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Bacterial Profile and Drug Susceptibility Patterns of Isolates from Surgical Site Infections, Healthcare workers' Hands and the Surrounding Environment at Surgical wards of Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Gelila Melaku, Entitled: Bacterial profile and drug susceptibility pattern of isolates from surgical site infection, health care worker's hand and the surrounding environment at surgical wards of Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Medical 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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List of abbreviations

AST:- Antimicrobial Susceptibility Test

ATCC:- American Type Culture Collection

BSI:- Bloodstream Infection

CDC:- Center for Disease control

CFU:- Colony forming unit

CLSI:- Clinical and Laboratory Standards Institute

CONS:- Coagulase Negative Staphylococci

DRERC:- Department research and ethical review committee

GNB:- Gram negative bacteria

GPB:-Gram positive bacteria

HCAI:- Health care associated infection

HCP:- Health care professional

HCW:- Health care worker

ICU:- Intensive care unit

MDR:- Multi drug resistance

NI:- Nosocomial infection

OR:- Operation room

QC:- quality control

SOP:- Standard Operating Procedures

SPSS :- Statistical Package for the Social Sciences

SPHMMC:- St. Paul's Hospital Millennium Medical College

Spp :- species

SSIs:- surgical site infections

UTI:- Urinary tract infection

WHO:- World Health Organization

Abstract

Background: Surgical site infections (SSIs) are infections affecting wounds from invasive surgery. Hospitals harbor many bacteria, including antibiotic-resistant strains, on frequently touched surfaces like work areas, medical equipment, and even furniture, which pose a significant risk for the development of SSIs.

Objective: To assess the bacterial profile and drug susceptibility patterns of isolates from SSIs, health care workers'(HCWs') hands and the surrounding environment at surgical wards of Saint Paul's Hospital Millennium Medical College (SPHMMC) Addis Ababa, Ethiopia.

Methods: A cross-sectional study, was conducted from June to October, 2024, and collected a total of 327 samples. All samples were inoculated on MacConkey and Blood agar. Gram staining and biochemical assays were used for identification of the bacteria. Antimicrobial susceptibility testing was done on Muller-Hinton agar by using the Kirby-Bauer disk diffusion technique and data analysis, was performed using SPSS version 26.

Results: The study revealed that out of total 327 samples 90.2 % were harboring bacterial growth. Only 9.8 % samples showed no bacterial growth. Mixed bacterial growth seen in 22.4%. A total of 368 bacterial isolates were identified. Gram-positive bacteria comprised 57.6 % (212) of the isolates, while Gram-negative bacteria accounted for the remaining 42.4% (156). High resistance rates were observed among Gram-negative isolates, including ceftriaxone resistant rate of 91.1%.Whereas, Gram-positive isolates showed high resistance against penicillin 89.7%. This study revealed a high prevalence of MDR, affecting 82.7% (177/214) of the isolates.

Conclusion and recommendation: This study highlights a high prevalence of bacterial contamination in SSIs, HCWs' hands, and surgical ward environment. *Klebsiella species*, *S.aureus,* and *E.coli* were identified as the major contributors to postoperative SSIs. It exposes critical concerns regarding antibiotic resistance in both Gram-positive and Gram-negative bacteria. These findings emphasize the urgent need for effective infection control measures and antibiotic stewardship.

Keyword: Surgical site infection, Health care worker, Environmental sample, Bacterial profile and drug resistance.

1. INTRODUCTION

1.1 Background

Surgical site infections (SSIs) represent a significant global burden to healthcare systems due to increased expenditures, patient morbidity, and mortality (1).The interpretation of clinical and laboratory results is necessary for the detection of SSIs (2). SSIs are caused by a variety of microorganisms colonizing and then invading the surgical incision, which can result in systemic infection and local inflammation. The most common microorganism cultured from SSIs is *S.aureus* (3). Additional investigation showed the presence of several clinically important bacteria, including *Enterobacter species, Klebsiella species, E. coli, E. faecalis,* and *P. aeruginosa* (4).

The surfaces that come into contact with hands in hospital settings are significant sources of various bacterial isolates, including *Staphylococcus aureus, Escherichia coli, Enterococcus, Acinetobacter, Klebsiella,* and *Pseudomonas species* (5).

Antibiotic-resistant bacteria cause significant nosocomial infections, and reservoirs of resistance genes are common and can be found on a variety of hospital surfaces, including work areas, medical equipment, housekeeping surfaces, and lobby furniture. The hospital environment provides these organisms with a new ecological home (6, 7).

One of the most crucial steps in preventing nosocomial infections, such as SSI and the spread of germs in hospitals, is hand hygiene with alcoholic hand rub solution (8, 9). In professional surgical practice, meticulous hand hygiene before putting on sterile gloves is standard procedure immediately prior to surgery (10, 11). Controlling nosocomial pathogens and resistant strains that contaminate hands, equipment, surfaces, and air has been a major focus of hospital design and hygiene measures (12).

The striking similarity between SSI-causing bacteria and environmental isolates as demonstrated above highlights a critical gap in our understanding. Further research directly comparing bacterial profiles from both SSI cases and their surrounding environments is urgently needed.

1.2 Statement of the problem

Healthcare-associated infections (HCAI) pose a major global risk to patient health and continue to be a formidable challenge for healthcare practitioners (13, 14). SSIs, in particular, are a common and serious complication, contributing significantly to illness and death worldwide. Microorganisms that are resistant to antibiotics are mostly responsible for SSI (15).

The 2019 HCAI information sheet report from the WHO states that 100 million patients worldwide are impacted annually (14). A meta-analysis of HCAI categories indicated that Respiratory tract infections, Urinary tract infections, SSIs, and Blood stream infections are the most widespread worldwide. Among these, SSIs were the most frequently reported in Africa, accounting for 51.1% (16). SSIs contribute to the spread of antibiotic resistance and endanger the lives of millions of patients every year (17). Compared to industrialized countries, developing countries have a greater prevalence of SSIs (18, 19). In Sub-Saharan Africa, the incidence of Nosocomial infections(NIs) has been reported to vary from 1.6% to 28.7% (20).

Ethiopia continues to have a significant HCAI burden. SSI was the most common type of HCAIs. The surgical, gynecology, and obstetrics wards had the highest total burden, followed by the surgical ward (14).

According to different researches, the most frequent causes of SSIs are insufficient availability of personal protective equipment, inadequate instruction in infection control procedures, a lack of an infection control policy in the hospital, and improper hand washing techniques (21, 22). Direct contact between a patient and an inanimate object without utilizing the required antisepsis or washing their hands properly can also result in infections (23).

Ethiopia also has a high risk of SSIs, with reported rates ranging from 10.9% in Bahirdar to 19.1% in Hawassa (24, 25). Another study conducted in Ethiopia on patients exhibiting clinical signs of

post-surgical wound infection found that 75% of patients had SSI that was confirmed by culture, and 82.9% of these patients had bacteria that were multi-drug resistant (26).

Continued research into the biology of infection at the surgical site, awareness of microorganisms associated with SSIs, and evaluation of antibiotic susceptibility patterns are crucial since the etiology of SSIs and their antibiotic susceptibility pattern are dynamic. SSI is known to be primarily transmitted through the hands of healthcare workers.

Many studies on the prevalence of SSIs have been carried out in Ethiopia. However, there were limited reports on the bacterial profile and drug resistance patterns of isolate from, SSIs, health care worker's hands and the surrounding environment at the same time for specific study area. Hence, this study will contribute to generating additional data for a better understanding of bacterial profile and drug susceptibility patterns of isolates from SSIs, health care worker's hand and the surrounding environment.

1.3 Significance of the study

These findings are crucial for designing targeted infection prevention protocols and for policymakers to formulate effective strategies to mitigate SSIs and other HCAIs. Understanding the distribution and antibiotic susceptibility of bacterial isolates from SSIs, HCWs hands, and surgical ward environment offers critical insights into the current microbial landscape within surgical settings. This knowledge informs clinicians about the effectiveness of common treatments, supporting the development of evidence-based prescribing strategies.

2. LITRATURE REVIEW

2.1. Prevalence of surgical site infections

A systematic review and meta-analysis involving data from 39 countries revealed a global pooled incidence of SSIs at 2.5%, with the African region exhibiting the highest rate at 2.7%. These findings emphasize the widespread challenge of SSIs and highlight the urgent need for targeted strategies to lower infection rates, particularly in high-risk areas (27).

A systematic review and meta-analysis has provided important new insights into SSIs in general surgery patients globally. The comprehensive study examined 57 high-quality research papers covering six different anatomical sites and analyzed data from nearly half a million patients (488,594). The research revealed that the 30-day SSI incidence rate was 11%, a finding that highlights how surgical infections continue to be a major challenge in modern healthcare and emphasizes the urgent need for strong infection prevention protocols across all surgical specialties (28).

A retrospective study carried out in India found that the Department of General Surgery had a 12.5 % prevalence of SSI. The most common of the three forms was superficial incisional SSI, which was followed by deep incisional SSI and organ / space SSI. Compared to 12.5 % of elective procedures, an alarming 17.7 % of SSI were linked to emergency surgery (29).

Another prospective study was conducted at the rural district hospital in Rwanda, in their reports, among 729 women who had a cesarean section and were qualified for follow-up, 550 (88.7%) went back for assessment. Patients with SSI are made up 10.9 % of the post-operative days, which included 60 women (30).

This systematic review and meta-analysis examined SSIs in Ethiopia, analyzing data from 18 studies. The research revealed that the overall prevalence of SSI in Ethiopian healthcare settings was 11.58 %, indicating that approximately one in nine surgical patients developed an infection.

Among patients who showed clinical signs and symptoms of SSI, 80.42 % had culture-confirmed bacterial infections (31).

Ethiopia also has a high risk of SSIs, with reported rates ranging from 10.9 % in Bahirdar to 19.1 % in Hawassa (24, 25). Another study conducted in Ethiopia on patients exhibiting clinical signs of post-surgical wound infection found that 75 % of patients had SSI that was confirmed by culture, and 82.9% of these patients had bacteria that were multi-drug resistant (26).

According to a cross-sectional study done in Ethiopia, SPHMMC in 2016, the prevalence of SSIs was found to be 24.6%. Patients who had undergone abdominal (30%) and orthopaedics (54.3%) procedures showed a significant prevalence (32).

2.2. Bacterial profile that causes SSIs

A cross-sectional study carried out in Iraq revealed that 113 (22.07%) of the patients examined had acquired SSI, 48 (9.38%) instances involved Gram negative bacteria, with *Klebsiella spp*. accounting for 21/48 (43.75%) of these cases. *E.coli,* 14/48 (29.17%) were the next most common causes. The bacteria with the lowest infection rate, 1/48 (2.08%), was *Citrobacter koseri* (33).

A retrospective observational analysis conducted in Germany in 2017 by Seven Johannes found that from total of 2,004,793 patients, 32,118 patients developed SSIs. In this investigation, *P. aeruginosa, Enterobacteriaceae, and Acinetobacter spp* were the most common bacteria that cause SSI (34).

According to a survey done in Egypt, 67.6% of patients have SSI. Superficial wounds were the most prevalent kind of wound infection, while contaminated wounds were the most common type of surgical wound. *S.aureus* was the most often isolated species (27.4%). 37.2% of cases had MDR isolates identified (35).

SSIs in Ethiopia were investigated in this systematic review and meta-analysis, which analyzed data from 18 studies. Overall, 11.58% of Ethiopian healthcare settings had SSI, according to the study. The microbiological analysis identified *S.aureus* as the predominant pathogen, causing 28.47% of infections, followed by *E.coli* at 15.93% (31).

S.aureus (30.3%) was the most common isolate in an Ethiopian, Harari region investigation that found that the overall prevalence of SSIs was 11.8%. Most of them are Penicillin-resistant *S. aureus* and *CONS* (36).

A prospective cohort study conducted in Jimma reported that SSIs affected 21.1% of cases, with 71.7% showing positive bacterial cultures. Gram-negative bacteria were the majority (78%), followed by gram-positive bacteria (11%), and 10% showed mixed growth. *E. coli* was the leading pathogen (21.43%), with *Pseudomonas aeruginosa* (19.05%) and *Proteus species* (14.29%) also prominent. *S. aureus* and *Klebsiella species* each accounted for 11.9% of infections (37).

A cross-sectional study at SPHMMC and Yekatit 12 Referral Hospital revealed that 90 out of 107 swabs (84.1%) were culture-positive, yielding 104 organisms. *E. coli* (23.1%) and MDR *Acinetobacter species* (22.1%) were the most common isolates, with 75% of Gram-negative bacteria showing multidrug resistance. Pan-antibiotic resistance was seen in 34.8% of *Acinetobacter* and 12.5% of *E. coli* isolates. Gram-negative bacteria predominated (73.1%), and while many antibiotics showed resistance, gentamicin and ciprofloxacin were relatively effective (38) .

2.3. Bacterial profile and drug susceptibility pattern of bacterial isolates that are found in hands of health care workers

A cross-sectional study was carried in Brazil on microbiological examinations of swabs taken from the hands of the 131 surgeons following the antisepsis process and 26 different species of

germs were reported following the hand washing technique. 25 strains of *Staphylococcus spp*. were found, accounting for 73.5 % of all identified bacteria. 24 of these isolates were CONS which were the most common types Additionally, *Micrococcus luteus* represented 12.2% of the overall total (39).

Most commonly isolated bacterial infections among 134 healthcare workers, as reported in a study conducted in Vietnam, were *A. baumannii, K. pneumoniae, and S. aureus.*(40).

According to a study done in Southern Italy, 5.41% (100/1,848) of the 1,848 HCWs in the hand sample tested positive with high frequency of CONS , *Enterobacteriaceae* were highly isolated from Gram-negative bacteria (41).

According to a prospective study by KD Pegu in 2021 on healthcare professionals, every hand showed growth, 95 % of the HCWs' hands held commensals, and 64 % held pathogens. 18 commensal and 21 pathogenic bacteria were identified (42).

According to a study done in Nigeria, CONS were the most common type of bacteria discovered on the hands of all 300 healthcare workers. Other bacteria found included *Enterobacteriaceae* (6%), *Streptococcus pyogenes* (2.7%), and *S.aureus* (23.7%). The isolates showed high levels of sensitivity to ceftriaxone (87.3%), augmentin (87.7%), and ofloxacin (96.7%) (43).

2.4. Bacterial profile and drug susceptibility pattern of bacterial isolates from hospital environment

A Southern Italian study found that 26.57 % (508/1,912) of the 1,912 environmental samples had positive results. A high frequency of *CONS* was observed in highly isolated *Staphylococcus species. Enterobacteriaceae* were highly isolated gram-negative bacteria (41).

Bacillus species, Klebsiella pneumoniae, Clostridium spp and *Staphylococcus aureus* were detected in a research on Cameron hospital environments that sampled 40 beds and 20 door handles. 42.5% of patient beds had *S. aureus* contamination. Nonetheless, the most prevalent

isolate from door handles was *C. perfringens* 35 %. *S. aureus* demonstrated a susceptibility rate of 95.2% to both gentamycin and azithromycin, while exhibiting complete resistance (100%) to bacitracin (44).

According to a cross-sectional study carried out at Wolaita Sodo University Teaching and Referral Hospital, Ethiopia, showed *CONS* (29.6%), *S.aureus* (26.3%), E*nterococci species* (16.5%) and *Acinetobacter species* (9.5%) were identified as medically significant bacterial pathogens from hospital environments. For every isolate, there found evidence of antibiotic resistance ranging from 7.5 to 87.5% (45).

In a study carried out at the University of Gondar Referral Hospital Environments, 42.10% of the isolates were *Klebsiella pneumoniae*, 35.09% were *E.coli*, and 7.01% were *Proteus mirabilis* (46).

Another cross-sectional study was carried out in Northwest Ethiopia on 356 environmental samples, 274 came from surfaces, and 82 from the air. Among the samples 39.6% exhibited bacterial growth, leading to a total of 190 isolates. Of these, 81.6% were classified as gram-positive isolates. The predominant isolates were *CONS* accounting for 44% followed by *S. aureus* (37.4%) *and Klebsiella species* (11.65). Furthermore, the isolates' profile of antimicrobial susceptibility revealed that roughly 75% of the detected isolates was MDR (47).

