ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMEN OF MEDICAL LABORATORY SCIENCES

Bacterial profile, antimicrobial susceptibility pattern and associated risk factors among children suspected for septicemia at St. Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia

By: Hindiya Lema (BSc)

Advisor(s): Mr. Dessie Abera (MSc)

Mr Melese Hailu (MSc)

A thesis submitted to department of Medical Laboratory Science, College of Health Sciences, and Addis Ababa University in partial fulfillment of the requirements for the degree of Masters in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology specialty).

July, 2022

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by HINDIYA LEMA , entitled: "Bacterial profile, antimicrobial susceptibility pattern and associated risk factors among septicemia suspected pediatrics 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.

Signed by the Examining Committee:

External Examiner _____Signature _____Date _____

Internal Examiner _____Signature _____ Date _____

Advisor _____Signature _____Date _____

Advisor _____Signature _____Date _____

_____

Chairperson of the Department of Graduate Program Coordinator

Acknowledgement

Firstly, I would like to express my gratitude to Department of Medical Laboratory Sciences and College of Health Sciences, AAU for providing me with the opportunity to conduct this research. Secondly, the microbiology department personnel and the pediatrics department of St Paul Millennium Medical College for their assistance in data collection data analysis and write up of this paper.

I also thanks to all the parents and guardians of the pediatrics who participated in this study for their contribution and also for making it possible.

My special thanks & gratitude goes to my Advisors, Mr. Dessie Abera (MSC) and Mr. Melese Hailu (MSc) for their great advice, direction, and support throughout the preparation of my research paper.

Table of Contents page no

List of Tables

page no

List of Figures **page no**

List of abbreviation

AAU:	Addis Ababa University
BSI:	Blood Stream Infection
CA:	Community acquire
CI:	Confidence Interval
CLSI:	Clinical and Laboratory Standards Institute
CONs:	Coagulate Negative Staphylococci
COR:	Crude odds ratio
DST:	Drug Susceptible Test
GNR:	Gram negative rods
GPC:	Gram positive cocci
HA:	Hospital acquired
HCA:	Health care-associated
HIV:	Human Immune Deficiency Virus
LBW:	Low Birth Wight
MDR:	Multi Drug Resistance
NICU :	Neonatal Intensive Care Unit
OPD:	Outpatient Department
SPSS:	Statistical package for social sciences
TSB:	Trypto soya broth
WHO:	World health organization

Abstract

Background: Septicemia in children is associated with a high rate of morbidity and mortality. As a result, the goal of this study was to find bacterial etiologies, antimicrobial resistance (AMR) profiles, and risk factors for septicemia and mortality in children aged 5 to 14.

Methods: Between August and December 2021, a hospital-based cross-sectional study was undertaken at St. Paul's Hospital Millennium Medical College. Each patient's socio-demographic and clinical information was gathered using a pre-designed questionnaire. A blood sample was taken aseptically from each patient and inoculated into Trypto Soya Broth. Each blood culture bottle was aerobically incubated for 7 days in a row. Blood agar, chocolate agar, and MacConkey agar were used for subculturing. Gram stain, colony features, and biochemical tests were used to describe positive cultures. Kirby-disk Bauer's diffusion method was used to test drug susceptibility. All data was entered into Microsoft Excel, exported, and analyzed with SPSS version 24 software. Statistical significance was defined as a P value of less than 0.05.

Results: Among 360 study participants, 21.3 %(n=77/360) showed bacterial growth, where 80.5 %(n=62/360) and 19.4 %(n=15/360) were gram negative and gram positive bacteria respectively. Commonly isolated organisms were *Klebsiella spp 54.8 %(n=34/360)*, *E. coli 30.6 %(n=19/360) and Coagulase negative Staphylococci* 17.7 %(n=11/360). Birth weight, underlying chronic disease, congenital anomalies, was associated with positive blood culture. Antibiotic resistance was found in both Gram positive and Gram-negative bacteria. Ciprofloxacin and Amikacin was the most effective antibiotic for gram negative bacteria while for gram positive bacteria Vancomycin, Clindamycin and Ciprofloxacin were effective drugs.

Conclusion: This study discovered a high frequency of antimicrobial resistance (AMR), particularly in gram negative bacteria. Gram-negative bacteria were the most common cause of Septicemia in children. The most prevalent pathogen in this investigation was Klebsiella pneumoniae.

Key words: septicemia, bacteriological profile, antimicrobial susceptibility pattern, children Addis Ababa Ethiopia

1. Introduction

1.1 Background

Septicemia is described as the presence of bacteria in the blood and is frequently accompanied with severe illnesses, earning it the nicknames "blood poisoning," "bacteremia with sepsis," and "systemic inflammatory response syndrome" [1]. Fever, trouble breathing, tachycardia, malaise, refusal of feeds/lethargy are all symptoms of septicemia in children, which can progress to significant complications such as shock, multi-organ failure, and DIC, all of which necessitate prompt antibiotic treatment. As a result, bloodstream infections are one of the most dangerous circumstances, and it is critical to identify the causal organism by blood culture (gold standard) as soon as possible [2].

Sepsis is a bloodstream illness caused by pathogenic bacteria that is usually diagnosed based on clinical suspicion [3]. In youngsters, however, the symptoms are frequently non-specific, and the clinical course can be fulminate, fast developing to a medical emergency requiring immediate intervention [4].

Sepsis is one of the most common causes of death in newborns and children around the world [5]. Bacteremia can range from self-limiting infections to life-threatening septicemia, all of which require urgent and sensible antibiotic therapy. Changing epidemiology, a lack of standard antimicrobial recommendations in the community, the evolution of antibiotic resistance, and a scarcity of excellent diagnostic facilities are important denominators for the rise in BSI-related morbidity and mortality in developing countries [5].

Because the bacteriologic profile of septicemia in children is always changing, it's important to keep track of the causing organisms and their drug susceptibility patterns. Despite the fact that blood cultures are not always positive in all cases of septicemia, they remain the gold standard for bacterial agent detection. Because of the significant frequency of multidrug-resistant bacterial strains, infections with gram negative bacteria pose a more serious therapeutic challenge, particularly in ICU patients [6].

Multiple organ failure, complications, and death may be predicted by epidemiological characteristics and clinical findings on admission. Identifying these factors could help to improve and update sepsis diagnosis and treatment protocols. In addition, knowing the present etiological profile of sepsis, as well as the potential influence of immunization programs, is critical for guiding antibiotic therapy and lowering the risk of death [7].

S. aureus and E.coli have been recognized as common causative agents for pediatric blood stream infection (BSI) in industrialized countries (Europe and the United States). S.aureus, Klebsiella spp, and Salmonella spp are the most prevalent causative agents of pediatric bloodstream infection in poor countries such as Sub-Saharan Africa [8].

1.2 Statement of the problem

Septicemia is particularly common in pediatrics' age groups and is one of the leading causes of morbidity and mortality worldwide [9]. Furthermore, most basic and secondary health care facilities do not routinely do bacterial culture to detect bacterial infection, complicating the challenge in underdeveloped nations [10].

In Africa, the epidemiology of pediatric bloodstream infection (BSI) is poorly understood. Despite the fact that there are few documented accounts of community-acquired sepsis in African children, information on hospital-acquired septicemia is scarce. Healthcare-associated septicemia is thought to be responsible for up to 25000 fatalities in African children each year. [24] Overall, developing-country rates of healthcare-associated infection are considered to be at least double those in high-income countries [11]. Its conclusive diagnosis is based on the presence of a positive blood culture, however only about half of all positive blood cultures indicate actual bloodstream infection.

In Ethiopia, data on community-acquired pediatric septicemia is so few that it is impossible to estimate the countrywide prevalence. According to three investigations conducted in Ethiopia, the general prevalence of septicemia among septicemia suspect children and the primary etiologic agent were 13%, CONs, k.pneumoniae [5][13], 27.9%, S.aureus & S.maecesence, and

32.1% K.ozaen & S.aureus [38]. In addition, according to a study conducted by Negussieet.al. in 2015, multi-drug resistance was found in the majority of isolates (92.7%) [13]

Because epidemiology differs by time and place, it necessitates frequent inspection and adjustment for each location. As a result, the goal of this study was to determine the bacterial etiologies, antibiotic resistance, and risk factors related with septicemia in children aged 5 to 14.

1.3 Significance of the study

.

Understanding the epidemiology and antimicrobial susceptibility patterns of prevalent bacteria in a specific place aids in antibiotic selection because septicemia is a life-threatening illness. The majority of bacterial isolates either gram positive or gram negative is determined by geographic location and changes in time, and the antibiotic susceptibility pattern is also impacted by geography and time. Determining the bacterial profile and drug sensitivity pattern could aid infection management and antibiotic use in this location. Understanding these variables can help prioritize resources and create techniques to lower the fatality rate associated with bloodstream infection.

Furthermore, this research may aid in identifying the risk factors for juvenile septicemia and, as a result, taking appropriate measures to mitigate those risks. Because today's government policy is focused on improving the health of children under the age of five, the study could provide useful information to policymakers. The research could also be used as a reference source for future research.with an infection in the bloodstream

As a result, this study will be conducted at SPHMMC to examine the many organisms that cause children septicemia, related risk factors and their antibiotic resistance patterns, as it will serve as important reference for clinicians starting empiric antibiotic therapy.

2. Literature review

Retrospective and prospective data with descriptive and observational cohort study was undertaken in the Hospital of Municipal Dr. Mário Gatti in the municipality of Campinas, São Paulo state (SP), Brazil pediatric ICU. From the beginning of 2011 to the end of 2013, Forty of the 115 patients had positive cultures. S. aureus (11/40), Klebsiella pneumoniae (7/40), Neisseria meningitidis (5/40), Pseudomonas aeruginosa (4/40), and E. coli (4/40) were the most prevalent community-acquired infectious agents [7].

From July 2015 to June 2016, during the 12-month period a prospective cohort study was conducted. Ujjain is a city in the Indian state of Madhya Pradesh. The SBI was 30% (n = 92) based on all positive cultures (blood, urine, and CSF), and 26% (n = 78) based on only positive blood culture; however, the SBI varied with clinical diagnosis. Bronchopneumonia (20%) was the most prevalent diagnosis, with an SBI rate of 93 percent. SBI proportions of 58 and 55 percent were found in meningitis and enteric fever, respectively. In 57 children with bronchopneumonia, the SBI was discovered [14].

