortality and morbidity worldwide (Leal *et al*., 2012; Alam *et al*., 2014).

A study conducted in the State of Amazonas reported that colonization by group *B Streptococcus* (GBS) in pregnant women increases the risk for neonatal sepsis in premature newborns by 15.2% (Pinheiro *et al*., 2007).

Other findings from retrospective studies report association between maternal infection by GBS in the urinary tract (62.1%) with prevalence of neonatal sepsis in 1/1,000 infants born alive admitted to neonatal intensive care unit, and that otherinfections originated during pregnancy are risk factors for neonatal sepsis (Goulart *et al*., 2006).

Other studies reveal increased incidence of this disease among infants born to teenage mothers who gave birth prematurely and who had fewer than six routine antenatal care appointments, which is recommended by the Ministry of Health (six appointments). This may contribute to the non-identification of key factors involved in the referred infectious process (Pinheiro *et al*., 2007).

Late onset sepsis is related to the stay of newborns in neonate intensive care units (NICU) where they are exposed to the use of peripherally inserted central catheter (PICC), mechanical ventilation and parenteral nutrition (Meireles *et al*., 2011; Marchant *et al*., 2013; Romanelli *et al*., 2014).

Other maternal factors that increase the risk of neonatal sepsis include chorioamnionitis, delivery before 37 weeks and prolonged rupture of membranes greater than 18 hours (Raymond *et al*., 2017).

Finally, incubators, intravenous nutrition and the use of PICC were also identified as risk factors for neonatal sepsis, and PICC was considered the most serious risk factor (Lin *et al*., 2012; Li *et al*., 2013)

2.2.4. Epidemiology

The epidemiology of neonatal sepsis has been changing with time (Bizzarro *et al*., 2005).

One in ten deaths associated with pregnancy and childbirth is due to maternal sepsis (Say *et al*., 2014) and the burden of sepsis is most likely highest in low- and middle-income countries (Fleischmann *et al*., 2016).

It is estimated that 3 million newborns and 1.2 million children suffer from sepsis globally every year (Fleischmann *et al*., 2018).

Three out of every ten deaths due to neonatal sepsis are thought to be caused by resistant pathogens (Laxminarayan*et al*., 2016).

The incidence of EOS has decreased since the 1990s due to the introduction of universal screening of group B streptococcus (GBS) in pregnant women and intrapartum antibiotic prophylaxis (IAP) (Van Dyke *et al*., 2009).

However, rates of LOS have remained relatively the same. *Escherichia coli* now accounts for more cases of EOS compared to GBS (Shane and Stoll, 2013). *Escherichia coli* and GBS were the most common causes of EOS in preterm and term babies respectively. Rates of all cause term and preterm EOS declined significantly as did preterm sepsis due to *E. coli*. While rate of sepsis due to early-onset GBS declined, this did not reach significance. Given the high proportion of preterm babies undergoing blood culture, it is unlikely that any EOS events were missed (Braye*et al*., 2019).

During the yearsof 1997, 2005, 2007, and 2010, CoNS remained the most frequent pathogen.The relative proportion of Gram-negative bacilli is substantial in preterm babies (Aldemir *et al*., 2019).

2.2.5. Transmission and causative agents

EOSis generally caused by the transmission of pathogens from the female genitourinary system to the newborn or the fetus, these pathogens can ascend the vagina, the cervix, and the uterus, and can also infect the amniotic fluid. Neonates can become infected in utero or during delivery as they pass through the vaginal canal. Typical bacterial pathogens for EOS include Group; GBS *Escherichia coli*, coagulase-negative Staphylococcus, *Haemophilus influenza*, or *Listeria monocytogenes* (Simonsen *et al*., 2014).

LOS usually occurs via the transmission of pathogens from the environment after delivery, such as contact from healthcare workers or caregivers. It may also be caused by a late manifestation of vertically transmitted infection. Infants that require intravascular catheter insertion, or other invasive procedure that disrupts the mucosa, are at increased risk for developing LOS. Preterm neonates are at higher risk for sepsis/infection than term neonates, as they tend to require more invasive procedures than term neonates. Coagulase-negative staphylococcal species, especially *Staphylococcus epidermidis* is the leading cause, responsible for greater than 50% of

LOS cases in industrialized countries, although many bacterial and viral pathogens can be associated with LOS (Meenakshi *et al*., 2019).

