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COLLEGE OF HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Bacteriological Profile, Antimicrobial resistance and Outcome of Neonatal Sepsis Among Patients at St.Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by MEREMA SHERIF, entitled: "Bacteriological Profile , Antimicrobial resistance and Outcome of Neonatal Sepsis Among Patients at St Paul Hospital Millennium Medical college" and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (diagnostic and public health microbiology) complies with the regulations of the University and meets the accepted standards concerning originality and quality.

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List of abbreviations

AAU:	Addis Ababa University
AMR:	Antimicrobial Resistance
CI:	Confidence Interval
CLSI:	Clinical and Laboratory Standards Institute
CONs:	Coagulase Negative Staphylococci
COR:	Crude odds ratio
EOS:	Early Onset of Sepsis
LBW:	Low Birth Wight
LOS:	Late Onset of Sepsis
MDR:	Multi Drug Resistance
NICU:	Neonatal Intensive Care Unit
SPSS:	Statistical Package for Social Sciences
SOP:	Standard Operational Procedures
SPHMMC:	St Paul Hospital millennium medical college
TSB:	Trypto Soya Broth
WHO:	World Health Organization

Abstract

Background: Neonatal septicemia is the presence of clinically associated bacteria or fungi in the blood of neonate's .It causes high morbidity and mortality worldwide especially in developing country.

Objectives: The study was designed to assess the bacteriological profile, antimicrobial resistance and outcome of neonatal sepsis at St. Paul's Hospital Millennium Medical College.

Methods: A hospital based cross sectional study design was conducted at St. Paul's Hospital Millennium Medical College from March 2020 to July 2020 G.C. Socio-demographic and clinical data was collected from each patient. 1-2ml of blood was drawn aseptically and inoculated at bedside on Trypto Soya Broth. Gram stain was performed and subcluturing was done every other day on blood agar, chocolate agar and Mac Conkey agar plates. For species identification, colony characteristics and biochemical tests are used if the culture is positive. All the isolates was tested for susceptibility test by using Kirby-Bauer's disk diffusion method. Outcome of neonates was assessed using checklists. Data was encoded in to Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS) version 20 software.

Results: Among 400 study populations, 84(21%) showed bacterial growth, 67 (79.8%) gram negative and 17 (20.23%) gram positive bacteria. Commonly isolated organisms were *Klebsiella spp 37* (44%), *E. coli 19* (21.6%) *and Coagulase negative Staphylococci* 13 (15.47%) were the leading causes of neonatal sepsis in our study. Antibiotic resistance was found in both Gram positive and Gram negative bacteria. Ciprofloxacin and Amikacin were the most effective antibiotic for gram negative bacteria while for gram positive bacteria Vancomycin, Clindamycin and Ciprofloxacin were effective drugs.

Conclusion: Bacterial isolates were not giving response to first and second line antimicrobials that are utilized for the treatment of sepsis in babies who are in the first month of life. Antibiotics in the third line are moderately successful against isolated bacteria. High utilization rate of antibiotics is the most important contributory factor for the development of AMR and continuous surveillance is needed in order to keep national guidelines on antimicrobial therapy updated.

Key words: Septicemia, Bacteriological profile, Antimicrobial susceptibility pattern, Blood culture

1. Introduction

1.1. Back ground

Neonatal septicemia is the presence of clinically associated bacteria or fungi in the blood of neonate's (1).Even within the same area, different organisms that cause sepsis in neonates varies significantly and show variation through time. This variation may affect the success of scientific management .*Group B streptococci (GBS), Escherichia coli, and Listeria monocytogenes* are leading causes of neonatal septicemia in developed countries. Although gram negative bacteria and *coagulase-negative staphylococci* are the leading in developing countries (2).

Neonatal sepsis is classified into two sub groups: early-onset (EONS) and late-onset (LONS), depending on either symptom occurs before or after 72 hours of life. EONS is described as the beginning of sepsis signs inside the limit of 72hour of birth and is caused by microorganisms occur in the maternal genital tract before or at the time of birth. LONS begins after 72 hour from birth, is maybe due to bacteria carried from the hospital or the society at the time of delivery (3).

Despite advances in neonatal care leading to improved survival rates and reduced complications in preterm infants, there has been little improvement in the prophylaxis, treatment, and adverse neurodevelopmental outcomes associated with neonatal sepsis over the last three decades. The risk of neonatal sepsis is inversely proportional to the gestational age (GA) and birth weight of the baby (BW).While ~20% of very low BW (<1,500 g; VLBW) infants suffer from one or more systemic infections during their hospital stay, the rate may reach up to 60 % in the most immature infants (4).

Due to difficulties in the early diagnosis of neonatal sepsis and the potentially devastating outcomes, empirical antibiotic treatment is usually initiated when sepsis is suspected. However, this empirical therapy is often ineffective, with needless broad-spectrum antibiotics and long treatment periods leading to a rise in multidrug-resistant microorganisms in NICUs and a high burden for developing countries (5).

1.2 Statement of the problem

Globally, Neonatal sepsis is the leading health problem. Sepsis caused by bacteria is mostly common in newborns, with a international prevalence of 1 to 10 per 1000 live births.In developed countries, Septicemia is lower when we compared with developing countries. The death rate associated with sepsis as high as 50% for not treated neonates (6).

Despite the burden of neonatal sepsis, high quality evidence in diagnosis and treatment is lacking. The susceptibility of population, lack of consensus in definitions and variability between regions hinder the development of clinical trial and global recommendation. Physicians carring for infected neonates face multiple challenges in diagnostic and treatment decisions. The situation in developing countries are further complicated by lack of reliable surveillance systems and high proportion of home births (7).

Neonatal sepsis is Ethiopia's leading cause of newborn mortality, covers on 33% of all neonatal mortality. As reported in 2016 Ethiopian Demographic and Health Surveys, neonatal death decreased from 49 deaths per 1,000 live births in 2000 to 29 deaths per 1,000 births in 2016, decreasing of 41% over the last 16 years. However, the causes of neonatal mortality in Ethiopia are not well known, former studies have stated neonatal sepsis, asphyxia, birth injury, tetanus, preterm birth, congenital malformations, and "unknown causes" were leading causes of neonatal death. From varies studies done in different countries described that among the neonates admitted to the NICU who were diagnosed with suspected sepsis ranged from 4.3% to 75.1%(8).

In low- and middle-income countries, neonatal sepsis remains a leading cause of neonatal hospital admission, morbidity, and mortality (LMICs).In this setting, bacterial infection, including bacteremia, is complicated by multi drug resistance, particularly related to healthcare acquired infection and effective management of neonatal sepsis is increasingly problematic. Recently, WHO has acknowledged the problem of antimicrobial resistance (AMR) as an endemic and widespread problem in LMICs. AMR in LMICs represents one of the biggest threats to global health and is one of the greatest current challenges in infectious disease research (9).

The reason why we did this study is to determine the recent changing pattern of Bacteriological profile, their antimicrobial susuptability test on neonatal patients and outcome of the neonates. Since the epidemiology varies in time and place, it needs a regular inspection and customization for a given locality. Furthermore, the impact of antimicrobial susceptibility and outcome in neonates in LMICs is also not well documented in the study sites. To address these gaps, We conducted this research to investigate the bacteriological profile, antimicrobial patterns and outcome of neonatal sepsis at SPHMMC.

1.3 Significance of the study

As septicemia is a life threatening emergency, the knowledge of epidemiological and antimicrobial susceptibility pattern of common pathogens in a given area helps to inform the choice of antibiotics. Predominance of either the gram positive or gram negative bacterial isolates is influenced by geographical location and changes in time. So the antibiotic susceptibility pattern influenced by location and time. The determination of the bacterial profile and their antibiotic sensitivity pattern could guide in the infection control and rational use of antibiotic in this locality. Understanding these variables would help to prioritize resources and plan strategies for decreasing the mortality associated with bloodstream infection.

Additionally this study could help to identify the etiologic agent of neonate septicemia and thereby to take effective measure on those etiologic agents. Since today's government policy has been focused on under five children health improvement, the study could help as one of the valuable input to the policy makers. This study could also serves as a reference material being as base line for related study.

Therefore, this study helps to isolate and identify the bacterial agents responsible for sepsis in neonates , to decide the antibiotic susceptibility pattern of isolates and the outcome of septicemia on neonates in St.Paul's Hospital Millennium Medical College.

2. Literature review

Since blood stream infection (septicemia) being one of the challenging problem, many research have been done in the world. These researches show the prevalence of septicemia etiologic agent and their antimicrobial resistant pattern has been changed from place to place and from time to time. So, it needs to update epidemiological data and information regarding to the etiologic agents and their AST for a given place and time.

According to a study conducted in Shanghai, China from 2013 to 2017 on Clinical features and antimicrobial susceptibility profiles of culture-proven neonatal sepsis in a tertiary children's hospital, retrospectively reviewed. From 976 culture-positive cases, the leading organisms accountable for neonatal sepsis were *Staphylococcus epidermidis (22.87%), Escherichia coli (9.68%), Alcaligenesxylosoxidans (9.38%) and Klebsiellapneumoniae (9.09%).* Most of Gram-positive bacteria isolates were susceptible to vancomycin, linezolid, minocycline, and tigecycline but over 90% of cases were immune to penicillin . Amikacin and imipenem sensitivity and resistance to ampicillin were establish in most of Gram-negative bacteria isolates (3).

A prospective observational study was carried out in NICU in a tertiary care hospital in West Bengal, Kolkata, India, From January 2017 to June 2017. The study population included 69 intramural (born in our study institution) neonates and 33 extramural (born elsewhere) neonates. The proportion of preterm neonates (80) is more than term neonates (22). Most babies (86) belong to <2.5 kg birth weight group. Overall higher incidence of Gram-negative bacterial septicemia (*Klebsiella spp, Burkholderia spp, Pseudomonas spp.*) was observed. Fungal (*Candida spp.*) septicemia was predominant in babies with LOS (10).