From January 2010 to June 2012, the Microbiology Laboratory of the FMIC in Kabul, Afghanistan, conducted another cross-sectional investigation. 212 (51.71 percent) of the 410 isolates were gram-negative bacilli, whereas 184 (44.88 percent) were gram-positive cocci. In addition, 14 Candida species (3.41 percent) were discovered. The Enterobacteriaceae family had 66 Klebsiella (16.1 percent), 42 Enterobacter (10.2 percent), 35 Escherichia (E.) coli (8.5 percent), and 16 Serratia (3.9 percent) species among the most frequently isolated gram-negative bacteria. In addition, 21 Pseudomonas species (5.12 percent) were isolated [6].

From July 2011 to January 2012, a hospital-based retrospective investigation of blood cultures from infants to children up to 14 years old who were brought to the Teaching Hospital Tamale's Neonatal Intensive Care Unit (NICU) and Pediatric Wards with a preliminary diagnosis of sepsis. The prevalence of confirmed bacterial sepsis was 25.9% (86/331) out of 331 blood specimens cultured. The NICU had a point prevalence of 44.4 percent (28/63) while the Pediatric unit had a point prevalence of 21.6 percent (58/268). Gram positive cocci (GPC) were the most

common isolates, accounting for 60.9 percent of all isolates with Coagulase positive (32.2 percent) and Coagulase-negative (28.7%) Staphylococci. Gram negative rods (GNR) accounted for 39.1% of all isolates, with Klebsiella, E.coli, and Salmonella being the most frequently isolated organisms [15].

A hospital-based cross-sectional study was conducted in Kathmandu, Nepal, between March 2015 and August 2016. 231 (7.48%) of 3,088 blood cultures were positive for substantial bacterial pathogen growth, indicating blood stream infection Trends of BSI in Pediatric and Adult Patients In comparison to adult patients (6.6 percent), more pediatric patients (9.3%) were found to have BSI, and this trend was statistically significant ($p = 0.008$, CI-99 percent). Infants ($n = 50$, 12.9 percent) had the largest proportion of culture confirmed bloodstream infections, followed by children ($n = 40$, 6.9 percent), adults ($n = 131$, 6.72 percent), and the elderly ($n = 10$, 5.5 percent) patients [16].

Another experiment was carried out in the laboratory of the Aminu Kano Teaching Hospital (AKTH). SCD was found in 68 (30.22%) of the 225 blood specimens tested, with the highest prevalence (16%) among individuals aged 1-2 years old. Salmonella typhi had the highest rate of occurrence in SCD positive children (39.71%), followed by Streptococcus pneumoniae (14.71%), Salmonella Group B (13.24%), Staphylococcus aureus (4.88%), and Escherichia coli 3 (4.41%). [17]

Another study was conducted in the Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G.Nagara, between April 2012 and March 2013. Culture positivity was detected in 26.9% of the total 252 clinically confirmed septicemia patients, which was consistent with Tiwari, Mehrotra, and Negussie's findings of 25%, 23.1 percent, and 27.9%, respectively. Higher rates of 43.78 percent, 44.4 percent, and 72.7 percent have been recorded by others [2].

Another retrospective descriptive cohort study was undertaken in Cape Town, South Africa, at a pediatric referral hospital. During the years 2011 and 2012. During the study period, 248 (35.8%) of the 693 distinct bacterial and fungal BSI cases were community-acquired (CA), 371 (53.5%) hospital-acquired (HA), and 74 (10.7%) healthcare-associated (HA) (HCA). 6.7 BSI incidents

per 1000 hospitalizations was the overall risk. CA-BSI was caused by Escherichia coli, Staphylococcus aureus, and Streptococcus pneumoniae, while HA-BSI was caused by Klebsiella pneumoniae, Acinetobacter baumanii, and S.aureus [18].

A multiple cross-sectional analytical study involving 950 children under the age of five years old in Tanzania's northwestern region was conducted from July 20, 2016 to October 4, 2017. The prevalence of BSIs among children was 14.2 percent (95 percent confidence interval: 12.1–16.6 percent), with specific prevalences of 8.3, 6.4, and 20.0 percent in district, regional, and tertiary hospitals, respectively. Klebsiella pneumoniae (55, 40.4 percent), Staphylococcus aureus (23, 17.0 percent), and Escherichia coli (17, 12.6 percent) were the most prevalent bacterial pathogens recovered from 135 culture-positive children [19].

Pediatric bloodstream infections from low- and middle-income countries between January 1, 1990 and October 30, 2019. The research comprised a total of 4836 bacterial isolates, with 2974 Gram-negative (63.9 percent [52.2–74.9]) and 1858 Gram-positive (35.8% [24.9–47.5]) germs. Salmonella typhi (26.2%) was the most commonly isolated pathogen in Asia, followed by Staphylococcus aureus (7.7%), but in Africa, S. aureus (17.8%) and Streptococcus pneumoniae (16.8%) were the most prevalent pathogens, followed by Escherichia coli (10.7%) [20].

At Hawassa University Comprehensive Specialized Hospital, data from another retrospective lab was retrieved to assess bacterial isolates and rates drug resistance. A total of 116 (35.9%) of the 323 blood cultures examined were positive for bacterial BSI. Because blood culture sensitivity decreases with age, neonates had a higher risk of bloodstream infection than other age groups (OR, 3; 95 percent CI 1.5–5.1; p = 0.001). The predominant gram-positive isolate CoN Sand S.aureus showed the highest level of resistance to penicillin (61.3%) and tetracycline (78.8%), whereas Meropenem (6.4%), Ceftriaxone (13%) and Doxycycline (13%) showed the lowest level of resistance. Despite the fact that most gram negative isolates were resistant to the medicines tested, K.pneumoniae showed less resistance to cefotaxime and chloramphenicol[21].

From October 2011 to February 2012, a cross-sectional study including 201 pediatric patients (aged 12 years) was undertaken at the pediatric units of TikurAnbessa Specialized Hospital and

Yekatit12 Hospital. Blood cultures were positive in 56 of the 201 blood samples analyzed (27.9 percent). Gram negative bacteria made up 29 percent (51.8 percent) and Gram positive bacteria made up 26 percent (46.4 percent). Staphylococcus aureus 13 (23.2 percent) was the most common pathogen detected, followed by Serratia marcescens12 (21.4 percent), CoNS 11 (19.6 percent), Klebsiella spp 9 (16 percent), and Salmonella spp 3 (5.4 percent) [22].

From June 5, 2016 to March 8, 2017, a hospital-based cross-sectional study was undertaken at Zewuditu Memorial Hospital. There was bacterial growth in 113 (36.5%) of the 309 samples, with 84 (74.3%) gram positive bacteria and 29 (25.3%) gram negative bacteria. Staphylococcus aureus 57(50.4%), Coagulase negative Staphylococci 25(22%), and Klebsiella pneumoniae21(18.5%) were the most commonly isolated organisms [23].

3. Risk factors

Children with major injuries and those on long-term antibacterial medication, as well as malnourished children, children with chronic medical conditions, and children on immunosuppressants, are all at risk for septicemia. Polymicrobial sepsis is linked to catheters, gastrointestinal disorders, neutropenia, and cancer in high-risk patients [24].

Gender, age groups, weight at enrolment, length of hospital stay before sampling, history of indwelling medical device, history of antibiotic use before sampling, presence of comorbidities, and some hematological parameters of the 201 study subjects were among the demographic and clinical characteristics we observed [14].

Septicemias were significantly more linked with age with low median weight compared to age with higher median weight [3.4 (2.5–8.0) kg versus7.5 (3.3–10.0) kg; p 0.001]. When weight was adjusted for age, however, there was no discernible difference between underweight and overweight children and those of normal weight. Other factors linked to septicemia based on univariate analysis [20].

According to a cross-sectional study conducted in Kenya by Ngarutya et al., "the prevalence of bacterimia in acutely malnourished children aged 2 to 59 months at Mbagathi District Hospital, Nairobi." Children with vomiting and diarrhea were more likely to have bacteremia [25].

Nigusse et al., 2015 published a cross-sectional study titled "Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Culture Isolates among Septicemia Suspected Children" in Ethiopia. Sepsis was ruled out based on the clinical symptoms of lethargy. In addition, septicemia was statistically linked with weight at enrolment [14].

4. Objectives

4.1. General objectives

To assess Bacterial profile, antimicrobial susceptibility pattern and associated risk factors among children suspected for septicemia at St. Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia

4.2. Specific objectives

- To assess the bacteriological causes of probable septicemia in children.
- To determine the antibiotic susceptibility pattern of commonly isolated septicemia in children aged 5 to 14.
- To assess the risk factors for septicemia in pediatric patients aged 5 to 14.

5. Materials and methods

5.1. Study area

The research was carried out at SPHMMC, which is located in Addis Ababa, Ethiopia's capital city. Although the medical school started in 2007 and the hospital was founded in 1968 by the late Emperor Haile Selassie, St Paul's Hospital Millennium Medical College was formed by a decision 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. The college employs around 2800 clinical, academic, administrative, and support personnel. Patients are referred from all around the country for medical specialized services. The hospital has 800 beds and serves around 370,000-400,000 people per year with diagnostic and therapeutic services. When it comes to these services, SPHMMC is also the most affordable.

5.2. Study design and study period

- A hospital based cross-sectional study was conduct from August to December 2021.

5.3. Population

5.3.1 Source Population

- All pediatrics patients who have been diagnosed as septicemia patient at SPHMMC during the study period

5.3.2. Study population

- All septicemia suspected pediatrics between 5-14 years and who gave assent, consent and volunteer to participate were included.

5.4 Inclusion and exclusion criteria

5.4.1 Inclusion criteria

- Patients age between 5-14 years old.

5.4.2 Exclusion criteria

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

5.5. Study Variables

5.5.1 Independent variables:

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

5.5.2 Dependent variables:

- Bacterial profile and Antimicrobial susceptibility pattern.

5.6. Sample size determination and Sampling technique

5.6.1. Sample size determination

This study's sample size was estimated using a single sample size estimation method. The earlier study by Daniel et al., 2017 on Bacterial profile, antimicrobial susceptibility pattern, and associated risk factors among children suspected for septicemia at Zewuditu Memorial Hospital, Addis Ababa, Ethiopia[47] yielded a p value of 36.5 percent.