2.2.6. Clinical manifestations

Neonatal sepsis is a clinical syndrome with hemodynamic changes and other systemic clinical manifestations resulting from the presence of pathogenic microorganisms (bacteria, viruses, or fungi) in normally sterile fluid, such as blood or cerebrospinal fluid (CSF) in the first month of life **(**Shane *et al*., 2017).It is an important cause of neurocognitive sequelae andneonatal mortality (Hentges*et al*., 2014; Liang *et al*., 2018).

The clinical signs can be grouped as follows: apnea, difficulty breathing, cyanosis; tachycardia or bradycardia, poor perfusion or shock; irritability, lethargy, hypotonia, seizures; abdominal distension, vomiting, food intolerance, gastric residue, hepatomegaly; unexplained jaundice; body temperature instability; petechiae or purpura. To take into account the clinical signs, ideally the newborn should show manifestations in three distinct systems, or two clinical signs indistinct systems associated with a maternal risk factor **(**Procianoy and Silveira, 2020).

2.2.7. Diagnosis

The clinical manifestations vary considerably and are non-specific, which makes the diagnosis of early neonatal sepsis difficult and predisposes to excessive antibiotic use.If early neonatal sepsis is suspected, blood culture and CSF samples should be collected. Urinalysis is not indicated, since urinary infection in early neonatal sepsis is unusual (Procianoy and Silveira, 2020).

Complete blood count (CBC) and serum C-reactive protein have a better negative predictive value than a positive predictive value. Correspondingly the most common CBC findings are immature to total neutrophil ratio (I/T ratio) >0.2, leukopenia (< 5000), or leukocytosis (>25,000). Additionally, serial low C-reactive protein levels (serum levels below 10 mg/L) help to rule out the diagnosis of neonatal sepsis in a newborn with negative blood culture **(**Procianoy and Silveira, 2020).

It is recommended to draw at least 1ml of blood as low level bacteremia may not be detected with smaller aliquots and is common in neonatal sepsis (Polin, 2012).

Lumbar puncture with cerebrospinal fluid (CSF) analysis and culture should be evaluated in any infant with positive blood culture or when clinical or metabolic abnormalities strongly suggest bacterial sepsis, as meningeal signs may not be present on the physical exam. It should be repeated if the patient fails to improve on antibiotic

treatment, if symptoms worsen or if bacteria growth from blood culture (simonsen *et al*., 2014).

Persistently normal CRP levels provide strong evidence against bacterial sepsis and antimicrobial agents can be safely discontinued. Other inflammatory markers, including procalcitonin, haptoglobin, and cytokines can also be obtained to support the diagnosis or to monitor during treatment. Radiography of the chest may be performed to look for any pulmonary findings. CT or MRI of the head may be warranted if concerns for hydrocephalus, infarction, or abscess exist (Wynn, 2016).

So, blood culture remains the best approach to identify the etiological microorganisms when a bloodstream infection is suspected but it takes long time because it relies on bacterial or fungal growth. The introduction in clinical microbiology laboratories of the matrix assisted laser desorption ionization time of flight mass spectrometry technology, DNA hybridization, microarrays or rapid PCR based test significantly reduce the time to results. Tests for direct detection in whole blood samples are highly desirable because of their potential to identify bloodstream pathogens without waiting for blood cultures to become positive (Marco, 2017).

2.2.8.Treatment andmanagement

Empiric treatment with antibiotics should be started as soon as sepsis is clinically suspected, even without confirmatory lab. data. In general, antimicrobial resistance patterns of common bacteria in the neonatal ICU should guide the initial choice of antibiotics. Typical treatment regimens include intravenous (IV) broad-spectrum penicillin and aminoglycosides to cover for the most common pathogens in neonates: GBS, *E. coli*, and *L. monocytogenes*. The combination of Ampicillin and Gentamicin is the most commonly used antibiotic regimen (Polin, 2012).

With LOS, nosocomial coverage should be provided for the hospital acquired pathogens such as coagulase negative *Staphylococcus*, *S. aureus* and *Pseudomonas* species. It is recommended to start these patients on a combination of Vancomycin and an Aminoglycoside (Cortese *et al*., 2016).

A third generation cephalosporin should be given if Gram-negative meningitis is suspected. It provides adequate penetration via blood-brain barrier and coverage for these pathogens. However, ceftriaxone should be avoided, as it can lead to hyperbilirubinemia(Sullins and Abdel-Rahman, 2013).

