ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Incidence of surgical site infection, predisposing factors and associated costs at Dessie Referral Hospital, Dessie, Ethiopia

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

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List of abbreviations

- BMI- Body Mass Index
- CDC- Center for Diseases Control
- CI- Confidence Interval
- CoNS- Coagulase Negative Staphylococci
- CLSI- Clinical Laboratory Standard Institute
- CS- Caesarean Section
- DRH- Dessie Referral Hospital
- ECDC- European Center for Disease Control and prevention
- ESBL- Extended Spectrum Beta Lactamase
- HAI- Hospital Acquired Infection
- ICD-CM- International Classification of Disease- Clinical Modification
- ICU- Intensive Care Unit
- INICC-International Nosocomial Infection Control Consortium
- LOS- Length Of hospital Stay
- MDR- Multi Drug Resistant
- MRCoNS- Methicillin resistant CoNS
- MRSA- Methicillin Resistant *Staphylococcus aureus*
- MSSA- Methicillin Sensitive *Staphylococcus aureus*
- NCCLS- National Committee for Clinical Laboratory standards
- NHIS- National Nosocomial Infection Surveillance
- OR- Odds Ratio
- QC- Quality Control
- SD- Standard Deviation
- SDD- Susceptibility Dose Dependent
- SOP- Standard operating procedure
- SPSS- Statistical Package for Social Science
- SSI- Surgical Site infection
- USA- United States of America

Operational definition

1. Wound class: An assessment of the degree of contamination of a surgical wound at the time of the operation. Wound class should be assigned by a person involved in the surgical procedure (e.g., surgeon, circulating nurse, etc.).

1.1. Clean Wound: A clean wound is an uninfected operative wound in which no inflammation is encountered and the respiratory, alimentary, genital or uninfected urinary tracts are not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage. Operative incisional wounds that follow non-penetrating trauma should be included in this category.

1.2. Clean-contaminated wounds: they are operative wounds in which the respiratory, alimentary, genital or uninfected urinary tracts are entered under controlled condition and without unusual contamination. Specifically operations involving the biliary tract, appendix, vagina and oropharynx are included in this category provided no evidence of infection or major break in technique is encountered.

1.3. Contaminated wounds: include open, fresh, accidental wounds. In addition operations with major breaks in sterile technique or gross spillage from the gastrointestinal tract, and incisions in which acute, nonpurulent inflammation is encountered are included in this category.

1.4. Dirty or infected wounds: include old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera. This definition suggests that the organisms causing postoperative infection were present in the operative field before the operation (1,2).

2. ASA: physical status classification of patients before surgery, developed by the American Society of Anesthesiology

2.1. ASA-1: Normally healthy patient

2.2. ASA-2: Patient with mild systemic disease

2.3. ASA-3: Patient with severe systemic disease that is not incapacitating

2.4. ASA-4: Patient with an incapacitating systemic disease that is a constant threat to life

2.5. ASA-5: Moribund patient who is not expected to survive for 24 hours with or without operation (1,2).

3. Elective surgery: an operation that was planned at least 24 hours in advance (1,2).

4. Emergency surgery: an operation that was not planned at least 24 hours in advance (1,2).

5. Episiotomy: a cut that is sometimes made at the opening of a woman's vagina to make the birth of a baby easier or safer.

6. Case definition

Post operative surgical site infection was defined according to CDC and ECDC criteria. SSI was classified as superficial, deep incisional or organ/space infection, with:

a. Purulent drainage with or without laboratory confirmation from the superficial or deep incision
b. Organism isolated from an aseptically obtained culture of fluid or tissue from superficial or deep incision or organ/space.
c. Sign or symptoms of infection: Pain and tenderness, localized swelling, or heat
d. Purulent drainage from the drain that is placed into the organ/space.
e. Diagnosis of SSI by surgeon or attending physician (1,2).

