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Incidence of post-Cesarean wound infections, bacterial profiles, resistant patterns, and associated costs among women delivering at public health facilities in Eastern Ethiopia: Multicenter study

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This is to certify that the thesis prepared by Akera Fekade, entitled: Incidence of post-Cesarean wound infections, bacterial profiles, resistant patterns, and associated costs among women delivering at public hospitals in Eastern Ethiopia: Multicenter study and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and public health microbiology) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAU	Addis Ababa University
AMR	Antimicrobial Resistance
AST	Antimicrobial sensitivity test
BMI	Body mass index
CDC	Center of Disease Control
CS	Cesarean section
CLSI	Clinical laboratory Standards Institute
CSI	Cesarean section infection
DRERC	Departmental research and ethical review committee
LSCS	Lower segment Cesarean section
MHA	Muller Hinton agar
MOH	Ministry of Health
Post-CS SSI	Post cesarean section surgical site infection
SOP	Standard Operating Procedure
SSI	Surgical site infection

Abstract

Background: Cesarean site infection is an infection (CSI) that takes place at the surgery site and occurs within 30 days after cesarean section. Post cesarean wound infection is a vital cause of complications after cesarean delivery.

Objective: To determine the incidence of post-cesarean wound infections, bacterial profiles, resistant patterns, and associated costs among women delivering at public hospitals in Eastern Ethiopia.

Methods: A hospital-based cross-sectional study conducted at Hiwot Fana Specialized University Hospital, Dil Chora Referral Hospital, Dechatu Health center and Sabian General Hospital from January 01 to March 2025. Out of 427 mothers who underwent cesarean section were included. Socio-demographic data was gathered by personal interviews using a pre-tested questionnaire. Pus samples were collected using sterile cotton swab and inoculated in appropriate culture media. The Kirby-Bauer disk diffusion technique was employed to determine the antimicrobial resistance patterns of bacterial isolates. SPSS version 26 used to enter and analyze the data.

Results: From a total of 427 CS delivered mothers, 56 (13.3%) developed Post-CS SSI. Out of 56 isolates, 31 (55.3%) isolates were gram-positive organism and 25 (44.4%) isolates were gram-negative organisms. The predominant bacteria isolates were *S. aureus* (47.3%), *K. pneumonia* (17.5%), and *E.coli* (14.2%). The majority of isolates were resistant to tetracycline (83.3%), trimethoprim-sulfamethoxazole (88.8%), and amoxicillin-clavulanic acid (46.5%). Previous CS ($p<0.001$), type of CS ($p=0.002$), obesity ($p=0.001$), hospital stay ($p<0.001$), post-operative hemoglobin level ($p<0.001$), residence ($p=0.002$), and premature rupture of membrane ($p<0.001$), showed statistically significant association with postoperative wound infection. The mean $\pm$ SD cost for laboratory, treatment, and hospital admission with post-CS SSI and without post-CS SSI were 910 ± 97.8 , 6183 ± 416.2 , and 3099 ± 299.8 ; 539.71 ± 161.536 , 3491.33 ± 1077.529 , and 1736.17 ± 343.473 respectively.

Conclusion: The incidence of post cesarean wound infection was 13.3 % and extra costs incurred on patients and health facilities. Implementation of a regular infection control policy along with microbiological work could reduce the incidence of CS-infection.

Key words: *surgical site infection, cesarean section, post-CS SSI, pregnant women*

1. Introduction

1.1 Background of the study

Cesarean section is a major surgical procedure that is applied to deliver the fetus through an incision in the mother's abdomen (1). It's an essential procedure to save lives when the vaginal procedures pose a risk. Based on the WHO report in 2021, caesarean section use continues to rise globally, accounted for more than 1 in 5 (21%) of all childbirths. Due to this, a continuous rise of infection is expected (2). Cesarean section is a major when complications occur in pregnancies, like excessive fetal weight, fetal mal presentation, multiple pregnancy, prolapses cord, placenta abruption and mother's age (3).

According to the Centers for Disease Control and Prevention (CDC) criteria, Post cesarean wound infection is an infection that takes place at the surgery site and occurs within 30 days after cesarean section. It is a serious public health issue which causes maternal morbidity and mortality, prolonged hospital stays, readmission, reoperation and high excess hospital costs (4).

The predisposing risk factors are responsible for the cause of post-cesarean wound infection. include age, premature rupture of membranes, prolonged labor, alcohol drinking habits, smoking habits, obesity, limited ANC follow-up, and previous CS, antibiotic prophylaxis, elective or emergency CS, extended hospital stay, anemia, hypertension, and diabetes mellitus(5).

Bacteria that cause surgical site infection mostly come from the mother's endogenous flora. The incidence of surgical site infection raises because of emergence antibiotic resistant pathogens like multidrug-resistant pathogens and methicillin-resistant *S.aures*. The risk of bacterial infection is five to twenty times higher for mothers who had cesarean delivery than for those who gave birth vaginally (6). Signs and symptoms of CSI include redness, swelling, pain around the surgical site, and fever (7).

The most common pathogenic bacteria isolated from infected site include *Staphylococcus aureus* (*S. aureus*), *Enterococcus faecalis* (*E. faecalis*), *K. pneumoniae*, *Escherichia coli* (*E. coli*) and *Coagulase negative Staphylococcus* (*CoNS*) (8). Post-CS SSI contributes to infection-associated costs result from prolonged hospitalization, treatment and other medical expenses (9).

Antimicrobial resistance (AMR) is the most common public health issue and 1.27 million global deaths occurred due to the bacterial AMR and contributed to 4.95 million deaths(10). Most bacteria develop a resistance to antimicrobial agents; this is because of the misuse of drugs and the bacteria's nature. Mothers who develop resistance to antibiotics raise the risk of morbidity, mortality, extended stay in hospital, and medical expenses (11).

There are a potential sources that increase the chance of getting infection were contaminated air, operating room team, crowded theatre environment, surgical gowns, Non-sterile equipment, and contaminated clinical setting (12).

Post-Cesarean wound infection is primary cause of maternal morbidity and mortality in Ethiopia. It remains a challenge for healthcare system and requires further investigation to assess associated risk factors of SSI. This study aims to identify microorganisms that are responsible for SSI, antimicrobial susceptibility pattern, assess associated risk factors and evaluate the cost expenditure related to SSI.

1.2 Statement of the problem

Worldwide, the number of mothers who develop post-cesarean wound infection is anticipated to rise, mainly because of the continuous increase in rates of cesarean deliveries in recent years (13). The meta-analysis conducted in 2024 found a 24 % pooled cesarean section rate in Eastern Africa, with Ethiopia having the highest rate at 28.3 % (14). The incidence rates of post-CS SSIs across different parts of the world range estimate from 1% to 20%, and maternal mortality could be as high as 3% globally (15).

The impact of cesarean section infection in Ethiopia has increased because of insufficient supply, failure to follow infection prevention protocols, and inadequately trained professional (16). In Ethiopia, the pooled estimate of cesarean section infection was 9.72% (9).

The rising rate of bacteria that are resistant to commonly used antibiotics is caused by improper usage of antibiotics, due to this post-cesarean wound infection continues to pose a significant challenge(17). Antimicrobial Resistance (AMR) is a major public health threat in 21 centuries. In 2019, 4.95 million deaths were encountered globally due to bacterial AMR(18).

SSIs can result in significant financial costs for both healthcare providers' and patients. For healthcare providers, the cost of treating SSIs can be substantial. A study conducted in England found that the median additional cost resulting from surgical site infections (SSI) was £5,239 (19). The additional cost for surgical site infection include extended hospital stays, laboratory tests, reoperation, antibiotics or other medications, and supplies for wound care (20).

The occurrence of CS infection increases in parallel with the rate of cesarean delivery (CD) increases (21). The mean length of CS-related infection hospitalization is high, estimated at 2.36 days. Because of postoperative complications in hospitals giving acute care lead to unplanned admission after patient discharge (22).

Although, there are data available on CS infection incidence in the Ethiopian context, the information is not adequate, moreover, as far as we know data is very scarce in relation to the cost associated with CS infection, particularly in the current study site. Hence we conducted the study to fill the existing gaps and update the information.

1.3 Significance of the study

This research might contribute to identifying the incidence level of post-cesarean infection. This study might give a clear understanding for the Ministry of Health to see the incidence level and take appropriate action to minimize the chance of developing SSI. Identifying the microorganisms that are responsible for causing SSI is vital for effective treatment, prevention, and monitoring the microorganisms helps healthcare facilities track infection trends and improve overall surgical safety protocols.

By conducting this research, healthcare providers can gain crucial insights into the frequency and causes of post-cesarean infections, which help them, implement better prevention and treatment strategies. Additionally, by exploring the economic impact of CS infections, the study can inform policy changes aimed at reducing healthcare costs for patients and the health system. The findings will be instrumental in improving maternal healthcare quality, informing training for healthcare professionals, and shaping protocols that can minimize infection risks and healthcare costs in public hospitals.

2. Literature Review

2.1. Based on the incidence of CSI and associated risk factors.

A review and meta-analysis was conducted on the global and regional incidence of post cesarean surgical site infection published from 2000 to 2023. The global incidence of Post-CS SSI was investigated in 180 studies, and 53 countries were included. The pooled global incidence estimate was 5.63 %. At continent level, Africa reported the highest incidence rate, estimated 11.91 %, and North America had the lowest incidence rate, 3.87 % (23).

The research was carried out by Charrier L, et al., in 2009 in Italy. The study had 430 women's who had been delivered by CS. There were 430 pregnant women's delivered by cesarean section (CS). The result of the research showed that the overall rate of infection was 4.7 % (20/430) and premature rupture of membrane was statistically significant factor contributing to SSI (24).