Furthermore, increasing antibiotic resistance is a concern for neonatal sepsis and treatment should always be de-escalated as soon as possible (Shane *et al*., 2017b).

2.2.8.1.Treatment Planning

The treatment regimen for neonatal sepsis varies based on various risk factors and conditions. The typical antibiotics used are discussed above, but the duration of therapy can vary based on the underlying etiology, isolated organisms, the presence of any neonatal complications, or other risk factors. Neonates with positive blood cultures typically respond to treatment within 24 to 48 hours and repeat cultures and studies are usually negative by 72 hours (Meenakshi *et al*., 2019).

Despite standard recommendations to discontinue antibiotics once cultures are negative, many clinicians will continue therapy for 10 to 14 days based on the organism, or 21 days if meningitis was suspected (Cortese *et al*., 2016).

Increasing the duration of antibiotics may be necessary for some situations. However, contributes to the increasing incidence of antibiotic resistance and puts the neonate at increased risk of complications including necrotizing enterocolitis or death (Dong and Speer, 2015).

The treatment for suspect EOS with negative cultures is also variable. Cultures can be negative for a variety of reasons, including maternal antibiotic use, initiation of antibiotics prior to obtaining cultures or false negative tests. Determining adequate antibiotic therapy without any positive cultures can make the determining duration of therapy difficult, and an empiric 10-day treatment course is completed, as long as the neonate's symptoms have improved (Simonsen *et al*., 2014).

2.2.9. Prevention and Control

There are two main steps to preventing sepsis; prevention of microbial transmission and infection prevention of the evolution of an infection to sepsis conditions.

Prevention of infection in the community involves using effective hygiene practices, such as hand washing, and safe preparation of food, improving sanitation and water quality and availability, providing access to vaccines, particularly for those at high risk, as well as appropriate nutrition, including breastfeeding for newborns (WHO, 2018).

Prevention of infection in health care facilities mainly relies on having functioning infection prevention and control (IPC) programs and teams, effective hygiene practices and precautions, including hand hygiene, along with a clean, well-functioning environment and equipment (WHO, 2018).

Prevention of the evolution to sepsis in both community and health care facilitiesrequires the appropriate antibiotic treatment of infection, including

reassessment for optimization, prompt seeking of medical care, and early detection of sepsis signs and symptoms. Scientific evidence has clearly demonstrated the effectiveness of infection prevention. For instance, improved hand hygiene practice in health care can reduce infection by as much as 50% (Luangasanatip *et al*., 2015), while, in community settings, it can cut the risk of diarrhea by at least 40% (UNICEF, 2018).

Water, sanitation and hygiene (WASH) improvements could result in a 10% reduction of the total burden of disease worldwide (Prüss-Ustün *et al*., 2014).

To prevent neonatal sepsis obstetric physicians are important in ensuring that screening for GBS and all other prenatal screening for infections is performed and properly treated during delivery. Nursery nurses are also important in preventing and managing neonatal sepsis as they can pick up and detect early signs of sepsis. Pediatricians, in-hospital and outpatient, also play a key role in detecting signs of sepsis through history and physical exam. In-hospital pediatricians are essential in managing the evolving treatment of neonatal sepsis and making adjustments as necessary. They are also important in reaching out to the proper consultants, such as pediatric surgeons, pharmacists, as needed (Singh and Gray, 2019).

2.3. Previous studies

In India, Gram negative organisms were more common (71.42%) than Gram positive (28.57%) among neonates with sepsis. Klebsiella was the most common pathogen (48.21%) in both early and late onset septicemia. Ceftazidime showed better results and active against *Klebseilla*, *E. coli*, pseudomonas and unidentified Gram negative bacilli. In aminoglycosides amikacin has much better results than gentamicin. All organisms except *E. coli* showed sensitivity to cefotaxime. Vancomycin had good activity against Gram positive organisms (Enterococcus, CONS, MRSA) (Verma, 2015).

Wagstaff *et al*., 2019 in Utah Hospital system, USA found that; out of 311 neonates in the LOS cohort, 62 (20%) were culture-confirmed, most culturing coagulase negative Staphylococci (46%). The use of Cefotaxime for unconfirmed EOS and LOS increased throughout the study period. Cefotaxime administration was associated with an increase in neonatal mortality (Wagstaff *et al*., 2019).