Another retrospective study conducted on Late-Onset Neonatal Sepsis: Overview of Risk Factors and Bacterial Etiology in a Tertiary Care Hospital in North India: Sixty-eight newborns with LOS were included in the study. In 36 , 26%, and 33.3 % of population- and hospital-acquired infection cases, respectively, *Staphylococcus* and *Klebsiella* were the most prevalent

bacteriological isolates. *Staphylococcus aureus* was susceptible to vancomycin in 100% cases while high resistance was seen for ampicillin in both the groups. Similarly, gentamicin was found to be resistant in both the groups for *Klebsiella* while imipenem was susceptible in 100% cases (11).

A cross-sectional prospective study was carried out in Bangladesh on Evaluation of hypoglycemic status and etiologic factors in neonatal septicemia, Among 106 neonates with sepsis, 68 showed EONS and 38 showed LONS. Neonates who were born below 2.5kg were 51, 18 were born below 1.5kg and 22 were born early (22).Studies of hypoglycemic status showed that there were varies types of septicemia in neonates, hypoglycemic neonates were 77 and between them EONS covered 59 and 18 taken by LONS. Positive blood culture for bacteria were found in 33 neonates. From the total isolates *Klebsiella spp*. accounts for 10 then *Acinetobacter spp.* 8 (12).

A retrospective study conducted in Tehran, Iran from 2007 to 2014 on Bacterial Etiology and Antibiotic Resistance Patterns in Neonatal Sepsis Ninety neonates with positive culture test were included. Fifty-three were male (58.9%). Most bacterial growths in culture were *Staphylococcus aureus* and *E. coli*. Antibiotic resistance rates for ceftriaxone, cefotaxim, and gentamycin were 5 percent, 30 percent, and 15 percent, respectively. There were 15 cases (16.7%) with resistance to imipenem (13).

A retrospective cross-sectional study conducted in Nepal, on Bacteriological profile and antibiotic susceptibility of neonate patients who were in NICU, 69 (20.5%) of the 336 neonates admitted to the NICU had culture-positive sepsis. Most of the cases were EONS (n = 54, 78.3%) and were between preterm neonates (n = 47, 68.1%). Most of bacterial isolates were gram-negative, with *Klebsiella* species responsible for 33.3% (n = 23). *Klebsiella* showed high resistance to commonly used antibiotics like ; Cefotaxime (90.5%), Gentamicin (75%), Ciprofloxacin (76.2%), Ofloxacin (72.2%) and Chloramphenicol (65%). From gram-positive species, *CONS* (n = 14, 20.3%) were leading. *CONS* showed high resistance to Oxacillin (80%), Cefotaxime (66.7%) and Meropenem (80%) but better susceptibility (100%) to Vancomycin and Linezolid. Multidrug-resistant rate was 73.9% (14).

Another cross-sectional study conducted in Bharatpur, Nepal on Changing trend of Neonatal Sepsis in neonate and antibiotic Susceptibility Pattern of Bacterial Isolates,, Bacterial growth were found in 56 specimens (10.8%) from 516 total specimens . Gram negative isolates were accountable for the most of septicemia in neonates 69.6 %. The most widely isolated organism was *Acinetobacter species* 32.1%, then *Staphylococcus aureus* 19.6%.*Acinetobacter species* 32.1% and *Staphylococcus aureus* 16% were the leading isolate in EONS. *Staphylococcus aureus* (19.6%) and *Acinetobacterspecies* (8.9%) were common in LONS. Amikacin, piperacillin/tazobactam, meropenem, ofloxacin, and gentamicin susceptibility was found in Gram-negative isolates (15).

A Prospective study conducted in Arab states on early onset neonatal septicemia, this study included5 neonatal care units which are found in United Arab Emirates, Saudi Arabia and Kuwait. EOS was occurred in 102 neonates from total of 67,474 live births. From 1000 live births the occurrence rate for EONS was 1.5 , with rates ranging from 2.64/1000 live births in Kuwait to 0.40/1000 live births in Saudi Arabia's . The leading EOS causative organism was *GBS* (60%), then *E.coli* (13%). The total incidence of invasive *GBS* disease was 0.90 per 1000 live births (16).

A cross sectional study was conducted from January to May, 2016 in Ghana on Bacteriological profile and antibiotic susceptibility pattern of common isolates of neonatal sepsis, Males covered (60.7 %) of the 150 clinically suspected neonatal sepsis cases. From 26 varies pathogens isolated, gram positive organisms covered 18 (69%) and gram negative organisms 8 (31%). *Staphylococcus epidermidis* was the leading organism which was responsible for 53.8% of the total. *Proteus mirabilis (4%) and Escherichia coli(4%)* were also identified. Ampicillin resistance was 100% for all identified isolates (17).

A prospective cross-sectional study conducted in Nigeria on Clinical features, clinical outcome, a etiology and antibiotic susceptibility pattern of neonatal sepsis, From 180 neonates, 85 were selected. EONS was found in 55 neonates, while the rest 30 was covered by LONS.Culture-proven septicemia shown in 22.4% neonates. The occurrence of culture-proven sepsis in the

hospital was 2.8/100 live-births. From total isolates gram-negative bacteria cover 78.9%. The leading bacterial isolates were *Klebsiellaspp (31.6%), Enterobacterspp (21.1%), and coagulase-negative Staphylococci (15.8%).* Great resistance rate to cefuroxime and ampicillin was recorded in gram-negative bacteria. The death rate was 26 % (18).

A cross sectional study was conducted in Zambia on Etiology, Antibiotic Resistance and Risk Factors for Neonatal Sepsis in a higher Referral Center .From 313 neonates with suspected sepsis, 33% (103/313) had positive blood cultures, of which 85% (88/103) were early-onset sepsis. The most common isolate was *Klebsiella species*, which accounted for 75% (77/103) of cases, followed by *coagulase-negative staphylococci* (6% (7/103), *Staphylococcus aureus* (6% (6/103), *Escherichia coli* (5% (5/103), and *Candida species* (5% (5/103) (19).

According to Prospective study conducted in Egypt Mansoura Hospitals NICU on three Egyptian Neonatal Network (EGNN) participants over a period of 18 months from March 2011 to August 2012. 357 neonates treated with suspected sepsis during the study period, the incidence between neonates who were admitted at three NICU was a 45.9% (357/778). *CONS* were the leading isolates, then *Klebsiella pneumoniae*. The majority of the bacterial isolates were resistant to common empiric antibiotics. Although, multidrug resistance was shown in 70.1% (89/127). Gram-positive isolates shown better susceptibility towards ciprofloxacin, imipenem, vancomycin, and amikacin (20).

Another retrospective study conducted in Egypt on Emerging antimicrobial resistance in early and late-onset neonatal sepsis, 314 neonates were diagnosed with sepsis. There were 166 positive findings from total. *Klebsiella pneumoniae (42%) and CONS (19%)* were the leading from isolates .Ampicillins (100%), cephalosporins (93%), and piperacillin-tazobactam (99%) were the most resistant antibiotics, whereas aminoglycosides (36%) and aminoglycosides (52%) were the least resistant. Vancomycin resistance was lowest in Gram positive isolates (18 %). Multidrug tolerance was shown in 92 (38%) of the cultures, with gram negative isolates responsible for the majority (78/92) (6).

A community-based prospective study conducted in Madagascar on Bacterial Infections in Neonates from 2012 to 2014, A total of 981 newborns in rural and urban areas of Madagascar were included. The incidence of culture-confirmed severe neonatal infections was high; 17.7 cases/1,000 live births. Most (75%) occurred during the first week of life. The most common (81%) bacteria isolated were gram-negative. Multidrug-resistant neonatal infection occurred 7.7 times per 1,000 live births (21).

According to study conducted in Asella teaching and referral hospital (ATRH)from April 2016 to May 2017 on Blood culture result and antimicrobial resistance pattern taken from reports of NICU, a total of 303 neonates with clinical sepsis were included. From this 88 (29.4%) of blood cultures show growth. *CoNS were 22 (25%), E.coli 18 (20.5%) and S.aureus 16 (18%)* were the leading isolates. Ampicillin and Gentamycin resistance of *E. coli* were 66.7% and 55.6% while the resistance rate against *Klebsiella spp*.against these two antibiotics is far higher 91% and 82% respectively. On the other hand, Gram-positive bacteria isolates shown better sensitivity to third-line antibiotics such as Clindamycin, Vancomycin, and Ciprofloxacin, whereas Gram-negative bacteria isolates shown a better sensitivity to Ciprofloxacin and Amikacin. (22).

A cross- sectional study was conducted in Gondar University Hospital on Bacterial profile and antimicrobial susceptibility pattern in septicemia suspected patients. From the total of 390 blood specimens, 71 (18.2%) positive for blood culture. *CONs* accounted for 42.3 % of the bacteria isolated, followed by *S. aureus (17 (23.9 %) Klebiesella spp 12.9 %, E. coli 7%*, *Pseudomonas aeroginosa 5.6 %and Salmonella spp. 4.2%*.The gram positive cover 69% while and gram negative bacteria found in 31% of the culture isolates. Most antibiotics tested showed high rates of resistance by the isolated bacteria. Gram positive bacteria resistance ranged from 23.5 % to 58.8 % while 20 % to 100% for gram negative bacteria (23).