The research was carried out by Abhishek Kumar and his collaborators at the Department of Microbiology in Western India. Out of 1895 pregnant women's who underwent cesarean section, 285 (14.7%) developed post cesarean wound infection. The incidence rate of post-cesarean surgical site infection was 14.7 % and contributing factors such as anemia, extended hospital stay, failure to receive antimicrobial prophylaxis, emergency CS, previous CS were increase the risk of developing SSI. *Staphylococcus aureus* (22.1%) were predominant bacteria from gram-positive organism and *K.pneumoniae* (24.4%) isolated from gram-negative organism (5).

Filbert J and his colleges carried out a prospective cohort study in 2014 in Mwanza, Tanzania. A total of 345 pregnant women delivered by CS were included. Only 34 patients developed CSI. Post cesarean wound infection significantly increase among women with prolonged labor, premature rupture of membrane, pregnancy induced hypertensive, and more vaginal examination. *Staphylococcus aureus* was the most frequently isolated organism (27.3%), succeeded by *Klebsiella pneumoniae* (22.7%) (25).

In 2017, in Iraq, Shaymaa Kadhim conducted a prospective study to assess the incidence rate and contributing risk factors for SSI. The study included 3036 pregnant women's who had undergone CS delivery, 191(6.3%) of them had post cesarean wound infection. Risk factors like emergency CS, vertical incision, Obesity, diabetes mellitus, and rupture of membrane before CS were significantly associated with SSI (26).

Rahel Mezemir and her colleagues carried out a prospective observational cohort research in 2022 at a referral hospital in Addis Ababa, Ethiopia. The study enrolled 741 pregnant women who had undergone CS delivery. Post cesarean wound infection occurred 11.6 % of cases and contributing factors like 2 or 3 antenatal care visits, membrane rupture before CS, and multiple vaginal examinations increase the risk of developing SSI (16).

In 2021, Tsegaw Alemye and his collaborators were carried out a research, in Harar, Ethiopia. 1069 medical records were collected and reviewed. According to the review the incidence of SSI was 12.3 %. In this study emergency-CS and rupture of membrane was significantly associated factor with post cesarean wound infection (27).

Diriba Ayala and his colleagues carried out a cross-sectional study in Nekemte, Ethiopia. The research was done to determine the magnitude and contributing factors of SSI. Surgical site infections occurred in 8.9% of cases. Mothers with the age above 35 years, pregnancy induced hypertension, prolonged labor, and mothers who received general anesthesia were lead to an increased surgical site infections (28).

The research was carried out by Hana Lijaemiro and her colleagues. This study was conducted to identify the frequency and predictors that contributes to cesarean section infections. The study included 175 women who gave birth by cesarean delivery in selected government hospitals in Addis Ababa, 25 (15%) of mothers developed postpartum infection. Risk factors such as age, weeks of gestations, surgery duration, and more than 5 times vaginal examinations were significantly associated risk factors for post-CS SSI (29).

The research was conducted in Debretabor, Ethiopia. The prevalence of cesarean section infection was about 8 %. Pregnancy induced hypertension, intrauterine infection, vertical abdominal incision, and anemia following surgery were significantly correlated with post cesarean wound infection (30).

2.2 Bacterial profile and antimicrobial resistance pattern in CS infection

A study conducted at Rafidia Hospital-Nablus, Palestine, in 2016 reported the prevalence of bacteria isolates as follows: *E. coli*, *S. aureus*, *Klebsiella spp*, *Enterobacter sp*, and *Acinetobacter sp* were 56.7%, 30%, 6.7%, 3.3%, and 3.3% respectively. Nalidixic acid (88.9%), Norfloxacin (77.8%), Amoxycillin/clavulanic acid (77.8%), Kanamycin (66.7%), and Ciprofloxacin (55.6%) were highly resistant for *S. aureus*. Additionally, 33.3% of the *S. aureus* isolates were classified as methicillin-resistant *S. aureus* (MRSA)(31).

In 2019, in Nigeria, Charles O and Amarachi N conducted a prospective study. 600 study participants who underwent a caesarean section were enrolled. Out of 600 participants, only 51 (8.5%) were developed a cesarean section infection (CSI). The major isolates were *S. aureus* (37.3%), *Klebsiella pneumonia* (27.1%), and *E. coli* (22.0%)(32).

Mulu W and her colleagues carried out a research in Bahirdar, Ethiopia, and 10.9% of participants had SSI. They found forty-two isolated bacteria, among them *S.aureus* was the predominant isolate by 26.2%, succeeded by *E. coli* and Coagulase-negative Staphylococcus species, each 21.4%. A maximum of 100% both Gram-positive and Gram-negative bacterial isolates were resistant to multiple drugs (33).

In 2018, Abebaw Bitew and his collaborators conducted a research in Gondar, Ethiopia. The study included 107 study participants, ninety bacteria isolates were confirmed. The most frequently isolated bacteria species were *S. aureus* (41.6%), *E. coli* (19.8%), *K. pneumoniae* (13.9%), coagulase-negative Staphylococcus (12.9%), and *Enterobacter spp* (4%). The majority of isolates were resistant to ampicillin, amoxicillin, and tetracycline but susceptible to ceftriaxone and amikacin. Multidrug-resistant bacterial species were isolated (8).

Evaluation of antimicrobial prophylaxis use and rate of SSI in Southern Ethiopia resulted in the rate of SSI being 16.5%. *K.pneumoniae* 60%, *S.aureus* 17.8%, *E.colli* 11.1%, and *P.aeruginosa* 11.1% were common bacteria responsible for SSI. From gram-negative bacteria, *E.coli* was resistant organism. *S. aureus* is 100 % sensitive to ceftriaxone and gentamycin. The isolated bacteria were resistant to chloramphenicol's 28.2% is the leading procedure followed by appendectomy, 21.2%, head and neck surgery,12.5% (34).

3. Objectives

3.1 General objectives

To determine the incidence of post-cesarean wound infections, bacterial profiles, antimicrobial susceptibility pattern, and healthcare costs among women delivered at public health facilities in Eastern Ethiopia.

3.2 Specific objectives

3.2.1 To determine the bacterial pathogens associated with cesarean section infection and antimicrobial resistance patterns of the isolated bacteria.

3.2.2 To identify risk factors associated with post cesarean wound infection.

3.2.3 To estimate costs expenditures of mothers related to post cesarean wound infection.

4. Hypothesis

- Ho: the incidence of post-cesarean wound infection has no significant difference from WHO infection rate guideline.

5. Materials and methods

5.1. Study area

This research carried out at Hiwot Fana Specialized University Hospital (HFSUH), Dil Chora Referral Hospital, Dechatu health center, and Sabian General Hospital. The study area was in two cities found in the Eastern region. The first, Dire Dawa, which is around 457 km kilometers away in the East from the capital city of Ethiopia, Addis Ababa. In Dire Dawa, there are two Hospitals and ten public sector health centers. The other city is Harar located at 510 km towards the east of Addis Ababa, Ethiopian. Three Hospitals and eight Health centers were found in Harar city.

5.2. Study design and period

Multi center hospital-based cross-sectional study was conducted from January 01 to March 01, 2025.

5.3. Population

5.3.1 Source population

All pregnant women had a cesarean section at selected public health facilities during the study period.

5.3.2 Study population

All women who had post cesarean wound infection at selected public health facilities during the study period.

5.4 Inclusion and exclusion criteria

5.4.1 Inclusion criteria

. All pregnant women who gave birth by cesarean section were enrolled in the study.

5.4.2 Exclusion criteria

Pregnant women who were not willing to enroll and received antibiotics seven days before cesarean delivery were excluded from the study.

5.5 Study variables

5.5.1 Dependent variables

- Bacterial Isolates from post-CS SSI.
- Antimicrobial susceptibility patterns of bacterial isolates from SSI.
- Cost related to CS infection

5.5.2 Independent variables

- ✓ Socio-demographic factors: age, residence educational background, marital status, occupation, and monthly income.
- ✓ Medical characteristics: Body Mass index, anemia, pregnancy induced hypertension, Gestational DM, Urinary tract Infection, post-operative hemoglobin, hospital Stays
- ✓ Obstetric characteristics: Maternal parity, frequency of antenatal care visits, previous CS, fetal birth weight, and type of CS

5.6 Sample size calculation and Sampling method

5.6.1 Sample size calculation

As demonstrated below, the sample size is determined using a single population proportion sample size calculation, with a value of P taken (50%).

$n = (z \cdot \sqrt{2})^2 \cdot p(1-p) / d^2$ were

n=sample size

z = statistic for level of confidence interval 95%.z score =1.96 p= estimated prevalence 50%

d= precision 5%

$z^2 p(1-P) / d^2 = 1.96^2 \times 0.5(1-0.5) / 0.05^2 = 384$ by considering 10% non-response rate

- The total sample size(n) will be 427

5.6.2 Sampling method

This study used a convenient sampling technique and proportional allocation formula was used for sample size determination based on the average monthly number of pregnant women delivered by CS in each hospitals and health center (Figure1).