- Sample Size is 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?

$$n = \frac{1.96^2 x\ 0.365\ (1 - 0.365)}{0.05^2} = 356$$

5.6.2. Sampling technique

A convenience sampling techniques was employed to include study participants who meet the consideration criteria until the specified test estimate is accomplished.

5.7 Data collection and laboratory processes

5.7.1 Data collection

Data was collected once the parents/guardians of the pediatrics gave their consent, and a standardized questionnaire was utilized to collect socio-demographic variables. laboratory technologists and Trained nurses took blood samples. The study's aim, as well as any potential risks and benefits, were described to the participants. By checking diferent medical records and conducting interviews, demographic data and additional information such as the presence of chronic illness, birth weight, indwelling medical devices pre-sample antibiotic history, and length of hospital stay data were gathered. Data on mothers' and children's nutritional status is gathered depending on their clinical diagnosis. Because taking antibiotics before collecting a blood sample could influence the culture outcome, participants who had taken antibiotics in the previous two weeks were excluded from the study. However, the prescribed antibiotics were documented after the blood sample was taken.

5.7.2 Specimen collection and transportation

Locates an appropriate vein in the arm using a pressure cuff. While cleaning the vein puncture site, deflate the cuff. Iodophor or iodine tincture is used as an antiseptic, followed by 70% isopropyl alcohol. For optimal antibacterial efficacy, iodophors require 1-2 minutes of contact time. Following the aforesaid aseptic approach, the sample was taken by medical practitioner and an experienced nurse. After collection, inoculate 1-2 ml of the sample on trypto soya broth (TSB) at the bedside and transport to the microbiological laboratory within 5-10 minutes.

5.7.3.1. Isolation and identification

After aseptically collecting the sample, it was inoculated on TSB at the bedside and incubated for up to 7 days or until growth was found at 37°C. On a daily basis, check the bottles for visible indicators of bacterial development, such as hemolytic, turbidity, gas production, or the creation of distinct colonies. Subcultures were created on Blood agar, MacConkey agar, and Chocolate agar after 24 hours, 48 hours, 72 hours, and finally 7 days, and incubated aerobically at 37°C for 24 hours and 48 hours with 5-10% CO2 regardless of the stage of bacterial development. A Gram stain will be employed for macroscopically positive blood samples. Those with growth on the cultural media can be detected by identifying the pure colony biochemical tests. Based on colony morphological features and biochemical test findings, the causative agent was identified. Gram positive bacteria will be tested using coagulase, catalase, and manitol salt agar, whereas gram negative bacteria will be tested using citrate utilization, manitole triple sugar iron, indole urea, oxidase, and motility tests.

5.7.3.2 Antimicrobial susceptibility test

Antimicrobial susceptibility testing for isolated organisms was carried out on MHA using Kirby-disk Bauer's diffusion, as per the Clinical and Laboratory Standards Institute guideline (CLSI 2016) [48]. The bacteria isolated were tested for antimicrobial susceptibility using antibiotic discs. Gentamicin (10g), clindamycin (2g), ciprofloxacin (5g), erythromycin (15g), chloroamphenicole (30g), Vancomycin (30g), Ceftazidme (30g), and cotrimoxazole (5g) were utilized for gram positive bacteria. Ampicilin (10g), Gentamicin (10g), Ciprofloxacin (5g), chloroamphenicole (30g), erythromycin (15g), Ceftazidme (30g), Cotrimoxazole (5g), and Amikacin were used to treat gram negative bacteria. For Acetinobacter and Enterobacter spp., however, chloroamphenicole and erythromycin were not used.

5 .8 Data Quality Assurance

The high quality of the data was due to the use of standardized data collection materials, pre-testing of questionnaires, adequate training before the start of data collection, and close monitoring throughout data collection by the principal investigator. For laboratory analysis, the pre-analytical, analytical, and post-analytical stages of quality assurance adopted in the Addis Ababa public health research and emergency management core procedure shall be strictly followed. Furthermore, the laboratory analysis technique was carried out by laboratory personael who were well-trained and experienced.

5.8.1. Pre-analytical phase

We first obtained verbal and written agreement from the individual before filling out the information on the pre-filled questionnaire. The patient's identifying number is written on the container, and the blood is extracted aseptically with a sterile syringe. Specimens are transferred to the microbiological laboratory within 5-10 minutes of collection.

5.8.2. Analytical phase

All materials, equipment, and operations are under strict supervision. Quality control of culture media was performed For sterility testing and the ability to grow control bacteria strains. For both media and antibiotics discs A barium sulfate (BaSO4), equivalent to a 0.5 McFarland standard, turbidity standard and standard reference strains of the American type culture collection (S. aureus, (E. coli (ATCC-25922),ATCC-25923),and P. aeruginosa (ATCC-27853)) will be used as Control bacteria strains to standardize the inoculums density of bacterial suspension for The microbiology laboratory at Addis Ababa public health research and emergency management core process followed SOPs to the letter, and the results were re-checked by experienced microbiologist personal.

5.8.3. Post-analytical phase

By using the patient's identification number The results were recorded. Before the results were delivered to the caregiver, the department head double-checked and analyzed the reporting to eliminate inaccuracies in the test results. The suitable action(s) were taken when a result had significant patient or public health consequences.

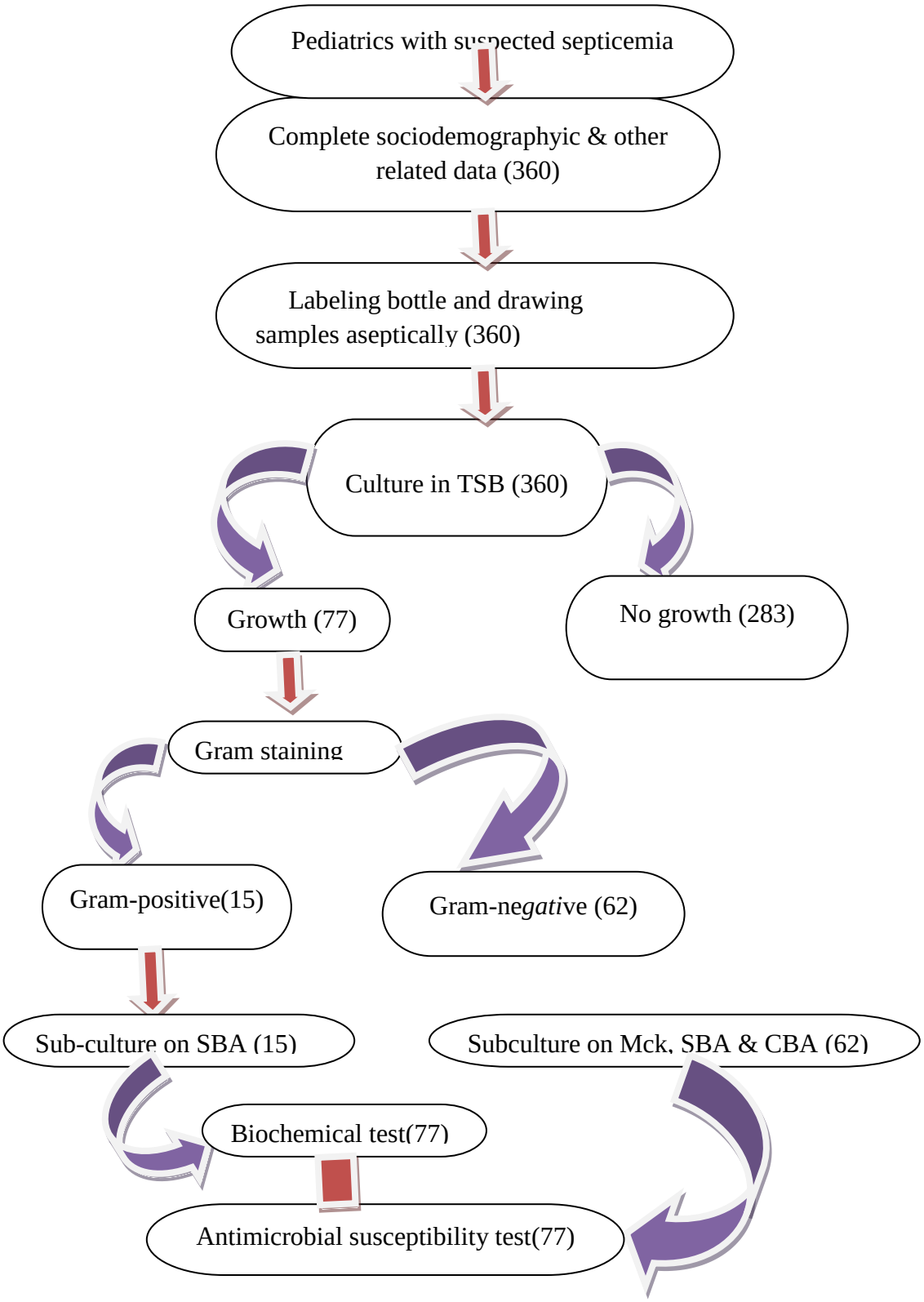

Figure 1 -Work flow of the study

5.9. Data Processing and Analyses

All data was entered into Microsoft Excel and analyzed with the Statistical Package for Social Sciences (SPSS) software version 24. Frequencies and percentages were employed as descriptive statistics. To examine the relationship between the dependent and independent variables, a binary logistic regression model was used. A statistically significant P value is less than 0.05. An odds ratio with a 95 percent confidence interval was used to assess the strength of the link. Finally, the findings are presented in the form of words, graphs, and tables.

5.10 Operational definitions

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

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

5.11. Ethical consideration

The Departmental Research and Ethics Review Committee "DRERC" of the Department of Medical Laboratory Sciences, CHS, AAU, gave its approval to the study. A permission letter was also obtained from the Addis Ababa Health Bureau, as well as SPHMMC and AAPHREMCP. Study participants were recruited after they were informed about the study's goals and purpose, and after they gave their informed consent. There was no risk involved with the sample process; it was the same as taking a specimen for culture and sensitivity testing in a regular laboratory. All confirmed cases of septicemia were reported to the study patients' responsible clinician. The study's findings were kept completely private.

