



St. Paul's Hospital Millennium Medical College

Graduate study program

Bacterial Etiology, Antimicrobial Susceptibility Profile, and Associated Risk Factors of Surgical Site Infections and Blood Stream Infections at Asella Teaching and Referral Hospital, South East Arsi, Ethiopia: Prospective Cohort Study.

By: Mr.Bekele Dabi (BSc, M.Sc. candidate)

A Research thesis submitted to the Department of Microbiology, Immunology and Parasitology; St. Paul Hospital Millennium Medical College in Partial Fulfillment of the Requirements for the Degree Master of Science in Medical Microbiology.

Addis Ababa, Ethiopia

February, 2026



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Approval by the board of examination

As a member of the examining board of the final MSc open defense, we hereby certify that we have read and evaluated the thesis entitled _ Bacterial Etiology, Antimicrobial Susceptibility Profile, and Associated Risk Factors of Surgical Site Infections, and Blood Stream Infections at Asella Teaching and Referral Hospital, South East Arsi, Ethiopia: A prospective cohort study presented by Mr. Bekele Dabi. The thesis has been examined and approved in its present form as fulfilling the requirements for the degree of Master of Science in Medical Microbiology.

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This is to certify that the thesis prepared and presented by Mr. Bekele Dabi: Bacterial Etiology, Bacterial Etiology, Antimicrobial Susceptibility Profile, and Associated Risk Factors of Surgical Site Infections and Blood Stream Infections at Asella Teaching and Referral Hospital, South East Arsi, Ethiopia, and submitted in fulfillment of the requirement for the degree of Master of Science (MSc) in Medical Microbiology complies with the regulations of the college and meets the accepted standards with respect to originality and quality.

Name: Signature:.....

Declaration

I, the undersigned, declare that the thesis entitled Bacterial Etiology, Antimicrobial Susceptibility Profile, and Associated Risk Factors of Surgical Site Infections and Blood Stream Infections at Asella Teaching and Referral Hospital, South East Arsi, Ethiopia, submitted for the degree of Master of Science (MSc) to St.Paul's Hospital Millennium Medical College is my own work and has not previously been submitted at another university. The materials obtained from other sources have been duly acknowledged and cited their sources.

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ABBREVIATIONS/ACRONYMS

ATRH	Asella Teaching Referral Hospital
CoNs	Coagulase Negative Staphylococcus aureus
GBs	Group B streptococcus
EOS	Early-onset
LOS	Late-onset
USA	Unitied State of America
ICU	Intensive Care Unit
NICU	Neonatal Intensive Care Unit
AMR	Anti-Microbial Resistance
MDROs	Multi Drug Resistant Organisms
CDC's	Communicable Disease Controls
AIC	Africa Inland Church
NHS	National Health Service
HAI	Hospital Acquired Infections
SSI	Surgical Site Infection
BSI	Blood Stream Infection
HPA	Health Protection Agency
ESBL	Extended Spectrum Beta Lactamase
MRSA	Methicillin Resistance Staphylococcus aureus
MRI	Magnetic Resonance Imaging
VAC	Vacuum Assisted Closure
NPWT	Negative Pressure Wound Therapy

HBOT	Hyperbaric Oxygen Therapy
CLABSI	Central Line Associated Blood Stream Infection
VRE	Vancomycin Resistant Enterococci
CPO	Carbapenemase Producing Organisms
ESBL	Extended Spectrum Beta Lactamase
DOTS	Directly Observed Treatment Short Course
TB	Tuberculosis
SPSS	Statistical Packaging for Social Science
IRB	Institutional Review Board
SPHMMC	St. Paul's Hospital Millennium Medical College
WHO	World Health Organization

ABSTRACT

Background: Blood Stream Infections (BSIs) are defined as the presence of viable infectious microorganisms in the bloodstream causing clinical illness. Local and regional antimicrobial resistance (AMR) surveillance contributes to national efforts, which in turn support global strategies to combat AMR. A Surgical Site Infection (SSI) is defined as an infection occurring within 30 days after surgery involving the skin, superficial incision, deep soft tissue of the incision. Surgical site infection accounts for one of the common healthcare associated infections occurring both in developed, and developing countries. SSIs continue to be a major cause of postoperative morbidity and mortality despite improvements in aseptic techniques, particularly in settings with limited resources.

Objective: To determine bacterial etiology, antimicrobial susceptibility profile and associated risk factors of surgical site infections and blood stream infections at Asella Teaching and Referral Hospital, South East Arsi, Ethiopia.

Methods: Prospective cohort study design was conducted from June, 2024 to June, 2025. Overall a total of 314 post operative admitted patients from the obstetrics and gynecology and general surgical wards were included, through consecutive sampling technique. Wound swabs were collected aseptically on the first day when patients presented with clinical evidence of surgical site infections after surgery. Socio-demographic data and clinical samples (Swab and Blood) were collected by using pre-tested standardized questionnaires and aseptic techniques, respectively. Bacterial isolation, and identification were done based on the standard microbiological tests. Phenotypic antimicrobial susceptibility profiles were performed using the Kirby-Bauer disk method. Interpretation of the zone diameters was done following the Clinical and Laboratory Standards Institute guidelines CLSI guideline 2025. Data were entered into Epi-info 7, cleaned, exported to Excel 2016, and loaded into SPSS version 26 software for analysis. Descriptive statistics were presented in tables and bar charts, and multivariate regression were done to see any significant association. A p value of less than or equal to 0.05 was considered as the presence of significant association.

Results: Out of 314 study participants, 56.4% (n = 177/314) were female, and 43.6% (n=137/314) were male. From surgical ward were 56.7% (n=178/314), from gynecology and obstetrics wards were 43.3% (n=136), with a mean age of 36.1 years (SD $\pm$ 15.6). Overall identified SSIs patients

those who developed SSI presumptive were 16.6% (n=52/314), from this SSIs confirmed culture positive were 63.5% (n=33/52). Out of a total of culture positive SSI, 60.6% (n=20/33) were developed presumptive BSIs after the follow up. From these presumptive BSI 65% (n=13/20) were confirmed bacterial culture positive in blood culture. Gram-positive bacteria 51.5% (n=17/33), gram-negative were 48.5% (n=16/33).

The predominant pathogens were *Staphylococcus aureus* from wound swabs 30.3 % (n=10/33), followed by *Escherichia coli* from blood culture 18.2% (n=6/33), from wound swabs culture 9.1% (n=3/33), and a total of sum were 27.3% (n=9/33). *Staphylococcus aureus* were resistant to Ampicillin 10 (100%) and Gentamicin 4(75%), while gram-negative bacteria showed an overall resistance rate of 48.5% (n=16), with *E. coli* resistant to cefepime 79% (n=3) and ceftriaxone 98% (n=6). *Klebsiella spp* were resistant to *Cefepime* 7(80%) and *ceftriaxone* 7(100%). Similarly, *klebsiella pneumonia* were showed resistant to cefepime 2(80%) and *trimethoprim-sulfamethoxazole* 2(80%). Almost all identified gram-negative bacteria showed susceptible to *Meropenem*, *Imipenem*, *Ertapenem*, and *Amikacin*. Multidrug resistance was observed in 56% (n=14) of isolates, with 72% (n=18) resistant to more than three antimicrobial classes.

CBC parameters likes WBC, Lymphocyte, Neutrophil, Platelets for SSI and BSI Platelet were compared platelets were significantly $P<0.01$ associated with BSI. Emergency surgical procedure was statistically significant when compared to other independent variables of risk factors with ($P<0.05$), for blood stream infections, BSI, [(AOR=4.9, 95% CI (0.07338_7.9409); $p=0.047$). The multivariate analysis show that diabetes mellitus, and smoking cigarette, alcohol intake ($P<0.05$) so those variables were statistically significant for SSIs and BSIs [AOR=1.66, 95% CI (0.36_7.6)]; $P=0.01$, AOR=6.1, 95% CI (1.1_36.7); $P=0.04$, and AOR=6.0, 95% CI (1.1_31.90); $P=0.034$] respectively.

Conclusion and Recommendation: SSIs and BSIs were predominantly caused by *Staphylococcus aureus* 30.5% and *Escherichia coli* 27.3%, respectively. To assess SSI and BSIs incidence rates, bacterial profiles, and antibiotic susceptibility patterns.

Key words: Antimicrobial Susceptibility, Antibiotic Resistance, MDR, Surgical Site Infection, Blood stream infections, Ethiopia

1. INTRODUCTIONS

1.1. Background

A surgical site infection (SSI) is defined as an infection occurring within 30 days after surgery involving the skin and subcutaneous tissue of the incision (superficial incision) and/or deep soft tissue (eg, fascia, muscle) of the incision (deep incision) and/or any part of the anatomy (eg organs and spaces) other than an incision opened or processed during surgery (1). Surgical site infections and blood stream infection accounts for 20% of all infections associated with healthcare, making it a growing global public health concern (2). Developing countries suffered the most, likely because they lacked the infrastructure, personnel, and tools necessary to handle the operational cases (3).

Hospitalcare associated infections (HAIs) continue to be the most common cause of post-operative wound infections, despite the existence of other possible causes. The hospital setting affects how frequently these infections occur. Bloodstream infections (BSI) are infectious diseases defined by the presence of viable bacterial or fungal microorganisms in the bloodstream (later demonstrated by the positivity of one or more blood cultures) that have elicited an inflammatory response characterized by the alteration of clinical, laboratory and hemodynamic parameters. In this sense, the definitions of BSI and that of sepsis are 2 sides of the same phenomenon, since sepsis is an infectious syndrome triggered by an infectious disease, while BSI is a sepsis triggered by viable microorganisms circulating in the bloodstream. Of course, BSI can be preceded, followed or be concomitant to a localized infectious disease, like endocarditis, pneumonia, UTI, meningitis and others. The interest in focalizing on BSI, instead of infection in general, is in the diagnostic certainty inherent a positive blood culture, although contamination is possible (4, 5).

Depending on the nature of wound, post-operative infections are categorized as either superficial or deep wound infections. The type of operation done, antibiotic dosage and use, anesthesia techniques, and the nature of the infecting organism are some of the factors that affect these infections (6).

The bacteria that cause various diseases originate from the endogenous flora of the patient's skin and mucous membranes (7). *S.aureus*, *Enterobacteriaceae*, Coagulase Negative *Staphylococci* (CoNS), *Enterococci species*, *E. coli*, *K.pneumoniae* and *P. aeruginosa* are the bacterial pathogens

that have been isolated in these illnesses (8, 9). According to previous incidence studies, *P. aeruginosa* and *S. aureus* accounted for 5–15% and 20–40% of surgical site infections, respectively, with the variance reflecting temporal and geographic correlations (10, 11). Nosocomial infections and post-operative wound blood stream infection have remained a major cause of morbidity and death among the operated patients (12). It is usually associated with hospital and community acquired bacterial infections including intravenous catheterization. Most of these risk factors are preventable through early diagnosis and timely appropriate clinical management (11, 12, 13).

According to global data from systematic studies, septic shock strikes roughly 11 out of every 100,000 people each year, whereas severe BSI affects 13 to 300 persons per 100,000. Every year, BSI affects 31 million individuals worldwide; in Taiwan, there are 285 instances for every 100,000 hospital admissions (14, 15). The incidence of blood stream infection in adult intensive care unit patients varies from 6.3% to 53%, according to studies conducted in the USA, Germany, and the Netherlands (16).

Septic shock is a highly aggressive condition that can cause progressive organ failure but is considered reversible if identified and treated early(17). An estimated 24 million new cases of septic shock are reported annually by the World Health Organization (WHO), with the burden being much higher in low- and middle-income nations (18). Despite advances in understanding its pathophysiology, diagnosis, and treatment, the incidence and mortality of severe sepsis and septic shock have increased in recent decades (19).

Blood stream infection can extend hospital stays by up to 75%, partly due to its potential to cause organ failure (20, 21). A German study reported hospital mortality of 44.6% among septic shock patients, with another 19.1% dying within the first year post-diagnosis. The factors contributing to mortality according to the study included age, nosocomial origin of sepsis, diabetes, cerebrovascular disease, and renal replacement therapy (22). While most septic shock studies have been conducted in developed countries, it is more prevalent in developing nations where data remain scarce (23). Studies in Rwanda and Ethiopia report high mortality rates of 82.1% and 50.9%, respectively, among septic shock patients admitted to surgery (24, 25).

Surgical site infection and blood stream infections remain a significant cause of morbidity and mortality, with *Staphylococcus aureus* and *E.coli* being the most commonly isolated organisms, accounting for 20–40% and 5–15% of infections, respectively, varying by geography and time (26, 27).

Surgical site infections (SSIs) are notably prevalent in developing countries due to risk factors such as low adherence to safety protocols at patient, procedural, hospital, and surgical team levels resulting in SSI risks that are 2 to 20 times higher than in developed countries (28). The pooled prevalence of hospital-acquired infections (HAIs) globally is around 15.5%, with SSIs leading at 5.6%, figures that notably exceed those in Europe and the USA (39). In Africa, SSI incidence ranges widely from 2.5% to 30.9% and is associated with prolonged hospital stays, increased treatment needs, disability, and death (29, 30).

The causative pathogens usually originate from the patient's own skin and mucous flora but can also come from the hospital environment, including contaminated medical equipment (31, 32). Methicillin and vancomycin resistant *S. aureus* strains have been isolated, complicating treatment further (33).

In Ethiopia, although some studies report SSI , and BSI prevalence between 11.0% and 25.5% respectively, updated data on bacterial causes and antimicrobial susceptibility are scarce, making it difficult to design targeted prevention strategies (34). Therefore, this study conducted at Arsi University College of Health Science Asella Teaching and Referral Hospital, Ethiopia, was aimed to determine the bacterial profile and antimicrobial susceptibility patterns among patients diagnosed with SSIs, and BSI in general surgery and gynecology/obstetrics departments (35).

1.2. Statement of the problem

SSIs, and BSIs are among the most common and serious complications following surgical procedures globally, posing considerable challenges to patient safety and health system efficiency. SSIs and BSI contribute substantially to increased morbidity, prolonged hospital stays, escalated healthcare costs, and elevated mortality rates, particularly in resource-limited settings (36). In Ethiopia, SSIs remain a significant public health problem, with a pooled national prevalence estimated at approximately 12.3%, which is markedly higher than in many developed countries (12.3%; 95% CI: 10.2–14.4%). This high burden reflects several compounding factors including insufficient infection prevention measures, limited surgical infrastructure, variable antibiotic prophylaxis practices, and inadequate postoperative care (37).

Despite the critical importance of addressing SSIs and BSI there were a shortage of localized, comprehensive data on the incidence and etiological agents responsible for these infections within referral hospitals such as Asella Teaching and Referral Hospital (ATRH). The risk and pattern of SSIs can vary markedly by geographic location, hospital setting, and patient population. ATRH, serving a broad regional population in Oromia, is a key center for surgical care in Ethiopia, yet specific information regarding the burden of SSIs in this context remains scarce. Without these data, it is challenging to implement targeted infection prevention strategies and tailor clinical management to the local microbial ecology.

The bacterial profile of SSIs and BSI in Ethiopia were dominated by both Gram-positive and Gram-negative organisms, with *Staphylococcus aureus* and *Escherichia coli* frequently identified as the leading pathogens. Evidence from multicenter studies across Ethiopia indicates *S. aureus* accounts for roughly 30% of SSI isolates while *E. coli* constitutes approximately 20%, followed by other species including *Klebsiella pneumoniae* and *Coagulase-negative Staphylococci*. Yet, pathogen distribution can differ by hospital setting, patient demographics, and nature of surgical procedures performed (38).

One of the most pressing challenges in managing SSIs and BSI in Ethiopia were the increasing prevalence of antimicrobial resistance (AMR) among the bacterial pathogens involved. High rates of multidrug resistance (MDR), especially among *Enterobacteriaceae* such as *E. coli* and *Klebsiella species*, have been reported, with some studies documenting MDR frequencies exceeding 90% in hospital isolates. This alarming trend limits the effectiveness of standard empirical antibiotic regimens, complicates treatment, and increases the risk of poor clinical outcomes. Factors contributing to the rise in AMR include indiscriminate use of antibiotics, lack of antimicrobial stewardship programs, and insufficient microbiological diagnostic capacity for guiding therapy (39).

Several risk factors have been identified as significantly associated with the development of SSIs and BSI in Ethiopian hospitals. Patient-related risk factors include older age, and comorbidities such as diabetes mellitus. Prolonged hospital stay before surgery (≥ 24 hours), longer operation duration (≥ 2 hours), and emergency surgery status have shown strong associations with SSI occurrence. Other key risk factors are clean-contaminated or contaminated wounds, open surgical sites, and malnutrition. In obstetric surgeries such as cesarean sections, additional factors increasing SSI risk include membrane rupture of 12 hours or more, anemia (hemoglobin less than 11 mg/dL), rural residence, and inadequate or absent antibiotic prophylaxis (40).

In this context, regular surveillance of the bacterial pathogens causing SSIs and BSI, their antimicrobial susceptibility patterns is essential to inform evidence-based clinical and public health interventions. However, at ATRH, data on the incidence rate of SSIs and BSI the bacterial species most commonly implicated, and their resistance patterns are not systematically documented. This knowledge gap poses a significant obstacle to optimizing patient management and infection control policies at the hospital level. Furthermore, understanding local susceptibility patterns is critical for guiding rational antibiotic prophylaxis and treatment, thereby minimizing the emergence and spread of resistant strains (41).

Additionally, the absence of localized data on SSI and BSI incidence and microbial cause limits the hospital's ability to benchmark against national and international standards or to measure improvements following implementation of infection prevention interventions. Given the resource constraints typical in referral hospitals in Ethiopia, evidence generated from localized studies is invaluable for prioritizing allocation of resources and designing targeted training for healthcare workers to improve surgical outcomes.

Therefore, this research seeks to address these gaps by determining the incidence of surgical site infections, and blood stream infections at ATRH, characterizing the bacterial pathogens involved, and evaluating their antimicrobial susceptibility profiles. The findings of this study are expected to provide key baseline data to inform clinicians, hospital administrators, and policymakers for improving SSI and BSI surveillance, antibiotic stewardship, and infection prevention practices in this setting. Ultimately, by generating evidence specific to ATRH, this study aims to contribute to reducing SSI and BSI-related morbidity and improving surgical care quality in Ethiopia.

1.3. Significance of the study

This study is significant because it provides valuable data that enable healthcare professionals to make informed decisions about empirical antibiotic therapy, thereby improving treatment outcomes and minimizing the use of unnecessary broad-spectrum antibiotics. By identifying risk factors associated with surgical site infections (SSIs) and blood stream infection (BSI), the study supports healthcare facilities in implementing targeted interventions such as enhanced surgical practices, strict hand hygiene protocols, and robust antibiotic stewardship programs to reduce infection rates. Additionally, comparing local data with national trends helps determine whether SSIs and BSI represent isolated issues or broader challenges. Which facilitate real-time improvements in infection prevention. Overall, the information generated will assist decision-makers in updating local antibiotic prophylaxis guidelines and monitoring bacterial resistance through antibiograms, ultimately benefiting both patients and healthcare workers by promoting more effective infection control strategies.

2. LITERATURE REVIEW

2.1. Surgical Site Infection, and Blood Stream Infection

2.1.1. Incidence

In different parts of the world, varied proportions of SSI have been reported so far. A global, multicenter study across 66 countries including low-income, middle-income, and high-income countries indicated that the overall incidence of SSI was 12.3% (42). A systematic review and meta-analysis in sub-Saharan Africa indicated that the pooled incidence of BSI was 14.8% (43). In Ethiopia, a similar study revealed the overall incidence of SSI to be 21.1% (44). The incidence of SSI and BSI in these studies showed that the problem is still worsening in developing countries compared to developed countries. A study conducted in low- and middle-income countries showed another rationale for the increasing burden of SSI and BSI in wide areas of those regions as well as globally (45).

According to the CDC's National Nosocomial Infection Surveillance System, 38% of all nosocomial infections in surgical patients are surgical site infections(46). According to a 2011 study of acute care hospitals in the United States, surgical site infections are the number one cause of infection after pneumonia (24). The incidence of surgical site infections has varied widely worldwide, ranging from 2.6% to 58% (47). The United States alone reports 160,000–300,000 cases annually, with an annual cost of SSI ranging from \$3.5–10 billion (48).

On the other hand, a 2013-2014 report of 16 European countries by the European Center for Disease Prevention and Control showed that 967,191 operations resulted in 18,364 surgical site infections and blood stream infections (58). The report revealed that SSI rates range from 0.6% to 9.5% per 100 operations (49). In Kenya, the highest incidence of SSI (37.7%) was observed in Kenyatta National Hospital, while the lowest (5%) SSI was observed in Africa Inland Church (AIC) Kijabe Hospital after a sharp decrease of 9.3% (50).

SSI and BSI in surgery is significant and ranges from socioeconomic to physical and psychological effects on the patient and health care. These effects include depression, pain, loneliness, anxiety, immobility due to lack of physical activity, mortality and the huge debt that patients accumulate after additional hospital days can cause financial difficulties for some patients (24).

In addition, in six European countries - Germany, France, the United Kingdom, Italy, the Netherlands, and Spain, the impact of SSI and BSI on health care found that SSI and BSI were associated with increased health costs due to treatment, investigations, and hospital costs (25).

According to data from Ethiopia or from low- and middle-income countries, SSI was the most common cause of nosocomial infections (51), and in some cases, up to one third of surgical patients may develop SSI despite preoperative preparations and antibiotic prophylaxis is practiced (52).