Abstract

Background: Surgical wound infection is a prototype of (Hospital Acquired Infection) HAI and constitutes a serious problem. Patients diagnosed with Surgical Site Infection (SSI) face a 2 to 11 times increased in mortality along with prolonged hospital stays, treatment associated risks, pain, suffering, delayed wound healing, revision of surgery, and potential long-term sequelae. The burdens posed by SSIs are also reflected in excess of health care costs. Advances in control of infections have not completely eradicated the problem because of development of resistance. There are very limited data in relation to this agenda particularly cost associated with SSI in Ethiopia as well as Africa.

Objective: The aim of this study was to assess the incidence of surgical site infection, predisposing factors and associated costs at Dessie Referral Hospital, Dessie, Ethiopia

Methods: A hospital based cross sectional study was conducted from July 22 – October 25, 2016 in Dessie referral hospital. About 338 surgical patients who fulfilled the inclusion criteria were included using consecutive convenient sampling technique. Information regarding socio-demographic status and past medical history of the respondent was collected using pre-structured questionnaire. Two pus swabs were taken when a patient was first presented with clinical evidence of infection. One of the swabs was processed for microscopic examination the other one was inoculated to MacConkey agar, Blood agar and Manitol salt agar following a standard operating procedure. Colony characteristics, Gram's reaction and biochemical tests were used to differentiate the organisms. Antibiotic susceptibility of the isolated bacteria was determined by Kirby-Bauer disc diffusion technique, following CLSI guideline (CLSI document M100-S24). The data was analyzed by SPSS version 20 and the results were presented by using tables and different graphs. Logistic regression analysis was used to determine the association between dependent and independent variables.

Results: out of the 338 patients included in this study 49 (14.5 %) were clinically suspected for SSIs. Forty one out of the 49 (83.7 %) wound swabs were culture positive; hence the overall

culture confirmed SSI was 12.1 %. More than half of the bacteria isolated were gram negative rods. About 79.2 % of all the isolates were Multi Drug Resistant (MDR). About 66.67 % and 100 % of *E. coli* and *K. pneumonia* were Extended Spectrum Beta Lactamase (ESBL) producers respectively. In this study, 55.6 % of the *Syaphylococcus species* were methicilin resistant (*MRSA*). Hypertension, ASA score-3, elective surgery and the age group 35-44 years were found to be statistically significant associated factors for SSIs. A more than two times increase of the cost and length of hospital stay was observed as a result of SSI in Dessie Referal Hospital.

Conclusion and recommendation: the rate of SSI, MDR, MRSA and ESBL producing bacteria was very high among patients with SSI. A hospital based survey should be conducted on a regular basis, evidence based information should be given to the surgical team in order to reduce the rate of SSIs and the use of some drugs should be limited due to a high degree of resistance.

Key terms: SSI, predisposing factors, drug susceptibility, cost

1. Introduction

1.1. Background

Nosocomial infections (also called hospital acquired infections (HAI)) occur worldwide and affect both developed and developing countries. The most frequent nosocomial infections are infections of surgical wounds, urinary tract infections and lower respiratory tract infections (3). A surgical site infection (SSI) is an infection that occurs after surgery in the part of the body where the surgery took place due to contamination during the time of operation (4). Any purulent discharge from a closed surgical incision, together with signs of inflammation of the surrounding tissues should be considered as wound infection (3,5), irrespective of whether micro-organisms can be cultured (5). Surgical site infections occur within thirty days after the operative procedure (except in case of added implants, when the duration extends to one year from operation) (6). However, wounds that are closed and primarily healed are not considered infected (5).

The Centre for Disease Control, (CDC), USA, further defines the surgical site infections according to a standard set of clinical criteria to classify into: (a) Superficial incisional SSI which involves only skin and subcutaneous tissue of incision, (b) Deep incisional SSI which involves deep soft tissues (e.g. fascia and muscle layer) of the incision, (c) Organ Space SSI includes infection apparently related to the operative procedure and infection involves any part of the body, excluding skin incision, fascia, muscle layer that is operated or manipulated during operative procedure (1,2,6–8). The infection is usually acquired during the operation itself; either exogenously (e.g. from the air, medical equipment, surgeons and other staff), or endogenously from the flora on the skin or in the operative site (3,9) or, rarely, from blood used in surgery (3).