Another cross- sectional study was conducted in Gondar Hospital from September/2015 to May/2016, On Bacterial etiologic agents causing neonatal sepsis and their associated risk factors, A total of 251 neonates were included in the study. 117 (46.6%) showed bacterial growths from total of 251 participants. Gram-positive bacteria were found in abundance 67.5%. *S. aureus* covered 40.8% from total isolate, then *coagulase negative Staphylococci* 21.6% and *K.*

pneumonia 15.8%. Total MDR of isolates was 78(65%),gram positive covered 56 (69.1%) while gram negative bacteria 22 (56.4%) (24).

A cross-sectional study was conducted in Wolaitasodo Town, southern Ethiopia, on Neonatal sepsis and associated risk factors among neonates in hospitals from April 22 to June 29, 2018 were included. Out of 275 newborns, this study found that 33.8% of the neonates had neonatal sepsis during admission. Age of mothers, multiple digital vaginal examinations, exclusive and immediate breastfeeding within an hour, kangaroo mother care (KMC) within an hour, and age of neonates, all the above mentioned had were statistically significant role in neonatal septicemia (25)

A prospective cross-sectional study was conducted in Wollega University Teaching and Referral Hospital, Western Ethiopia, on Clinical Treatment Outcomes of Neonatal Sepsis in NICU. Total of 306 neonates participated in the study, of them 43.46% were males and 92.5% had the weight of 2.5 kg- 4 kg. EONS were found in 75.5% of total participants and 24.5% were LONS. Most of the neonates (96.08%) were diagnosed with empirically. Neonates born through vagina were 66.66%andall of them were taken the mixture of ampicillin and gentamicin .Most of neonates (90.19%) were recovered and discharged. A total of 12 (3.92%) death was recorded (26).

3. Objectives

3.1 General objective

- To assess the Bacteriological profile, antimicrobial resistance pattern and outcome of neonatal sepsis at St Paul Hospital Millennium Medical College.

3.2 Specific objectives

- To determine the bacteriological profile of neonatal sepsis at St Paul Hospital Millennium Medical College.
- To determine antibiotic resistance pattern of the bacteria among neonates.
- To assess the neonatal sepsis outcome among neonates in St Paul Hospital Millennium Medical College.

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4. Materials and methods

4.1. Study area

This study was conducted in SPHMMC which is located in Addis Ababa, the capital city of Ethiopia. Although the medical school opened in 2007 and the hospital was founded in 1968 by the late Emperor Haile Selassie, Millennium Medical College was created by a decree of the Council of Ministers in 2010. For its undergraduate medical education, the College created Ethiopia's first integrated modular and hybrid problem-based curriculum. More than 2800 clinical, research, administrative, and support staff members work at the college to provide medical specialty services to patients from all over the world. The hospital has a total of 800 beds. From this, 25 beds for pediatrics medical ward,16 bed for pediatrics surgical ward and 4 beds for pediatrics ICU.SPHMMC gives diagnostic and treatment services for about 370,000-400,000 patients per year. From this 12,000-14,400 are pediatrics who are visiting pediatrics OPD. SPHMMC also offers the lowest cost for these services when compared to the private hospitals.

4.2. Study design and study period

- A hospital based cross-sectional study was conducted from March 2020 to July 2020 G.C.

4.3. Population

4.3.1 Source Population

- All neonatal patients who visited SPHMMC during the study period

4.3.2. Study population

- All neonatal patients who suspected of having septicemia according to the inclusion criteria.

4.4. Inclusion and exclusion criteria

4.4.1 Inclusion criteria

- Patients less than or equal to 28 days.
- Patient's family or care-givers who agreed to participate and give informed consent.

4.4.2 Exclusion criteria

- Patients who are taking antibiotics for the last two weeks during data collection.

4.5. Study Variables

4.5.1 Dependent variables:

- Etiologic agent, Antimicrobial susceptibility pattern and outcome of neonates.

4.5.2 Independent variables:

- Age, sex, underlying chronic disease, congenital anomalies, indwelling medical devise, weight, length of hospital stay and hospital ward.

4.6. Sample size determination and Sampling technique

4.6.1. Sample size determination

- The required sample size for this study was calculated based on the study done at Gondar specialized hospital with prevalence 46.6 %.
- Sample Size was determined by the following formula: $n = Z^2_{\alpha/2} P (1- P)/ d^2$

Where:

P -is the estimated proportion.

Z- reflects the confidence interval; we will use 95 % confidence interval so the value of zα/2 will be 1.96 - d is the margin of error, here it is 0.05.

α -is the level of error?

$$n = \frac{1.96^2 x\ 0.466\ (1 - 0.466)}{0.05^2} = 382$$

4.6.2. Sampling technique

- Convenience sampling techniques were employed to include study participants who meet the inclusion criteria until the required sample size is achieved.

4.5.7 Data collection and laboratory processes

4.7.1 Data collection

Data collectors (experienced nurse and laboratory technologist) were identified, trained and informed to collect the data as the pre-structured questionnaire. The purpose of the study as well as any related harm and benefit were explained to the study participants accordingly. Demographic data and other information including presence of chronic disease, indwelling medical devise, birth weight and pre-sample antibiotic history data were collected by reviewing different medical records and interview. Data on nutritional status of the mothers and neonates collect based on their clinical diagnosis. Since antibiotic drug treatment before taking the blood sample could compromise the culture result, those who were taking antibiotics in the last two weeks excluded from the study. But after taking the blood sample, the prescribed antibiotics were recorded. Outcome of neonates assessed using checklists.

4.7.2Specimen collection and transportation

Using a pressure cuff, locates a suitable vein in the arm. Deflate the cuff while disinfecting the Vein puncture site. The antiseptic preparations are Iodophor or Iodine tincture followed by 70% Isopropyl alcohol. Iodophors require 1-2 minutes of contact time for maximum antiseptic effect. The sample was taken by experienced nurse or medical doctor following the above aseptic technique. After collection, 1-2 ml of the sample inoculate at the bed side on tryptosoya broth (TSB) and then transported to the microbiology laboratory within 5-10 minutes.

Figure 1; - TSB for adult and neonates

Figure 2; 1-2 ml of whole blood add to12.5 ml of TSB bottle, sample photograph taken during the study time .

4.7.3. Laboratory processes

4.7.3.1. Isolation and identification

After the sample has been collected aseptically, it was inoculated at bed-side on TSB and incubate at 37°C for up to 7 days or until growth detected. Bottles observed macroscopically daily for visible evidence of bacterial growth such as hemolysis, turbidity, gas production, or formation of discrete colonies. Regardless of the state of bacterial growth subcultures were made after 24 hr, 48 hr, 72 hr and finally at the 7 days onto Blood agar, and MacConkey agar, then incubated aerobically at 37°C for 24 hr and Chocolate agar incubate at 37°C for 48 hr at 510% CO. Gram stain was performed for macroscopically positive blood samples. For those having growth on the subcultured media, by isolating the pure colony biochemical tests preceded. Based on the colonial morphological characteristics and biochemical test results identified the etiologic agent. For gram positive bacteria coagulase, catalase and manitol salt agar and for gram negative indole, citrate utilization, triple sugar iron, urea, manitol, oxidase and motility test were performed and the organisms identified as per the standard procedures.

4.7.3.2 Antimicrobial susceptibility test

Antimicrobial susceptibility testing's were performed for isolated organisms on Kirby-Bauer's disk diffusion on MHA according to Clinical and Laboratory Standards Institute guideline (CLSI 2016).Antibiotic discs for antimicrobial susceptibility test were used for the bacteria isolated.

Accordingly for gram positives, Ampicilin, Gentamicin,clindamycin,ciprofloxacin, Ceftriaxone ,Cloxacillin, erythromycin, chloroamphenicole ,Vancomycin, Ceftazidme and cotrimoxazole used. For gram negatives, Ampicilin, Gentamicin,Cefotaxime, Ciprofloxacin,chloroamphenicole,erythromycin, Ceftazidme, Cotrimoxazole and Amikacin used. But chloroamphenicole and erythromycin were not used for Acetinobacter and Enterobacter spp.

4.8 Data Quality Assurance

Data quality was ensured through use of standardized data collection materials, pretesting of the questionnaires, proper training before the start of data collection and intensive supervision during data collection by the principal investigator. For laboratory analysis pre-analytical, analytical and post-analytical stages of quality assurance was considered that are found in SOPs of the microbiology laboratory of SPHMMC. In addition, well-trained and experienced laboratory professionals have participated in the laboratory analysis procedure.

4.8.1. Pre-analytical phase

First we asked the participant verbally and by written consent for their willingness and then we fill all the information on the preformed questionnaire. Labeling the bottle with patient's identification number and then aseptically drawn by using sterile syringe. Following collection, specimens transported to the microbiology laboratory within 5-10 minutes.

4.8.2. Analytical phase

All materials, equipment and procedures adequately controlled. Quality control of culture media was done for sterility test and the ability to grow the control bacteria strains. For the susceptibility test the inoculum density of bacterial suspension standardized by a barium sulfate (BaSO4) turbidity standard, equivalent to a 0.5 McFarland standard used and standard reference strain of American type culture collection(S. aureus (ATCC-25923), E. coli (ATCC-25922) and P. aeruginosa (ATCC-27853)) will be used as Control bacteria strains for both media and antibiotics discs. Standard operating procedure (SOPs) of the microbiology laboratory of SPHMMC strictly followed and the results were checked by the senior microbiologist.

4.8.3. Post-analytical phase

The results were recorded with the patients' identification number. In order to avoid the errors in the results of the test, the reporting was repeatedly checked and evaluated by the head of the department before the results given to the caregiver. Appropriate action was taken when a result has serious patient or public health implication.