A cross-sectional study conducted in Ethiopia at Tikur Anbessa Specialized Hospital, reported that Out of the 164 swabbed samples, 141 (86%) were positive for bacterial growth. The predominant bacteria identified from ORs and ICUs were *S.aureus* (23 % vs 11.5%), *Acinetobacter baumannii* (3.8% vs 17.5%) and CoNS (12.6 % vs 2.7 %) respectively. Among the items assessed in the hospital, linens were found to be the most contaminated, exhibiting a contamination rate of 14.8%. Gram-positive bacteria had significantly high resistance levels to penicillin (92.8 %), cefoxitin (83.5%), and erythromycin (53.6%). On the other hand, Gram-negative bacteria revealed the highest resistance levels to ampicillin (97.5%), ceftazidime (91.3%), and ceftriaxone (91.3%).

Additionally, a relatively low resistance rate was observed for amikacin at 25%, followed by ciprofloxacin at 37.5% (48).

3. OBJECTIVES

3.1. General objective

- To assess the bacterial profile and drug susceptibility patterns of bacterial isolates from surgical site infection, health care worker's hands and the surrounding environment at surgical wards of SPHMMC, Addis Ababa, Ethiopia.

3.2. Specific objectives

- To determine the bacterial profile of bacterial isolates from surgical site infection, health care workers' hands and environmental samples.
- To determine drug susceptibility patterns of bacterial isolates from surgical site infection, health care workers' hands, and environmental samples.
- To determine MDR bacterial isolates from surgical site infection, health care workers' hands and environmental samples.

4. MATERIALS AND METHODS

4.1. Study area

The study was carried out in Addis Ababa, Ethiopia, at St. Paul's Hospital Millennium Medical College. The hospital was established in 1968. Currently one of Ethiopia's tertiary public hospitals, it has approximately 700 beds and treats both ambulatory and hospitalized patients from all over the country. It serves an annual average of 400,000 patients from a catchment area of over 5 million, performing approximately 12,650 surgical procedures each year (49). Samples from the orthopedic ward were collected at AaBET (Addis Ababa Burn Emergency and Trauma) Hospital, as the orthopedic wing of St. Paul's Hospital is situated there.

4.2. Study design and period

A hospital-based cross-sectional study was conducted from June to October, 2024.

4.3. Population

4.3.1. Source population

All admitted patients, all hospital environment and all HCWs working at SPHMMC during the study period serve as the source of population.

4.3.2. Study population

Patients admitted to the surgical ward, all surgical ward environment and all HCWs who are working in the surgical ward fulfilling the inclusion criteria were served as the study populations.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

All patients who underwent surgery and were suspected of surgical site infection and HCWs were assigned to surgical wards during the study period.

4.4.2. Exclusion criteria

- Patients with postoperative infections were referred from other hospitals for admission at SPHMMC.

4.5 Study variables

4.5.1. Dependent variables

- Bacterial isolates from SSIs, HCWs, and from the surgical ward environmental culture.
- Antimicrobial susceptibility pattern of bacteria isolated from SSI, HCWs and environmental samples from surgical wards

4.5.2. Independent variables

- Socio-demographic data of the patient (Age,Sex, Address)
- Socio-demographic data of HCWs (Age,Sex, Address, Profession, Level of qualification)
- Clinical data of the patient(Surgical procedure performed, Type of surgery, Nature of surgery)
- Different in animate objects(bed, chair, door handle, floor, iv pole, oxygen cylinder, sink, sphygmomanometer, stethoscope, suction jar, table, and water tap) in the surgical wards.

4.6 Sample size calculation and sampling method

4.6.1 Sample size calculation

As demonstrated below, the sample size of SSI suspected patients were determined using a single population proportion sample size calculation, with a value of P taken from a previous study ,conducted in Addis Ababa with a prevalence of 10.2% (50).

$n=(z \propto/2)^2 * p\ (1-p)/d^2$

Where

- n=sample size
- z = statistic for level of confidence interval 95%.z score =1.96
- p= estimated prevalence 10.2%
- d= precision 5%

$z^2\ p*(1-P)/d^2 = 1.96^2 x\ 0.102(1-0.102)/0.05^2=141$

To get the final sample size

Assuming 10% non-response rate: **n/(1-10% non -respondent)**

141/(1-0.1)=141/0.9=156.72~157

When we add 10% non-responsive rate, the sample size was 157 SSI suspected patients and other samples were taken from 65 HCWs those who work in the surgical ward and 105 samples from the surgical ward environment. Totally 327 samples were collected from three sources.

4.6.2. Sampling methods

Using consecutive sampling techniques, 157 samples were taken from SSI suspected patients and the other samples were taken from 65 HCWs hands those who work in the surgical ward and 105 samples from the surgical ward environment. Environmental samples were collected over 15 weeks, with 7 samples taken randomly per week.

4.7 Measurement and data collection

4.7.1 Data and sample collection procedure

Swabs from HCWs hands

Each HCW was required to sign an informed consent form and was told the research aims prior to the start of sample collection. In addition, socio-demographic questionnaire data regarding their age, sex, and place of residence were collected. Hand samples were taken during routine medical procedures.

Sterile cotton swabs soaked in sterile 0.85 % saline solution were used to collect samples from both hands of the HCW's. The samples were taken from the finger pulp, inter-digital region, palms, back of hands, and wrist. The swabs were immediately placed in Amies transport medium and transported to the laboratory.

Swabs from patients

Before starting data collection, permission from the ward's consultant was sought. Each patient/attendant was required to sign an informed consent/assent form and was told the research aims prior to the start of sample collection. A well-structured questionnaire [Appendix] was used to collect data. The researcher obtained bio data from the patients through questionnaire and obtained data on prescribed antibiotics from the patient records.

After speaking with the patient, sterile cotton swabs were used to take the samples from the wound. It was gathered after wound cleansing, labeled, and delivered to the lab for further analysis in less than an hour. At every stage, we took precautions to avoid cross-contamination.

Samples from environment

The sample was taken in fifteen rounds, from inanimate objects in the surgical ward that could be touched by staff members, patients, or visitors were checked for bacterial contamination, the samples were taken at random from different surgical wards.

Settle plate techniques were used for the air sampling process. Six sealed plastic bags holding Petri dishes containing Blood and Mac-Conkey's agar media were delivered to surgical wards. The sample number, collection date, and time were written on the plates. The plates were exposed for fifteen minutes at predetermined locations in the surgical wards, each about one meter above the floor. Following this exposure, the air sample plates had their lids replaced before being transported in sealed plastic bags to the laboratory, where they were incubated for 24 to 48 hours at 35 -37°C.

Samples were taken using a swab dipped in sterile normal saline from a variety of surfaces, including the bed, chair, door handle, floor, IV pole, oxygen cylinder, sink, sphygmomanometer, stethoscope, suction jar, table, and water tap. Every sample was correctly labeled and sent to the SPHMMC Microbiology laboratory for processing.

Transportation of samples

After collection, the specimens were sent to the SPHMMC Microbiology laboratory for processing by being placed individually in sterile test tubes with Amies transport medium. The air sample plates were placed in sealed plastic bags and transported to the laboratory with their lids on.

4.7.2 Inoculation and Identification of bacteria

The collected samples were inoculated immediately on blood agar and MacConkey agar plates and incubated at 37°C. Blood agar plates were incubated in 5% Co2 jars; on the other hand, MacConkey was incubated aerobically. Growth was observed after 24 and 48 hours of incubation. The colonies on the positive plates were preliminarily characterized by colony characterization and a Gram-stain reaction. The isolated organisms underwent conventional biochemical testing for species identification. Gram-negative bacteria were identified using oxidase, triple sugar iron agar, citrate utilization, urea, hydrogen sulfide and gas production, indole, and motility tests.

Gram-positive bacteria were identified using catalase, slide and tube coagulase, PYR, novobiocin susceptibility test, and bile esculin tests. [Appendix]

4.7.3 Antimicrobial Susceptibility Tests (AST)

The ASTs of each isolate were tested on Mueller–Hinton agar (Liofilchem, Italy) using the standard Kirby–Bauer's disc diffusion method, which was based on the Clinical and Laboratory Standard Institute (CLSI) 2023 M100 guidelines. Before streaking to the agar plate, the inoculum was adjusted to a turbidity equivalent to a 0.5 McFarland standard by placing the tubes in the DEN-1B densitometer. After the appropriate antibiotic discs were applied and incubated for 15 minutes, the plates were inverted and incubated at 37°C. After 18–24 hours of incubation, each plate was examined, and the diameter of the zone of inhibition was measured in millimeters using a calibrated ruler and interpreted as per CLSI 2023 breakpoints. After the diameter of the inhibition zone was measured, the organisms were reported as susceptible, intermediate, or resistant.

For *Enterobacteriaceae* we used Ampicillin(AMP-10µg), Ceftriaxone (CFT-30µg) or Cefotaxime(CTX-30µg),Gentamicin (GN-10µg), Augmentin(AUG-20/10µg) (Amoxicillin-20µg and clavulanate-10µg), Trimethoprim-Sulfamethoxazole (TSX-1.25/23.75µg), Ampicillin-sulbactam (SAM-10/10µg), Ciprofloxacillin (CIP-5µg), Ceftazidime (CFZ-30µg), Cefepime (CFP-30µg), Tazobactem/piperaciline (TZP-10/100µg), Chloramphenicol (CAF-30µg), Amikacine (AK-30µg), Meropenem (MEM-10µg) or Imipenem (IPM- 10µg).

For *pseudomonas aeruginosa* we used Ceftazidime (CFZ-30µg), Cefepime (CFP-30µg), Ciprofloxacillin (CIP-5µg), Meropenem (MEM-10µg), Amikacine (AK-30µg), Tazobactem/piperaciline (TZP-10/100µg), and Tobramycin (TM-10µg).

Acinetobacter spp were tested against Ampicillin-sulbactam (SAM-10/10µg), Ceftazidime (CFZ-30µg), Tazobactem/piperaciline (TZP-10/100µg), Trimethoprim-Sulfamethoxazole (TSX-1.25/23.75µg), Ceftriaxone(CFT-30µg) or Cefotaxime (CTX-30µg), Cefepime (CFP-30µg), Amikacine (AK-30µg), Ciprofloxacillin (CIP-5µg), Gentamicin (GN-10µg), Meropenem (MEM-10µg) or Imipenem (IPM-10µg).

For *Staphylococcus* spp, we use Clindamycin (CM-2µg), Azithromycine (AZM-15µg) or clarithromycin (CLR-15µg) or Erythromycin(ER-15µg), cefoxitin (FOX-30µg), Ciprofloxacillin (CIP-5µg), Gentamicin (GN-10µg), Chloramphenicol (CAF-30µg), oxcycline (OX-30µg) or Doxycycline (DO-30µg), Tetracycline (TTC-30µg), Trimethoprim-Sulfamethoxazole (TSX-1.25/23.75µg) and penicillin(P-10µg).

For *Enterococcus*, we use penicillin (P-10µg), ampicillin (AM-10µg), Chloramphenicol (CAF-30µg), Tetracycline (TTC-30µg) and vancomycine (VA-30µg).

For *Sterptococcus* spp. β-hemolytic we use Clindamycin(CM-2µg), Azithromycine (AZM-15µg) or Erythromycin(ER-15µg) , ampicillin (AM-10µg), penicillin(P-10µg), and Tetracycline(TTC-30µg) (51).

4.8 Quality control and data quality assurance

Standard Operating Procedures were meticulously followed to ensure that the media met CLSI quality control standards. Visual inspections were conducted to check for issues such as contamination, uneven filling, hemolysis, bubbles, signs of freezing, and fractures in the media or plastic petri dishes. Media quality was checked for sterility and then tested using ATCC strains to confirm it supported bacterial growth. Antimicrobial drug effectiveness was assessed using a disk diffusion assay with ATCC strains; the diameter of the zone of inhibition around the drug disks indicated the drug's potency. Before being used, every fresh lot shall undergo quality control

testing using standard strains of *E. Coli* ATCC 25922 , *P. aeruginosa* ATCC 27853 , *E. faecalis* (ATCC 29212 or 33186) and *S. aurous* ATCC 25923. A pretest was conducted on 5 % of the participants.

4.8.1 Pre-analytical phase

The patient's identifying number is written on the container, and sterile cotton is used to collect an aseptically wound sample, HCWs hand and environmental sample. The samples were delivered to the microbiology laboratory at SPHMMC for analysis.

4.8.2 Analytical phase

A standard strain of *E. Coli* ATCC 25922 , *P. aeruginosa* ATCC 27853,*E. faecalis* (ATCC 29212 or 33186) and *S. aurous* ATCC 25923 were used as the control bacteria strains for both media and antibiotics discs, and the inoculum was adjusted to a turbidity equivalent to a 0.5 McFarland standard by placing the tubes in the DEN-1B densitometer to standardize the inoculum density of bacterial suspension for the susceptibility test. The senior microbiologist closely followed the SOPs and the results were verified.

4.8.3 Post-analytical phase

The outcomes documented together with the patient's unique number. Following a successful isolation and identification of the bacteria during the analytical phase of the study, the isolated bacteria are then stored in a mixture of glycerol and tryptosoya broth, and then labeled with the study code, date, and isolated bacteria and finally keeping them in a refrigerator between -20°C and -80°C. Before the results are presented to the caregiver, the department head thoroughly reviews and verifies the reporting to ensure that there are no inaccuracies in the test results.

4.9. Data analysis and interpretation

All data have been entered, checked, cleaned, and analyzed using the Statistical Package for Social Sciences (SPSS) version 26 software. Descriptive statistics of frequency and percentage were used to present the bacterial profile, the resistance pattern of the isolate to antibiotics and for variables of socio-demographic distribution using tables, charts, graphs and texts.

4.10. Operational definitions

Wound class: The wounds were classified as Dirty / Infected, Contaminated, Clean, and Clean-Contaminated. The presence of purulent discharge, severe contamination, pre-operative infection, etc. was among the specific classification criteria. Using a consistent technique, the operating surgeon or a blinded observer made the classification.

Clean wounds; are characterized by the absence of inflammation and the avoidance of entry into the genital, alimentary, respiratory, or urinary tracts.

Clean-contaminated; are when the respiratory, alimentary, vaginal, or urinary tracts are penetrated under carefully monitored circumstances and without unusual contamination, the wound is said to be clean-contaminated.

Contaminated wounds ; refer to acute, non-purulent inflammation or recent, unintentional open wounds.

Dirty/infected wounds; are described as a wound that had substantial microbiological contamination at the time of surgery, such as purulent discharge, obvious foreign material contamination, a pre-operative infection that was evident at the time of surgery, or an infection that was proven by culture. This definition adheres to the CDC's(Centers for Disease Control and Prevention) classification rules (52).

Multi-drug resistance; is defined as a bacterial isolate, that is resistant to three or more classes of antibiotics (53).

4.11 Ethical considerations

Ethical clearance was obtained from the Department of Medical Laboratory Science, College of Health Sciences, Addis Ababa University's Department Research and Ethical Review Committee (DRERC), DRERC/760/24/MLS on 09/02/24 and from SPHMMC institutional review board (IRB) (PM23/1140) on 17/04/2024. The study sites were also provide a letter of permission. Throughout the study period, participants were receive an explanation of the goals and methods of the investigation. The attending physicians were informed of the patient's outcome. In order to maintain the confidentiality of participant information, no personal identifiers were documented in the pre-designed client information extraction form, and the data secured from participant records was accessible solely to the principal investigator.

4.12 Dissemination of the results

This work will be submitted to Addis Ababa Univeristy, Department of Medical Laboratory Sciences and SPHMMC. Additionally, the manuscript will be prepared and submitted to a national or international publication for peer review, and it will also be presented to the scientific community in other settings. It will also be presented at relevant scientific conferences, workshops, and seminars.