6. Results

Socio demographic characteristics

74.7 percent (n=269/360) of the 360 study participants were aged 5 to 8, while 25.3 percent (n=91/360) were aged 9 to 14. About 54.7 percent (n=197/360) were males and 45.3 percent (n=163/360) were females. In this study, 78.6% (n= 283/360) of the participants had a gestational age (week) more than 37 and 21.4 percent (n=77/360) had a gestational age (week) less than or equal to 37. About 73.9 percent of the pediatricians (n= 266/360) had a weight greater than 2.5 kg, while 26.1 percent (n= 94/360) had a weight less than or equal to 2.5 kg.

Table 1: Socio-demographic characteristics of pediatrics patients at SPHMMC from August 2021 to December 2021

Variables	Categories	Frequency %
Age	5-8	269(74.7)
	9-14	91(25.3)
	Male	197(54.7)
	Female	163(45.3)
Gestational age (week)	>37	283(78.6)
	<=37	77(21.4)
Birth weight (KG)	>2.5	266(73.9)
	<=2.5	94(26.1)

Risk factors associated with septicemia

In this study, 66.6 percent of participants (n=240/360) had a body temperature above 37 degrees Celsius. 7.7% (n=28/360) were malnourished, according to the clinical diagnostic. (Refer to table 2)

As seen in the graph below, around 55.8% (n=201/360) of the children were inpatient (NICU and IPD) and the rest were outpatient (Emergency and OPD). In this study, 29.7% of the children (n=107/360) had a chronic illness.

Only 11.6 percent (n= 42/360) of the pediatric patients were hospitalized for more than 10 days. (See the table below.)

As demonstrated in table 2, there was a statistically significant difference between septicemia in pediatrics and ward visited using a binary logistic regression model [COR: 1.6, 95 percent CI: 1.561-13.082, P=0.005].

Also, when compared to individuals without septicemia, the length of hospital stay in days [COR: 0.15, 95 percent CI: 0.076-0.296, P=0.000].

Table 2: Risk factors with septicemia SPHMMC from August 2021 to December 2021

Variable		Total	Bactermia (N=77)	No bactermia (N=283)	COR	95%(CI)	P value
Age	5-8	269	51(18.9%)	218(81.9%)	0.585	0.338-1.011	0.055
	9-14	91	26(28.5%)	65(71.4%)			
Sex	Male	197	40(20.3%)	157(79.6%)	1.153	0.696-1.909	0.581
	Female	163	37(22.6%)	126(77.3%)			
Gestational age(week)	>37	283	60(21.2%)	223(78.7%)	1.053	0.573-1.937	0.868
	<=37	77	17(22.0%)	60(77.9%)			
Birth weight(kg)	>2.5	266	53(19.9%)	213(80%)	1.378	0.793-2.395	0.256
	<=2.5	94	24(25.5%)	70(74.4%)			
Body temperature c	>37	120	32(26.6%)	88(73.3%)	0.635	0.378-1.066	0.086
	<37	240	45(18.7%)	195(81.2%)			
HIV status	Positive	15	2(13.3%)	13(86.6%)	1.806	0.399-8.178	0.443
	Negative	345	75(21.7%)	270(78.2%)			
Ward visited	PICU	25	10(40%)	15(60%)	1.6	0.666-3.847	0.293
	IPD	176	41(23.2%)	135(76.7%)	2.058	0.941-4.501	0.07
	EPD	89	17(19.1%)	72(80.8%)	4.519	1.561-13.082	0.005
	POD	70	9(12.8%)	61(87.1%)			
Congenital anomalies	Yes	15	4(26.6%)	11(73.3%)	0.738	0.228-2.86	0.612
	No	345	73(21.1%)	272(78.8%)			
Underlying Chronic Disease	Yes	107	18(16.8)	89(16.8%)	1.504	0.838-2.697	0.171
	No	253	59(23.3%)	194(76.6%)			
Nutritional Status	Malnourished	28	6(21.4%)	22(78.5%)	1.003	0.392-2.567	0.996
	Non-malnourished	332	71(21.3%)	261(78.6%)			
Indwelling Medical Device	Yes	338	76(22.4%)	262(77.5%)	0.164	0.022-1.240	0.08
	No	22	1(4.54%)	21(95.4%)			
Length of hospital stay in days	>10	42	24(57.1%)	18(42.8%)	0.15	0.076-0.296	0.000
	<10	318	53(16.6%)	265(83.3%)			

7. Bacterial etiologies of septicemia among pediatrics

A total of 21.3% (n=77/360) of the 360 participants in the study exhibited bacterial growth, with 40 men and 37 women. 19.4% (n=15/360) of culture positives were gram positive, while 80.5% (n=62/360) were gram negative. *K.pneumoniae* was the most commonly isolated bacterium (44.1%) (n=34/360), followed by *E.coli* (24.6%) (n=19/360) and CONs (14.2%) (n=11/360), respectively.

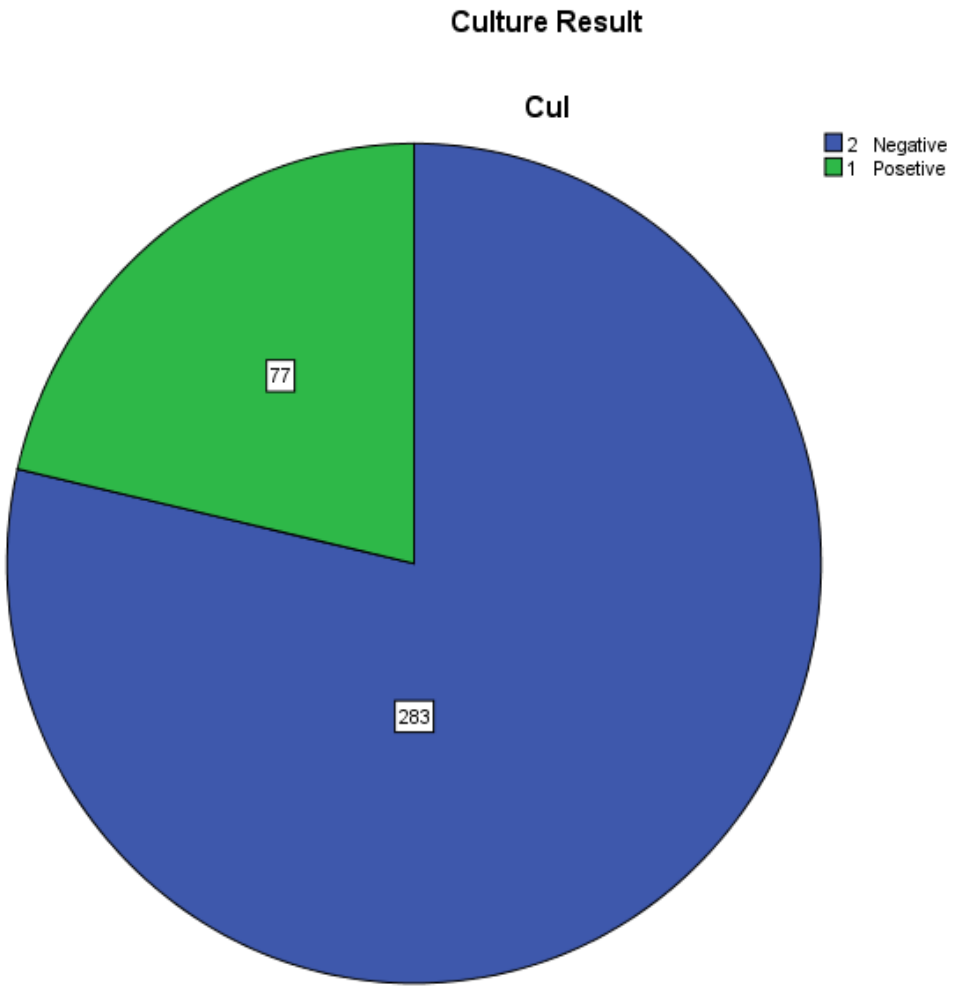

Figure 1. Blood culture result of septicemia suspected pediatrics at St Paulo's Hospital, from August 2021 to December 2021).

Bacterial isolation based on age classification (Table 3) shows that the quantity of bacteria isolated was inversely proportional to the age of children. *K.pneumoniae* and *E.coli* were the most commonly isolated bacteria. IPD isolates the majority of bacteria seen in patients (41). *K.pneumoniae* and *E.coli* were the most common bacteria in all of the wards. (See table 3 for details.)

Table 3: Bacterial distribution with sex and age among septicemia suspected pediatrics at SPHMMC August 2021 to December 2021

Variable		Organisms isolated					
		S.aureus	*CONs*	*K.pneumonia*	*E.coli*	*Acinitobacter*	*Entrobacter*
Age	5-8	1(25%)	8(72.7%)	22(64.7%)	14(73.6%)	4(66.6%)	2(66.6%)
	9-14	3(75%)	3(27.2%)	12(35.2%)	5(26.3%)	2(33.3%)	1(33.3%)
Sex	Male	1(25%)	6(54.5%)	21(61.7%)	6(31.5%)	3(50%)	3(100%)
	Female	3(75%)	5(45.4%)	13(38.2%)	13(38.2%)	3(50%)	0(0)
Ward	PICU	0(0)	1(9.0%)	6(17.64%)	2(10.52%)	1(16.6%)	0(0)
	IPD	3(75%)	6(54.54%)	15(44.1%)	13(68.42%)	4(66.6%)	0(0)
	EPD	0(0)	2(18.18%)	9(26.4%)	3(15.7%)	1(16.6%)	2(66.6%)
	POPD	1(25%)	2(18.18%)	4(11.76%)	1(5.26%)	0(0)	1(33.3%)
	Total	4(100%)	11(100%)	34(100%)	19(100%)	6(100%)	3(100%)

8. Antibiotic resistance pattern of the isolates

8.1 Gram-positive bacteria

CONs were the most frequently isolated bacteria, and they were resistant to ampicillin, cotrimoxazole, and gentamycin (81.8 percent). S.aureus, the second most commonly isolated bacterium, was likewise resistant to ampicillin, cefuroxime, and gentamycin (75%). Antibiotics such as ciprofloxacin, chloramphenicol, vancomycin, and clindamycin were found to be effective against *S.aureus* and *CONs.*

Table 4: Antimicrobial resistance patterns of isolated gram positive bacteria at SPHMMC; August 2021 to December 2021

Antibiotics	Resistant pattern of Gram- positive isolates	
	CoNS n= (11%)	*S.aureus* n= (4%)
Ampicillin	9(81.8%)	3(75%)
Ciprofloxacin	3(27.2%)	0(00)
Cotrimoxazole	9(81.8%)	1(25%)
Chloramphenicol	5(45.4%)	0(00)
Erythromycin	6(54.5%)	2(50%)
Amikacin	0(00)	0(00)
Ceftazidime	7(63.6%)	4(100%)
Cefuroxim	7(63.6%)	3(75%)
Vancomycin	2(18.1%)	0(00)
Clindamycin	2(18.1%)	0(0)
Gentamycin	9(81.8%)	3(75%)

8.2 Gram-negative bacteria

K.pneumoniae was the most usually isolated gram negative bacteria, and it was resistant to a wide range of medications. It has ampicillin resistance (97.0%), gentamycin resistance (94.1%), erythromycin resistance (82.3%), and cotrimoxazole resistance (82.3%). (70.5%). Ciprofloxacin and amikacin were effective against *K.pneumoniae*. (See fig. 5.)