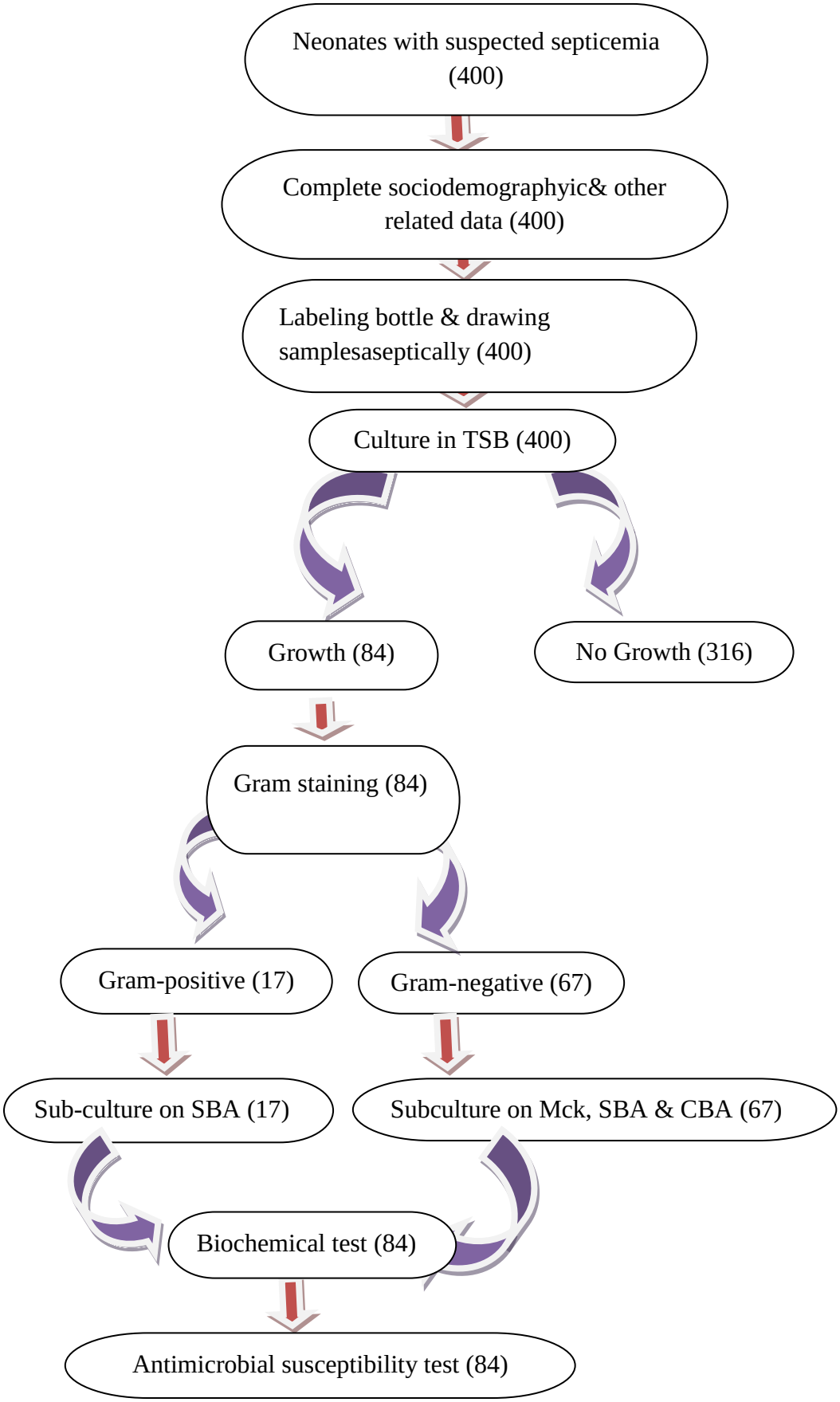

Figure 3 :-Work flow of the study

4.9. Data Processing and Analyses

All data were encoded in to Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS) version 20 software. We used descriptive statistics, such as frequencies and percentages. Binary logistic regression model was performed for factors associated with culture isolates and neonatal outcome. In all cases, P-value less than 0.05 considered as statistically significant. The strength of the association was interpreted using an odds ratio in a 95% confidence interval. Finally, the results presented on words, graphs and tables.

4.10. Operational definitions

Sensitivity (S): Zone of inhibition radius is wider than, equal to, or not more than 3mm smaller than the control.

Intermediate (I): Zone of inhibition radius is more than 3mm smaller than the control but not less than 3mm.

Resistant (R): No zone of inhibition or zone radius measure 2mm or less than the control.

Septicemia: -defined as the presence of bacteria or fungi in the blood.

Multi Drug Resistance: - bacterial resistance for three or more antibiotics of different classes

Neonatal outcome:- Can be recovered or death outcome.

4.11. Ethical consideration

Ethical clearance was obtained from Department of Research and Ethical Review Committee of Medical laboratory Science, College of Health Science, Addis Ababa University and from institution review board of SPHMMC research directorate. Study subjects recruited after they become informed about the objectives and use of the study and then after they gave informed consent. All confirmed cases of septicemia were reported to the study subjects' responsible clinician.All the information contained within the study was confidential.

5. Results

Among 400 study populations, 246 (61.5%) were males and 154(38.5%) were females. Regarding the weight of the neonates, about 41.5% of neonates had the weight of less than or equals 2.5 kg were as the remaining 58.5% of neonates had weight of greater than 2.5 kg. From the total study population 118 (29.5) were born preterm .The age of 364 (91%) neonates was below one week (Table 1).

Table 1: Socio-demographic characteristics of neonatal patients at SPHMMC from March 2020 to July 2020

Variables	Categories	Frequency (%)
Gender	Male	246(61.5)
	Female	154(38.5)
Gestational age	Term	282(70.5)
	Preterm	118(29.5)
Birth weight(kg)	≤ 2.5	166(41.5)
	>2.5	234(58.5)

As shown in table 2, using binary logistic regression model, there was a statistical significant difference between septicemia in neonates with birth weight (p=0.000), HIV status (p=0.005) and clinical manifestations (p=0.000) compared to those without septicemia.

Table 2: Socio-demographic characteristics and back ground information with septicemia at SPHMMC.

		Total	Bacteremia (N=84)	No Bacteremia (N=316)	COR	(95% CI)	P-value
Age	0-7 days	364	79	285			
	8-28 days	36	8	28	1.031	0.452-2.350	0.943
Sex	Male	246	56	190	0.855	0.522-1.401	0.535
	Female	154	31	123			
Underlining chronic disease	No	228	44	184	0.717	0.445-1.156	0.172
	Yes	172	43	128			
Indwelling Medical device	No	4	0	4			
	Yes	396	87	309	0.000	0.000-0.000	0.999
Birth Weight	≤2.5kg	166	86	80	0.004	0.001-0.0029	0.000
	>2.5kg	234	1	233			
Gestational Age	≤37 week	118	87	31	0.000	0.000-0.000	0.993
	>37 week	282	0	282			
Nutritional Status	Malnutrited	14	6	8	0.354	0.119-1.049	0.061
	Non malnutited	386	81	305			
HIV Status	Negative	385	79	306	4.427	1.558-12.576	0.005
	Positive	15	8	7			
Clinical manifestation	EOS	319	64	255	0.863	0.798-0.952	0.000
	LOS	81	23	58			

5.1 Patterns of isolated organism

Gram- negative bacteria accounted for 67 (79.78%) while the remaining 17 (20.23%) were Gram- positive bacteria. Majority of culture proven sepsis were of early onset (65 vs 19). Gram-negative bacteria were found in 59 (90.76%) of EONS isolates, while Gram-positive bacteria were found in 11 (57.89%) of LONS isolates (Table 2). When disaggregating to specific bacteria pathogen; *Klebsiella spp 37 (44.04%) and E. coli 19 (22.61%)* were by far the leading causes of neonatal sepsis in this study.

Table 3:The distribution of microbial isolates according to the time of infection.

Organism Identified	**EOS(N=65)**	**LOS(N=19)**	**Total**
Klebsiella spp	34	3	37
Escherchia coli	16	3	19
Acitenobacter spp	6	2	8
Staphylococcus aureus	3	1	4
Coagulase neagative Staphylococci	3	10	13
Enterobacter Spp	3	0	3

5.2 Antibiotic resistance pattern of the isolates

5.2.1 Gram-positive bacteria

The majority of Gram-positive bacteria isolates came from LONS, suggesting that they were hospital-acquired infections.First-line, second line antibiotics (Ampicillin and Gentamycin) and third generation cephalosporins were highly resisted by most gram positive bacteria. The resistance rates of *CoNS and S. aureus* against Ampicillin were 11(84.6%) and 3(75%) and respectively (Table 4). Similarly, the resistance rates of these two organisms to Gentamycin were

9 (69.23) and 3(75%) respectively. Isolated Gram-positive bacteria showed better susceptibility patterns for Vancomycin, Clindamycin, Ciprofloxacin and Chloramphenicol (Table 4).

Table 4: Antimicrobial resistance patterns of isolated gram positive bacteria at SPHMMC**;** March 2020 –July 2020 G.C

	CoNS 13 n (%)	*S.aureus* 4 n (%)
Ampicillin	11(84.6)	3(75)
Gentamycin	9(69.23)	3(75)
Ceftriaxone	7(53.84)	3(75)
Ciprofloxacin	3(23.07)	0(00)
Cotrimoxazole	10(76.92)	1(25)
Vancomycin	3(23.07)	0(00)
Chloramphenicol	6(46.15)	0(00)
Clindamycin	2(15.38)	0(00)
Erythromycin	6(46.15)	2(50)
Cloxacillin	13(100)	3(75)
Ceftazidme	8(61.53)	4(100)

5.2.2 Gram-negative bacteria

In the current study, isolated Gram-negative bacteria were also highly resistant to commonly used empiric antibiotics (Table 5). *E. coli* and *Klebsiella* species were extremely resistant to Ampicillin [13 (68.4) and 36 (97.2) respectively]. Similarly, these bacteria were also highly resistant against Gentamycin [11 (57.8) and 35 (94.59) respectively]. *E. coli* and *Klebsiella* resistance rates against Cefotaxime, one of the commonly used third- generation Cephalosporin in our neonatal departement were also high. Chloramphenicol, Ciprofloxacin and Amikacin showed more effectiveness against identified Gram-negative bacteria.