5. RESULTS

5.1. Socio-demographic characteristics of SSI-suspected patients and HCWs

This study included 157 patients suspected of having SSIs. The patients were predominantly female (54.1%, 85/157), with most patients (51.6%, 81/157) aged between 40 and 59 years. Over half (51.0%, 80/157) were from rural areas. The study also involved 65 HCWs, with a slightly higher proportion being male (64.6%, 42/65). Most HCWs (66.2%, 43/65) were aged between 20 and 30 years, and nurse practitioners represented substantial portion (58.5%, 38/65) of this group. Adult and pediatric surgery wards accounted for the majority of HCWs 43.1% (28/65) and 24.6% (16/65), respectively (Table 1).

Table 1: Socio-demographic characteristics of SSI-suspected patients (n=157) and HCWs (n=65)

Variable	Subcategory	Frequency(n)	Percent (%)
Gender (Patients)	Female	85	54.1%
	Male	72	45.9%
Age in Years (Patients)	<20	12	7.6%
	20–39	28	17.8%
	40–59	81	51.6%
	≥60	36	22.9%
Residence (Patients)	Rural	80	51.0%
	Urban	77	49.0%
Surgical Ward (HCWs)	Adult surgical ward	28	43.1%
	Gynecology surgical ward	11	16.9%
	Orthopedic surgical ward	10	15.4%
	Pediatric surgical ward	16	24.6%
Sex (HCWs)	Female	23	35.4%
	Male	42	64.6%

Variable	Subcategory	Frequency(n)	Percent (%)
Age (HCWs)	20–30	43	66.2%
	31–40	22	33.8%
Profession (HCWs)	Nurse	38	58.5%
	Physician	18	27.7%
	Student	9	13.8%
Education (HCWs)	BSc	27	41.5%
	General Practice (GP)	1	1.5%
	Intern	11	16.9%
	MSc	15	23.1%
	Resident (R1–R4)	11	16.9%

5.2. Bacterial Isolates from SSI suspected patients, HCWs and surgical ward environment

5.2.1. Total bacterial isolates

This study analyzed 327 swabs collected from three sources: surgical ward environments (105, 32.11%), HCWs (65, 19.88%), and patients with suspected SSIs (157, 48.01%). A remarkable 90.2% (295/327) of samples yielded at least one bacterial isolate, with the highest contamination rate observed among suspected SSI patients (152, 51.52%), followed by surgical ward environments (94, 31.86%) and HCWs hands (49, 16.61%). Only 32 (9.8 %) samples showed no bacterial growth. of those 32 samples 5(15.6%) samples were from SSI suspected patients, 16(50%) were from HCWs hands sample, and the remaining 11(34.4%) samples were from the surrounding environment.

A total of 368 distinct bacterial isolates were identified, with Gram-positive bacteria predominating (212, 57.6%) over Gram-negative bacteria (156, 42.4%). Interestingly, 22.4% (66/295) of the contaminated samples exhibited multi-bacterial contamination. *CONS* was the most prevalent bacterial species, accounting for 124 (33.7%) of all isolates. These findings emphasize the widespread bacterial contamination in surgical

ward environments, highlighting the potential for transmission from environmental surfaces and HCWs to patients.

Gram-positive isolates were largely dominated by *CONS*, representing 58.5% (124/212) of all Gram-positive isolates. *Staphylococcus aureus* (21.2%, 45/212) was the second most prevalent Gram-positive species, with *Bacillus species*, *Micrococcus species*, and other *Staphylococcus species* (*S. lugdunensis*, and *S. epidermidis*) and *S. pyogenes*, found at lower frequencies (Figure 1).

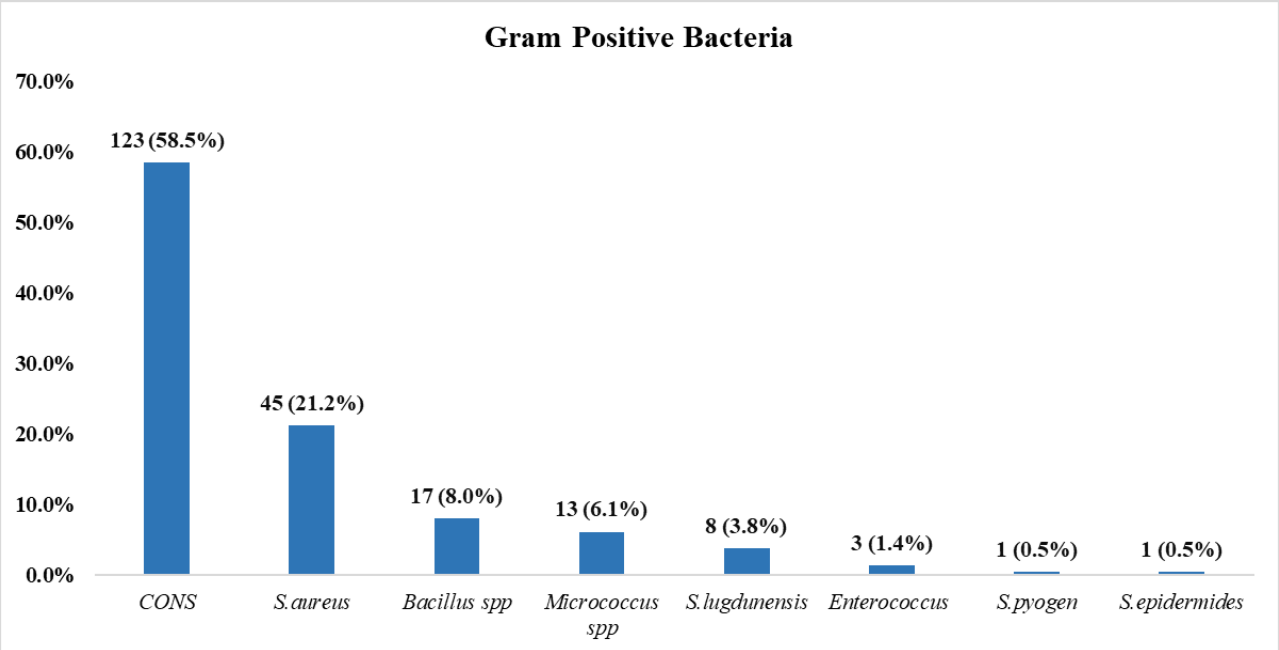

Figure 1: Total percentage of gram-positive isolated bacteria from surgical ward environmental samples, HCW's hand, and SSI suspected patients.

On the other hand, Gram-negative isolates were predominantly *Klebsiella spp* (35.9 %, 56/156), followed by *E. coli* (29.5%, 46/156), *Acinetobacter spp*. (14.1%, 22/156), and *Pseudomonas aeruginosa* (13.5%, 21/156). Other Gram-negative species, including *Enterobacter spp.*, *Citrobacter spp.*, and *Proteus spp.*, were identified at lower frequencies (Figure 2).

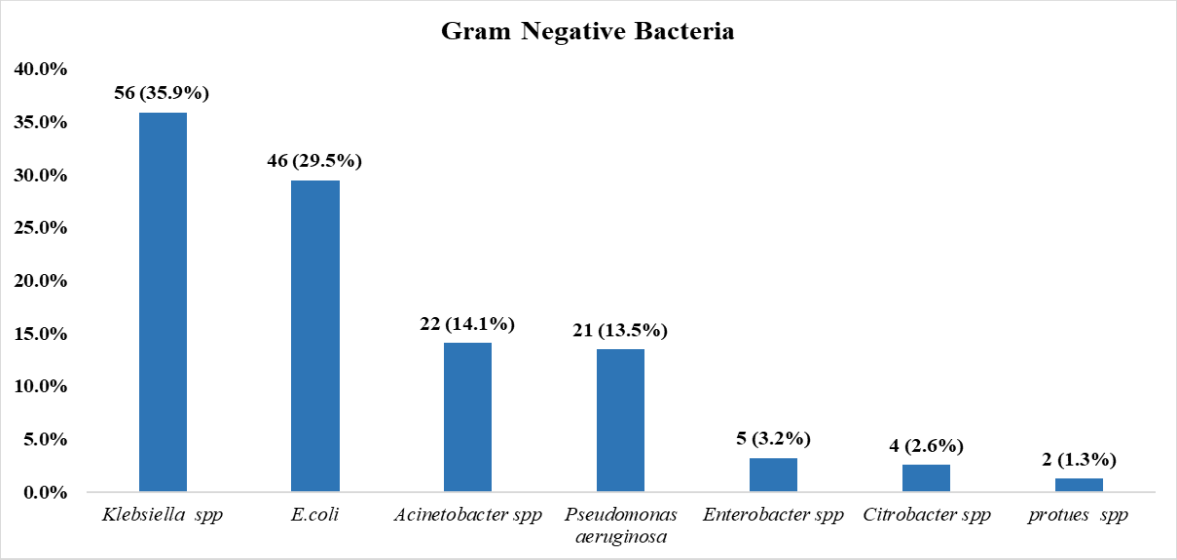

Figure 2; Total percentage of gram-negative isolated bacteria from surgical ward environmental samples, HCW's hand, and SSI suspected patients.

5.2.2 Procedural and Clinical Characteristics of SSI suspected patients and isolated bacteria

The majority of SSI suspected patient samples come from adult surgical wards (51.0%, 80/157 patients), followed by orthopedic (24.8%, 39/157), gynecology (17.8%, 28/157), pediatric (5.1%, 8/157), and urology (1.3%, 2/157) surgical wards (Table 2). Among the 157 patients, gastrointestinal surgeries represented the largest surgical category (22.9 %, 36 /157), followed by gynecological (17.8 %, 28/157) and orthopedic (17.8%, 28/157) procedures. A considerable number of surgeries, (59.2%, 93/157), were performed as emergency procedures. Furthermore, the majority of patients (52.2%, 82/157) presented with clean-contaminated wounds followed by contaminated surgeries at (34.4%, 54/157). Dirty surgeries represented (8.9%, 14/157) of the cases, while clean surgeries were the least common, accounting for only (4.5%, 7/157) of the total.

Analysis of 157 SSI patient samples revealed bacterial growth in 152 (96.8%) samples, yielding a total of 163 isolates. Gram-negative bacteria significantly outnumbered Gram-positive bacteria (71.8%, 117/163 vs. 28.2%, 46/163). *Klebsiella spp., E. coli,*

and *Staphylococcus aureus* were the most frequently identified species, each accounting for approximately 20% of the isolates (Table 2). A small percentage of samples (7.0%, 11/163) showed mixed bacterial infections. Five samples (3.1%) exhibited no bacterial growth within a 24-hour incubation period.

Table 2; Total bacterial isolate from SSI suspected patients with their proportion of each surgical wards of SPHMMC, 2024.

Type of bacterial isolates	**Adult surgical ward n=80(51%)**	**Pediatrics surgical ward n=8(5.1%)**	**Gynecology surgical ward n=28(17.8 %)**	**Orthopedics surgical ward n=39(24.8 %)**	**Urology surgical ward n=2(1.3%)**	**Total number of sample N=157(100 %)**
Gram positive bacteria	**20(25.3%)**	**2(18.2%)**	**8(28.6%)**	**16(37.2%)**	**0(0%)**	**46(28.2%)**
S.aureus	15(19%)	2(18.2%)	4(14.3%)	12(27.9%)	0(0%)	33(20.2%)
S.lugdunensis	5(6.3%)	0(0%)	1(3.6%)	2(4.7%)	0(0%)	8(4.9%)
S.pyogen	0(0%)	0(0%)	0(0%)	1(2.3%)	0(0%)	1(0.6%)
S.epidermides	0(0%)	0(0%)	0(0%)	1(2.3%)	0(0%)	1(0.6%)
Enterococcus	0(0%)	0(0%)	3(10.7%)	0(0%)	0(0%)	3(1.8%)
Gram negative bacteria	**59(74.7%)**	**9(81.8%)**	**20(71.4%)**	**27(62.8%)**	**2(100%)**	**117(71.8%)**
Klebsiella spp	20(25.3%)	3(27.3%)	3(10.7%)	8(18.6%)	1(50%)	35(21.5%)
E.coli	20(25.3%)	1(9.1%)	6(21.4%)	6(14%)	0(0%)	33(20.2%)
Acinetobacter spp	8(10.1%)	2(18.2%)	2(7.1%)	7(16.3%)	0(0%)	19(11.7%)
Pseudomonas aeruginosa	9(11.4%)	2(18.2%)	5(17.9%)	5(11.6%)	0(0%)	21(12.9%)
Enterobacter spp	1(1.3%)	1(9.1%)	3(10.7%)	0(0%)	0(0%)	5(3.1%)
Citrobacte	1(1.3%)	0(0%)	1(3.6%)	0(0%)	0(0%)	2(1.2%)

r spp						
Protues spp	0(0%)	0(0%)	0(0%)	1(2.3%)	1(50%)	2(1.2%)
Total number of isolates	**79(48.5%)**	**11(6.7%)**	**28(17.2%)**	**43(26.4%)**	**2(1.2%)**	**163(100%)**

5.2.3 Bacterial profile from HCW hands

Hand swabs from 65 HCWs were cultured, revealing bacterial growth in 75.4 % (49/65) of samples after 24 hours. Analysis of the resulting 60 bacterial isolates showed a strong predominance of Gram-positive bacteria (85%, 51/60), with Gram-negative bacteria accounting for the remaining 15% (9/60). *CONS* were the most common isolate, representing 73.3% (44/60) of all isolates. Mixed bacterial colonization was presented in 15.4% (10/65) of samples. No bacterial growth was observed in 16 (24.6%) of the samples after 48 hours of incubation period (Table 3).

Table 3; Total bacterial isolate from HCWs hands with their proportion across surgical wards of SPHMMC, 2024.

Type of bacterial isolates	**Adult surgical ward n=28(43.1%)**	**Pediatrics surgical ward n=15(23.1%)**	**Gynecology surgical ward n=10(15.4%)**	**Orthopedics surgical ward n=8(12.3%)**	**Urology surgical ward n=4(6.2%)**	**Total number of sample N=65(100%)**
Gram positive bacteria	**15(75%)**	**15(88.2%)**	**6(100%)**	**10(83.3%)**	**5(100%)**	**51(85%)**
S.aureus	2(10%)	2(11.8%)	0(0%)	2(16.7%)	1(20%)	7(11.7%)
CONS	13(65%)	13(76.5%)	6(100%)	8(66.7%)	4(80%)	44(73.33%)
Gram negative bacteria	**5(25%)**	**2(11.8%)**	**0(0%)**	**2(16.7%)**	**0(0%)**	**9(15%)**
Klebsiella spp	3(15%)	0(0%)	0(0%)	0(0%)	0(0%)	3(5%)
E.coli	2(10%)	2(11.8%)	0(0%)	2(16.7%)	0(0%)	6(10%)
Total number of isolates	**20(33.33%)**	**17(28.33%)**	**6(10%)**	**12(20%)**	**5(8.33%)**	**60(100%)**

5.2.4 Bacterial profile from surgical ward environmental sample

This study examined 105 environmental samples from a surgical ward, encompassing various frequently touched surfaces: beds (11.4 %, 12/105), tables (9.5%, 10/105), IV poles (9.5%, 10/105), sphygmomanometers (9.5%, 10/105), and door handles (3.8%, 4/105). Three air samples were also collected. Sample distribution across surgical wards included adult (26.7%, 28/105), pediatrics (25.7%, 27/105), gynecology (19.0%, 20/105), orthopedics (22.9%, 24/105), and urology (5.7%, 6/105) wards (Table 4). A high proportion (89.5%, 94/105) of samples yielded bacterial growth, resulting in 145 isolates. Gram-positive bacteria significantly predominated (79.3%, 115/145), with *CONS* being the most prevalent (55.2%, 80/145). *Klebsiella spp.* and *Bacillus spp.* followed at lower frequencies (12.4%, 18/145 and 11.0%, 16/145, respectively). Nearly half (47.9%, 45/94) of the positive samples showed mixed bacterial growth, while 10.5 % (11/105) showed no growth after 24 hours of incubation.