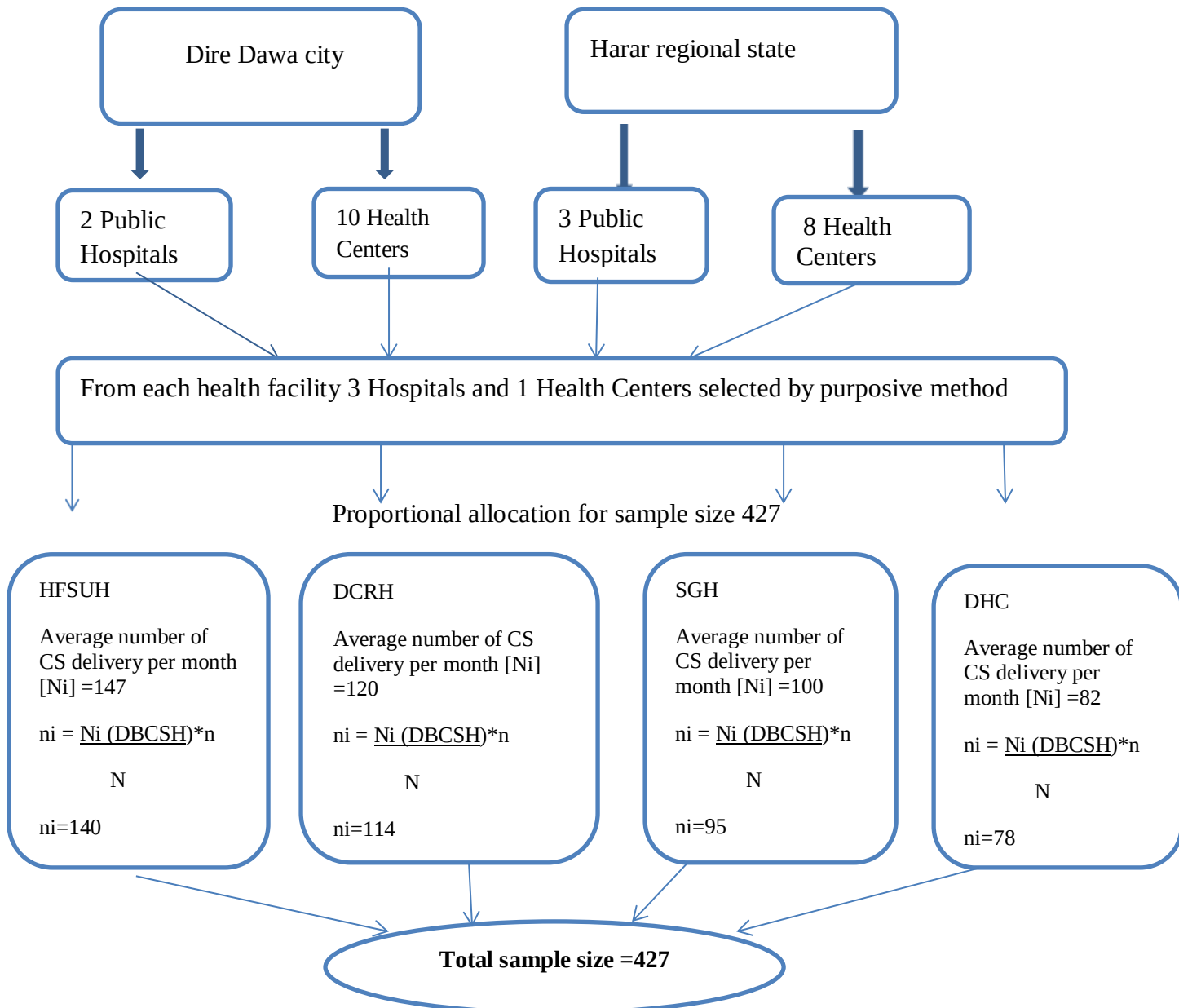


Figure 1: Schematic presentation of sampling procedure and sample size allocation for public health facilities in Eastern, Ethiopia, 2025.

5.7 Measurement and data collection

5.7.1 Data collection procedure

8 Nurse and 4 physicians were trained for data collection and data was collected following written consent from each participant. Participants were asked to give their consent if they are willing to participate in the research after we explained the aim of the study to them. Next, a nurse employed a semi-structured questionnaire to gather clinical and socio-demographic data from all pregnant women delivered by cesarean section. After that, physicians took a wound sample by using sterile cotton swabs. It was gathered prior to wound cleansing, labeled, and delivered to the lab for further analysis in less than an hour. We took care to prevent cross-contamination at every turn.

Then information's regarding financial costs was collected from the hospital finance and hospital liaisons and by asking health professionals allocated in the maternity wards regarding the type of drugs and materials used for the diagnosis of CSI with consideration of the cost of drugs, service, investigations, diagnosis and other costs paid for the treatment and diagnosis of CSI. The financial cost of mothers who had not developed CSI was compared with the financial loss of due to CSI.

Transportation of samples: the samples were transported by STUART media to Hiwot Fana Specialized University Hospital (HFSUH).

5.7.2 Isolation and identification of bacteria

Pus sample was collected using a sterile swab from the infected site. The swab samples were inoculated in MacConkey and blood agar medium. A candle jar was used to incubate the culture media at 37°C for 24 hours. The microorganisms were isolated based on colony morphology and gram stain. The obtained pure colonies were used for biochemical tests, antibiotic sensitivity test, and implementing the suggested standard operating procedures.

5.7.3 Antimicrobial Susceptibility Tests (AST)

Antimicrobial susceptibility test were done by using the Kirby Bauer Disc Diffusion method. Zones of inhibition of growth around the appropriate sensitivity discs will be measured and categorized as resistant or sensitive after being compared to a standard.

According to CLSI 2024 34th edition guide line for Enterobacteriaceae we use AMP-Ampicillin (10µg), CZ-cefazolin (30µg), CFT-Ceftriaxone(30µg) or CTX-cefotaxime(30µg), TM-Tobramycin (10µg), AMX- Amoxicillin and clavulanate (20/10µg), TSX-Trimethoprim-Sulfamethoxazole, SAM-Ampicillin-sulbactam (10µg), GN-Gentamicin(10µg), CIP-Ciprofloxacin (5µg),TTC-Tetracycline (30µg) ,MEM-Meropenem (10µg) and IPM-Imipenem (10µg).

According to CLSI 2024 34th edition guide line for *Pseudomonas aeruginosa* we use CFZ-Ceftazidime (30µg), CFP-Cefepime (30µg), CIP-Ciprofloxacin (5µg), LVX-Levofloxacin (5µg), MEM-Meropenem (10µg) and IPM – Imipenem (10µg).

According to CLSI 2024 34th edition guide line for *Staphylococcus* spp we use CM-Clindamycin(10µg), AZM-Azithromycin (15µg) or CLR-clarithromycin (15µg) or ER-Erythromycin(15µg), FOX-cefoxitin, DO-Doxycycline, MI-Minocycline, TTC-Tetracycline, TSX-Trimethoprim-Sulfamethoxazole (16µg), LNZ-Linezolid (30µg), LVX-Levofloxacin (5µg), VA-vancomycin (MIC), and (P)penicillin(10µg).

According to CLSI 2024 34th edition guide line for *Enterococcus* we use P-penicillin(10µg) ,AM- ampicillin(10µg), LVX-Levofloxacin (5µg), VA-Vancomycin (30µg), and GN-Gentamicin (10µg) (35).

5.8 Data Quality Assurance

CLSI quality control requirements were followed to confirm that media fulfills Standard Operating Procedure [SOP]. Visual examinations for contamination, uneven fill, hemolysis, bubbles, freezing evidence, and fractures in the media or plastic Petri dishes were carried out.

Quality control was carried out to verify the quality of mediums. Before being used, every fresh lot were undergo quality control testing using standard strains of *E. coli* [ATCC 25922], *P. aeruginosa* [ATCC 27853], *K. pneumoniae* [ATCC 700603], *E. faecalis* [ATCC 29212 or 33186], and, *S. aureus* [ATCC 25923]. A pretest that was given to 5% of participants was used to evaluate a questioner for completeness, readability, and ease of understanding. The SOPs were followed for collecting the samples. Data quality was carefully followed prior to analysis.

5.8.1 Pre-analytical phase

Prior to filling out the completed questionnaire, we started request the participant's willingness both orally and in writing. The questionnaires were initially prepared in English, translated into Amharic and Afan Oromo for participants to understand the questions. The patient's identifying number was written on the container, and sterile cotton is used to collect an aseptically wound sample. Specimens were delivered to microbiology laboratory at Hiwot Fana Specialized University Hospital (HFSUH) within a cold chain.

5.8.2 Analytical phase

Every process, piece of machinery, and material is properly managed. Quality control of the culture media consists of test for contamination and ability to cultivate control strains of bacteria. A standard strains of *E. Coli* [ATCC 25922], *K. pneumoniae* [ATCC 700603], *P. aeruginosa* [ATCC 27853], *E. faecalis* [ATCC 29212 or 33186] and *S. aureus* ATCC [25923] were utilized as control strains of bacteria for culture media and AST discs, and turbidity standard for barium sulfate (BaSO₄), equivalent to a 0.5 McFarland standard, was used to standardize the concentration of bacteria suspension for AST. The senior microbiologist was closely following the standard operating procedures (SOPs) and the results were verified.

5.8.3 Post-analytical phase

The results were documented with their patient's unique number. Before the results are presented to the caregiver, the department head reviewed and verified the reporting to ensure that there are no inaccuracies in the test results.

5.9 Data analysis and interpretation

Before analysis, the data was entered into IBM-SPSS version 26. The descriptive data like frequency, percentage, mean and standard deviation was calculated. Independent 't' test was applied to compare mean $\pm$ SD of costs for treatment, laboratory and hospital admission. Bivariant and multivariate logistic regression analyses were used to assess the possible risk factors of surgical site infection. The level of significance P value <0.25 in bivariate analysis were included in multivariate logistic regression. Odds ratio with 95% confidence interval was calculated to determine degree of association between dependent and independent variables. A P-value below 0.05 was considered statistically significant for all cases. The

research outcomes reported in words, charts, graphs, and tables.

5.10 Operational definitions

Post-cesarean surgical site infection: an infection occurred within 30 days after surgery and purulent discharge came out from the incision site.

Cesarean delivery: an operational procedure used to give birth via an incision in the abdomen and uterus.

Multidrug Resistance: is defined as non-susceptibility to at least one agent in three or more antimicrobial categories

Financial Cost: The total amount of cost needed from the hospital to treat SSI infection happened during CS delivery.

Cesarean section infection: presence of infection at the site of surgery following cesarean delivery.

5.11 Ethical considerations

Before data collection, written consent was obtained from the Research and Ethics Review Committee (RERC) of Addis Ababa University College of Health Science, Department of Medical Laboratory Science (DRERC/778/24/MLS), and permission letter was submitted to Hiwot Fana Specialized University Hospital, Dil Chora Referral Hospital, Dechatu Health center and Sabian General Hospital. Furthermore, informed written consent was obtained from each study participant before the actual data collection and the purpose of the study was explained to the study participant and they were informed about the information obtained in course investigation will be kept confidential using codes and they do have right to get their results without payment. The test results of study participants were communicated to their attending physician.