BSIs are linked to high rates of morbidity and death. Over 1200,000 BSI episodes and 157,000 deaths were reported annually in Europe, while 575,000–677,000 BSI episodes and 79,000–94,000 deaths were reported annually in North America. Every organ in the body is at risk from microorganisms in circulating blood, whether they are present continually, sporadically, or momentarily. Additionally, organ failure and sepsis are potentially fatal consequences of BSI. It has been demonstrated that in patients with BSIs, early diagnosis and timely delivery of the proper antibiotic medication enhance patient outcomes and lower mortality. The most reliable method for diagnosing BSIs is blood culture. Microorganisms' prevalence and susceptibility to antibiotics differ by geographic location and are impacted by things like patterns of antibiotic use. Multidrug-resistant organisms (MDROs) have emerged and spread due in large part to the overuse and inappropriate use of antibiotics. Lengthening hospital stays, raising mortality rates, and necessitating the administration of more costly antibiotics. Antibiotic resistance is one of the top 10 health risks, according to the World Health Organization. Research from Nepal has also shown that BSI pathogens are becoming more resistant (81, 82, 87, 98).

Blood culture is the gold standard test for the diagnosis of BSIs. The prevalence and antimicrobial susceptibility of microorganisms vary by geographical region and are influenced by factors such as antibiotic usage patterns. Over the years, the excessive and irrational use of antibiotics has been widely recognized as a major contributor to the emergence and spread of multidrug-resistant organisms (MDROs). The rising trend of antibiotic resistance has led to limited treatment options, increasing the likelihood of inadequate therapy. Moreover, infections caused by MDROs worsen

clinical outcomes by prolonging hospital stays, increasing the risk of death, and requiring the use of more expensive antibiotics. World Health Organization has identified antibiotic resistance as one of the top ten health threats. Studies from Nepal have also reported increasing resistance among BSI pathogens. Accurate and up-to-date information on the microbiological and antimicrobial susceptibility profiles linked to BSIs is crucial for directing the right course of treatment and enhancing patient outcomes. The 10-year prevalence of bacteremia in the current study was 12.1%, which is in line with earlier research from Nepal that reported rates ranging from 10.3 to 13.5%. Similar and varying prevalence rates were observed by studies from nearby developing nations, such as 13.6% in Bangladesh and 14.2–17.1% in India (101,102,107).

2.2. Bacteria Etiology of SSI, and BSI

Most studies show that the distribution of pathogens isolated from SSIs had been not changed significantly over the last decade. *Staphylococcus aureus*, coagulase-negative *staphylococci*, *Enterococcus species*, and *Escherichia coli* were still the most commonly isolated bacteria. A large body of evidence indicates that an increasing proportion of SSIs were caused by multidrug-resistant bacteria, *methicillin-resistant Staphylococcus aureus*, and ESBL producers (26). A National Health Service (NHS) England hospital surveillance report (2012) shows that *Staphylococcus aureus* isolates from orthopedic and spine surgeries account for more than 36% of infections. However, *coagulase-negative staphylococci* and *enterobacteriae* predominate in patients who will undergo cardiac bypass and colonic surgical procedures. *Staphylococcus aureus* also was account for SSIs among patients admitted due to the decline of MRSA (2).

In a separate report by the Health Protection Agency (England), *Enterobacteriaceae* was accounted for all bacteria, followed by *Staphylococcus aureus*, MRSA all *Staphylococcus species*, and *Staphylococcus aureus*. In gastrointestinal procedures, poly antimicrobials was accounted for orthopedic, while monomicrobials were generally high in clean surgery (3). In addition, several researchers was found that *Staphylococcus aureus* becomes the dominant SSI bacterium, followed by *Pseudomonas aeruginosa*, *E.coli*, *Klebsiella*, *Citrobacter*, and *Acinetobacter species*(53). However, in a separate project, the most common SSI isolate will be found to be *Pseudomonas species*, followed by *Klebsiella species* (5). According to various reports by researchers in Nigeria, India, Switzerland, and Greece, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*,

Enterococcus, *Pseudomonas aeruginosa*, and *staphylococci coagulase-negative* are the most common isolates in surgical site infections (54).

Studies by other researchers in two different countries indicate that SSI is also caused by anaerobic bacteria such as *Bacteroides fragilis*, *Clostridium perfringens* and atypical pathogens such as *Mycoplasma hominis* and *Urea plasma species*(7). On the other hand, a study of SSI and BSI pathogens in Ethiopia was found an overall bacterial prevalence of *S. aureus* and *E. coli* as the most common bacterial isolates (55).

In a separate analysis of postoperative wound infection in Ethiopia, 111 pathogenic bacteria was isolated from clinical swab samples, dominated by *staphylococcal species* and gram-negative *E. coli*. In this analysis, coagulase-negative *staphylococci* was isolated commonly, followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* from different locations in the hospital environment. During the project, MRSA levels was recorded, and clinical and environmental samples, respectively(56). The predominant bacterial isolates in SSIs and BSI in Tanzania and Kenya were caused by gram-positive *enterococci* and *Staphylococcus aureus*(57).

In Ethiopia, the most common isolated organism for SSI and BSI were *Staphylococcus aureus* (*S. aureus* (30.4%), *coagulase-negative staphylococcus* (*CONS*) (11.7%), *E. coli* (9.4%), *E. faecalis* (5.9%), *Pseudomonas aeruginosa* (5.5%), *Enterobacter species* (4.0%) and *Klebsiella species* (4.0%) were also isolated (58).

2.3. Antibiotic resistance Bacterial Causing SSIs

The incidence of SSI has increased significantly due to the emergence of multidrug-resistant pathogens and methicillin-resistant *Staphylococcus aureus* (59). Moreover, the work of another Nepalese researcher showed that 64 (66.67%) of the bacteria were resistant to multiple drugs, while 26 (27.08%) of the isolates were resistant to a single antibiotic, and only 6 (6, 25%) of the isolates. It was resistant to one antibiotic and sensitive to all tested antibiotics. Variable resistance of Gram-negative rods and Gram-positive bacterial isolates to broad-spectrum antibiotics such as cephalosporin, chloramphenicol, and penicillin was observed in several studies (60).

2.4. Risk factors of SSI, and BSIs

Depending on the related comorbidity, different factors affect SSI and BSI in different patients. These characteristics are extrinsic (associated to the technique) and intrinsic (connected to the patient). Internal variables include both non-modifiable characteristics like advanced age and modifiable ones like obesity, excessive alcohol consumption, smoking, and immunosuppressive disorders. Inadequate skin preparation, poor sterilization, improper hair removal, contaminated instruments, the presence of foreign materials (implants, drains, catheters, and urinary/nasogastric tubes), the length of the procedure, previous colonization with resistant bacteria, and the hospital environment are examples of external variables. Intraoperative variables (hypothermia, hypoxia, stress-induced hyperglycemia, shock, heavy bleeding, major blood transfusions, hypovolemia (low blood volume), arterial lines, prolonged mechanical breathing, and abnormal fluid accumulations (hematoma). Factors related to the health system, such as poor surgical skill, non-compliance with infection prevention recommendations, or insufficient antibiotic prophylaxis (timing/choice) (61).

2.4.1. Cigarette smoking

Due to the thousands of harmful chemicals like nicotine, tar, and carbon monoxide that harm almost every organ and impair bodily functions, smoking cigarettes is a major risk factor for many serious health issues, including heart disease, stroke, various cancers (lung, mouth, throat, etc.), Chronic Obstructive Pulmonary Disease (COPD), diabetes complications (vision loss, kidney failure), and weakened immunity. A vicious cycle of increasing health concerns, from early aging to serious organ damage and early death, is created by the addictive nature of nicotine, which encourages ongoing usage. Cigarette smoke contains nicotine, a vasoconstrictor that hindered wound healing by lowering blood flow to the surgical site. The inflammatory cascade may be attenuated as a result of reduced blood supply to injured tissues (62).

2.4.2. Gender

Gender is a significant factor in the development of postoperative site infection in surgical patients, according to a few study. Sex hormones like estrogen and testosterone are associated with this component. By altering the genes in charge of inflammation and epithelium regeneration, they prevent wounds from healing. Gender risk factors include biological differences (e.g., hormonal

changes during menopause affecting heart health or autoimmune risk) and sociocultural factors (e.g., stereotypes influencing career choices, increased risk of gender-based violence in patriarchal systems, and different health-seeking behaviors) that disproportionately or uniquely affect men and women, affecting opportunities, safety, and health. Heart disease, autoimmune diseases, mental health issues (such as depression in women), obesity, and social violence are important issues that are frequently connected to negative stereotypes and economic inequality (63).

2.4.3. Age

Research suggests that following surgery, the elderly and young were more susceptible to SSI. Age-related risk factors for infection in surgical patients have not been thoroughly studied. However, a review of factors influencing the healing process of wounds in the old revealed that altered inflammatory responses cause wound infections in the elderly. Heart disease, diabetes, obesity, and dementia are among the many conditions that increase with age. These conditions are often caused by changes in the body (less effective cholesterol removal, decreased muscle) and lifestyle factors (poor diet, sedentary habits), which are exacerbated by stress and social isolation. However, controlling diet, exercise, sleep, and stress can reduce these risks and encourage healthy aging. Chronic illnesses (CVD, diabetes), cognitive decline (dementia), obesity, and social isolation are major age-related concerns; unhealthy behaviors (smoking, inactivity) exacerbate these conditions (64).

2.4.4. Comorbidities

It was shown that conditions including diabetes and anemia, which impair immunity, were linked to an increased incidence of SSI. This is due to the fact that people with these illnesses have compromised immunity, making it more difficult for the body to successfully eradicate the infection at the location of the wound. Genetics (primary), chronic diseases (diabetes, cancer), infections (HIV), malnutrition, aging, drugs (steroids, chemotherapy), and lifestyle choices (smoking, alcohol) all weaken the immune system and cause immunosuppressive conditions. On the other hand, factors like being female, having a family history, and certain infections increase the risk of autoimmune problems, which frequently call for immune-dampening treatments (65).

2.4.5. Diabetes Mellitus

Surgery is severely impacted by chronic metabolic diabetes mellitus. An important part of this effect is the complex connection between diabetes and surgical site infections (SSI). Diabetes has a major impact on wound healing because of compromised immunological responses, reduced collagen production, and poor microvascular circulation. SSI is more common in diabetic patients because to peripheral vascular disease, obesity, neuropathy, and hyperglycemia. Bacterial infections, such as those caused by *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus* species, are often the cause of SSI in diabetics. Procalcitonin and C-reactive protein are two important biomarkers that this analysis identifies as indicators for SSI in diabetics. These infections require immediate and customized treatment because of their severe consequences, which include deep tissue involvement, bloodstream infection, and extended hospitalization. Medical experts that focus on both diabetes control and SSI prevention may enhance the outcome for diabetic patients having surgery. This is because both illnesses place a heavy load on both patients and the medical system (66).

2.4.6. Hypertension

In addition to diabetes and obesity, hypertension is a major, independent, and frequently controllable risk factor for bloodstream infections (BSI) and surgical site infections (SSI). It raises the danger by interfering with immunological response, tissue perfusion, and, if left unchecked, organ damage. The main causes of hypertension, which in turn raises the risk of infection, are as follows, per epidemiological research and health recommendations: Physical inactivity: A sedentary lifestyle is closely linked to the onset of hypertension. Obesity/Abdominal Fat: Because adipose tissue can reduce tissue oxygenation and create the perfect conditions for SSI, high body mass index (BMI) and increased abdominal circumference are significant contributors. Low consumption of fruits and vegetables and a high intake of salt are examples of dietary habits. Family History: Having close ancestors with high blood pressure and a genetic tendency.

Smoking and excessive alcohol use are examples of lifestyle behaviors. Stress: Both both emotional and work-related stress. One important independent risk factor for the development of SSI has been found to be hypertension. Perioperative Complications: A history of hypertension is

a significant, unique risk factor for post-operative infection in spine surgery. **Tissue Hypoxia:** Poor tissue perfusion and oxygenation are caused by uncontrolled hypertension and related vascular disorders, which impedes healing and raises the risk of bacterial infection. **Immune Dysfunction:** Patients with comorbid conditions linked to hypertension, such as diabetes or chronic kidney disease, may have compromised immune systems, which increases their risk of developing bacteremia. Chronic high blood pressure can harm blood arteries, creating focal sites or entry locations for infections. SSI risk can be decreased by controlling blood pressure to desired values (<140/90 mmHg) prior to surgery (67).

2.5. Management, and Treatment

Management Principles: The "4 C's of wound care" are essential: Cleanse (remove debris), Control (manage bleeding/infection), Cover (protect with dressings), and Check (regular monitoring). Pain management with analgesia is also crucial during dressing changes. **Cleaning/Irrigation:** Gentle cleansing with saline solution to remove debris, often using pressurized irrigation (approx. 8 psi) to avoid damaging tissue. **Debridement:** Removal of dead, damaged, or infected tissue via surgical (scalpel), enzymatic (gels), or sharp methods to facilitate healing. **Dressings & Bandages:** Maintaining a moist, protected wound bed is critical. Common types include alginates (for high exudate), foams (for moderate exudate), and hydrogels (for dry, sloughy wounds). **Infection Control:** Use of topical antibiotics, antiseptic agents, or systemic antibiotics to manage bioburden. **Pressure Management (Offloading):** Using special shoes, casts, or cushions to reduce pressure on foot or pressure ulcers. **Advanced Wound Care Therapies**
Negative Pressure Wound Therapy (NPWT): A vacuum dressing system that pulls out fluid and reduces infection. **Hyperbaric Oxygen Therapy (HBOT):** Breathing 100% oxygen in a pressurized chamber to enhance healing in chronic wounds. **Biological dressings:** Use of maggots (larval therapy) or skin substitutes for severe or chronic non-healing wounds. **Compression Therapy:** Essential for venous leg ulcers to improve blood flow (68).

2.6. SSI and BSI Case Definitions (CDC/NHSN Criteria)

Superficial Incisional SSI: Occurs within 30 days, involves only skin and subcutaneous tissue. Must include at least one of: purulent drainage, organisms from fluid/tissue, or symptoms (pain, swelling, heat) with the incision opened by a surgeon.

Deep Incisional SSI: Occurs within 30-90 days, involves deep soft tissues (fascia/muscle). Includes purulent drainage from the deep incision, spontaneous dehiscence, or an abscess/evidence of infection on imaging or direct exam.

Organ/ Space SSI: Occurs within 30-90 days, involves any part of the anatomy (organs/spaces) opened or manipulated during the procedure.

Lab confirmed BSIs can be classified as primary or secondary to an infection at another body site. A primary laboratory-confirmed BSI in an admitted patient with a central line in place for greater than 2 calendar days and onset occurred during their critical care+ stay or CLABSI occurred on the day of transfer or the next day after transfer out of critical care+ OR 2. A Primary or Secondary laboratory-confirmed antibiotic-resistant organism (MRSA, VRE, CPO or ESBL) from a blood culture in an admitted patient. Inclusion criteria. BSI case identified in the emergency department in patients who are subsequently admitted to a facility under surveillance; this includes patients who may be transferred from one emergency department to another emergency department. All BSIs positive for MRSA, VRE, and CPO identified while admitted to a facility under surveillance. Hospital acquired BSIs with ESBL identified while admitted to a facility under surveillance. CLABSI attributed to adult and pediatric critical care. CLABSI is already present on admission to critical care+ Blood cultures with only a viral pathogen. BSI with an antibiotic-resistant organism (69).

If the same organism is isolated from a subsequent blood culture. Relapse if less than or equal to 10 days from a negative culture or less than or equal to 10 days from completion of appropriate antibiotic therapy, consider as a relapse and do not report. New: If greater than 10 days from a negative culture (if culture was done) and Greater than 10 days from completion of appropriate antibiotic therapy, report as a new infection. If multiple organisms are identified in a blood culture, report all organisms as one New BSI record, unless one of the organisms is a secondary BSI, then

report both as Primary and Secondary BSI. If a subsequent blood culture is identified with different organisms than the initial New BSI record entered, report as a New BSI record (70).

Surgical wounds are classified by the CDC into four categories based on infection risk, ranging from Class I (Clean) to Class IV (Dirty). This system predicts risk of SSI. Classification is usually determined at the end of a procedure, accounting for contamination levels and tissue involvement. Clean: No inflammation, no hollow viscus entered

Class I - Clean (0–2% infection risk): Uninfected, closed wounds without inflammation. They do not enter the respiratory, alimentary, genital, or urinary tracts. Examples: Thyroidectomy, hernia repair.

Class II - Clean-Contaminated (<10% infection risk): Operative wounds in which the respiratory, alimentary, genital, or urinary tracts are entered under controlled conditions without unusual contamination. Examples: Appendectomy, cholecystectomy.

Class III - Contaminated (<20% infection risk): Open, fresh, accidental wounds, or surgical wounds with major breaks in sterile technique or gross spillage from the gastrointestinal tract. Examples: Open wound, bile spillage, and penetrating injury.

Class IV - Dirty-Infected (>20% infection risk): Old traumatic wounds with retained devitalized tissue, or wounds involving existing clinical infection or perforated viscera. Examples: Abscess drainage, fecal contamination.

Conceptual frame work

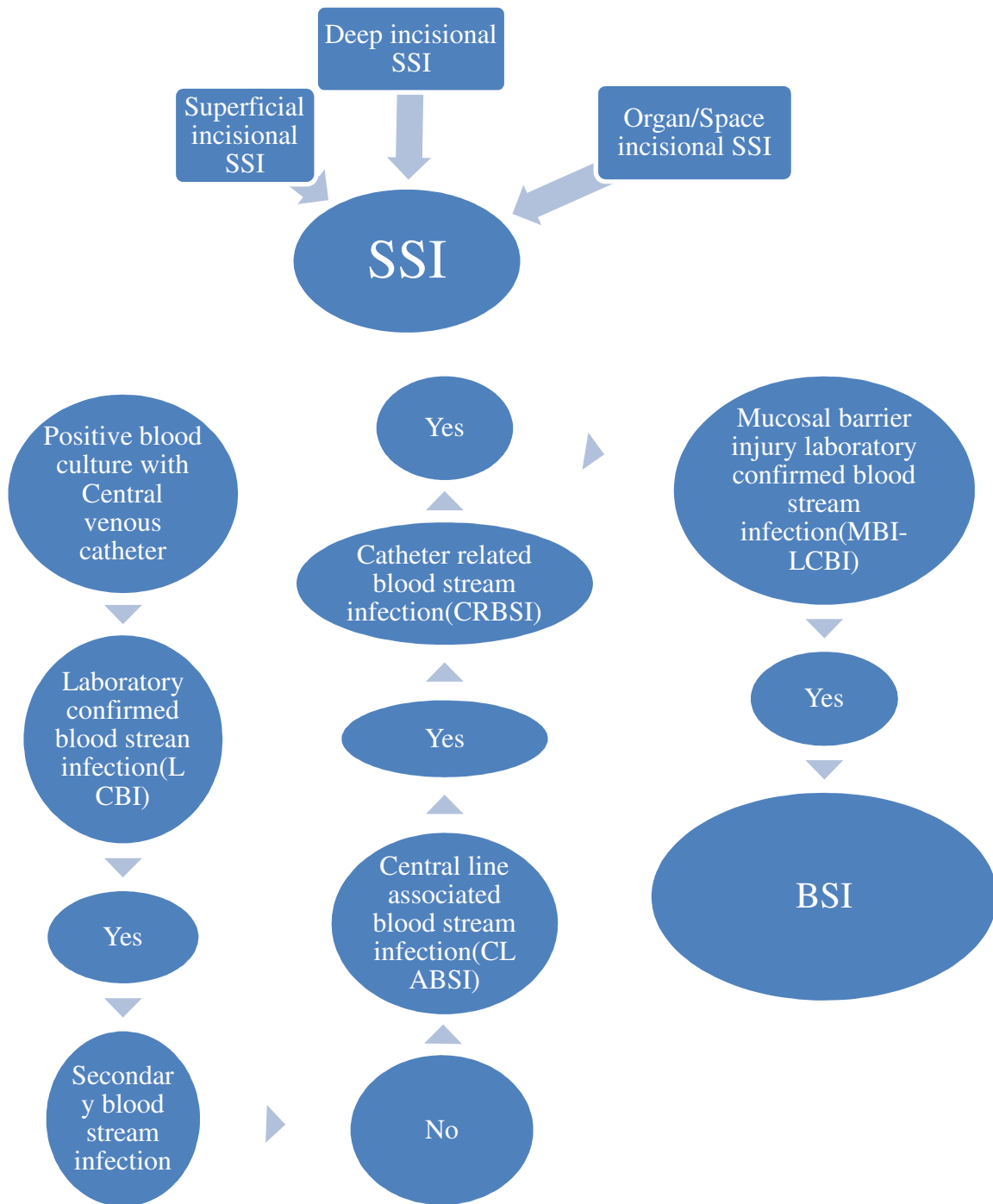


Figure 1. Conceptual frame work of SSI and BSI

3. OBJECTIVES

3.1. General objective

- To determine bacterial etiology, antimicrobial susceptibility profile and associated risk factors of SSIs and BSIs at Asella Teaching and Referral Hospital, South East Arsi, Ethiopia

3.2. Specific objectives

- To determine the incidence of bacterial pathogens causing SSIs and BSIs
- To identify the bacterial pathogens causing SSIs and BSIs
- To determine the antimicrobial susceptibility patterns of bacteria causing SSIs and BSIs
- To identify risk factors associated with the development of SSIs and BSIs

4. MATERIALS AND METHODS

4.1. Study area

The study was conducted at Asella teaching and referral hospital. The city is located 159 km away from Addis Ababa, the capital city of Ethiopia. The hospital was founded in 1964 and is now part of Arsi University and its College of Health Sciences. It is a tertiary hospital serving as a referral center for the Arsi Zone, which has a population of over 3.5 million people. The hospital provides a range of specialized services, including Laboratory service Unit, Emergency Room, Internal Medicine, Pediatrics, Gynecology and Obstetrics with a Fistula Unit, Surgery, ICU, Urology Unit, Dental Medicine, NICU, Orthopedic Unit and Ophthalmology. Recently, Neurology Unit was also established to enhance brain, spinal cord and nerves care services. The hospital has a sophisticated diagnostic laboratory with sections for immunohematology, clinical chemistry, quality regulation, molecular biology, and microbiology.

