ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Hospital acquired infections, antimicrobial drug resistance patterns and associated risk factors among under five children attending Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia.

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A research thesis submitted to the Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology)

June , 2018.

Addis Ababa, Ethiopia

Addis Ababa University

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This is to certify that the thesis prepared by Zerihun Woldesenbet, entitled:

Hospital acquired infections, antimicrobial drug resistance patterns and associated risk factors among under five children attending Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia 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 with respect to originality and quality.

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Acknowledgement

First of all I would like to pass my gratitude to Addis Ababa University, Department of Medical Laboratory Sciences for providing me good inspiration in the scientific teaching and for providing me a chance to conduct research which is a chance to learn and to contribute.

Secondly I want to honor my advisors, Kassu Desta (MSc, PhD candidate) my lifetime instructor, for his insightful, unreserved advice and material support, Dr Aster Tsegaye (MSc, PhD) sharing me her tremendous research experience and Dr Merertu Temesgen(MD, pediatrician) for supporting me through clinical investigation and follow up of study participants. Following this I want to thank Yekatit 12 Hospital Medical College for sponsoring my graduate study and supporting me with different materials and chemicals for my research. I am also grateful to staffs at neonatal intensive care unit, Berla pediatric unit and neonatal septic wards of Yekatit 12 hospital medical collage for their collaboration on specimen collection and completing questionnaire, and all laboratory and management staffs for their humble reception and support. In addition, I want to pass my appreciation to the guardian/parents or care givers of the children who accept to participate in this study.

Lastly, I want to appreciate my family(Hana Seboka and Rekik Zerihun) for sharing their love and compassion unconditionally.

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Abbreviations

AAU:	Addis Ababa University
AMR:	Antimicrobial Resistance
AST:	Antibiotic Susceptibility Testing
ATCC:	American Type Culture Collection
CBC:	Complete Blood Count
CDC:	Centers for Disease Control and Prevention
CI:	Confidence Interval
CLSI:	Clinical Laboratory Standards Institute
CRP:	C - Reactive Protein
CSF:	Cerebro- spinal fluid
DRERC:	Departmental Research and Ethics Review Committee
EDTA:	Ethylene Diamine Tetra Acetic acid
HAI:	Hospital Acquired Infections
LBW:	Low Birth Weight
MDR:	Multiple Drug Resistance
MHA:	Mueller-Hinton Agar
MRSA:	Methicillin-Resistant Staphylococcus aureus
NI:	Nosocomial Infections
NICU:	Neonatal Intensive Care Units
PLT:	Platelet
RBC:	Red Blood Cell
VAP:	Ventilator-Associated Pneumonia
VRE:	Vancomycin-Resistant *Enterococcus*
WBC:	White Blood Cell
WHO:	World Health Organization

Abstract

Hospital acquired infection (HAI) is an infection which develops 48 hours after hospital admission, that was not incubating at the time of admission to hospital. In patients with HAI, costs as well as the use of antibiotics increase with an extended hospitalization. There is limited information on the magnitude of HAI in Ethiopia particularly in pediatrics patient groups.

Objective: The aim of this study is to assess the incidence of hospital acquired infections, antimicrobial drug resistance patterns and associated risk factors among under five children attending Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia.

Methods: This was a prospective cohort study that was conducted at City –Government of Addis Ababa, Yekatit 12 Hospital Medical College from January to April 2018. All admitted patients who did not have antibiotic and stay at least 48 hours during sample collection period were included with the outcome variable being the prevalence of hospital acquired infection. Blood, urine, body fluids, stool, and swabs were cultured on corresponding media(brain heart infusion, chocolate agar, blood agar, MacConkey…etc) for isolation and identification of the bacteria and Kirby- Bauer method for antibiotic susceptibility testing. Data were analyzed using SPSS version 23. Chi-square tests were performed to determine risk factors associated with HAI.

Results: A total of 720 under five children were admitted during the study period (from January 2018 to April 2018). Out of this 435 children fulfilled the study criteria and were included in the study. Of the 435 children, 255(58.7%) were males and 180(41.3%) females and resulting in male to female ratio of 1.41:1. About 259 were neonates and the remaining 176 were children age between 21days and 5 years. The overall incidence of hospital acquired infections in this study was 8.5% with the most common isolate being *Klebsiella pneumonia* (n = 19) followed by *S. aureus* (n=7). *Klebsiella pneumoniae* has shown high level of resistance (100%) to most of third generation cephalosporins and *S. aureus* has shown high resistance to penicillin and cotrimoxazole. Prevalence of MDR seen among *K. pneumonia* which is 100%, followed by *Acinetobactor species* in this case we have 66.7% MDR. The presumed predisposing factors were not found statistically associated.

Conclusion: This study showed that hospital acquired infections remains high especially in neonatal intensive care unit complicating disease of already prone children. Therefore, appropriate infection prevention has to be strengthened.

Key terms: Hospital acquired infection, multiple drug resistance and under five children.

1. Introduction

1.1 Background

Hospital acquired infections (HAI) is an infection which develops 48 h after hospital admission, that was not incubating at the time of admission to hospital. Interchangeably the term "Nosocominal" is used for any disease acquired by patient under medical care [1]. It is an infection acquired by patient during hospital stay. For the following reasons infections are not believed as nosocomial are: 1. infections that were present at the time of admission and become complicated, nevertheless pathogens or symptoms change resulting to a new infection; 2. infections that are acquired trans-placentally due to some diseases like toxoplasmosis, rubella, syphilis or cytomegalovirus and appear 48 h after birth [2].

One of the earliest records of hospital infections are perhaps those found in an Egyptian papyrus written around 3000 B.C. Again the famous Hindu physician Charaka and surgeon Sushuruta (400 B.C.) have also emphasized the need for prevention of infection in clinical practice. The records of Herodatus on the conditions that prevailed in Greek and Roman hospitals in the period 1000 to 600 B.C., and the Hipprocrates treatise (400 BC) testifying the existence of hospital acquired infection[3]. Despite progress in public health and hospital care, infections continue to develop in hospitalized patients, and may also affect hospital staff. Hospital acquired infections occur worldwide and affect both developed and resource-poor countries. They are a significant burden both for the patient and for public health. [4].

Hospital-acquired infections appeared before the origination of hospitals and became a health problem during the miraculous antibiotic era. Due to these infections, not only the costs but also the use of antibiotics increased with an extended hospitalization. This resulted in elevated morbidity and mortality. Studies conducted in different parts of the world show that in North America and Europe 5%–10% of all hospitalizations result in nosocomial infections, while Latin America, Sub-Saharan Africa and Asia show more than 40% hospitalizations with nosocomial infections [5]. Nosocomial infections (NIs) are one of the major causes of mortality and morbidity in the pediatric wards and neonatal intensive care units (NICUs) [6]. They have an impact on the healthcare system as they increase the use of medical resources, duration of hospitalization, as well as cost of treatment in both developed and developing countries[7].

As a pressing threat to international health, antimicrobial resistance is of increasing importance. Resistance to antimicrobials threatens nearly a century of gains made since the discovery of antibiotics. It also undermines the contribution of these drugs to improvements in childhood survival in the developing world, particularly among neonates[8]. Antimicrobial resistance has been reported in both community-acquired and health-care associated infections worldwide. However, in low and middle-income countries, surveillance is often inconsistent because of insufficient integration and non-representativeness of local data, and scarce microbiological diagnostic facilities[9]. In clinics and hospitals, scarce diagnostic resources and consequent therapy based on clinical syndromes also drive antibiotic consumption, which is a key factor in promotion of resistance. Syndromic management is a sensitive (rather than specific) method for serious bacterial infections, therefore, likely to capture viral, parasitic, and self-limiting illnesses [9].

Hospital acquired infections are caused by many microbes and each one can cause infection in healthcare settings. Bacteria are responsible for about 90% HAIs, whereas protozoans, fungi, viruses and mycobacteria are less contributing compared to bacterial infections[10]. The agents that are usually involved in hospital-acquired infections include *Streptococcus spp., Acinetobacter spp., Enterococci, Pseudomonas aeruginosa* (*P. aeruginosa*), coagulase-negative staphylococci, *Staphylococcus aureus (S. aureus), Bacillus cereus (B. cereus), Legionella* and *Enterobacteriaceae* family members including *Proteus mirablis*, *Klebsiella pneumonia* (*K. pneumonia*), *Escherichia coli* (*E. coli*), *Serratia marcescens*. Out of these *Enterococci, P. aeruginosa*, *S. aureus* and *E. coli* have a major role[10].

The predisposing factors for hospital acquired infections may be due to ordinary risks or peculiar to the environment of a hospital, and both intrinsic and extrinsic factors play an important role in the development of nosocomial infections. They include the duration of hospitalization, type of ward, underlying disease, medical procedures and devices, antimicrobial therapy and negligence of laid down hygienic and sanitary procedures. The widespread and indiscriminate use of antibiotics has a selective pressure which gradually replaces antibiotic sensitive strains with those resistant to multiple antibiotics. Such strains are resistant to normal concentrations of disinfectants commonly used in the hospitals. The emergent antimicrobial resistant strains are commonly involved in the causation of nosocomial infections[11]. Ethiopia is also among the

developing countries where the impact of HAI in causing morbidity and mortality is very significant.

1.2 Statement of the Problem

According to the World Health Organization (WHO), hospital acquired infections are one of the major infectious diseases and have a huge economic impact worldwide. These infections affect about 2 million people annually resulting in 5-15% of them requiring hospitalization. In addition, a WHO study conducted in 55 hospitals of 14 countries representing four WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) showed that an average of 8.7% of hospitalized patients contracted nosocomial infections with the highest frequencies noted from patients in the South-East Asian and Eastern Mediterranean Regions (10.0 and 11.8%) respectively[12]. However, the prevalence of Healthcare Associated Infections in developing countries varied from 5.7% to 19.1%. In line with this, various prevalence rates have been recorded for the following countries: Mongolia 5.4%, Latvia 5.7%, Thailand 6.5%, Lebanon 6.8%, Indonesia 7.1%, Cuba 7.3%, Islamic Republic of Iran 8.8%, Lithuania 9.2%, and Albania 19.1%[12].

The above reports suggest that HAI infections are widespread in sub Saharan Africa with surgical sites being the most common. The prevalence rates of the infections reported varied between 2.5% - 14.8% in Burkina Faso, United Republic of Tanzania and Senegal[13]. The peak report is the finding of 5.7-45.8% in Nigeria and Ethiopia[14,15] with the former having an incidence as high as 45.8% . However, reliable estimates of the global burden are hampered by a paucity of data adequately describing endemic infections at national and regional levels, particularly in resource-limited settings[16]. In countries where less than 5% of the gross national product is spent on health care, and workforce density is less than five per 1000 population, other emerging health problems and diseases take priority[17]. The epidemiological gap leading to the absence of reliable estimates of the global burden is mainly because surveillance of Hospital acquired infections expends time and resources and needs expertise in study design, data collection, analysis, and interpretation[18].

Generally, the above data suggest that not only risks of hospital acquired infection are significantly higher in developing countries but also that the effect on patients and health-care systems is severe and greatly underestimated. In Ethiopia there is limited data on hospital acquired infection of under five children[29]. But there are some patchy information available in

some part of the country, some on neonatal intensive care the others on surgical site infections. In addition to this there is no updated or current information on HAI. Therefore, this study provided comprehensive (neonates plus under five children) and updated information on HAI.

1.3. Significance of the study

The study was carried out to gain insight concerning incidence of hospital acquired infection, pathogens involved, associated risk factors and antibiotic susceptibility pattern of bacterial pathogens among under five children. The patient's benefit from the study because more intensive investigations were done which have allowed detecting and treating an infection earlier than otherwise. Hospital acquired infections are important in wide-ranging concern in the medical field. The benefit for the patient was being part of this study which helps to create safe hospital environment for the patient and the community as a whole. The finding of this study will be used by the hospital administration and pediatric unit to enhance infection control and for planning. This study generated data on HAI infection in children that will serve as additional information for further studies and to develop prevention interventions by policy makers/ stakeholders. The finding of antimicrobial susceptibility pattern helps to choose appropriate antibiotic for empiric treatment.

2. Literature Review

2.1. Epidemiology of hospital acquired infection

Over 1.4 million people worldwide suffer from HAI at any given time. Hospital wide prevalence of HAI varies from 5.7% to 19.1%, with a pooled prevalence of 10.1%[19].On the same report, in developed countries, HAI concerns 5–15% of hospitalized patients and can affect 9–37% of those admitted to intensive care units. Recent studies conducted in Europe reported hospital-wide prevalence rates of patients affected by HAI ranging from 4.6% to 9.3%[20].

In another review conducted in 1149 hospitals in European Union Member states, from 29 countries data for 17 273 children and adolescents were analyzed. From them 770 health-care-associated infections(pathogens) were reported in 726 children and adolescents, corresponding to a prevalence of 4·2% (95% CI 3·7–4·8)[20]. Apart from this, a meta-analysis done in Southeast Asia has shown that the burden of Healthcare-Associated Infections in a pooled prevalence of overall HAIs was 9.0% (95% CI, 7.2 –10.8)[21]. In another prospective HAI surveillance conducted in Cambodian pediatric hospital the incidence of HAI was 4.6/1,000 patient-days (95% confidence interval 3.8–5.6) and rates were highest amongst neonates[22]. In another study on the targeted surveillance of nosocomial infection in intensive care units of 177 hospitals in Jiangsu Province of China, the incidence of NI appeared to decrease, and the incidence of NI per 1000 patient-days and adjusted incidence were 25.63‰ and 7.41‰ in 2010, and 9.73‰ and 2.76‰ in 2015, respectively [23].

In a systematic review conducted in Iranian burn patients it was shown that the overall incidence of HAI was 20.9%[24].Systematic review of literatures from different African countries revealed that the hospital-wide prevalence of HAI in Africa varied between 2.5% and 14.8%; in surgical wards, the cumulative incidence ranged from 5.7% to 45.8%[25]. In point prevalence survey conducted in Morocco the prevalence of HAI was 10.3%. Intensive care units were the most affected wards (34.5%)[26].In addition to those a prospective cohort study conducted in Egypt, has shown that 38.5% developed NI [27]. In similar study conducted in Egypt the overall rate of HAI was 5.2/100 admissions [28]. In Ethiopia, according to a case control study done on 111 cases from pediatrics wards of Tikur Anbessa Hospital Nosocomial infection rate was 5 per 100 discharges[29].