Table 4; Total Bacterial isolate from surgical ward environment with their proportion of each surgical wards SPHMMC, 2024.

Type of bacterial isolates	Adult surgical ward n=28(26.7 %)	Paediatrics surgical ward n=27(25.7 %)	Gynaecology surgical ward n=20(19%)	Urology surgical ward n=6(5.7%)	Orthopaedics surgical ward n=24(22.9 %)	Total number of sample N=105(100 %)
Gram positive	**35(79.5%)**	**29(78.4%)**	**19(86.4%)**	**6(75%)**	**26(76.5%)**	**115(79.3%)**
CONS	20(45.5%)	18(48.6%)	16(72.7%)	4(50%)	22(64.7%)	80(55.2%)
Bcillus spp	9(20.5%)	6(16.2%)	1(4.5%)	0(0%)	0(0%)	16(11%)
Micrococcus spp	6(13.6%)	5(13.5%)	2(9.1%)	1(12.5%)	0(0%)	14(9.7%)
S.aureus	0(0%)	0(0%)	0(0%)	1(12.5%)	4(11.8%)	5(3.4%)
Gram negative	**9(20.5%)**	**8(21.6%)**	**3(13.6%)**	**2(25%)**	**8(23.5%)**	**30(20.7%)**
Klebsiella spp	6(13.6%)	6(16.3%)	0(0%)	1(12.5%)	5(14.7%)	18(12.4%)
E.coli	2(4.5%)	0(0%)	2(9.1%)	1(12.5%)	2(5.9%)	7(4.8%)
Acinetobacter spp	1(2.3%)	0(0%)	1(4.5%)	0(0%)	1(2.9%)	3(2.1%)
Citrobacter spp	0(0%)	2(5.4%)	0(0%)	0(0%)	0(0%)	2(1.4%)
Total number of isolates	**44(30.3%)**	**37(25.5%)**	**22(15.2%)**	**8(5.5%)**	**34(23.4%)**	**145(100%)**

5.3 Antimicrobial susceptibility patterns

Gram negative bacterial isolates from SSI suspected patients, HCW hands, and the surgical ward environments showed high resistance against ceftriaxone (91.1%), ampicillin (80.7%), and ceftazidime (70.5%). In contrast, significantly lower resistance was observed for amikacin (12.2%) and chloramphenicol (29.2%) (Table5). Similarly, Gram-positive isolates exhibited high resistance to penicillin (89.7%), azithromycin (69.1%), and tetracycline (69%), while chloramphenicol resistance remained comparatively low (22.8%)(Table 6).These findings highlight the substantial challenge posed by multi-drug resistant bacteria in this hospital setting and underscore the need for targeted infection control strategies and prudent antibiotic stewardship.

Table 5; AST patterns of Gram-negative isolates.

Antibiotics	**Result**	***Klebsiella spp* n=56**	***Acinetobacter spp* n=22**	***Citrobacterer spp* n=4**	***P.aeruginosa* n=21**	***E. Coli* n=46**	***Enterobacter spp* n=5**	***protues spp* n=2**	**Total number of isolate N=156**
CTX	S	n=3 5.4%	n=0	n=0	ND	n=2 4.3%	n=2 40%	n=0	**7/135 5.2%**
	I	n=3 5.4%	n=0	n=0	ND	n=0 0%	n=1 20%	n=1 50%	**5/135 3.7%**
	R	n=50 89.3%	n=22 100%	n=4 100%	ND	n=44 95.7%	n=2 40%	n=1 50%	**123/ 135 91.1%**
GN/TN	S	n=21 37.5%	n=9 40.9%	n=2 50%	n=15 71.4%	n=25 54.3%	n=4 80%	n=0	**76/156 48.7%**
	I	n=4 7.1%	n=1 4.5%	n=0	n=0 0%	n=4 8.7%	n=1 20%	n=0	**10/156 6.4%**
	R	n=31 55.4%	n=12 54.5%	n=2 50%	n=6 28.6%	n=17 37%	n=0	n=2 100%	**70/156 44.9%**
CIP	S	n=24 42.9%	n=6 27.3%	n=2 50%	n=12 57.1%	n=18 39.1%	n=3 60%	n=1 50%	**66/156 42.3%**
	I	n=1 1.8%	n=2 9.1%	n=0	n=5 23.8%	n=7 15.2%	n=0	n=0	**15/156 9.6%**
	R	n=31 55.4%	n=14 63.6%	n=2 50%	n=4 19%	n=21 45.7%	n=2 40%	n=1 50%	**75/156 48.1%**
AMP	S	ND	ND	n=1 25%	ND	n=3 6.5%	n=0	n=1 50%	**5/57 8.8%**
	I	ND	ND	n=0	ND	n=5 10.9%	n=1 20%	n=0	**6/57 10.5%**
	R	ND	ND	n=3 75%	ND	n=38 82.6%	n=4 80%	n=1 50%	**46/57 80.7%**
AUG	S	n=12 21.4%	ND	n=0	ND	n=12 26.1%	n=0	n=1 50%	**25/113 22.1%**
	I	n=6 10.7%	ND	n=0	ND	n=4 8.7%	n=0	n=0	**10/113 8.85%**
	R	n=38 67.9%	ND	n=4 100%	ND	n=30 65.2%	n=5 100%	n=1 50%	**78/113 69%**
TSX	S	n=14 25.0%	n=8 36.4%	n=0	ND	n=23 50%	n=4 80%	n=0	**49/135 36.3%**
	I	n=8 14.3%	n=3 13.6%	n=0	ND	n=0	n=0	n=0	**11/135 8.5%**
	R	n=34 60.7%	n=11 50%	n=4 100%	ND	n=23 50%	n=1 20%	n=2 100%	**75/135 55.5%**
SAM	S	n=7	n=4	n=0	ND	n=4	n=3	n=0	**18/135**

		12.5%	18.2%			8.4%	60%		**13.3%**
	I	n=11	n=3	n=1	ND	n=6	n=0	n=1	**22/135**
		19.6%	13.6%	25%		13%		50%	**16.3%**
	R	n=38	n=15	n=3	ND	n=36	n=2	n=1	**95/135**
		67.9%	68.2%	75%		78.3%	40%	50%	**70.4%**
MER	S	n=29	n=8	n=2	n=10	n=41	n=5	n=2	**97/156**
		51.8%	36.4%	50%	47.6%	89.1%	100%	100%	**62.2%**
	I	n=5	n=0	n=0	n=1	n=2	n=0	n=0	**8/156**
		8.9%			4.8%	4.3%			**5.1%**
	R	n=22	n=14	n=2	n=10	n=3	n=0	n=0	**51/156**
		39.3	63.6%	50%	47.6%	6.5%			**32.7%**
FEP	S	n=7	n=3	n=0	n=6	n=3	n=3	n=0	**22/156**
		12.5%	13.6%		28.6%	6.5%	60%		**14.1%**
	I	n=15	n=1	n=0	n=4	n=6	n=0	n=0	**26/156**
		26.8%	4.5%		19%	13%			**16.7%**
	R	n=34	n=18	n=4	n=11	n=37	n=2	n=2	**108/15**
		60.7%	81.8%	100%	52.4%	80.4%	40%	100%	**6**
									69.2%
CAZ	S	n=12	n=1	n=0	n=9	n=13	n=1	n=0	**36/156**
		21.4	4.5%		42.9%	28.3%	20%		**23.1%**
	I	n=3	n=3	n=0	n=0	n=3	n=1	n=0	**10/156**
		5.4%	13.6%		0%	6.5%	20%		**6.4%**
	R	n=41	n=18	n=4	n=12	n=30	n=3	n=2	**110/15**
		73.2%	81.8%	100%	57.1%	65.2%	60%	100%	**6**
									70.5%
TZP	S	n=22	n=5	n=0	n=12	n=28	n=4	n=2	**73/156**
		39.3%	22.7%		57.1%	60.9%	80%	100%	**46.8%**
	I	n=3	n=2	n=0	n=1	n=6	n=0	n=0	**12/156**
		5.4%	9.1%		4.8%	13%			**7.69%**
	R	n=31	n=15	n=4	n=8	n=12	n=1	n=0	**71/156**
		55.4%	68.2%	100%	38.1%	26.1%	20%		**45.5%**
AK	S	n=40	n=18	n=3	n=19	n=40	n=5	n=2	**127/15**
		71.4%	81.8%	75%	90.5%	87%	100%	100%	**6**
									81.4%
	I	n=6	n=1	n=0	n=0	n=3	n=0	n=0	**10/156**
		10.7%	4.5%			6.5%			**6.4%**
	R	n=10	n=3	n=1	n=2	n=3	n=0	n=0	**19/156**
		17.9%	13.6%	25%	9.5%	6.5%			**12.2%**
CAF	S	n=24	ND	n=1	ND	n=35	n=5	n=1	**66/113**
		42.9%		25%		76.1	100%	50%	**58.4%**
	I	n=8	ND	n=1	ND	n=5	n=0	n=0	**14/113**
		14.3%		25%		10.9%			**12.4%**
	R	n=24	ND	n=2	ND	n=6	n=0	n=1	**33/113**
		42.9%		50%		13%		50%	**29.2%**

Key; ND =Not done ,CTX- ceftraxione,GN-gentamycine, TN-tobramycine only for p.aeruginosa,CIP-Ciprofloxacin,AMP-ampiciline,AUG-augmentine,TSX-Trimethoprim/Sulfamethoxazole,SAM-ampiciline sulbactam, MER-meropenem, FEP-cefepime, CAZ-ceftazidime,TZP-Tazobactem/piperaciline,AK-amikacine,CAF-chloramphenicole

Table 6; AST patterns of Gram-positive isolates.

Antibiotic	Result	*S.aureus* N=45	*S.lugdunensis* N=8	*Enterococcus spp* N=3	*S.pyogen* N=1	*S.epidermides* N=1	Total number of isolate N=58
CIP	S	n=20 44.4%	n=1 12.5%	ND	ND	n=1 100%	**n=22/54 40.7%**
	I	n=3 6.7%	n=1 12.5%	ND	ND	n=0	**n=4/54 7.4%**
	R	n=22 48.9%	n=6 75%	ND	ND	n=0	**n=28/54 51.9%**
GN	S	n=19 42.2%	n=0	ND	ND	n=0	**n=19/54 35.2%**
	I	n=2 4.4%	n=0	ND	ND	n=1 100%	**n=3/54 5.6%**
	R	n=24 53.3%	n=8 100%	ND	ND	n=0	**n=32/54 59.3%**
TSX	S	n=17 37.8%	n=4 50%	ND	ND	n=1 100%	**n=22/54 40.7%**
	I	n=5 11.1%	n=4 50%	ND	ND	n=0	**n=9/54 166.7%**
	R	n=23 51.1%	n=0	ND	ND	n=0	**n=23/54 42.6%**
CAF	S	n=19 42.2%	n=6 75%	n=3 100%	ND	n=0	**n=28/57 49.1%**
	I	n=13 28.9%	n=2 25%	n=0	ND	n=1 100%	**n=16/57 28.1%**
	R	n=13 28.9%	n=0	n=0	ND	n=0	**n=13/57 22.8%**
FOX	S	n=18 40%	n=0	ND	ND	n=0	**n=18/54 33.3%**
	I	n=1 2.2%	n=0	ND	ND	n=0	**n=1/54 1.85%**
	R	n=26 57.8%	n=8 100%	ND	ND	n=1 100%	**n=35/54 64.8%**
AZT/ER	S	n=11 24.2%	n=0	ND	n=1 100%	n=1 100%	**n=13/55 23.6%**
	I	n=3 6.7%	n=1 12.5%	ND	n=0	n=0	**n=4/55 7.3%**
	R	n=31 68.9%	n=7 87.5%	ND	n=0	n=0	**n=38/55 69.1%**
CD	S	n=19 42.2%	n=5 62.5%	ND	n=1 100%	n=1 100%	**n=26/55 47.3%**

	I	n=7 15.6%	n=0	ND	n=0	n=0	**n=7/55 12.7%**
	R	n=19 42.2%	n=3 37.5%	ND	n=0	ND	**n=22/55 40%**
PN	S	n=2 4.4%	n=0	n=2 66.7%	n=1 100%	n=0	**n=5/58 8.6%**
	I	n=0	n=0	n=0	n=0	n=1 100%	**n=1/58 1.72%**
	R	n=43 95.6%	n=8 100%	n=1 33.3%	n=0	n=0	**n=52/58 89.7%**
TTC	S	n=15 33.3%	n=0	n=0	n=0	n=0	**n=15/58 25.9%**
	I	n=2 4.4%	n=0	n=0	n=1 100%	n=0	**n=3/58 5.2%**
	R	n=28 62.2%	n=8 100%	n=3 100%	n=0	n=1 100%	**n=40/58 69%**
AMP	S	ND	ND	n=3 100%	n=1 100%	ND	**n=4/4 100%**
	I	ND	ND	n=0	n=0	ND	**n=0**
	R	ND	ND	n=0	n=0	ND	**n=0**
VAN	S	ND	ND	n=3 100%	ND	ND	**n=3/3 100%**
	I	ND	ND	n=0	ND	ND	**n=0**
	R	ND	ND	n=0	ND	ND	**n=0**
DN	S	ND	ND	ND	ND	n=1 100%	**n=1 100%**
	I	ND	ND	ND	ND	n=0	**n=0**
	R	ND	ND	ND	ND	n=0	**n=0**
OX	S	ND	ND	ND	ND	n=1 100%	**n=1 100%**
	I	ND	ND	ND	ND	n=0	**n=0**
	R	ND	ND	ND	ND	n=0	**n=0**

Key:CAF-chloramphenicole,GN-gentamycine,CIP-Ciprofloxacin,TSX-Trimethoprim /Sulfamethoxazole,FOX-cefoxitine,PN-peniciline,TTC-tetracycline,AZT-azithromycine,ER-erythromycine,CD-clindamycine,AMP-ampiciline,VAN-vancomycine,DN-doxycycline,OX-oxycycline,,ND =Not done

5.3.1 Multidrug-resistance pattern

This study revealed a high prevalence of MDR, affecting 82.7% (177/214) of the isolates. Among Gram-negative bacteria, MDR was exceptionally prevalent (84%, 131/156), with 100 % MDR rates observed in *Citrobacter spp.,* and *Proteus spp.* High MDR rates were also detected in *E. coli* (89.1%, 41/46) and *Klebsiella spp.* (94.6%, 53/56), while *Pseudomonas aeruginosa* showed a lower MDR rate (38.1%, 8/21). Gram-positive bacteria also exhibited a high MDR prevalence (79.3%, 46/58), with 100 % MDR rates observed in *S. lugdunensis* and *S. epidermidis. Staphylococcus aureus* showed a substantial MDR rate (82.2%, 37/45), while *Enterococcus* displayed a lower MDR rate (0%, 0/3) (Table 7). These findings highlight the significant challenge posed by MDR pathogens in this study population.

Table 7; Proportion of MDR bacteria from isolated bacterial species.