Post-operative wound infections have been a problem in the field of surgery since time of immemorial (10). Surgical site infection infections are the commonest nosocomial infection and responsible for the increasing financial burden, morbidity and mortality related to surgical operations (9–11). Despite an improved understanding of the patho-physiology and improved methods of prevention and prophylaxis, SSI remain the most common cause of post operative morbidity and mortality (10,11).

There are many factors that are thought to affect the susceptibility of surgical site infection, some of which strongly predispose to wound infection (5). The main risk factor is the extent of contamination during the procedure (clean, clean contaminated, contaminated, dirty), which is to a large part dependent on the length of the operation, and the patient's general condition (3,5). Other factors include the quality of surgical technique, the presence of foreign bodies including drains, the virulence of the microorganisms, concomitant infection at other sites, the use of preoperative shaving, the two extremes of age, immune status, high dose steroids, diabetes mellitus, morbid obesity, cancer and the experience of the surgical team (3,12).

The infecting microorganisms are variable, depending on the type and location of surgery, and antimicrobials received by the patient (3). Hundred years ago the *Streptococcus* was the most frequent pathogen encountered in the surgical wounds, but replaced by the *Staphylococcus* twenty years ago (9). In recent years, there has been a growing number of post-operative wound infections due to Gram negative organisms (9,11). In general the most commonly isolated aerobic microorganisms includes *S. aureus, CoNS, Enterococci, E. coli, P. aeurginosa, Klebsiella pneumonia, Enterobacter, Pr. mirabilis, other Streptococci, Candida, and Acinetobacter* (11,13). In addition the types of bacteria present in such kind of wound infections could vary with geographical locations (11).

The wound infection and other postoperative infections continue to be a problem even though the antibiotics have reduced their frequency. This is because of the widespread and indiscriminate usage of the antibiotics results in multidrug resistant bacteria. Inspite of better advances made in the aseptic precautions, antimicrobial agents, sterilization and operation techniques, the postoperative wound infection still continues to be the major problem in the surgical specialties (9). Therefore, isolation of the microorganisms and identifying their susceptibility pattern to different kinds of antibiotics and antimicrobial helps in the better management of postoperative wound infection.

1.2. Statement of the problem

A nosocomial infection can be defined as: An infection acquired in hospital by a patient who was admitted for a reason other than that infection. Despite progress in public health and hospital care, infections continue to develop in hospitalized patients, and may also affect hospital staff. Many factors promote infection among hospitalized patients: decreased immunity among patients; the increasing variety of medical procedures and invasive techniques creating potential routes of infection; and the transmission of drug-resistant bacteria among crowded hospital populations, where poor infection control practices may facilitate transmission. They are a significant burden both for the patient and for public health. At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital (3).

SSIs are ranked among the most common HAI, along with pneumonia, urinary tract infections and blood stream infections (14). SSI are second most common type of nosocomial infections (13). Patients diagnosed with SSI face a 2 to 11 times increase in mortality along with prolonged hospital stays, treatment associated risks, pain, suffering, delayed wound healing, revision surgery, and potential long-term sequelae (14–16). Surgical wound infection is a prototype of HAI and constitutes a serious problem. It is therefore used as a good index of nosocomial infection (14).

Intact skin is an innate immune barrier to control microbial populations that live on the skin surface and to prevent underlying tissue from becoming colonized and invaded by potential pathogens. Any damage to the skin (wound) exposes the subcutaneous tissue and provides a moist, warm, and nutritious environment that is conducive to microbial colonization and proliferation. Hence any wound is at some risk of becoming infected. Infection in wound constitutes a major barrier to healing and can have an adverse impact on the patient's quality of life as well as on the healing rate of the wound (17,18). Infected wounds are likely to be more painful, hypersensitive and odorous, resulting in increased discomfort and inconvenience for the patient (17).