2.2. Etiologic agents involved in hospital acquired infection

In a review of Hospital-Acquired Infections in united states of America the most commonly incriminated pathogens that cause HAIs were Methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile*, and vancomycin-resistant *Enterococcus* (VRE)[30].In a similar review study conducted in 1149 hospitals in European Union Member states, from 29 countries,392 microorganisms were reported for 342 health-care-associated infections, with *Enterobacteriaceae* being the most frequently found (113 [15%])[20].In another study on the targeted surveillance of nosocomial infection in intensive care units of 177 hospitals in Jiangsu Province of China, the six most common pathogen-caused NIs found were *Acinetobacter baumannii*, *K pneumoniae*, *P aeruginosa*, *S aureus*, *Candida albicans*, and *Escherichia coli* (*E. coli*)[23].

According to a systematic review conducted on literatures of Iranian burn patients, the most common prominent isolates were *P aeruginosa*(30.39%), *K pneumonia* (17.54%), *Acinetobacter*(17.47%), and *S aureus*(14.98%) with high antibiotic resistance isolated from the cultures of different sites of infections including burn wound[24]. In a similar study conducted on literatures from different African countries, the isolated pathogens, by order of decreasing frequency, were *Pseudomonas aeruginosa*, *Escherichia coli*, *K. pneumoniae* and *Enterobacter* spp. Another study reported *Enterobacter cloacae* as the most common pathogen followed by *E. coli*, *S. aureus* sand *P. aeruginosa*[25]. In the same article, report on HAI cumulative incidence in surgical patients showed the following distribution: *K. pneumoniae*(38.7%); *E. coli* (22.7%); *P. aeruginosa*(16.8%) and *S. aureus*(10.7%).

In a point prevalence survey conducted in Morocco *Staphylococcus* was the organism most commonly isolated (18.7%)[26].In another cross-sectional study conducted in Tanzania a wide range of bacteria was isolated, the most predominant being gram-negative bacteria: Proteus spp. (n = 48, 12.7%), *Escherichia coli* (n = 44, 11.7%), *Pseudomonas spp*. (n = 40, 10.6%) and *Klebsiella spp* (n = 38, 10.1%)[31].In addition to those, a prospective cohort study conducted in Egypt identified that most of the infections were caused by *Klebsiella spp* (34.2%), *S. aureus* (26.1%), *P. aeruginosa* (14.9%), *E. coli* (11.2%), *Proteus spp* (7.4%), group B *Streptococcus* (3.7%), and *C. diversus* (2.5%).)[27]. Lastly, in a case control study done on 111 cases from pediatrics wards of Tikur Anbessa Hospital Ethiopia,14 different type of bacteria were found. *E*

coli, *Klebsiella pneumoniae* and pseudomonas species were the most frequently isolated organisms[29].

2.3. Predisposing factors for hospital acquired infection

The use of instrumentation or devices for intubation, delivery of therapeutic agents, or drainage of body fluids during patient care as supportive measures has been identified as important predisposing factors to HAIs in United States of America. According to this review study most are device-related infections, i.e. catheter associated urinary tract infections, vascular catheter associated infections, and ventilator-associated pneumonias. Unlike other hospital-acquired infections, device-related infections are linked directly to medical care[30]. In a similar review study conducted in 1149 hospitals from 29 countries of European Union Member states, bloodstream infections were the most common type of infection (343 [45%]), followed by lower respiratory tract infections (171 [22%]), gastrointestinal infections (64 [8%]), eye, ear, nose, and throat infections (55 [7%]), urinary tract infections (37 [5%]), and surgical-site infections (34 [4%]). The prevalence of infections was highest in paediatric intensive care units (15·5%, 95% CI 11·6–20·3) and neonatal intensive care units (10·7%, 9·0–12·7). Independent risk factors for infection were age younger than 12 months, prolonged length of stay, and the use of invasive medical devices[20].

In a meta-analysis conducted in Southeast Asia the pooled incidence density of ventilator-associated pneumonia, central line–associated bloodstream infection, and catheter-associated urinary tract infection was 14.7 per 1000 ventilator-days (95% CI, 11.7–17.7), 4.7 per 1000 catheter-days (95% CI, 2.9–6.5), and 8.9 per 1000 catheter-days (95% CI, 6.2–11.7), respectively[21].A targeted surveillance of nosocomial infection in intensive care units of 177 hospitals in Jiangsu Province of China, have identified central line-associated bloodstream infection, catheter-associated urinary tract infection, and ventilator-associated pneumonia (VAP) as predisposing factors, though overall incidence is decreased over time [23].

Moreover, according to a prospective cohort study conducted in Egypt, low birth weight (LBW) and prematurity were reported to be important risk factors for NI[27].In a similar study conducted in Egypt mechanical ventilation, invasive device utilization, neonatal age, neutropenia, Intensive Care Unit residence and hospital stay for > 7 days were the commonest risk factors significantly associated with HAI in that hospital[28].Age less than one year,

malnutrition, admission to orthopedics unit, peripheral intravenous line and prolonged hospitalization were significantly associated with nosocomial infection as per a case control study done on 111 cases from pediatrics wards of Tikur Anbessa Specialized Hospital, Ethiopia, [29].

2.4. Antimicrobial susceptibility of pathogens isolated from hospital acquired infection

Antimicrobial resistance (AMR) is becoming a serious global threat and HAIs are among major contributors. For example, according to surveillance of nosocomial infection in intensive care units of 177 hospitals in Jiangsu Province of China, the isolation rate of carbapenem-resistant gram-negative bacilli were as follows: carbapenem-resistant *Acenetobactor bounboi* 80.53%, carbapenem-resistant *Pseudomonas aroginosa* 39.94%, carbapenem-resistant *Kelbsiela pneumonia* 24.86%, and carbapenem-resistant *E. coli* 9.23%. The isolation rate of methicillin-resistant *S. aureus*(MRSA) was 66.30%[23]. In another prospective HAI surveillance conducted in Cambodian pediatric hospital the resistance to third generation cephalosporins was common, supporting the use of more expensive carbapenem drugs empirically in HAI cases[22].

In a prospective cohort study conducted in Egypt, all of the isolated bacteria were resistant by 100.0% for Cefotaxime. While all bacteria were sensitive by 100.0% to Imipenem except *Klebsiella spp*(78.2%) and *S. aureus* (24.8%)[27].A point prevalence survey conducted in Morocco has shown that methicillin-resistant was detected in 50% of cases [26]. In another cross-sectional study conducted in Tanzania all *Staphylococcus aureus* tested were resistant to penicillin (n = 22, 100%) while susceptible to vancomycin. Significant resistance to cephalosporins such as cefazolin (n = 62, 72.9%), ceftriaxone (n = 44, 51.8%) and ceftazidime (n = 40, 37.4%) was observed in Gram-negative bacteria, as well as resistance to cefoxitin (n = 6, 27.3%) in *S. aureus*[31].In Ethiopia, a case control study done on 111 cases from pediatrics wards of TikurAnbessaHospital Ethiopia, reported that the resistance to ampicillin was 91.9% , gentamycin 67.2%, ceftriaxone 50%, norfloxacin18.3%, and ciprofloxacin 15.4%[29].

Taken together, the above reviews show that the prevalence of HAI varied according to the resource availability(developed versus developing countries). And the etiologic factors, antibiotic susceptibility pattern and the risk factors also varied according to the study area or country. With the increasing trend of antibiotic resistance, which is a global public health threat, there is a need to monitor the magnitude of HAIs and sensitivity pattern of the identified

microorganisms and provide data for hospital managers and policy makers for appropriate action. The pediatric age group especially the neonatal group is less investigated in our country, necessitating for such studies to filling the gaps.

2.5. Conceptual framework

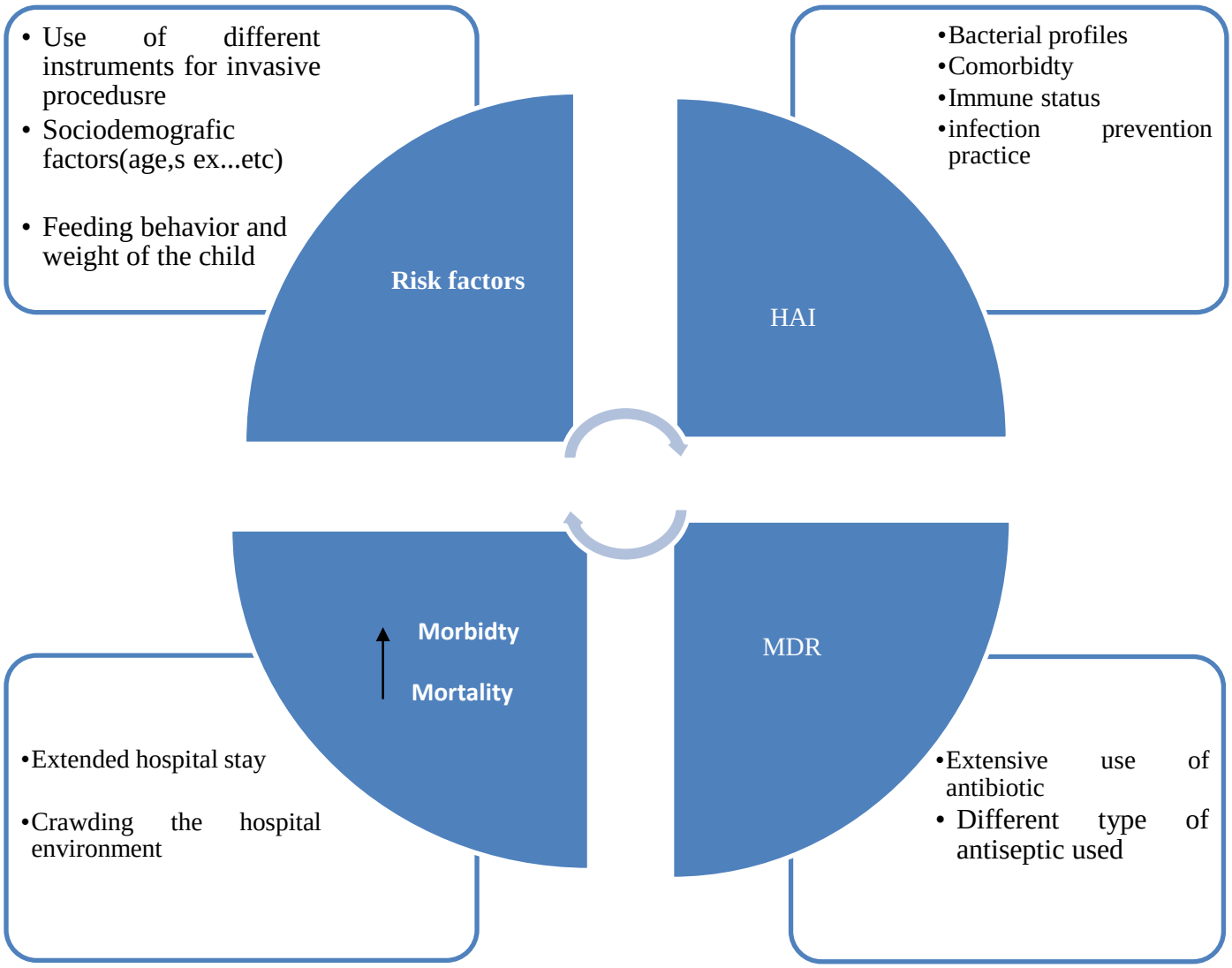


Fig 1. Conceptual framework(Based on information found in literatures[1-29])

3. Objectives

3.1. General objective:

To determine the incidence of hospital acquired infections, antimicrobial drug resistance patterns and associated risk factors among under five children attending Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia, from January 2018 to April 2018.

3.2. Specific objectives

- To determine the incidence of hospital acquired infections ,
- To assess the bacterial profiles associated with HAI among under five children
- To determine antibiotic resistance patterns of pathogens involved in HAI among under five children
- To determine the associated risk factors for hospital acquired infection

4. Hypothesis

The prevalence of nososcominal infections in under 5 years pediatric population and associated etiologic factors are the same with the finding of study conducted in Tikur Anbesa Hospital Ethiopia.

5. Materials and methods

5.1. Study area

The study was conducted in Yekatit 12 hospital medical college, Addis Ababa, Ethiopia. The hospital is a teaching Hospital managed by Addis Ababa City Administrative Health Bureau. It is located in Arada Sub-City of City Government of Addis Ababa. The Hospital is established in 1915 with a total of 25 beds and 37 health professionals. According to the data obtained from the hospital, currently the hospital has 725 health professionals and 375 administrative staff with around 272 beds and provides different medical services for around 4 million people. It is the only hospital under the city Administrative Health Bureau of Addis Ababa with high number of neonatal and pediatric beds and providing services for high number neonates and pediatric patients. It is also the only hospital under the city Administrative Health Bureau of Addis Ababa, where there are culture and drug susceptibility test services available. According to current HIMS; pediatrics and NICU have totally 86 beds and have around 165 admissions per month.

5.2. Study design and period

A prospective cohort study were carried out at the Neonatal Intensive Care Unit(NICU) and pediatric wards of Yekatit 12 hospital medical college in Addis Ababa, Ethiopia, over a period from January 2018 to April 2018.

5.3. Population

5.3.1. Source population

All clients who come to receive different medical services at the department of pediatrics and Neonatal Intensive Care Unit(NICU) of Yekatit 12 hospital medical college.

5.3.2. Study Population

All infants delivered in the department of obstetrics and gynecology and admitted to NICU, and those under five children referred to Yekatit 12 Hospital Medical College for admission and full filling the eligibility criteria were the study population.

5.4. Inclusion and exclusion criteria

5.4.1. Inclusion criteria

The criteria include under five children without any sign of infection at admission and remained hospitalized for at least 48 hours at Berla pediatric III, NICU, septic ward and other wards(eg. Burn, psychiatry, Berla pediatric Unit II).

5.4.2. Exclusion criteria

Children who used antibiotic before 10 days and or during data collection as well as any neonate who died or discharged before 48 h were excluded.

5.5. Study variables

5.5.1. Dependent variables

- Incidence of hospital acquired infections(HAI)
- Bacterial profiles associated with hospital acquired infections
- Drug susceptibility patterns of the pathogens

5.5.2. Independent variables:

- Socio-demographic variables such as age. sex, … etc
- Feeding behavior and weight of the child
- Device utilization
- Reason of admission
- Admission wards
- Antibiotic consumption
- Use of different instruments (catheter, central lines, respiratory support , canulla etc, surgery, previous hospitalization
- Complete blood count
- C-reactive proteins

5.6. Sample size calculation and Sampling method

5.6.1. Sample size calculation

The sample size was calculated using the formula for single proportion sample size calculation by taking a 95% confidence level and a 5% margin of error [32]. And proportion or prevalence form previous study which is 5% was 73 which are very small. Therefore, in this study we included all pediatric patients admitted to pediatric wards, NICU and other wards who full fill the inclusion criteria during the study period.