5.12 Dissemination of the results

The study finding was submitted to Addis Ababa University, College of Health Sciences Department of Medical Laboratory Sciences. Furthermore, the findings of the study submitted to national or international peer reviewed journal for publication. The findings were presented at national and international scientific conferences.

6. Results

6.1 Descriptive characteristics of study participants

6.1.1. Socio-demographic characteristics of study participants

The research was carried out on a total of 427 pregnant women who gave birth by cesarean section were included. Of these, 56 (13.3 %) developed post cesarean wound infection. Most of the participants were in the age group of 18-27 years (250, 58.5 %) with a median age of 26.9 (SD $\pm$ 5.3) years. Many of the respondents 277 (64.9 %) were lived in urban area. A total of 172 (40.3%) participants were completed higher education, 335 (78.5%) married and 220 (51.5%) housewife. Most of the participants, 229 (53.6%), had monthly income ranging from 1501-5000 birr. More than half of the respondents 267 (62.5%) didn't engage in physical exercise, 285 (66.7%) had never consumed alcohol and 305 (71.4%) never smoked cigarettes (Table 1).

Table 1: Socio-demographic characteristics of women who gave birth by cesarean section at public hospitals in Eastern, Ethiopia 2025, n=427

Variables	Categories	Frequency	Percent %
Age of the mothers (Years)	18-27	250	58.5
	28-37	163	38.2
	>37	14	3.3
Residence	Urban	277	64.9
	Rural	150	35.1
Occupation	Housewife	220	51.5
	Government employee	101	23.7
	Private business owner	66	15.5
	Student	40	9.4

Educational Background	No formal education	56	13.1
	Primary education	67	15.7
	Second education	132	30.9
	Higher education	172	40.3
Marital Status	Married	335	78.5
	Divorced	35	8.2
	Single	51	11.9
	Widowed	6	1.4
Monthly Income in birr (\$)	<1500 (11)	48	11.2
	1501-5000 (11-36.6)	229	53.6
	5001-10000 (36.6-73.5)	132	30.9
	>100001 (73.5)	18	4.2
Habit of Physical exercise	Never	267	62.5
	Sometimes	130	30.4
	Regularly	30	7
Alcohol Drinking habit	Never	285	66.7
	Occasionally	126	29.5
	Regularly	16	3.7
Smoking Habit	Never	305	71.4
	Occasionally	108	25.3
	Regularly	14	3.3

6.1.2. Clinical characteristics of study participants

Majority of the respondents, 220 (51.5 %), had BMI greater than 25 kg/ m². Among the study participants, 139 (32.6 %) had anemia and only 36 mothers (8.4 %) had gestational diabetic mellitus. Forty (9.4 %) of participants had pregnancy induced hypertension and 74 (17.3 %) had a history of urinary tract infection. More than half of the respondents, 239 (56 %), had hemoglobin levels less than 11gm/dl after operation, and 76 (17.8 %) of mothers had an extended hospital stays exceed 7 days (Table 2).

Table 2: Clinical characteristics of women who gave birth by cesarean section at public hospitals in Eastern, Ethiopia 2025, n=427

Variables	Categories	Frequency	Percent%
Body Mass index	>25 Kg/ m ²	220	51.5
	< 25 kg/ m ²	207	48.5
Pre-operative anemia	Yes	139	32.6
	No	288	67.4
Pregnancy induced Hypertension	Yes	40	9.4
	No	387	90.6
Gestational DM	Yes	36	8.4
	No	391	91.6
Urinary tract Infection	Yes	74	17.3
	No	353	82.7
Post-operative hemoglobin	<11gm/dl	239	56
	>11gm/dl	188	44
Hospital Stays	<7days	351	82.2
	>7days	76	17.8

6.1.3 Obstetrics characteristics of the study participants

Out of 427 mothers, 238 were multipara, 180(42.2%) primipara and 9(2.1%) were grand multipara. Majority of the participants 373(87.3%) had a follow up care in health facilities and 240(56.2%) had a previous CS scar. The mean fetal weight was 3400 gm. The minimum and maximum birth weights were 1700 gm and 5200 gm. About 251(58.8%) of mothers had emergency surgery and 176(41.2%) had elective surgery. Based on the type of abdominal incision, 292(68.4%) was transverse incision and 135(31.6%) was vertical incision. Less than half of the respondents 146(34.2%) had rupture of membrane before CS (Table 3).

Table 3: Obstetric characteristics of women who gave birth by Cesarean section at public hospitals in Eastern, Ethiopia 2025, n=427

Variables	Categories	Frequency	Percent%
Parity	Primipara	180	42.2
	Multipara	238	55.7
	Grand multipara	9	2.1
Antenatal care follow up	Yes	373	87.4
	No	54	12.6
Previous CS	Yes	240	56.2
	No	187	43.8
Fetal weight	<2400 gm	15	3.5
	2400-4000gm	386	90.4
	>4000gm	26	6.1
Type of CS	Emergency	251	58.8
	Elective	176	41.2
Incision type	Transverse	292	68.4
	Vertical	135	31.6
Premature rupture of membrane	Yes	146	34.2
	No	281	65.8

6.1.4 Isolation of pathogenic bacteria from post-CS SSI

60 pus samples were obtained from the wound site and processed during the study period. Of these, 56 pathogenic bacteria were isolated and the remaining 4 specimens had no bacteria growth. Out of 56, 31 (55.35 %) were gram-positive and 25 (44.9 %) were gram-negative. Among gram-positive bacteria, *Staphylococcus aureus* 27 (48.2 %) was a predominant bacteria. The predominant isolated bacteria from gram-negative was 10(17.8 %) *k.pneumonia*, 8 (14.02 %) *E.coli*, and 6 (10.71 %) *Citrobacter spp*. The least isolated bacteria were *Enterococcus spp*, and *Pseudomonas spp* were 4 (7.4 %), 1 (1.78 %) respectively (Table 4).

Table 4: Isolation of pathogenic bacteria from post-CS SSI at public hospitals in Eastern, Ethiopia 2025, n=56

Category	Bacterial isolate	Frequency	Percent (%)
Gram-positive	<i>S,aureus</i>	27	48.21
	<i>Enterococcus spp</i>	4	7.14
	Total	31	55.35
Gram-negative	<i>k.pneumoniae</i>	10	17.8
	<i>Pseudomonas spp</i>	1	1.78
	<i>E.coli</i>	8	14.2
	<i>Citrobacter spp</i>	6	10.71
	Total	25	44.49

6.1.5. Distribution of bacterial isolates in different public health facilities

The highest bacteria isolate was found from Hiwot Fana Specialized University Hospital (HFSUH) 23 (41.07%), followed by Dil Chora Referral Hospital 21 (37.5%). The least isolates were from Dechtau Health Center 7 (12.5%) and Sabian General Hospital 5 (8.9%) (Table5).

Table 5: Distribution of bacterial isolate among different public health facilities in Eastern, Ethiopia 2025, n=56

Category	Bacterial Isolate	HFRH	DCRH	SBG	DHC
		N (%)	N (%)	N (%)	N (%)
Gram Positive	<i>S.aureus</i>	12	10	3	2
	<i>Enterococcus spp</i>	2	2	0	0
Gram Negative	<i>k.pneumoniae</i>	5	4	1	0
	<i>Pseudomonas spp</i>	0	1	0	0
	<i>E.coli</i>	3	2	0	3
	<i>Citrobacter spp</i>	1	2	1	2
Overall incidence		23 (41.07%)	21 (37.5%)	5 (8.9%)	7 (12.5%)

6.1.6. Cost analysis for Post-CS SSI.

The result of independent ‘t’ test showed that overall mean costs of patients with post-CS SSI were [9985.7 ± 541.9] compared to patients without post-CS SSI [5767 ± 1175.5]. The mean ± SD value of medication costs among women who had developed SSI was [6183.3 ± 4162], laboratory costs [910.5 ± 97.8], and hospital admission costs [3099.9 ± 299.8]. The mean difference in treatment costs, laboratory costs, and hospital admission costs among women who developed SSI and those who had not developed SSI were statistically significant ($p < 0.001$), ($p = 0.016$), and ($p < 0.001$) (Table 6).

Variables	SSI present N=56, Mean $\pm$ SD	SSI absent N=371, Mean $\pm$ SD	P value
Cost For Laboratory	910.5 $\pm$ 97.8	539.03 $\pm$ 161.4	<0.001
Cost for treatment	6183.3 $\pm$ 416.2	3491.57 $\pm$ 1082.7	<0.001
Cost for hospital admission	3099.95 $\pm$ 299.8	1736.9 $\pm$ 355.0	0.016
Total cost	9985.7 $\pm$ 541.9	5767.53 $\pm$ 1175.5	<0.001

Table 6: Cost analysis for post-CS SSI at public hospitals in Eastern, Ethiopia 2025, n=427



Figure 2: Box plot of cost among mothers who had SSI and who had not at public hospitals in Eastern Ethiopia 2025, n=427

6.1.7. Factors associated with post-CS SSI.

The level of significance P value <0.25 in bivariate analysis were included in multivariate logistic regression. Multivariable logistic regression contained residence, BMI, prior CS, type of CS, rupture of membrane before surgery, anemia after surgery, and extended hospital stays

were strong association with post-CS SSI at $p \leq 0.05$. Mothers from rural areas were 2.37 times more likely to acquire SSI compared to those mothers from urban areas (AOR = 2.53, 95% CI: 1.308, 4.92). Mothers with previous CS scar were 3.916 times more likely to have SSI than mothers with no scar (AOR = 3.916, 95% CI: 1.78, 8.590). Mothers who stayed longer than 7 days in hospital show increased chance of having post-CS SSI by 5.86 in contrast to those who hadn't (AOR = 5.86, 95% CI: 2.56, 13.43). A two-fold increase in post-CS SSI showed in women's who had post-operative hemoglobin less than 11mg/dl (Table 7).