Wound infections have been a problem in the field of surgery for a long time (19). Surgical site infections are a major global problem in the field of surgery leading to many complications. Most post-surgical wound infections are hospital acquired and vary from one hospital to the other (20). Advances in control of infections have not completely eradicated the problem because of the development of resistance to antibiotics (19). The situation is even more serious in developing countries due to irrational prescriptions of antimicrobial agents (20).

The incidence of SSIs has varied from a low of 2.5 % to a high of 41.9 % (21)**.** SSIs are the most common type of healthcare-associated infection in the United States, affecting more than 500,000 patients and cause 8000 deaths annually (15). Nationwide surveillance in Japan revealed that 6-8 % of patients undergoing a surgical procedure develop an SSI every year (22). In the most recent point prevalence survey of inpatients in England, SSI was again the third most frequently occurring HAI, causing 15.7 % of reported infections (23). A nation-wide study conducted by the Brazilian Ministry of Health, in 1999, obtained a rate of SSI of 11 % of the total surgical procedures assessed (24). In Ethiopia, different studies reported that the prevalence of post surgical wound infection ranges from 14.8 % -60 % (20).

The burden posed by SSIs are also reflected in excess health care costs (16). In United States SSI associated costs can range from $400 for superficial SSI to upward of $30,000 for organ/space SSIs leading to system-wide excess costs of more than $7 billion per year (15). A recent review in England comparing studies of magnitude of costs due to SSI estimated that the healthcare cost for a patient with SSI is likely to be approximately twice that of one without (23).

The impact of SSI is even higher in developing countries, because most hospitals in developing countries especially Africa, have rudimentary and highly compromised infection control programmes. This could be due to lack of awareness of the problem, lack of personnel , poor water supply, erratic electricity supply, poor laboratory back up and funding (18). Since the etiology of SSIs and their antibiotic susceptibility pattern changes over time, continued study in understanding the biology of infection at the surgical site, knowledge of SSI associated pathogens & assessment of antibiotic sensitivity pattern is very important.

1.3. Significance of the study

Since the etiology of SSIs differs from country to country and from hospital to hospital even within the same region, accurate information on the incidence and etiology of surgical site infection acquired within a hospital is therefore essential for articulation of effective preventive measures. Data from drug susceptibility tests are very important to specifically select therapeutic options to patients with post surgical wound infections. Providing a cost data is a critical step in convincing organizations to allocate scarce resources to infection control programmes. That is providing cost data could demonstrate that these interventions will not only reduce the rate of infection, but will also result in savings that exceed the cost of preventive strategies.

So far there is inadequate data regarding bacteriology, antibiotic sensitivity, factors affecting surgical site infection and associated costs in the study area, therefore, this study was conducted to elucidate the above issues and suggest timely recommendations for empirical antimicrobial therapy. This study also helps the hospital to improve its infection prevention and control program by giving evidence based information. This study, specially the cost agenda can also be used as baseline information for other studies.

2. Literature review

2.1. Incidence of SSI and culture positivity rate

The rate of SSI varies from as low as 1.77 to as high as 25.2 % in different studies done at different part of the world. In a study conducted in tertiary care teaching hospital in India over a period of one year a total of 619 patients who underwent surgical procedure were included. Of these 156 (25.20 %) were clinically suspected of SSI and microbiological tests performed, 79 (50.64 %) of them became culture positive and a total of 123 bacteria isolated (10). A study was conducted on 168 patients who underwent surgery in AL-Najaf teaching hospital, Iraq and 26 (15.5 %) of patients developed SSI (12).