4.2. Study design and Period

An institutional based prospective cohort study design was conducted from June 2024 to June 2025.

4.3. Source of population

All patients those who surgery were done and admitted at obstetrics, gynecology and surgery wards Asella Teaching and Referral Hospital during study period.

4.4. Study population

All patients who were admitted after surgery for follow up at obstetrics, gynecology and surgery wards for presenting with clinical features of surgical site infections, at wards Asella Teaching and Referral Hospital.

4.5. Eligibility

4.5.1. Inclusion criteria

All patients those who surgery were done, and admitted at Obs/Gyn, surgery wards those developed clinical features of surgical site infections, at Asella Teaching University Hospitals during the study period.

4.5.2. Exclusion criteria

- Patients who were not give consent
- Parents/ legal guardians were not available for interview
- Patients who was not developed a surgical site infection during the study period

4.6. Sample size determination

The sample size was determined by using single population proportion formula by considering the prevalence of SSIs (24.6%) from a study carried out in Hawassa University comprehensive specialized hospital, southern Ethiopia (4)

By using the formula $n = \frac{Z^2 p(1-p)}{d^2}$ $P=0.246$; from a previous study; $Z = 1.96$

(i.e., for a 95% C.I.) And $d=0.05$

This gives 285. The sample size was corrected $N = \frac{1}{1-\text{non response rate}} \times n$

With a 10% non-response rate, a total of 314 patients were included in the study

4.7. Sampling technique

A consecutive sampling technique was applied. When the patients had presented with clinical evidence of surgical site infection

4.8. Study variables

4.8.1. Dependent variables

- Magnitude of bacterial etiology
- Antimicrobial susceptibility profile

4.8.2. Independent variables

- Age
- Gender
- Cigarette smoking
- Alcohol intake
- Comorbidities (DM, HTN, CLD, CVD, Renal Failure, Rheumatoid Arthritis)
- Surgical Ward (General Surgery, Gynecology/Obstetrics)
- Surgical Procedures (Elective, Emergency, Minor, Major)
- Swelling
- Fever
- Delayed healing

4.9. Data Collection

Structured questionnaire was used to collect socio demographic data, clinical characteristics and associated risk factors surgical site infection, and blood stream infections. In addition, patients' information such as comorbidity, indwelling medical device, were collected by reviewing medical records. Outcome were assessed using checklists.

4.10. Specimen Collection, and Laboratory Testing

4.10.1. Blood Collection and Transportation

After proper cleansing by 70% isopropyl alcohol and 2% tincture iodine, 10ml blood samples were collected from peripheral vein. The blood samples were inoculated into Tryptone soya broth and transported to the microbiology laboratory within 5–10 min at 25°C.

4.10.2. Wound swab Collection, and Transportation

A wound sample was taken aseptically from an appropriate site of infection using a sterile cotton swab. Where possible specimens should be collected before starting any antimicrobial therapy. If the area to be swabbed is dry, moisten the swab with sterile water or clean the wound with saline to remove surface contaminants. Sample a representative area of the wound by moving the swab across the wound surface in a zigzag motion, at the same time as rotating it between the fingers. (Care should be taken to ensure that the swab only comes into contact with the wound surface, avoid touching the swab on surrounding skin). Immediately after collection the swab should be returned to its plastic sheath (placed into the transport medium). Label the sample with at least three identifiers (Name, D.O.B & NHS or Hospital No.) and place into a specimen bag. Transport to the laboratory as soon as possible with Amies Medium. If transport is delayed, refrigeration is preferable to storage at ambient temperature.

4.10.3. Bacterial culture and Identification

The blood culture bottles were incubated at 35–37 °C under aerobic conditions for 24hrs and inspected daily for 7days until growth detected. Bottles were observed macroscopically daily for visible evidence of bacterial growth such as hemolysis, turbidity/cloudiness, and visible gas bubbles or distension/bulging of the bottle's cap. Subculture was done on MacConkey agar and incubated aerobically at 35–37 °C for 24 hrs. Whereas, sub-culture was done on sheep blood agar and chocolate agar and incubated at 5% CO₂ for 48–72hrs. The same procedure was repeated until the seventh day before the blood cultures considered to be negative. Bacterial identification was done through colony morphology, Gram stain and biochemical tests. Biochemical tests were performed on sub cultured media by isolating the pure colonies. For gram-positive bacteria, catalase, coagulase, and mannitol fermentation were performed. For gram-negative bacteria, indole, citrate utilization, triple sugar iron, urea, mannitol, oxidase, nitrate reduction, lysine decarboxylase, lysine deaminase and motility test were performed.

Microscopic Examination (Gram Stain): A gram stain is performed on the sample to differentiate bacteria into Gram-positive (purple) or Gram-negative (pink) based on their cell wall structure. Gram stains can be performed to evaluate the relative numbers of neutrophils and squamous epithelial cells. Clinically infected wounds almost always produce a pyogenic reaction and many

polys should be seen in Gram stains. The presence of abundant epithelial cells indicates a superficial sample or contamination from the skin of the wound margins.

Culture and Isolation: The swab is streaked on media, such as blood agar or MacConkey agar, and incubated to grow colonies, which are then analyzed. **Identification Tests:** Biochemical Tests: Catalase (distinguishes *Staphylococci*), coagulase (identifies *S. aureus*), and oxidase tests are commonly used.

4.10.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility test was performed for each bacterial isolate based on Clinical and Laboratory Standard Institute guideline CLSI guidelines 2025 by using disc diffusion method on Muller Hinton agar. Approximately 3–5 pure colonies of the test organism were collected using a sterile wire loop and emulsified in 2 ml of normal saline to achieve a turbidity equivalent to the 0.5 McFarland standard. A sterile cotton swab was dipped into the standardized bacterial suspension, and excess fluid was removed by squeezing the swab against the wall of the tube. The swab was then used to evenly inoculate the surface of MHA by streaking in three directions to ensure a uniform lawn of bacterial growth. After 15 minutes, Antibiotic-impregnated paper disks were placed on MHA surface, ensuring a minimum distance of 24 mm between disks to prevent overlapping zones of inhibition. The plates were incubated at 37°C for 24 hours. The diameter of the zones of inhibition was measured to the nearest millimeter using a calibrated ruler. Grades of susceptibility pattern were interpreted by comparison of the zone of inhibition according to CLSI guideline 2025 (98). Susceptibility testing was conducted using commonly prescribed antibiotics. The following antibiotic disks were tested: Amoxicillin-clavulanic acid (20/10 µg), Amikacin (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Trimethoprim-sulfamethoxazole (25 µg), Ertapenem (10 µg), Meropenem (10 µg), Imipenem (10 µg), Chloramphenicol (30 µg), Ampicillin (10 µg), Ceftazidime (30 µg), Cefepime (30 µg), Ceftriaxone (30 µg), and Piperacillin-Tazobactam (100/10 µg). Results are categorized into Susceptible (S) (high likelihood of therapeutic success), Intermediate (I) (uncertain effect, requires higher dosage), or Resistant (R) (high likelihood of treatment failure) based on CLSI standards (71).

4.10.5. Storage of Isolates

Colonies from a 24-hour culture were picked and added to cryovials containing 1.8 ml of storage media Tryptic Soy Broth with 20% glycerol. The cryovials were then vortexed to ensure proper mixing and stored in a deep freezer (- 80°C) for further testing.

4.10.6. Operational definition

Multidrug Resistance (MDR): in bacteria is formally defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. This standard definition, often used for healthcare-associated infections, implies that the microorganism has developed resistance to multiple, structurally unrelated drugs with different mechanisms of action. Non-susceptibility to ≥ 1 agent in ≥ 3 antimicrobial classes.

Surgical site infections (SSIs): are infections occurring within 30 days of an operation (or up to 1 year for implants) involving skin, subcutaneous tissue, or deep tissues/organs manipulated during surgery, characterized by pus, fever, pain, or localized swelling. CDC surveillance criteria define them as superficial, deep incisional, or organ/space. Timing: Within 30 days of procedure (day 1 = day of surgery) or 90 days-1 year if an implant is left in place.

Superficial Incisional SSI: Involves only skin and subcutaneous tissue. Requires purulent drainage, organism(s) isolated from cultured tissue, or symptoms (pain, swelling, heat) plus deliberate opening by a surgeon.

Deep Incisional SSI: Involves deep soft tissues (fascia/muscle). Requires purulent drainage from deep incision, spontaneous dehiscence, or abscess identified on examination or imaging.

Organ/Space SSI: Involves any part of the body deeper than fascial/muscle layers, specifically manipulated during the procedure.

Blood Stream Infection(BSI): is standardly defined as the presence of viable microorganisms (bacteria or fungi) in the blood, confirmed by at least one positive blood culture, often accompanied by systemic signs of infection like fever, chills, or hypotension. BSIs are classified as primary (no identifiable source) or secondary (originating from a localized infection).

Laboratory-Confirmed BSI (LCBI): A patient with a recognized pathogen cultured from 1+

blood cultures, OR a common skin contaminant cultured from 2+ blood cultures drawn on separate occasions along with symptoms (fever, chills, and hypotension).

CLABSI: is a laboratory-confirmed bloodstream infection (LCBI) in a patient with a central line in place for >2 days on the date of the event, which is not related to an infection at another site. The central line must have been in use within the 48-hour period before the infection.

HAI: A Healthcare-Associated Infection (HAI), also known as a nosocomial or hospital infection, is defined as an infection that a patient acquires while receiving care for another condition in a hospital or other healthcare facility. The infection was not present or incubating at the time of admission. Typically, this refers to infections occurring >48 hours after admission, though it can appear after discharge.

Colonization: The establishment, survival, and reproduction of microorganisms on the external or internal surfaces of a host (such as the skin, mucosa, or wounds) without causing tissue damage, injury, or an immune response. A colonized individual shows no signs or symptoms of sickness.

Infection: An infection is the invasion, survival, and multiplication of harmful microorganisms—such as bacteria, viruses, fungi, or parasites—within body tissues, resulting in tissue destruction and a host inflammatory response (fever, pain, white blood cell increase).

4.10.7. Data Quality Assurance

Quality control of the culture media was performed for new batch of media. Visual inspection was done to check cracks in media, hemolysis, and evidence of freezing and presence of air bubbles. The sterility of culture media was ensured by incubating 5% of each batch of the prepared media at 37 °C for 24 hr. All prepared culture media were also checked by inoculating known strains such as *E. coli* (ATCC 25922) gram-negative bacteria, *S. aureus* (ATCC 25923) for gram-positive bacteria and *N. gonorrhoeae* (ATCC 49226) for fastidious bacteria (72).

4.10.8. Statistical analysis

All data were encoded in Microsoft Excel 2016 and exported to Statistical Package for Social Sciences (SPSS) version 26 software. Descriptive statistics, such as frequencies and percentages was used to describe the proportion of bacterial isolates and associated risk factors of surgical site infection, and blood stream infection. Binary logistic regression model was performed to determine the association between post-operative blood stream infection and associated factors. Moreover, all variables with a p-value <0.25 in the bivariable analysis were included in multivariable analysis to account for possible confounding variables. P-value less than 0.05 considered as statistically significant. The strength of the association was interpreted using an odds ratio in a 95% confidence interval. Finally, the results presented in graphs and tables.

4.10.9. Ethical considerations

The study was approved by Institutional Review Board (IRB) of St. Paul's Hospital Millennium Medical College (SPHMMC) Reference No: (PM23/1206), and Asella Teaching, and Referral Hospital were given to me an official permission letter. The purpose and procedures of the study were explained to the study participants. Patients who give their informed consent was selected and included as study participants. All information obtained from study participants was kept confidential and samples collected from patients was used only for this research. There is no direct payment for participation in the study. However, any positive findings were sent to the attending physician about appropriate care and treatment.

5. RESULTS

5.1. Socio-demographic characteristics of the study participants

Among 314 surgical site infection patients were, 137 (43.6%) were males and 177 (56.4%) were females. Of these patients, 178 (56.7%) were admitted to the surgical ward, while 136 (43.3%) were admitted to the gynecology and obstetrics ward at ATRH. The mean age of the participants was 36.1 years with a standard deviation of 15.6 years (Table 1).

Table 1. Socio-demographic characteristics of the study participants in ATRH, from June 2024 to June2025 (n=314)

Variables	Categories	Frequency (%)
Gender	Male	137(43.6)
	Female	177(56.4)
Age category	10-30	88(28)
	31-50	128(40.8)
	51-70	85(27.1)
	>71	13(4.1)
Occupation	Government Employeers	30(9.6)
	Private Employeers	43(13.7)
	Merchant	40(12.7)
	Housewife	107(34.1)
	Others	94(29.9)
Marital status	Married	234(74.5)
	Unmarried	80(25.5)
Residence place	Urban	155(49.4)
	Rural	159(50.6)
Educational status	No formal education	39(12.4)
	Primary school	140(44.6)
	Secondary school	72(22.9)
	Preparatory school	29(9.2)

	College	24(7.6)
	University	10 (3.2)
Wards	Surgical ward	178(56.7)
	Gyn/Obs ward	136(43.3)
Hospital stay	1-7days	64(20.4)
	8-14days	116(36.9)
	15-20days	96(30.6)
	21-30days	38(12.1)
Comorbidity	Yes	33(10.5)
	No	281(89.5)
Family income	Median	10,500(6000-15000)

5.2. Incidence Proportion of Bacterial Causing SSIs and BSIs

The incidence rate measures the speed at which new cases occur, calculated as the number of new cases divided by the total person-time at risk, and number of new cases were 33 and Total person –time at risk 314. The formula is:

Incidence Rate (IR) = Number of New cases / Total person time at risk X100

$$IR = 33/314 \times 100 = 10.5$$

5.3. Proportion of bacterial isolates

Clinically confirmed cases of surgical site infections accounted for 52/314 (16.6%) patients, and Culture confirmed surgical site infections were 33/52 (63.5%), and bacteria culture confirmed blood stream infections were 13/20 (65%). The most predominant causes of postoperative blood stream infections that culture confirmed in this study were *Staphylococcus aureus*, representing 10 of 33 isolates (30.3%), and followed by *Escherichia coli*, representing 9 of 33 isolates (27.3%) (Fig. 1).

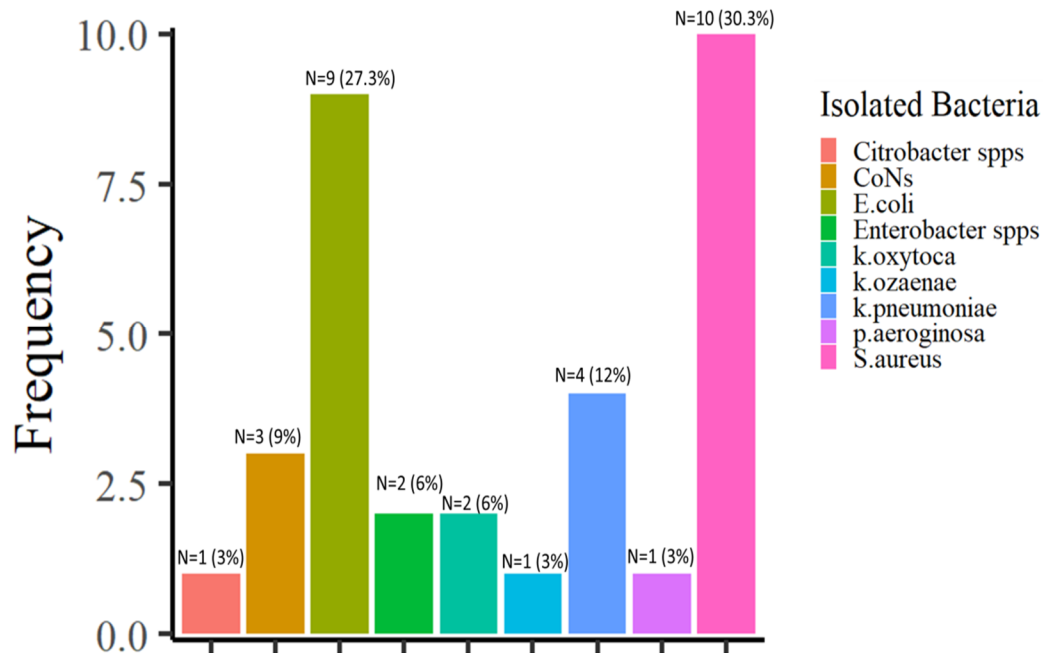


Figure 2. .Distribution of pathogenic bacterial isolates among patients who developed SSI and BSI, at ATRH, Surgery, and Gyn/Obs wards, June 2024 to June 2025

5.4. Bacterial Isolation from Blood and Wound Swabs

Out of (n=52/314) 16.6% presumptive SSI patients from obstetrics and gynecology and general surgical wards, (n=33/52) 63.5% of them were positive in wound swab culture and blood collected aseptically, (n=20/33) 60.6% were presumptive for BSI, and (n=13/20) 65% of them were confirmed positive blood culture (Fig 3).

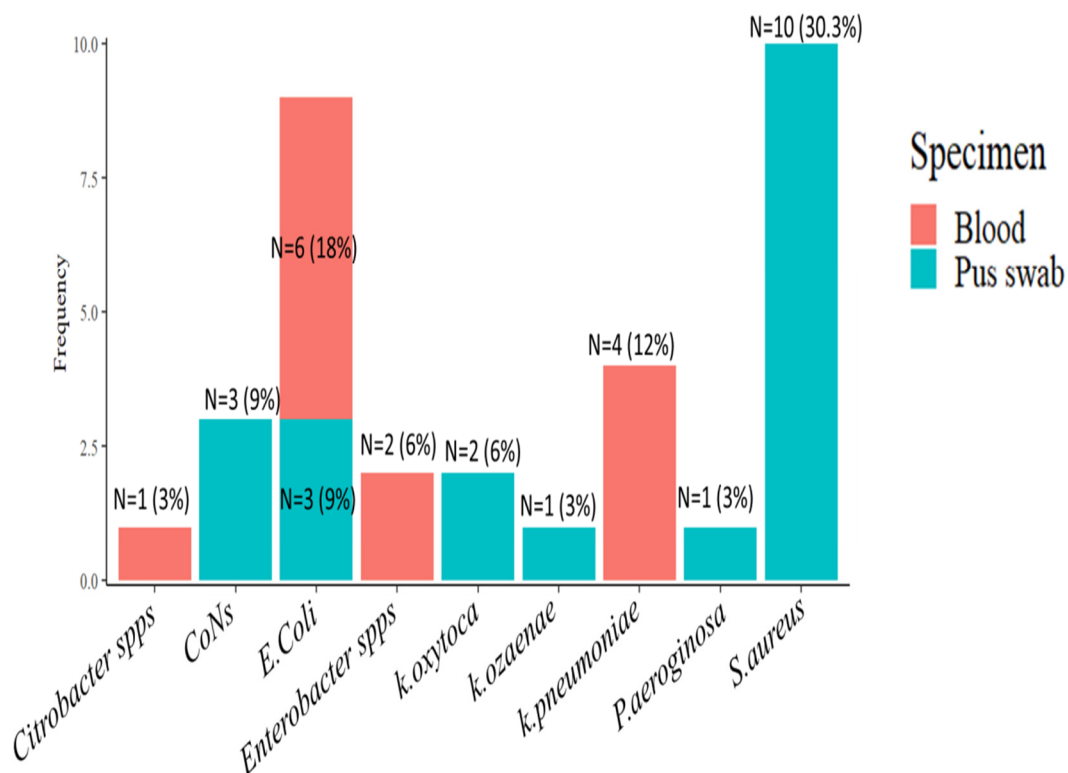


Figure 3. *Bacterial Isolation from Blood and Wound Swab at ATRH, Surgery, and Gyn/Obs wards, June 2024 to June 2025*

5.5. Multivariate analysis of associated factors with SSI and BSI

Bivariate and Multivariate logistic regression analysis were conducted to identify those variables statistically significant association with surgical site infections. The multivariate analysis show that diabetes mellitus, and smoking cigarette, alcohol intake ($P < 0.05$) so those variables were statistically significant for surgical site infection and blood stream infections. [AOR=1.66, 95% CI (0.36_7.6)], [AOR=6.1, 95% CI (1.1_36.7), and [AOR=6.0, 95% CI (1.1_31.90] respectively.

Table 2: Multivariate analysis to identify risk factors associate with SSI, and BSI at ATRH, Surgery, and Gyn/Obs wards, June 2024 to June 2025.