Gram negative Isolated bacteria	R_0 N (%)	R_1 N (%)	R_2 N (%)	R_3 N (%)	R_4 N (%)	R_5 N (%)	R_6 N (%)	R_7 N (%)	R_8 N (%)	R_9 N (%)	R_{10} N (%)	MDR N (%)
***Klebsiella spp*(56)**	0(0.0%)	1(1.8%)	2(3.6%)	6(10.7%)	10(17.9%)	13(23.2%)	18(32.1%)	6(10.7%)	0(0.0%)	0(0.0%)	0(0.0%)	**53(94.6%)**
***E. Coli* (46)**	0(0.0%)	0(0.0%)	5(10.9%)	8(17.4%)	17(37.0%)	12(26.1%)	4(8.7%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	**41(89.1%)**
***Acinetobacter spp* (22)**	0(0.0%)	0(0.0%)	2(9.1%)	2(9.1%)	5(22.7%)	8(36.4%)	5(22.7%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	**20(90.9%)**
***P. Aerugin osa* (21)**	2(9.5%)	5(23.8%)	6(28.6%)	3(14.3%)	1(4.8%)	4(19.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	**8(38.1%)**
***Enterobacter spp* (5)**	0(0.0%)	1(20.0%)	1(20.0%)	2(40.0%)	1(20.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	**3(60%)**
Citrobacter spp	0(0.0%)	0(0.0%)	0(0.0%)	1(25.0%)	1(25.0%)	0(0.0%)	0(0.0%)	2(50.0%)	0(0.0%)	0(0.0%)	0(0.0%)	**4(100%)**

	(4)											
	***Proteus spp* (2)**	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(50.0%)	0(0.0%)	1(50.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	**2(100%)**
Gram positive	***S. Aureus* (45)**	1(2.2%)	6(13.3%)	1(2.2%)	3(6.7%)	2(4.4%)	3(6.7%)	3(6.7%)	9(20.0%)	9(20.0%)	8(17.8%)	0(0.0%)	**37(82.2%)**
	***S. Lugdunensis* (8)**	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	2(25.0%)	4(50.0%)	2(25.0%)	0(0.0%)	0(0.0%)	**8(100%)**
	***Enterococcus spp* (3)**	0(0.0%)	2(66.7%)	1(33.3%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	**0(0%)**
	***S. Pyogenes* (1)**	0(0.0%)	1(100%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	**0(0%)**
	***S. Epidermidis* (1)**	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(100%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	**1(100%)**

6. Discussion

6.1 Bacterial profile and drug susceptibility pattern of isolates from SSI suspected patients

Postoperative SSIs remain a major cause of morbidity among surgical patients, significantly impacting patient outcomes. SSIs necessitate extended hospital stays, increased nursing care and wound management, potential readmissions, and the possibility of additional surgical interventions. Effective treatment hinges on accurate identification of the causative bacterial pathogens and the selection of appropriate, targeted antibiotics (37).

This study demonstrated a high rate (96.8%) of positive bacterial cultures among patients suspected of SSIs, yielding 163 isolates. This finding aligns with similar studies conducted in rural Uttarakhand, India (96.4%) (54), Western Rajasthan (86.3%) (55), Addis Ababa, Ethiopia (84.1%) (38).

However, it contrasts significantly with lower rates reported in other studies from Southwest Ethiopia, Mizan tepi (12.6 %) (57), jimma, Southwest Ethiopia, (71.7%) (37), Northern Jordan (60%) (58). These variations likely reflect differences in geographic location, study populations, microbiological techniques, and infection control practices across various healthcare settings.

This study found Gram-negative bacteria to be the predominant causative agents (71.8%) in postoperative SSIs, exceeding Gram-positive bacteria (28.2%), a finding consistent with previous researches (55, 57, 59-65). This predominance may be linked to the high proportion of abdominal surgeries in the study population, as Gram-negative bacteria are frequently associated with intra-abdominal infections (66).

The *Enterobacteriaceae* family, particularly *Klebsiella spp.,* emerged as the most common Gram-negative isolate, aligning with prior findings (37, 55, 63, 64, 67, 68), While *E. coli* was the

second most prevalent Gram-negative isolate in this study, other studies have reported it as the most frequent isolate (54, 64, 69).

Several studies have shown that *S. aureus* is the most prevalent isolate in SSI, those studies are conducted in Uttarakhand state, India, in Ethiopia, in lucy hospital, Kenya,(54, 55, 63, 69-71) in this investigation, *S. aureus* is still the second most isolated strain comparable to *E. coli.* Since *S. aureus* is a component of the skin and nasal flora, infections with it are most frequently linked to endogenous sources. Exogenous sources include contamination from the environment, surgical tools, or healthcare personnel's hands (72). Similarly, the common occurrence of *E. coli*, a component of normal intestinal flora, suggests an endogenous source, particularly given the high number of gastrointestinal procedures in the study population.

This study revealed high resistance rates among Gram-negative isolates to commonly used empirical antibiotics, including ceftazidime (74.4%), ceftriaxone (73.5%), and cefepime (64.1%). This highlights the significant challenge of treating these infections with frequently prescribed agents and underscores the need for evidence-based antibiotic prescribing guidelines. However, the study also identified amikacin (90.6%), meropenem (57.3%), and piperecilline tazobactam(48.7 %) as effective antibacterial agents against Gram-negative bacteria, consistent with previous findings (37, 54, 73)

Gram-positive isolates, particularly *Staphylococcus aureus*, demonstrated high resistance to penicillin (95.6%), azithromycine (68.9 %), and tetracycline (62.2%). Conversely Ciprofloxacin exhibited the highest efficacy (44.4%), closely followed by chloramphenicol, clindamycin, and gentamicin, all demonstrating similar levels of effectiveness (around 42%),aligning with previous research conducted on jimma (37). These results highlight the significance of selecting antibiotics according to local resistance patterns and precise pathogen identification.

6.2 Bacterial profile and drug susceptibility pattern of isolates from surgical ward environments

A large part (89.5%) of inanimate surfaces in the surgical ward showed bacterial contamination. CONS was found most often, making up 55.2 % (80/145) of all isolates, which matches earlier research in Gondar (74). *Staphylococcus species*, along with Gram-negative and Gram-positive rods, were commonly found, supporting results from previous studies (75, 76). These results highlight the need for strict cleaning protocols in surgical areas to reduce the chance of hospital-acquired infections.

The widespread occurrence of *Staphylococci species* in surgical ward settings is likely attributed to their presence in both healthy and sick individuals, enabling their spread through hand contact and respiratory droplets (77). As a result, their extraordinary capacity to endure for extended periods of time on dry, inadequately cleaned surfaces explains their existence in these settings (78).

Gram-positive bacteria significantly dominated the environmental isolates (79.3%), compared to Gram-negative bacteria (20.7%), consistent with a previous study conducted in University of Gondar Hospital, Northwest Ethiopia (74). The adult surgical ward showed the highest contamination rate (30.3%), followed by pediatric (25.5%) and orthopedic (23.4%) wards, with *CONS* consistently identified as the primary contaminant across all wards ,these results aligned with those from previous studies conducted on university of Gonder in Ethiopia and Kathmandu, Nepal (74, 79).

Gram-negative isolates demonstrated susceptibility to meropenem (70 %), gentamicin (50 %), amikacin (46.7%), ciprofloxacin (46.7 %), and chloramphenicol (46.7 %), while exhibiting high resistance to ceftriaxone (96.6 %), augmentin (89.7%), and ampicillin-sulbactam (82.8%). The other study conducted on Tikur anbesa specialized hospital (TASH) also found high resistance to ceftriaxone and ampicillin. However, our study indicates better susceptibility to meropenem,

gentamicin, amikacin, ciprofloxacin, and chloramphenicol compared to this study (48). These differences may stem from variations in study area. Our findings highlight alarming antibiotic resistance among bacterial isolates colonizing surgical ward surfaces, indicating a potential reservoir for nosocomial infections resistant to commonly used treatments within the institution.

6.3 Bacterial profile and drug susceptibility pattern of isolates from HCW hands

This study highlights a concerning rate of bacterial contamination on the hands of HCWs, with *CONs* detected on 73.3% of HCWs. This contamination likely arises from direct patient contact, exposure to bodily fluids, and contact with contaminated surfaces within the surgical ward environment. This prevalence is notably higher than that reported in a study from Iran (39.3%) (80). but surpasses the lower rate observed in India (17.8%) (81). The elevated contamination rate observed in this study may be attributed to inadequate disinfection practices and infrequent hand washing among HCWs, emphasizing the crucial need for improved hygiene protocols in healthcare settings.

While this study found a lower rate of bacterial contamination on healthcare workers' hands (75.4%) compared to studies in Gondar (90%) and Malaysia (97%) (74, 82). the findings still highlight a significant concern. The sample collection, conducted during routine medical procedures without strict standardization. This enables us to obtain a precise image of the amount of bacteria present on hands.

Although most isolates represented typical skin flora, a notable proportion (26.7%) contained bacteria associated with SSIs, including *S. aureus* (11.7%), *E. coli* (10%), and *Klebsiella spp*.

(5%). This aligns with a European study that, while dominated by *CONs*, also identified *Staphylococcus aureus* (10.5%) and Gram-negative rod (14.5%). These results emphasize the potential for HCWs to transmit HCAIs despite the prevalence of skin commensals (83).

This study identified a MDR Gram-negative bacterial prevalence of 100% among healthcare workers, This rate is significantly greater than a finding of 15.8% reported in a study at Democritus University of Thrace,in Greece (84). and what was reported by O'Fallon et al. in a US healthcare worker population (7.7%), highlighting the significant regional variation in MDR bacterial prevalence (85).

7. Strength and Limitation of the study

Our strength is the comprehensive picture of bacterial contamination in the surgical ward that was provided by the samples that were gathered from a variety of sources, including the environment, HCW's hands, and SSI patients. Our investigation was limited by the fact that all sample swabs taken from those three sites were not subjected to anaerobic bacterial cultures and molecular tests because there were insufficient microbiology lab facilities available during the study period. In this context, more prospective research can be conducted.

8. Conclusion

This study revealed a high prevalence of bacterial contamination across SSIs suspected patients, HCWs hands, and the surrounding environment. *Klebsiella* species, *Staphylococcus aureus*, and *Escherichia coli* were identified as the major contributors to postoperative SSIs. The study also highlighted significant antibiotic resistance among both Gram-positive and Gram-negative isolates, particularly to commonly used antibiotics like ceftriaxone, ampicillin, ceftazidime, penicillin, azithromycin, and tetracycline. Significant bacterial contamination was also detected on the hands of HCWs and various environmental surfaces within the surgical ward, with *CONS*

being a frequently identified contaminant. These findings highlight critical deficiencies in infection control practices, contributing to the spread of antibiotic-resistant bacteria.

9. Recommendation

Future research should explore the molecular traits of bacterial isolates to understand their virulence and resistance mechanisms. Studies on anaerobic bacteria are vital to completing the SSIs landscape. Additionally, investigating the clonal relationships between bacterial strains from SSIs, HCWs' hands, and surgical environments is crucial.

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10. APPENDICES

Annex I: Participants information sheet

English participant's information sheet

Title of the Research Project: Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, health care worker hands and the surrounding environment at surgical wards of SPHMMC, Addis Ababa, Ethiopia, 2024.

Principal investigator: Gelila Melaku (BSc, MSc candidate)

Name of the Organization: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

Introduction

I'm a student at Addis Ababa University studying medical laboratory science with a focus on diagnostic and public health microbiology. The objective of this research is to determine the Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, health care worker hands and the surrounding environment at surgical wards of SPHMMC, Addis Ababa, Ethiopia, 2024.

I thus ask that you grant me permission to take a swab from the wound and respond to the questions. The sample will undergo laboratory analysis to identify the underlying cause of the infection and potential treatment medications.

Your participation is voluntary

This permission form provides study details. You will be required to sign or mark this form once you have acknowledged receipt of the information and agreed to participate. Your hospital care will not be impacted if you choose not to participate in the study, and you are free to leave at any time.

Risk and discomfort

Except for a tiny touch from the cotton swab at the site of swabbing, there is no risk associated with taking the wound swab.

Benefits

You will receive information from the clinicians regarding wound infection prevention, and the wound infection will be successfully managed with the help of the findings.

Confidentiality

All efforts will be made to keep your personal information confidential.

Inquiries or questions

For any questions, inquiries or research related injury, please contact: Principal investigator, Gelila Melaku

Principal investigator address

Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Mobile: 0915 -95-92-82

Email: gelilamelaku773@gmail.com

Waraqaa odeeffannoo hirmaattotaa.

Mata duree Qorannoo: “Profaayilii baakteeriyaa fi haala saaxilamummaa qorichaaf baakteeriyaa infekshinii bakka baqaqsanii hodhuu, harka hojjetaa eegumsa fayyaa fi naannoo naannoo isaa irraa adda baafaman kutaalee baqaqsanii hodhuu SPHMMC, Addis Ababa, Ethiopia,2024.”

Qorataa mumme: Galiilaa Malaakuu (BSc, kaadhimamaa MSc).

Maqaa Dhaabbatichaa: Kutaa Saayinsii Laaboraatoorii Meedikaalaa, Kolleejjii Saayinsii Fayyaa, Yuunivarsiitii Addis Ababa

Seensa

Ani Yunivarsiitii Addis Ababaatti barattuu saayinsii laaboraatoori meedikaalaa yoon ta’uu xiyyeeffannon kiyyas maaykiroobaayoloojii fayyaa haawaasaa fi diyaagnoostikiidha. Kaayyoon qorannoo kanaas profaayilii baakteeriyaa fi haala saaxilamummaa isaan qorichaaf qaban baakteeriyoota infekshinii bakka baqaqsanii yaaluu, harka hojjetaa ogeessa fayyaa fi naannoo kutaalee baqaqsanii yaaluu hoospitaal qulqulluu phaawloos irraa addda baafanaman qo’achuudha. Haaluma kanaan bakka madaa irraa akkan saamuda fudhadhuu fi gaafilee aramaan gadiif akka deebii naaf laattan hayyama keessan isin gaafadha. Saamuda kana xiinxala laabraatoorii taasifamuun sababa bu’uuraa infekshinichaa fi qoricha wal’aansaaf ta’uu danda’u adda baasuun ni hojjetama.

Hirmaannaan keessan fedhiidhaan ta’a.

Unkaan hayyamaa kun ibsa qo’annoo ni kenna. Erga odeeffannoo gahaa argattanii fi hirmaachuuf eeyyamamaa taatan booda unka kana mallatteessuu ykn mallattoo kaa’uun isin irraa eegama. Yoo qorannoo kana irratti hirmaachuu dhiisuuf filattan yaaliin hospitaala keessatti isiniif taasifamu irratti dhiibbaa hin geessisu. Erga hirmaattannii booda yeroo barbaaddanitti qorannoo keessaa ba’uuf mirga qabdu.

Balaa fi miira namaa hin tolle

Tuqaatii xinnaa yeroo saamuda fidhatamuun ala jiruu irraa kan hafe, balaan saamuda fudhachuu wajjin walqabatu hin jiru.

Faayidaalee

Ittisa infekshinii madaa ilaalchisee ogeeyyii fayyaa irraa odeeffannoo ni argattu, raga qorannoo kana irraa argamuun yaaliin madaa keessanif taasifamu ni daaggarama.

Iccitii eeguu

Odeeffannoon dhuunfaa keessan iccitii akka ta'uuf carraaqqiin hundi ni taasifama.

Gaaffii

Gaaffii miidhaa qorannoo wajjin walqabatu kamiyyuu yoo qabaattan qorattuu mummee kan taate Galiilaa Malaakuu qunnamuu dandeessu.

Teessoo qorattuu mummee

Kutaa Saayinsii Laaboraatoorii Meedikaalaa, Koolleejjii Saayinsii Fayyaa, Yuunivarsiitii Addis Ababaa, Itiyoophiyaa.

Lakk. bilbilaa: 0915 -95-92-82

Email: gelilamelaku773@gmail.com

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

የጥናቱ ርዕስ: “የባክቴሪያ ፕሮፋይል እና የመድኃኒት ተጋላጭነት ሁኔታ ከቀዶ ሕክምና ቦታ ኢንፌክሽን ፣የጤና አጠባበቅ ሠራተኛ እጆች እና አካባቢው በ SPHMMC የቀዶ ጥገና ክፍሎች ፣ አዲስ አበባ ፣ ኢትዮጵያ ፣ 2024”

ዋና ተመራማሪ፡ ገሊላ መላኩ

የድርጅቱ ስም፡ በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና የላቦራቶሪ ሳይንስ ትምህርት ክፍል

መግቢያ

በአዲስ አበባ ዩኒቨርሲቲ የህብረተሰብ ጤና ማይክሮባዮሎጂ እና ምርመራ ላይ ትኩረት በማድረግ የህክምና ላብራቶሪ ሳይንስ በመማር ላይ እገኛለው፡፡

የዚህ ጥናት ዓላማ ከቀዶ ሕክምና ቁስል፣ ከጤና ባለሙያ እጆች እና በቅዱስ ጳውሎስ ሆስፒታል ቀዶ ጥገና ክፍል አከባቢዎች ላይ የሚገኙ የባክቴሪያ አይነቶች እና የመድኃኒት ተጋላጭነት ሁኔታን ማጥናት ነው፡፡ ስለዚህ ከቁስሉ ላይ በጥጥ ናሙና እንድወስድ እና ለጥያቄዎቹ ምላሽ ለመስጠት ፍቃደኛ እንዲሆኑ እጠይቃለሁ። ናሙናው የኢንፌክሽኑን ዋና መንስኤ እና ሊሆኑ የሚችሉ የሕክምና መድሃኒቶችን ለመለየት የላብራቶሪ ምርመራ ይደረግበታል.