Table 7: Factor associated with post- CS SSI at public hospitals in Eastern, Ethiopia 2025, n=427

Covariates	Category	SSI		Bivariate Analysis		Multivariate Analysis	
		Yes N (%)	No N (%)	COR (95% CI)	P	AOR(95% CI)	P
Residence	Rural	26(9.3)	251(90.2)	2.41 (1.36-4.26)	0.002	2.537(1.308-4.96)	0.006
	Urban	30(20)	120(80)	1		1	
Body Mass Index	>25Kg/m2	41(18.6)	179(81.3)	2.93(1.569-5.480)	0.001	3.99(1.92-8.30)	<0.001
	< 25 kg/m2	15(7.2)	192(92.7)	1		1	
Previous CS	Yes	45(18.75)	195(81.25)	3.69(1.852-7.361)	<0.001	3.915(1.78-8.543)	0.001
	No	11(5.88)	176(94.1)	1		1	
Type of CS	Emergency	42(17.2)	201(82.7)	2.82(1.472-5.434)	0.002	2.745(1.318-5.71)	0.007
	Elective	14(7.6)	170(92.3)	1		1	

Premature rupture of membrane	Yes	33(24.09)	104(75.9)	4.01(2.246-7.199)	<0.001	3.55(1.8-7.03)	<0.001
	No	23(7.9)	267(92.0)	1		1	
Post-operative hemoglobin	<11 mg/dl	43(18.9)	184(81.05)	3.76(1.928-7.361)	<0.001	2.53(1.193-5.361)	0.015
	>11 mg/dl	13(6.5)	187(93.5)	1		1	
Hospital stay	<7 days	40(10.69)	334(89.3)	1		1	
	>7 days	16(30.1)	37(69.8)	3.611(1.84-7.070)	<0.001	5.86(2.567-13.43)	<0.001

6.1.8. Antibacterial resistance pattern for Gram-positive bacteria

Gram-positive organism highly resistant to penicillin, tetracycline, cefotaxime, and ciprofloxacin were 71.4 %, 58.3 %, 73.6 %, and 50 % respectively. In contrast, clindamycin (55 %), vancomycin (100 %), and linezolid (100 %) were susceptible to gram-positive organism (Table 8).

Table 8: Antimicrobial resistance patterns for Gram-positive bacteria among women with post-caesarean wound infections at public hospitals in Eastern, Ethiopia 2025, n=56

List of antibiotics	AST	Bacteria Isolated from Gram-positive organisms N (%)		
		<i>S.aureus</i> N (%)	<i>Enterococcus spp</i> N (%)	Total N (%)
CXT	S	5(26.3)	NA	5(26.3)
	I	NA	NA	NA
	R	14(73.6)	NA	14(73.6)
CIP	S	9(40.9)	3(75)	12(46.1)
	I	1(4.5)	NA	1(3.8)
	R	12(54.5)	1(25)	13(50)
COT	S	3(27.2)	NA	3(27.2)

	I	NA	NA	NA
	R	8(72.7)	NA	8(72.7)
CD	S	11(55)	NA	11(55)
	I	NA	NA	NA
	R	9(45)	NA	9(45)
GEN	S	1(50)	NA	1(33.3)
	I	NA	NA	NA
	R	1(50)	1(100)	2(66.6)
P	S	4(21.5)	2(100)	6(28.5)
	I	NA	NA	NA
	R	15(78.9)	NA	15(71.4)
TTC	S	8(38.9)	NA	8(33.3)
	I	NA	2(66.6)	2(8.3)
	R	13(61.9)	1(33.3)	14(58.3)
VA	S	NA	2(100)	2(100)
	I	NA	NA	NA
	R	NA	NA	NA
LNZ	S	3(100)	NA	3(100)
	I	NA	NA	NA
	R	NA	NA	NA
LVX	S	3(21.4)	2(66.6)	5(29.4)
	I	2(14.2)	NA	2(11.7)
	R	9(64.2)	1(33.3)	10(58.8)
SXT	S	4(40)	NA	4(36.3)
	I	NA	1(100)	1(9.09)
	R	6(60)	NA	6(54.5)

*CIP: ciprofloxacin, COT: cotrimoxazole, GEN: gentamycin, TTC: tetracycline, LNZ: linezolid, VA: Vancomycin, SXT: trimethoprim-sulfamethoxazole, LVX: levofloxacin, CXT: cefotaxin, CD: clindamycin, P: penicillin

6.1.9. Antibacterial resistance pattern for Gram-negative bacteria

Gram-negative bacteria isolates susceptible to cefoxitin, ceftazidime, Imipenem, Ertapenem, and Meropenem were 66.6 %, 64.2 %, 92.8 %, 94.4%, and 95 % respectively. In contrast, trimethoprim-sulfamethoxazole (88.8 %), tetracycline (83.3%), Amoxicillin/ clavulanic acid (46.6%), cefepime (57.1%), ceftriaxone (89.4%), cefotaxime (66.6%), and cefuroxime (66.6%) were highly resistant to gram-negative bacteria (Table 9).

Table 9: Antimicrobial resistance patterns for Gram-negative bacteria among women with post-caesarean wound infections at public hospitals in Eastern, Ethiopia 2025, n=56

List of antibiotics	AST	Bacteria Isolated from Gram-negative organisms N (%)				
		<i>Klebsella spp</i> N (%)	<i>Enterobacter spp</i> N (%)	<i>Citrobacter spp</i> N (%)	<i>Pseudomonas spp</i> N (%)	Total N (%)
CXT	S	4(66.6)	4(80)	2(50)	NA	10(66.6)
	I	NA	1(20)	NA	NA	1(6.6)
	R	2(33.3)	NA	2(50)	NA	4(26.6%)
AMC	S	1(12.5)	3(60)	1(33.3)	NA	5(33.3)
	I	1(12.5)	1(20)	2(66.6)	NA	4(26.6)
	R	6(75)	1(20)	NA	NA	7(46.6)
CAZ	S	7(77.7)	NA	1(25)	1(100)	9(64.2)
	I	NA	NA	2(50)	NA	2(14.2)
	R	2(22.2)	NA	1(25)	NA	3(21.4)
FEP	S	5(71.4)	NA	1(16.6)	NA	6(35.2)
	I	1(14.2)	NA	2(33.3)	NA	3(17.6)
	R	1(14.2)	3(100)	3(50)	1(100)	8(47.01)
CRO	S	1(11.1)	NA	1(16.6)	NA	2(11.1)
	I	NA	NA	NA	NA	NA
	R	8(88.8)	3(100)	5(83.3)	NA	16(88.8)
CTX	S	1(33.3)	3(60)	NA	NA	4(33.3)
	I	NA	NA	NA	NA	NA
	R	2(66.6)	2(40)	4(100)	NA	8(66.6)

CXM	S	1(50)	2(40)	NA	NA	3(33.3)
	I	NA	NA	NA	NA	NA
	R	1(50)	3(60)	2(100)	NA	6(66.6)
CIP	S	6(66.6)	NA	NA	1(100)	7(53.8)
	I	2(22.2)	NA	NA	NA	2(15.3)
	R	1(11.1)	3(100)	NA	NA	4(30.7)
IPM	S	5(83.3)	4(100)	4(100)	NA	13(92.8)
	I	NA	NA	NA	NA	NA
	R	1(16.6)	NA	NA	NA	1(7.14)
MEM	S	5(83.3)	7(100)	7(100)	1(100)	19(95)
	I	NA	NA	NA	NA	NA
	R	1(16.6)	NA	NA	NA	1(5)
TTC	S	1(25)	NA	NA	NA	1(16.6)
	I	NA	NA	NA	NA	NA
	R	3(75)	2(100)	NA	NA	5(83.3)
LVX	S	6(85.7)	3(60)	1(50)	NA	10(71.4)
	I	NA	NA	NA	NA	NA
	R	1(14.2)	2(40)	1(50)	NA	4(28.5)
SXT	S	1(25)	NA	NA	NA	1(11.1)
	I	NA	NA	NA	NA	NA
	R	3(75)	3(100)	2(100)	NA	8(88.8)
EPM	S	6(85.7)	7(100)	4(100)	NA	17(94.4)
	I	NA	NA	NA	NA	NA
	R	1(14.2)	NA	NA	NA	1(5.5)

*CTR: ceftriaxone, CXT: cefotaxin, LVX: levofloxacin, FEP: cefepime, CIP: ciprofloxacin, CPM: cefepime, AMC: amoxicillin-clavulanic acid, IPM: imipenem, TTC: tetracycline, CRO: ceftriaxone, CTX: cefotaxime, CXM: cefuroxime, CAZ: ceftazidime, MEM: meropenem. EPM: erapipenem, SXT: trimethoprim-sulfamethoxazole.

7. Discussion

Post cesarean wound infection rise maternal mortality, morbidity, prolonged hospital stay, and increase health care cost(4). In this study the major etiologic agent that significantly contributes to the cause the maternal mortality and morbidity were isolated. This will help to decrease the occurrence and effect of post cesarean wound infection.

The incidence of Post-CS SSI in this study was 56 (13.3%). It is aligned with previous researches undertaken in Harar, Ethiopia (12.3%), Nigeria (12.5%), and Dhulikhel Hospital, Nepal (12.6%) (27, 36, 37). The WHO surgical site infection rate guidelines were up to 20% in Africa, which is in line with this study (38). According to this study, the incidence of post-CS SSI was higher in comparison to previous research undertaken in Assela, Ethiopia (9.4%), Hawassa University Teaching Hospital, Ethiopia (11%), Estonian University Hospital (6.2%), and Regional Referral Hospital, Oman (2.66%) (39-42). This variation in the incidence of post-CS SSI might result from socioeconomic status, study area, health care delivery system, and study population. The difference may arise from lack of adequate sterilization technique, and quality of surgical care provision.