Table 5: Antimicrobial resistance patterns of isolated gram-negative bacteria, SPHMMC; August 2021 to December 2021

Antibiotics	*Resistance pattern of Gram-negative isolates*			
	Klebsiella spp N=34%	*E.coli* N=19%	*Acetinobacter spp* N=6%	*Enterobacter spp* N=3%
Ampicillin	33(97.0)	13(68.4)	6(100)	2(66.6)
Ciprofloxacin	8(23.5)	5(26.3)	2(33.3)	1(33.3)
Cotrimoxazole	24(70.5)	10(52.6)	6(100)	2(66.6)
Chloramphenicol	18(52.9)	8(42.1)	(NA)	(NA)
Erythromycin	28(82.3)	13(68.4)	(NA)	(NA)
Amikacin	12(35.2)	4(21.0)	2(33.3)	1(33.3)
Ceftazidime	23(67.6)	19(100)	6(100)	3(100)
Gentamycin	32(94.1)	11(57.8)	6(100)	2(66.6)

- NA-not applicable

8.3 Multidrug-resistant (MDR) bacterial isolates

The predominatent 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 in 3 (75) *S. aureus,9(81.8)CONs, 13*(68.4%) *E.coli*, 33(97.0%) of *Klebsiella spp*, 6 (9.68%) of *Acitinobacter spp* and 2(66.6%) of *Enterobacter spp*.

Table 6: Multiple drug resistance patterns of bacterial isolate among children suspected for septicemia at SPHMMC

Bacterial isolate	Anti-microbial resistance (%)					
	Total	R0	R1	R2	R3	R4
Gram Positive	**15(19.48%)**	0(0)	1(6.66)	2(13.33)	11(73.33)	1(6.66)
S.aureus	4(26.66)	0(0)	0(0)	1(25)	3(75)	0(0)
CONS	11(73.33)	0(0)	1(9.09)	3(27.27)	6(54.54)	1(9.09)
Gram Negative	**62(80.51%)**	0(0)	1(1.61)	5(8.06)	48(77.41)	8(13.0)
E.coli	19(30.64)	0(0)	1(5.26)	3(15.79)	11(57.9)	4(21.05)
Klebsiellaspp	34(54.84	0(0)	0(0)	1(2.94)	28(82.35	5(14.7)
Acitinobacterspp	6(9.68)	0(0)	0(0)	0(0)	5(83.33)	1(16.66)
Entrobacterspp	3(4.84)	0(0)	0(0)	1(33.33)	2(66.6)	0(0)
Total	**77(100)**	0(0)	2(2.6)	7(9.09)	59(76.62)	9(11.68)

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

9. Discussion

To avoid complications and reduce mortality from BSI, prompt diagnosis and appropriate therapies are required. Empiric therapy is initiated based on prior knowledge of common causal agents and their susceptibility to prescription antibiotics, and then changed based on the ultimate culture and susceptibility result. The findings of this investigation revealed the profile of septicemia-causing bacteria isolates, as well as the related risk factors for pediatric septicemia and their susceptibility to the most regularly used antimicrobial drugs.

Males outnumbered females in this study, with 54.7% (n=197/360) and 45.3% (n=163/360) respectively, which was comparable with earlier studies in Ethiopia [14], Nigeria [26], Tanzania [27], Iran [28], and Kenya [25]. Blood culture verified septicemia was found in 21.3% (n=77/360) of the 360 children in our research. These findings were similar to those reported in earlier studies conducted in Lahore (27.9%) [29], Cameroon (28.3%) [30], and Nigeria (22%) [31], but were greater than those reported in Tanzania (13.4%) [32], Uganda (17.1%) [33], Nepal (4.2%) [34], and Ethiopia (7.7%) [35]. In our study, males had a higher positivity rate (40%) than females (37%) in septicemia with sex, whereas in another study conducted in Ethiopia [14], males had a higher positivity rate (40%) than females (37%) in septicemia with sex.

Vomiting (30.0%), fever (24.7%), and reluctance to eat were the most common signs and symptoms in this study (16.4%). In a comparable study [36], fever, unwillingness to feed, lethargy, and trouble breathing were the most common symptoms; in this study, 7.7% of the pediatricians were malnourished, which was slightly lower than the Tanzanian study (15.5%) [9]. A total of 29.7% (n=107/360) of the study participants had underlying chronic disease, with 16.8% (n=18/360) being culture positive.

In our investigation gram negative was the predominant etiologic agent of septicemia, although in most studies, such as those conducted in India [37] and Saudi Arabia [38], the predominant etiologic agent for BSI was gram positive. In this study, the most common gram positive bacteria were CONs [35], and the most common gram negative bacteria were K.pneumoniae, which is (The overwhelming etiologic specialist for BSI in our examination was gram negative, in spite of

the fact that in most thinks about, such as those conducted in India [37] and Saudi Arabia [38], the transcendent etiologic operator for BSI was gram positive. In this consider, the foremost common gram positive bacteria were CONs[35], and the foremost common gram negative microbes were K.pneumoniae, which is) consistent with studies conducted in India [39], Tanzania [27], and Nigeria [40], and the most common gram negative bacteria were E.coli [41]. The observed differences could be attributed to a variety of factors, including underlying clinical conditions, bacterial pathogen virulence factors, and patient immune status [2].

The type of isolates found in this study are comparable to those seen in other African investigations. An initial report from South Africa shows that S. aureus and Klebsiella [39] are the most common isolates found in blood cultured samples of children. CoNS and S.aureus [42] became prevalent once again in Zimbabwe. This could indicate a nosocomial infection as a result of the placement of a medical device into the youngster during the procedure. In line with this study, a systematic review study conducted in resource-poor nations found that Gram-negative organisms, particularly Klebsiella pneumoniae[28], were the most common isolation from child blood cultures[19].

Antimicrobial resistance was found to be high not only to the first-line medications (ampicillin and gentamicin), but also to the second-line recommended therapy (3rd generation cephalosporins and amikacin). Our findings showed that isolated Gram-positive bacteria are more susceptible to Vancomycin, Clindamycin, and Ciprofloxacin, which is also corroborated by a study from India and other reports [43].

E. coli and Klebsiella spp., the two most common Gram-negative bacteria isolates in our collection, were both highly resistant to Ampicillin and Gentamycin. Furthermore, ciprofloxacin (23.5%) exhibited moderate sensitivity for gram negative bacteria. Ampicillin and cotrimoxazole, which are commonly administered antibiotics, exhibited high percentages of resistance (97.0%) and 70.5%, respectively, for gram negative bacteria, which was consistent with studies from Uganda and India [36, 37]. Furthermore, K.pneumoniae was cotrimoxazole resistant, which was consistent with prior research from Tanzania and Iraq [44, 45]. Cephalosporins were resistant to K.pneumoniae except for ceftraxion, although they were

vulnerable to K.pneumoniae in another trial done in India [39]. The ciprofloxacin sensitivity of K.pneumoniae was similar to that of a Nigerian investigation [46].

Poor infection control procedures and incorrect antibiotic use were the key factors driving the rise in antimicrobial resistance in bacteria. Antibiotic usage tactics such as antibiotic limitation, combination therapy, and antibiotic cycling may aid in the reduction or prevention of resistance.

10. Limitation

- Due to the difficulty of sampling in pediatrics, we do not employ a two-sided sample to boost sensitivity and specificity.
- Due to a lack of equipment in the lab, we were unable to identify anaerobic fungus and bacteria.
- Due to a budget shortage, the antimicrobial drugs used in this study are insufficient.

11. Conclusion

The prevalence of septicemia in our study overall was 21.3%. The commonest causative agent of children septicemia was Gram negative. CONs and *K.pneumoniae gram* positive and gram negative bacteria respectively were the predominantly isolated agent in this study. Congenital anomalies, underlying chronic disease, age of the children, and Birth weight were indictor of septicemia. Multi drug resistances of the isolate were extremely high and also gratlytall of the isolate indicate high resistance for most of the antibiotics. In general, the result of this study consider the require for persistent assessment of local antibiotic sensitivity designs of pathogens for the definition of a judicious antibiotic approach.

12. Recommendation

Bacteria and their AST pattern must be monitored on a regular and ongoing basis. Based on the findings, each facility's reasonable antibiotic use must be adjusted. Even if the government is attempting to enhance the health of children under the age of five, this study shows that the government still encompasses a huge work to do in terms of children health.

13. References

1. Al-Rawazq HS, Mohammed AK, Al-Zubaidy RH**.** Bacterial Isolates in Blood Culture of Children with Septicemia. *J Fac Med Baghdad* 2012; 54(1):96-9.

2. Hanumantha S, Tabaseera N. Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Culture Isolates among Septicemia Suspected Children in a Rural Tertiary Care Hospital. Int. J. Curr. Microbiol. App. Sci. 2017;6(11):1167-71.

3. Daniels R: Surviving the first hours in sepsis: getting the basics right (an intensivist's perspective). J Antimicrob Chemother 2011, 66(suppl 2):ii11–ii23.

4. Muller-Pebody B, Johnson AP, Heath PT, Gilbert RE, Henderson KL, Sharland M: Empirical treatment of neonatal sepsis: are the current guidelines adequate? Arch Dis Child Fetal Neonatal Ed 2010, 96(1):F4–F8.