Based on the information from HIMS, the hospital has 165 admissions in a month, and the sample collection time was four months. Form this we have an estimated sample size of 660 of total admission. From this according to inclusion criteria the study subjects were selected.

5.6.2. Sampling Method

For this research convenient sampling technique was employed to collect samples within the specified period in the above mentioned site.

5.7. Measurement and data collection

5.7.1. Data collection procedure

5.7.1.1. Socio-demographic factors, risk factors complete history

For socio-demographic factors, risk factors and complete history standard questionnaires were used. For neonate to detect any risk factors of infection either natal or postnatal factors were assessed, the variables included were admission date, gestational age, birth weight, and sex. Complete obstetric history to detect risk factors of sepsis such as: mode of delivery, and symptoms of sepsis as lethargy and poor feeding. In addition to antibiotic administration and medical devices used (endotracheal tube/mechanical ventilation, central venous catheter, urinary catheter, peripheral arterial/venous catheter, and feeding tube), data on secondary bacteriemia, antimicrobials prescribed were collected.

5.7.1.2. Clinical examinations

The complete clinical assessment were carried out to all study participants in the units by neonatologist(for neonates)and pediatricians (in different pediatric wards) on duty, and standard data collection form were filled. Birth weight was measured in addition to clinical assessment for signs of sepsis such as: respiratory dysfunction (apnea, signs of respiratory distress), circulatory dysfunction (poor peripheral circulation, hypotension, and prolonged capillary refill), Gastro Intestinal Tract dysfunction (abdominal distension, feeding intolerance, hepatomegaly and jaundice) and neurological dysfunction (irritability, hypotonia, lethargy).

5.7.2. Laboratory methods

5.7.2.1. Laboratory method selection

All study participants admitted to the units(pediatric ward and neonatal intensive care) were subjected to the following investigations at the time of admission to exclude infection: complete blood count (CBC), C-reactive protein(CRP), blood and urine culture. On the third day of admission, all of the studied participants were re-evaluated clinically to detect nosocomial infection. Blood specimens were collected and bacteriological studies were done for participants in which infection is suspected clinically including: full blood count, C-reactive protein, microbiological confirmation of diagnosis by: blood culture, culture of other specimens (according to the site of infection), urine, pus and tracheal aspirate cultures were added when needed. Cerebro spinal fluid (CSF) culture was performed in all participants who have clinical signs of meningitis or bacterial growth in blood culture. Specimens also were collected from different medical equipments or supplies and environment utilized to care for the study participants to determine risk factors. All specimens were cultured on specific media for identification of the organism and antibiotic susceptibility.

5.7.2.2. Sample collection

Aseptic techniques were employed during collection of all samples to prevent the introduction of micro-organisms into the patient's anatomical space, and to prevent the sample from being contaminated during the process of collection. All samples were regarded as potentially infectious and standard precautions guidelines were followed by all healthcare workers during sample collection and handling. Specimens were collected before administering antimicrobial

agents when possible with as little contamination from indigenous flora. Appropriate collection devices, sterile equipment were utilized, and aseptic technique was followed to collect specimens. All swabs were kept moist in a normal saline after the specimen is collected. As the specimens were inoculated immediately no transport medium was used .The specimen container was clearly labeled with code. Generally, 3-5ml blood(3ml for culturing(in brain heart infusion broth(Oxoid, UK)) and 2ml (in EDTA tube)for CBC and CRP) was collected (refer annex IX) for amount of blood collected from neonates which is based on the weight of the children. And 1-10ml urine, swabs from different body sites and equipments(environment or medical devices) different types of body fluids amount not less than 1ml were collected. The specimen source and/or specific site were identified correctly so that proper culture media were selected during processing in the Laboratory. All specimens were transported to the laboratory promptly. This ensures the survival and isolation of fastidious organisms and prevents overgrowth by more hardy bacteria. It also shortens the duration of specimen contact with some local anesthetics used in collection procedures that may have antibacterial activity.

5.7.2.3. Isolation and identification of bacteria

The collected specimens were transported to the laboratory as quick as possible. Based on the sample type appropriate media were selected(for blood culture 30ml brain heart infusion, as primary and sub-cultured on chocolate agar, blood agar, and MacConkey agar; for swabs of different types blood agar, and MacConkey agar and manitol salt agar, for urine culture blood agar, and MacConkey agar, for different body fluids chocolate agar, blood agar, and MacConkey agar and stool cultured on Deoxycholate citrate agar and MacConkey agar). All the culture media were from Oxoid UK. Then after, the samples were inoculated on to the appropriate medium and incubated at 35 ^{o}C -37^{o}C overnight. The principal method for the detection of bacteria from clinical specimens is by culture on solid culture media. Bacteria grow on the surface of culture media to produce distinct colonies. Different bacteria produce different but characteristic colonies, allowing for early presumptive identification and easy identification of mixed cultures. Bacteria can also be grown in liquid media (broth). Bacterial growth is easy to detect as the clear liquid turns turbid, usually within 24–48 hr, but incubation was extended to 7 days, within seven days turbidity and hemolysis observed, for those with hemolysis and turbidity subcultured and gram stain performed, then based on type of colony identification carried out [36].

Identification of bacteria

Identification of bacteria was based on growth characteristics (such as the time required for growth to appear or the atmosphere in which growth occurs), colony and microscopic morphology, Grams reaction, battery of biochemical, physiologic, and, in some instances, serologic characteristics[36].

Interpretation of culture results

For *Staphylococcus species* : Catalase , Coagulase, DNAse were performed and interpreted according to flow chart(Annex VII).

For gram negative bacteria: Oxidase, carbohydrate utilization test(on Kiligler iron agar), indole test on nutrient broth, mannitol fermentation test, decarboxylation on lysine iron agar, citrate utilization on Simon's citrate agar, motility test, and urea utilization test were performed and interpreted according to flow chart(Annex VI).

5.7.2.4. Antibiotic susceptibility testing(AST)

The AST test was performed by Kirby Bauer test which is a qualitative assay. Once isolated colonies are available from an organism that has been identified as a potential pathogen, the following steps followed; colonies selected, inoculums suspension prepared in saline, inoculums suspension standardized(turbidity measured by densitometer(0.5MF)), allow a Mueller-Hinton Agar (MHA) plate to warm to room temperature so that any excess moisture will be absorbed into the medium. Inoculated on plate, antimicrobial disks added, plate incubated(16-18hours), inhibition zones measured[38].

Interpretation of AST

1. Zone of inhibition interpreted in-comparison with the break points in the standard (CLSI guideline 2017)[37].
2. Measuring Unusual Zones: i. Double zone: the innermost zone measured
   ii. Interpreting Zone with Feathered Edge: Measure the point at which an obvious demarcation between growth and no growth.
   iii. Swarming due to Proteus mirabilis: Measure the obvious zone. Ignore the swarm even if it covers the zone. measuring unusual zones adopted from manual of antimicrobial susceptibility testing[38].

Antibiotics tested for gram negative organisms

Table1. Showing antibiotics tested for of Gram negative bacterial pathogens

Antibiotic	Concentration(µg)	Antibiotic	Concentration(µg)	Antibiotic	Concentration(µg
Ampicilin	10	Cefoftaxim	30	Cefuroxim	30
Gentamycin	10	Ceftriazone	30	Chloramphenicol	30
Augmentin	10/20	Cefepim	30	Ciprofloxacilin	5
Norfloxacilin	10	Aztronam	30	Nitrofurantoin	300
Tobramacin	10	Ceftazidime	30	Cefixim	5
Amikacin	30	Cotrimoxazol	1.25/23.75	Meropenem	10
Imipenem	10	Doxycycline	30		

Antibiotics tested for gram positive organisms

Table 2. Showing antibiotics tested of Gram positive bacterial pathogens

Antibiotic	Concentration	Antibiotic	Concentration(µg)	Antibiotic	Concentration(µg)
Penicillin	10	Erythromycin	15	Augmentin	10/20
Nitrofurantoin	300	Cotrimoxazol	1.25/23.75	Ceftazidim	30
Vancomycin	30	Gentamycin	10	Cefuroxime	30
Ceftriazone	30	Cefotaxime	30	Azitromycin	30
Cefoxitin	30	Clindamycin	2	Amoxilin	10
Ampicilin	10	Tobramacin	10		

5.7.2.5. Detection of Carbapenem Resistance

All the carbapenem (meropenem and imepenem) resistant or intermediate isolates were checked for the presence of carbapenemase using modified Hodges test(MHT) also known as the clover leaf test as per the CLSI 2017. The presence of indentation indicates a positive test and the isolate is a carbapenemase producing strain. No growth of the ATCC *E.coli* 25922 along the organism growth streak indicates a negative test and the isolate is not carbapenemase producer.

5.7.2.6. Detection of extended spectrum beta-lactamase

Initial screening for extended spectrum beta-lactamase(ESBL) was done by the diameter of zones of inhibition produced by ceftazidime(30µg) , ceftriaxone(30µg) and cefotaxime (30µg) are the screening criteria within the CLSI 2017. These breakpoints are indicative of ESBL production is: for CAZ $\leq$ 20mm, CRO$\leq$25mm and CTX$\leq$ 27mm.

5.7.2.7 Confirmation tests for ESBL

5.7.2.7.1 Combined disk test

In this test ceftazidime (30µg) disk and cefotaxime (30µg) disk were used alone and their combination with clavulanic acid(30µg). An increase of zone of inhibition $\geq$ 5mm for either of the disks mentioned above and their combination were interpreted as ESBL producer. This screening test was used for comparing double disk synergy method.

5.7.2.7.2 Double disk synergy test

The organism tested was spread on to a Mueller-Hinton agar plate. The antibiotic disks used are ceftriaxone(30µg), cefotaxim(30µg) ceftazidime(30µg), Aztronam(30µg) and amoxillin/clavunilic acid (20/10µg). The four antibiotics were placed at a distance of 20mm from amoxillin/clavunilic acid disk which is placed at the center. After 24 hr incubation enhanced zone of inhibition observed for either of the antibiotics and amoxillin/clavunilic acid were observed, and the test is positive.

5.7.2.8 Subsidiary tests

5.7.2.8.1 **Complete blood count**

Complete hematological parameters including Diff, WBC, RBC, and PLT were performed using Abbott Hematology Analyzer CELL-DYN ®1800(Abbott, USA). The Cell-DYN 1800 System is a bench-top analyzer consisting of the main analyzer with data module, display station and with external printer. The main analyzer and display station are housed in a single chassis. The instrument is open system, equipped to aspirate blood from collection tube that is opened and held under the open aspiration. Four independent measurements are performed by the analyzer: WBC, RBC, and PLT measured in electrical impedance channel and hemoglobin measured by spectrophotometer. Finally, differential data are measured in the optical flow channel [39].

During each measurement cycle, the sample is aspirated, diluted and mixed, and the measurement for each parameter is performed. 30µl of EDTA blood was aspirated by the instrument, and diluted in the respective counting plates (WBC and RBC counting plates).The instrument automatically, counts the WBC, RBC, PLT, WBC differential and measures hemoglobin concentration. The result for patient is displayed on the screen and data is recorded automatically[39].

5.7.2.8.2 **C-reactive protein**

The C - reactive protein test contains a suspension of polystyrene latex particles which have been coated with antibody to human C-reactive protein. The CRP produced in response to inflammation or necrosis binds to the specific antibody coating the latex particles. In specimens having abnormally high levels of CRP, this binding is evident by rapid agglutination of the latex[40]. Agglutination (positive reaction) indicates the level of C-reactive protein is above normal (approximately 1.0mg/dl or greater). The lack of agglutination (negative reaction) indicates the level of C-reactive protein is within the normal range [40].

5.8. Data and laboratory Quality Assurance

The reliability of study findings is guaranteed by implementing quality control measures throughout the whole process of data collection and laboratory works.

5.8.1. Data collection tool

A standardized questionnaire was used to collect data of socio-demographic characteristics for determining the prevalence of HAI. Laboratory samples of urine, sputum, wound swabs; fecal specimens, throat swabs, nasal swabs, and blood samples were collected. Medical records and consultation with the person in charge of the patient is the gold standard for the identification of the infection. Data were collected based on the signs and symptoms and the specific site criteria, as recommended by CDC [33].

5.8.2. Pre-analytical

The processes of selecting appropriate tests, ordering, collecting, identifying and labeling(three label system i.e PID, CODE, LSN), handling, and transporting biological samples were performed as per the standard guideline at each ward. The Process of accepting samples by the laboratory, centrifuging, aliquoting, diluting, and sorting the biological samples all the process of pre-analytical steps were performed according to standard operating procedure. Aseptic techniques were implemented in all the steps of specimen collection and inoculation on to culture media to minimize contamination.

5.8.3. Analytical

All materials equipment and procedures were adequately controlled. All culture media was prepared according to the direction of the manufacturer. Culture media was tested for sterility and performance. Sterility of media was checked by incubating overnight at 37^{o}c. Performance of MaConkey agar plate and blood agar plate was tested using the control strains *E.coli* ATCC 25922, *P.mirables* ATCC 35659, and *S aureus* ATCC 25923, *S. pneumonia*(patient strain); respectively. International control bacterial strains: *Escherichia coli*(ATCC 25922), *S.aureus* (ATCC 25923), *Pseudomonas aeroginosa*(ATCC27853) were used in controlling the potency of the drugs. For biochemical test media, the media were inoculated with bacterial species of known positive and negative reactions. Moreover; culture growth, biochemical test and antimicrobial susceptibility test results were confirmed by specially trained experienced microbiologists working in the microbiology unit of the study site.

5.8.4. Post-analytical

All of the extracted information (filled questionnaire and laboratory findings) were checked for legibility, completeness, consistency and placed in secure location. Cross-checking and data cleaning were done. During data cleaning and cross-checking missing information was obtained by going back to the questionnaire and laboratory records. The data were stored in a CD as a backup. All laboratory isolates were stored as per the SOP of the study site.