Variables	Culture results of SSI and BSI				COR (95% CI)	P-value	AOR (95% CI)	P-value
	Neg	Pos	Neg	Pos				
Gender								
Female	154(87%)	23(12.9%)	180(57.3)	8(2.6)	1.89(0.87- 4.13)	0.107	1.21(0.45-3.22)	0.702
Male	127(92.7%)	10(7.29%)	121(8.5)	5(1.6)	1.57(0.65-3.02)	0.124	1.02(0.25-2.54)	0.116
Delayed Wound Healing								
Yes	56(65%)	30(35%)	66(21)	10(3.2)	41.1(11.2-43)	3.18	39.2(11.2-136)	8.2
No	225(98.6%)	3(1.3%)	235(4.8)	3(0.96)	-	-	-	-
Diabetes Mellitus								
Yes	8(66.7%)	4(33.3%)	313	1(0.32)	4.7(1.33-16.6)	0.02	1.66(0.36-7.6)	0.01
No	273(90.4%)	29(9.6%)	313(9.7)		-	-	-	-
Hypertension								
Yes	7(77.8%)	2(22.2%)	80(25.5)	4(1.3)	0.39(0.078-1.9)	0.245	0.38(0.04-3.17)	0.37
No	274(89.8%)	31(20.2%)	2289(72.6)	2(0.64)	-	-	-	-
Smoking								
Yes	6(54.5%)	5(45.5%)	56(17.8)	15(4.8)	8.18(2.3-28.5)	0.0009	6.1(1.1-36.7)	0.04

No	275(90.8 %)	28(9.2%)	238(7 5.8)	5(1.6)	-	-	-	-
Alcohol intake								
Yes	47(94%)	3(6%)	62(19 .7)	3(0.9 6)	2.1(0.58-6.8)	0.025	6(1.1-31.9)	0.034
No	234(88.6 %)	30(11.4%)	247(7 8.7)	2(0.6 4)	-	-	-	-

5.6. Associated Risk Factors of SSIs and Blood Stream Infections

Logistic regression analysis was conducted to identify variables statistically significant associated with surgical site infection, and blood stream infections. Culture positive results of SSI were (n=33), and culture positive results BSI were (n=13) Gynecology and Obstetrics, surgery wards. $p < 0.05$ it is statistically significant. Emergency surgical procedure was statistically significant when compared to other independent variables of risk factors with ($P < 0.05$), for blood stream infections, BSI, $p\text{value} = 0.04725$, [(AOR=4.9, 95% CI (0.07338_7.9409))]

Table 3: Analysis of variables, associated risk factors with blood culture and wound swab culture results of SSI and blood stream infections at ATRH, Surgery, and Gyn/Obs wards, June 2024 to June 2025.

Variables	Culture result of SSI			Culture result of BSI		
	Negative	Positive	Pvalue	Negative	Positive	Pvalue
Surgical Ward						
General Surgery	165(52.5)	13(4.14)	-	171(54.5)	7(2.2)	-
Gyn/Obs	126 (40.1)	10(3.2)	2.1905	130(41.4)	6(1.9)	0.08696

Surgical Procedure

Elective	87(27.7)	20(6.4)	-	92 (29.3)	0 (0)	-
Emergency	136(43.3)	6(1.9)	0.052	133(42.4)	3(0.96)	0.0473
Minor	10(3.2)	0 (0)	-	10(3.2)	0 (0)	-
Major	48(15.3)	7(2.2)	-	66(21)	10(3.2)	-
Comorbidity						
None	286 (94.7)	16 (5.3)	-	294 (97.4)	8(2.6)	-
CLD	1(100)	0 (0)	-	0 (0)	1(100)	-
CVD	1(100)	0 (0)	-	0 (0)	1(100)	-
DM	0 (0)	1(100)	18.108	1(100)	0 (0)	2.20
Hypertension	6(100)	0 (0)	-	3(50)	3(50)	-
Renal Failure	2(100)	0 (0)	-	0 (0)	2(100)	-
Rheumatoid Arthritis	1(100)	0 (0)	-	0 (0)	1(100)	-
Swelling						
No	237 (100)	0(0)	-	237 (100)	0(0)	-
Yes	60(77.9)	17(22.1)	5.531	61(79.2)	16(20.8)	2.032
Fever						
No	187 (99.5)	1 (0.5)	-	187(99.5)	1(0.5)	-
Yes	110 (87.3)	16(12.7)	7.06	111(88.1)	15(11.9)	1.05
Delayed healing						
No	226 (99.1)	2 (0.9)	33.46	227(99.6)	1 (0.4)	-
Yes	71 (82.6)	15(17.4)	9.93	71 (82.6)	15(17.4)	2.93

5.7. Antibiotic Susceptibility Pattern of Bacterial Isolate

Antimicrobial susceptibility testing was performed on 33 SSI and BSI isolates. The isolates showed high susceptibility to imipenem 33 (89.3%), meropenem 33 (88%), ertapenem 25 (85.5%), amikacin 16 (96.4%), gentamicin 15 (89.3%), and chloramphenicol 23 (92.9%). Additionally, Isolates shows resistants were Ampicillin 33 (100%), cefepime 25 (75.8%) and Ceftriaxone 30 (96.9%), Ceftazidime 10 (38.4%) (Table 4).

Table 4. Antibiotic susceptibility pattern from patients SSI, and BSI *at ATRH, Surgery, and Gyn/Obs wards, June 2024 to June 2025* (n=314)

Antibiotic	Sensitive (n %)	Intermediate (n, %)	Resistance (n %)
Ampicillin	0 (0)	0 (0.0)	33 (100)
Ceftriaxone	3(3.1)	0 (0.0)	30 (96.9)
Cefepime	2 (7.6)	3 (16.9)	25 (75.8)
Ceftazidime	5 (18.9)	18 (42.7)	10 (38.4)
Piperacillin- Tazobactam	23(61.3)	8 (22.3)	2 (11.4)
Amoxicillin- Clavulanate	20 (65.3)	5(14.7)	8(19.4)
Imipenem	20 (87.5)	10(10.5)	3 (2.0)
Meropenem	26 (89.6)	7 (10.4)	0 (0.0)
Ertapenem	23 (89.0)	8 (8.4)	2 (2.6)
Amikacin	30 (96.8)	3 (3.2)	0 (0.0)
Gentamicin	6 (26.4)	17 (42.6)	10 (31.0)
Ciprofloxacin	20 (75.4)	9 (20.1)	4 (4.5)
Chloramphenicol	26 (91.0)	3 (3.2)	4 (5.3)
Trimethoprim- Sulfamethoxazole	10(33.2)	0(0.0)	23(66.8)

Key: S (Sensitive), I (Intermediate), R (Resistant), NA (Not Applicable)

5.8. Antibiotic Susceptibility Testing Pattern of Isolates by Bacterial Species

Species specific AST analysis of the study indicates that among isolates, 19 (98%) of *E. coli* were resistant to ceftriaxone and 16 (79%) to cefepime. All *k. pneumonia*, *K. Ozanae* and *K. ocytoa* isolates (100%) were resistant to *ceftriaxone* and *cefepime*. *Carbapenem* and *aminoglycoside* susceptibility was high: *E. coli* showed 75 (89.3%) susceptibility to imipenem and 9 (89.3%) to amikacin 33 (96.4%), while all *klebsiella* species were 100% susceptible to both antibiotics (Table 5).

Antibiotic	Susceptibility Pattern	<i>S.aureus</i> (n=10)	<i>E. coli</i> (n = 9)	<i>K. pneumoniae</i> (n = 4)	<i>K.oxytoca</i> (n =2)	<i>CONS</i> (n=3)	<i>Enterobacter</i> spp n=2	<i>K. ozanae</i> n=1	<i>Citrobacter</i> spp n=1	<i>P.aeruginosa</i> n=1
Ampicillin	S	0(0.0)	0 (0.0)	N/A	NA	0(0.0)	0(0.0)	NA	0(0.0)	NA
	I	0(0.0)	0 (0.0)	N/A	NA	0(0.0)	0(0.0)	NA	0(0.0)	NA
	R	10(100)	9(10)	N/A	NA	3(100)	2(100)	NA	1(100)	NA
Ceftriaxone	S	NA	1 (2.0)	0 (0.0)	0 (0.0)	NA	0(0.0)	0(0)	0(0.0)	0(0.0)
	I	NA	0 (0.0)	0 (0.0)	0 (0.0)	NA	1(1.6)	0(0)	0(0.0)	0(0.0)
	R	NA	8(98.0)	4 (100.0)	2(100)	NA	1(98.4)	1(100)	1(100)	1(100)
Cefepime	S	NA	0 (0.0)	0 (0.0)	0 (0.0)	NA	1(92.2)	0(0.0)	1(100)	1(100)
	I	NA	2 (21.0)	1 (20.0)	0 (0.0)	NA	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	R	NA	7(79.0)	3 (80.0)	2(100)	NA	1(7.8)	1(100)	0(0.0)	0(0.0)
Ceftazidime	S	NA	1 (13.0)	0 (0.0)	0 (0.0)	NA	2(100)	0 (0.0)	0(0.0)	1(100)
	I	NA	5 (45.0)	3 (60.0)	0 (0.0)	NA	0(0.0)	0 (0.0)	0(0.0)	0(0.0)
	R	NA	3 (42.0)	1 (40.0)	2(100.0)	NA	0(0.0)	1 (100)	1(100)	0(0.0)
Piperacillin-Tazobactam	S	NA	6 (73.0)	2 (40.0)	0 (0.0)	NA	1(94)	0 (0.0)	0(0.0)	1(100)
	I	NA	2 (19.0)	2 (60.0)	2 (100)	NA	1(6)	1(100)	0(0.0)	0(0.0)
	R	NA	1 (8.0)	0 (0.0)	0 (0.0)	NA	0(0.0)	0 (0.0)	1(100)	0(0.0)
Amoxicillin-Clavulanate	S	NA	5 (66.6)	2 (60.0)	2(100.0)	NA	0(0.0)	0 (0.0)	0(0.0)	NA
	I	NA	2 (16.7)	0 (0.0)	0 (0.0)	NA	1(5.5)	0 (0.0)	0(0.0)	NA
	R	NA	2 (16.7)	2 (40.0)	0 (0.0)	NA	1(94.5)	1 (100.0)	1(100)	NA
Imipenem	S	10 (97)	6 (89.3)	4 (100.0)	2(100.0)	NA	1(1.8)	1 (100.0)	1(100)	1(100)
	I	0(0.0)	1 (2.4)	0 (0.0)	0 (0.0)	NA	0(0.0)	0 (0.0)	0(0.0)	0(0.0)
	R	0(0.0)	2 (8.3)	0 (0.0)	0 (0.0)	NA	1(98.2)	0 (0.0)	0(0.0)	0(0.0)
Meropenem	S	8(90.0)	6 (88.1)	3 (80.0)	2(100.0)	NA	0(0.0)	1 (100.0)	1(100)	1(100)
	I	0(0.0)	1 (2.4)	0 (0.0)	0 (0.0)	NA	0(0.0)	0 (0.0)	0(0.0)	0(0.0)
	R	2(20.0)	2 (9.5)	1 (20.0)	0 (0.0)	NA	2(100)	0 (0.0)	0(0.0)	0(0.0)

Ertapenem	S	8(85.5)	5 (87.0)	4 (100.0)	2(100.0)	NA	0(0.0)	1 (100.0)	1(100)	1(10 0)
	I	2(14.5)	1 (3.5)	0 (0.0)	0 (0.0)	NA	0(0.0)	0 (0.0)	0(0.0)	0(0. 0)
	R	0(0.0)	3 (9.5)	0 (0.0)	0 (0.0)	NA	2(100)	0 (0.0)	0(0.0)	0(0. 0)
Amikacin	S	N/A	6 (96.4)	4 (100.0)	2 (100.0)	NA	2(100)	1 (100.0)	1(100)	1(10 0)
	I	N/A	3 (3.6)	0 (0.0)	0 (0.0)	NA	0(0.0)	0 (0.0)	0(0.0)	0(0. 0)
	R	N/A	0 (0.0)	0 (0.0)	0 (0.0)	NA	0(0.0)	0 (0.0)	0(0.0)	0(0. 0)
Gentamicin	S	0(0.0)	7 (89.3)	4(100.0)	2 (100.0)	0(0.00	1(78.6)	1 (100.0)	1(100)	1(10 0)
	I	1(25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0(0.00	0(0.0)	0 (0.0)	0(0.0)	0(0. 0)
	R	9(75.0)	2 (10.7)	0 (0.0)	0 (0.0)	3(100)	1(21.4)	0 (0.0)	0(0.0)	0(0. 0)
Ciprofloxacin	S	0(0.0)	3 (38.1)	3 (75.0)	2(100.0)	2(82.5)	1(97.2)	1 (100.0)	0(0.0)	1(10 0)
	I	1(10.5)	1 (20.2)	1 (25.0)	0 (0.0)	0(0.0)	0(0.0)	0 (0.0)	1(100)	0(0. 0)
	R	9(89.5)	5 (41.7)	0(0.0)	0 (0.0)	1(16.5)	1(2.8)	0 (0.0)	0(0.0)	0(0. 0)
Chloramphenicol	S	4(40.5)	7 (92.9)	5 (100.0)	2 (100.0)	NA	0(0.0)	0 (0.0)	0(0.0)	0(0. 0)
	I	3(29.8)	1 (3.6)	0 (0.0)	0 (0.0)	NA	0(0.0)	0 (0.0)	0(0.0)	0(0. 0)
	R	3(29.8)	1 (3.6)	0 (0.0)	0 (0.0)	NA	2(100)	1 (100.0)	1(100)	1(10 0)
Trimethoprim-Sulfamethoxazole	S	6(84.5)	18 (21.4)	1 (20.0)	3 (75.0)	2(76.5)	NA	0 (0.0)	1(100)	0(0. 0)
	I	2(8.5)	0 (0.0)	0 (0.0)	0 (0.0)	1(23.5)	NA	0 (0.0)	0(0.0)	0(0. 0)
	R	2(7.0)	66 (78.6)	3 (80.0)	1 (25.0)	0(0.0)	NA	1(100.0)	0(0.0)	1(10 0)

Table 5. Antibiotic Susceptibility Patterns by Bacterial Species at ATRH, June 2024 to 2025 (n, %)

5.8. Multidrug resistance pattern of bacterial isolates

About 11 (55%) of the bacterial isolates were resistant to three, four, and more classes of antimicrobial agents. 26 isolates were showed resistance to three different antimicrobial classes (100%), 10 isolates were (40%) resistant to two classes, and 3 isolates were (5%) demonstrated resistance to one antimicrobial class. Microorganisms, typically bacteria that are resistant to at least one agent in three or more antimicrobial categories.

Table 6. Multidrug resistance pattern of bacterial isolates at ATRH, Surgery, and Gyn/Obs wards, June 2024 to June 2025 (n=314)

Bacterial isolates	Degree of resistance (%)						
	R0	R1	R2	R3	R4	≥R4	Total (%)
<i>S.aureus</i> (n=10)	0(0.0)	0(0)	4(60)	2(20)	1(10)	1(10)	100%
<i>E.coli</i> n=9	0(0.0)	2(15)	3(30)	2(35)	1(20)	0(0.0)	100%
<i>K.pneumoniae</i> (n=4)	0(0.0)	0(0.0)	2(40)	1(25)	1(35)	0(0.0)	100%
<i>Enterobacter spp</i> (n=2)	0(0.0)	0(0.0)	1(50)	1(50)	0(0.0)	0(0.0)	100%
<i>Citrobacter spp</i> (n=1)	0(0.0)	1(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	100%
Total (n=26)	0(0.0)	3(5)	10(40)	6(35)	3(15)	1(5.0)	100%

Key: R0=no Resistant in a class, R1=Resistant to 1drug in a class, R2=Resistant to 2drug in classes, R3=Resistant to 3drug in classes, R4=Resistant to 4drug in classes, ≥R4=Resistant to greater than 4drugs or equal to 4drug in classes.

Antimicrobial class used to define MDR- *Penicillin* (*Ampicillin*, *Amoxicillin-Clavulanate*, and *Piperacillin-Tazobactam*), *Cephalosporin* (*Ceftazidime*, *Ceftriaxone*, and *Cefepime*) *Carbapenem* (*Ertapenem*, *Meropenem*, and *Imipenem*), *Aminoglycoside* (*Amikacin*, *Gentamicin*), and *SulfonamideandTrimethoprim* (*Trimethoprim-Sulfamethoxazole*), *Amphenicol* (*Chloramphenicol*), *Fluoroquinolone* (*Ciprofloxacin*).

5.9. Association of CBC measurement to SSI and BSI

When we compare the different laboratory parameters, WBC Count, Neutrophil percentage Lymphocyte percentage were not associated with BSI. Where as Platelet count was significantly ($P<0.05$) associated with BSI. Similarly the WBC Count, Neutrophil percentage Lymphocyte percentages as well as Platelet count were not associated with SSI.

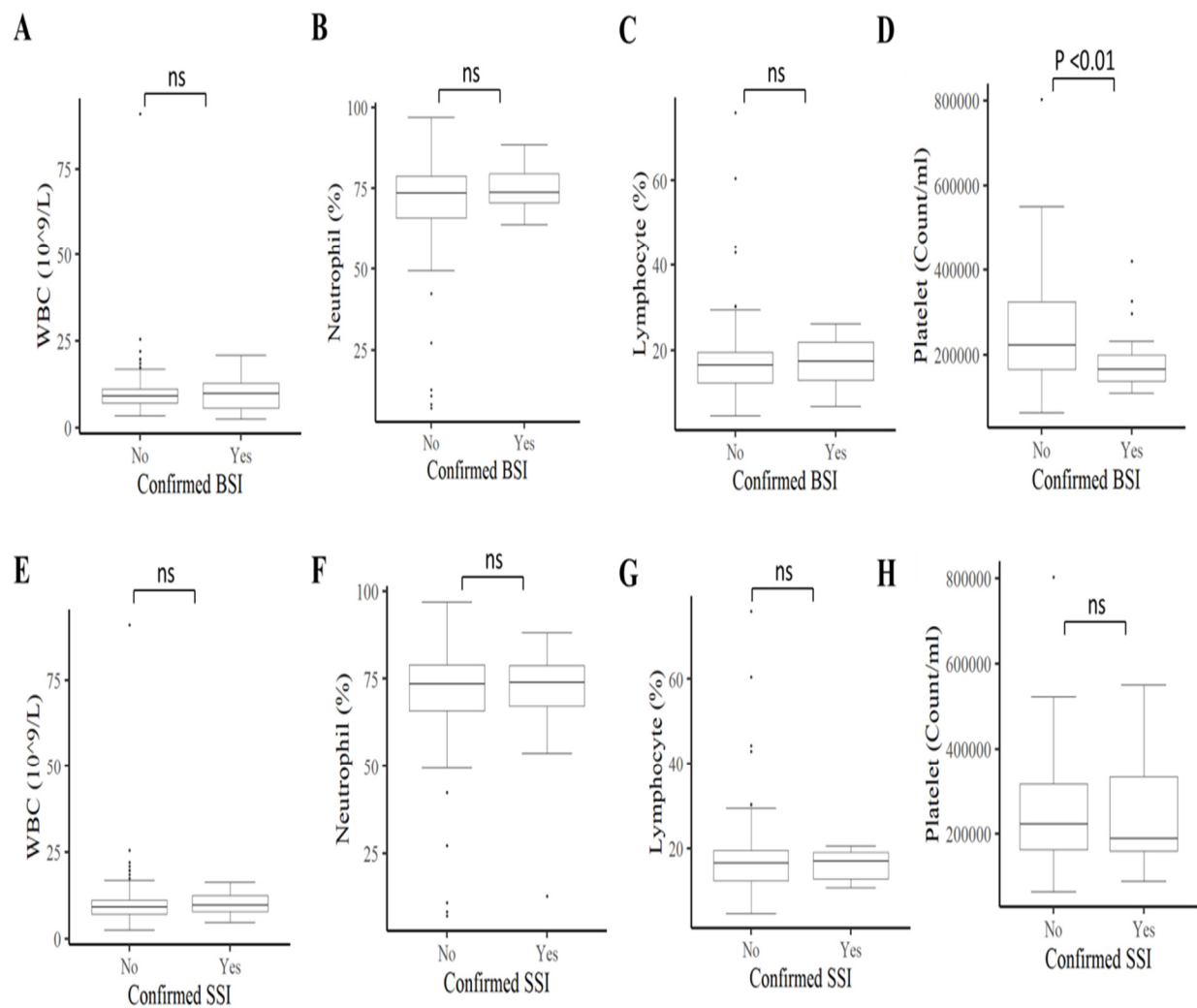


Figure 4: Distribution and patterns of WBC, Neutrophil, Lymphocyte, and Platelet analysis of measurements of SSI and BSI at ATRH, Surgery, and Gyn/Obs wards, June 2024 to June 2025

6. DISCUSSION

The main objectives of this studies were to determine the incidence of bacterial pathogens causing surgical site infections and blood stream infections. In this study, bacteria were isolated out of 314 participants about patients were presumptive SSI were 16.6%, from this culture positive SSI isolates were SSI 63.5%, and follow up culture positive SSI patients a bacteria that suspected for BSI were 60.6%, and confirmed blood culture positive BSI 65% were identified. The predominant organism in this study was *S. aureus* 30.3%), followed by *E. coli* (27.3%), *k. pneumoniae* (12.1%), respectively. This predominance of *S. aureus* agrees with findings from previous studies in Addis Ababa (37%), Arbaminch (76%), and Harari (30.3%), Ethiopia as well as studies from other countries, including India (45.3%), and Egypt (27.4%) (73, 74, 75).

In the present study, the incidence rate of SSI were 10.5 % different bacteria were isolated. This result is consistent with studies conducted in Addis Ababa (11.1%), Harari (11.8%) , Mizan-Tepi (12.6%), Wolaita Sodo (13%) (76, 77). Similar findings have been reported in other countries, such as Sierra Leone (11.5%) and India (20.8%). However, the results of this study were lower than those of other studies conducted in Dessie (23.4%), Arba-Minch (29.4%), and Egypt (67.6%) (78). conversely, the results were slightly higher than those of similar studies conducted in the USA (7.8%), and Mexico (7.9% (79, 80).