Premature rupture of membrane was significantly associated ($p < 0.001$) with post-CS SSI. Mothers who had rupture of membrane before CS contribute to high chance of developing SSI by Three times more than who had not [AOR = 3.8, CI: (1.734-6.62)]. It is aligned with previous research done in Nigeria (OR = 4.45) and US (OR = 3.0; 95% CI, (3.24–3.5))(43, 44). The amniotic fluid in pregnant women played as a barrier for pathogenic bacteria but if the fetal membrane ruptured early, the protective effect of the fluid gradually decreases over time. The pathogenic bacteria get access to enter in amniotic cavity through cervical canal may result in SSI.

Mothers from rural areas were 2.37 times more likely to acquire SSI compared to those mothers from urban areas (AOR = 2.37, 95% CI: 1.23, 4.56). There was a study undertaken in Tigray and US also shown significant association (43, 45). This may be caused by limited access to health care and poor hygiene affects a good practice throughout the surgery process.

This study showed that the risk of developing Post-CS SSI after CS were 2.5 times higher among women who had emergency CS compared to those had elective CS (AOR = 2.501, 95% CI: 1.226-5.10). Similar findings were reported from Nigeria showed that 11% who had emergency procedure got post-CS SSI than elective CS(32). This could be caused by limited time for preparation can lead to poor sterility condition and imposing the patient at greater risk of bacterial contamination.

A significant correlation was found between post-CS SSI and obesity. The study revealed that having a body mass index more than 25kg/m² has four times higher risk for having post-CS SSI {(AOR = 4.06, 95% CI: 1.97-8.37). Current study align with a prospective study performed in Iraq (26). This might be due to thick subcutaneous tissue, impaired immunity, and limited penetration of prophylactic antibiotics in adipose tissue. This study also found that mothers with post-operative hemoglobin level were less than 11 g/dl were 2.3 times higher risk of having post-CS SSI. This research supported by the studies undertaken in Nigeria and Ethiopia(36, 46).This is because decrease amount of hemoglobin results impaired transportation of oxygen which leads to hinder wound healing and weakened immune system.

The risk of post-CS SSI was 5.6 times greater among women had a hospital stay longer than 7 days (AOR = 4.06, 95% CI: 2.47-12.73). This study aligns with research done in different settings (16, 27, 47).This might be due to the long the mothers stay the more to get infection because hospital areas assumed to be contaminated by medical staff, patients, and attendants. In addition, women's who had previous scar is more likely to develop post-CS SSI than those had not (48, 49). This might be due to presence of prior scars had intraoperative adhesion, weaker scar and poor healing.

Previous studies showed that hospitalization was prolonged for mothers who developed post-CS SSI compared to those who had not (50). This cause significant increase in cost of the following medications, laboratory, and hospital admissions. The mean cost of treatment or medication with post-CS SSI was 6176 birr and 3485 birr for patients without SSI. Likewise, Mean cost on hospital admission were (3106.9 birr) with SSI compare to (1732 birr) patients without SSI. Laboratory cost mean differed in mothers with SSI (910.5 birr) to mothers without SSI (538 birr). The cost of treatment, laboratory, and hospital admission cost were significantly associated with post-CS SSI $p<0.001$, $p=0.016$, and $p<0.001$ respectively. It agrees with a study done in Germany (50). The overall mean cost for patients who had post-

CS SSI was (9985 birr) and was therefore higher than for mothers without SSI (5756 birr); $p < 0.001$. This finding is consistent with a study in Berlin and others (50, 51).

In current study, Gram-positive organisms were 56.1% and 42.1% were Gram-negative organisms. *S. aureus* is a predominant from gram-positive organisms by (47.3%) subsequent to *K. pneumoniae* were (17.5%), *E. coli* (14.3%), *Citrobacter* (10.5%), *Enterococcus spp* (7.01%), *CoNs* and *Pseudomonas spp* were similar (1.75%). This is supported by the research done in Ethiopia, the most frequently isolated bacteria were *S. aureus* (41.6%), *E. coli* (19.8%), *K. pneumoniae* (13.9%), coagulase-negative *Staphylococcus* (12.9%), and *Enterobacter spp* (4%) (8). Based on research conducted in Pakistan, A total of 153 organisms were identified, of which *Staphylococcus aureus* (50.32%), followed by *Pseudomonas aeruginosa* (16.3%), *Escherichia coli* (14.37%), and *Klebsiella pneumoniae* (11.76%) (52).

In other previous study, revealed that a high proportion of Gram-negative organism than Gram-positive organisms. According to study done in Ethiopia, 55.9% were accounted for gram negative organisms and 44.1% for gram positive bacteria. *Escherichia coli* was the major bacteria isolate from gram negatives (24.3%) subsequent to *Staphylococcus aureus* (23.4%) in gram positives (53). This difference in the pattern of distribution of bacterial isolates in different setups may be due to diversity of the study participants and inappropriate usage of local antimicrobial agent lead to emergence of bacteria that resist to commonly used antibiotics.

In present study, linezolid (100%), vancomycin (100%), and clindamycin (55%) were susceptible for gram-positive bacteria. The current research is consistent to studies in Ethiopia, and India (54, 55). Levofloxacin (71.4%), ciprofloxacin (53.8%), Imipenem (92.8%), cefotaxime (66.6%), and ceftazidime (64.2%) were susceptible for isolated bacteria from gram-negative. This aligns with research conducted in India, Nepal, and Ethiopia (55-57). Many of the gram-negative bacteria were resistant to ceftriaxone, cefuroxime sodium, amoxicillin/clavulanic acid, cefotaxime, cefepime, trimethoprim-sulfamethoxazole, and tetracycline which are consistent with different studies worldwide (7, 58, 59). This could be due to inappropriate or irrational usage of antibiotics causes the antibiotic to be ineffective for pathogens.

Staphylococcus aureus was the number one bacteria isolated from wound site, which was resistant to Tetracycline, Penicillin, and Co-trimoxazole were 61.9%, 78.9%, and 72.75% respectively. This findings is consistent with study undertaken in Bahirdar, Ethiopia showed that the overall resistance rate of Tetracycline, Chloramphenicol, Penicillin and Ampicillin were 62% (60).

E.coli was highly resistant to cefepime (100%), ceftriaxone (100%), ciprofloxacin (100%), and tetracycline (100%). This is in agreement to study done in Oman (42). *K.pneumonia* was resistant to amoxicillin/clavulanic acid (75%), ceftriaxone (88.8%), and Tetracycline (75%). This aligns with a study conducted in Tanzania (25). High incidence of antibiotic resistant occurred because frequent usage of drug without prescription.

8. Strength and limitation of the study

8.1 strength of the study

- The data had adequate representation of the research because conducted in 3 hospitals and 1 health center.
- This study's source of data was primary data, so the quality of the data is good.

8.2 limitation of the study

- This research can't determine the cause and effect because of the cross-sectional nature of the study.
- Fungus and strict anaerobic bacteria were not identified in current study so this can increase negative culture results. This can underestimate the burden of the infection.

9. Conclusion and recommendation

9.1 Conclusion

The incidence of post cesarean wound infection was 13.3% and the frequently isolated bacteria were *S. aureus*, *K. pneumonia*, *E. coli*, *Citrobacter spp*, and *Enterobacter spp*. As observed in this study, both gram positive and gram negatives, have developed resistant to widely used antibiotics such as tetracycline and trimethoprim-sulfamethoxazole. Patients with multi-drug resistance infections cause an increase in hospital stays and health care costs. Previous CS, type of CS, obesity, hospital stay, post-operative hemoglobin level, residence, and premature rupture of membrane, all this contributing risk factors associated with postoperative wound infections.

9.2 Recommendation

To reduce the incidence rate of post-cesarean wound infection, strict adherence to infection prevention control in all surgery process, and all medical staff involved in the surgery should take training on aseptic technique. Health bureaus, health professionals, and researchers should work collaboratively to strengthen infection prevention, enhance microbiological surveillance, promote rational antibiotic use, and conduct further studies on additional risk factors and their economic impact. Ministry of Health should implement a microbiology laboratory in Dire Dawa because to track down easily common pathogenic bacteria and drug resistance bacteria to take appropriate action. To prevent impact of multi-drug resistance, patients should take antibiotics rationally.

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11. Annexes

Annex I. Participant information sheet

A. English Version

Principal investigator: Akera Fekade

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

Project title: Incidence of post-Cesarean section infections, bacterial profiles, resistant patterns, and healthcare costs among women delivered at public hospitals in Eastern, Ethiopia, 2025.

Background: I am Akera Fekade, currently enrolled in graduate program at Addis Ababa University, College of Health Sciences, and specializing in microbiology. Now, I am going to conduct a research project on incidence of post- Cesarean wound infections, bacterial profiles, resistant patterns, and healthcare costs among women delivered at public hospitals in Eastern, Ethiopia, 2025.

Purpose of the investigation: The goal of the research is to determine the incidence of post-Cesarean wound infections, bacterial profiles, resistant patterns, and healthcare costs among women delivered at public hospitals in Eastern, Ethiopia.

Procedures and anticipated participation: If you are willing to enroll on this study, you need to give your consent. After consent, you will give a wound sample, information about the cost they spend in hospital, and personal interview to fill the questionnaire. All the data kept confidentially and performed by using codes. Besides this you have the right to know your result without any payment. Consequently, if significant result seen, further diagnosis and treatment will be made by linking with the concerned health professionals. Since participation is volunteer bases, you have the right to participate or not to participate but your participation on the study will benefit you as well.

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Annex II: Consent Form (study participants)

A. English version

Card No. (Code) _____

I confirm that I have read and understand the information about the project as provided in the participant information sheet. I have been informed about the nature of this study and willingly consent to take part in it. I understand that the content of the interview will be kept confidential. I have been informed about the risks and benefits involved. Furthermore, I have been assured that any future questions that I may have will also be answered by a member of the research team. I voluntarily agree to take part in this study.