የእርስዎ ተሳትፎ በፈቃደኝነት ላይ የተመሰረት ነው

ይህ የፍቃድ ቅጽ የጥናቱን ዝርዝር ሁኔታዎችን ያትታል። መረጃው እንደደረስዎት እውቅና ከሰጡ እና ለመሳተፍ ከተስማሙ በኋላ ይህን ቅጽ መፈረም ወይም ምልክት ማድረግ ይኖርቦታል። በጥናቱ ላይ ላለመሳተፍ ከመረጡ የሆስፒታል እንክብካቤዎ ላይ ተጽእኖ አይኖረውም ፤ እንዲሁም በማንኛውም ጊዜ ከጥናቱ መልቀቅ ይችላሉ፡፡

ስጋት እና ምቾት ማጣት

በጥጥ ጫፍ ከተሰራው ናሙና መቀባያ ከሚኖረው ትንሽ ንክኪ በስተቀር ከናሙና አወሳሰድ ጋር ተያይዞ ምንም አይነት አደጋ የለውም፡፡

ጥቅሞች

የቁስል ኢንፌክሽን መከላከልን በተመለከተ ከህክምና ባለሙያዎች መረጃ ያገኛሉ እና በግኝቶቹ መሰረት የቁስል ህክምናው በተሳካ ሁኔታ ይደረጋል፡፡

ሚስጥራዊነት

የግል መረጃዎን በሚስጥር ለመጠበቅ ሁሉም ጥረቶች ይደረጋሉ። ለማንኛውም ጥያቄዎችወይም ከምርምር ጋር የተያያዘ ጉዳት እባክዎን ዋና ተመራማሪ የሆኑትን ገሊላ መላኩን ያነጋግሩ

ዋና መርማሪ አድራሻ

የሕክምና የላቦራቶሪ ሳይንስ ትምህርት ክፍል, የጤና ሳይንስ ኮሌጅ, አዲስ አበባ ዩኒቨርሲቲ, አዲስ አበባ, ኢትዮጵያ፡፡

ስልክ ቁጥር: 0915 -95-92-82

ኢሜል፡ gelilamelaku773@gmail.com

Annex II: Informed Consent form

Statement of consent and signatures

I have been made aware of the following study plans: Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, health care worker hands and the surrounding environment at surgical wards of SPHMMC, Addis Ababa, Ethiopia, 2024

I received a brief explanation of the study's purpose and application. Additionally, I am told that all of the information on the questionnaire will be kept private. Furthermore, I've been made fully aware of my rights to withhold information, object to participation, and withdraw from the study at any time. None of these decisions will have an impact on my general health treatment.

I so freely consented to provide the researcher with my wound samples for the aforementioned study after fully comprehending the circumstances. I consented to the specimen's testing for antibiotic pattern and bacterial identification.

Thus, I, the undersigned, thus give my consent to take part in the research “Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, health care worker hands and the surrounding environment at surgical wards of SPHMMC, Addis Ababa, Ethiopia, 2024.

Participant code number ……

Date ……

Signature ……

For those who can’t read the information

Advisor health care worker name …….

Date……

Signature ……..

Ibsa hayyamaa fi mallattoo

Waa'ee qorannoo " Profaayilii baakteeriyaa fi haala saaxilamummaa qorichaaf baakteeriyaa infekshinii bakka baqaqsanii hodhuu, harka hojjetaa eegumsa fayyaa fi naannoo naannoo isaa irraa adda baafaman kutaalee baqaqsanii hodhuu SPHMMC, Addis Ababa, Ethiopia, 2024." irratti hubannoon naaf kennamee jira. irgairga odeeffannoo dhorkuu, hirmaannaa mormuu fi yeroo kamiyyuu qorannoo irraa bahuu koo guutummaatti akkan hubadhu taasifameera. Murtoowwan kun tokkollee wal'aansa fayyaa waliigalaa koo irratti dhiibbaa kan hin geessisne ta'uus hubadheera.

Haala jiru guutummaatti erga hubadhee booda, qorannoo armaan olitti ibsame kanaaf saamuda madaa kiyya irraa qorannoo kanaaf akka fudhatamuu hayyameera. Qorannoon saamuda kanaa akkaataa antibaayootikii fi adda baasuu baakteeriyaaf akka oolu hayyameera.

Haala kanaan, ani maqaan kiyya armaan gaditti kan eerame qorannoo "Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, health care worker hands and the surrounding environment at surgical wards of SPHMMC, Addis Ababa, Ethiopia, 2024."jedhu irratti hirmaachuuf eeyyama kiyya laadheera.

Koodi hirmaataa .....

Guyyaa ……

Mallattoo ……

Hirmaattoota odeeffannoo kana dubbisuu hin dandeenyeef

Ogeessa fayyaa gorsa laate: .....

Guyyaa……

Mallattoo ……..

የተሳታፊዎች ስምምት ማረጋገጫ

ስለ ‘‘የባክቴሪያ ፕሮፋይል እና የመድኃኒት ተጋላጭነት ሁኔታ ከቀዶ ሕክምና ቦታ ኢንፌክሽን ፣የጤና አጠባበቅ ሠራተኛ እጆች እና አካባቢው በ SPHMMC የቀዶ ጥገና ክፍሎች ፣ አዲስ አበባ ፣ ኢትዮጵያ ፣ 2024’’

ጥናት ላይ በቂ ገለጻ ተደርጎልኛል።

የጥናቱ ዓላማ እና አተገባበር አጭር ማብራሪያ ደረሰኝ። በተጨማሪም፣ በመጠይቁ ላይ ያሉት መረጃዎች በሙሉ በሚስጥር እንደሚጠበቁ ተነግሮኛል። በተጨማሪም፣ በማንኛውም ጊዜ መረጃን የመከልከል፣ ያለመሳተፍ እና ከጥናቱ የመውጣት መብቶቼን ሙሉ በሙሉ እንዳለኝም ተነግሮኛል። ከእነዚህ ውሳኔዎች ውስጥ አንዳቸውም በእኔ አጠቃላይ የጤና ህክምና ላይ ተጽዕኖ አይኖራቸውም።

ለተመራማሪው ሁኔታዎቹን ሙሉ በሙሉ ከተረዳሁ በኋላ ለተጠቀሰው ጥናት የቁስል ናሙናዬን ለመስጠት በነጻነት ተስማምቻለሁ። የናሙናውን የአንቲባዮቲክ ሁኔታ እና የባክቴሪያ አይነት መለያን ለመፈተሽ ለሚደረገው ምርመራ ተስማምቻለሁ።

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

የተሳታፊ መለያ ቁጥር .....

ቀን .....

ፊርማ .....

መረጃውን ማንበብ ለማይችሉ

አማካሪ ጤና ባለ ሙያ ስም .....

ቀን .....

ፊርማ .....

Annex III;- Information sheet for parents / guardians

Title of the Research Project: Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, health care worker hands and the surrounding environment at surgical wards of SPHMMC, Addis Ababa, Ethiopia, 2024.

Principal investigator: Gelila Melaku (BSc, MSc candidate)

Name of the Organization: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

Introduction

I'm a student at Addis Ababa University studying medical laboratory science with a focus on diagnostic and public health microbiology. The objective of this research is to determine the Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, health care worker hands and the surrounding environment at surgical wards of SPHMMC, Addis Ababa, Ethiopia, 2024.

I thus ask that you grant me permission to take a swab from the wound and respond to the questions. The sample will undergo laboratory analysis to identify the underlying cause of the infection and potential treatment medications.

Your participation is voluntary

This permission form provides study details. You will be required to sign or mark this form once you have acknowledged receipt of the information and agreed to participate. Your hospital care will not be impacted if you choose not to participate in the study, and once you are, you are free to leave at any time.

Risk and discomfort

Except for a tiny touch from the cotton swab at the site of swabbing, there is no risk associated with taking the wound swab.

Benefits

You will receive information from the clinicians regarding wound infection prevention, and the wound infection will be successfully managed with the help of the findings.

Confidentiality

All efforts will be made to keep your personal information confidential.

Inquiries or questions

For any questions, inquiries or research related injury, please contact: Principal Gelila Melaku

Principal investigator address

Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Mobile: 0915 -95-92-82

Email: gelilamelaku773@gmail.com

Waraqaa odeeffannoo warra / guddistootaaf

Mata duree Pirojektii Qorannoo: Profaayilii baakteeriyaa fi haala saaxilamummaa qorichaaf baakteeriyaa infekshinii bakka baqaqsanii hodhuu, harka hojjetaa eegumsa fayyaa fi naannoo naannoo isaa irraa adda baafaman kutaalee baqaqsanii hodhuu SPHMMC, Addis Ababa, Ethiopia, 2024.

Qorataa ijoo: Gelila Melaku (BSc, kaadhimamaa MSc)

Maqaa Dhaabbatichaa: Kutaa Saayinsii Laaboraatoorii Meedikaalaa, Kolleejjii Saayinsii Fayyaa, Yuunivarsiitii Addis Ababa

Seensa

Ani Yunivarsiitii Addis Ababaatti barattuu saayinsii laaboraatoori meedikaalaa yoon ta'uu xiyyeeffannon kiyyas maaykiroobaayoloojii fayyaa haawaasaa fi diyaagnoostikiidha. Kaayyoon qorannoo kanaas profaayilii baakteeriyaa fi haala saaxilamummaa isaan qorichaaf qaban baakteeriyoota infekshinii bakka baqaqsanii yaaluu, harka hojjetaa ogeessa fayyaa fi naannoo kutaalee baqaqsanii yaaluu hoospitaal qulqulluu phaawloos irraa addda baafanaman qo'achuudha. Haaluma kanaan bakka madaa irraa akkan saamuda fudhadhuu fi gaafilee aramaan gadiif akka deebii naaf laattan hayyama keessan isin gaafadha. Saamuda kana xiinxala laabraatoorii taasifamuun sababa bu'uuraa infekshinichaa fi qoricha wal'aansaaf ta'uu danda'u adda baasuun ni hojjetama.

Hirmaannaan keessan fedhiidhaan ta'a.

Unkaan hayyamaa kun ibsa qo'annoo ni kenna. Erga odeeffannoo gahaa argattanii fi hirmaachuuf eeyyamamaa taatan booda unka kana mallatteessuu ykn mallattoo kaa'uun isin irraa eegama. Yoo qorannoo kana irratti hirmaachuu dhiisuuf filattan yaaliin hospitaala keessatti isiniif taasifamu irratti dhiibbaa hin geessisu. Erga hirmaattannii booda yeroo barbaaddanitti qorannoo keessaa ba'uuf mirga qabdu.

Balaa fi miira namaa hin tolle

Tuqaatii xinnaa yeroo saamuda fidhatamuun ala jiruu irraa kan hafe, balaan saamuda fudhachuu wajjin walqabatu hin jiru.

Faayidaalee

Ittisa infekshinii madaa ilaalchisee ogeeyyii fayyaa irraa odeeffannoo ni argattu, raga qorannoo kana irraa argamuun yaaliin madaa keessanif taasifamu ni daaggarama.

Iccitii eeguu

Odeeffannoon dhuunfaa keessan iccitii akka ta'uuf carraaqqiin hundi ni taasifama.

Gaaffii

Gaaffii miidhaa qorannoo wajjin walqabatu kamiyyuu yoo qabaattan qorattuu mummee kan taate Galiilaa Malaakuu qunnamuu dandeessu.

Teessoo qorattuu mummee

Kutaa Saayinsii Laaboraatoorii Meedikaalaa, Koolleejjii Saayinsii Fayyaa, Yuunivarsiitii Addis Ababaa, Itiyoophiyaa.

Lakk. bilbilaa: 0915 -95-92-82

Email: gelilamelaku773@gmail.com

ለወላጆች/አሳዳጊዎች የመረጃ ወረቀት

የጥናቱ ርዕስ: ‘‘የባክቴሪያ ፕሮፋይል እና የመድኃኒት ተጋላጭነት ሁኔታ ከቀዶ ሕክምና ቦታ ኢንፌክሽን ፣የጤና አጠባበቅ ሠራተኛ እጆች እና አካባቢው በ SPHMMC የቀዶ ጥገና ክፍሎች ፣ አዲስ አበባ ፣ ኢትዮጵያ ፣ 2024’’

ዋና ተመራማሪ፡ ገሊላ መላኩ

የድርጅቱ ስም፡ በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና የላቦራቶሪ ሳይንስ ትምህርት ክፍል

መግቢያ

በአዲስ አበባ ዩኒቨርሲቲ የህብረተሰብ ጤና ማይክሮባዮሎጂ እና ምርመራ ላይ ትኩረት በማድረግ የህክምና ላብራቶሪ ሳይንስ በመማር ላይ እገኛለው፡፡

የዚህ ጥናት ዓላማ ከቀዶ ሕክምና ቁስል፣ ከጤና ባለሙያ እጆች እና በቅዱስ ጳውሎስ ሆስፒታል ቀዶ ጥገና ክፍል አከባቢዎች ላይ የሚገኙ የባክቴሪያ አይነቶች እና የመድኃኒት ተጋላጭነት ሁኔታን ማጥናት ነው፡፡ ስለዚህ ከቁስሉ ላይ በጥጥ ናሙና እንድወስድ እና ለጥያቄዎቹ ምላሽ ለመስጠት ፍቃደኛ እንዲሆኑ እጠይቃለሁ። ናሙናው የኢንፌክሽኑን ዋና መንስኤ እና ሊሆኑ የሚችሉ የሕክምና መድሃኒቶችን ለመለየት የላብራቶሪ ምርመራ ይደረግበታል.

የእርስዎ ተሳትፎ በፈቃደኝነት ላይ የተመሰረት ነው

ይህ የፍቃድ ቅጽ የጥናቱን ዝርዝር ሁኔታዎችን ያትታል። መረጃው እንደደረስዎት እውቅና ከሰጡ እና ለመሳተፍ ከተስማሙ በኋላ ይህን ቅጽ መፈረም ወይም ምልክት ማድረግ ይኖርቦታል። በጥናቱ ላይ ላለመሳተፍ ከመረጡ የሆስፒታል እንክብካቤዎ ላይ ተጽእኖ አይኖረውም ፤ እንዲሁም በማንኛውም ጊዜ ከጥናቱ መልቀቅ ይችላሉ፡፡

ስጋት እና ምቾት ማጣት

በጥጥ ጫፍ ከተሰራው ናሙና መቀባያ ከሚኖረው ትንሽ ንክኪ በስተቀር ከናሙና አወሳሰድ ጋር ተያይዞ ምንም አይነት አደጋ የለውም፡፡

ጥቅሞች

የቁስል ኢንፌክሽን መከላከልን በተመለከተ ከህክምና ባለሙያዎች መረጃ ያገኛሉ እና በግኝቶቹ መሰረት የቁስል ህክምናው በተሳካ ሁኔታ ይደረጋል፡፡

ሚስጥራዊነት

የግል መረጃዎን በሚስጥር ለመጠበቅ ሁሉም ጥረቶች ይደረጋሉ። ለማንኛውም ጥያቄዎችወይም ከምርምር ጋር የተያያዘ ጉዳት እባክዎን ዋና ተመራማሪ የሆኑትን ገሊላ መላኩን ያነጋግሩ

ዋና መርማሪ አድራሻ

የሕክምና የላቦራቶሪ ሳይንስ ትምህርት ክፍል, የጤና ሳይንስ ኮሌጅ, አዲስ አበባ ዩኒቨርሲቲ, አዲስ አበባ, ኢትዮጵያ፡፡

ስልክ ቁጥር: 0915 -95-92-82

ኢሜል፡ gelilamelaku773@gmail.com

Annex IV:- Assent form for parents /guardians for children less than 7 years old

I have been made aware of the following study plans: Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, health care worker hands and the surrounding environment at surgical wards of SPHMMC, Addis Ababa, Ethiopia, 2024

I received a brief explanation of the study's purpose and application. Additionally, I am told that all of the information on the questionnaire will be kept private. Furthermore, I've been made fully aware of my rights to withhold information, object to participation, and withdraw from the study at any time. None of these decisions will have an impact on my general health treatment.