This variation may be due to differences in study populations, time periods, and sample sizes. The studies involved larger and more diverse groups of participants and had better facilities with enhanced infection control, such as modern surgical procedures, improved ventilation in operating rooms, advanced sterilization methods, and proper patient care (81). Additionally, variations might come from inadequate preoperative wound care and insufficient infection control techniques, often caused by poor hygiene and lack of water. In developing countries, poor-quality equipment, inadequate infrastructure, shortages of advanced supplies and trained personnel, and financial constraints frequently lead to a higher incidence of surgical site infections (SSIs) and compromised patient care. This supports the observation that most organisms isolated from SSIs are derived from the patient's flora and environment, where *S. aureus* is a common isolate. According to this study, *E.coli* the second predominant isolate, accounting for approximately (27.3%) of cases, followed by *K. pneumoniae*. However, other studies conducted in Addis Ababa (23.1%), Mizan-Tepi, Ethiopia (29.3%), and Mexico (27.5%), reported *E. coli* as the most predominant isolate (82, 83).

The results of this study showed that the overall culture positivity rate of confirmed (BSI) identified from general surgery, gynecology, and obstetrics ward patients was (39.4%) align with study conducted in different area (79). The alignment with findings from Nepal (12.6%) and, India (14.2%) (84, 85).

Suggests a degree of global comparability in BSI prevalence within certain settings (86). However, the significant divergence from previously reported rates within Ethiopia itself (5.4–11.3%), as well as Tanzania (3.2%) and Iran (9.6%), the lower prevalence reported in Tanzania and Iran may reflect differences in diagnostic capabilities, healthcare infrastructure, and the overall burden of infectious diseases within those specific regions (87, 88, 89).

Conversely, the higher prevalence rates documented in other Ethiopian studies (18.2–31.9%) and across various low and middle-income countries including India (31.2%), Cameroon (16%), and Nigeria (19.2%), and even reaching alarmingly high levels in specific settings within Tanzania (38.9%), Nigeria (43.5%), Ethiopia (50.6%), and Ghana (51.4%) (90, 91, 92, 93).

In the current study Gram positive organisms were more frequently isolated than Gram negative organisms (94). A similar finding was observed in the study conducted in Gondar, Ethiopia and in Cape Town, South Africa (95). *Staphylococcus aureus* was the most prevalent etiological agent for blood stream bacterial infections and this finding is comparable with other studies conducted in different areas (95). *Coagulase negative staphylococci (CONS)* was the other common cause of bacterial infection in the BSI which was consistent with other studies conducted in India, Egypt and Ethiopia (96, 97, 98).

The high prevalence of CONS might be in association with an increasing burden of nosocomial infection in hospitalized patients (99). The pathogen was previously considered as a culture contaminant but is now recognized to be an important cause of SSI (100). In the present study *E.coli* was the predominant pathogen among Gram negative organisms followed by *K.pneumoniae*. Similar findings was observed in studies conducted in Ethiopia and Nigeria (101). In the current study different factors were found to be associated with bacteremia (102).

In the present study all Gram positive organisms showed resistance Ampicillin, Ceftriaxone, Gentamycin, Ciprofloxacin for *Staphylococcus aureus* showed resistant (103). Gram-negative bacteria had an overall resistance rate of 69.7%. *Klebsiella spp* and *K. pneumoniae* were notably

resistant to gentamicin and trimethoprim-sulfamethoxazole (104). *E. coli* showed resistance to ceftazidime 4(45%) and cefepime 6(79%) (105). Most gram-negative bacteria were susceptible to Imipenem, Meropenem, Ertapenem, and amikacin (106). Similar findings were observed in studies conducted in Egypt and Nigeria (107). In the current study the majority of isolated organisms of Gram positive and Gram negative bacteria were resistant to amoxicillin-clavunate and ampicillin, similar to studies conducted in Egypt and Ghana (108).

Most of the Gram positive organisms were resistant to Ampicillin and Ciprofloxacin in the present study. A similar finding was observed in a study conducted in Egypt (109). Most of the Gram negative organisms were resistant to Ampicillin, cephalosporins, gentamicin, and amoxicillin-clavulanate which showed similarity to studies conducted in Nigeria and India (110). *P. aeruginosa* was only sensitive to gentamicin and the isolate was resistant to the other tested drugs in the present study (111).

However, this study finding was different from a study conducted in Gondar, Ethiopia that showed *P. aeruginosa* was resistant to gentamycin (112). The observed difference may be due to difference in the use of antibiotic drugs and the presence of microbiology laboratory (113). In the current study multi-drug resistance was observed in (55%) of the bacterial isolates were resistant to three or more classes of antimicrobial agents (114).

The present study had a higher multi-drug resistance rate compared with several other studies (116) All *S. aureus*, *K. pneumoniae*, *E. coli*, *P. aeruginosa* strains showed multi-drug resistance similar to studies conducted in Ethiopia and Nepal (103).

The variables that were statistically significant in the study were, found for participants who underwent emergency surgery and developed BSI. Similar results were obtained; this is lower than the prevalence reported in Europe (6.5%), Asia (9.0%), and the US (4.0%) (113,114).

Additionally, this rate is greater than the HAIs of 15.5% (107), Addis Ababa 18.4%, Oromia 17.4%, Amhara 13.27%, Tigray 27.6%, and SNNPR 21.4% (108) that have been reported in developing nations. While unadjusted studies indicated a significantly greater incidence of SSI in drinkers compared to moderate drinkers, the connection was not statistically significant after controlling for variables like age and smoking. In this investigation, alcohol consumers were

higher than non-users. The correlation may result from long-term alcohol use weakening the immune system and delaying wound healing. According to Bacterial Etiology, Antimicrobial Susceptibility Profile, and Associated Risk Factors of Surgical Site Infections and Blood Stream Infections at Asella Teaching and Referral Hospital, South East Arsi, Ethiopia: Prospective Cohort Study, patients with diabetes mellitus had an OR of 16.6% for bloodstream infections and surgical site infections compared to those without the disease. The results are similarly, to research done in Bahir-dar (10.2%), Dire-Dawa (6.2%), Africa (8.84%), Malaysia (8.9%), and Germany (8.2%) respectively (109,114,115,116).

According to Bacterial Etiology, Antimicrobial Susceptibility Profile, and Associated Risk Factors of SSIs and BSIs the OR for SSI was 6.1% among individuals who smoked cigarettes. The prevalence of this study was lower than that of studies in Eastern Ethiopia (28%), Madagascar (28.5%), and Bangladesh (23.19%) (117), but it was comparable to studies in China (31.8%) and Tunisia (30.4%) (118). consequently, those patients had diabetes mellitus, and smoked cigarettes, all of which were statistically significantly linked with the development of SSI and BSI ($p < 0.05$) compared to other independent variables (119).

7. STRENGTH AND LIMITATION OF THE STUDY

7.1. Strength of the study

The primary advantage is that the exposures were measured before to the outcome, which is crucial for establishing causation.

To the best of our knowledge, this is one of the few research in Ethiopia that aims to concurrently identify the antimicrobial susceptibility patterns of risk variables related with SSI and BSI and the incidence of bacterial etiology.

This provides useful baseline information for local AMR monitoring.

Another advantage of doing the study at ATRH was that its high volume of patients and function as a referral center provided an ideal environment for producing findings that were pertinent to the area and locally.

This allows researchers to directly quantify the risk of outcomes and the incidence of diseases (new cases). Low recall bias and the effects of a single exposure on a variety of outcomes can be assessed in a single research.

7.2. Limitations of the Study

In this study, molecular techniques were not done, to provide more information to indicate specific antibiotic resistance at the level of genes, have not been performed.

The current study was conducted in a single institution, but it would be more representative if it was conducted in several health facilities.

We have done the study only in general surgery and gynecology and obstetrics wards, but it would be good if we included other wards in the study area.

In addition to that there is also a lack of microbiology laboratory facilities, we could not isolate anaerobic bacterial pathogens from the study participants.

Wound infections are caused by a number of microorganisms which include bacteria, fungi, and viruses. This study was only able to address blood stream infection caused by aerobic bacteria.

8. CONCLUSION AND RECOMMENDATIONS

8.1. Conclusion

According to Bacterial Etiology, Antimicrobial Susceptibility Profile, and Associated Risk Factors of Surgical Site Infections and Blood Stream Infections, multi-drug resistance organisms accounted for 75.8% (n=25/33) of the surgical site infection and blood stream infection at Arsi University School of Health Science Asella Teaching Referral Hospital. The most predominant pathogens were *S. aureus*, and followed by *E. coli* respectively. Patients underwent emergency surgery, diabetes mellitus, and smoked cigarettes, all of which were statistically significantly to development of surgical site infections, and blood stream infections. There revealed frightening MDR values and significant antibiotic resistance. Strong connections between BSI and SSI, presentation in specific clinical settings, such as obstetrics, gynecology, and surgical wards, were

also discovered by the study. *Staphylococcus aureus*, *E. coli*, *K. pneumoniae*, *Coagulase Negative staphylococcus* (CONS), *Enterobacter species*, *Citrobacter species*, *K. oxytoca*, *K. ozannae*, and *Pseudomonas aeruginosa* were the most frequently isolated bacterial pathogens. Despite the widespread usage of antibiotics, the rate of wound infection was inversely connected with the incorrect medication selection.

8.2. Recommendations

The high incidence of bacterial pathogens causing surgical site infections and blood stream infections outstanding to the organisms' high level of antibiotic resistance, sensitivity tests should be conducted on a regular basis to improve the sensible were used antibiotics, and the selection of antibiotics should have been based on the sensitivity patterns. The patient's treatment sheet should provided a clear indication of the dosage and duration of the prescription antibiotics once they were administered. The hospital formulary and sensitivity patterns should serve as the foundation for treatment recommendations on the used antibiotics. To ensure that antibiotics were used rationally, this should have been reviewed periodically. To lower the risk of hospital-acquired infections, prolonged hospital stays should have been avoided. To determine whether the suggested standard practices were being followed when addressing wounds, research should have been done. Antimicrobial susceptibility testing revealed widespread resistance to commonly used antibiotics, underscoring the urgent need for effective antimicrobial stewardship.

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10. Annex

I. Research Participant Information Sheet

Title of the study: Bacterial Etiology, Antimicrobial Susceptibility Profile, and Associated Risk Factors of Post-Operative Surgical Site Infections and Blood Stream Infection at ATRH, South East Arsi, Ethiopia: A Longitudinal Prospective Study Design

Investigator: Mr.Bekele Dabi

Organization: College of Health Science, Arsi University

Sponsor: Arsi University, Ethiopia

You were invited to be involved in a research on surgical site infection, and blood stream infection which is very life threatening and common in this area. Before deciding to participate in the research, it was better to understand the information in this form because it briefs your rights and our responsibilities to you. This information and consent or assent form consists of purpose, examinations, possible advantages, risks and inconveniences related to this study. Your right to resign from taking part in the research was also explained below. You had been the right to ask any question at any time related to the research. Your participation is voluntary. You may talk to anyone you fill joyful and you may take time to decide whether to participate or not.

Purpose of the study

The purpose of this study is to assess bacterial etiology, antimicrobial susceptibility profile, and associated risk Factors of post-operative surgical site infections and blood stream infection at, among patients suspected for SSI in ATRH, South East Arsi, Ethiopia.

SSI was one of the commonest diseases which were a huge burden to the world. It was more prevalent in resource limited countries than high income counties. SSI was particularly devastating in the African including Ethiopia. It was caused by mostly bacteria. However, it was prevalence and severity depends on several factors, such as socio-demographic, season, age, sex, immunity, underlining disease, health system quality and etc. So that patients at ATRH was participate in this research.

Study Procedure: if you were willing to participate in this research and are eligible, you was asked the following information.

- 1) Small interview, which includes your socio demographic characteristics

2) Collection of samples for laboratory investigations: A total volume of 10ml of blood and, wound swab was collected.

Any of the procedures performed specific for this study are fee free.

Risks and inconveniences

There is no risk involved in taking the wound swab except for a slight touch of the cotton swab that will be felt at the point of swabbing.

Venous blood draws may cause brief discomfort, bruising, and hematoma at the site. There was also a small risk of infection or fainting. I was collected blood using sterile technique to minimize risks.

Collection of catheter tips poses no additional risk, as they are routinely removed as part of dialysis care.

Environmental swabbing involves minimal risk, as sterile swabs were used.

Participation was require your time for the interview and sample collection during your surgery visits. We were coordinated with your surgery schedule to minimize inconvenience.

There were a small risk of loss of confidentiality of your information. We were used strict security measures to protect your data and privacy.

Participation in this research is voluntary. You may experience discomfort in answering some of the questions. You could decline to answer any questions and withdraw at any time.

There may be risks that were currently unknown or unforceseable. Please contact the study team at any time if you have any concerns.

Let the study staff know about any discomfort, hematoma, bruising or other issues after the blood collection. We were provide care or recommend you follow up with your physician.

Please ask the study team if you have any other questions or concerns about the potential risks or inconveniences. We were dedicated to making study participation as safe and comfortable for you as possible.

Benefit

The clinicians give you the information on wound infection prevention and the results obtained had been used to effectively manage the wound infection.

The information learned from this study may help improve infection control and prevention practices in surgery units in Ethiopia and beyond. This can lead to reduced rates of dangerous surgery-related infections for other patients.

Your participation were contributed to better understanding of the types of bacteria causing infections, improving diagnosis and treatment approaches.

Identifying risk factors could allow screening of patients at high risk and targeting of infection control measures.

Understanding antimicrobial resistance patterns could help shape better antibiotic prescription practices.

Overall, this study aims to guide evidence-based policies to enhance the quality and safety of surgery care in Ethiopia.

You were received the results of any blood culture or other sample testing done as part of this study, which may aid your care. However, no other individual results had been provided.

There is no payment for participating in this study. However, your contributions were provided valuable data to improve patient care, infection prevention, and surgery practices.

Please let the study team know if you have any other questions about the potential benefits or value of the study. Your willingness to participate may help protect other patients undergoing surgery.

Compensation

All study-related examinations were free of charge to you.

Protection of your private life

We were went everything we could to protect your privacy. Information retrieved from your medical record would be treated as strictly confidential. The information would be identified by a code and not by your name. The documents where your name is mentioned would not be shared

with anyone, except the study researchers, and your doctor who have to keep it confidential. By signing this informed consent or assent form, you agree with this access to your records.

The findings of the study would be published in medical journals and i could be shared in an international database. Your name were not appeared in any database, report or publication resulting from this study.

Ethics committee

IRBC of St. Paul's Hospital Millennium Medical College, Arsi University College of health science were reviewed and approved the study before the start of it. The committee were also perform ongoing review of the study to make sure it is carried out in the safest way possible.

When clinical relevant results were discovered, the clinician would immediately be informed, who can trace back the patient identity when needed.

Voluntary participation

Your participation in this research was totally voluntary. Your participation was dependent on your choice. Your decision to participate or not to participate in the research would not have any effect on the services you get from the hospital. You have a full right to with draw from the research at any time, even after signing the consent or assent form. You do not have to give a reason for wanting to stop from being part of the study.

Contact person in case of questions

If you have any questions concerning your participation in this study, your rights or if you think you have been harmed as a result of the study, you may contact the researcher, now, during, or after the study whose contact information are provided below.

<u>Name</u>	<u>Phone no.</u>	<u>Email address</u>
1. Mr.Bekele Dabi	+251924066208	bekeledabi19@gmail.com
2. Dr.Anteneh Mehari	+251912060506	antenehmehari@gmail.com

II. Consent/assent Form

Title of the study: Bacterial Etiology, Antimicrobial Susceptibility Profile, and Associated Risk Factors of Surgical Site Infections and Blood Stream Infection at ATRH, South East Arsi, Ethiopia: A Prospective Cohort Study Design

Part which is destined only to the participant or his legal representative

I understood and I have been informed about the study and the participant informed consent or assent form sheet copy is given to me.

The study was explained to me in a simple and clear language with enough information. Sufficient time was given to me to realize and ask questions, to which I got satisfactory answers. I am freely willing to be part of the study and cooperate in the study activities. I am willing to give information concerning my medical history, clinical examination and participate in giving blood and wound swab samples. If I want to discontinue participating, even after signing the informed consent, I know I can do so. I agreed that my doctor and other healthcare professionals involved in my diagnosis were informed about my participation in this study.

☐

Yes

☐

No

To be completed by the participant

Date:

Name of the participant:

Signature (or thumbprint) of participant:

To be completed by the witness (in case the participant/legal guardian is illiterate

Date:

Name of witness:

Signature of witness:

Note: If the participant (or legal guardian) is unable to read and/or write, an impartial witness should be present during the informed consent discussion. After the written informed consent form is read and explained to the participant and after (s) he has orally consented to participation in the study, and has provided fingerprint, the witness should complete the name of the participant, add the date of fingerprint, and sign, and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by the participant (or legal guardian), and that informed consent was freely given.

To be completed by the person obtaining the informed consent

I, undersigned, ... confirm that I have informed the participant (and/or the legal guardian) about all the relevant aspects of this study. I confirm that he/she has consented voluntarily to participate in the study.

Part which is destined only to the participant or his legal representative

I understood and I have been informed about the study and the participant informed consent or assent form sheet copy is given to me.

The study was explained to me in a simple and clear language with enough information. Sufficient time was given to me to realize and ask questions, to which I got satisfactory answers. I am freely willing to be part of the study and cooperate in the study activities. I am willing to give information concerning my medical history, clinical examination and participate in giving blood and wound swab samples. If I want to discontinue participating, even after signing the informed consent, I know I can do so. I agree that my doctor and other healthcare professionals involved in my diagnosis are informed about my participation in this study.

☐

Yes /

☐

No:

To be completed by the participant

Date:

Name of the participant:

Signature (or thumbprint) of participant:

Note: the fingerprint replaces the signature, ONLY for illiterate participants (accompanied by a witness)

To be completed by the legal guardian of the participant (in case the participant is 0 to 18 years old)

Date:

Name of legal guardian:

(In case there is no 'legal' guardianship, consider asking to add the relationship of the guardian with the participant here as well)

Signature (or thumbprint) of legal guardian:

Note: the fingerprint replaces the signature, ONLY if the legal guardian was illiterate (accompanied by a witness)

To be completed by the witness (in case the participant/legal guardian is illiterate)

Date:

Name of witness:

Signature of witness:

Note: If the participant (or legal guardian) was unable to read and/or write, an impartial witness should be present during the informed consent discussion. After the written informed consent form is read and explained to the participant and after (s) he has orally consented to participation in the

Study, and has provided fingerprint, the witness should complete the name of the participant, add the date of fingerprint, and sign, and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information

was accurately explained to, and apparently understood by the participant (or legal guardian), and that informed consent was freely given. study, and has provided fingerprint, the witness should complete the name of the participant, add the date of fingerprint, and sign, and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by the participant (or legal guardian), and that informed consent was freely given.

To be completed by the person obtaining the informed consent

III. Amharic Version Form

I, undersigned, ... confirm that I have informed the participant (and/or the legal guardian) about all the relevant aspects of this study. I confirm that he/she has consented voluntarily to participate in the study.

Date:

Signature:

አባሪ I.