5. Hermon MM, Etmayr T, Brandt JB, Sadeghi K, Burda G, Golej J. Pediatric infection and sepsis in five age subgroups: single-center registry. Wiener Medizinische Wochenschrift. 2021; 171(1):29-35.

6. N. Obeng-Nkrumah, A.-K. Labi, N. O. Addison, J. E. M. Labi, and G. Awuah-Mensah "Trends in pediatrics and adult bloodstream infections at a Ghanaian referral hospital:retrospective study," *Annals of Clinical Microbiology and Antimicrobials*,2016;

7. Tariq TM. Bacteriologic profile and antibiogram of blood culture isolates from a children's hospital in Kabul. J Coll Physicians Surg Pak. 2014 : 1;24(6):396-9.

8. São Pedro TD, Morcillo AM, Baracat EC. Etiology and prognostic factors of sepsis among children and adolescents admitted to the intensive care unit. Revista Brasileira de terapia intensiva. 2015 ; 27(3):240.

9. Mehdinejad M, Khosravi DA, Morvaridi A. Study of Prevalence and Antimicrobial Susceptibility Pattern of Bacteria Isolated from Blood Cultures. Journal of Biological Sciences2009; 9: 249-253.

10. Singh AK, Venkatesh V, Singh RP, Singh M. Bacterial and antimicrobial resistance profile of bloodstream infections: A hospital-basedstudy. Chrismed J Health Res 2014; 1:140-4.

11. Shahian M, Pishva N, Kalani M. Bacterial Etiology and Antibiotic Sensitivity Patterns of Early-Late Onset Neonatal Sepsis among Newborns in Shiraz, Iran 2004-2007. Iran J Med Sci December 2010; 35(4):293-98.

12. Dramowski A, Cotton FM, Rabie H, Whitelaw A. Trends in pediatric bloodstream infections at a South African referral hospital. BioMed Central 2015; 15:33.

13. Okon KO, Askira UM, Ghamba PE, Isyaka TM, Hamidu IM, Kankop JW et al. Childhood Septicemia; Retrospective Analysis of Bacterial Pathogens and Antimicrobial Susceptibility Pattern in Maiduguri, Nigeria.N Y Sci J 2014; 7(6):9-13.

14. Negussie A, Mulugeta G, Bedru A , Ali I, Shimeles D. , Lema T. et al. Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Culture Isolates among Septicemia Suspected Children in Selected Hospitals Addis Ababa, Ethiopia. Int J Biol Med Res.2015; 6(1):4709-4717.

15. Pathak A, Upadhayay R, Mathur A, Rathi S, Lundborg CS. Incidence, clinical profile, and risk factors for serious bacterial infections in children hospitalized with fever in Ujjain, India. BMC infectious diseases. 2020 ;20(1):1-1

.

16. Acquah SE, Quaye L, Sagoe K, Ziem JB, Bromberger PI, Amponsem AA. Susceptibility of bacterial etiological agents to commonly-used antimicrobial agents in children with sepsis at the Tamale Teaching Hospital. BMC infectious diseases. 2013 : 1;13(1):89.

17. Parajuli NP, Parajuli H, Pandit R, Shakya J, Khanal PR. Evaluating the trends of bloodstream infections among pediatric and adult patients at a teaching hospital of Kathmandu, Nepal: role of drug resistant pathogens. Canadian Journal of Infectious Diseases and Medical Microbiology. 2017 : 1;2017.

18. Folgori L, Bielicki J. Advances in pediatric septic shock: future challenges in pediatric and neonatal sepsis: emerging pathogens and antimicrobial resistance. Journal of pediatric intensive care. 2019 ;8(1):17.

19. Lochan H, Pillay V, Bamford C, Nuttall J, Eley B. Bloodstream infections at a tertiary level paediatric hospital in South Africa. BMC infectious diseases. 2017 : 1;17(1):750.

20. Seni J, Mwakyoma AA, Mashuda F, Marando R, Ahmed M, DeVinney R, Pitout JD, Mshana SE. Deciphering risk factors for blood stream infections, bacteria species and antimicrobial resistance profiles among children under five years of age in North-Western Tanzania: a multicentre study in a cascade of referral health care system. BMC pediatrics. 2019 1;19(1):32.

21. Droz N, Hsia Y, Ellis S, Dramowski A, Sharland M, Basmaci R. Bacterial pathogens and resistance causing community acquired paediatric bloodstream infections in low-and middle-income countries: a systematic review and meta-analysis. Antimicrobial Resistance & Infection Control. 2019 1;8(1):207.

22. Alemayehu TA, Agegnehu AA, Ali MA, Mitiku EM, Azerefegne EA. Under-five children's Bacterial Blood Stream Infection in Southern Ethiopia.

23. Negussie A, Mulugeta G, Bedru A, Ali I, Shimeles D, Lema T, Aseffa A. Bacteriological profile and antimicrobial sensitivity pattern of blood culture isolates among septicemia-suspected children at Tikur Anbessa specialized hospital and Yekatit 12 hospital, Addis Ababa, Ethiopia. Critical Care. 2013 ;17(4):1-59.

24. Enrione MA, Powell KR. Sepsis, septic shock and systemic inflammatory response syndrome. In: Kleigman RM, Behraman RE, Jenson HB, Stanton BF, editors. Nelson textbook of paediatrics. Philadelphia: WB Saunders; 2007. p. 1094.

25. Ngaruiya KW**.** The prevalence of bacterimia in the severly malnourished children aged 2 to 59 months at Mbagathi District Hospital Nairobi (Msc thesis).*College of health Sciences, University of Nairobi* (Cited Dec 2015).Available from-erepository.uonbi.ac.ke/.../

26. Meremikwu MM, Nwachukwu EC, Asuquo AE, Okebe UJ, Utsalo JS. Bacterial isolates from blood cultures of children with suspected septicaemia in Calabar, Nigeria. *BMC infectious diseases* 2005; 5:110.

27. Blomberg B, ManjiKP, UrassaWK, TamimBS, MwakagileDSM, Jureen R, et al. *BMC Infectious Diseases 2007; 7:43.*

28. Shahian M, Pishva N, Kalani M. Bacterial Etiology and Antibiotic Sensitivity Patterns of Early-Late Onset Neonatal Sepsis among Newborns in Shiraz, Iran 2004-2007. *Iran J Med Sci December 2010; 35(4):293-98* .

29. Latif S, Anwar MS, Ahmad AI. Bacterial pathogens responsible for blood stream infection (BSI) and pattern of drug resistance in A Tertiary Care Hospital of Lahore. Biomed BiochimActa. 2009; 25:101-5.

30. Kamga HLF, Njunda Al, Nde PF, Assob JCN, Nsagha DS, Weledji P. Prevalence of Septicemia and Antibiotic Sensitivity Pattern of Bacterial isolates at the U n ive r s i t y Te a c h i n g H o s p i t a l Ya o u n d é , C a m e ro o n . A f r J ClnExperMicrobiol2011; 12(1): 2-8.

31. Omoregie R, Egbe CA, Ogefere HO, Igbarumah, Omijie RE. Effects of Gender and Seasonal Variation on the Prevalence of Bacterial Septicemia among Young Children in Benin City, Nigeria. Libyan J Med 2009; 4:153-157.

32. Moyo S, Aboud S, Kasubi M, Maselle SY. Bacteria isolated from blood stream infections at a tertiary hospital in Dar es Salaam, Tanzania – antimicrobial resistance of isolates. S Afr Med J 2010; 100: 835-838.

33. Bachou H, Tylleskär T, Kaddu-Mulindwa DH, Tumwine JK. Bacteraemia among severely malnourished children infected and uninfected with the human immunodeficiency virus-1 in Kampala, Uganda. BMC Infectious Diseases 2006; 6:160.

.

34. Karki S, Rai GK, Manandhar R. Bacteriological Analysis and Antibiotic Sensitivity Pattern of Blood Culture Isolates in Kanti Children Hospital. JNepal PaediatrSoc2010; 30(2):94.

35. Ghiorghis B. Neonatal sepsis in Addis Ababa, Ethiopia: a review of 151 bacteremic neonates. Ethiop Med J 1997; 35(3):169-76.

36. Mugalu J, Nakakeeto MK, Kiguli S, Kaddu-mulindwa D. H.Aetiology, risk factors and immediate outcome of bacteriologically confirmed neonatal septicaemia in Mulago hospital, Uganda. *Afr Health Sci. 2006 Jun;* 6(2): 120–126.

37. Prabhu K, Bhat S, Rao S. Bacteriologic Profile and Antibiogram of Blood Culture Isolates in a Pediatric Care Unit. *Journal of Laboratory Physicians* 2010; 2 (2):85-8.

38. Abo-Shadi AM, Al-Johani AA,Bahashwan AA**.** Antimicrobial Resistance in Pathogens Causing Pediatrics Bloodstream Infections in a Saudi Hospital.*British Microbiology Research Journal* 2012; 2(4): 212-227.

39. Sudharshan RC, Pradeep RM, Neelima A. Bacteriological Profile of neonatal sepsis in a tertiary care hospital. *World Journal of Pharmacy and Pharmaceutical Sciences* 2013;2(6) 5709-5717.

40. Okon KO, Askira UM, Ghamba PE, Isyaka TM, Hamidu IM, Kankop JW et al. Childhood Septicemia; Retrospective Analysis of Bacterial Pathogens and Antimicrobial Susceptibility Pattern in Maiduguri, Nigeria.*N Y Sci J* 2014; 7(6):9-13.

41. Karki S, Rai GK, Manandhar R. Bacteriological Analysis and Antibiotic Sensitivity Pattern of Blood Culture Isolates in Kanti Children Hospital.*J. Nepal Paediatr. Soc*. 2010; 30 (2):94-7.

42. Watson SR, Carcillo AJ, Linde-Zwirble TW, Clermont G, Lidicker J, Angus CD. The Epidemiology of Severe Sepsis in Children in the United States.*Am J Respir Crit Care Med*2003; 167: 695–701.

43. Moges F, Eshetie S, Yeshitela B, Abate E. Bacterial etiologic agents causing neonatal sepsis and associated risk factors in Gondar, Northwest Ethiopia. BMC pediatrics. 2017 ;17(1):137.