Table 5: Antimicrobial resistance patterns of isolated gram-negative bacteria, SPHMMC**;** March 2020 –July 2020 G.C

	Klebsiella spp *N=37, n (%)*	*E.coli* *N=19 ,n (%)*	*Acetinobacter spp* *N=8 n (%)*	*Enterobacter spp* *N=3 n (%)*
Ampicillin	36(97.2)	13(68.4)	8(100)	2(66.66)
Gentamicin	35(94.59)	11(57.8)	8(100)	2(66.66)
Cefotaxime	35(94.59)	13(68.4)	6(75)	1(33.33)
Ciprofloxacin	9(24.32)	5(26.3)	2(25)	1(33.33)
Cotrimoxazole	27(72.9)	10(52.63)	8(100)	2(66.66)
Chloramphenicol	20(54.05)	8(42.1)	NA	NA
Amikacin	13(35.13)	4(21)	2(25)	1(33.33)
Erythromycin	30(81.08)	13(68.4)	NA	NA
Ceftazidme	25(67.56)	19(100)	7(87.5)	3(100)

- NA-not applicable

5.2.3 Multidrug-resistant (MDR) bacterial isolates

The majority of bacterial isolates from blood cultures were found to be multidrug-resistant, with resistance to first- and second-line antibiotics being the most common.MDR found in15 (78.94%) *E. coli*, 36 (97.29%) of *Klebsiella spp*.,8 (11.94%) of *Acitinobacter spp*,2(66.67%) of *Enterobacter spp*, 3(75%) of *S. aureus* and 9(69.23%) of *CONS*.

Table 6: Multi drug resistance (MDR) level of the bacterial isolate from blood among septicemia suspected neonates patients at SPHMMC Hospital Medical College. (March 2020 –July 2020 G.C)

Bacterial isolate	***Anti-microbial resistance*** (%)					
	Total	R0	R1	R2	R3	R4
Gram Negative	**67(79.78%)**	0(0)	1(1.49)	5(7.46)	51(76.12)	10(14.92)
E.coli	19(28.36)	0(0)	1(5.26)	3(15.79)	11(57.90)	4(21.05)
Klebsiellaspp	37(55.22)	0(0)	0(0)	1(2.70)	31(83.78)	5(13.51)
Acitinobacterspp	8(11.94)	0(0)	0(0)	0(0)	7(87.5)	1(12.5)
Entrobacterspp	3(4.48)	0(0)	0(0)	1(33.33)	2(66.66)	0(0)
Gram Positive	**17(20.23%)**	0(0)	1(5.88)	4(23.53)	11(67.70)	1(5.88)
S. aureus	4(23.52)	0(0)	0(0)	1(25)	3(75)	0(0)
CONS	13(76.47)	0(0)	1(7.69)	3(23.08)	8(61.54)	1(7.69)
Total	**84(100)**	0(0)	2(2.38)	9(10.71)	62(73.80)	11(13.09)

R0- No antibiotic resistance, R1- Resistance to one, R2-Resistance to two, R3-Resistance to three, R4- ≥Resistance to four

6. Clinical outcomes of neonates

Outcome measure noted were recovery and death due to illness during hospital stay. Majority of the patients 372 (93%) were recovered from their condition with improvement. A total of 28 (7%) mortality was recorded (Figure 5).Out of 28 deaths premature 21 (75%) and low birth weight babies 23 (82.14%) constitute major group.

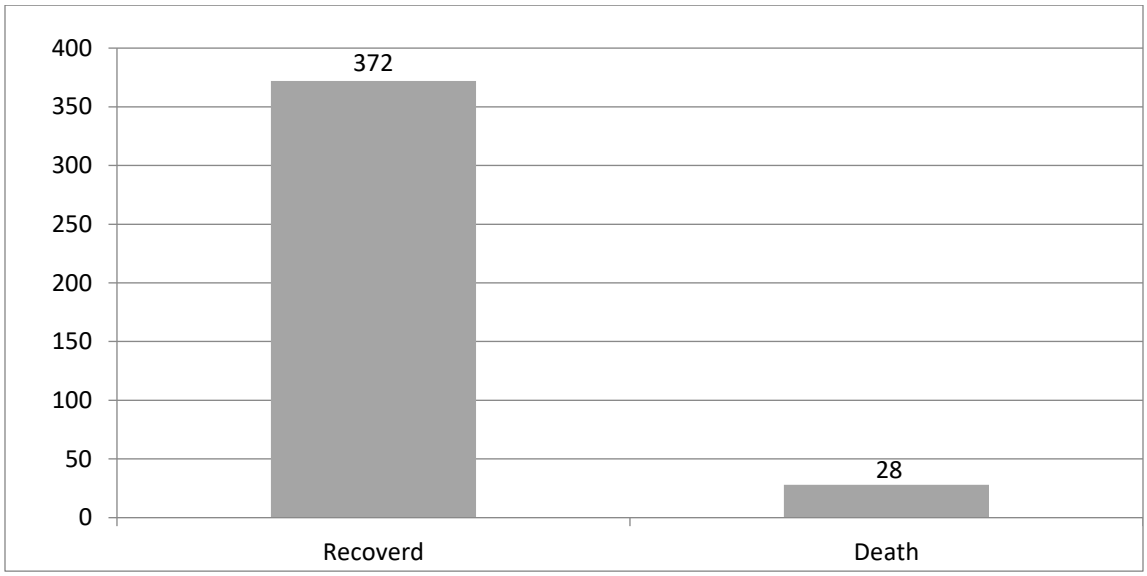

Figure 4: Clinical outcomes of neonatal sepsis at St Paulo's Hospital, from March 2020 to July 2020G.C.

Among neonatal patients who showed chronic infection 91.27% were recovered and the rest 8.72% dead. Neonates who are connected with indwelling Medical device 92.92% were recovered and 7.07% were dead. Neonates with birth weight of ≤2.5kg show high number of death (13.85%) than from neonates who were in weight >2.5kg(2.13%). Number of neonates who were born with ≤37 week were 118, from those neonates 21 were dead because of sepsis. On the other hand, neonates with LONS showed high number of death (17.28%) than neonates who showed EONS (4.38%). (Table 7)

Table 7: Socio-demographic characteristics and back ground information with outcome at SPHMMC.

		Total	Recovered (N=372)	Death (N=28)	COR	(95% CI)	P-value
Age	0-7 days	364	341(93.68%)	23(6.31%)			
	8-28 days	36	31(86.11%)	5(13.88%)	2.391	0.850-6.729	0.099
Sex	Male	246	230(95.83%)	16(6.50%)	0.823	0.378-1.791	0.624
	Female	154	142(92.20%)	12(7.79%)			
Birth weight	≤2.5kg	166	143(86.14%)	23(13.85%)	7.366	2.739-19.813	0.000
	>2.5kg	234	229(97.86%)	5(2.13%)			
Gestational age	≤37 week	118	97(82.20%)	21(17.79%)	8.505	3.506-20.632	0.000
	>37 week	282	275(97.51%)	7(25%)			
Nutritional status	Malnutrited	14	7(50%)	7(50%)	17.381	0.5.580-54.142	0.000
	Non malnut	386	365(94.55%)	21(5.44%)			
HIV status	Negative	385	361(93.76%)	24(6.23%)	0.183	0.054-0.617	0.006
	Positive	15	11(73.33%)	4(26.66%)			
Clinical manifestation	EOS	319	305(95.61%)	14(4.38%)	0.220	0.100-0.482	0.000
	LOS	81	67(82.71%)	14(17.28%)			

As shown in table 7, using binary logistic regression model, there was a statistical significant difference between dead neonates with birth weight (p=0.000), Gestational age (p=0.000), Nutritional status (p=0.000), HIV status (p=0.006) and clinical manifestations (p=0.000)compared to those recovered.

7. Discussion

Neonatal sepsis is widespread and contributes to mortality among neonates admitted to St Paul's Hospital, according to the findings of this report. In this study, the prevalence of positive blood culture sepsis was 21%.The prevalence of this study was less than reports from hospital-based and cross-sectional research in Ethiopia (9, 24, 26) and other developing countries (19, 20).

In this study, 65 (77.8%) and 19 (22.61%) neonates were presented with EOS and LOS respectively. The study showed that EOS was more common than LOS, which is in agreement with former studies done in Ethiopia (27,28) and other countries, including Madagascar and Tehran (23 ,13) . On the other hand LONS were more common in India, according to other studies (6,10).

The most common signs and symptoms in our study was respiratory distress (58.5%), fever (12.3%) and Jaundice (10.5%).Fever, refusal to feed, respiratory distress, poor activity, and neonatal jaundice were the most common symptoms in a similar study (29), although other studies (30, 31 and 32) recorded fever, refusal to feed, respiratory distress, poor activity, and neonatal jaundice as the frequent symptoms. Gram Negative bacteria were the most commonly isolated organisms causing neonatal sepsis in this finding which is in congruent with study reports from Nigeria, Madagascar, India & Nepal (20,23 and 33) but not congruent with Tehran , Ghana, and China (13,19, and 34)

In EONS gram-negative bacteria were account for majority cases while gram-positive bacteria were common in LONS. This is similar with former study in Ethiopia and other developing countries including Nigeria. Majority of the cases of neonatal sepsis were caused by *Staphylococcus aureus, E. coli, or Klebsiella spp*., according to findings from a study of neonatal sepsis in 19 developing countries.*GBS* had reported as the leading pathogen causing neonatal sepsis in Malawi (21, 25).