5.9. Data analyses and interpretation

Data was entered into computer by using Microsoft Access (office 2007). Frequency and percentages were computed to present all categorical variables including sex, hospital acquired infections and type pathogens involved. Data were analyzed using SPSS (the Statistical package for Social Sciences for Windows) version 23. Descriptive statistics such as count, percent, mean and standard deviation were used for children, maternal characteristic, admission diagnosis and manipulations were done for these patients. Multivariate analysis was performed for risk factors associated with HAI such as longer length of hospital stay, presence of comorbidity, invasive devices and antibiotic use. Chi square test were performed and P value less than 0.05 was used to determine significance levels and strength of associations.

5.10. Ethical considerations

Ethical clearance was obtained from Departmental Research and Ethics Review Committee(DRERC) of the department of Medical Laboratory Sciences of Addis Ababa University and from Addis Ababa Public Health Research and Emergency Management Core process. Permission was obtained from Yekatit 12 Hospital Medical College. The purpose and procedure of the study was explained to the parents or guardian of the study participants in the study site. Those who agreed to participate were asked to sign consent form. Written and informed consent were obtained. All needed official permissions were obtained. In addition the clinical specimens collected during the study period were used only for the stated objectives. For the participants found to have significant bacterial isolates, results were sent to the responsible clinician as soon as possible. The confidentiality of the information collected was maintained by using code numbers for participants.

5.11. Dissemination of the result

The findings of this study are forwarded to Department of Medical laboratory Sciences, School of Allied Health Sciences, College of Health Sciences Addis Ababa University. The findings will be communicated to Addis Ababa city administration health bureau and Yekatit 12 hospital medical college. The findings will also be presented in different conferences and manuscript will be sent to peer reviewed journals for publication.

5.12. Operational definitions

Hospital acquired infections (HAI): is defined as a localized or systemic condition that results from an adverse reaction to the presence of an infectious agent(s) or its toxin(s) and occurring 48 hours or more after hospital admission that was not incubating at the time of admission [34].

Multiple drug resistance (MDR):

In this study it is defined as the resistance of bacteria to multiple antimicrobial agents, classes or subclasses of antimicrobial agents i.e. resistance to at least to one antibiotic in three antibiotics class or sub classes[35].

Under five children

A young human being whose age ranges from date of birth to 59 months.

6. Results

6.1. Sociodemographic and clinical characteristics

Total admission during the study period(from January 2018 to April 2018) were 720 patients (340 neonates in neonatal intensive care unit(NICU), 200 children in Berla pediatric unit and 180 neonates were in neonatal septic ward. The initial diagnosis during admission were analyzed and is shown in the following graph(Figure 2).From this figure it was shown that the most common admission diagnosis were sepsis (early or late), pneumonia and meningitis and hence were excluded from the study based on the study criteria. Therefore, out of the 720 admitted children, 435 children fulfill the study criteria and were included in the study. Accordingly, the socio-demographic characteristics of the selected children are summarized in Table 3.

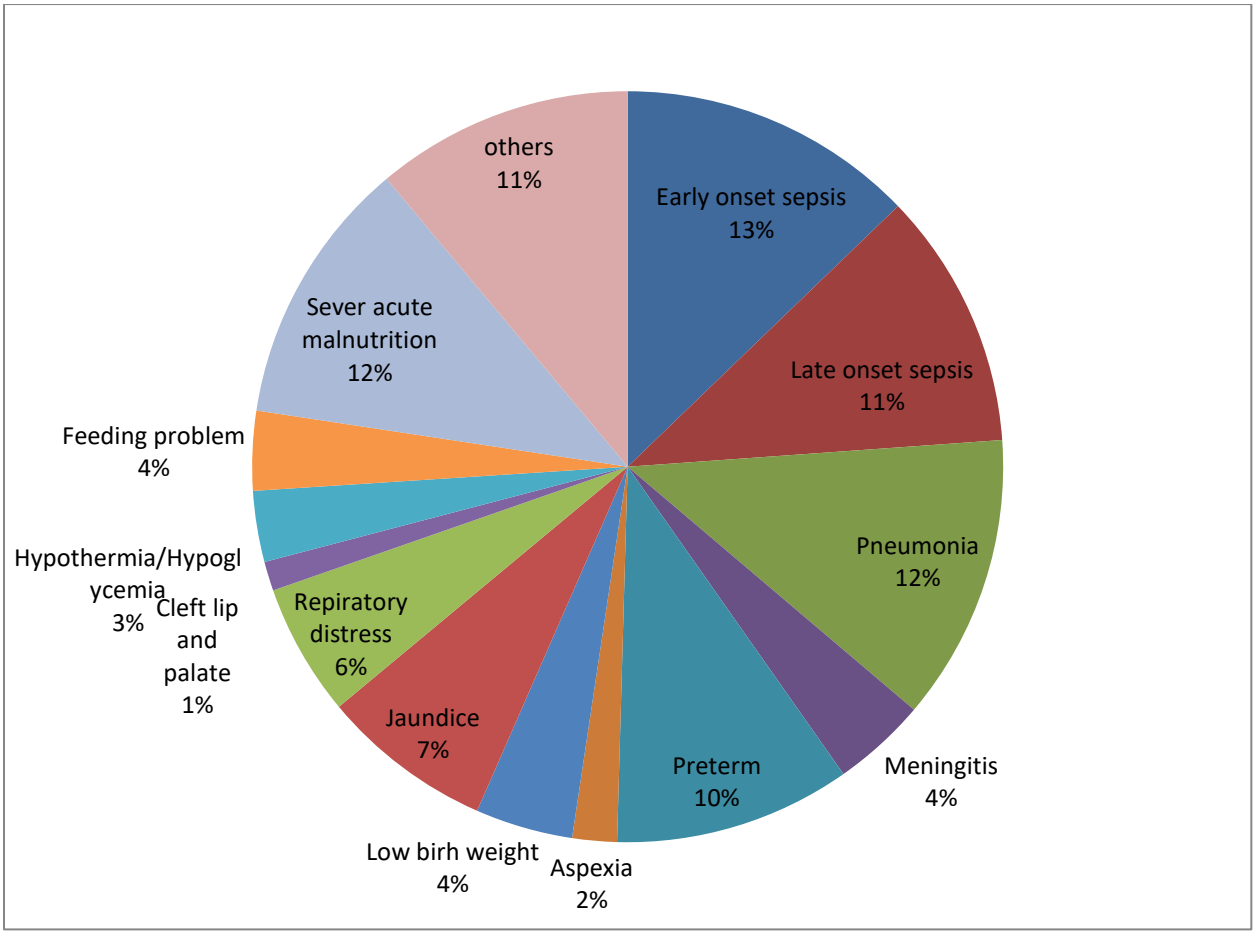


Fig 2. Admission diagnosis for the sampled children at Yekatit 12 Hospital Medical College from January to April, 2018

Out of the 435children, 255(58.7%) were males and 180(41.3%) were females giving a male to female ratio of 1.41:1. From the 435 participants 259 were neonates and the remaining 176 were children age between 21days and 5 years. When analyzed by the admission wards, from neonatal intensive care unit (NICU) 152 neonates, neonatal ward (septic ward)107 neonates and in Berla pediatric unit III(BPCIII) 176 children who fulfill the study criteria were admitted.

The gestational age for the 258 neonates were as follows, preterm birth 27.1 %, term birth 51.6%, and post term birth were 21.3%. For neonates mode of delivery 85.3 %(220) were through spontaneous vaginal delivery and the remaining 14.7%(38) were through cesarean section(CS). Regarding weight of the neonates, 32.8 %(84)were low birth weight and the remaining 67.2%(172) were normal(Table 3).

The device utilization and duration among all study participants were also presented in table 3. As can be seen from the table, from 435 study participants devices which come to direct contact to their body were not used only in 72cases. The commonest device being used was intravascular line (IVL)(used by 291patients) followed by IVL and mechanical respirator combination used by 78 patients. The frequent device utilization duration was 2-4 day(194 patients) followed by 5-8 day duration by 116 patients.

Table 3. Characteristics of studied neonates and under five children at Yekatit 12 Hospital Medical College, January-April, 2018 (n=435)

Characteristics	Categories	No	Percent
Gender	Male	255	58.6
	Female	180	41.4
Gestational age	Preterm(<37wks)	133	51.5*
	Term(37-39eks)	70	27.1*
	Post term(>39wks)	55	21.4*
Mode of delivery	SVD**	220	85.3*
(for neonates only)	Cesarean section	38	14.7*
Birth weight	LBW (<2.5kg)	84	32.6*
(for neonates only)	Normal wt(≥ 2.5kg)	172	66.4*
Average length of hospital stay	Culture negative	7.6(in days)	91.5
	Culture positive	13.7(in days)	8.5
Device used	No device	72	14.3
	IVL	291	66.9
	IVL And Respirator	78	17.9
	Respirator	4	9
Duration of device utilization	Only 1 day	25	5.7
	2-4 days	194	44.6
	5-8 days	116	26.7
	Greater or equal to 9 days	28	6.4

*The percentage is among neonates **SVD spontaneous vaginal delivery

6.2 Bacteriological findings of the under five children associated with HAI results

A total of 435 blood specimens, 366 urine, 50 cerebrospinal fluid, 10 wound swabs, 22 sputum and 56 stool samples were cultured. Over all 3.9% of the cultured specimens had positive growth after overnight incubation: these were 0.2% of wound swabs(n=2 cultures) and 3.7% of blood samples (n = 34).We obtained a total of 37 bacterial isolates. There was a wide range of bacterial isolates, the majority being Gram-negative isolates, with *Klebsiella spp*(n = 19,), *Enterobacter species*(n =4), *Acinetobacter spp*(n=3), *Pseudomonas spp* (n = 1) and *Citrobacter* spp(n=3),

being predominant, together accounting for 30 (81% of all pathogens isolated). Among Gram-positive only *S. aureus* were isolated(n=7).

The overall incidence of hospital acquired infections (HAI) in this study was 8.5%(infections among 37 participants). *K. pneumoniae* was the highest isolate *4.4%* (19 patients) followed by *S.aureus* 1.6%(7patientes infected). Of them, 12 isolates of *K. pneumonia* (out of 19) found in male participants and the remaining 7 *K. pneumoniae*(out of 19) were isolated from female participants. Out of the seven isolates of *S. aureus*, five were detected in males and two in females. Of the three detected *Acinetobactor species, two* were in males and one in female, only one *P aeroginosa* detected and it was in female. Of the four *Enterobacter species* detected two each were in male and female, and of the three *Citrobacter species* detected one was in male and two in female.

In this study, nine *K. pneumonia*e isolated from NICU, 5 from BPCIII, and 5 from septic ward, out of seven *S. aureus*2 from NICU, 4 from BPCIII, and 1 from septic ward, *Acinetobactor species* three only in BPC III, *Enterobacter species*2 from NICU, one from BPCIII and one from septic ward, *P aeroginosa* only 1 from BPCIII, *Citrobacter species*(3 isolates)2 from NICU and 1 from septic ward. From this we can conclude that most of the pathogens were isolated from neonates. In newborns with low birth weight, 9/19 *K.pneumonia, 2/4 Enterobacter species* were detected but the other pathogens were not occurred. But more than two third of the pathogens occurred in newborns with normal weight (Table 4).

Table 4. Bacterial profiles associated with HAI among neonates and under five children at Yekatit 12 Hospital Medical College January-April, 2018

Pathogens	Frequency	Percent
K pneumonia	19	4.4
S. aureus	7	1.6
Acinetobactor species	3	0.7
Enterobactor species	4	0.9
P.aeroginosa	1	0.2
Citrobactor species	3	0.7
Total	37	8.5

6.3. Hematological and CRP profile of study participants in relation to bacterial profile

The laboratory profile of the study participants were presented in table 4. The average white blood cell count was 12.7cells/mm^3, RBC 4.67milion cells/mm^3, platelet 268cells /mm^3 and mean hemoglobin was 14gm/dl. Among 21 patients with low RBC only two were infected(1 *K. pneumonia* the other *Citrobacter species*), with low WBC count only two were infected(1 *K. pneumonia* the other *Citrobacter species*), with low platelet count five were infected(2*K. pneumonia, two with S.aureus* and one with *Citrobacter species*), with low hemoglobin concentration 8 were infected(3*K. pneumonia, 2S.aureus,* 1*Acinetobacterpecies, 1 Enterobacter species,* and1*Citrobacter species*). But all hematological profiles did not have statistically significant association(P value at 95%CI for WBC 0.17, RBC 0.998, Platelet 0.45, and Hemoglobin 0.27). Most of the participants also fall within acceptable normal range for all the parameters mentioned. Most participants were negative for C-reactive protein (CRP) 87.6%, and the remaining 12.4% were positive. Except 1 *K. pneumonia*and two *S.aureus* infections the rest happened in C-reactive protein positive patients (34/37 total isolates).C-reactive protein positive were statistically significant in patients with culture positive results(p value at 95%CI were 0.001)(Table 5).

Table 5. Selected hematological and C-reactive protein profiles of neonates and under five children at Yekatit 12 Hospital Medical College, January-April, 2018 (n=435)

Characteristics		Frequency	Percent
Hemoglobin	Less than 11gm/dl	73	16.8
	Greater or equal to 11gm/dl	362	83.2
White blood cell	<5000cells/mm^3	17	3.9
	5000-10000cells/mm^3	249	57.2
	>10000 cells/mm^3	169	38.9
Red blood cell	<3.8 million cells/mm^3	22	4.8
	3.8-5 million cells/mm^3	346	79.5
	>5 million cells/mm^3	67	15.4
Platelet	<150000 cells/mm^3	59	13.6
	150000-300000cells/mm^3	272	62.5
	>300000cells/mm^3	104	23.9
C-reactive protein	Non reactive	381	87.6
	Reactive	54	12.4

6.4 Frequency of prescribed antibiotics in the study period and AMR

6.4.1 Frequency of prescribed antibiotics in the study period

Of the 435 under five children, 101 (23.2%) children were not treated with antibiotics. Among 334 children treated with antibiotics, on average a single child had been treated with two antibiotics. The details are shown in table 6. And the most frequently used antibiotics were

ampcillin and gentamycin combination followed by ceftriaxone. But most of the antibiotics were prescribed based on symptoms/signs without laboratory confirmation of the causative agent of the disease. Though the rate of utilization was small all the costliest antibiotics like amikacin, meropenum, augmentin were also prescribed without any confirmation of the pathogen as well as whether the drug is susceptible or not to that particular condition of the patient(Table 6).