44. Christopher A**,** Mshana SE, Kidenya BR, Hokororo A, Morona D. *Italian Journal of Pediatrics 2013; 39:27.*

45.Ibrahim AS, Rahma S**.** Microbiological Profile of Neonatal Septicemia. *The Iraqi postgraduate medical journal* 2012;11(1):13-8.

46. Nwadioha IS, Nwokedi POE, Kashibu E, Odimayo SM, Okwori EE.A review of bacterial isolates in blood cultures of children with suspected septicemia in a Nigerian tertiary Hospital.*African Journal of Microbiology Research* 2010; 4 (4):222-225.

47. Daniel et al. on Bacterial profile, antimicrobial susceptibility pattern and associated risk factors among septicemia suspected pediatrics patients at Zewuditu Memorial Hospital, Addis Ababa, Ethiopia 2017.

48. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition. *CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute;* 2012.

Annexes

Annex 1: English version of participant information sheet, assent, consent & questionnaire

I. Participant information sheet

Department of Medical Laboratory Science, Collage of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Title of the Research Project: Bacteriological Profile, Antimicrobial Susceptibility Pattern and Associated Risk factor of Septicemia Suspected Pediatrics at SPHMMC, Addis Ababa, Ethiopia. First and first, I want to express my gratitude in advance for your cooperation and consent to participate in this study. Please read or listen to the study's general information as it is read to you. Please feel free to ask any questions you may have about the study.

Background information

Background: Bloodstream infections are extremely common in children and are one of the leading causes of morbidity and mortality in this age group. Fever, trouble breathing, tachycardia, malaise, refusal of feeds/lethargy are all symptoms of septicemia in children, which can progress to significant complications such as shock, multi-organ failure, and DIC, all of which require prompt and vigorous antibiotic therapy.

Aim of the study

The goal of this study is to evaluate the Bacteriological Profile, Antimicrobial Susceptibility Pattern, and Associated Risk Factors of Septicemia in children aged 14 and under at SPHMMC in Addis Ababa, Ethiopia.

Benefits for participants

Participants in this study will not receive any money or other incentives in exchange for their participation. However, you will be treated as needed based on the diagnosis. Most importantly, the findings of the study will aid in the development of efficient septicemia prevention and control measures for children. As a result, you are indirectly assisting other patients as well as society.

Risks and complication

There are no known hazards associated with your participation. A blood sample will be obtained from your peripheral vein once as part of a regular laboratory procedure. You may experience some discomfort during sample collection, but it is not painful.

Confidentiality

There will be no sensitive questions about your social desirability asked of you, but any information gathered in conjunction with this study that can be linked to you will be kept private. Participants will not be barred from stopping or withdrawing from the study at any point. Only those who are interested in learning more about their lab results can do so by entering their code number. Numbers will be used to code the information we collect about you. Personal information will not be given to a third party or included in any report resulting from this study.

Assurance of Principal Investigator

I sign below to certify that I am taking over responsibility for the scientific, ethical, and technical conduct of the research project, as well as the submission of progress reports to all study stakeholders.

Hindiya Lema(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: Hindiya Lema: Department of Medical Laboratory Sciences, Collage of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: hindiyabeshir@gmail.com Mobile-0910551721

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

II. **Assent form**

We are doing this research to find out more about pediatrics blood stream infection and the choice that you have to take part in it is up to you. We would still take good care of you no matter what you decide. We want you to ask us any questions that you have any time. If you

decide to be in the research, you may need a needle poke so we could test some of your blood. We would ask you to read questions on a piece of paper. Then you would mark your answers on the paper. A person on the research team would ask you questions. Then you would say your answers out loud. We will look at your past doctor visits and use information about your care. Some of the questions might be hard to answer. You can say 'no' to what we ask you to do for the research at any time and we will stop. This research will not help you and you would not be paid. But we hope it will help other kids who have disease like you do. It is also OK to say yes and change your mind later. You can stop being in the research at any time. If you want to stop, please tell the researcher. Take the time you need to make your choice. If you want to be in the research after we talk, please sign below. We will write our name too. This shows we talked about the research and that you want to take part. I _____

III. Consent form

At SPHMMC Addis Ababa, Ethiopia, I was informed about a study that aims to determine the Bacteriological Profile, Antimicrobial Susceptibility Pattern, and Associated Risk Factors of Septicemia Suspected Pediatrics. The study's purpose and implementation were briefly described to me. Furthermore, I have been told of my right to refuse information, refuse to comply, and withdraw from the study at any time, and none of my decisions will have any impact on my child's overall health care. As a result, I freely consented to grant informed consent to the researcher to supply my child's blood for the aforementioned study after fully comprehending the issue. I consented to have the sample checked for septicemia. I I've had the chance to ask questions regarding the project and have received satisfactory answers in a language I understand. I was also told that the findings of the blood study would be forwarded to the doctor who was monitoring my child, and that I may request the information if I so desired.

I

Participant code: _____ Signature: _____ Date: _____

IV. Questionnaire

Addis Ababa University Collage of Health Sciences, School of Allied Health Science 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 ☐

II. Septicemia associated questions

3. Ward of the pediatrics

a. OPD ☐ b. EPD ☐ c. PICU ☐

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

5. Body temperature

a. <36.5°C ☐ b. >38.5°C ☐

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

a. Yes ☐ b. No ☐

7. If you say yes for "6" specify_____

8. HIV status of the pediatrics

a. Positive ☐ b. Negative ☐ c. unknown ☐

9. Is there any indwelling medical device?

a. Yes ☐ b. No ☐

10. If you say yes for no.9, what kind of device?

a. Intravenous devices ☐ b. Urinary catheters ☐ e. Other ☐

c. Endotracheal ☐ d. Ventilator support ☐

11. Antibiotics given after sampling_____

12. Nutritional status of the pediatrics?

a. Malnourished ☐ b.Non malnourished ☐

13. What is his/her birth weight?

a.<2.5 Kg ☐ b.> 2.5 Kg ☐

14. Gestational age

a. <37 weeks ☐ b.>37 weeks ☐

15. Is there any congenital anomali • • •

a. Yes ☐ b. No ☐

V. Laboratory Data

20. Date of specimen collection_____

21. Media used _____

22. Gram stains result _____

23. Biochemical test_____

24. Organism isolated _____

25. Drug susceptibility pattern

a. Sensitive to _____

b. Intermediate to _____

c. Resistance to _____

VI. 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, 2- 3 ml for infants aged 1 month to 2 years, 3-5 ml for older children.

5. Insert the needle through the rubber liner of the bottle cap and dispense accordingly

6. 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.

-Swab the area starting at the spot with a 10% tincture of iodine with a circular motion.

-Clean an area of roughly 50 mm in diameter with 70% ethanol. Allow to dry naturally.

3. Remove the protective cover or lift back the tape from the top of the culture bottle (s). Using an ethanol-ether swab, wipe the top of the bottle.

4. Withdraw around 1-2 ml of blood each culture for neonates, 2- 3 ml for infants aged 1 month to 2 years, and 3-5 ml for older children using a sterile syringe and needle.

5. Insert the needle into the bottle cap's rubber liner and distribute as needed.

6. Pour the tryptosoya broth culture medium bottle into the tryptosoya broth culture medium bottle.

7. Wipe the top of each with a fresh ethanol-ether swab.

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.

1. Clearly labeling the slides with the date as well as the patient's name and number.

2. Smears are made by equally coating an area on a slide with a diameter of 15-20mm.

3. Smear Drying After making smears, leave the slide in a safe area to air dry, away from insects and dust.

4. Use heat or alcohol to fix the dried smear (methanol).

5. Apply crystal violet stain on the fixed smear for 30-60 seconds.

6. Use clean water to quickly remove the discoloration. Use filtered water or clean boiled rainwater if the tap water is contaminated.

7. Drain all of the water and apply lugol's iodine on the smear for 30-60 seconds.

8. Rinse the iodine away 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 theH2O2solution.
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. aureus* which 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

Interpretation

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 OC for up to 48 h.
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 color 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 OC 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 H2S 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 37OC 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 OC for 24 hr

3. Look for yellow color in the medium

Interpretation

Yellow color……..Positive manitol test

Red color …….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 OC 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 pneumoniae* 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 OC 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.

Oxides test

Principle

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

Procedure

1. Place a piece of filter paper in a clean petri dish and add 2 or 3 drops of freshly prepared oxides 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 color within a few seconds.

Interpretation

Blue-purple color . . . . . Positive oxides test (within 10 seconds)

No blue-purple color . . . . . Negative oxides test (within 10 seconds)

Controls

Positive oxides control: *Pseudomonas aeruginosa*

Negative oxides 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 OC 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, assent, consent & questionnaire

የተሳታፊዎች የመረጃ ቅፅ

I. አዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የህክምና ላብራቶሪ ሳይንስ ዲፓርትመንት

አርዕስት፡-

በአዲስ አበባ ከተማ በቅዱስ ጳውሎስ ሆስፒታል የህጻናት የደም ውስጥ በሽታ አምጭ ተህዋስያን ስርጭት፤ለተለያዩ ጸረባክቴሪያ ያላቸው አይበገሬነትና የበሽታውን አጋላጭ ሁኔታዎችን ስለማጥናት

አጠቃላይ መረጃ፡-

በጥናቱ በመሳተፍዎ ከልብ እያመሰገንን ከመወሰንዎ በፊት ይህን ቅፅ በትክክል አንብቡ ወይም ሲነበብልዎ በትክክል ያዳምጡ፤ እንዲሁም ግልጽ ያልሆነልዎትን ነገር በሙሉ በነፃነት ይጠይቁ

ስለጥናቱ መረጃ፡-

የህጻናት የደም ውስጥ ተህዋስያን በሽታ በአለም አቀፍ ደረጃ በተለይም በታዳጊ ሀገራት ከፍተኛ የሆነ ህመምና ሞት ያስከትላል፡፡በተደረጉ ጥናቶች የተለያዩ አጋላጭ ሁኔታዎች እንዳሉ ለማወቅ ተችለል፡፡ ስለዚህ የበሽታውን አምጭ ተህዋስያን ስርጭትና ለጸረተህዋስያን ያላቸውን ግትርነት ማወቅ በሽታውን ለመቆጣጠርና ለመከላከል እንዲሁም ተገቢውን መድሃኒት ለማዘዝ ይረዳል፡፡