According to the study done by the International Nosocomial Infection Control Consortium (INICC), to assess SSI rate in four Mexican cities, in 16 cities in Turkey, and in 4 Colombian cities, the overall SSI rate was 5.5 %, 4.3% and 3.8 % respectively (16,25,26). A study performed in Italy showed an overall SSI rate of 2.6 % (7). A 1o year's retrospective cross sectional survey in Oman showed that the overall SSI was 2.66 %, 1.5 % and 1.16 % for CS, emergency and elective surgeries respectively (27).

In different areas of the world variable number bacteria were isolated from culture of surgical site infection samples. According to a study conducted in a tertiary care hospital in India 32 out of 50 or 64 % of surgical site infection samples showed growth on culture. Out of the 32 samples which were culture positive a total of 35 pathogens isolated (11). In another study performed in India, pus samples from 100 clinically suspected surgical site infection patients were processed. Out of these 27 samples became culture positive (21). A research done in Taiwan on postoperative spinal deep wound infection showed that out of 30 suspected samples 19 gave culture positive result (28). Another study performed in a general teaching hospital, Iraq showed that out of 100 postoperative wound samples 88 gave culture positive result (29). According to a hospital based study conducted between February and April 2003 in Uganda on a total of 94 samples from patients who underwent surgery, 56 specimens (59.6 %) had bacterial growth

within 48-hours of incubation (19). A study conducted in Nigeria isolated 161 bacteria from 153 swab samples of postoperative wound (5).

A study conducted in Ayder Teaching and Referral Hospital, Mekelle, Ethiopia assessed aerobic bacteria in post surgical wound infections and pattern of their antimicrobial susceptibility. In this study 610 successive patients who underwent surgery were followed and 128 (20.1 %) of patients fulfilled the clinical criteria for surgical site infection. Bacterial growth was seen in 96/128 (75 %) of the patients yielding a total of 123 bacterial isolates (20). Another study performed in Felege Hiwot Referal Hospital Bahirdar, Ethiopia showed an overall SSI rate of 10.9 % (30,31).

2.2. Bacterial etiology of SSI

Three independent studies in India revealed that the two major bacterial groups in postoperative wound infection were gram positive cocci (20.32-37 %) and gram negative bacilli (63-79.67 %) (9–11). In one of the above studies, *E. coli* was most frequently isolated (24.3 %) followed by *S. aureus* (21.6 %), *Pseudomonas spp* (20.7%), *Klebsiella spp* (13.5 %) and *Coagulase negative Staphylococci (CoNS)* (7.2 %) (9). Another study in India showed that the predominant organism was *S.aureus* 11 (40.7 %), followed by *Klebsiella species* (25.9 %), and *P. aeruginosa* (11.1 %) (13).

According to a study in Iraq, the bacteria isolated from clean wounds were *S. aureus and S. albus* (two each), whereas in contaminated wound the predominant organism was *E. coli* (six in number) followed by two *S. aureus* and two *Streptococci*. In dirty wounds the majority of bacteria detected was *P. aeruginosa* (eight in number) followed by *E. coli* and *P. mirabilis* (five each), then three *S. aureus*, three *Enterobacter species*, two *Enterococci* and two mixture of *Klebsiella* and *P. mirabilis* (12). In another study in Iraq, the isolated bacteria in their decreasing order were; *S. aureus* (38.6 %), *E. coli* (31.8 %), *P. aeruginosa* (27.3 %), *Klebsiella pneumonia* and *P. vulgaris* (15.9 % each), *S. pneumonia* (13.6 %) and *S. pyogens* (4.5 %) (29).

S. aureus was the predominant bacteria isolated (53.4 %) in a study conducted in Nigeria, followed by *E. coli*, *S. epidermidis*, *P. aeruginosa*, *Klebsiella* and *Proteus species* with their respective percentage 23 %, 11.2 %, 5 %, 3.7 %, and 3.7 % (5). In Uganda too, *S. aureus* was reported to be the most common bacterial isolate (45.1 %), followed by *Coliforms* (16.9 %), *P. mirabilis* (11.3 %), *P. aeruginosa* (9.9 %), *K. pneumonia* and *E. coli* (7 % each), and *Enterobacter species* (2.8 %) (19).