Table 6. Frequently prescribed antibiotics in the study period used to treat the participants at Yekatit 12 Hospital Medical College, January-April, 2018

Antibiotics used	Frequency	Percent
Ceftriaxone	43	9.9
AmpcilinCefotaxim	27	6.2
CeftriaxoneMetrondazole	7	1.6
Cefotaxim Ceftriaxone	12	2.8
Ampcilin Gentamycin	144	33.1
Ampcilin,CloxacillinCefotaxime	5	1.1
Ampcilin,Cefotaxime Gentamycin	14	3.2
Amoxacillin	12	2.8
Ceftriaxone, Gentamycin	5	1.1
Ampcilin	9	2.1
Cloxacillin	7	1.6
Others	49	11.3
No antibiotic	101	23.2

6.4.2 Antimicrobial Resistance pattern in the study period

Analysis of antimicrobial sensitivity pattern revealed that, there was high antimicrobial resistance in bacterial isolates in this study; *Klebsiella spp* and *Enterobacter Spp*. exhibited relatively high resistance to all drugs tested among Gram-negative isolates. Ampicillin resistance was frequent among *Klebsiella spp* (n = 19, 100%), *Enterobacter Spp.* (n = 4, 100%), *AcinetobacterSpp*(n=3, 100%) and S. aureus(n=7,100%).Resistance to cefotaxim by *Klebsiellaspp, Enterobacter Spp.*, and *Acinetobacter Spp.*,was (n = 19, 94%), (n = 4, 100%),(n=3, 100%),respectively. Resistance to third-generation cephalosporins (ceftriaxone and ceftazidime) was as well observed for *Klebsiella spp* (n = 19, 94.1% for both) *Enterobacter Spp.* (n = 4, 94.1% &100%)and *AcinetobacterSpp*(n = 3, 100% for both).

Among the Gram-positive isolates, *S. aureus* was the only species, which demonstrated detectable resistance to penicillin (n = 7, 100%), erythromycin (n = 7, 43%),trimethoprim–sulfamethoxazole (n = 7, 85.7%). As can also be seen from table 6 all pathogens have shown 100% resistance to ampcillin and more than 95% percent resistance to third generation cephalosporins by the gram negative bacteria. But 100% sensitivity was demonstrated for Amikacin. And all of the *S. aureus* isolates were sensitive to cefoxitin(suggesting that all were sensitive to methicillin)(Table 7).

Table 7. Antibioticresistance pattern of isolated pathogens from infected neonates and under-five children at Yekatit 12 Hospital Medical College, January-April, 2018

Antibiotics	Isolated pathogens and resistance in percent					
	K.pneumonia	*S aureus*	*Acinetobactor*	*Citrobactor*	*Enterobactor*	*P. aeroginosa*
Amikacin	None	None	None	None	None	None
Ampcillin	100%	100%	100%	100%	100%	100%
Augmentin	64.7%	None	100%	33.3%	50%	100%
Cefotaxim	94.1%	None	100%	None	100%	100%
Ceftazdim	94.1%	100%	100%	None	94.1%	100%
Ceftriaxone	94.1%	None	100%	None	100%	100%
Ciprofloxacillin	17.6%	None	33.3%	None	50%	None
Clindamycin	NA	None	NA	NA	NA	NA
Cefoxitin	NA	None	NA	NA	NA	NA
Erythromycin	NA	28.5%	NA	NA	NA	NA
Gentamycin	17.6%	NA	None	33.3%	25%	None
Meropenum	5.9%	NA	33.3%	None	25%	None
Penicillin	NA	100%	NA	NA	NA	NA
Tobramycin	29.4%	NA	33.3%	None	25%	None
Amoxacillin	100%	43%	100%	100%	100%	100%
SXT	100%	85.7%	50%	100%	25%	100%

Note: NA not applicable

6.5. Extended spectrum beta-lactamase(ESBL) and carbaphenemase producers

In this study out of a total 30Gram-negative bacterial isolates 80%(n=24) were ESBL positive as screened by combined disk test and double disk synergy test. Regarding carbapenemase from all Gram-negatives only 3.3%(n=1) were positive as tested by modified Hoges test.

6.6. Prevalence of methicillin resistant S. aureus(MRSA)

From a total of seven isolates of *S. aureus*none were resistant to cefoxitin. This implies to all the isolates were sensitive to methicillin(no MRSA detected).

6.7. Prevalence of multiple drug resistance (MDR) bacteria

The details of the prevalence of multiple drug resistance is presented in table 8. As can be seen from the table the high prevalence of MDR was seen among *K.pneumoniae* which is 100%, followed by *Acinetobactor species*, in this case 2/3 were MDR (Table 8).

Table 8. Prevalence of MDR among neonates and under-five children at Yekatit 12 Hospital Medical College, January-April, 2018

Isolates	**Multiple drug resistance**	
	Number	**Percent**
K.pneumoniae	19	100%
S. aureus	3	42.9%
Acinetobactor species	2	66%
Enterobactor species	1	33.3%
P.aeroginosa	1	100%
Citrobactor species	1	33%

6.8. Findings from environmental samples

Environmental specimens were also analyzed from the respective wards where the children have been treated for different medical conditions. Only *E.coli* were isolated from labor ward but there were no pathogen isolated from different utensils such as incubator, O_2cylinders, respirators, suction machines, irradiant warmer, water tank and the sink. But for neonatal intensive care unit analysis of the whole environment performed including the floor and the air in addition to the utensils mentioned above. In this ward different pathogenic bacteria isolates were identified and the detail were described in table 9. From Berla pediatric unit(BPCII) only *Enterobacter spp* were identified otherwise no pathogen was detected from other utensils (Table 9).

Table 9. Types of isolates from environmental samples of neonatal and pediatric wards Yekatit 12 Hospital Medical College, January-April, 2018

Isolated pathogens	Type of utensil /environment									
	Sink	Water tank	Irradiant warmer	The air*	floor	Suction machine	Beds	Incubator	O_2cylinders	Respirators
K.pneumonia	✓	✓	✓	✓	No	No	No	No	No	No
S. aureus	✓		No	No	No	No	No	No	No	No
E.coli	✓	✓	No	✓	✓	No	✓	No	No	No
Acinetobactor species	✓	No	No	✓	✓	No	No	No	No	No
Enterobactor spcies	✓	No	No	✓	✓	No	No	No	No	No

*by open plate technique

6.9. Hospital acquired infections with respect to admission wards

Rates of hospital acquired infections (HAIs) in different Pediatric wards and Units are presented in table 10. As can be seen from the table, the highest positivity rate was seen in neonatal intensive care unit(4.7%) followed by Berla pediatric unit(BPCIII) (3.8%) which was slightly higher than Neonatal ward (septic ward)(3.6%) (Table 10).

Table 10. Rates of HAIs in different Pediatric wards and Units of Yekatit 12 Hospital Medical College, January-April, 2018

Wards	**Total admission No. (%)**	**Infected patients No. (%)**	**Positive cultures/Total cultures(% of positive)**
NICU	152(34.9)	15(9.9)**	4.7%*
Berla pediatrics III	176(40.5)	14(8)**	3.8%*
Septic ward	107(24.6)	8(7.5)**	3.6%*

*positive cultures are computed from admitted patients at that ward. **within the wards

6.10. Hospital acquired infections with respect to sites of infection

Frequency of different sites of infection in various units were displayed in figure3 below. From the figure it was shown that blood stream infection was the most frequent mode of infection in all of the wards included in the study. There were no pathogens detected in urine (Figure .3)

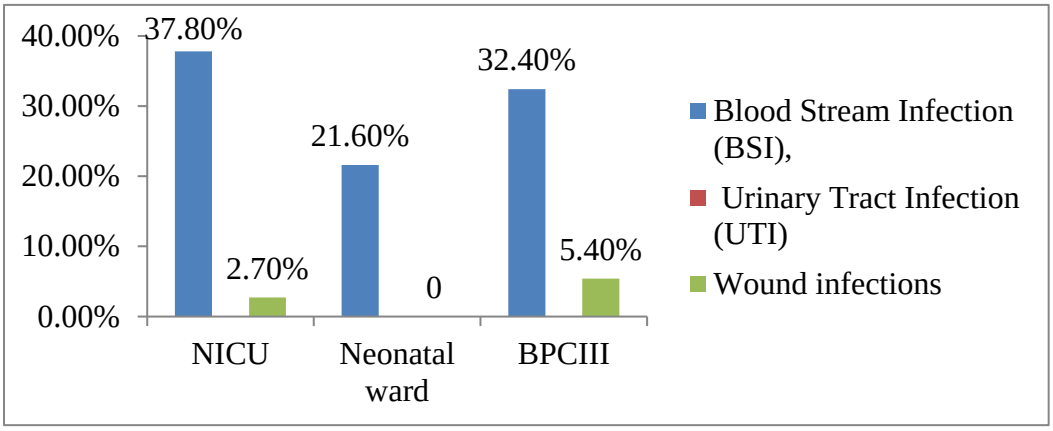


Fig 3. Frequency of different sites of infection in various units

6.11. Associated risk factors

The details of predisposing factors were shown in table 11. The possible risk factors assessed in this study include gender, age, admission ward, invasive device utilization, hospital stay longer than seven days, thrombocytopenia, leukocytosis, and leucopenia. None of the analyzed presumed associated risk factors were statistically significant (Table 11). Apart from statistical insignificance, the occurrence of HAIs in a presumed risk factors such as: admission ward (eg NICU), age less than 28 days, invasive device utilization, hospital stay longer than seven days still has relatively high incidence of HAIs.

With respect to device utilization all infections with *K. pneumonia* occurred in patients who have Venous Intravascular catheter (IVL) and/or both IVL and patients put on tube mechanical respirator similarly 5/7 *S.aureus* infections, all 3 by *Acinetobacter species* infections, 4 *Enterobacter species* infections were associated with device utilization. Concerning the duration of device utilization most infections occurred in devices stayed between 2-8days(19*K. pneumonia,4/7S.aureus, 2/3Acinetobacter species* infections, 4/4*Enterobacter species infections*).

Table 11. Risk factors related to the occurrence of infection among hospitalized children at Yekatit 12 Hospital Medical College, January-April, 2018

Variables	Category	No nosocomial infection (n(%))	Nosocominal Infection (n(%))	P value
Gender	Male	234(91.4)	22(8.6)	0.951
	Female	164(91.6)	15(8.4)	0.951
Age	≤28days	236(91.1)	23(8.9)	0.438
	>28 days	162(92%)	14(8)	0.438
Wards/Units	NICU	137(90)	15(10)	0.749
	Septic ward	99(92.5)	8(7.5)	0.749
	BPCIII	162(92)	14(8)	0.749
Invasive device utilization	Yes	330(90.9)	33(9.1)	0.721
	No	105(98)	4(2)	0.721
1. Under weight	No	73(87)	11(13)	0.88
	Yes	163(94.8)	9(5.2)	0.86
Neonates 2. GA	AGA	119(89.5)	14(10.5)	0.697
	Preterm	64(91.4)	6(8.6)	0.686
	Post term	52(94.5)	3(5.5)	0.709
3.Mode of delivery	CS	35(92.1)	3(7.9)	0.907
	SVD	200(90.9)	20(9.1)	0.821
Hospital stay longer than 7 days	No	259(93.2)	19(6.9)	0.244
	Yes	139(88.5)	18(11.5)	0.244
Thrombocytopenia	No	54(91.5)	5(8.5)	0.429
	Yes	344(91.4)	32(8.6)	0.429
Leukocytosis	No	244(91.7)	22(8.3)	0.846
	Yes	154(91.1)	15(8.9)	0.846
Leucopenia	No	383(91.6)	35(8.4)	0.846
	Yes	15(88.2)	2(11.8)	0.846

6.12. Outcomes of the under five children

Analysis of outcomes of the participants revealed that, from 435 study participants 91.3 % (397 participants) improved(cured), 7.6%(33participants died) and 1.1%(5 participants) were referred. Among participants infected by *K.pneumoniae*, 11 out of 19 cured and the remaining eight died, participants infected by *S aureus* all improved(Table 12).

Table 12. Outcomes of the under five children by bacterial profile at Yekatit 12 Hospital Medical College, January-April, 2018

Pathogen	DISCHARGE			Total
	IMPROVED	DIED	REFFER	
K.pneumonia	11	8	0	19
S aureus	7	0	0	7
Acinetobactor species	3	0	0	3
Enterobactor species	4	0	0	4
P. aeroginosa	1	0	0	1
Citrobactor species	2	1	0	3
No pathogen	369	24	5	398
Total	397	33	5	435

7. Discussion

7.1. Incidence of Hospital acquired infection

Yekatit 12 Hospital Medical College is a referral teaching hospital serving a catchment of approximately 4 million people in Addis Ababa, the capital city of Ethiopia. The study aimed to determine the incidence of Hospital acquired infections, antimicrobial drug resistance patterns and associated risk factors among under five children who were admitted at NICU, neonatal wards and Berla pediatric unit in this hospital.

In this study, the overall HAI incidence rate was 8.5%which is higher than what was found in several countries in Europe, which reported HAI prevalence of 3.7-4.8%[20]. This may be due to difference in practice of infection prevention and resource availability for infection prevention. In our setup there is crippling shortage of resources for infection prevention and also poor adherence to infection prevention practices[29]. However, this rate was comparable to that reported in some developing countries such as Southeast Asia that showed the burden of Healthcare-Associated Infections in a pooled prevalence to be 9.0% (95% confidence interval [CI], 7.2–10.8)[21]. Similarly it is also comparable to the hospital-wide prevalence of HAI in Africa(systematic review) which varied between 2.5% and 14.8%; in surgical wards, the cumulative incidence ranged from 5.7% to 45.8%[25].

However, these comparisons are purely illustrative because the methods used (definitions and types of HAI identified, methods of case finding, exclusion of imported HAI or not) and hospitals, or patients included are different following the surveys. The high prevalence rate found in the current study can be explained firstly by the absence of a national strategy to prevent HAI in Ethiopian hospitals; and secondly by the highly developed healthcare systems that teaching hospitals offer including invasive medical and surgical procedures. It should also be noted that prevalence studies are certainly less expensive faster and easier to achieve, but the results are subject to seasonal fluctuations and can sometimes coincide with epidemic peaks.

The overall incidence of 20.9% of HAIs found in Iranian burn patients [24] is very high as compared to the finding of this research. And this may be due to the methodology deference between this study and the Iranian study, as the Iranian study considered only burn patients which have high probability to acquire nosocominal pathogens. The finding of this research is also lower than that of the finding in Morocco which was 10.3%[26]. This difference may be

explained by difference in methodology as theirs is based on point prevalence. In addition to those, the overall rate of HAI in Egypt was 5.2/100 admissions [28] which is lower than the finding of this research which may be due to difference in the setup and study population. Finally, the incidence of HAIs in this research is higher than the finding at Tikur Anbessa Specialized Hospital, which reported nosocomial infection rate of 5 per 100 discharges [29]. This may be due to the difference in methodology (case control), the time or season of the year the research was conducted.