የምርምር ተሳታፊ የመረጃ ወረቀት

የጥናቱ ርዕስ:- የባክቴሪያ ኤቲዮሎጂ፣ ፀረ-ተህዋሲያን ተጋላጭነት መገለጫ እና ከቀዶ ጥገና በኋላ የሚመጡ በሽታዎች እና የደም ዝውውር ኢንፌክሽን ተያያዥነት ያላቸው ምክንያቶች በ ATRH ደቡብ ምስራቅ አርሲ፣ ኢትዮጵያ

መርማሪ: አቶ በቀለ ዳቢ

ድርጅት: የጤና ሳይንስ ኮሌጅ: የአርሲ ዩኒቨርሲቲ

ስፖንሰር: አርሲ ዩኒቨርሲቲ: ኢትዮጵያ

በዚህ አካባቢ በጣም ለሕይወት አስፈጻሚ በሆነው በቀዶ ሕክምና ቦታ ኢንፌክሽን ላይ በሚደረገው ጥናት ላይ እንዲሳተፉ ተጋብዘዋል። በጥናቱ ውስጥ ለመሳተፍ ከመወሰንዎ በፊት በዚህ ቅጽ ውስጥ ያለውን መረጃ መረዳት የተሻለ ነው ምክንያቱም መብቶችዎን እና ለእርስዎ ያለንን ሀላፊነቶች ያብራራል። ይህ የመረጃ እና

የስምምነት ወይም የስምምነት ቅጽ ዓላማን፣ ምርመራዎችን፣ ሊሆኑ የሚችሉ ጥቅሞችን፣ አደጋዎችን እና ከዚህ ጥናት ጋር የተያያዙ ችግሮችን ያካትታል። በጥናቱ ላይ ከመሳተፍ የመልቀቂያ መብትዎ ከዚህ በታች ተብራርቷል። ከጥናቱ ጋር በተገናኘ በማንኛውም ጊዜ ማንኛውንም ጥያቄ የመጠየቅ መብት አልዎት። የእርስዎ ተሳትፎ በፈቃደኝነት ነው። ደስተኛ የሚሰማዎትን ማንኛውንም ሰው ማነጋገር ይችላሉ እና ለመሳተፍ ወይም ለመሳተፍ ለመወሰን ጊዜ ሊወስዱ ይችላሉ።

የጥናቱ ዓላማ

የዚህ ጥናት ዓላማ የበክቴሪያ ኤቲኦሎጂ፣ ፀረ-ተሕዋስያን ተጋላጭነት መገለጫ እና ከቀዶ ሕክምና በኋላ የሚደረጉ ኢንፌክሽኖች እና የደም ዝውውር ኢንፌክሽን ተጋላጭነቶችን በ ATRH, ደቡብ ምሥራቅ አርሲ, ኢትዮጵያ ውስጥ በ SSI ከተጠረጠሩ ታካሚዎች መካከል።

SSI በዓለም ላይ ትልቅ ሽክም ከሆኑ በጣም የተለመዱ በሽታዎች አንዱ ነው። ከፍተኛ ገቢ ካላቸው አውራጃዎች ይልቅ በንብረት ውስን አገሮች ውስጥ በብዛት ይታያል። SSI በተለይ ኢትዮጵያን ጨምሮ በአፍሪካ እጅግ አስከፊ ነው። በአብዛኛው የሚከሰተው በበክቴሪያዎች ነው። ይሁን እንጂ የስርጭቱ እና የክብደቱ መጠን በበርካታ ሁኔታዎች ላይ የተመሰረተ ነው፣ ለምሳሌ በሶሻሎ-ስነ-ሕዝብ, በወቅት, በእድሜ, በጾታ, በበሽታ መከላከያ, በበሽታ, በጤና ስርዓት ጥራት እና ወዘተ. ስለዚህ በ ATRH ውስጥ ያሉ ታካሚዎች በዚህ ምርመራ ውስጥ ይሳተፋሉ።

የጥናት ሂደት:

በዚህ ጥናት ላይ ለመሳተፍ ፍቃደኛ ከሆኑ እና ብቁ ከሆኑ የሚከተለውን መረጃ ይጠየቃሉ።

- 1) ትንሽ ቃለ መጠይቅ፣ እሱም የእርስዎን የሶሻሎ ስነ-ህዝብ ባህሪያትን ያካትታል
- 2) ለላቦራቶሪ ምርመራዎች ናሙናዎች መሰብሰብ: አጠቃላይ 10ml ደም እና የቁስል እጢ ይሰበሰባል። ለዚህ ጥናት የሚደረጉ ማንኛውም ሂደቶች ከክፍያ ነጻ ናቸው።

ስጋቶች እና አለመመቻቸ

በጥጥ በተሰራው ቦታ ላይ የሚሰማውን የጥጥ ሳሙና ትንሽ ከመንካት በስተቀር የቁስሉን እጥበት ለመውሰድ ምንም አይነት አደጋ የለም። የቪነስ ደም መሳብ በቦታው ላይ አጭር ምችት፣ መጎዳት እና ሄማቶማ ሊያስከትል ይችላል። በተጨማሪም ትንሽ የመያዝ ወይም ራስን የመሳት አደጋ አለ። የሰለጠኑ ሰራተኞች ስጋቶችን ለመቀነስ የጸዳ ቴክኒኮችን በመጠቀም ደም ይሰበስባሉ። የካቴተር ምክሮችን መሰብሰብ ምንም ተጨማሪ አደጋ አያስከትልም፣ ምክንያቱም በመደበኛነት እንደ የዲያሊስስ እንክብካቤ አካል ስለሚወገዱ። የንጽሕና መጠበቂያዎች ጥቅም ላይ ስለሚውሉ የአካባቢን ማጠብ አነስተኛ አደጋን ያካትታል። ተሳትፎ በቀዶ ጥገና ጉብኝቶች ወቅት ለቃለ መጠይቁ እና ለሳሙና ማሰባሰብ ጊዜዎን ይጠይቃል። ምችትን ለመቀነስ ከቀዶ ጥገና መርሃ ግብርዎ ጋር እናስተባብራለን። የመረጃዎን ሚስጥራዊነት የማጣት ትንሽ አደጋ አለ። የእርስዎን ውሂብ እና ግላዊነት ለመጠበቅ ጥብቅ የደህንነት እርምጃዎችን እንጠቀማለን። በዚህ ጥናት ውስጥ መሳተፍ በፈቃደኝነት ነው። አንዳንድ ጥያቄዎችን ለመመለስ ምችት ሊሰማዎት ይችላል። ማንኛውንም ጥያቄ ለመመለስ እምቢ ማለት እና በማንኛውም ጊዜ ማንሳት ይችላሉ።

በአሁኑ ጊዜ የማይታወቁ ወይም የማይቻሉ አደጋዎች ሊኖሩ ይችላሉ። እባክዎን የሚያሳስብዎት ነገር ካለ በማንኛውም ጊዜ የጥናት ቡድኑን ያግኙ። ከደም መሰብሰብ በኋላ ስለ ማንኛውም ምችት፣ ሄማቶማ፣ ስብራት ወይም ሌሎች ጉዳዮች ለጥናቱ ሰራተኞች ያሳውቁ። እንክብካቤ እንሰጥዎታለን ወይም ከሐኪምዎ ጋር እንዲከታተሉት እንመክራለን። እባክዎን የጥናት ቡድኑን ስለ ሊኖሩ ስለሚችሉ አደጋዎች ወይም ችግሮች ሌላ ጥያቄዎች ወይም ስጋቶች ካሉዎት ይጠይቁ። የጥናት ተሳትፎን በተቻለ መጠን ለእርስዎ አስተማማኝ እና ምቹ ለማድረግ ቆርጠን ተነስተናል።

ጥቅማ ጥቅሞች

የሕክምና ባለሙያዎች ስለ ቁስል ኢንፌክሽን መከላከያ መረጃ ይሰጡዎታል እና የተገኘው ውጤት የቁስሉን ኢንፌክሽን በተሳካ ሁኔታ ለመቆጣጠር ጥቅም ላይ ይውላል። ከዚህ ጥናት የተማረው መረጃ በኢትዮጵያ እና ከዚያም በላይ ባሉት የቀዶ ህክምና ክፍሎች የኢንፌክሽን ቁጥጥር እና መከላከያ አሰራሮችን ለማሻሻል ይረዳል። ይህ ለሌሎች ታካሚዎች አደገኛ የቀዶ ጥገና-ነክ ኢንፌክሽኖች መጠን እንዲቀንስ ሊያደርግ ይችላል። የእርስዎ ተሳትፎ የኢንፌክሽን መንስኤ የሆኑትን የባክቴሪያ ዓይነቶች በተሻለ ሁኔታ ለመረዳት፣ የምርመራ እና የሕክምና ዘዴዎችን ለማሻሻል አስተዋፅኦ ያደርጋል። የአደጋ መንስኤዎችን መለየት ከፍተኛ ተጋላጭነት ላይ ያሉ ታካሚዎችን መመርመር እና የኢንፌክሽን መቆጣጠሪያ እርምጃዎችን ማነጣጠር ያስችላል።

የፀረ-ተህዋሲያን መከላከያ ዘዴዎችን መረዳት የተሻሉ የአንቲባዮቲክ ማዘዣ ልምዶችን ለመቅረጽ ይረዳል። በአጠቃላይ ይህ ጥናት በኢትዮጵያ የቀዶ ጥገና ህክምናን ጥራት እና ደህንነትን ለማሳደግ በማስረጃ ላይ የተመሰረተ ፖሊሲዎችን ለመምራት ያለመ ነው። እንደ የዚህ ጥናት አካል የተደረገ ማንኛውንም የደም ባህል ወይም ሌላ የናሙና ምርመራ ውጤት ያገኛሉ፤ ይህም እንክብካቤዎን ሊረዳ ይችላል። ሆኖም፣ ሌላ የግለሰብ ውጤቶች አይቀርቡም። በዚህ ጥናት ውስጥ ለመሳተፍ ምንም ክፍያ የለም። ነገር ግን፣ የእርስዎ አስተዋጽኦ የታካሚ እንክብካቤን፣ የኢንፌክሽን መከላከልን እና የቀዶ ጥገና ልምዶችን ለማሻሻል ጠቃሚ መረጃን ይሰጣል። እባክትን የጥናቱ ጥቅማጥቅሞች ወይም ጠቀሜታ በተመለከተ ሌላ ማንኛውም ጥያቄ ካሎት ለአጥኚው ቡድን ያሳውቁ። ለመሳተፍ ፈቃደኛ መሆን ሌሎች በቀዶ ጥገና ላይ ያሉ ታካሚዎችን ለመጠበቅ ሊረዳ ይችላል።

ማካካሻ

ሁሉም ከጥናት ጋር የተያያዙ ፈተናዎች ለእርስዎ ከክፍያ ነጻ ናቸው።

የግል ሕይወትዎ ጥበቃ

የእርስዎን ግላዊነት ለመጠበቅ የምንችለውን ሁሉ እናደርጋለን። ከህክምና መዝገብዎ የተወሰደ መረጃ እንደ ጥብቅ ሚስጥራዊ ይቆጠራል። መረጃው በስምህ ሳይሆን በቡድን ነው የሚታወቀው። ስምዎ የተጠቀሰባቸው ሰነዶች ከአጥኚዎቹ ተመራማሪዎች እና ሚስጥራዊ ከሆኑ ዶክተርዎ በስተቀር ለማንም አይገኝም። ይህን በመረጃ የተደገፈ የስምምነት ወይም የፍቃድ ቅጽ በመፈረም፣ በዚህ የመዝገቦች መዳረሻ ተስማምተዋል። የጥናቱ ግኝቶች በሕክምና ጀርድሎች ውስጥ የሚታተሙ ሲሆን በዓለም አቀፍ የውሂብ ጎታ ውስጥ ሊካፈሉ ይችላሉ። በዚህ ጥናት ውስጥ በማንኛውም የውሂብ ጎታ፣ ሪፖርት ወይም ህትመት ውስጥ ስምዎ አይታይም።

የሥነ ምግባር ኮሚቴ

የአርሲ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የቅዱስ ጳውሎስ ሆስፒታል ሚሊኒየም ሕክምና ኮሌጅ የሥነ ምግባር ኮሚቴ ጥናቱን ከመጀመሩ በፊት ገምግሞ ያፀድቃል። ኮሚቴው ጥናቱ በተቻለ መጠን አስተማማኝ በሆነ

መንገድ መካሄዱን ለማረጋገጥ ቀጣይነት ያለው ግምገማ ያደርጋል። ከሊኒካዊ አግባብነት ያላቸው ውጤቶች ሲገኙ፣ የሕክምና ባለሙያው ወዲያውኑ ይነገራል፣ አስፈላጊ ሆኖ ሲገኝ የታካሚውን ማንነት ማወቅ ይችላል።

በፈቃደኝነት ተሳትፎ

በዚህ ጥናት ውስጥ ያለዎት ተሳትፎ ሙሉ በሙሉ በፈቃደኝነት ነው። የእርስዎ ተሳትፎ እንደ ምርጫዎ ይወሰናል። በጥናቱ ላይ ለመሳተፍ ወይም ላለመሳተፍ ያደረጉት ውሳኔ ከሆስፒታል በሚያገኙት አገልግሎት ላይ ምንም አይነት ተጽእኖ አይኖረውም። የስምምነት ወይም የስምምነት ቅጹን ከፈረሙ በኋላም ቢሆን በማንኛውም ጊዜ ከጥናቱ የመውጣት ሙሉ መብት አለዎት። የጥናቱ አካል ከመሆን ለማቆም የምትፈልግበትን ምክንያት መስጠት አያስፈልግም።

በጥያቄዎች ጊዜ ሰውን ያነጋግሩ

በዚህ ጥናት ውስጥ ስለመሳተፍዎ፣ ስለመብትዎ ወይም በጥናቱ ምክንያት ጉዳት ደርሶብዎታል ብለው የሚያስቡ ከሆነ፣ ጥያቄዎች በሚነሱበት ጊዜ የሚያነጋግሩት ሰው፣ በጥናቱ ውቅት፣ ወይም ከጥናቱ በኋላ ማንኛውም ሰው ማነጋገር ይችላሉ። የእውቂያ መረጃ ከዚህ በታች ቀርቧል።

ስም	ስልክ ቁጥር.	የ ኢሜል አድራሻ
1 በቀለ ዳቢ	+251924066208	bekeledabi19@gmail.com
2 ዶ/ር አንተነህ መሃሪ	+2519 12060506	antenehmehari@gmail.com
3 ዘነበ ገ/ዮሐንስ	+2519 13294377	zenebezoha@gmail.com

II. የስምምነት/የእውቅና ቅጽ

የጥናቱ ርዕስ፡- የባክቴሪያ ኢቲዮሎጂ፣ ፀረ-ተህዋሲያን ተጋላጭነት መገለጫ እና ተያያዥ አደጋዎች የቀዶ ጥገና ጣቢያ ኢንፌክሽን እና የደም ስርጭት መንስኤዎች በATRH፣ ደቡብ ምስራቅ አርሲ፣ ኢትዮጵያ፡ የወደፊት የቡድን ጥናት ንድፍ

ለተሳታፊው ወይም ለህጋዊ ወኪሉ ብቻ የተወሰነ ክፍል

እኔ የተረዳሁት ለተሳታፊው ወይም ለህጋዊ ተወካዩ ብቻ የተወሰነ ክፍል እና ስለ ጥናቱ ተነግሮኛል እናም ተሳታፊው በመረጃ የተደገፈ ስምምነት ወይም የፍቃድ ቅፅ ቅጂ ተሰጠኝ። ጥናቱ በቂ መረጃ ባለው ቀላል እና ግልጽ ቋንቋ ተብራርቶልኛል። ለመገንዘብ እና ለመጠየቅ በቂ ጊዜ ተሰጥቶኝ አጥጋቢ መልስ አግኝቻለሁ። የጥናቱ አካል ለመሆን እና በጥናት እንቅስቃሴዎች ውስጥ ለመተባበር በነፃነት ፈቃደኛ ነኝ። የሕክምና ታሪኬን ፣ ክሊኒካዊ ምርመራዬን በተመለከተ መረጃ ለመስጠት እና የደም እና የቁስል እብጠት ናሙናዎችን ለመስጠት ፈቃደኛ ነኝ ። መሳተፍን ማቋረጥ ከፈለግኩ፣ በመረጃ የተደገፈ ስምምነት ከፈረምኩ በኋላም ቢሆን፣ ይህን ማድረግ እንደምችል አውቃለሁ። በምርመራዬ ውስጥ የተሳተፉት ሀኪሜ እና ሌሎች የጤና አጠባበቅ ባለሙያዎች በዚህ ጥናት ውስጥ ስለመሳተፍበት መረጃ እንደተሰጣቸው እስማማለሁ።

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በአሳታፊው የሚጠናቀቅ

ቀን:

የተሳታፊው ስም.....

የተሳታፊው ፊርማ (ወይም የጣት አሻራ):.....

በምስክሩ መሞላት (ተሳታፊው/ህጋዊ ሞግዚቱ ማንበብና መጻፍ የማይችል ከሆነ)

ቀን:

የምሥክር ስም:

የምሥክር ፊርማ:

ማስታወሻ፡ ተሳታፊው (ወይም) ከሆነ ህጋዊ ሞግዚት) ማንበብ እና/ወይም መፃፍ አይችልም፤ በመረጃ ላይ የተመሰረተ የስምምነት ውይይት በሚደረግበት ጊዜ ገለልተኛ ምስክር መገኘት አለበት። በጽሑፍ የተገለጸው የስምምነት ቅጽ ከተነበበ በኋላ ለተሳታፊው ከተገለጸ በኋላ (ዎች) በጥናቱ ለመሳተፍ በቃል ከፈቀዱ እና የጣት አሻራ ካቀረቡ በኋላ ምስክሩ የተሳታፊውን ስም መሙላት፤ የጣት አሻራ ቀን መጨመር እና መፈረም እና የፍቃድ ቅጹን በግል ቀን ያድርጉ። የስምምነት ቅጹን በመፈረም ምስክሩ በስምምነት ቅጹ ላይ ያለው መረጃ እና ማንኛውም ሌላ የጽሁፍ መረጃ በትክክል የተብራራለት እና በተሳታፊው (ወይም ህጋዊ ሞግዚት) የተረዳ እንደሆነ እና በመረጃ ላይ የተመሰረተ ስምምነት በነጻ መሰጠቱን ያረጋግጣል።

በመረጃ የተደገፈ ፈቃድ ባገኘ ሰው መሞላት አለበት

እኔ የተስተካከለ, ... የዚህ ጥናት ተዛማጅ ገጽታዎች. እሱ/ሷ በጥናቱ ለመሳተፍ በፈቃዳቸው መስማማታቸውን አረጋግጣለሁ። እኔ የተረዳሁት ለተሳታፊው ወይም ለህጋዊ ተወካዩ ብቻ የተወሰነ ክፍል እና ስለ ጥናቱ ተነግሮኛል እናም ተሳታፊው በመረጃ የተደገፈ ስምምነት ወይም የፍቃድ ቅፅ ቅጂ ተሰጠኝ። ጥናቱ በቂ መረጃ ባለው ቀላል እና ግልጽ ቋንቋ ተብራርቶልኛል። ለመገንዘብ እና ለመጠየቅ በቂ ጊዜ ተሰጥቶኝ አጥጋቢ መልስ አግኝቻለሁ። የጥናቱ አካል ለመሆን እና በጥናት እንቅስቃሴዎች ውስጥ ለመተባበር በነፃነት ፈቃደኛ ነኝ። የሕክምና ታሪኬን ፣ ክሊኒካዊ ምርመራዬን በተመለከተ መረጃ ለመስጠት እና የደም እና የቁስል እብጠት ናሙናዎችን ለመስጠት ፈቃደኛ ነኝ ። መሳተፍን ማቋረጥ ከፈለግኩ፣ በመረጃ የተደገፈ ስምምነት ከፈረምኩ በኋላም ቢሆን፣ ይህን ማድረግ እንደምችል አውቃለሁ። በምርመራዬ ውስጥ የተሳተፉት ሀኪሜ እና ሌሎች የጤና አጠባበቅ ባለሙያዎች በዚህ ጥናት ውስጥ ስለመሳተፍበት መረጃ እንደተሰጣቸው እስማማለሁ።

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በአሳታፊው የሚጠናቀቅ

ቀን፡

የተሳታፊውስም.....

የጣት አሻራው ፊርማውን ይተካዋል፤ ማንበብ ላልቻሉ ተሳታፊዎች ብቻ (በምስክር የታጀበ)

በተሳታፊው ህጋዊ ሞግዚት መሞላት (ተሳታፊው ከ 0 እስከ 18 ዓመት ዕድሜ ላይ ከሆነ)

ቀን:

ህጋዊ ሞግዚት ስም:

(‘ህጋዊ’ ሞግዚትነት ከሌለ፣ የአሳዳጊውን ግንኙነት ለመጨመር መጠየቅ ያስቡበት) እዚህ ያለው ተሳታፊም)

የህጋዊ ሞግዚት ፊርማ (ወይም አውራ ጣት):

ማስታወሻ: የጣት አሻራ ፊርማውን ይተካዋል፤ ህጋዊ ሞግዚቱ ማንበብና መጻፍ የማይችል ከሆነ ብቻ (ከምሥክር ጋር)

በምስክሩ መሞላት (ተሳታፊው/ህጋዊ ሞግዚቱ ማንበብና መጻፍ የማይችል ከሆነ)

ቀን:

የምሥክር ስም:

የምሥክር ፊርማ:

ማስታወሻ: ተሳታፊው (ወይም) ከሆነ ህጋዊ ሞግዚት) ማንበብ እና/ወይም መጻፍ አይችልም፤ በመረጃ ላይ የተመሰረተ የስምምነት ውይይት በሚደረግበት ጊዜ ገለልተኛ ምስክር መገኘት አለበት። በጽሑፍ የተገለጸው የስምምነት ቅጽ ከተነበበ በኋላ ለተሳታፊው ከተገለጸ በኋላ (ዎች) በጥናቱ ለመሳተፍ በቃል ከፈቀዱ እና የጣት አሻራ ካቀረቡ በኋላ ምስክሩ የተሳታፊውን ስም መሙላት፣ የጣት አሻራ ቀን መጨመር እና መፈረም እና የፍቃድ ቅጹን በግል ቀን ያድርጉ። የስምምነት ቅጹን በመፈረም ምስክሩ በስምምነት ቅጹ ላይ ያለው መረጃ እና ማንኛውም ሌላ የጽሁፍ መረጃ በትክክል የተብራራለት እና በተሳታፊው (ወይም ህጋዊ ሞግዚት) የተረዳ መሆኑን እና በመረጃ ላይ የተመሰረተ ስምምነት በነጻ መሰጠቱን ያረጋግጣል። የጣት አሻራ ተሰጥቷል፤ ምስክሩ የተሳታፊውን ስም መሙላት፣ የጣት አሻራውን ቀን ማክል እና መፈረም እና የፍቃድ ቅጹን በግል መወሰን አለበት። የስምምነት ቅጹን በመፈረም ምስክሩ በስምምነት ቅጹ ላይ ያለው መረጃ እና ማንኛውም ሌላ የጽሁፍ መረጃ በትክክል የተብራራለት እና በተሳታፊው (ወይም ህጋዊ ሞግዚት) የተረዳ እንደሆነ እና በመረጃ ላይ የተመሰረተ ስምምነት በነጻ መሰጠቱን ያረጋግጣል።

በመረጃ የተደገፈ ፈቃድ ባገኘ ሰው መሞላት አለበት

III. የአማርኛ ቅጂ ቅፅ

እኔ የተስተካከለ, ...