In neighboring country in Yemen, 90 (57%) cases were yielded positive cultures. EOS showed higher positive culture results (61.7%) than LOS (32%). Gram negative bacteria constituted 97.8% of the total isolates, of which *Klebsiella pneumoniae* was

the predominant pathogen (36.7%), followed by Pseudomonas species (30.0%). All Gram negative bacterial isolates were sensitive to imepenem and some isolates were sensitive to fourth generation cephalosporins, but most isolates were highly resistant to the majority of other antibiotics tested as reported by Al-Shamahy (2012).

Abdelmoneim and kheir (2014) in a Tertiary Neonatal Unit,in Sudan investigated 62 babies diagnosed clinically with sepsis and they found the prevalence of sepsis was 17.5% and the mortality was 14.5%. Moreover, blood culture was positive in 61.3% of babies and C- reactive protein was positive in 44.7% of babies with positive blood culture. In another study carried out in Khartoum, one hundred twenty neonates were studied. Sepsis was confirmed by clinical and laboratory measures. In which sixty-seven (55.8%) neonates were males and 53(44.2%) were females. Fourteen (11.7%) were preterm, 99 (82.5%) were full term and 7(5.80%) were postdated. EOS was detected in 79 (65.8%) neonates while LOS was detected in 41 (34.2%) neonate (Ahmed and Omer, 2015).

Also in Soba University Hospital, Sudan; out of 119 blood samples investigated, only 37.8% (45/119) were found to be positive for neonatal septicemia and all cases was EOS. The frequency of Grampositive and Gram negative bacteria was57.8% 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 isolated G+ve were sensitive to Vancomycin and resistant to Ciprofloxacin, Amoxyclav, Erythromycin, and Oxacillin (Babiker *et al*., 2018).In a more recent study carried at Omdurman Maternity Hospital, out of 202 positive blood cultures, 130 cases (64.4%) were at EOS and72 cases (35.6%) were recorded for LOS. Gram-negative pathogens approaching was123(60.9%). *Staphylococcus aureus* was the most common organism in both groups of neonatal sepsis being isolated from (71, 35.7%), followed by *Klebsiella pneumoniae* (43, 21.2%). Gram-negative organisms were sensitive to Imepenem (97.3%) and Meropenem (80.5%) and resistant to third generation Cephalosporins (65.3%) and Amoxicillin/Clavulanic acid (91.4%). Gram-positive organisms were resistant to Cefotaxime (75%), Amoxicillin/Clavulanic acid (65.4%), and Clindamycin (68.2%); 91.6% of Gram-positive isolates were sensitive to Vancomycin (Abdelaziz1 *et al*., 2019).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

3. MATERIALS AND METHODS

3.1. Study design

This is a descriptive, cross -sectional, hospital based study.

3.2. Study area

This study was conducted in Asia Specialist Hospital and Omdurman Maternity Hospital in Omdurman locality.

3.3. Study duration

This study was carried out from August 2019 to September 2020**.**

3.4. Study population

Neonates less than 28 days, with different sex, onsets and race, with low blood sugar(reduced movements and sucking), diarrhea, breathing problems body temperature, changes seizures (slow or fast heart rate) who were diagnosed clinically to have septicemia.

3.5. Ethical Considerations

The ethical approval was obtained from Scientific Research Committee, College of Medical Laboratory Science, Sudan University of Science and Technology, Khartoum State. Also, permission was taken from hospitals and its laboratories involved in the study.

3.6. Sample size

A total of 150 blood specimens was collected.

3.7. Data Collection

Data were obtained via medical records.

3.8. Sampling technique

Non –probability, convenience sampling technique.

3.9. Laboratory processing

3.9.1. Collection of blood samples

Blood samples were taken under aseptic conditions. the area of vein puncture was cleaned by 70% alcohol and dried. Then tenure iodine was applied to site of the vein puncture and left it to dry.

Using sterile disposable syringe 2-3ml of blood was collected. The top of blood culture bottle was cleaned with 70% alcohol and left to dry. The needle was replaced with anew sterile needle and aseptically blood was injected in culture bottle. Bottles were labeled with patient's data and time of collection.