In a study conducted at Ayder teaching and referral hospital, Mekele, Ethiopia, 58(47.2 %) of the isolates were Gram positive and 65 (52.8 %) were Gram negative. *S. aureus* 40 (32.5 %) and *Klebsiella species* 29(23.6 %) were the predominant isolates followed by *CoNS* (14.6 %), *Proteus spp.* (12.2 %), *P. aeruginosa* (8.9 %), *E. coli* (4.9 %), and *Citrobacter spp.* (3.2 %). Single bacterial isolates were recovered from 73/96 (76.05 %) patients whereas 23/96(23.95 %) had polymicrobial infections (20). In another study performed in Felege Hiwot referral hospital, Bahirdar, Ethiopia, the predominant organisms isolated were *S. aureus* (26.2 %), followed by *E. coli* and *CoNS* (21.4 % each), *P. aeruginosa* (11.9 %), *P. mirabilis* (9.5 %), *K. pneumonia* and *E. aerogenes* (4.8 % each) (30).

The rate and type of organisms isolated from various kinds of surgical procedure varies between procedures and in different studies. A study in Italy showed that, the highest rate of infection occurred in patients who undergone rectal surgery (8.9 %), followed by colon surgery (8.3 %) (7). According to a study in Turkey, the highest rate of SSI was found in Ventricular shunt and colon surgery (11.4 % each), limb amputation (9.5 %) and thoracic surgery (6.6 %) comes next (25). In a study conducted in India, majority of SSIs developed among patients of renal surgeries (28.57 %), followed by Laparatomy (27.27 %), Appendicectomy (23.18 %) and hepatobiliary surgeries (20.58 %) (10). In one Ugandan study, most of the organisms 26(36.6 %) were isolated from surgical sites after incision and drainage (19).

2.3. Predisposing factors associated with SSI

A number of preoperative, perioperative and postoperative predisposing factors have been studied and found to be associated risk factors for development of post operative SSIs. Preoperative antibiotic prophylaxis, advanced age (>50 years), level of wound contamination, diabetes, hypertension, obesity, general condition of the patient at the time of surgery (ASA score) and steroid usage were found to be independent associated risk factors in different studies (7,13,21,27,29,32). A study conducted in India showed that surgical wounds with drains were found to be more infected 23/67 (34.3 %) than without drains 5/33 (15.2 %) (21).

A study in Iraq showed that, as the duration of the operation increased the rate of SSI also increased, that is the rate of infection was 65.4 %, 26.9 % and 7.7 % for surgeries lasting for more than 1 hour, 30 minute-1 hour and less than 30 minute (12). In a study conducted in Italy the rate of SSI was higher in patients who stayed in hospital for more than two days before the operation (3.6 %) as compared to those who stayed less than two days (1.7 %) (7). According to a study in Taiwan on postoperative wound and diabetic mellitus, the rate of infection was very high among diabetic patients (10.3 %) as compared to non diabetic patients (0.7 %) (33). According to a study in Ayder teaching and referral hospital, Mekele, Ethiopia, the rate of SSI increased in association with the Level of wound contamination, that is the rate was 9.4 %, 17.7 %, 32.2 % and 42.7 % for class I, II, III and IV wounds. This study also showed increased rate of SSI (63.5 %) in emergency surgeries (20).