I so freely consented to provide the researcher with my wound samples for the aforementioned study after fully comprehending the circumstances. I consented to the specimen's testing for antibiotic pattern and bacterial identification.

Thus, I, the undersigned, thus give my consent to take part in the research “Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, health care worker hands and the surrounding environment at surgical wards of SPHMMC, Addis Ababa, Ethiopia, 2024.

Signature ……

For those who can’t read the information

Advisor health care worker name …….

Date……

Signature ……..

Guardians/child code number _____

Guardians signature/fingerprint_____

date _____

Unka hayyamaa warra /guddistoota daa'imman waggaa 7 gadi ta'aniif

Waa'ee qorannoo " Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, health care worker hands and the surrounding environment at surgical wards of SPHMMC, Addis Ababa, Ethiopia, 2024." irratti hubannoon naaf kennamee jira. irgairga odeeffannoo dhorkuu, hirmaannaa mormuu fi yeroo kamiyyuu qorannoo irraa bahuu koo guutummaatti akkan hubadhu taasifameera. Murtoowwan kun tokkollee wal'aansa fayyaa waliigalaa koo irratti dhiibbaa kan hin geessisne ta'uus hubadheera.

Haala jiru guutummaatti erga hubadhee booda, qorannoo armaan olitti ibsame kanaaf saamuda madaa kiyya irraa qorannoo kanaaf akka fudhatamuu hayyameera. Qorannoon saamuda kanaa akkaataa antibaayootikii fi adda baasuu baakteeriyaaf akka oolu hayyameera.

Haala kanaan, ani maqaan kiyya armaan gaditti kan eerame qorannoo "Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical ssardsards of SPHMMC, Addis Ababa, Ethiopia, 2024."jedhu irratti hirmaachuuf eeyyama kiyya laadheera.

Koodi hirmaataa .....

Guyyaa ……

Mallattoo ……

Hirmaattoota odeeffannoo kana dubbisuu hin dandeenyeef

Ogeessa fayyaa gorsa laate: .....

Guyyaa……

Mallattoo ……..

ዕድሜያቸው ከ7 ዓመት በታች ለሆኑ ሕፃናት ለወላጆች/አሳዳጊዎች የማረጋገጫ ቅጽ

ስለ “የባክቴሪያ ፕሮፋይል እና የመድኃኒት ተጋላጭነት ሁኔታ ከቀዶ ሕክምና ቦታ ኢንፌክሽን ፣የጤና አጠባበቅ ሠራተኛ እጆች እና አካባቢው በ SPHMMC የቀዶ ጥገና ክፍሎች ፣ አዲስ አበባ ፣ ኢትዮጵያ ፣ 2024”

ጥናት ላይ በቂ ገለጻ ተደርጎልኛል።

የጥናቱ ዓላማ እና አተገባበር አጭር ማብራሪያ ደረሰኝ። በተጨማሪም፣ በመጠይቁ ላይ ያሉት መረጃዎች በሙሉ በሚስጥር እንደሚጠበቁ ተነግሮኛል። በተጨማሪም፣ በማንኛውም ጊዜ መረጃን የመከልከል፣ ያለመሳተፍ እና ከጥናቱ የመውጣት መብቶቼን ሙሉ በሙሉ እንዳለኝም ተነግሮኛል። ከእነዚህ ውሳኔዎች ውስጥ አንዳቸውም በእኔ አጠቃላይ የጤና ህክምና ላይ ተጽዕኖ አይኖራቸውም።

ለተመራማሪው ሁኔታዎቹን ሙሉ በሙሉ ከተረዳሁ በኋላ ለተጠቀሰው ጥናት የቁስል ናሙናዬን ለመስጠት በነጻነት ተስማምቻለሁ። የናሙናውን የአንቲባዮቲክ ሁኔታ እና የባክቴሪያ አይነት መለያን ለመፈተሽ ለሚደረገው ምርመራ ተስማምቻለሁ።

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

የተሳታፊ መለያ ቁጥር .....

ቀን .....

ፊርማ .....

መረጃውን ማንበብ ለማይችሉ

አማካሪ ጤና ባለ ሙያ ስም .....

ቀን .....

ፊርማ .....

Annex V;- Informed Assent form for children 7-18 years old

I am gelila melaku from Addis Ababa University. I am doing a study on Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, health care worker hands and the surrounding environment at surgical wards of SPHMMC, Addis ababa,Ethiopia ,2024.It is because of your parent's recommendation that we are requesting you to participate in this research project. We will interview you for this study and then collect a wound sample for analysis in the lab. We promise to keep all of your responses confidential; we won't share them with your instructor, friends, or parent(s)/guardian. They will only be seen by those working on the study.

Your participation is voluntary

This permission form provides study details. You will be required to sign or mark this form once you have acknowledged receipt of the information and agreed to participate. Your hospital care will not be impacted if you choose not to participate in the study, and once you are, you are free to leave at any time.

Risk and discomfort

Except for a tiny touch from the cotton swab at the site of swabbing, there is no risk associated with taking the wound swab.

Benefits

You will receive information from the clinicians regarding wound infection prevention, and the wound infection will be successfully managed with the help of the findings.

You should know that:

- You do not have to be in this study if you do not want to.
- You won’t get into any trouble with parent/guardian, your doctor, the school or me if you say no.
- You may stop being in the study at any time. [If there is a question you don’t want to answer, just leave it]

- Your parent[s]/guardian[s] were asked if it is OK for you to be in this study. Even if they say it's OK, it is still your choice whether or not to take part.
- You can ask any questions you have, now or later. If you think of a question later ,you or your parents can contact the following researchers or institution;

Confidentiality

All efforts will be made to keep your personal information confidential.

Sign this form only if you:

- Have understood what you will be doing for this study,
- Have had all your questions answered,
- Have talked to your parent[s]/legal guardian about this project, and
- Agree to take part in this research

Guardians /Child code number _____

Guardians Signature/fingerprint_____

Child Signature/fingerprint: _____

date_____

Unka Hayyama Odeeffannootiin Daa'imman waggaa 7-18 ta'aniif

Ani gelila melaku Yunivarsiitii Addis Ababa irraati. Kutaa baqaqsanii hodhuu SPHMMC, Addis ababa,Ethiopia ,2024 irratti Bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, harka hojjetaa eegumsa fayyaa fi naannoo naannoo isaa irratti qorannoo hojjechaa jira.Sababa gorsa warra keessaniiti akka nuti pirojektii qorannoo kana irratti akka hirmaattan isin gaafachaa jiru. Qorannoon kanaaf gaaffii fi deebii isiniif goona, sana booda saamuda madaa walitti qabnee mana yaalaa keessatti xiinxaluuf. Deebii keessan hunda iccitii akka ta'u waadaa seennaa; barsiisaa, hiriyoota, ykn warra (warra)/guddistuu keessaniif hin qoodnu. Isaanis warra qorannicha irratti hojjetan qofatu argatu.

Hirmaannaan keessan fedhiidhaan ta'a.

Unkaan hayyamaa kun ibsa qo'annoo ni kenna. Erga odeeffannoo gahaa argattanii fi hirmaachuuf eeyyamamaa taatan booda unka kana mallatteessuu ykn mallattoo kaa'uun isin irraa eegama. Yoo qorannoo kana irratti hirmaachuu dhiisuuf filattan yaaliin hospitaala keessatti isiniif taasifamu irratti dhiibbaa hin geessisu. Erga hirmaattannii booda yeroo barbaaddanitti qorannoo keessaa ba'uuf mirga qabdu.

Balaa fi miira namaa hin tolle

Tuqaatii xinnaa yeroo saamuda fidhatamuun ala jiruu irraa kan hafe, balaan saamuda fudhachuu wajjin walqabatu hin jiru.

Faayidaalee

Ittisa infekshinii madaa ilaalchisee ogeeyyii fayyaa irraa odeeffannoo ni argattu, raga qorannoo kana irraa argamuun yaaliin madaa keessanif taasifamu ni daaggaram.

Akkas beekuu qabda:

- Yoo hin barbaanne qo'annoo kana keessa ta'uun si hin barbaachisu.
- Yoo lakki jette warra/guddistuu, hakiima kee, mana barumsichaa ykn ana waliin rakkoo tokkollee hin seentu.
- Yeroo barbaaddetti qo'annoo keessa jiraachuu dhiisuu dandeessa. [Gaaffiin deebii kennuu hin barbaanne yoo jiraate dhiisii qofa]

- Warri kee/guddistoonni kee qorannoo kana keessatti argamuun kee sirrii ta’uu isaa gaafatamaniiru. Yoo tole jedhanii illee hirmaachuu fi dhiisuun filannoo kee ti.
- Gaaffii qabdan kamiyyuu ammas ta'e booda gaafachuu dandeessu. Booda gaaffii yoo yaadde ,ati ykn warri kee qorattoota ykn dhaabbata armaan gadii qunnamuu dandeessa;

Iccitii eeguu

Odeeffannoon dhuunfaa keessan iccitii akka ta’uuf carraaqqiin hundi ni taasifama.

Unka kana mallatteessi yoo:

- Qo’annoo kanaaf maal akka hojjettu hubatteetta,
- Gaaffiiwwan kee hundi deebii argatteetta,
- Waa’ee pirojektii kanaa warra[wwan]/guddistuu seeraa kee wajjin haasa’uu, fi
- Qorannoo kana irratti hirmaachuuf walii galuu

Eegdota /Lakkoofsa koodii daa'imaa _____ .

Eegdota Mallattoo/ashaaraa qubaa_____ .

Mallattoo/ashaaraa qubaa Mucaa: _____ .

guyyaa_____

ከ7-18 አመት ለሆኑ ህጻናት በመረጃ የተደገፈ የድጋፍ ቅጽ

ገሊላ መላኩ ነኝ ከአዲስ አበባ ዩኒቨርሲቲ። በባክቴርያ ፕሮፋይል እና የመድኃኒት ተጋላጭነት ጥለት ከቀዶ ሕክምና ቦታ ኢንፌክሽን፣የጤና አጠባበቅ ሠራተኛ እጅ እና አካባቢው አካባቢ በቀዶ ሕክምና ክፍል SPHMMC, Addis Ababa, Ethiopia ,2024 ጥናት እያደረግሁ ነው። በዚህ የምርምር ፕሮጀክት ላይ እንድትሳተፉ እየጠየቁ ነው። ለዚህ ጥናት ቃለ መጠይቅ እናደርግልዎታለን ከዚያም በቤተ ሙከራ ውስጥ ለመተንተን የቁስል ናሙና እንሰበስባለን. ሁሉንም ምላሾችዎን በሚስጥር ለመጠበቅ ቃል እንገባለን; ከአስተማሪህ፣ ከጓደኞችህ፣ ወይም ከወላጅ(ዎች)/አሳዳጊዎች ጋር አናጋራቸውም። በጥናቱ ላይ ለሚሰሩ ሰዎች ብቻ ይታያሉ.

የእርስዎ ተሳትፎ በፈቃደኝነት ነው።

ይህ የፍቃድ ቅጽ የጥናት ዝርዝሮችን ይሰጣል። መረጃው እንደደረሰህ እውቅና ከሰጠህ እና ለመሳተፍ ከተስማማህ በኋላ ይህን ቅጽ መፈረም ወይም ምልክት ማድረግ ይኖርብሃል። በጥናቱ ላይ ላለመሳተፍ ከመረጡ የሆስፒታል እንክብካቤዎ ላይ ተጽእኖ አይኖረውም, እና አንዴ ከሆናችሁ, በማንኛውም ጊዜ ለመልቀቅ ነጻ ይሆናሉ.

ስጋት እና ምቾት ማጣት

በጥጥ በተሰራው ቦታ ላይ ከጥጥ የተሰራውን ትንሽ ንክኪ በስተቀር, የቁስሉን እብጠት ከመውሰድ ጋር የተያያዘ ምንም አደጋ የለም.

ጥቅሞች

የቁስል ኢንፌክሽን መከላከልን በተመለከተ ከህክምና ባለሙያዎች መረጃ ያገኛሉ, እና የቁስሉ ኢንፌክሽን በግኝቶቹ እርዳታ በተሳካ ሁኔታ ይስተናገዳል.

ይህን ማወቅ አለብህ፡

- ካልፈለግክ በዚህ ጥናት ውስጥ መግባት የለብህም።
- አይሆንም ከተባለ ከወላጅ/አሳዳጊ፣ ከዶክተርዎ፣ ከትምህርት ቤቱ ወይም ከኔ ጋር ምንም አይነት ችግር ውስጥ አይገቡም።
- በማንኛውም ጊዜ በጥናቱ ውስጥ መሆንዎን ማቆም ይችላሉ። [መመለስ የማትፈልገው ጥያቄ ካለ ተወው]

- ወላጅዎ/አሳዳጊዎ በዚህ ጥናት ውስጥ መሆንዎ ምንም ችግር እንደሌለው ተጠይቀዋል። ምንም እንኳን ደህና ነው ቢሉም፣ መሳተፍ አለመሳተፍ አሁንም የእርስዎ ምርጫ ነው።
- ያለዎትን ማንኛውንም ጥያቄ አሁን ወይም በኋላ መጠየቅ ይችላሉ። በኋላ ላይ አንድ ጥያቄ ካሰቡ፣እርስዎ ወይም ወላጆችዎ የሚከተሉትን ተመራማሪዎች ወይም ተቋም ማግኘት ይችላሉ።

ሚስጥራዊነት

የግል መረጃዎን በሚስጥር ለመጠበቅ ሁሉም ጥረቶች ይደረጋሉ።

ይህንን ፎርም ከፈረሙ ብቻ፡-

- ለዚህ ጥናት ምን እንደምታደርጉ ተረድተዋል፣
- ለጥያቄዎችዎ ሁሉ መልስ አግኝተዋል፣
- ስለዚህ ፕሮጀክት ከወላጆችዎ/ህጋዊ አሳዳጊዎ ጋር ተነጋግረዋል፣ እና
- በዚህ ጥናት ውስጥ ለመሳተፍ ይስማሙ

አሳዳጊዎች/የልጆች ኮድ ቁጥር _____

የአሳዳጊዎች ፊርማ/አሻራ _____

የልጅ ፊርማ/የጣት አሻራ፡ _____

ቀን_____

Annex VI: Questioner English version

Code ……

Date…….

1. Gender: 1, Male 2,Female
2. Age in years 1,<20 2,20-39 3,40-59 4,60=/<
3. Residence(address)? 1, rural 2, urban
4. Surgical department in which patient admitted or attending…….
5. duration of surgery In min 1. 0-30 2. 31-60 3. 61-90 4. >91
6. Surgical procedure performed……
7. Type of surgery: (1) Clean surgery (2) Clean contaminated surgery (3) Contaminated surgery (4) Dirty surgery
8. Nature of surgery (1) Emergency surgery (2) Elective surgery
9. Antibiotic prophylaxis: (1) yes (2) no
10. If yes, type of the antibiotic prophylaxis given

(1) Ceftriaxone (2) Gentamycin (3) Metronidazole (4) Others, mention_____

11. Antibiotics prescribed ……
12. Postoperative antibiotics given with their respective quantity

A

B

C

D

Laboratory results record sheet

1. Gram staining result after culturing the bacteria (bacterial morphology and staining reaction)

   ……

   ……

2. Organisms isolated ……
3. Sensitivity pattern of isolated organisms ……..
4. Isolated organism……

List of anti-biotics					
Diameter					
Interpretation					

Gaaffii

Koodii .....