3.9.2. Automated blood culture

Each blood specimen bottle was incubated in the automated blood culture machine (Render, China) which continuously every 10 minutes monitor the presence of CO_2 that indicate for bacterial growth. In the broth of the diphasic medium there is resin which neutralize antibiotics and increase the detection sensitivity. The Released CO_2 during the microorganism growth detected by detection filter (CO_2 only) to the sensor and the background of the smear made from broth with resin is clearer than that with active carbon.Detected colorchange due to CO_2 in thebottle bottom was considered positive (Render, 2018).

3.9.3. Sub culture from blood culture bottle

Regular observation of bottle color, turbidity, Gram stain, and subculture weredone to detect the positive growth. Regular subcultures were done after one day of incubation, then after 2 days and then 3 days later. Each sample was sub-cultured on blood agar, MacConkey's agar, and Chocolate agar and incubated aerobically at 37°C for 5 days. No change in the media or negative subculture for up to 7 days, considered negative.

3.9.4. Identification of isolates

Identification was done based on colonial morphology, Gram stain, and standard biochemical tests.

3.9.4.1. Gram's stain

Smear was prepared by using sterile slide for each single colony isolated. One drop of sterile distilled water (D.W) applied to the each slide, chosen a single colony by sterile wire loop, full chosen colony emulsified on the slid by the D.Wcircular, the smear was left to dry by air, fixed by exposure to flam three times then stained using crystal violet as basic stain for 1 minute, washed. Iodine was added for 1 minute, washed and decolorized by acetone alcohol, then neutral red or saffranin was added as counter stain for 2 minutes.Finally washed, examined under microscope using ×100 oil immersion to morphological appearance and Gram reaction. The results of Gram's stain was reported (Carter and Cole, 2012).

3.9.4.2. Biochemical tests

Gram positive cocci were identified by using the following biochemical tests: coagulase, manitol fermentation, optichin disk, and novobiosin susceptibility (Colle *et al*., 2011). While Gram negative rods were identified using carbohydrate

fermentation, triple sugar iron agar test (TSI), gas production,oxidase, urease, indole, motility media (SIM) and Simmon's citrate (Mahon *et al*.,2008).

3.9.4.2.1. Biochemical tests of Gam positive cocci

3.9.4.2.1.1. Catalase test

Two ml of 3% hydrogen peroxide (H_2O_2) was poured in a sterile in a sterile glass test tube, then colony was taken using a sterile wooden stick and immerged into the H_2O_2, active bubbles indicated positive catalase enzyme released by Staphylococcus which produce O_2 gas. On the other hand the absent ofit, indicated for negative test (Cheesbrough, 2006).

3.9.4.2.1.2. Coagulase test

Coagulase causes plasma to clot by converting fibrinogen to fibrin. Place one drop of physiological saline on end of a slid, Emulsify a colony of organism on the drop to make suspension then add EDTA (ethylenediamine tetraacetic acid) anti-coagulated human plasma positive coagulase enzyme detect by granular appearance of organism from true coagulase clumping, or clot tube method dilute the plasma 1 in 10 in physiological slain (mix 0.2 ml of plasma with 1.8ml of slain) test is 18-24 hour's broth culture, pipette 0.5ml of the diluted plasma in each tube, add 5 drops(0.1ml)were added of test organism to tube, after mixing, incubated the three tubes at 35-37 C°, examine for clotting after 1 hours up to 6 hours(Cheesbrough, 2008).

3.9.4.2.1.3. Manitol salt agar media (MSA)

Bacterial colonies were inoculated in MSA by wire loop under aseptic conditions by streaking manner, incubated for 14 days at 37°C.*S.aureus* produced yellow colonies, whereas other *Staphylococci* produced a small red or pink colonies with no color change to the medium (Colle, 2011).

3.9.4.2.1.4. Optochin disc susceptibility

It used for the presumptive identification of the *Streptococcus pneumoniae* which is optochin sensitive, from other alpha hemolytic *Streptococci* such as *viridans Streptococcus* which are optochin resistant.

Optochin disc was used in culture techniques as primary disc and applied gently on the streak well of blood agar using sterile forceps under aseptic technique and incubated for 24hours at37°C, for presumptive identification of the *streptococcus pneumonia*which is optochin sensitive from other alpha hemolytic *streptococci* such as *streptococcus viridans* which are optochin resistant , the growth of bacteria that

were optochin sensitive there is inhibition around an optochin disc giving inhibitory zone while the optochin resistant one grown normally without inhibitory zone (Abdulla, 2019).