የዚህ ጥናት ተዛማጅ ገጽታዎች. እሱ/ሷ በጥናቱ ለመሳተፍ በፈቃዳቸው መስማማታቸውን አረጋግጣለሁ።

ቀን:

ፊርማ:

IV. Afaan Oromoo Version Form

I, undersigned, ...confirm
that I have informed the participant (and/or the legal guardian) about all the relevant aspects of
this study. I confirm that he/she has consented voluntarily to participate in the study.

Date:

Signature:

I.Waraqaa Odeeffannoo Hirmaattota Qorannoo

Mata duree qorannichaa: Taateewwan Infeekshinii fi Infeekshinii Ujummoo Dhiigaa, Etiology, Qoricha Dandamachuu piroofiyilii Baakteeriyaa, Saaxilamummaa Farra Maayikiroobiyaanii fi Qabxiilee Balaa dhukkubsattoota tajaajila Baqasanii yaala guddaa Infeekshinii madaa fi malaa uumuu jala jiran biratti, Arsii Hoospitaala Rifaraala Asallaa Kallattii Kibba Bahaa, Itoophiyaa: Qo’annoo garee Fulduraa Giddugala Hedduu

Qorataa: Obboo Baqqala Dhaabi (MLT, Kaadhimamaa Digrii Lammaffaa)

Dhaabbata: Arsii Univarisiitii Kollejii Saayinsii Fayyaa

Ispoonsara: Arsii Univarisiitii

Dhukkubsattoota dhukkuba infeekshinii madaa fi walitti dhufeenya malaa uumuu qabaniif yaala lubbuu baraaruu Kan ta’e baqasanii yaalu irratti akka hirmaattan affeeramtaniittu. Qorannicha irratti hirmaachuuf murteessuu dura odeeffannoo bifa kanaan jiru hubachuun gaariidha. Sababni isaas mirga kee fi itti gaafatamummaa nuti siif qabnu gabaabsa waan ta’eef. Odeeffannoo fi unki hayyamaa Kun kaayyoo, qormaata, faayidaa, balaa fi rakkoolee qorannoo kanaan walqabatan ta’uu danda’an of keessaa qaba. Mirgi qorannoo kana irratti hirmaachuu irraa aangoo gadhiisuu keessanis akka armaan gadiitti ibsameera. Gaaffii qorannoo kanaan walqabatee yeroo barbaaddetti gaafachuuf mirga qabda. Hirmaannaan keessan fedhii keessaniini. Yeroo guuttu Nama kamiyyuu wajjin haasa’uu dandeessa. Akkasumas, yeroo fudhattee, hirmaachuu fi dhiisuu murteessuu dandeessa.

Kaayyoo qorannichaa

Qorannoon kun dhukkubsattoota wal’aansa Qoricha Dandamachuu piroofiyilii Baakteeriyaa, Saaxilamummaa Farra Maayikiroobiyaanii fi Qabxiilee Balaa dhukkubsattoota tajaajila Baqasanii yaala guddaa Infeekshinii madaa fi malaa uumuu jala jiran biratti, Arsii Hoospitaala Rifaraala Asallaa Kallattii Kibba Bahaa, Itoophiyaa fudhataman gidduutti taatee, sababoota, fi sababoota balaa dhukkuba kanaan qabaman qorachuuf kan akeekedha.

II. Unka Hayyamaa / Hayyama

Taateewwan Infeekshinii fi Infeekshinii Ujummoo Dhiigaa, Etiology, Qoricha Dandamachuu piroofiyilii Baakteeriyaa, Saaxilamummaa Farra Maayikiroobiyaanii fi Qabxiilee Balaa dhukkubsattoota tajaajila Baqasanii yaala guddaa Infeekshinii madaa fi malaa uumuu jala jiran biratti, Arsii Hoospitaala Rifaraala Asallaa Kallattii Kibba Bahaa, Itoophiyaa: Qo’annoo garee gara fuula duraa Giddugala Hedduu

Kutaa hirmaataa ykn bakka bu’aa seeraa isaa qofaaf murtaa’e

Hubadheera, waa’ee qorannichaas naaf beeksiseera, hirmaataan hayyama ykn hayyama beekumsa qabu waraabbii waraqaa unkaa naaf kennama.

Qorannoon Kun afaan salphaa fi ifa ta’een odeeffannoo gahaa ta’een naaf ibsame. Yeroon gahaan akkan hubadhuu fi gaaffii gaafachuuf naaf kenname, kanaafis deebii quubsaa argadhe. Qaama qo’annichaa ta’ee hojiiwwan qo’annichaa irratti tumsuuf bilisaan fedhii qaba. Seenaa yaalaa Koo, qorannoo kilinikaa ilaalchisee odeeffannoo kennuu fi saamuda dhiigaa fi madaa kennuu irratti hirmaachuuf fedhii qaba. Yoon hirmaachuu addaan kutuu barbaade, erga hayyama beekumsa qabu mallatteessee boodas, kana gochuu akkan danda’u Nan beeka. Doktorri Koo fi ogeeyyiin fayyaa biroo qorannoo kana keessatti hirmaatan waa’ee hirmaannaa Koo qorannoo kana irratti akka beeksisan walii gala.

☐

Eeyyee

☐

Lakki

Unka Hirmaattotaan guuttamu

Guyyaa:

Maqaa Hirmaataa:

Mallattoo hirmaataa ykn Ashaara:

Hubachiisa: - Kan Ashaaraadhaan mallatteessuu danda’an namoota barnoota idilee hin barannee qofaaf tajaajila.

Unka Guddiftuu seeraatiin Kan guuttamu (hirmataan daa'imman 0-18 gadi yoo ta'e

Guyyaa:

Maqaa maati Guddiftuu daa'ima:

Warri guddiftuu Kan yoo seeraa jala to'ataman taanani isaan hirmaachiisu qabna.

Mallattoo ykn Ashaara Guddiftuu:

Unka Nama ragaatiin guuttamu guddiftuun seeraa yoo dubbisu fi barreessuu hin dandeenye

Guyyaa:

Maqaa Ragaa:

Mallattoo Ragaa.....

Hubachiisa: Hirmaataan ykn guddiftuu seera barreessuu erga hin dandeenye raga kenname irratti Nama wabii of danda'e Ni barbaachiisa.hirmaata qorannoo keessatti hirmachiisuuf guddiftuu seeraa ykn immoo ashaara kennu yoo danda'e wabiilee isaatiin raggaasiise sirriitti xinxallu isaa baramu qaba.

Abbaan hirmaatuu suni Unka waliigaltee argate guutuu qaba.

Ani.....namoota hirmaatan hundumaaf beeksiise wantoota qo'annoo kana keessatti argaman baafata barbaachiisoo akka raggaasiisan mirkaneessi.

Guyyaa:

Mallattoo:

V. Structured, an Interviewer Administered Questionnaires

Arsi University Medical College Department of Medical Microbiology, Questionnaire on the Socio Demographic Data for Bacterial profile and Drug Resistance pattern study among Wound infection Associated with Septicemia at Assela Referral and Teaching Hospital In 2023.

Questionnaire to assess socio demographic characteristics and health condition of participant

Study number: _____ Date of interview: ____/____/____

DD MM YYYY

1. Study site _____
2. Study ID from 001 to 150 _____
3. Medical Record Number _____
4. Ward _____
5. Does the patient meet the criteria of inclusion (Patients who are suspected to have blood stream infections, Patients who are volunteer) ☐ Yes ☐ No
6. Note: if the patient does not meet the inclusion criteria, please exclude the patient from the study
7. Date of interview in Gregorian calendar (dd/mm/yy) _____

I. Socio-demographic information

8. Gender ☐ Male ☐ Female
9. Age in year's _____
10. Residence ☐ Rural ☐ Urban

11. Nationality ☐ Ethiopian ☐ Non Ethiopian
12. Religion ☐ Orthodox ☐ Muslim ☐ Protestants ☐ Wakefeta ☐
Other specify
13. Marrital status ☐ Married ☐ Unmarried ☐ Divorced ☐ Widowed
14. Educational status No formal education ☐ 1-4 ☐ 5-9 ☐ 10-12 ☐
above.....
15. Occupation ☐ Farmer ☐ Shopkeeper ☐ Government employee
☐ Daily laborer ☐ Student ☐ Others, please, specify.....
16. How many are you in a house? Please, write family size in number _____

II. Questions on the patient clinical symptoms

17. How many days have you been sick? Please, write number of day's _____
18. Have you contact with any one with SSI? ☐ Yes ☐ No
19. What is the cause of the wound? ☐ Surgical ☐ Burns ☐ Accident ☐ Bites
☐ others specify.....
20. Duration of wound?
21. Surgical procedure performed.....
22. Do you have redness of the skin? ☐ Yes ☐ No
23. Have you sudden fever, chills, and pain? ☐ Yes ☐ No
24. Have you delayed healing process of wound? ☐ Yes ☐ No
25. Have you tenderness and warmth? ☐ Yes ☐ No

26. Have you swelling? ☐ Yes ☐ No
27. Have you produce pus discharge from the wound site during incisional? ☐ Yes ☐ No
28. Have you loss of appetite? ☐ Yes ☐ No
29. Have you fatigue and weakness? ☐ Yes ☐ No
30. Had you nausea and vomiting? ☐ Yes ☐ No
31. Have you rapid heart rate? ☐ Yes ☐ No
32. Have you feeling cold? ☐ Yes ☐ No
33. Have you photophobia or light sensitive? ☐ Yes ☐ No
34. Have you sleepiness or difficulty of waking? ☐ Yes ☐ No
35. Have you irritability? ☐ Yes ☐ No
36. Have you general pain and discomfort? ☐ Yes ☐ No
37. Are you pregnant? (If female age of > 18 years) ☐ Yes ☐ No
38. Have you a follow up for diabetes mellitus? ☐ Yes ☐ No
39. Do you smoke cigarretes? ☐ Yes ☐ No
40. Do you have exposure to smokers? ☐ Yes ☐ No
41. Have experienced invasive devices and procedures? ☐ Yes ☐ No
42. Have been long time hospilalized? ☐ Yes ☐ No

43. Have you taken any antibiotic before 30 days? ☐ Yes ☐ No
44. Do you take drugs prescribed for you appropriately and completely? ☐ Yes ☐ No
45. Do you use broad spectrum drugs frequently? ☐ Yes ☐ No
46. Did you take antimicrobials for long time? ☐ Yes ☐ No
47. We are going to take blood and swab sample, would you give us? ☐ Yes ☐ No
48. Sample is taken? ☐ Yes ☐ No

49. Antibiotics prescribed

.....

.....

.....

.....

III. Clinical Information Based on Physical Examination and Patient Chart

50. Body temperature of the patient in degree centigrade _____
51. Is there any underlining disease? If so, please, specify _____

የጥናት ቃለ መጠይቅ ቅጽ

1. የጥናት ቦታ _____
2. የጥናት መታወቂያ ከ 001 እስከ 150 _____
3. የሕክምና መዝገብ ቁጥር _____

4. ዋርድ _____

5. በሽተኛው የመደመር መስፈርቱን አሟልቷል (የደም ዝውውር ኢንፌክሽን በሽታ ያለባቸው ታማሚዎች፣

በጎ ፈቃደኞች የሆኑ ታካሚዎች) አዎ አይደለም

6. ማሳሰቢያ፡ በሽተኛው የማካተት መስፈርቱን ካላሟላ፣ እባክትን በሽተኛውን ከጥናቱ ማግለል

7. የቃለ መጠይቁ ቀን በጎርጎርያን ካላንደር (ቀን/ሚያዝያ/ዓ) _____

I. ማህበራዊ-ስነ-ህዝብ መረጃ

8. ጾታ ወንድ ሴት

9. እድሜ ዓመታት _____

10. የመኖሪያ ገጠር ከተማ

11. ብሄረሰብ ኢትዮጵያዊ ያልሆነ

12. ሀይማኖት ኦርቶዶክስ ሙስሊም ፕሮቴስታንቶች ዋቄፈታ

ሌላም ይገልጻሉ በላይ

15. ስራ ገበሬ ባለሱቅ የመንግስት ሰራተኛ የቀን ሰራተኛ ተማሪ

ሌሎች፣ እባክዎን ይግለጹ.....

16. ቤት ውስጥ ስንት ናቸው? እባክዎን የቤተሰብ ብዛትን ቁጥር _____

II ይጻፉ በታካሚው ክሊኒካዊ ምልክቶች ላይ ጥያቄዎች

17. ስንት ቀናት ታምመዋል? እባክዎን የቀኖችን ቁጥር ይጻፉ _____

18. ከ SSI ጋር ግንኙነት ኖረዋል? አዎ አይደለም

19. የቁስሉ መንስኤ ምንድን ነው? የቀዶ ጥገና ቃጠሎ አደጋ

ሌሎችን ይገልጻል

20. የቁስሉ ቆይታ?

21. የቀዶ ጥገና ስራ ተከናውኗል.

22. የቆዳ መቅለት አለብዎት? ☐ አዎ ☐ አይደለም

23. ድንገተኛ ትኩሳት፣ ብርድ ብርድ ማለት እና ህመም አለቦት? ☐ አዎ ☐ አይደለም

24. የቁስል ፈውስ ሂደት ዘግይተሃል? ☐ አዎ ☐ አይ

25. ርህራሄ እና ሙቀት አለዎት? ☐ አዎ ☐ አይደለም

26. እብጦት አለብዎት? ☐ አዎ ☐ አይደለም

27. በቁርጥማት ወቅት ከቁስሉ ቦታ የሚወጣ ፈሳሽ ፈጥረዋል? ☐ አዎ ☐ አይደለም

28. የምግብ ፍላጎት ማጣት አለብዎት? ☐ አዎ ☐ አይደለም

29. ድካም እና ድክመት አለብዎት? ☐ አዎ ☐ አይደለም

30. ማቅለሽለሽ እና ማስታወክ ነበረዎት? ☐ አዎ ☐ አይደለም

31. ፈጣን የልብ ምት አለዎት? ☐ አዎ ☐ አይደለም

32. ቅዝቃዜ ይሰማዎታል? ☐ አዎ ☐ አይደለም

33. የፎቶፊብያ ወይም የብርሃን ስሜት አለህ? ☐ አዎ ☐ አይደለም

34. እንቅልፍ አለቦት ወይም መንቃት ይከብዳል? ☐ አዎ ☐ አይደለም

35. ንዴት አለብህ? ☐ አዎ ☐ አይደለም

36. አጠቃላይ ህመም እና ምችት አለቦት? ☐ አዎ ☐ አይደለም
37. ነፍሰ ጡር ነህ? (የሴት ዕድሜ > 18 ዓመት ከሆነ) ☐ አዎ ☐ አይደለም
38. ለስኳር በሽታ ሜላሊትስ ክትትል አለህ? ☐ አዎ ☐ አይደለም
39. ሲጋራ ታጫሳለህ? ☐ አዎ ☐ አይደለም
40. ለአጫሾች ተጋላጭነት አለህ? ☐ አዎ ☐ አይደለም
41. ወራሪ መሳሪያዎችን እና ሂደቶችን አጋጥሞታል? ☐ አዎ ☐ አይደለም
42. ለረጅም ጊዜ ሆስፒታል ገብተሃል? ☐ አዎ ☐ አይደለም
43. ከ 30 ቀናት በፊት ማንኛውንም አንቲባዮቲክ ወስደዋል? ☐ አዎ ☐ አይደለም
44. ለእርስዎ የታዘዙ መድሃኒቶችን በትክክል እና ሙሉ በሙሉ ይወስዳሉ? ☐ አዎ ☐ አይደለም
45. ሰፊ ስፔክትረም መድሃኒቶችን በብዛት ይጠቀማሉ? ☐ አዎ ☐ አይደለም
46. ፀረ ጀርም መድሃኒቶችን ለረጅም ጊዜ ወስደዋል? ☐ አዎ ☐ አይደለም
47. ደም እና ናሙና እንወስዳለን, ይሰጡናል? ☐ አዎ ☐ አይደለም
48. ናሙና ተወስዷል? ☐ አዎ ☐ አይ

49. የታዘዙ አንቲባዮቲኮች

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III. በአካላዊ ምርመራ እና በታካሚዎች ሰንጠረዥ ላይ የተመሰረተ ክሊኒካዊ መረጃ

50. የታካሚው የሰውነት ሙቀት በዲግሪ ሴንቲግሬድ _____

51. ከስር ያለው በሽታ አለ? ከሆነ፣ እባክዎን ይግለጹ_____

VI. Laboratory Tests, Principles and Procedures

Gram Stain

Gram Staining is the common, important, and most used differential staining techniques in microbiology. It differentiates the bacteria into Gram Positive and Gram Negative Bacteria.

Principle: When the bacteria is stained with primary stain Crystal Violet and fixed by the mordant, some of the bacteria are able to retain the primary stain and some are decolorized by alcohol. The cell walls of gram positive bacteria have a thick layer of protein-sugar complexes called peptidoglycan and lipid content is low. Decolorizing the cell causes this thick cell wall to dehydrate and shrink which closes the pores in the cell wall and prevents the stain from exiting the cell. So the ethanol cannot remove the Crystal Violet-Iodine complex that is bound to the thick layer of peptidoglycan of gram positive bacteria and appears blue or purple in colour.

In case of gram negative bacteria, cell wall also takes up the crystal violet (CV)-Iodine complex but due to the thin layer of peptidoglycan and thick outer layer which are formed of lipids, CV-Iodine complex gets washed off. When they are exposed to alcohol, decolorizer dissolves the lipids in the cell walls, which allows the crystal violet-iodine complex to leak out of the cells. Then when again stained with safranin, they take the stain and appear red in color.

Reagents Used in Gram Staining

Crystal Violet, the primary stain

Iodine, the mordant

A decolorizer made of acetone and alcohol (95%)

Safranin, the counterstain

Procedure of Gram Staining

1. Take a clean, grease free slide.
2. Prepare the smear of suspension on the clean slide with a loop full of sample.
3. Air dry and heat fix
4. Pour Crystal Violet and keep for about 30 seconds to 1 minute and rinse with water.
5. Flood the gram's iodine for 1 minute and wash with water.
6. Then, wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water.
7. Add safranin for about 1 minute and wash with water.
8. Air dry, Blot dry and Observe under Microscope.

Interpretation

Gram-Positive: Blue/Purple Color

Gram Negative: Red Color.

Bacteria Culture Tests

Principle: Clean and aseptic environments are required for pure culture isolation. An inoculating loop is used to streak on agar culture media to create streak plates. Reusable loops are made of wire to withstand heat introduced during the sterilization process. It is important to sterilize the loop prior to use to prevent the growth of unwanted microbes that are present on the loop. The loop is sterilized by dipping it into ethanol, and then flame it to redness. The loop will be allowed to cool before sampling the bacterial inoculum. This is to transfer bacteria aseptically from an inoculum source onto a culture medium. Hence, any tools that could carry out such a task could be used. An agar medium is streaked gently with continuous horizontal lines from the top of the plate

to about a quarter of the way downwards. By rotating the medium at 90 ° and with the cooled sterilized loop will be dagged from the inoculum previous streak line to an uninoculated part of

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the plate. This step will be repeated twice and the final streak will be brought into the center of the plate.

Preparation of Blood Agar

- 1.Suspend 38g of nutrient agar powder in 1 litre of distilled water.
- 2.Heat this mixture while stirring to fully dissolve all components.
- 3.Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.
- 4.Once the nutrient agar has been autoclaved, allow it to cool but not solidify.
- 5.When the agar has cooled to 45-50 °C, Add 5% (vol/vol) sterile defibrinated blood that has been warmed to room temperature and mix gently but well.
- 6.Avoid Air bubbles.
- 7.Dispense into sterile plates while liquid.

Interpretation:

Alpha hemolysis

- Alpha hemolysis is defined by a greenish-grey or brownish discoloration around the colony as a result of the partial lysis of the red blood cells.

- During α -hemolysis, H_2O_2 produced by the bacteria causes hemoglobin present in the RBC of the medium to be converted into methemoglobin.

- Some of the α -hemolytic species are a part of the human normal flora, but some species like *Streptococcus pneumoniae* causes pneumonia and other such severe infections.

Beta hemolysis

- Beta hemolysis is defined by a clear zone of hemolysis under and around the colonies when grown on blood agar.

- The clear zone appears as a result of the complete lysis of the red blood cells present in the medium, causing denaturation of hemoglobin to form colorless products.

- β -hemolytic bacteria include the Group A streptococci like *S. pyogenes* and Group B, *S. agalactiae*, both of which are associated with severe infections in humans.

Gamma hemolysis

- Gamma hemolysis is also called non-hemolysis as no lysis of red blood cells occurs.

- As a result, no change of coloration or no zone of hemolysis is observed under or around the colonies.

- Species like *Neisseria meningitidis* are non-hemolytic or gamma-hemolytic.

Chocolate agar

chocolate agar is essentially as same as blood agar except RBCs are lysed when they are added into a molten agar base. Lysis of RBCs release growth stimulating factors hemin (factor X) and NAD (factor V) that will be used by fastidious organisms. It is used mostly to isolate *N.gonorrhoeae* and *Hemophilus* spps.

Principle: When supplements are added to chocolate agar, fastidious organisms grow best while over growth by contaminating organisms is inhibited. Essential elements to the growth of fastidious organisms are from the digested caesin and animal tissues. Products of the digestion will provide nitrogenous nutrients, amino acids and other necessary elements for growth of organisms.

The cornstarch in the media neutralizes toxic metabolites such as fatty acids which can kill sensitive organisms like *Nisseria* spp. Potassium phosphate keep unoform Ph and sodium chloride maintains osmotic balance. As a variant of blood agar, chocolate agar contains lyzed RBCs by slowly heating at 80 OC. Heating inactivates enzymes degrading NAD. Supplements provided growth factors, X and V.