Date _____

Signature _____

Thank you for your participation!

Annex III: Questionnaire Socio-demographic characteristics and clinical information of the participants

Project Title: Incidence of post-Cesarean section infections, bacterial profiles, resistant patterns, and healthcare costs among women delivered at public hospitals in Eastern, Ethiopia 2025.

A. English Version

Code number _____

No	Variable	Response
1.	Age	
2.	Residence	1. Rural 2. Urban
3.	Occupation	1. House wife 3. Private business owner 2. Government employee 4. Student 5.If other please specify_____
4.	Marital status	1. Married 3. Single 2. Divorced 4. Widowed
5.	Education Status	1. Primary Education 2. Secondary Education 3.Higher Education
6.	Monthly income	1. <1500 Birr 3. 5001-10000 Birr 2.1501-5000 Birr 4. >10001 Birr
7.	Physical exercise	1. Never 2.Sometimes 3.regularly
8.	Alcohol drinking habit	1. Never 2. Sometimes 3.regularly
9.	Smoking habit	1.Never 2.Sometimes 3.regularly

10.	Does the patient have to follow up condition?	A, yes B, no
11.	Hospital Stays	1.>7 days 2.<7 days
12.	Have you taken any antibiotic for Prophylaxis before CS?	A yes B no
13.	BMI	1.>25 Kg/ m2 2.< 25 kg/ m2
14.	Post-operative hemoglobin	1.<11kg/dl 2.>11Kg/dl
15	If yes please record antibiotic type?	
16	Does the patient take any antibiotic after 30 min of CS?	A yes B no.
17	If yes please record antibiotic type?	
18	Type of CS	A. emergency B elective
19	Gestational Diabetes Mellitus	A Yes B no
21	Premature rupture of membrane	A. yes B.no
22	Anemia	A. yes B, no
23	Pregnancy induced hypertension	A. yes B, no
24	History of urinary tract infection?	A yes B no
25	Incision type?	A. Transverse B. vertical

26	Parity	1.Primipara 2.Multipara 3.Grand multipara
27	Antenatal care follow up	1.Yes 2.No
28	Previous CS	1.Yes 2.No
29	Fetal weight	1.<2400 gm 2.2400-4000gm 3.>4000gm

Annex I. Barreeffama Odeeffannoo Hirmaattota

A. Afaan Ingiliffaa

Qorataa Ol'aanaa: Akeru Fekade

Mekaanizimii: Yuunivarsitii Finfinnee, Kollejii Saayinsii Fayyaa, Kutaalee Saayinsii Labooraatorii Fayyaa

Mata Duree Qorannoo: Hojiiwwan waraqaalee cabsuuf baay'ee qaama buqqisu fi waraabbii rakkoo fi qorannoo bulchiinsa fayyaa haala waraqaa cabsu irraa faayidaalee baay'ee qabu waliin waliigaltee Gadaameessaa fi Qabiyyee rakkoo Saayinsii Rakkoo fi Faayidaalee waggaa keessaa irratti wal- qixxummaa irratti ilaalchaa hin fudhannee sagantaalbsa:

Hirmaattota qorannoo kabajamoo: Ani barataa MSc ti kan Yuunivarsitii Finfinnee, Kulliyoota Saayinsii Labooraatorii Fayyaa keessa barachuudhaan. Akkasumas, hirmaattotni isinii qabiyyee ilaalchisee waraqaa walgahii hojii rakkoo akkasuma yaalii fi faayidaa keessa jira.

Kaayyoo Qorannoo: Kaayyoon qorannoo kanaa qorannoo rakkoowwan waraqaalee cabsuuf baay'ee saayinsii rakkoo hirmaataan faayidaa sirreessitootis fi irkannaa yaada bu'aa fi barbaachisummaa yaadaa waliin madaalchise keessa jira, sadarkaan hojii yeroo gabaabaan mara keessaa dhufee **Yeroo:** Yeroo qorannoo kanaaf yeroon isaa ji'a 2-3 fudhata.

Tartiiba fi hirmaannaa eegamu: Yoo qorannoo kana keessatti hirmaachuuf fedhii qabaattan, hayyama keessan kennuu qabdu. Hayyamni erga kennamee booda, sammun cinaa irraa fudhatama, baasiwwan ispoortii hospitaala keessatti kaffaltan, fi gaaffilee irratti deebii kennuudhaan qorannoo guutuu irratti hirmaattu. Odeeffannoon argame hundi sirriitti eeggama, fi lakkoofsa koodii fayyadamuun hojjetama. Akkasumas, bu'aan keessan kaffaltii malee isinitti himama. Yoo bu'aan barbaachisaa ta'e argame, qorannoo dabalataa fi yaalii wal'aanummaa ogeeyyii fayyaa waliin ta'uun ni taasifama. Hirmaannaan keessan fedhii keessan irratti hunda'ee ta'uu isaa hubadhaa; hirmaachuun keessan qorannoo kana keessatti isin fayyada.

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Annex II: Foomii Eeyyama (hirmaattota qorannoo)

A. Afaan Ingiliizii

Qorataa Ijoo: Akera Fekade

Mata Dureewwan Qorannoo: Incidence of post-Cesarean section infections, bacterial profiles, resistant patterns, and healthcare costs among women delivered at public hospitals in Eastern, Ethiopia 2025. Ani barreeffama kana dubbisee ykn afaan ani hubadhu keessatti akka naaf dubbatameetti dubbisuu koo ni mirkaneeffadha, akkasumas kaayyoo, adeemsa, fi balaa qorannoo kana keessatti ibsame akka hubadhe ni beeka. Yeroo kamiyyuu qorannoo kana irraa haa ba’u yoo barbaade sababa tokko malee ba’uu dandeenya. Gaafiiwwan gaafachuuf carraa argadhee, deebiiwwan argadheefis nan gammada. Akka hirmaataan qorannoo kanaa ta’uu kootiif kaffaltii addaa tokko hin jiru beekuu kootiif, hirmaachuuf ammaan dura akka waliigaltee kanaatti ni waliigala.

Barreeffama kana mallatteeffamee naaf kennamuu akka beeku nan hubadha.

Maqaa hirmaataa _____ Umurii Teessoo _____ Mallattoo _____ Guyyaa _____

Maqaa Gaafataa _____ Mallattoo _____ Maqaa _____

Qorataa Ijoo: Akera Fekade Mallattoo _____

Galatoomaa hirmaannaa keessaniif!

Annex III: Questionnaire Socio-demographic characteristics and clinical information of the participants

Gaaffii: Dhimmoota Saayinsii-fi-Soshaal-Demokraatikii fi Odeeffannoo Kliinikaa Hirmaatoota Mata-duree Qorannoo: Baay'inni Dhukkuba Gochaa Dhiibbaa Dhiigaa Erga Dhiibbaan Siizaarii (Cesarean) booda, Akkaataa Baakteriyaa, Mallattoolee Qorannoo fi Sirna Dadhabbii isaanii, fiKharraawwan Tajaajilaa manneen yaalaa uummata bakka bu'oota biyya Itoophiyaa gara bahaatti bara 2025.

A. Afaan Ingiliffaatti

Lakkoofsa koodii _____ Lakkoofsa Kaardii Hospitaalaa _____

Naannoo _____ Lakkoofsa bilbilaa fi lakkoofsa bilbila biraa _

Yaadachiisa: Deebii sirrii ta'e gara fuula dhiyaateetti koodii dhiibbaadhaan barreessitanii ykn gubbaan eeruu kennaa. Siin filatamte ta'uudhaan qorannoo kana keessatti hirmaachuuf ni gaafatama, deebii sirrii akkasumas gorsa gaaffii kennamee akkuma ta'eetti kennuuf ni gorfama.

Lakk.	Variable	Deebii
1	Umuri	
2	Bakka Jireenya	Baadiyyaa 2. Magaalaa
3	Hojii	1. Haadha manaa 2. Hojjetaa mootummaa 3. Hojjetaa daldala dhuunfaa 4. Barataa
4	Haala fuudhaa heerumaa	1. Heerume 2. Addaan cite 3. Dhiira/dubartii takkaa 4. Waliin jiraachu dhabuu
5	Sadarkaa Barnootaa	Barreessuu fi dubbisuu hin danda'u Sadarkaa jalqabaa 3. Sadarkaa lammaffaa 4. Diiplomaa fi ol
6	Galii ji'aa	1. <1500 Birrii 2. 1501-5000 Birrii 3. 5001-10000 Birrii 4. >10001 Birrii

7	Shaakala qaamaa	1. Hin shaakalu 2. Eeyyee, nan shaakalaa. Shaakala kee torbanitti sa'aatii meeqa? _____
8	Amala dhugaatii Alkoolii	1. Hin dhugu. 2. Dhuga. Torbanitti yeroo meeqa dhugda? _____
9	Amala tamboo xuuxuu	1. Hin xuuxu. 2. Xuuxu. Guyyaa sigara/paakeejii meeqa xuuxda? _____
10	Dhukkuba hordoffii barbaadu qaba?	A. Eeyyee B. Lakkii
11	Yoo eeyyee ta'e, kun hospitaala kana keessatti moo hospitaalota biroo keessatti raawwata?	
12	Isheen kun dhalchuu ishee jalqabaa dha?	A. Eeyyee B. Lakkii
13	Yoo hin ta'in, hamma meeqaa dhalatte?	
14	Kun dhiibbaa jalqabaa hojjachuu isheetii dha moo miti?	A. Eeyyee B. Lakkii
15	Yoo hin ta'in hamma meeqaa hojjatte?	
16	Dhiibbaan dura hospitaala keessatti guyyoota meeqaa turte?	