3.9.4.2.1.5. Novobiocin susceptibility

Novobiocin disc was used to separate coagulase-negative *Staphylococci*especially*S. saprophyticus* which is novobiocin sensitive. Novobiocinwas used as primary disc, applied gently on the streak well of blood agar using a sterile forcepsunder aseptic technique and incubated for 24 hours at 37°C. Inhibitory zone means resistance for novobiocin as in optochin disc test(Abdulla, 2019).

3.9.4.2.2. Biochemical tests of Gam negative

3.9.4.2.2.6. Kliger iron agar (KIA)

A small part of the tested colonywas picked off using straight loop and inoculated in KIA media. First stabbing the butt, then streaking the slope in the zigzag pattern, and then incubated at 37°C aerobically overnight. Then the result were interpreted as following:

A yellow but red – pink slope indicated the fermentation of glucose only.

A yellow slope and butt indicated the fermentation of lactose and glucose.

A red –pink slope and butt indicated no fermentation of glucose andlactose.

Blacking along the stab line or throughout the medium indicated H_2Sproduction. Cracks and bubbles in the medium indicated gas production from glucose fermentation (Taher,2019)

3.9.4.2.2.7. Indole test

The tested colony was inoculated in sterile peptone water using a sterile wire loop and then incubated at 37°Caerobically, overnight. Few drops of Kovac's reagent were added to medium and shaken gently to test for indole. A positive result was indicated by production of red ring in the surface layer within 10 minutes (Taher, 2019).

3.9.4.2.2.8. Citrate utilization test

Slope of Simmon's citrate agar medium were prepared.The slope was streaked by using sterile straight wire. Then incubated overnight at 37°C,aerobically. A positive reaction was indicated by the change in color of the medium into blue color and growth while the negative reaction was indicated by no change in the color and no growth (Abdulla, 2019).

3.9.4.2.2.9. Urease test

The tested colony was inoculated on the surface of the slope of christensen'surea agar medium by a sterile straight loop in zigzagging manner and then incubated over night at 37°C aerobically. The positive reaction was indicated by the color change in the indicator (phenol red) to pink color and negative reaction as indicated by no change in the color (Taher, 2019).

3.9.4.2.2.10. Motility test

Inoculate the liquid bacterial culture to the test tube motility slant medium using the stab technique. Incubate at the relevant temperature for 24-48hr examine the test tube slant for the presence or absence of growth along the line of the stab inoculation; inoculation is with a straight wire/needle that is stabbed two-thirds of the way into the media. Care should be taken to ensure that the wire/needle is in the exact same line when removed from the medium as it was when it was initially inserted for inoculation(UK Standards for Microbiology Investigations,2018).

3.9.4.2.2.11. Oxidase test

A piece of filter paper was placed on clean Petri dish and three to four drops of freshly oxidase reagent (tetra methyl paraphenylene diamine dihydrocholorid) were added using sterile Pasteurpipette;A wooden stick was used to pick a colony of the tested organism and placed on filter paper. The positive reaction was indicated by production of blue –purple color within10 seconds (Taher, 2019)

3.9.5. Disk diffusion test for antimicrobial susceptibility

3.9.5.1. Preparation of bacterial suspension

The inoculum density was compared with McFarland standard solution of $BaSO_4$(0.1ml of 1%$Bacl_2$ +9.9mlof 1% H_2SO_4). The suspension was stored in the refrigerator at 4°C until used.

3.9.5.2. Modified Kirby Bauer method

Three to five colonies of similar appearance were tough and emulsified in 3to4 ml of normal saline or nutrient broth, in good light the turbidity of the suspension were matched with turbidity of McFarland standard against piece of paper. Muller Hinton agar was seeded by using sterile cotton swab and the surface of the media allowed to dry, then by sterile forceps apply the disc about 15mm from the edge and 25mm, then the plate was incubated in incubator at 37˚C for 18-24 hrs. Interpretation of zone by

interpretative chart either to be sensitive, intermediate and resistant (Cheesbrough,2008).

3.9.5.3. Antimicrobial susceptibility testing

The applied antibiotics for Gram+vecocci include Tetracycline, Erythromycin, Clindamycin, Co-trimexazon, Vancomycin, and Oxycillin to check MRSA(Jyothi*et al*., 2013).

For Gram negative rods includes Ampicillin+Amoxyclav+Gentamycin, Cephalexin, Cefitazidime, Ciprofloxacin, Cefuroxime, Ceftriaxone, and Imipenem(CLSI, 2011).