Guidelines on neonatal sepsis management in most centers (35, 36) recommend Ampicillin and Gentamycin as first-line empiric therapy. Unfortunately, most of the bacteria found in this study

were highly resistant.Egypt and India (35, 37) showed high resistance rates of isolated bacteria against Ampicillin (85–95%) and Gentamycin (57.3–72%). The high rate of antimicrobial resistance (AMR) may indicate that the named drugs were overused as empiric treatment for most other common neonatal problems that were not infectious in nature. Furthermore, the majority of neonates with bacteremia confirmed by culture were born in a health facility, where the majority of neonatal sepsis is caused by hospital-acquired infections.

Resistance rates of isolated Gram-positive bacteria against third generation Cephalosporines were also high in our study which is consistent with studies from Nigeria, Tanzania and other developing countries (36, 38).These findings highlight the importance of conducting ongoing reviews of empirical antibiotics used in the treatment of neonatal sepsis to ensure that antimicrobial use is optimized.

Our study demonstrated a better susceptibility of isolated Gram-positive bacteria against Vancomycin, Clindamycin and Ciprofloxacin, which is also supported by the study from India and other reports (24). This may be clarified by the fact that these antibiotics are used less often for two reasons: First, antibiotics are only used as a last resort, showing that they are underutilized in the NICU. Second, Ciprofloxacin has not been validated for use in younger children unless a benefit-risk study justifies its use, and Amikacin is not readily available in most clinics, indicating a lower rate of utilization making most isolated bacteria better susceptible to these two antibiotics. *S. aureus* were found to be sensative for vancomycin which is in agreement with study findings from Vietnam and Egypt showed no resistance strains of *S. aureus* against Vancomycin (35, 39). This may be explained by an increase in the use of this antibiotic as a third-line antibiotic as most first- and second-line antimicrobial agents have failed, which is consistent with other research findings. (39,40,41).

The two most common Gram-negative bacteria isolates in our sample, *E. coli* and *Klebsiella spp*., were highly resistant to Ampicillin, Gentamycin, and third-generation Cephalosporines. Better susceptibility of *E. coli* and *Klebsiella spp*. for Ciprofloxacin and Amikacin were reported in my study which have been also demonstrated in other study findings (42). These two drugs could be a potential antibiotic of choice for empiric treatment of neonatal sepsis in the future.

Finally, the outcome of neonates with infections is highly dependent on timely diagnosis and treatment Diagnosing neonatal infection, however, is a challenge, since 28 (93%) died in the hospital. The current study's mortality rate was comparable to results from other Ethiopian studies (27). However, in India, extremely high rates of mortality have been recorded (41, 42).

8. Limitations

- Because of the occurrence of corona virus in our country, the data collection and analysis were delayed.
- This study did not determine associated risk factors for neonatal sepsis.
- A multidisc used for the culture did not contain methicillin. As a result, sensitivity tests for S. aureus were not possible.
- Anti-microbial drugs used in the current study are not enough due to budget shortage.

9. Conclusion

According to our findings, the most common causes of neonatal sepsis was *CoNS, S. aureus, E. coli,* and *Klebsiella spp.* These bacteria isolates were particularly resistant to the first- and second-line empiric antimicrobials used in neonatal departments while neonatal sepsis was being treated. Antibiotics in the third line are moderately successful against isolated bacteria. Among the total neonate, 65(77.38) were diagnosed as EONS . Most of the patients with 372 (93%) were recovered and discharged.

10. Recommendation

Antibiotic overuse is the most significant contributory factor in the production of AMR, and continuous monitoring is needed to keep national antimicrobial therapy guidelines up to date. To minimize neonatal morbidity and mortality, it is recommended that coexisting infections be investigated using a selection of other clinical samples in addition to blood samples.

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Annex

Annex 1: English version of participant information sheet for gurdians

I. Participant information sheet

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Title of the Research Project: The Bacteriological profile, Antimicrobial resistance and outcome of neonatal sepsis among patients at SPHMMC Addis Ababa, Ethiopia.

First of all I would like to thank you in advance for your cooperation and consent in participation in this study. Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely.

Background information

Background: Neonatal septicaemia causes high morbidity and mortality worldwide especially in developing country. Therefore the knowledge of the causative agents of neonatal septicaemia and antimicrobial susceptibility pattern will be helpful in the control and selection of empiric antimicrobial therapy.

Aim of the study:

The purpose of this study is to determine the Bacteriological profile, Antimicrobial resistance and outcome of neonatal sepsis among patients at SPHMMC Addis Ababa, Ethiopia.

Benefits for participants

Study participants will not have any financial incentives or other inducements from participating on this study. However, based on the diagnosis result you will be treated accordingly. Most importantly, the result of the study will be beneficial to design effective prevention and control measure for neonatal septicaemia. Hence, you are indirectly benefiting other patients and the society in this respect.

Risks and complication

There are no anticipated risks to your participation. As routine laboratory procedure blood sample will be taken once from your peripheral vein. During sample collection you may feel some discomfort but this does not produce serious pain.

Confidentiality

There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential. Participants will not be prohibited to stop or withdraw at any time from the study. Only interested participants can retrieve their own lab result using their code number. The information collected about you will be coded using numbers. No personal information will be disclosed to third party or will not appear in any report from this study.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Merema sherif (PI)

Signature: _____ Date: _____

Note: If you have any questions about this study, you should feel free to ask now or anytime throughout the study by contacting

PI Address: Merema sherif: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: Meryemsh20@gmail.comMobile-0919199601

Department of medical laboratory sciences, CHS, AAU, Tel-0112755170

II. Consent form

I have been informed about the study which plans to determinethe Bacteriological profile, Antimicrobial resistance and outcome of neonatal sepsis among patients at Addis Ababa, Ethiopia.The objective and the application of the study were briefly explained to me. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my child‘s overall health care. It is therefore with full understanding of the situation that I agreed to give the informed assent voluntarily to the researcher to give my child‘s blood for the mentioned study. I agreed that the specimen would be tested for septicaemia. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I was also informed that results for the analysis of blood will be given to the Doctor who follow my child and that I may ask the information if I want. I _____ hereby give my assent for giving of the requested information and my child‘s blood for this study.

Participant code:_____ Signature: _____ Date: _____

III. Questionnaire

Addis Ababa University College of Health Sciences, Department of Medical Laboratory Science.

Questionnaire for the demographic characteristics and assessment of risk factors of pediatrics septicemia who visited SPHMMC.

Patient Identification: - _____

Facility name _____ Year _____ Participant code _____

Participants address (Sub city) _____ Telephone _____ signature_____

Name of the ward _____Block_____

Data collector name _____date_____signature_____

I. Socio- Demographic Characteristics of the Study participants.

1. Age _____

2. Sex a) Male ☐ b) Female ☐

3. Number of Sisters and Brothers_____

4. Monthly income per individual (in Ethiopian birr) _____

II. Septicemia etiologic agent questions

5. Ward of the neonate a) OPD ☐ b).EPD ☐ c). NICU ☐

6. Date of admission (in the hospital)(dd/mm/yy)_____

7. When did the patient manifest the clinical future of septicemia?

a) 48 hr before admission ☐ b) 48 hr after admission ☐

8. Clinical features of the pediatrics

a, Abdominal distension. ☐ b, Difficulty in breathing ☐ c, Refusal of feeds ☐

d, Tachycardia ☐ e, Lethargy ☐ f, Jaundice ☐

g, Chills ☐ h. Shock ☐ i, Fever . ☐

j, Vomiting ☐

9. Body temperature a) <36.5 ☐ b).>38.5°C ☐

10. Is there any underlying chronic disease (pneumonia, wound infection, anemia etc…) °C

a)Yes ☐ b). No ☐

11. If you say yes for "9" specify_____

12. HIV status of the neonate a). Positive ☐ b). Negative ☐

13. Is there any indwelling medical device? a). Yes ☐ b). No ☐

14. If you say yes for no.13, what kind of device?

a). Intravenous devices ☐ b). Endotracheal ☐

c). Urinary catheters ☐ d). Ventilator support ☐

e). Other ☐

15. Antibiotics given after sampling_____

16. Nutritional status of the mother

a). Malnourished ☐ b). Non malnourished ☐

17. Weight at birth? 1) <2.5 Kg ☐ 2) > 2.5 Kg ☐

18. Gestational age _____

1) <37 weeks ☐ 2)>37 weeks ☐

19. Is there any congenital anomalies a) Yes ☐ b) No ☐

20. Neonatal outcome a, Good ☐ b, Poor ☐

III. Laboratory Data

21. Date of specimen collection_____

22. Media used _____

23. Gram stains result _____

24. Biochemical test_____

25. Organism isolated _____

26. Drug susceptibility pattern

a. Sensitive to _____

b. Intermediate to _____

c. Resistance to _____

IV. Comments

Name of principal investigator _____

Signature _____

Date _____

Annex 2: Procedure for specimen collection, processing and result interpretation

I. Laboratory procedure for collection and culturing of Blood

1. Using a pressure cuff, locate a suitable vein in the arm. Deflate the cuff while disinfecting the vein puncture site.
2. Wearing gloves, thoroughly disinfect the vein puncture site as follows:

-Using 10% tincture of iodine and a circular action, swab the area beginning at the point. -Using 70% ethanol, cleanse an area about 50 mm in diameter. Allow to air-dry.
3. Lift back the tape or remove the protective cover from the top of the culture bottle(s). Wipe the top of the bottle using an ethanol-ether swab.
4. Using a sterile syringe and needle, withdraw about 1-2 ml of blood per culture for neonates.
5. Insert the needle through the rubber liner of the bottle cap and
6. Dispense accordingly into the trypto soya broth culture medium bottle.
7. Using a fresh ethanol-ether swab, wipe the top of each culture bottle and replace the tape or protective cover(s). Without delay, mix the blood with the broth.