17	Dhiibbaan booda hospitaala keessatti guyyoota meeqaa turte?	
18	Dursee antibayootikii fudhatte?	A. Eeyyee B. Lakkii
19	Yoo eeyyee ta'e, gosa antibayootikii galmeessi.	
20	Dhiibbaa booda daqiiqaa 30 keessatti antibayootikii fudhatte?	A. Eeyyee B. Lakkii
21	Yoo eeyyee ta'e, gosa antibayootikii galmeessi.	
22	Gosa dhiibbaan Hojjate	A. Muddama B. Filatamaa
23	Seenaa dhiibbaa sukkaaraa qaba?	A. Eeyyee B. Lakkii
24	Yoo eeyyee ta'e, gosa sukkaaraa:	a) Sukkaara gosa 1 b) Sukkaara gosa 2 c) Sukkaara ulfaa
25	Iddoo urgoffii yeroo dheeraa (>12 sa'aatii) qaba?	A. Eeyyee B. Lakkii
26	Baay'inni dhiiga xiqqaadha?	A. Eeyyee B. Lakkii

27	Seenaa dhiibbaa dhiigaa qaba?	A. Eeyyee B. Lakkii
28	Yoo eeyyee ta'e, maaliif ta'aa?	A. Dhaloota dura B. Dhaloota booda
29	Seenaa dhukkuba uumaan urgooftuu qaba?	A. Eeyyee B. Lakkii
30	Yoo eeyyee ta'e, gosa qoricha antibayootikii galmeessi?	
31	Gosa cinaachaa?	A. Bal'inaa B. Giddu-galeessa
32	Qorannoo laaboraatoorii irratti maallaqa meeqa kaffalte?	
33	Yaalii yookin qoricha irratti maallaqa meeqa kaffalte?	
34	Hordoffii hospitaala irratti maallaqa meeqa kaffalte?	
35	Maallaqa birraa biroo irratti kaffalte?	

B. Amharic Version

[illegible]

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Annex II: 同意書 (Consent Form)

A. 研究の目的と意義 (Purpose and Significance of the Study)

本研究の目的は、_____

本研究は、_____ (Participant Information Sheet) _____
_____ _____
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研究のリスクと利益: _____

研究の進捗と結果の報告: _____

研究の倫理審査委員会による承認を得ています。

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Annex IV: Laboratory Test Procedures

Wound sample collection: to avoid contamination we use aseptic technique for collection in order to separate normal flora.

Gram stain: used to differentiate bacteria based on their cell wall structure.

Procedure of Gram's Stain

1. Fix the dried smear with heat by gently passing it over spirit lamp or Bunsen burner.
2. Cover the fixed smear with crystal violet stain for 30 – 60 seconds
3. Rapidly wash off the stain with clean water
4. Tip off all the water, and cover the smear with Lugol's iodine for 30 – 60 seconds
5. Wash off the iodine with clean water
6. Decolorize rapidly with acetone-alcohol for 30 seconds. Wash immediately with clean water.
7. Cover the smear with Neutral red or Safranin for 2 minutes
8. Wash off the stain with clean water.
9. Wipe the back of the slide clean, and place it in a draining rack for the smear to air dry
10. Examine the smear microscopically, first with the 40x objective to check the staining and to see the distribution of material, and then with oil immersion objective to report the bacteria and cells.

Results

- Gram positive bacteria Purple
- Gram negative bacteria Pale to red

Culture media: artificially prepared media containing the required nutrients used for propagation of microorganisms.

Detailed Procedure

1. Bring culture media to RT and label plates with appropriate information (e.g., Patient ID, specimen type, date).
2. Prepare a Gram stain smear using one swab.
3. Inoculate the sample using a second swab:
 - Roll the swab onto the first quadrant.
 - Rotate the plate and streak subsequent quadrants, touching the previous quadrant 2-3 times.
4. Incubate plates aerobically at 35-37°C for 18-24 hours.
5. If no growth is observed after 18-24 hours, record a preliminary result and re-incubate for another 18-24 hours.
6. If no growth is observed after 48 hours, discard plates and issue a final report.
7. If growth is observed, perform biochemical identification and antimicrobial susceptibility testing (AST) per specific SOPs.

Procedural Notes: The skin is colonized with normal flora such as coagulase negative staphylococci, diphtheroids and alpha-haemolytic streptococci. The isolation of these organisms from wounds is generally considered to be contamination. However, when isolated from tissues and deep wounds especially those associated with prosthetic devices, these organisms may be significant. Consult with the physician in these circumstances.

Result Reporting

- **Positive Results:**
 - Identify bacteria to species level and report with AST results.
 - Report the presence of significant organisms detected in Gram stain in association with polymorphonuclear leukocytes (PMNs).

- **Negative Results:**

- "No growth within 48 hours" for samples with no growth.
- "No pathogenic bacteria isolated" for samples with normal flora growth.

Result Interpretation

- Significant growth includes *S. aureus*, beta-hemolytic streptococci, *Pseudomonas aeruginosa*, and other potential pathogens with PMN association.
- Growth of ≥ 3 types of coliforms or Gram-negative bacilli may indicate contamination (report as "mixed Gram-negative bacilli). Normal skin commensals can be reported as "commensal flora."

Biochemical tests for gram-positive bacteria

1. **Catalase test**- catalase enzyme produces by microorganism in order to neutralize the killing effect of hydrogen peroxide and used to differentiate Staphylococci from streptococci.

Principle:

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

Procedures: Place one drop of hydrogen peroxide reagent on slide and observe immediately for effervescence.

If colony is from BAP, use care not to pick up blood

Result Reporting: Positive: report as catalase positive

Negative: report as catalase negative

Result Interpretation: Positive: shows immediate appearance of bubbles.

Negative: shows no bubbles or a few bubbles after

2. Coagulase test

The coagulase test is used to differentiate *Staphylococcus aureus*, the most pathogenic of the staphylococci, from other commonly isolated staphylococci. Coagulase is a thermostable thrombin-like substance that activates fibrinogen to form fibrin, resulting in a fibrin clot. This is demonstrated in the test tube by the formation of a clot when plasma is inoculated with *S. aureus*.

Procedure

Drop a saline on microscope slide that have pus sample and add loop-full plasma the look for clumping within 10 seconds.

Result

Positive-----Confirm with tube and report based on tube coagulase reporting mechanism

Negative-----Coagulase Negative *Staphylococcus*

Biochemical test for gram-negative bacteria

1. Oxidase test

To differentiate isolated bacteria that produce cytochrome c oxidase from oxidase negative. The role of the enzyme is to transport electron in oxygen molecule. Oxidase positive are *Pseudomonas*, *Neisseria*, and *Vibrio*.

Principle

tetramethyl-p-phenylenediamine dihydrochloride used as a reagent and play as artificial electron donor. Dark purple colors appear after the bacteria oxidize the reagent.

Procedure

Take a small colony from the culture media on filter paper or oxidase test strip

Add a drop of oxidase reagent and look for color change within 10 to 30 seconds

Result

Positive results: dark purple color appears

Negative results: No color change seen

2. Urease test

This test is used to detect the enzyme urease, which breaks down urea into ammonia.

Testing for urease enzyme activity is important in differentiating enterobacteria.

Brucella and *Proteus* strains are strong urease producers.

Principle

Urea convert to ammonia by the enzyme called urease and the release of ammonia changes the pH to alkaline medium then pH indicator change color if it is positive.

Procedure

Inoculate the test organism in to urea broth and incubate for 24 hours then check for color change.

Results

Positive results: pink color appears

Negative results: No color change seen

3. Motility test

Motility test doesn't look for metabolic properties of the bacteria then it isn't biochemical test.

- Rather, this test can be used to check for the ability of bacteria to migrate away from a line of inoculation.
- To perform this test, the bacterial sample is inoculated into motility media using inoculating straight wire.
- Simply stab the media in as straight a line as possible and withdraw the needle very carefully to avoid destroying the straight line.

After incubating the sample for 24-48 hours, observations can be made.

- Check to see if the bacteria have migrated away from the original line of inoculation.

- If migration away from the line of inoculation is evident then you can conclude that the test organism is motile (positive test).
- Lack of migration away from the line of inoculation indicates a lack of motility (negative test result).

Antimicrobial sensitivity testing

Procedure: A laboratory test to determine the effectiveness of antimicrobial agents against a specific bacterium.

Principle

AST using the disk diffusion method estimates in vitro susceptibility. A standardized bacterial inoculum is spread on MHA plates, and antimicrobial disks are placed on the agar surface. Inhibition of bacteria occurs when antimicrobial agent diffuses in the media at the time of incubation, creating a zone of inhibition. Zone of inhibition diameter is measured and interpreted as susceptible, intermediate, or resistant using CLSI guidelines.

Detailed Procedures

1. Prepare bacterial suspension:
 - Touch 4-5 isolated colonies with a loop and suspend in TSY broth or saline.
 - Adjust to 0.5 McFarland standards using a nephelometer or black-lined paper.
2. Prepare MHA plates:
 - Ensure plates are contamination-free, have a uniform agar depth (4 mm), and are not overly wet or dry.
3. Inoculate plates:
 - Dip a sterile cotton swab into the suspension and remove excess inoculum by pressing against the tube wall.
 - Streak the swab over the entire MHA surface three times, rotating the plate 60° after each streak.
 - Run the swab around the plate's rim to ensure even coverage.

4. Apply antimicrobial disks:
 - Place disks on the agar surface using a dispenser or forceps.
 - Ensure complete contact between disks and agar.
5. Incubate plates:
 - Invert plates and incubate at $36\pm 1^{\circ}\text{C}$ for 16-18 hours (24 hours for *S. aureus*).
6. Measure zones of inhibition:
 - Measure the diameter of the clear zone around each disk in millimeters.
 - Record the diameter and interpret results using CLSI guidelines.

Result Reporting

Report results as Susceptible (S), Intermediate (I), or Resistant (R) based on CLSI guidelines.

Declaration

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

M.Sc. candidate

Signature

Date of submission

Akera Fekade (Bsc)

This Thesis has been submitted with our approval as advisors;

Advisors

Signature

Date

Dr. kassu Desta (MSc, PHD)

Biruk Zerfu (MSc, PHD candidates)

. Date:_____Place: Addis Ababa, Ethiopia