The most affected ward in the current study was NICU. This finding is consistent with the finding in Morocco [26]and can be explained by the frequency of severe disease occurring in NICU, the prescription of broad spectrum antibiotics and also use of devices and invasive procedures. This study identified two main sites of HAI: blood stream infection and wound infection (burn wound).These sites are the most frequently reported in prevalence surveys [21, 23].

7.2. Bacterial etiology associated with HAI

The culture results revealed a higher positive growth rate on blood specimens (n =34, 7.8%), compared to others. Both gram-negative and positive species were observed. These findings are in agreement with other studies in 29 countries of Europe where most of etiologic agents incriminated with hospital acquired infections tend to be gram-negatives(*Enterobacteriacie*) and gram positive species with a variety of bacterial pathogens [20]. Similarly in China Jiangsu province gram-negative bacterial pathogens were the most frequently encountered[23]. The bacterial spectrum observed from this study showed a high diversity of gram-negative bacilli such as *Klebsiella spp* (4.4%), *Enterobacter species*(0.9%) and *Acinetobacter Species*(0.7%), with *Staphylococcus aureus* (1.6%)being the only gram-positive isolate. The majority of these gram-negative bacilli were from blood stream infections rather than from other disease conditions. This predominantly gram-negative infection pattern, as also observed in other studies [24, 25, 26,27,29, 31], is different from that most commonly reported from North America[30]. The reasons for this are not entirely known, but the suggested reasons could be difference in geographical location and climate. The present findings do, however, emphasize that we cannot use knowledge obtained in North America directly for clinical care and empirical treatment in sub-Saharan Africa. In Morocco *Staphylococcus* was the organism most commonly isolated (18.7%)[26]. This has some similarity with North America but different from the current finding.

For this there is no clear explanation but difference in methodology(point prevalence), so the finding may be coincidence.

7.3. Antimicrobial susceptibility patterns

Proper identification and determination of antimicrobial resistance of the bacterial pathogens is crucial to help physicians to provide proper treatment promptly. In addition to this, prudent use is essential in controlling antimicrobial resistance, which has now become one of the major challenges for medical progress.

The most frequently used drugs were Ampcillin 36.74%, gentamycine 29.5%, cefotaxim 9.9%, ceftriaxone (11.5%),metronidazole (2.7%), cloxacillin (3.8%) all were used mostly in combination with other antibiotics. Along with this, the observed resistance patterns of all bacterial isolates tested on drugs such as amikacin showed no resistance, whereas, ampicillin 100%, augmentin 69.6%, cefotaxim 98.5%, ceftazidme 97.4%, ceftriaxone 98.5%, ciprofloxacillin 33.6%, Erythromycin 43%, gentamycin 25.3%, penicillin 100%, amoxacillin 100%, cotrimoxazole 100%, but all of *S. aureus* isolates were sensitive to cefoxitin. The finding is different from other study conducted in Ethiopia which indicated resistance of ampicillin was 91.9%, gentamycin 67.2%, ceftriaxone 50%, norfloxacin 18.3%, and ciprofloxacin 15.4%[29]. This deference may be due the deference in the type of pathogens isolated, in our study *Klebsiella spp* were common but on study at Tikur Anbesa specialized Hospital, the common pathogen were *E.coli* which is most sensitive than *Klebsiellaspp*[41].

Of note, the most commonly ordered antibiotics like ampicillin was 100% resisted by all identified isolates, both gram negatives and gram positives. As ceftriaxone is a third-generation cephalosporin, it is expected that its use to be controlled, yet it was the most commonly used drug. Moreover, convincing percentages of resistant strains of *Klebsiella species* to first and third-generations of cephalosporins have been broadly noted, more than 90%. *Staphylococcus aureu*s was the predominant species among gram-positive isolates. It accounted for no observed resistances to cefoxitin but has shown high resistance to other antibiotics such as penicillin, erythromycin, and trimethoprim–sulfamethoxazole. Cefoxitin sensitivity is different from other studies [22, 23, 26, 27], this may be due to in this study the number of isolates are very small which may have led to failure to detect the resistance.

The MDR rates in this study for gram negative pathogens were 83%, ESBL 80%, and carbapenemase were 3.3%. The MDR rate is higher than the finding at Tikur Anbesa Specialized hospital(73.9% for gram negative) this difference may be due method(post operation wound) and the type and number of isolates(few in this study)[42]. The ESBL found in this study were higher than the finding in Gondar Referal Hospital(average ESBL 25%). This difference is may be due to the variety of isolates, in this study *K. pneumoniae* common but in Gondar Referral Hospital *E.coli* were common[43]. The finding of ESBL from neonates and children(average 94%) in Ethiopia is higher than the finding of this study these may be due to the difference in the method and the number of isolates[44].

7.4. Predisposing factors

Predisposing factors of HAIs identified in other studies such as from United States of America[30], review studies in 29 European Union Member states[20] were use of instrumentation or devices for intubation, delivery of therapeutic agents, prolonged length of stay, and admission in Intensive care units, and age younger than 12months. Also the main risk factor in Southeast Asia [21], and Jiangsu Province of China[23] were similar to that reported elsewhere. But these study findings were completely different from the result of this study. But in this study the risk factors were admission ward, invasive device utilization, hospital stay longer than seven days, were not statistically significant risk factors. This difference may be due to irrespective of the clinical diagnosis the admitted patients may be placed into any of the antibiotics including third generation cephalosporines. This prevents the possibility of acquiring infections irrespective of the conditions around the patients.

It is better to keep in mind that though the findings were statistically insignificant, the occurrence of HAIs in the mentioned risk factors in this study was still higher. In another study conducted in Egypt, low birth weight (LBW), mechanical ventilation, invasive device utilization, neonatal age, neutropenia, Intensive Care Unit residence and hospital stay for > 7 days were the risk factors significantly associated with HAI in that hospital. Prematurity were reported to be important risk factors for HAI[27]. As mentioned above these risk factors were not found in this study. These differences may be due to the reasons stated above or other reasons this study cannot explain. Even in study done in Tikur Anbessa Specialized Hospital Ethiopia, age less than one year,

malnutrition, admission to orthopedics unit, peripheral intravenous line and prolonged hospitalization were significantly associated with nosocomial infection[29]. This is different than the finding of this study, which may be due to the same reason mentioned earlier. This implies that the requirement to establish and capacitate microbiology laboratory to detect anaerobic bacteria which are highly associated with hospital acquired infections and extensive investigations to identify predisposing factors.

8. Strengths of the study

- In this study more than 200 neonates were included which were very difficult to find specimen of different types.
- This study identified the prevalence of ESBL and carbapenemase which is risk for availability of antibiotic options.
- This study tried to observe hematological profile in association with hospital acquired infection though not associated statistically
- This study also tried to identify nosocominal pathogens from different equipments and hospital environment

9. Limitation of the study

This study did not detect hospital acquired viral, parasitic, fungal and anaerobic bacterial pathogens.

10. Conclusion and recommendations

The study has revealed a wide range of causative agents, with an alarming rate of resistance to the commonly used antimicrobial agents. Furthermore, the bacterial spectrum differs from those observed in high-income countries. Ultimately, the prevalence of HAI was high(8.5%) in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. This study represents basic information for future monitoring of HAI. Based on our findings the following recommendations were forwarded:

- Enhancement of basic microbiology services and quality control of antimicrobial susceptibility testing will be beneficial for obtaining valid and reliable HAI surveillance information. Availability of published data will increase awareness among health authorities and may be used to engage policymakers to prioritize infection prevention and control. This will lead to better funding opportunities for prevention and control programs.
- It is necessary to review the current infection control practices in all hospitals particularly hospital with high workloads. The training and retraining of health care givers on principles of infection control is strongly recommended.
- Strengthen collaborations and linkages between national health institutions and other major health facilities in the areas of nosocomial infections prevention, control and surveillance.
- Since it is well understood that preventive medicine is superior to curative medicine, health personnel have no excuse of allowing hospitals to be health hazard areas by harboring pathogens. However, in the event that an outbreak occurs, the source(s) of infection such as hospital personnel, patients or inanimate objects, such as water, air, beddings, surfaces and food should be identified and the pathogen eliminated. Thus, continuous monitoring of HAI and their drug resistance pattern is recommended.
- The bacterial profiles associated with HAI among under five children in this study varied, but the finding is not exhaustive. Therefore, we recommend exhaustive bacterial identification associated with HAI including anaerobic once.
- The antibiotic resistance patterns of pathogens involved in HAI among under five children were very high including ESBL and carbapenems, therefore we recommend utilization of antibiotics based on sensitivity data rather than syndromic approaches.

References

1. Prakash K. Nosocomial infection-an overview.[Online] (Accessed: 8 November, 2017), Available from: http://www.researchgate.net/publication/18951524
2. Festary A, Kour V, Correa B, Verdasquera D, Roig T, Couret P. Cytomegalovirus and herpes simplex infections in mothers and newborns in a Havana maternity hospital. *MEDICC Rev*. 2015; 17(1): 29-34.
3. Prakash K. Nosocomial infections: An overview for the surgeon Proceedings of the XVII National Continuing Medical Education Program in Surgery, 2000,132-136.
4. Emmerson M . The second national prevalence survey of infection in hospitals — overview of the results. *J Hosp Infect,* 1996; 32:175–190.
5. World Health Organization. Prevention of hospital-acquired infections: a practical guide. Geneva: World Health Organization; 2002. [Online] Available from: http://www.who.int/csr/resources/
6. Borguesi A, Stronati M. Strategies for the prevention of hospital acquired infections in the neonatal intensive care unit. *J Hosp Infect*, 2008;68:293–300.
7. Zaidi M, Huskins C, Thaver D . Hospital-acquired neonatal infections in developing countries. *Lancet*, 2005;365:1175–88.
8. Laxminarayan R, Matsoso P, Pant S. Access to effective antimicrobials: a worldwide challenge. *Lancet*, 2016; 387: 168–75.
9. World Health Organization. Antimicrobial resistance. Global report on surveillance. Geneva: *World Health Organization*. 2014.
10. Horan C, Andrus M, Dudeck A. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control*, 2008; 36(5): 309-32.
11. Vatopoulos C, Kalapothaki V, Legakis J. Risk factors for nosocomial infections caused by gram-negative bacilli. *J. Hosp. Infect*, 1996; 34:11-22.
12. World Health Organization. Report on the Burden of Endemic Health Care- Associated Infection Worldwide. Geneva: *WHO*. 2011;1-34.
13. Nejad B, Allegranzi B, Syed B,Benjamin B, Pittet D. Health-care associated infection in Africa: A systematic review. *Bull. World Health Organ*. 2011; 89:757-765.
14. Akande A. Emerging and reemerging infectious agents of nosocomial diseases – The need for review of hospital policy and control strategies. *Bayero J. Pure App. Sci*. 2012;5:19–25.

15. Mulu W, Kibru G, Beyene G, Damtie M. Post-operative nosocomial infections and antimicrobial resistance pattern of bacteria isolates among patients admitted at Felege Hiwot Referral Hospital, Bahirdar,Ethiopia. *Ethiopian J. Health Sci*. 2012;22:6.
16. Allegranzi B, Pittet D. Preventing infections acquired during health-care delivery. *Lancet*, 2008; 372: 1719-1720.
17. World Health Organization. The world health report: working together for health. Available from http://www.who.int/whr/2006/en/.
18. World Health Organization. The global burden of disease: 2004 update. Available from http://www.who.int/healthinfo/global_burden_disease/GBD_report_2004update_full.pdf.
19. World Health Organization. Report on the burden of endemic HAI world-wide; a systematic review of literature. Geneva. 2011.
20. Zingg W, Hopkins S, Ageron A, Holmes A, Sharland M, Suetens C. Health-care-associated infections in neonates, children, and adolescents: an analysis of paediatric data from the European Centre for Disease Prevention and Control point-prevalence survey. *Lancet Infect Dis.* 2017; 17: 381–89.
21. Ling M , Apisarnthanarak A, and Madriaga G. The Burden of Healthcare-Associated Infections in Southeast Asia: A Systematic Literature Review and Meta-analysis. *Clinical Infectious Diseases*, 2015;60(11):1690–9.
22. Hearn P, Miliya T, Seng S, Ngoun C, Day N, Lubell Y, et al. Prospective surveillance of healthcare associated infections in a Cambodian pediatric hospital. *Antimicrobial Resistance and Infection Control*, 2017; 6:16.
23. Li Y, Cao X, Ge H, Jiang Y, Zhou H, Zheng W. A study on the targeted surveillance of nosocomial infection in intensive care units of 177 hospitals in Jiangsu Province of China, *Journal of Hospital Infection*, 2017;10:009.
24. Rezai M, and Shahmohammadi S. Nosocomial Infections in Iranian Pediatric Patients with Burn Injuries: A Review. *J Pediatr Rev*. 2015; 3(2):e680.
25. Nejad S, Allegranzi B, Syed S, Ellis B & Pittet D: Health-care-associated infection in Africa: a systematic review. *Bull World Health Organ*. 2011;89:757–765.
26. Razine R, Azzouzi A, Barkat A, Khoudri I, Almontacer F, Chefchaouni C, and Abouqal R. Prevalence of hospital-acquired infections in the university medical center of Rabat, Morocco. *International Archives of Medicine*, 2012; 5:26.