Guyyaa…….

1. Saala, Dhiira Dubartii
2. Umurii waggaadhaan
3. Bakka jireenyaa(teessoo)? 1, baadiyyaa 2, magaalaa
4. Kutaa baqaqsanii yaaluu dhukkubsataan itti seene ykn keessa ciisee jiru.....
5. yeroo baqaqsanii yaaliif itti fudhate Daqiiqaadhaan 1. <1hrs 2. 1-2hrs 3. 2-3 hrs 4. 3-4 hrs 5.>4hrs
6. Adeemsa baqaqsanii hodhuu raawwatame.....
7. Gosa baqaqsanii yaaluu: (1) Baqaqsanii yaaluu qulqulluu (2) Baqaqsanii yaaluu faalame qulqulleessuu (3) Baqaqsanii yaaluu faalame (4) Baqaqsanii yaaluu xuraa'aa
8. Haala baqaqsanii yaaluu (1) Baqaqsanii yaaluu hatattamaa (2) Baqaqsanii yaaluu filannoo
9. Ittisa antibaayootikii: (1) eeyyee (2) lakki
10. Yoo eeyyee ta'e gosa ittisa farra baakteeriyaa kenname

    1)Seeftriyaakson (2) Jeentaamaayisin (3) Meetroonidaazool (4) Kanneen biroo, kaasuun_____ .
11. Qorichoota farra baakteeriyaa ajajaman_____
12. Antibaayootikii baqaqsanii yaaluu boodaa kenname

    A

    B

    C

Laboratory results record sheet

1.Gram staining result after culturing the bacteria (bacterial morphology and staining reaction)

……

……

……

……

2.Biochemical test ----------.

3.Organisms isolated

……

…….

4.Sensitivity pattern of isolated organisms ……..……

5.Other remarks --------

List of anti-biotics					
Diameter					
Interpretation					

Questionnaire Amharic version (የአማረኛ መጠይቅ)

መለያ ቁጥር .....

ቀን.....

1. ጾታ 1, ወንድ 2,ሴት

2. እድሜ 1,<20 2,20-39 3,40-59 5,60=/<

3. መኖሪያ (አድራሻ)? 1, ገጠር 2, ከተማ

4. ታካሚ የተኛበት ክፍል .....

5. ቀዶ ጥገናው የወሰደው ጊዜ በሰዓታት 1) <1 ሰአት 2) 1-2 ሰአት 3) 2-3 ሰአት 4) 3-4 ሰአት 5)>4 ሰአት

6. የተሰራልዎ የቀዶ ጥገና አይነት.....

7. የቀዶ ጥገናው የብክለት መጠን ሀ) ንጹህ ቀዶ ጥገና ለ) ንጹህ የተበከለ ቀዶ ጥገና ቀዶ ጥገና ሐ) የተበከለ ቀዶ ጥገና መ) ቆሻሻ ቀዶ ጥገና

8. የቀዶ ጥገና ሁኔታ ሀ) አስቸኳይ ቀዶ ጥገና ለ) የታቀደ ቀዶ ጥገና

9. ከቀዶ ጥገና በፊት የተወሰደ መድሀኒት አለ ወይስ የለም ሀ) አለ ለ) የለም

10. ለጥያቄ ቁጥር 9 መልሱ አዎ ከሆነ የተሰጠው የመድሀኒት አይነት ሀ) ሴፍትሪያግዞን ለ) ጀንታማይሲን ሐ) ሜትሮኒዳዞል መ) ሌላ ከሆነ ይጠቀስ

11. አንቲባዮቲኮች የታዘዙ

12. ከቀዶ ጥገና በሁዋላ የተሰጡ መድሀኒቶች አይነት እና ብዛት

1,.....

2,.....

3,.....

4,.....

Laboratory results record sheet

1.Gram staining result after culturing the bacteria (bacterial morphology and staining reaction)

……

……

……

……

2. Biochemical test ----------.

3. Organisms isolated ……

4. Sensitivity pattern of isolated organisms ……..

5. Other remarks --------

List of anti-biotics					
Diameter					
Interpretation					

Annex VII ;- Environmental sample collection form

1. Source (object) where sample taken ----------.
2. Code number----------
3. Surgical ward ----------
4. Media used ----------
5. Gram staining result after culturing the bacteria (bacterial morphology and staining reaction)

……

6. Biochemical test ----------.
7. Organisms isolate……
8. Drug Sensitivity pattern of isolated organisms ……..
9. Other remarks --------

List of antibiotics					
Diameter					
Interpretation					

Annex VIII:Questionnaire for Health Care Workers

Hello! My name is Gelila Melaku. I'm studying for my second degree at Addis Ababa University. The objective of this research is to assess the bacterial profile and drug susceptibility pattern of bacterial isolates from surgical site infection, health care worker hands and the surrounding environment at surgical wards of SPHMMC,Addis Ababa, Ethiopia ,2024

You are in direct contact with the patient on daily work and this is why I am interested in determining the bacterial profile and drug susceptibility pattern of bacterial isolates from HCW hands. Data collected will be used for research purposes only. I requisite you to complete it anonymously. We would appreciate it if you answer all the questions and answer them as honestly as possible.

Do you agree? □ yes □ no

code: _____

Please circle on the number you select

NO	Socio-Demographic	Response
1	Sex	1,Female 2, Male
2	Age	1.20-30 2.30-40 3. 40-50 4. above 50
3	Profession	1. Nurse 2. Physician 3. student 4. Other (specify)_____
4	Level of qualification	1. MSc 2. Dr. 3. BSc 4. Diploma 5. Other (specify) ____
5	surgical ward	1. adult surgical ward 2. pediatric surgical ward 3. orthopaedic surgical ward 4. gynecology

		surgical ward 5. urology surgical ward

Laboratory results record sheet

1.Gram staining result after culturing the bacteria (bacterial morphology and staining reaction)

……

……

……

……

2. Biochemical test ----------.

3. Organisms isolated ……

4. Drug Sensitivity pattern of isolated organisms ……..

5. Other remarks --------

List of anti-biotics					
Diameter					
Interpretation					

Annex IX: Laboratory protocol

1. Identified micro-organisms

Gram positive bacteria

Bacteria	**Isolate [tick where applicable]**
Staphylococcus spp	
Streptococcus spp	
Enterococcus	
Micrococcs	
Bacillus spp	

Gram negative bacteria

Bacteria	**Isolate [tick where applicable]**
Pseudomonas aeruginosa	
Escherichia coli	
Klebsiellaspp	
Proteus spp	
Citrobacter spp	
Acinetobacter spp	
Enterobacter spp	

2. Antimicrobial sensitivity testing Disc diffusion method.

A disc of blotting paper is impregnated with known volume and appropriate concentration of an antimicrobial. The disc is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism.

The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to the edge of the disc. To ensure reproducibility and comparability of results, the modified Kirby-Bauer diffusion technique will be used.

Modified Kirby-Bauer susceptibility testing technique

A sterile medium were prepared according to the manufacturer's instructions. The PH of the medium were set at 7.2-7.4.The media were poured into a 90mm sterile petridish to a depth of 4mm [about 25ml per plate]. These were done on a level surface so that the depth of the medium is uniform. NB If the media is too thin the inhibition zone will be falsely large and if too thick the zones will be falsely small. Each new batch of agar was controlled using E. faecalis [ATCC 29212 or 33186] and cotrimoxazole disc. The zone of inhibition should be 20mm or more in diameter. The plates were stored at 2-8^{o}C in sealed plastic bags. For use the plates were dried with their lids slightly raised in 35-37^{o}C incubator for about 30minutes.About one hour before use, the working stock of the discs were allowed to warm to room temperature, protected from direct sunlight.

Method

1, Using a sterile wire loop, touch 3-5 well isolated colonies of similar appearance to the test organism and emulsify in 3-4ml of sterile physiological saline or nutrient broth.

2, Before streaking to the agar plate, the inoculum were adjusted to a turbidity equivalent to a 0.5 McFarland standard by placing the tubes in the DEN-1B densitometer.

3, Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by rotating and pressing the swab against the side of the tube above the level of the

suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 120^{o} to ensure even distribution.

4, With the petri dish lid in place, allow 3-5 minutes [no longer than 15minutes] for the surface of the agar to dry.

5, Using sterile forceps, needle mounted in a holder, or multidisc dispenser, place appropriate antimicrobial discs, evenly distributed on the inoculated plate. The discs should be 15mm from the edge of the plate and no closer than about 25mm from disc to disc. No more than eight discs will be applied on each petri dish. Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved in one place.

6, With in 30minutes of applying the discs, invert the plate and incubate it aerobically at37^{o}C for 24 hours.

7, After overnight incubation, examine the control and the test plates to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm. the endpoint of inhibition is where growth starts.

3. Characterizations

3.1 Gram stain

1. This were used to differentiate Gram positive [appears purple] and Gram negative [appears pink] bacteria. The following steps will be followed.
2. Fixing the dried smear by passing over a flame three times.
3. The fixed smear were covered with crystal violet for 30-60 seconds.
4. The stain were rapidly washed with clean water.
5. All the water were tipped off and the smear covered with grams iodine.
6. The iodine were washed with clean water.
7. The smear were decolorized rapidly [in a few seconds] with acetone alcohol, then washed with clean water.
8. The smear were covered with neutral red stain (safranin) for two minutes.
9. The stain were then be washed off with clean water.

10. The back of the slide were wiped clear and placed in a draining rack for the smear to air dry.

11. The smear were then be examined microscopically first with 40x objective to check the staining and see the distribution of materials and then in oil immersion objective to look for bacteria and cells.

3.2 Biochemical

1. Indole test

These were used to identify Enterobacteriaceae. Most strains of Enterobacteriaceae break down the amino acid tryptophan, releasing indole.

Method

Kovács Reagent Method (Tube Test):

Add Reagent:

After incubation, add Kovács reagent (isoamyl alcohol, para-dimethylamino benzaldehyde, concentrated hydrochloric acid) to the broth culture.

Observe for the development of a red or reddish-violet color in the surface alcohol layer, indicating a positive result (indole production). A yellow or orange color indicates a negative result.

2. Bile Esculin

Bile Esculin Agar (BEA) were used as a differential medium for the isolation and presumptive identification of group D streptococci and enterococci from clinical specimens.

Method

Enterococci & Streptococci (Group D)

1. The media were allowed to reach room temperature.
2. From a primary plate, suspect isolates of enterococci were picked.
3. Incubate aerobically at 35°C.
4. were Examined after 2-6 hours for esculin as a positive colonies.
5. Hold up to 24 hours before reporting as negative.

3. Catalase test

were used to differentiate the bacteria that produce the enzyme catalase such as staphylococci from non catalase producing bacteria such as streptococci.

Method

i. 2-3ml of hydrogen peroxide solution were poured into a test tube.

ii. Using a wooden stick or a glass rod several colonies of the test organism were removed and immersed in the hydrogen peroxide solution.

iii. Active bubbling indicates a positive catalase test.

4. **Coagulase test**

This test were used to identify *staphylococcus aureus* which produces coagulase. Both tube test and slide test will be employed.

Method Slide test [detects bound coagulase]

i. A drop of human plasma were placed on two separate slides.

ii. A colony of the test organism will be emulsified in each of the drops to make two thick suspensions.

iii. Clumping of the organisms were occur within 10 seconds if the organism is *Staphylococcus aureus*.

Tube test [detects free coagulase]

i. Plasma were diluted in the ratio of 1:10.

ii. Three small test tubes were availed and labeled; test organism, positive control and negative control.

iii. 0.5ml of the diluted plasma were pipetted into each tube.

iv. Five drops [about 0.1ml] of the test organism were added into the labeled positive and 5drops of the *Staphylococcus aureus* culture were added to the tube labeled positive and 5 drops of sterile broth in the tube labeled negative.

v. The tubes were incubated at 35-37C after mixing gently. Clotting were occur after one hour, if no clotting occurs after one hour examination were repeated after every30minutes for up to 6hours.

vi. Clotting is indicative of *Staphylococcus aureus.*

5. Oxidase test

This test was used to identify Pseudomonas spp.

Method

1] Apiece of oxidase impregnated paper were placed in a petri dish.

2] Using a piece of stick or glass rod, a colony of the test organism were then be smeared on the filter paper.

3] Development of blue- purple color within a few seconds indicates positive oxidase test.

6. Urease test.

The urease test detects bacteria's ability to hydrolyze urea using the enzyme urease, indicated by a color change from yellow to pink in a urea medium containing a pH indicator like phenol red. This test will be used to identify Proteus spp Method.

Urea Medium: Use a urea medium, which contains urea and the pH indicator phenol red. Urea medium can be in the form of agar slants or broth.

Inoculation: Inoculate the agar slant surface or broth with a well-isolated colony of the test organism. The color of the specimen changes from yellow (negative) to red (positive).

7. PYR Test

PYR test were used for the detection of pyrolidonyl arylamidase (also called pyrrolidonyl aminopeptidase) activity in *Streptococcus pyogenes* (group A strep), *Enterococcus* spp., some coagulase-negative staphylococci, and some *Enterobacteriaceae*.

Disk Method (Rapid)

1. Wet the PYR test disc on the strip with 10 µl sterile distilled water or deionized water. Note: Do not flood the disk.
2. Put 5-10 colonies of the tested strain from 18-24 hours culture on the surface of the disc with a loop and smear them lightly on it.
3. Incubate the disc for 1-2 minutes at room temperature.
4. After incubation, add 1 drop of N, N-dimethylaminocinnamaldehyde.
5. Observe for red color development within 1-2 minutes.

8. Bacitracin test

This test was used to identify Streptococcus pyogenes.

Method

i. Bacitracin disk were placed on a culture plate inoculated with the organism and incubated at 35-37°c overnight.

ii. A zone of inhibition around the disc was indicative of Streptococcus pyogenes.

9. **Triple Sugar Iron (TSI) Agar Slant**

Triple sugar iron agar is used for the differentiation of enteric pathogens by ability to determine carbohydrate fermentation and hydrogen sulphide production. using a sterile inoculating needle, stab the butt of the LIA slant twice then streak back and forth along the surface of the agar with the organism. Incubate at 370ºc for 18 to 24 hours. If acid slant–acid butt (yellow–yellow): glucose and sucrose and/or lactose fermented. If alkaline slant–acid butt (red–yellow): glucose fermented only. If alkaline slant–alkaline butt (red–red): glucose not fermented. The presence of black precipitate (butt) indicates hydrogen sulfide production, and presence of splits or cracks with air bubbles indicates gas production.

10. Citrate utilization test citrate using Simmon's agar

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the color change of a pH indicator. Pick a single isolated colony and lightly streak the surface of the slant, then incubated at 37o c aerobically for 18 to 48 hours. Blue color indicates a positive reaction and green color indicate negative reaction.

11. Lysine decarboxylase (LDC)

The acids produced by the bacteria from the fermentation of glucose will initially lower the pH of the medium and cause the pH indicator to change from purple to yellow. The

acid pH 56 activates the enzyme that causes decarboxylation of lysine to amines and the subsequent neutralization of the medium. This results in another color change from yellow back to purple. Bacteria that decarboxylate lysine turn the medium purple. In addition bacteria that produce H2S appear as black colonies.

12. Motility

Spreading of turbidity in the medium was a positive proof.

Antibiotic sensitivity table Bacteria……

Antibiotic	Resistant	Intermediate	Sensitive
Amoxicillin/ Clavulanate			
Ceftriaxone			
Imipenem			
Ciprofloxacin			
Cefoxitin			

DECLARATION

The undersigned declares that this thesis complies with the regulations of the University and meets the accepted standards with respect to originality and quality. PI also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

M.Sc. candidate: Gelila Melaku (B.Sc.)

Signature:_____

Date of submission:_____

This proposal has been submitted with our approval as advisor.

Advisor: Kassu Desta (MSc, PHD, Associate Professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor:- Asegedech Asmamaw (MSc, PhD fellow)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor:- Zenebe Gebreyohannes (MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.