E. coli ATCC 25922was used as Control strain and test each time when susceptibility testing were performed.Zone diameters of each of the antibiotic was interpreted as per CLSI recommendation(CLSI,2011)

3.10. Data analysis

Data were analyzed by Statistical Package for Social Sciences (SPSS) software version 20. Frequencies were expressed in form of tables.

CHAPTER IV

RESULTS

CHAPTER IV

4. RESULTS

In this study, 150 blood specimens were collected from neonates with septicemia and their age ranged from 1 to 28 days with mean age 5.51±5.52S.D.

Out of 150 specimens, there were 80 (53.3%) yielded growth (positive blood culture) and 70 (46.7%) were showed no growth (table 4.1)

Table 4-1: Frequency of bacterial growth among neonates with septicemia

Percentage	Frequency	Growth
53.3%	80	Yes
46.7%	70	No
100%	150	Total

According to onset of disease; there were 31/80 (38.8%) with early onset and 49 (61.2%) with late onset as displayed in table 4.2.

Table 4-2: Distribution of neonates according to onset of disease

Percentage	Frequency	Onset of disease
38.8%	31	Early onset
61.2%	49	Late onset
100%	80	Total

Out of 80 growth; there were 20/80 (25%) G+ve cocci, while Gram-negative isolates were 60/80 (75%) as showed in table 4.3.

Table 4-3:Frequency of isolates among neonates according to Gram's stain

Percentage	Frequency	Gram's stain
25%	20	G-ve rods
75%	60	G+ve cocci
100%	80	Total

According to onsetof the disease it was observed that; *Klebsiellapneumoniae* was the most common isolatedbacteria (32/80 (40%)) in both onsets of neonatal sepsis, followed by *Pseudomonas aeruginosa* (21/80 (26.3%)) and *Staphylococcus aureus* (16/80 (20%)).

Table 4-4:Frequency of isolates according to onset of disease

Total (%)	Late Onset	Early Onset	Isolates
16 (20%)	9 (56.2%)	7 (43.8%)	*Staphylococcus aureus*
4 (5%)	2 (50%)	2 (50%)	*Enterococcus faecalis*
21 (26.3%)	15 (71.4%)	6 (28.6%)	*Pseudomonas aeruginosa*
32 (40%)	19 (59.4%)	13 (40.6%)	*Klebsiella pneumonia*
4 (5%)	3 (75%)	1 (25%)	*Escherchia coli*
2 (2.5%)	0(0%)	2 (2.5%)	*Citrobacterspp*
1 (1.2%)	1(1.25%)	0 (0%)	*Proteus mirabilis*
80 (100%)	49 (61.3%)	31 (38.7%)	Total

The antimicrobial susceptibility testing results showed that; Cefotaxime had the highest sensitivity (51(85%)) while Imipenem yielded the highest resistant (41(68.3%)) among Gram-negative rods.

Table 4-5: Frequency of sensitive and resistant isolates among Gram negative rods

Total	Resistant	Sensitive	Antimicrobial agents
60 (100%)	41(68.3%)	19 (31.7%)	Imipenem
60 (100%)	38 (63.3%)	22 (36.7%)	Meropenem
60 (100%)	14 (23.3%)	46 (76.7%)	Ceftazidime
60 (100%)	9 (15%)	51(85%)	Cefotaxime

All the isolated G+vecocci were sensitive to vancomycin (100%) and the highest resistant to Penicillin(90%).

Table 4-6: Frequency of sensitive and resistant isolates among Gram positive cocci

Total	Resistant	Sensitive	Antimicrobial agents
20 (100%)	18 (90%)	2 (10%)	Penicillin
20 (100%)	0 (0%)	20 (100%)	Vancomycin
20 (100%)	11 (55%)	9 (45%)	Ciprofloxacin
20 (100%)	9 (45%)	11 (55%)	Gentamicin

CHAPTER V

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

CHAPTER V

5. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

In this study blood culture results exhibited 80/150 (53.3%) positive that was similar to a study conducted in Yemen by Al-Shamahy 2012 (90/158 (57%)) and differ from Sorsa (2019) in South East Ethiopia who reported bacterial growth was 88/303 (29%) of blood cultures. This variations may be due to the lacking of hygienic measures during and after delivery.