27. Mohammed D , Seifi O. Bacterial nosocomial infections in neonatal intensive care unit, Zagazig University Hospital, Egypt. *Egyptian Pediatric Association Gazette,* 2014; 62: 72–79.
28. Hassan R, Eldegla H, Elmorsy F, Eldars W. Clinical and microbiological characteristics of healthcare-associated infections in a tertiary care pediatric hospital. *Egyptian Pediatric Association Gazette,* 2017;09:001.
29. Demissie M, Lulsesed S. The Prevalence of Nosocomial Infections and Associated Risk Factors in Pediatric Patients in Tikur Anbessa Hospital. *Ethiopian journal of pediatrics and child health,*2009;5:1-17.
30. Reed D, Kemmerly S. Infection Control and Prevention: A Review of Hospital-Acquired Infections and the Economic Implications. *The Ochsner Journal,* 2009;9:27–31.
31. Kumburu H, Sonda T, Mmbaga B, Alifrangis M, Lund O, Kibiki G et al. Patterns of infections, etiological agents and antimicrobial resistance at a tertiary care hospital in northern Tanzania. *Tropical Medicine and International Health,* 2017;22(4): 454–464.
32. Daniel W. Biostatistics: A foundation for analysis in the health sciences. 7th edition New York ; John Wiley and sons. 1999; 224-260.
33. CDC/NHSN. CDC/NHSN. Surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control*. 2008;(36):309–332.
34. Garner S, Jarvis R, Emori G, Horan C, Hughes M. CDC definitions for nosocomial infections, 1988. *Am J Infect Control*, 1988;16(3):128–140.
35. Carey R, Carmeli Y, Falagas M, Giske C, Harbarth S, Hindler J, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection*, 2012; 18(3): 268–281.
36. Baron E, Miller M, Weinstein M, Gilligan R, Thomson R. ..et al. Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: Recommendations by the Infectious Diseases Society of America and the American Society for Microbiology. *Clin Infect Dis.* 2013; 57(4): e22–e121.
37. Patel J, Weinstein M, Eliopoulos G, Jenkins S, Lewis J, Limbago B …et al. Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute (CLSI). 2017.
38. Cavalieri S, Harbeck R, McCarter Y, Ortez J, Rankin I, Sautter R …et al. Manual of antimicrobial susceptibility testing. *American Society for Microbiology*,2005.

39. Kendal R, Benoit E. Cell-Dyn 1800 system. Operators manual. Abbott Laboratories USA. 2006.
40. Claus D, Osmond P, Gewurz J. Accutex C-Reactive Protein (CRP) Latex Test. Thermo Scientific. Kit inserts,2008.
41. Mohammed R, Kalam A, Singh N.*E. coli* and *Klebseila pneumonia* sensitivity/resistance pattern towards antimicrobial agents. *Indian medical asso.* 2018.
42. Asres G, Legese M, Woldearegay G. Prevalence of Multidrug Resistant Bacteria in Postoperative Wound Infections at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. *Arch Med.* 2017:9, 4:12.
43. Engda T, Moges F, Gelaw A, Eshete S and Mekonnen F. Prevalence and antimicrobial susceptibility patterns of extended spectrum beta‐lactamase producing *Entrobacteriaceae* in the University of Gondar Referral Hospital environments, northwest Ethiopia. *BMC.* 2018: 11,335
44. Desta K, Woldeamanuel Y, Azazh A, Mohammod H, Desalegn D, Shimelis D et al. High Gastrointestinal Colonization Rate with Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae in Hospitalized Patients: Emergence of Carbapenemase-Producing K. pneumoniae in Ethiopia. *PLoS ONE. 2016:*11(8): e0161685.

Annex I. General information for the study participants (English version)

Introduction

My name is _____and I am MSc student of Addis Ababa University, School of Medical Laboratory Sciences. I am doing research entitled **Hospital acquired infections, antimicrobial drug resistance patterns and associated risk factors among under five children.** With advances in health care system, threat to Hospital Acquired Infections (HAIs) still remains. Currently HAIs continue to affect hospitalized patients and results in morbidity, mortality and additional costs as different study indicates. So this study will indicate Yekatit 12 hospital Medical College patients HAI, commonly isolated bacteria, antimicrobial drug resistance patterns and associated risk factors.

What is the reason of this study? The objective of this research is to study hospital acquired infections, antimicrobial drug resistance patterns and associated risk factors among under five children. If you agree to participate in the study, about 8ml blood for culturing, CBC, and CRP test drawn, 10-20ml urine will be collected for urine culture, different swabs and body fluids will taken as per the standard. Additionally some clinical information will be extracted from your log book(card).

Will the information be confidential? All the data obtained will be kept confidential by placing in looked rooms/ cabinets, only the study personnel will have accesses to the files. Anonymous testing will be undertaken, that means samples will be coded and positive results will not be identified by names.

What are the costs? All the investigations performed for the participants of this study will be free of charge.

What about compensation? You will not be compensated for your participation in this study but the patient may benefit from the study because more intensive investigations will be done which may allow detecting and treating an infection earlier than otherwise.

What about my rights to decline participation or withdraw from the study?

Your participation in this study is purely voluntary, and you may stop the participation or you may refuse to answer some of the questions if you feel uncomfortable. You are free to participate

in this study or you can withdraw your consent anytime, which will not incur/involve any penalty or loss of benefits to which you are entitled.

What about the harm which may happen in the study?

This research involves several tests that are done routinely in this hospital. There are no major risks. The swabs, blood draw and some body fluids (if collected) may cause some slight discomfort but otherwise will cause the patient no harm.

Assurance of the 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 reports for all stakeholders of the research project.

Zerihun Woldesenbet(PI): signature_____ Date_____

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

PI Address: Zerihun Woldesenbet: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa Ethiopia.

E-mail: Zooloozudi@gmail.com: Tele: +251913141480

Annex II. Information sheet (Amharic version)

መግቢያ

ስሜ _____ በአዲስ አበባ ዩኒቨርስቲ በጤና ሳይንስ ኮሌጅ በሜዲካል ላቦራቶሪ ትምህርት ክፍል ማስትሬት ዲግሪ ተማሪ ስሆን ከ5 ዓመት በታች በሆኑ ልጆች ላይ፣ ከሆስፒታል የሚያዙ በሽታዎች ፡ አሚጭ ተዋስያን፡ ለበሽታዉ የሚያጋልጡ መንስኤዎችና ተዋስያን ለመድሀኒት ያላቸዉ ምላሽ ላይ ምርምር እሰራለሁ። በአሁኑ ወቅት የሆስፒታል አገልግሎት ዘመናዊ ቢሆንም ከሆስፒታል የሚያዙ በሽታዎች የስጋት ምንጭ ናቸዉ። ይህም ሁኔታ በሆስፒታል የሚታከሙ ህሙማንን ለሌላ በሽታና ሞት ከመዳረጉም በላይ ለብዙ ወጭ ህሙማንን እንደሚዳርግ በብዙ ጥናቶች ተረጋግጧል። ስለዚህ ይህ ጥናት በየካቲት 12 ሆስፒታል ሜዲካል ኮሌጅ ላይ ያለዉን ሁኔታ ለማየት ይረዳናል።

የጥናቱ ምክንያት

የጥናቱ አላማ ከ5 ዓመት በታች በሆኑ ልጆች ላይ፣ ከሆስፒታል የሚያዙ በሽታዎች ፡ አሚጭ ተዋስያን፡ ለበሽታዉ የሚያጋልጡ መንስኤዎችና ተዋስያን ለመድሀኒት ያላቸዉ ምላሽ ላይ ነዉ። ከተስማሙ በዚህ ጥናት ላይ ለመሳተፍ ፈዋደኛ ከሆኑ ወደ 8ሚሊ ለደም ዉስጥ ተዋሲያን ምርመራና ማወቂያ፣ ለሙሉ ደም ቆጠራ፣ CRP የተባለ ፕሮቲን መመርመርያ ሲሆን ከ5-10ሚሊ ሽንት ለሽንት ዉስጥ ተዋሲያን ምርመራና ማወቂያ ይሰጣሉ። ስዋብና የሰዉነት ፈሳሽ ከተለያዩ የሰዉነት ክፍሎች ላይ ልወሰድ ይችላል። በተጨማሪ አንዳንድ መረጃዎች ከካርዱ ላይ ልወሰዱ ይችላሉ።

የጥናቱ ሚስጥራዊነት

የተገኘዉ መረጃ ሁሉ ሚስጥራዊነት በተቆለፈ ክፍል ወይም ሳጥን ዉስጥ በማስቀመጥ የጥናቱ ባለበት ብቻ እንዲያገኘዉ ይደረጋል። በምርመራ ወቅት የተለየ ኮድ በመጠቀም የተመርማሪዉ ስም እንዳይታወቅ እንከላከላለን።

የክፍያ ጉዳይ

በጥናቱ ላይ በሚሳተፉ ላይ ለሚደረጉ ሁሉም አይነት የላቦራቶሪ ምርመራዎች ወጭ ከክፍያ ነፃ ሆኖ በተመራማሪዉ ይሸፈናል።

የማካካሻ ጉዳይ

በጥናቱ ላይ ስለተሳተፉ ምንም ዓይነት ማካካሻ አያገኙም ነገር ግን ሀሙማኑ ከሚደረገዉ ጥልቅ ምርመራ በሽታዉ በጊዜ ተገኝቶለት በፍጥነት ይታከማል።

ከጥናቱ ላይ ያለመሳተፍና የመገለል መብት ጉዳይ

እርሶ በጥናቱ ላይ የተሳተፉት ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነዉ። ስለዚህ እርሶ በማንኛዉም ሰዓት በጥናቱ ላይ መሳተፍ ማቆም ይችላሉ። ያለመሳተፍም መብትዎ ነዉ፤ አንዳንድ ጥያቀዎች ካልመቾት ያለመመለስም መብት አለዎት። ይህን በማድረግዎ ምንም አይነት ቅጣት አያስከትልቦዎትም፤ ማግኘት የሚገባዎትን ሁሉነም ነገር አያሳጣም።

ከጥናቱ ጋር የተያያዙ ተጓዳኝ አደጋ ጉዳይ

ይህ ጥናት በሆስፒታል ዉስጥ የሚሰሩ ብዙ ምርመራዎችን ያካትታል። ሆኖም የተለያዩ ናሙናዎች ስወሰዱ ከሚሰማዉ ጥቅት ያለመመቸት በስተቀር ምንም አይነት የከፋ ጉዳት በጥናቱ ምክንያት አይከሰትም።

የተመራማሪዉ ማረጋገጫ

እኔ ከስር ፊርማየን ያስቀመጥኩት ሳይንሳዊ ጥናቱን በግብረገብ በተሟላ መልኩ እንደማካህድና የጥናቱን ርፖርት ለሚመለከታቸዉ ባለድርሻ አካላት እንደማቀርብ በፊርማየ አረጋግጣለሁ።

ዘሪሁን ወልደሰንበት(ዋና ተመራማሪ)፡ ፊርማ_____ቀን_____

ማስታወሻ፡ ስለጥናቱ ማንኛዉም ጥያቄ ካለዎት በሚቀጥለዉ አድራሻ ነፃ ሆነዉ ይጠይቁን፡

የዋና ተመራማሪ አድራሻ፣ ዘሪሁን ወልደሰንበት: በአዲስ አበባ ዩኒቨርሲቲ፣ የጤና ሳይንስ ኮሌጅ፣የህክምና ላቦራቶሪ ትምህርት ክፍል፤ አዲስ አበባ ኢትዮጲያ። ኢ ሜል: Zooloozudi@gmail.com: ስልክ: +251913141480

Annex III. Consent form for parents/guardians(English version)

I have been informed about the study which plans to determine Hospital acquired infections antimicrobial drug resistance patterns and associated risk factors among under five attending Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia. The objective and the application of the study were briefly explained to me. I have been well informed of my right to refuse information, decline to cooperate and dropout 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 consent voluntarily to the researcher to give my child's blood, urine swab and other body fluids for mentioned study. I agreed that the specimen would be tested for bacterial pathogens, CBC and CRP. I have had the opportunity to ask questions about the project and received clarifications to my satisfaction in a language I understand. I was also informed that the findings of the sample will be given to the health facility and that I may ask the information if I want.

If literate

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy) _____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ ____/____/_____ (dd/mm/yy) _____

Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy) _____

Participant code_____

Annex IV. Consent form for parents/guardians(Amharic version)

በየካቲት 12 ሆሲፒታል ሜዲካል ኮሌጅ ከ5 ዓመት በታች በሆኑ ልጆች ላይ፣ ከሆስፒታል የሚያዙ በሽታዎች ፡ አሚጭ ተዋስያን፡ ለበሽታዉ የሚያጋልጡ መንስኤዎችና ተዋስያን ለመድሀኒት ያላቸዉ ምላሽ ላይ ስለሚደረገዉ ምርምር በቂ መረጃ አግንቻለሁ። የጥናቱ አላማና አተገባበር በመጠኑ ተገልፆልኛል። በጥናቱ ላይ ያለመተባበር፤ ያለመሳተፍና ከተሳተፍኩማ በኋላ የማቋረጥ መብት እንዳለኝ ተረድቻለሁ። እኔ ባለመተባበረ ምክንያት ምንም አይነት በደል በልጅ ጤና ላይ እንደማደርስብኝ ተገልፆልኛል። ስለዚህ ያለዉን ሁኔታ ሙሉበሙሉ ተረድቸ በሙሉ ፈቃደኝነት ልጅ በጥናቱ ላይ በማሳተፍ የህመምተኛዉን/ዋን የሽንት ናሙና፣ ሰገራ ናሙና፣ የደም ናሙና፣ የተለያዩ የቆዳ ላይ ፈሳሽና የተለያዩ የሰዉነት ዉስጥ ፈሳሾችን በተጠየቅሁ ጊዜ የሚሰጥ መሆንና ፈቃደኛ መሆነን አረጋግጣለሁ። የተወሰዱት ናሙናዎችም ለሙሉ ደም ቆጤራ፣ ሲ.አር.ፒ ምርመራና በሽታ አምጭ ተዋሲያንን ለመመርመርና ተዋሲያኑ ለመድሃንት ያላቸዉን ምላሽ ለማወቅ እንደሆነ አዉቄ ተስማምቻለሁ። ጥያቄዎችን የማቅረብ እዲል አግኝቻለሁ ጥያቄየም እኔ በሚረዳዉ ቋንቋ ባግባቡ ተመልሰልኛል። ከዚህም በተጨማሪ የናሙናዎቹ ዉጤት ለህክምና ተቋሙ እንደሚሰጥ ተነግሮኛል ስለዚህ መረጃዉን ባስፈለገኝ ጊዜ ማግኘት እንደሚችል አዉቀያለሁ።

መፃፍ የማይችሉ ከሆነ የጣት አሻራዎን ያኑሩልን

ስም_____ቀን_____ፊረማ _____

መፃፍ የሚችሉ ከሆነ ስምና ቀን ፅፈዉ ይፈርሙ

ስም_____ቀን_____ፊረማ _____

የተመራማሪዉ ስምና ፊርማ

ስም_____ቀን_____ፊረማ _____

የተሳታፊዉ ኮድ፡_____

Annex V. Questionnaires

1. **Details of the person who filled in this questionnaire**

Name

Title/position

Contact numbers: Telephone Fax

Note: If you have given your contact details on another questionnaire, just give your name

2. **Interview Data collection techniques use**

2.1. Patient detail information

Date of survey:

Age: Sex: M F

Region: Zone: Wereda: Kebele:

Patient code:

2.2. Patient reason of admission information

Type of admission: ○ Elective ○ Emergency

Other, what is it? _____

Date of admission: Date: Time:

Diagnosis of Admission:

2.3. Patient recent admission history before admitted in the hospital

Has the patient transferred (Referred)? ○ Yes ○ No , if no go to section 3

If yes from which facility? ○ Health center ○ Government Hospital ○ Private Hosp

If yes, How long stay there?