Preparation of Chocolate Agar

- 1.Heat-lyse a volume of horse or sheep blood that is 5% of the total volume of media being prepared very slowly to 56°C in a water bath.
- 2.Dispense 20 ml into 15×100 mm Petri dishes. Allow the media to solidify and condensation to dry.
- 3.Place the plates in sterile plastic bags and store at 4°C until use.
- 4.As a sterility test, incubate an uninoculated plate for 48 hours at 35-37°C with ~5% CO₂ (or in a candle-jar).

Chocolate agar slants

- 1.Dispense 4 mL of the medium into 16X125 mm screw-cap tubes
- 2.Keep the tubes in slanted position and let them solidify

Chocolate agar slants appear brown to brownish-red color. They should be stored at 4°C when not in use and warmed to room temperature (25°C) before use.

Result interpretation

Colony characteristics in chocolate agar

- 1.*Neisseria meningitidis*: Growth on chocolate agar is greyish, non-hemolytic, round, convex, smooth, moist, glistening colonies with a clearly defined edge.
- 2.*Neisseria gonorrhoeae*: Colonies on Chocolate agar are pinkish-brown and translucent, exhibit smooth consistency and defined margins, and are typically 0.5-1 mm in diameter.
- 3.*Haemophilus influenzae*: Non-hemolytic, opaque cream-to-grey colonies (accompanying Sheep blood agar shows no growth).

Limitations of Chocolate Agar

1. For complete identification of a pathogen, additional biochemical and/or serological tests are required from pure culture.
2. Non-pathogenic bacteria may overgrow the pathogenic bacteria as it is an enriched medium. Therefore, nonselective media may be needed to run side by side.
3. The presence or absence of *N. gonorrhoeae* in a specimen does not rule out the possible presence of other pathogenic organisms.

Modified Thayer-Martin medium (MTM)

Principle: MTM media is a modification of chocolate agar. It is modified by the addition of certain chemicals or drugs to initiate the growth of specific organisms. Dextrose is added to enhance the growth of the gonococci. Hemoglobin provides X factor (hemin). IsovitaleX enrichment provides V factor (NAD), vitamins, amino acids, coenzymes, dextrose, ferric ion and other factors which improve the growth of pathogenic *Neisseria*. These selective media contain the antimicrobial agents, vancomycin, colistin and nystatin to suppress the normal flora. Vancomycin is active primarily against gram-positive bacteria. Colistin inhibits gram-negative bacteria, including *Pseudomonas* species.

Modified Thayer Martin agar contains nystatin to inhibit *Candida albicans*. Trimethoprim lactate refrains swarming of *Proteus* species. It is supplemented with vancomycin, nystatin and colistin to discourage the growth of normal flora including non-pathogenic *Neisseria* spps and to promote selective growth of *N. gonorrhoeae* and *N. meningitidis*. When bacitracin is added to chocolate agar, it inhibits growth of *H. influenzae* by inhibiting growth of other mixed bacteria. Guanine-cytosine bases and growth supplements provide especial growth requirements (factor X and V) that support growth of fastidious organisms when added into chocolate agar for the isolation of fastidious organisms such as *H. influenzae* when incubated at 35-37°C in a 5% CO₂ atmosphere.

Recommended Procedure

1. Allow medium to reach room temperature.

2. Using an inoculum from the specimen, perform a four-quadrant streak to obtain well-isolated colonies. Alternatively, if the specimen is contained on a swab, roll the swab across part of the medium in a Z pattern so that an adequate amount of the sample is transferred. Then taking a sterile loop streak the plate to disperse the sample throughout the medium.

3. Incubate at 35°C in a 5 to 10% CO₂ atmosphere.

4. Examine after 18-24 hours and again at 48 and 72 hours.

Interpretation of Results

1. *Neisseria* species grow while the majority of other organisms are inhibited. *N. gonorrhoeae* produces small, grey to white, mucoid colonies.

2. *N. meningitidis* produces larger bluish-grey, mucoid colonies

Uses

1. To isolate pathogenic *Neisseria* spp from mixed flora of bacteria and fungi

Limitations

☐ Additional tests are needed for confirmation (biochemical, serological)

☐ Pathogenic *Neisseria* spp are incubated at 3-7% of CO₂. this may inhibit other pathogens

☐ Selective media for pathogenic *Neisseria* may inhibit other pathogenic bacteria, e.g., *Haemophilus*.

MacConkey Agar

MacConkey agar is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae and the genus *Pseudomonas*.

Principle: It is used to isolate gram negative enteric bacteria and differentiate lactose fermenters from non lactose fermenters. Components from its composition: Pancreatic digest of gelatin and

peptones (meat and casein) provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms and lactose monohydrate is the fermentable source of carbohydrate. Its selectivity is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Sodium chloride maintains the osmotic balance in the medium. Neutral red is a pH indicator that turns red at a pH below 6.8 and is colorless at any pH greater than 6.8. Agar is the solidifying agent.

Uses of MacConkey Agar

1. Isolation of gram-negative enteric bacteria.
2. Differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria.
3. Isolation of coliforms and intestinal pathogens in water, dairy products and biological specimens.

Preparation of MacConkey Agar

1. Suspend 49.53 grams of dehydrated medium in 1000 ml purified/distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. Cool to 45-50°C.
5. Mix well before pouring into sterile Petri plates.

Result Interpretation on MacConkey Agar

1. Lactose fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile. The red color is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8.
2. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless and transparent and typically do not alter appearance of the medium. *Y. enterocolitica* may appear as small, non-lactose fermenting colonies after incubation at room temperature.

Biochemical Tests

Identification of the bacteria will be done after isolating a pure culture. Biochemical tests for bacterial identification are based on the concept that different bacteria produce unique enzymes (their 'thumbprints') that are capable of catalyzing different biochemical reactions. The procedure for most biochemical tests involves inoculating a pure culture of bacteria onto or into differential media and observing for indicators such as a color change in media. Usually, a series of biochemical tests are performed to accurately identify a bacterial species.

Oxidase Test- Principle, Procedure and Results

Oxidase test is used to determine the ability of an organism to produce cytochrome oxidase enzyme.

Principle: The test specifically detects the presence of cytochrome C oxidase enzyme in aerobic respiration. Cytochrome C oxidase is composed of many enzymes which alternatively oxidize and reduce each other by donating or accepting electrons derived from H₂. Reagent tetramethyl-p-phenylenediamine dihydrochloride impregnated in filter disk is used to determine ability of an organism to produce the cytochrome C oxidase. The reagent serves as an artificial substrate donating electrons and thereby becoming oxidized to a deep purple compound in the presence of the enzyme oxidase and free O₂. Development of pink, then maroon and finally dark purple colouration after rubbing the organism in the oxidase disc containing the reagent indicates positive reaction. The positive reaction involves the conversion of colourless, reduced tetramethyl-p-phenylenediamine to oxidized form into deep purple colour in presence of Cytochrome C oxidase. No colour change is indicative of the negative test result.

Procedure of oxidase test

1. Take a filter paper soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride.
2. Moisten the paper with sterile distilled water.
3. Pick the colony to be tested with wooden or platinum loop and smear in the filter paper.
4. Observe inoculated area of paper for a color change to deep blue or purple within 10-30 seconds.

Or

1. Take a commercially available oxidase disc containing the reagent.
2. Pick the isolated colony to be tested and rub in the disc.
3. Observe for colour change within 10 seconds.

Positive test: Development of deep purple color within 10 seconds

Negative test: Absence of color

Limitations of oxidase tests

1. Auto-oxidization and hence false positive result may be obtained.
2. Nickel, steel, and other wire loops may give false-positive results, so it is important to use only platinum wire.
3. Bacteria grown on media containing high concentrations of glucose show inhibited oxidase activity, so it is recommended to test colonies grown on media without excess sugar, such as nutrient agar.

Oxidase test tube method

1. Grow a fresh culture (18 to 24 hours) of bacteria in 4.5 ml of nutrient broth (or standard media that does not contain a high concentration of sugar)
2. Add 0.2 ml of 1% α -naphthol, and then add 0.3 ml of 1% p-aminodimethylaniline oxalate (Gaby and Hadley reagents).
3. Observe for color changes.

Result interpretation

- ☐ Microorganisms are oxidase positive when the color changes to blue within 15 to 30 seconds.
- ☐ Microorganisms are delayed oxidase positive when the color changes to purple within 2 to 3 minutes.
- ☐ Microorganisms are oxidase negative if the color does not change.

Catalase Test- Principle, Uses, Procedure, Result Interpretation

It is used to differentiate those bacteria that produce an enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci. The catalase test is a biochemical test for aerobic organisms that detects the production of catalase enzyme in the organism. The enzyme decomposes H_2O_2 releasing water and oxygen. The enzyme protects pathogenic organisms from oxidative damage of the reactive oxygen species and it neutralizes bactericidal effects of H_2O_2 . Under the aerobic condition, 3% H_2O_2 is used, whereas 15% H_2O_2 is used under anaerobic conditions.

Principle of Catalase Test: The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old.

Bacteria thereby protect themselves from the lethal effect of Hydrogen peroxide which is accumulated as an end product of aerobic carbohydrate metabolism.

Procedure of Catalase Test

Tube Method

1. Pour 1-2 ml of hydrogen peroxide solution into a test tube.
2. Using a sterile wooden stick or a glass rod, take several colonies of the 18 to 24 hours test organism and immerse in the hydrogen peroxide solution.
3. Observe for immediate bubbling.

Result interpretation

Positive reactions are evident by immediate effervescence (bubble formation).

No bubble formation (no catalase enzyme to hydrolyze the hydrogen peroxide) represents a catalase-negative reaction

Catalase Test (slant) method procedure

1. Add 1.0 ml of 3% H₂O₂ directly onto an 18- to 24-hour heavily inoculated pure culture grown on a nutrient agar slant.

2. Place the tube against a dark background and observe for immediate bubble formation.

Result interpretation

Positive reactions are evident by immediate effervescence (bubble formation)

No bubble formation (no catalase enzyme to hydrolyze the hydrogen peroxide) represents a catalase-negative reaction

Reporting result

- The catalase test separates staphylococci (positive) from streptococci and enterococci (negative).
- *Bacillus* is catalase-positive, and *Clostridium* spp. is catalase-negative.
- The test is useful to separate among the fastidious Gram-negative rods.
- *Neisseria gonorrhoeae* produces an enhanced elaboration of bubbles not seen with other members of the genus due to superoxol.

Uses of catalase test

- For differentiating catalase-positive Micrococcaceae and Staphylococcaceae from catalase-negative Streptococcaceae.
- Differentiating aerobic and obligate anaerobic organisms.
- Presumptive differentiation among certain Enterobacteriaceae.
- Differentiation of aerotolerant strains of *Clostridium*, which is catalase-negative, from *Bacillus*, which are catalase positive.

Limitation of catalase test

- RBCs contain catalase, and thus, in order to avoid false-positive results, blood agar should not be picked up with the colony.
- The test should not be tested from Mueller-Hinton agar.

- Collecting colonies with metal bacteriological loop materials might yield false-positive results; however, platinum loops do not yield false-positive results.
- Because the enzyme is present in viable cells only, colonies that are older than 24 hours should not be used. Older cultures may give false-negative results.
- Reversing the order of adding the reagent to the colony might result in false-negative results.
- The reagent and the colony should not be mixed.
- Some strains of *S. aureus* may appear catalase-negative by drop method so the test should be repeated with the tube method.
- 30% H₂O₂ is extremely caustic to the skin. If contact occurs, wash immediately with 70% ethyl alcohol, not water.

Coagulase Test- Principle, Procedure, Types, Interpretation

Introduction to Coagulase Test

Coagulase test is used to differentiate *Staphylococcus aureus* which produce the enzyme coagulase, from *S. epidermis* and *S. saprophyticus* which do not produce coagulase. i.e Coagulase Negative *Staphylococcus* (CONS).

Principle of Coagulase Test: Coagulase is an enzyme-like protein and causes plasma to clot by converting fibrinogen to fibrin. *Staphylococcus aureus* produces two forms of coagulase: bound and free.

Bound coagulase (clumping factor) is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in an alternation of fibrinogen so that it precipitates on the staphylococcal cell, causing the cells to clump when a bacterial suspension is mixed with plasma. This doesn't require coagulase-reacting factor.

Free coagulase involves the activation of plasma coagulase-reacting factor (CRP), which is a modified or derived thrombin molecule, to form a coagulase-CRP complex. This complex in turn reacts with fibrinogen to produce the fibrin clot.

Procedure and Types of Coagulase Test

Slide Test (to detect bound coagulase)

1. Place a drop of physiological saline on each end of a slide, or on two separate slides.
2. With the loop, straight wire or wooden stick, emulsify a portion of the isolated colony in each drop to make two thick suspensions.
3. Add a drop of human or rabbit plasma to one of the suspensions, and mix gently.
4. Look for clumping of the organisms within 10 seconds.
5. No plasma is added to the second suspension to differentiate any granular appearance of the organism from true coagulase clumping.

Coagulase test procedure

Tube Test (to detect free coagulase)

1. Dilute the plasma 1 in 10 in physiological saline (mix 0.2 ml of plasma with 1.8 ml of saline).
2. Take 3 small test tubes and label as T (Test), P (Positive Control) and N (Negative Control). Test is 18-24 hour broth culture, Positive control is 18-24 hr *S. aureus* broth culture and Negative control is sterile broth.
3. Pipette 0.5 ml of the diluted plasma into each tube.
4. Add 5 drops (0.1 ml) of the Test organisms to the tube labelled "T", 5 drops of *S. aureus* culture to the tube labelled "P" and 5 drops of sterile broth to the tube labelled "N".
5. After mixing, incubate the three tubes at 35-37 Degree Celsius.
6. Examine for clotting after 1 hour. If no clotting has occurred, examine at 30 minutes intervals for up to 6 hours.

Interpretation of coagulase test

Positive: fibrin clot of any size

Negative: no clot.

Fermentation Test – Principle, Procedure and Interpretation

Different fermentation media are used to differentiate organisms based on their ability to ferment carbohydrates incorporated into the basal medium.

Purple broth is used for studying carbohydrate fermentation reactions, particularly in the identification of gram-negative enteric bacteria with desired carbohydrates added.

Principle: The principle of carbohydrate fermentation states that the action of organism on a carbohydrate substrate results in acidification of the medium, detected by a pH indicator or dye. Carbohydrate fermentation is the process microorganisms use to produce energy. A fermentation medium consists of a basal medium containing a single carbohydrate (glucose, lactose, sucrose, mannitol etc.) for fermentation. However, the medium also contains various pH indicators. In addition to a pH indicator to detect the production of acid from fermentation, a Durham tube is placed in each tube to capture gas produced by metabolism. The carbohydrate fermentation patterns shown by different organisms are useful in differentiating among bacterial groups or species.

When the media are inoculated with an organism that is able to ferment the carbohydrate present, acid or acid and gas are produced. A Durham tube is provided in tubed broth media to collect the gas produced during fermentation. The indicator in the media changes from purple to yellow when the amount of acid produced by carbohydrate fermentation is greater than the alkaline end products from peptone utilization. If the carbohydrate is not fermented, the color will remain unchanged or become more alkaline (darker purple) due to degradation of the amino acids in the medium.

Fermentation Test method

1. Allow medium to warm to room temperature prior to inoculation.
2. Inoculate the purple broth (with carbohydrate of choice) with isolated colonies from an 18-24 hour pure culture of the organism.
3. Inoculate a control tube of Purple Broth Base in parallel with the carbohydrate based media.

4. Incubate inoculated media aerobically at 35-37°C for 3-5 days.

Note: Increased incubation up to 30 days may be necessary for some microorganisms.

5. Observe daily for development of a yellow color in the medium.

Result interpretation

- Positive: The development of a yellow color in the medium is indicative of a positive carbohydrate fermentation reaction.
- Negative: Lack of yellow color development is indicative of a negative carbohydrate fermentation reaction.
- Gas formation is indicated by the appearance of gas bubbles in the Durham tube [120].

Motility Test – Principle, Procedure and Interpretation

Motility is the ability of an organism to move by itself by means of propeller-like flagella unique to bacteria or by special fibrils that produce a gliding form of motility. Motile bacteria move by using flagella, thread like locomotor appendages extending outward from the plasma membrane and cell wall either single flagellum or multiple flagella. Motility is an important taxonomic tool and biological characteristic of microorganisms. The presence of flagella occurs primarily in bacilli but there are a few flagellated cocci, thus motility is a very important means of identification in the family Enterobacteriaceae.

Principle: Motility by bacterium is mostly demonstrated in a semi solid agar medium. In semi-solid agar media, motile bacteria 'swarm' and give a diffuse spreading growth that is easily recognized by the naked eye. The medium mainly used for this purpose is SIM medium (Sulphide Indole Motility medium) which is a combination differential medium that tests three different parameters, Sulfur Reduction, Indole Production and Motility. This media has a very soft consistency that allows motile bacteria to migrate readily through them causing cloudiness. The inoculum is stabbed into the center of a semisolid agar deep. Bacterial motility is evident by a diffuse zone of growth extending out from the line of inoculation. Some organisms grow throughout the entire medium, whereas others show small areas or nodules that grow out from the

line of inoculation. The non-motile bacteria will only grow in the soft agar tube and only the area where they are inoculated.

Motility test method

- 1.Touch a straight needle to a colony of a young (18- to 24-hour) culture growing on agar medium.
- 2.Stab once to a depth of only 1/3 to ½ inch in the middle of the tube. Be sure to keep the needle in the same line it entered as it is removed from the medium.
- 3.Incubate at 35°-37°C and examine daily for up to 7 days.
- 4.Observe for a diffuse zone of growth flaring out from the line of inoculation.

Result interpretation

- Positive: Diffuse, hazy growths that spread throughout the medium rendering it slightly opaque.
- Negative: Growth that is confined to the stab-line, with sharply defined margins and leaving the surrounding medium clearly transparent.

VII. Antimicrobial Susceptibility Testing

Quality control when performing Kirby –Bauer disc diffusion technique (based on CLSI, 2025)

- 3.Purchase Mueller Hinton agar from a reliable source.
- 4.Check its PH
- 5.Ensure plates contain the correct amount of agar
- 6.Check new batches using *E. faecalis* control strain (ATCC 29212 or 33186) and co –trimoxazole disc
- 7.Prepare carefully the Inoculum of the test and control organisms to ensure growth is confluent.
- 8.Much the turbidity of suspension with turbidity standard.
- 9.Renew the turbidity standard every few months.

10. Use discs containing the correct amount of antimicrobial and store discs correctly and do not use them beyond their expiry date. Alternatively, use stable antimicrobial tablets
11. Place discs or tablets correctly on the plate, not too close to each other or to the rim of the plate.
12. Use appropriate control strains
13. Regularly check the temperature of the incubator to ensure test are incubated at 35-37°C for 18-24 hours.
14. Measure inhibition zones carefully. Zone diameters with control strains should be within the limits published by CLSI.

VIII. Quality control of microbiological media

Culture media are used for isolation, identification and sensitivity test of different pathogens. To check the quality of the media and its ability to give satisfactory results, growth supporting characteristics, physical characteristics and sterility testing are mandatory.

Testing of Physical and Chemical Parameters

It includes color of the prepared medium, thickness, formation of excessive bubbles, uneven surface, shrinking of the media, cracks or crystallization in the media and chemical parameters such as pH of the medium should also be checked. Media performance is checked by inoculating standard strains and incubating it at the desired temperature. Whenever a new batch of media is prepared, 10% of samples should be projected to sterility testing.

Growth and Sterility testing

Every media must be sterile if prepared following strict quality control parameters. At least 5-10% of a new batch prepared should be incubated at 37°C for 18-24 hours to ascertain that there is no growth or contamination. If any colonies develop after incubation, the whole batch needs to be discarded.

After sterility testing, media are tested for supporting growth and giving desired reactions. For this purpose standard control strains of organisms are inoculated into the test medium and observed for typical reactions.

The results should be examined both qualitatively and quantitatively. A list of quality control (QC) strains to be tested against routinely used culture media are as follows. However, one strain can be inoculated on various other media.

Table 7: List of Quality Control Strains to be tested against routinely used Culture Media

S.no	Quality control strain	Media	Expected output
1	<i>Escherichia coli</i> (ATCC® 25922™)	MacConkey agar	Lactose fermenting pink colonies
2	<i>Acinetobacter baumannii</i> (ATCC® 19606™)	MacConkey agar	Non- Lactose fermenting pale colonies
3	<i>Staphylococcus aureus</i> (ATCC® 25923™)	Mannitol Salt agar	Golden Yellow colonies
4	<i>Streptococcus pyogenes</i> (ATCC® 19615™)	Blood agar	Beta haemolytic, grey colonies
5	<i>Streptococcus pneumoniae</i>	Blood agar	Alpha haemolytic, small greenish colonies
6	<i>Shigella sonnei</i> (ATCC® 25931™)	XLD agar	Red pink colonies
7	<i>Salmonella enterica</i> subsp. <i>enterica</i> (ATCC® 14028™)	XLD agar	Red pink colonies with black centre
8	<i>Pseudomonas aeruginosa</i> (ATCC® 27853™)	Mueller hinton Agar	Flat serrated greenish colonies
9	<i>Neisseria gonorrhoeae</i> (ATCC® 49226™)	Thayer martin agar	Water droplet like colonies
10	<i>Enterococcus faecalis</i> (ATCC® 29212™)	Bile esculin agar	Small transparent colonies with brown-black halos.
11.	<i>Escherichia coli</i> (ATCC® 25922™)	EMB agar	Blue-black bull's eye like colonies