Important: The blood must not be allowed to clot in the culture media because any bacteria will become trapped in the clot.

8. Clearly label each bottle with the name and number of the patient, and the date and time of collection.
9. As soon as possible, incubate the inoculated media. Protect the cultures from direct sunlight until they are incubated.

II. Laboratory procedure for Gram staining technique

1. Labeling the slides clearly with the date and patient's name and number.
2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.
3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.
4. Fix the dried smear by using heat or alcohols (methanol).
5. Cover the fixed smear with crystal violet stain for 30-60 seconds.
6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.
7. Tip off all the water, and cover the smear with lugol's iodine for 30-60 seconds.
8. Wash off the iodine with clean water.
9. Decolorize rapidly (few seconds) with 3% acetone alcohol. Wash immediately with clean water.
10. Cover the smear with neutral red or safranine stain for 2 minutes.
11. Wash off the stain with clean water
12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.
13. Examine the smear microscopically, first with the 40 X objective to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Result

- Gram positive bacteria ----------dark purple
- Gram-negative bacteria ----------pale to dark red

III. Laboratory procedure for Biochemical testing

Biochemical tests for gram positive bacteria:

Gram-positive cocci were identified based on their gram reaction, catalase, coagulase and manitol salt agar tests results.

Catalase test

Catalase test to differentiate staphylococci which produce the enzyme catalase from streptococci which are non- catalase producing.

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer.

Procedure

1. Pour 2-3 ml of 3% hydrogen peroxide to a test tube
2. Using a sterile wooden stick take the test organism & immerse into theH_2O_2solution.56
3. Look for immediate bubbling

Interpretation

Active bubbling . . . . . Positive catalase test

No bubbles . . . . . .Negative catalase test

Control

Positive catalase control: Staphylococcus species

Negative catalase control: Streptococcus species

Coagulase test

This test is used to identify S. aureuswhich produces the enzyme coagulase

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

Procedure

1. Place a drop of physiological saline on two separate slides
2. Emulsify the test organism in each of the drop to make thick suspension
3. Add one drop of plasma to one of the suspensions and mix gently. Look for clumping of the organism within 10 seconds

Interpritation

Clumping within 10 secs . . . . . S. aureus/ S.lugdnesis

No clumping within 10 secs . . . . . No bound coagulase

Controls

Positive coagulase control: Staphylococcus aureus/ S.lugdnesis

Negative coagulase control: Escherichia coli.

Manitol salt agar test

Principle

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Beef Extract provide the nitrogen, vitamins, and carbon in Mannitol Salt Agar. D-Mannitol is the carbohydrate source. In high concentrations, Sodium Chloride inhibits most bacteria other than staphylococci. Phenol Red is the pH indicator. Agar is the solidifying agent.Bacteria that grow in the presence of a high

salt concentration and ferment mannitol produce acid products, turning the Phenol Red pH indicator from red to yellow. Typical pathogenic *staphylococci* ferment mannitol and form yellow colonies with yellow zones. Typical non-pathogenic staphylococci do not ferment mannitol and form red colonies.

Procedure

Inoculate specimen on medium as a primary isolation or inoculate isolated colonies onto medium for differentiation**.** Incubate at 37ºC for 24 hour. Look for colony morphology.

Result

Positive: yellow colony and may have a yellow halo around the colony.

Negative: no growth of bacteria (*E.coli*) or growth with colorless or pink colony (CONs)

Biochemical test for gram negative bacteria Identification of gram negative bacteria will be based on their test result with a series of biochemical tests.

Indole test:

Principle The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethyl aminobenzaldehyde. This reacts with the indole to produce a red coloured compound. Kovac's reagent is recommended in preference to Ehrlich's reagent for the detection of indole from enterobacteria.

Method

1. Inoculate the test organism in a bijou bottle containing 3 ml of sterile tryptone water.
2. Incubate at 35–37 ^{O}C for up to 48 hr.
3. Test for indole by adding 0.5 ml of Kovac's reagent. Shake gently. Examine for a red colour in the surface layer within 10 minutes.

Interpretation

Red surface layer . . . . . Positive indole test

No red surface layer . . . . . Negative indole test

Urease test (Christensen's (modified) urea broth):

Principle The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red.

Procedure

1. Inoculate heavily the test organism in a bijou bottle containing 3 ml sterile Christensen's modified urea broth.
2. Incubate at 35–37 ^{O}C for 3–12 h (preferably in water bath for a quicker result).
3. Look for a pink color in the medium.

Interpretation

Pink color . . . . . Positive urease test

No pink color . . . . . …. . . Negative urease test

Triple Sugar Iron (TSI) Agar Slant

Principle

TSI agar tests are used to determine whether gram negative bacilli utilize glucose and lactose or sucrose fermentative and produce hydrogen sulfide. It contains 1% lactose, 1% sucrose and0.1% glucose and peptone. Phenol red and ferrous sulphate serves as an indicator for acidification of medium and H_2S production.

Procedure

1.Using a sterile inoculating needle, stab the butt of the TSI agar slant twice then streak back and forth along the surface of the agar with the organism.

2. Incubate at 37^{O}C for 18 to 24 h.

3. Look for the color change and gas production.

Interpretation

If acid slant–acid butt (yellow–yellow): glucose and sucrose and/or lactose fermented.

If alkaline slant–acid butt (red–yellow): glucose fermented only.

If alkaline slant–alkaline butt (red–red): glucose not fermented.

The presence of black precipitate (butt) indicates hydrogen sulfide production, and

Presence of splits or cracks with air bubbles indicates gas production.

Manitol test

Principle

The test organism is cultured on a medium which contains manitol. The microbe can ferment the carbohydrate (sugar) manitol as a carbon source. If manitol fermented to produce acid end product, the ph indicator phenol red changes to yellow.

Procedure

1. Inoculums from pure colony inoculated in a test tube of manitol broth.

2. Incubate at 35-37 ^{O}C for 24 hr

3. Look for yellow color in the medium

Interpretation

Yellow colour……..Positive manitol test

Red colour …….Negative manitol test

Citrate utilization test using Simmon's citrate agar

Principle The medium contains citrate as the sole source of carbon and inorganic ammonium salt as the sole source of nitrogen. Bacteria that can grow on this medium produce an enzyme, citrate-permease, capable of converting citrate to pyruvate. Pyruvate can then enter the organism's metabolic cycle for the production of energy. Growth is indicative of utilization of citrate, an intermediate metabolite in the Krebs cycle.

Procedure

1. Streak the slant back and forth with light inoculums picked from the center of a well isolated colony.
2. Incubate aerobically overnight at 35–37 ^{O}C for up to 4-7days.
3. Observe a color change from green to blue.

Interpretation

Blue. . . . . Positive citrate test

Green. . . . . …….. . Negative citrate test

Controls

A positive citrate test reaction is obtained with *Klebsiella pneumonia* and a negative reaction with *Escherichia coli*.

Motility Test (using motility agars):

Principle Motility agar will be prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility will be examined after 35^{O}C for 24 hour. Motility will be indicated by the presence of diffuse growth (appearing as coloring of the medium) away from the line of inoculation. But if the bacteria are non-motile, the growth of the bacteria will be along the stab, diffusion will not occur.

Oxidase test

Principle

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be used When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour.

Procedure

1. Place a piece of filter paper in a clean petri dish and add 2 or 3 drops of freshly prepared oxidase reagent.
2. Using a piece of stick or glass rod (not an oxidized wire loop), remove a colony of the test organism and smear it on the filter paper.
3. Look for the development of a blue-purple colour within a few seconds.

Interpretation

Blue-purple color . . . . . Positive oxidase test (within 10 seconds)
No blue-purple color . . . ….. . Negative oxidase test (within 10 seconds)

Controls

Positive oxidase control: *Pseudomonas aeruginosa*
Negative oxidase control: *Escherichia coli*

III. Laboratory procedure for Antimicrobial sensitivity testing

Procedure

Emulsify colonies of similar appearance in small volume of nutrient broth. Match the turbidity of the suspension against the turbidity standard which has a similar appearance to an overnight broth culture.

1. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).
2. Spread the inoculum evenly over the Muller-Hinton agar plate with the swab
3. Using a similar inoculation technique, inoculate an overnight broth culture of the Control organism evenly across the upper and lower third of the plate.
4. Using a sterile forceps or needle , place the antimicrobial disc on the inoculated plate
5. Incubate the plate aerobically at 35 ^{O}C for 18-24 hours.
6. Read the tests after checking that the bacterial growth of the test and control organism is neither too heavy nor too light.
7. Measure the radius of the inhibition zone. Interpret result based on the inhibition zone.

Sensitivity (S): Zone of radius is wider than, equal to, or not more than 3mm smaller than the control.

Intermediate (I): Zone radius is more than 3mm smaller than the control but not less than 3mm.

Resistant (R): No zone of inhibition or zone radius measure 2mm or less.