3. **Observational Data collection techniques use**

3.1. Patient admission ward Information

Which ward has the Patient admitted?

○ Emergency ○ NICU ○ Neonatal ward ○ BPCII ○ BPC III

Ward number: Bed number:

3.2. Additional Neonatal information

3.2.1. Birth weight_____

3.2.2. Gestational age

3.2.3. Obstetric history

A. Mode of delivery B. Symptoms of sepsis as lethargy and poor feeding.

4. Use medical records to fill the following question

4.1. Medical risk factor(Comorbid) of the patient(Medical records)

What medical risk factor has the patient have? Circle one or more of the risk factor that are listed below.					
○	Major trauma	○	malnourished	○	Vascular disease
○	Obesity	○	Peptic ulcer disease	○	HIV/AIDS
○	Renal disease	○	Mild liver disease	○	Rheumatologic disease
○	COPD	○	Moderate or severe liver disease	○	Diabetes without chronic complications
○	Diabetes with chronic complications	○	Dementia	○	Immunological and Hematological disease
○	Congestive heart failure	○	Peripheral vascular disease	○	Cerebrovascular disease
○	Myocardial infarction	○	Other(state)		Allergic :……..
○		○		○	Other immunosuppression
○	Any malignancy, including leukemia and lymphoma	○	Metastatic solid tumor	○	Other(state)

4.2. Patient therapy information

Has the patient taking any therapy?	○ Yes	○ No ,
What type of therapy has the patient taking?		
○ Steroid	What?1, _____ for how long? _____	
	What?2, _____ for how long? _____	
	What?3, _____ for how long? _____	
○ NSAID	What?1, _____ for how long? _____	
	What?2, _____ for how long? _____	
○ chemotropic(cytotoxic)	What?1, _____ for how long? _____	

		What?2, _____ for how long? _____
		What?3, _____ for how long? _____
○	Antibiotics	What?1, _____ for how long? _____
		What?2, _____ for how long? _____
		What?3, _____ for how long? _____

4.3. Patient Medical care device use

Has the patient use any medical care device?	○	Yes	○	No , if no go to section 6.3

If yes, what type of device used?

		Date of device procedure start:	Time	Date of device procedure termination	Time	Average date
○	Suprabubic catheter					
○	urethral catheter					
○	venous intravascular catheter					
○	arterial intravascular catheter					
○	central intravascular catheter					
○	tube ventilator(Respirator)					
○	mask ventilator(Respirator)					
	Other, what type is it, write here_____					

4.4. Surgical or non-surgical wound cleaning or dressing information

		Date of cleaning start	Frequency cleaning	Dressing or cleaning termination Date
	Surgical site cleaning or dressing			

Has the patient undergoes any form of surgery? ○ Yes ○ No, if no go to section 4.5

If yes, what type of surgery? Write here the type of surgery served

_____

4.5. Health care device provider (health professional) information

Who provide the health care device service to the patient?			
○	Senior doctors	○	Nursing/midwives students
○	Resident medical staff	○	Allied health professionals
○	Medical students	○	Health care assistants or equivalent
○	Nurses/midwives		

5. Clinical evaluation data collection technique use

5.1. Patient infection information after 48 hours admission and above.

Has the patient develop infection?	○ Yes	○ No , if no go to next section

If yes, what type of infection has the patient developed?

○	SSI	□	superficial infections	□	under the skin, organs, or implanted material	□	Bone and Joint (including spine)
○	Pneumonia	□	Ventilator associated	□	None VAP	□	
○	UTI	□	With urethral catheter	□	Without indwelling device	□	
○	Septicemia	□	Meningitides	□	GI. infection	□	

If other than the list , write here:_____

Has the patient non-surgical break in the skin? ○ Yes ○ No

If yes, what type of skin break has?

○ Vascular ulcer	○ Diabetic ulcer	○ Other	○ Pressure sore
○ Vascular pressure	If other, what: _____		

Date and time of infection diagnosed:	Date: _____	Time: _____

6. Laboratory result Data collection

6.1. Microbiology laboratory Result

What type of sample collected for Diagnosis?

○ Wound swab	○ Sputum	○ Midstream Urine	○ Blood	○ CSF	○ Stool
○ Ear swab	○ Pleural fluid	○ Suprapubic Urine	○ Pericardial fluid		○ Ascites fluid
○ Eye swab	○ Throat swab	○ Catheter urine			
○ Urethral swab	○ Vaginal swab				

7. Use Medical records to fill the following information

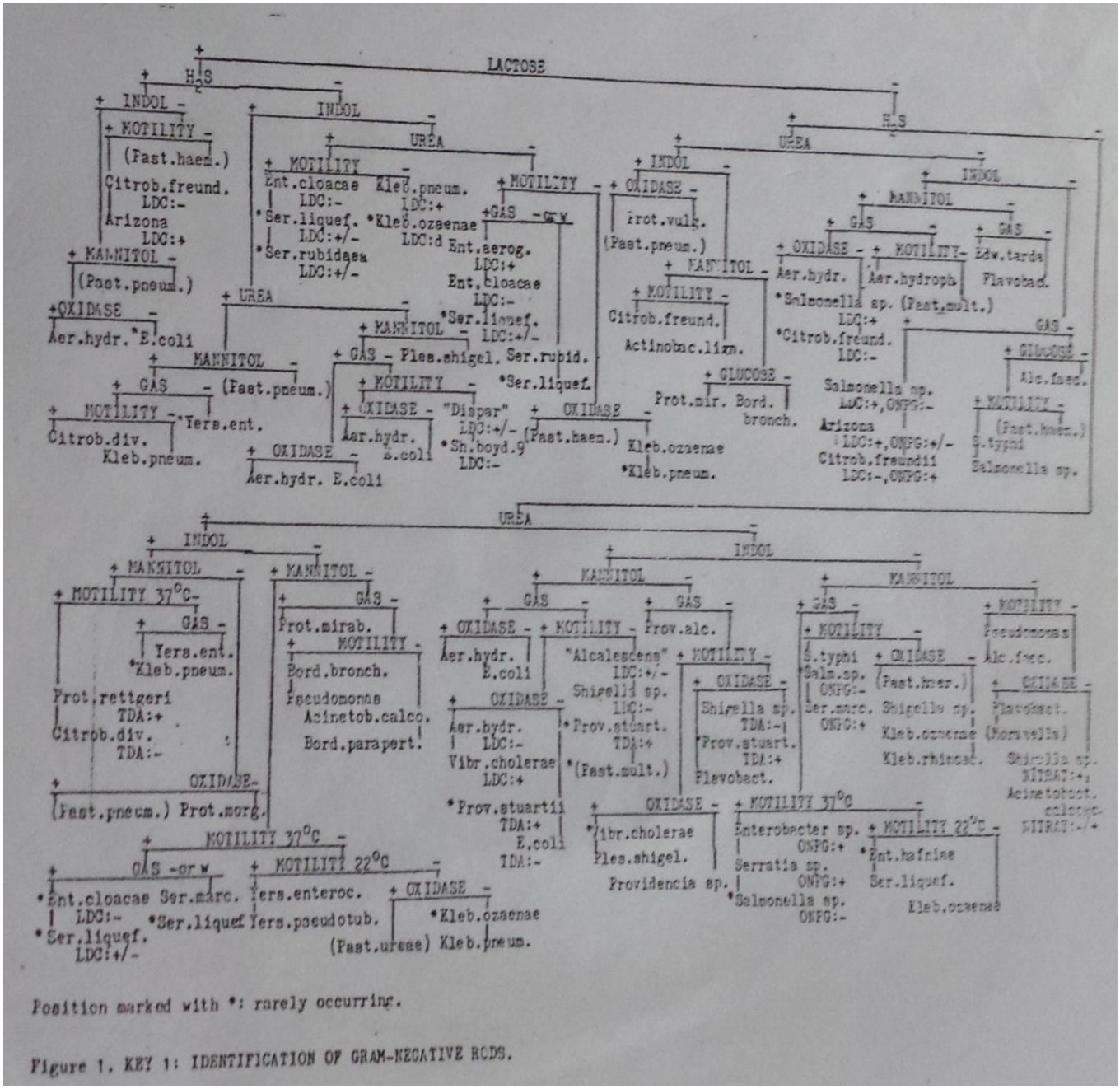
7.1. Prescribed patient antibiotic

Has the patient been prescribed antibiotic		○ Yes	○ No		
If yes, what type of antibiotic prescribed?	1				
	2				
	3				
Rout of administration	PO	IV	IM	ID	Annals
If no, write the reason					

7.2. Patient discharge or expired information

Date of discharge	Time of discharge
Reasoned of discharge or expired	

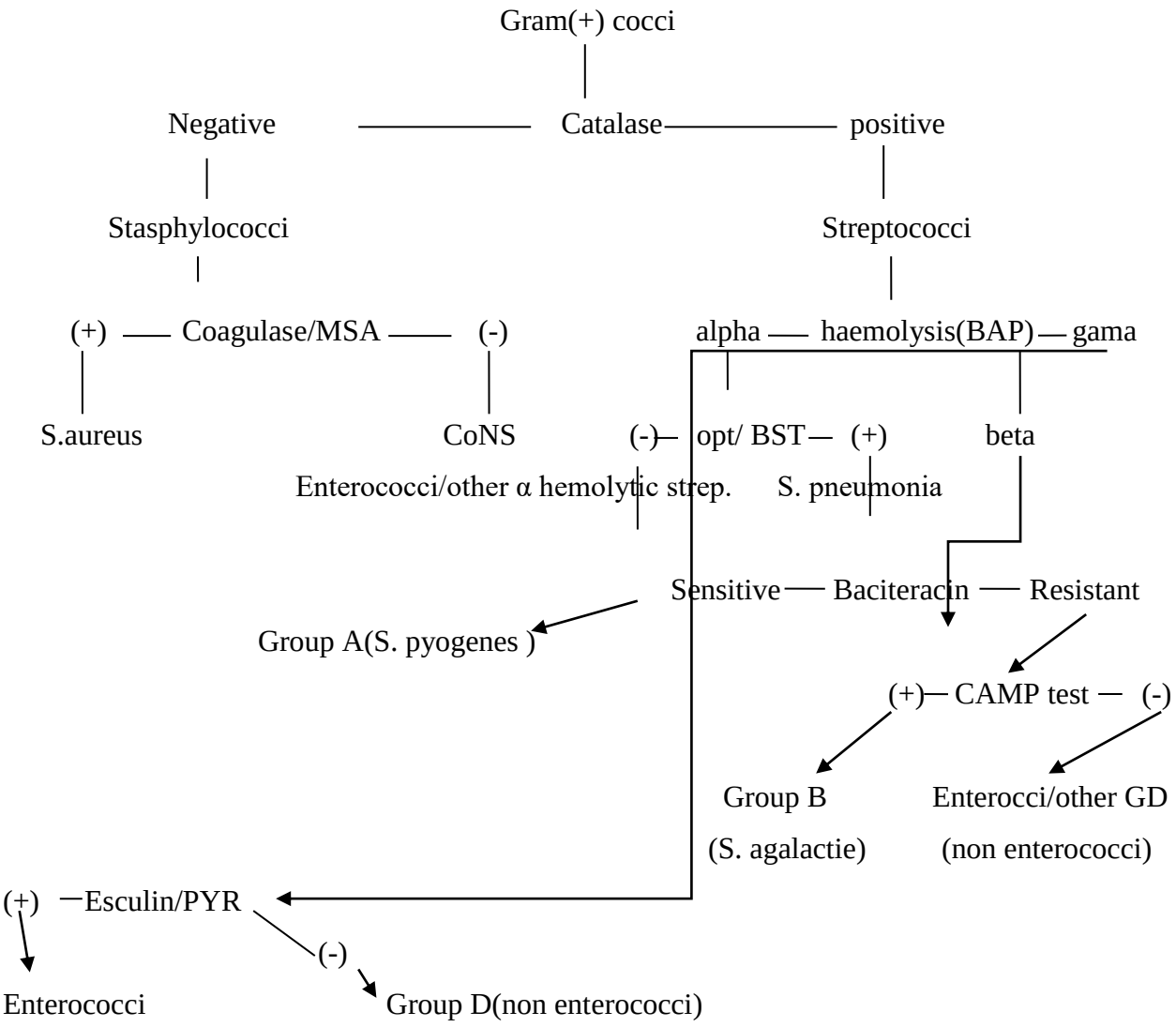
Annex VI. Flow chart for identification of Gram negative bacteria



Position marked with *: rarely occurring.

Figure 1. KEY 1: IDENTIFICATION OF GRAM-NEGATIVE RODS.

Annex VII. Flow chart for identification of Gram positive bacteria



Annex VIII. Simplified criteria for surveillance of hospital acquired infection

Type of hospital acquired infection	**Simplified criteria**
Surgical site infection	Any purulent discharge, abscess, or spreading cellulitis at the surgical site during the month after the operation
Urinary infection	Positive urine culture(1 or 2 species) with at least 105 bacteria/ml, with or without clinical symptoms
Respiratory infection	Respiratory symptoms with at least two of the following signs appearing during hospitalization: — cough— purulent sputum — new infiltrate on chest radiograph consistent with infection
Vascular catheter Inflammation	lymphangitis or infection purulent discharge at the insertion site of the catheter
Septicaemia	Fever or rigors and at least one positive blood culture

Note: the table sited from WHO 2002

Annex IX. Recommended Volumes of Blood for Culture in Pediatric Patients

Weight of Patient (kg)	Total Patient Blood Volume (mL)	Recommended Volume of Blood for Culture (mL)	Blood Volume for other tests (mL)	Total volume of blood collected (ML)
≤ 2	50–99	2	1	3
2.1–12.7	>200	3	2	5
12.8–36.3	>800	4	4	8

Note: The table sited from a guide to utilization of the microbiology laboratory for diagnosis of infectious diseases[36]

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Zerihun Woldesenbet (BSc.)

Signature:_____

Date of submission:_____

This proposal has been submitted with our approval as advisors.

Advisor: Kassu Desta (MSc, PhD candidate)

Signature: _____

Date:_____

Place: Addis Ababa, Ethiopia.

Advisor: Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Merertu Temesgen(MD, Pediatrician)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.