Consider onset of disease, the EOS and LOS were 31 (38.8%) and 49 (61.2%) respectively. This result was agreed to a study conducted in University of Utah Hospital System in United States, which reported that EOS was 23(2.3%) and LOS was 60 (20%) (Wagstaff *et al*., 2019).

However, it was lower than a study conducted in a Maternity Hospital in Omdurman, Sudan by Abdelaziz1 *et al*., (2019) who reported that; 64.4% cases were at early onset and 35.6% were at late onset sepsis, Ahmed, (2015) in Khartoum North Teaching Hospital, who described that early onset sepsis was detected in 79 (65.8%) neonates while late onset sepsis was detected in 41 (34.2%) neonate and in Al-Thawra University Hospital, Sana'a, Yemen, in which early onset sepsis showed higher positive culture results (61.7%) than late-onset sepsis (32%) (Al-Shamahy, 2012).

The differences may be as result of insufficient of antenatal care, poor breastfeeding which was a marker for serious bacterial infection.

In the present study, Gram negative rods and Gram positive cocci were encountered 75% and 25% respectively, which was similar to a study conducted in a Maternity Hospital in Omdurman, Sudan by Abdelaziz *et al*. (2019) which showed that; Gram negative and Gram positive organisms were 60.9% and 30.1% respectively in India by Verma, (2015) who reported that Gram negative were 71.42% and Gram-positive organisms were 28.57%. It was lower than a study conducted in Yemen, in which Gram negative bacteria constituted 97.8% of the total isolates as reported by Al-Shamahy (2012).

The above finding was mismatched with the study carried in Soba University Hospital, Sudan in which the frequency of Gram-positive and Gram negative bacteria was 57.8% and 42.2% respectively (Babiker *et al*., 2018).

Regarding to isolates, *Klebsiella pneumonia* was the most common isolate(32 (40%)) in both EOS and LOS, that was harmonized to a study conducted in India by Verma (2015) (48.21%)and in Yemen (36.7%) by Al-Shamahy (2012).

It was in consistent to study conducted in South East Ethiopia that showedthe predominant isolated bacteria were coagulase negative *staphylococci* (CoNS) 22 (25%), *Escherichia coli* 18 (20.5%) and *Staphylococcus aureus* 16 (18%) (Sorsa, 2019) and from study conducted in University of Utah Hospital System in United States in whichcoagulase negative staphylococci was 46% (Wagstaff *et al*., 2019).

In the current study, Gram-negative rods showed the highest sensitivity to Cefotaxime (51(85%))which was dissonant to Abdelaziz and his colleagues (2019) in Maternity Hospital in Omdurman, Sudan in which the highest sensitivity showed to Imipenem 110 (97.3%), Babiker *et al*. (2018b) in Sudan, Soba University Hospital which that showed the best sensitive to Imipenem 19(100%) and in India Department of Pediatrics, SP Medical College, Bikaner, Rajasthan by Verma (2015).

For the Gram-positive cocci, the uppermost sensitivity was displayed for Vancomycin 20 (100%) and most of these isolateswere highly resistant toPenicillin 18 (90%). This resultwas similar to a local studydone at Maternity Hospital in Omdurman, Sudan, which showed that; the highest sensitivity to Vancomycin 24 (92.3%) and the highestresistant to Ciprofloxacin 25 (96.2%) (Abdelaziz *et al*., 2019).

5.2.Conclusion

This studywas concluded that; positive blood culture was significant from neonatal sepsis and mostly was with late onset of disease. Also Gram negative rods were the most frequent isolated bacteria represented by *Klebsiella pneumoniae* while among Gram positive cocci; *Staphylococcus aureus* was the most common isolate.

Cefotaxime and Ceftazidimecan be used for empirical treatment of bacterial sepsis caused by Gram negative isolates while vancomycin is the best choice for Gram positive isolates.

5.3. Recommendations

- Further study with large sample size and more accurate tests such as PCR should be used to determine the rate of infection.
- Nurseries should periodically review their bacterial sensitivity pattern and the antibiotic policy.
- Monitoring treatment efficacy and it's relation with sensitivity, resistance over period of time.
- Standard and efficient antenatal care must be done for all pregnant women.
- Good hygiene must be followed when look after neonates.
- Encourage breast feeding to reduce the rate of infection.

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Appendix

Color plates

Render automated blood culture

- Reagents

B) Instrument reagents

C) System Operation