Annex 3: Amharic version of participant information sheet, consent & questionnaire

I. የተሳታፊዎችየመረጃቅፅ

አዲስአበባዩኒቨርሲቲየጤናሳይንስኮሌጅየህክምናላብራቶሪሳይንስዲፓርትመንት

አርዕስት፡-
በአዲስአበባከተማቅዱስጳውሎስሆስፒታልየህጻናትየደምውስጥበሽታአምጭተህዋስያንስርጭት፤ ለተለያዩጸረባክቴሪያያላቸውአይበገሬነትናየበሽታውንአጋላጭሁኔታዎችንስለማጥናት

አጠቃላይመረጃ፡-

በጥናቱበመሳተፍዎከልብእያመሰገንንከመወሰንዎበፊትይህንቅፅበትክክልአንብቡወይምሲነበብልዎበ ትክክልያዳምጡ፤እንዲሁምግልጽያልሆነልዎትንነገርበሙሉበነፃነትይጠይቁ

ስለጥናቱመረጃ፡-

የህጻናትየደምውስጥተህዋስያንበሽታበአለምአቀፍደረጃበተለይምበታዳጊሀገራትከፍተኛየሆነህመምና ሞትያስከትላል፡፡በተደረጉጥናቶችየተለያዩአጋላጭሁኔታዎችእንዳሉለማወቅተችለል፡፡ስለዚህየበሽታ ውንአምጭተህዋስያንስርጭትናለጸረተህዋስያንያላቸውንግትርነትማወቅበሽታውንለመቆጣጠርናለ መከላከልእንዲሁምተገቢውንመድሃኒትለማዘዝይረዳል፡፡

የጥናቱአላማ፡-

በቅዱስጳውሎስሆስፒታልየህጻናትየደምውስጥበሽታአምጭተህዋስያን(ባክቴሪያ)ስርጭት፤ለተለያዩጸ ረባክቴሪያያላቸውግትርነትናየበሽታውአጋላጭሁኔታዎችንማጥናትናማወቅነው

ጥናቱለተሳታፊዎችያለውጥቅም፡-

በጥናቱለሚሳተፉፍቃደኛተሳታፊዎችምንምአይነትየገንዘብክፍያየለምነገርግንበምርመራውውጤትመሰረትየመታከምእድልይኖሮታልበተጨማሪምየጥናቱውጤትየደምውስጥህመምለመቆጣጠርናለመከላከልስለሚጠቅምበተዘዋዋሪመንገድሌላህመምተኛእንዲሁምህብረተሰቡንየመጥቀምእድልያገኛሉ፡፡

በጥናቱተሳታፊዎችላይያለውጉዳትናተዛማጅችግር

በዚህጥናትበመሳተፍሊደርስብዎየሚችልአንድምጉዳትአይኖርምለዚህጥናትየሚያገለግልየደምናሙናየሚወሰድሲሆንከመጠነኛየህመምስሜትበስተቀርበጤናዎላይምንምጉዳትአይደርስም፡፡

የመረጃሚስጥራዊአጠባበቅ

መረጃበሚሰጡበትወቅትምሆነከዛበኋላባሉትጊዜያትሙሉበሙሉሚስጥራዊነቱየሚጠበቅናመረጃውምየሚያዘውበስምሳይሆንበመለያቁጥርይሆናልበጥናቱላይእያለበፊለጉትጊዜየማቆምወይምየማቋረጥመብትአልዎትየላብራቶሪውጤትዎንማወቅከፊለጉየመለያቁጥሮንበመጠቀምበሚሰጥዎየቀጠሮጊዜመውሰድይችላሉ፡፡

ጥናቱንየሚያካሂደውሰውማረጋገጫ

ለዚህጥናትሃላፊነቱንለመውሰድና፣ማናቻውንምጥናቱየሚመለከቱጉዳይክትትልለማድረግናለሚመለከተውአካልመግለጫለመስጠትበፊርማዬአረጋግጣለሁ፡፡

ፊርማ ---------- ቀን ----------

ማንኛውንምጥያቄመጠየቅለሚሸየሚቀጥለውንአድራሻዬንመጠቀምይችላሉ፡፡

ኢሜልMeryemsherif@gmail.comተንቀሳቃሽስልክ 0919199601

የህክምናላቦራቶሪሳይንስትምህርትክፍልስልክ፡ 0112755170

II. የፈቃደኝነትማረጋገጫቅጽ (ለቤተሰብ/አሳዳጊ)

በአዲስአበባከተማበቅዱስጳዉሎስሆስፒታልየህጻናትየደምውስጥበሽታአምጭተህዋስያንስርጭ
ትለተለያዩጸረባክቴሪያያላቸውአይበገሬነትናየበሽታውአጋላጭሁኔታዎችንለማጥናትበሚልርእ
ስላይበሚደረገውጥናትላይለመሳተፍሲሆንየጥናቱአላማናጥቅምበሚገባተገልያልኛልበመጠይቁላ
ይየሚሞላውየኔሙለመረጃምበሚስጥርእንደሚያዝተነግሮኛልበተጨማሪምበጥናቱውስጥልጄን
አለማሳተፍመብቴእንደሆነናበማንኛውምጊዜከጥናቱበራሴውሳኔመውጣትእንደምችልናበዚህም
ምክንያትምንምአይነትመጉላላትበልጄላይእንደማይደርስበሚገባተረድቻለሁስለሆነምሁኔታውን
በሚገባበማጤንበፍቃደኝነትልጄንበምርምሩላይለማሳተፍለተመራማሪውፍቃደኝነቴንሰጥቻለሁ
፡፡በተጨማሪምልጄየሚሰጠውየደምናሙናለተጠቀሰውጥናትብቻእንደሚውልተነግሮኝተስማም
ቻለው፡፡ማንኛውምያልገባኝንነገርየመጠየቅእድልተሰጥቶኝበሚገባኝቋንቋመልስአግኝቻለሁ፡፡
በተጨማሪምየሁለምየላብራቶሪምርመራውጤቶችበወቅቱክትትልለሚያደርገውየጤናባለሙያእ
ንደሚሰጥእናውጤቱንማወቅከፈለኩማግኘትእንደምችልተነግሮኛል፡፡

እኔ----------

የተባልኩግለሰብይህንሁለበመገንዘብበምርምሩላይስለልጄመረጃናየደምናሙናእንዲወሰድተስ
ማምቻለሁ፡፡

ፊርማ ---------- ቀን ----------

III. መጠይቅ

በአዲስአበባዩኒቨርሲቲየጤናሳይንስኮሌጅየሕክምናላቦራቶሪዲፓርትመንት

የጤናተቃሙስም----------ዓ.ም----------የጥናቱተሳታፊመለያቁጥር----------

አድራሻ፡ክ/ከ----------ስልክ----------ፊርማ----------

የታየበትክፍል----------ሀንጻ----------

የደምናሙናየወሰደውግለሰብስም----------ቀን----------ፊርማ----------

እባክዎንለጥናቱመሳካትያግዘንዘንድጥያቄዎችንበጥንቃቄእንዲሞለልንበትህትናእንጠይቃለን፡

1.ዕድሜ ☐

2.ጸታ

1.ወንድ ☐

2.ሴት ☐

3. የህፃኑየእህትናወንድምቁጥር

.....

4.የቤተሰብወርሀዊገቢ ☐

5.የታየበት/የተኛበትክፍል

1.ተመላላሽህክምናክፍል ☐

2.ተኝቶታካሚክፍል ☐

3.የህጻናትዕኑህሙማንክፍል ☐

4.ድንገተኛ ☐

6.አልጋ የያዘበትቀን----------

7.የበሽታውህመምናስሚትመታየትየጀመረውመቼነው

1. **አልጋከመያዙ ከ** 48 **በፊት** ☐

2. **አልጋከያዘከ** 48 **ሰዓትበኃላ** ☐

8.የበሽታውገጽታ

1.ትኩሳት ☐ 5.መዝለፍለፍ ☐ 8.የአተነፋፋ ☐

2.ማስመለስ ☐ 6. የሆድመነፋት ☐ 9.መልፈስፈስ/መዝለፍለፍ ☐

3.ብርድብርድማለት ☐ 7.አለመመገብ ☐ 10.እራስንመሳት ☐

4.የልብምትመፍጠን ☐

9.የሰውነትሙቀትመጠን

1. ከ 36.5 ሴንቲግሬድበታች ☐

2. ከ 37.5 ሴንቲግሬድበላይ ☐

10.ለብዙጊዜየቆየተያያዥበሽታአለ

1.አዎ ☐ ☐.የለም

11.ለአስረኛውጥያቄመልሱአዎከሆነምን----------

12.የ HIV ውጤት

1.ፖዝቲፍ ☐ 2.ኔጌቲፍ ☐

13.ወደሰውነትህየገባ/የተሰካ/ የተለጠፈየህክምናመሳሪያአለ

1.አዎ ☐ 2.የለም ☐

14.ለ 11ኛው ጥያቄመልሱአዎከሆነምንአይነትመሳሪያ

1. የደምቱቦመርፌ ☐ 3. የሽንትማሸኛቱቦ5.ለ☐ ☐

2. የአየርቱቦ4.☐ማናፈሻ ☐

15.የደምናሙናከተወሰደበኃላየተሰጠጸረተህዋሲያን----------

16.የእናትየዋየስርአተምግብሁኔታ

1. በምግብእጥረትየተጎዳ ☐

2. በምግብእጥረትያልተጎዳች ☐

17.ህጻኑእንደተወለደ/ች የሰውነትክብደት

1. ከ 2.5 ኪ.ግበታች ☐
2. ከ 2.5 ኪ.ግበላይ ☐

18.ከጽንሰትእስከውልደትያለእድሜ

1. ከ 37 ሳምንትበታች ☐
2. ከ 37 ሳምንትበላይ ☐

19.አብሮየተወለደየጤናችግርአለ

1.አዎ ☐
2.የለም ☐

20.የህፃኑየመጨረሻየህክምናውጤት

1. ጥሩ ☐
2. መጥፎ ☐

Declaration

Declaration I, the undersigned agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office.

M.Sc. candidate: **Merema sherif (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor:

Kassu Desta (MSC, PhD Candidate) **DessieAbera (MSC)**

Signature: _____ Signature: _____

Date: _____ Date: _____

Place: _____

Addis Ababa, Ethiopia.