የጥናቱአላማ፡-

በቅዱስ ጳውሎስ ሆስፒታል የህጻናት የደም ውስጥ በሽታ አምጭ ተህዋስያን(ባክቴሪያ) ስርጭት፤ ለተለያዩ ጸረባክቴሪያ ያላቸው ግትርነትና የበሽታው አጋላጭ ሁኔታዎችን ማጥናትና ማወቅ ነው

ጥናቱ ለተሳታፊዎች ያለው ጥቅም፡-

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለም፣ነገር ግን በምርመራው ውጤት መሰረት የመታከም እድል ይኖሮታል፡፡ በተጨማሪም የጥናቱ ውጤት የደም ውስጥ ህመም

ለመቆጣጠርና ለመከላከል ስለሚጠቅም በተዘዋዋሪ መንገድ ሌላ ህመምተኛ እንዲሁም ህብረተሰቡን የመጥቀም እድል ያገኛሉ፡፡

<u>በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳትና ተዛማጅ ችግር</u>

በዚህ ጥናት በመሳተፍ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም ለዚህ ጥናት የሚያገለግል የደም ናሙና የሚወሰድ ሲሆን ከመጠነኛ የህመም ስሜት በስተቀር በጤናዎ ላይ ምንም ጉዳት አይደርስም፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

መረጃ በሚሰጡበት ወቅትም ሆነ ከዛ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘው በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አልዎት፡፡ የላብራቶሪ ውጤትዎን ማወቅ ከፈለጉ የመለያ ቁጥሮን በመጠቀም በሚሰጥዎ የቀጠሮ ጊዜ መውሰድ ይችላሉ፡፡

ጥናቱንየሚያካሄደውሰውማረጋገጫ

ለዚህ ጥናት ሃላፊነቱን ለመውሰድና፣ ማናቻውንም ጥናቱ የሚመለከቱ ጉዳይ ክትትል ለማድረግና ለሚመለከተው አካል መግለጫ ለመስጠት በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ ---------- ቀን ----------

ማንኛውንም ጥያቄ መጠየቅ ለሚሹ የሚቀጥለውን አድራሻዬን መጠቀም ይችላሉ፡፡

ኢሜል hindiyabeshir@gmail.com **ተንቀሳቃሽ ስልክ** 0910551721

የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል **ስልክ፡** 0112755170

I. **. የፍቃደኝነት መጠየቂያ (ከ 7 ዓመት በላይ ለሆኑ ህፃናት)**

ይህ ጥናት የህጻናት የደም ውስጥ በሽታ አምጭ ተህዋስያን ጥናት የሚገለጽ ሲሆን መሳተፍ ከፈለግህ/ሽ ምርጫው ባንተ/ች የሚወሰን ነው፡፡ምንም አይነት ውሳኔ ብትወስን/ኚ ከዚህ በፊት የሚደረግልህ/ሽ እንክብካቤ አይቀንስም፡፡በማንኛውም ሰአት ማንኛውንም ጥያቄ መጠየቅ ትችላለህ/ሽ፡ ፡በጥናቱ ለመሳተፍ ከወሰነክ/ሽ ለምርመራ ጥቂት የደም ናሙና በመርፌ ከጅህ/ሽ ላይ ይወሰዳል፡፡ ወረቀቱ ላይ ለሰፈሩት ጥያቄዎች አንብበህ/ሽ ተገቢውን ምላሽ ወረቀቱ ላይ መላ/ይ፡፡ከጥናት ቡድኑ አባል ለሚጠይቅህ/ሽ ድምፅህን/ሽን ከፍ አድርገህ/ሽ መልስ ስጥ፡፡ከዚህ በፊት የነበረውን የጤና መረጃህን/ሽን ለጥናቱ እንጠቀምበታለን፡፡ምናልባት ለምትጠየቃቸው አንዳንድ ጥያቄዎች ምላሽ መስጠት ብትቸገር/ሪ ምንም አይነት ጉዳትና ችግር እንደማደረስብህ አናረጋግጥልሃለን፡፡በማንኛውም ሰአት በጥናቱ አልሳተፍም የማለት መብት አለህ/ሽ፡፡በዚህ ጥናት ምንም አይነት የገንዘብ ክፍያና የተለየ ጥቅም አታገኝም/ኚም ሆኖም ግን በዚህ የጥናት ዉጤት እንዳንተ/ቺ የታመሙ ህጻናት ይጠቀማሉ፡፡በማንኛውም ሰአት ጥናቱን ማቆም ትችላለህ/ያለሽ፡፡ምርጫህን/ሽን ለማሳወቅ ጊዜ ወስደህ/ሽ አስብበት፡፡በመጨረሻም ለመሳተፍ ከወሰንክ/ሺ ስምና ፊርማህን/ሽን ከታች ባለው ክፍት ቦታ ላይ አስፍር/ሪ፡፡

እኔ ---------- የተባልኩ ግለሰብ ይህን ሁሉ በመገንዘብ በምርምሩ ላይ መረጃና የደም

ናሙና እንዲወሰድ ተስማምቻለሁ፡፡

ፊርማ---------- ቀን----------

||. የፈቃደኝነት ማረጋገጫ ቅጽ (ለቤተሰብ/አሳዳጊ)

በአዲስ አበባ ከተማ በቅዱስ ጳውሎስ ሆስፒታል የህጻናት የደም ውስጥ በሽታ አምጭ ተህዋስያን ስርጭት፤ለተለያዩ ጸረባክቴሪያ ያላቸው አይበገሬነትና የበሽታው አጋላጭ ሁኔታዎችን ለማጥናት በሚል ርእስ ላይ በሚደረገው ጥናት ላይ ለመሳተፍ ሲሆን፤ የጥናቱ አላማና ጥቅም በሚገባ ተገልፆልኛል፡፡ በመጠይቁ ላይ የሚሞላው የኔ ሙሉ መረጃም በሚስጥር እንደሚያዝ ተነግሮኛል፡፡ በተጨማሪም በጥናቱ ውስጥ ልጄን አለማሳተፍ መብቴ እንደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ውሳኔ መውጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት በልጄ ላይ እንደማይደርስ በሚገባ ተረድቻለሁ፡፡

ስለሆነም ሁኔታውን በሚገባ በማጤን በፍቃደኝነት ልጄን በምርምሩ ላይ ለማሳተፍ ለተመራማሪው ፍቃደኝነቴን ሰጥቻለሁ፡፡ በተጨማሪም ልጄ የሚሰጠው የደም ናሙና ለተጠቀሰው ጥናት ብቻ እንደሚውል ተነግሮኝ ተስማምቻለው፡፡ ማንኛውም ያልገባኝ ንነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ውጤቶች በወቅቱ ክትትል ለሚያደርገው የጤና ባለሙያ እንደሚሰጥ እና ውጤቱን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡

እኔ ---------- የተባልኩ ግለሰብ ይህን ሁሉ በመገንዘብ በምርምሩ ላይ ስለልጄ መረጃና የደም ናሙና እንዲወሰድ ተስማምቻለሁ፡፡

ፊርማ ---------- ቀን ----------

V.መጠይቅ:

በአዲስ አበባዩ ኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ዲፓርትመንት

የጤና ተቃሙ ስም----------ዓ.ም----------የጥናቱ ተሳታፊ መለያ ቁጥር----------

I. **አድራሻ፡ክ/ከ----------ስልክ----------ፊርማ----------**

የታየበት ክፍል----------ሀንጻ----------

የደም ናሙና የወሰደው ግለሰብ ስም----------ቀን----------ፊርማ----------

እባክዎን ለጥናቱ መሳካት ያግዘን ዘንድ ጥያቄዎችን በጥንቃቄ እንዲሞሉልን በትህትና እንጠይቃለን፡፡

1. **ዕድሜ**----------

2. **ጾታ** 1) **ወንድ** ☐ 2) **ሴት** ☐

3. **የታየበት/የተኛበት ክፍል**

   1.**ተመላላሽ ህክምና ክፍል** ☐

   2.**ተኝቶ ታካሚ ክፍል** ☐

   3.**የህጻናት ፅኑ ህሙማን ክፍል** ☐

   4.**ድንገተኛ** ☐

4.**አልጋ የያዘበት ቀን**----------

5. **የሰውነት ሙቀት መጠን**

   1. **ከ** 36.5 **ሴንቲግሬድ በታች** ☐ 2. **ከ** 37.5 **ሴንቲግሬድ በላይ** ☐

6. **ለብዙ ጊዜ የቆየ ተያያዥ በሽታ አለ**

   1.**አዎ** ☐ 2.**የለም** ☐

7. **ለ ስድስተና ጥያቄ መልሱ አዎ ከሆነ ምን**----------

8. የ HIV ውጤት

1.ፖዝቲፍ ☐ 2.ኔጌቲፍ ☐ 3. አታወቅም ☐

9. ወደ ሰውነትህ የገባ/የተሰካ/የተለጠፈ የህክምና መሳሪያ አለ

1.አዎ ☐ 2.የለም ☐

10. ለ 9 ኛው ጥያቄ መልሱ አዎ ከሆነ ምን አይነት መሳሪያ

1. የደም ቱቦ መርፌ ☐ 3. የሽንት ማሸኛ ቱቦ ☐

2. የአየር ቱቦ ☐ 4.የአየር ማናፈሻ ☐ 5. ሌላ ☐

11.የደም ናሙና ከተወሰደ በኃላ የተሰጠ ጸረ ተህዋሲያን----------

12. የህጻኑ የስርአተ ምግብ ሁኔታ

1. በምግብ እጥረት የተጎዳ ☐ 2. በምግብ እጥረት ያልተጎዳ ☐

13. ህጻኑ እንደተወለደ/ች የሰውነት ክብደት

1. ከ 2.5 ኪ..ግ በታች ☐ 2. ከ 2.5 ኪ..ግ በላይ ☐

14. ከጽንሰት እስከ ውልደት ያለ እድሜ

1. ከ 37 ሳምንት በታች ☐ 2. ከ 37 ሳምንት በላይ ☐

15. አብሮ የተወለደ የጤና ችግር አለ

1.አዎ ☐ 2.የለም ☐

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: **Hindiya Lema (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor:

Dessie Abera (MSC) **Melese Hailu (MSC)**

Signature: _____ Signature: _____

Date: _____ Date: _____

Place: _____

Addis Ababa, Ethiopia