2.4. Antibiotic susceptibility pattern

Recent studies revealed that most of the isolates from SSI demonstrate a high frequency of antimicrobial resistance to commonly prescribed antibiotics (5). According to a study in India, *S. aureus* was resistant to penicillin in 95.8 % of the isolates, ciprofloxacin (91.7 %), cephalexin (79.2 %) and erythromycin (79.2 %). *CoNS* were found to be 100 % resistant to Cotrimoxazole, followed by Cephalexin (87.5 %), Penicillin, Gentamicin, Ciprofloxacin (75 % each). *Enterococcus spp* were 100 % resistant to Penicillin followed by Gentamicin (75 %). All the

gram positive cocci exhibited 100 % sensitivity to Vancomicin. Most gram negative bacilli species showed greater resistance (>70 %) to almost all antibiotics tested (9). In another study in India, 70.6 % of *S. aureus* isolates were resistant to oxacillin *(MRSA)* (10).

A study in Iraq showed that, Susceptibility of *S. aureus* was 76.5 %, 64.7 %, 23.5 %, 11.8 % and 5.9 % for Ciprofloxacin, Gentamycin, Streptomycin, Ampicillin and Amoxicillin respectively. *S. pneumonia* was 100 % sensitive for Ciprofloxacin and Gentamycin followed by Streptomycin 83.3 %. All the 4 *S. pyogenes* were susceptible for Ciprofloxacin. Most of the gram negative bacilli were 55-85 % sensitive to Ciprofloxacin and Gentamycin, but *P. vulgaris* was exceptionally 100 % susceptible for Gentamycin (29).

In a study conducted in Uganda, All the Gram-positive isolates were *S. aureus.* The majority of them 28 (87.5 %) were sensitive to gentamicin, 24 (75 %) were sensitive to methicillin and 22 (68.7 %) were sensitive to ciprofloxacin. Only 1 (3 %) was sensitive to ampicillin. Among the *S. aureus* isolates 8 (25 %) were Methicillin- Resistant *Staphylococcus aureus (MRSA)*. The majority of the *P. aeruginosa* isolated (85.7 %) were sensitive to both gentamicin and ceftazidime while only 42.8 % were sensitive to ciprofloxacin. Out of the thirty- two Gram-negative and oxidase negative isolates 27 (84.4 %), 20 (62.5 %), 59.4 %, 9.4 %, 3.1 % were sensitive to ceftazidime, gentamicin, ciprofloxacin, ampicillin and amoxicillin respectively. No Gram-negative isolate was sensitive to chloramphenicol (19). *P. aeruginosa* was completely resistant to Augmentin, Chloramphenicol, Tetracycline, Amoxicillin, and Erythromycin in a study conducted in Nigeria (5).

A study in Ayder teaching and referral hospital, Mekele, Ethiopia showed that, the drug resistance of isolated Gram negative bacteria was 92.3 % to Ampicillin, 92.3 % to Tetracycline and 92.3 % to Amoxicillin, 81.5 % to Ceftriazone, 69.2 % to Amoxicillin Clavunilic acid, 46.2 % for Ciprofloxacin, 26.2 % to Erythromycin and 16.9 % for Gentamicin. Klebseilla species showed 100 %, 93.1 %, 89.7 % and 86.2 % resistance for Amoxicillin, Tetracycline and Ceftriazone, respectively. *P. aeruginosa* isolates were 100 % resistant for Ceftriazone, Amoxicillin, Amoxicillin clavunilic acid and Tetracycline. All *P. aeruginosa* isolates were;

however, 100 % sensitive to Gentamicin. Resistance by *S.aureus* was 36/40 (90 %) to Tetracycline, Ceftriazone and Ampicillin, and 34/40 (85 %) to Cloxacilline. All of the isolates of *S.aureus* 40(100 %) were sensitive for Vancomycin. High resistance rate of *CoNS* was observed for Amoxicillin, Amoxicillin-clavunilic acid, Ampicillin and Tetracycline, 88.9 %, 77.8 %, 77.8 % and 77.8 %, respectively. All isolates of *CoNS* 18(100 %) were however, sensitive for Vancomycin (20).

2.5. LOS and Costs associated with SSI

Nosocomial infections cause high costs due to prolonged hospitalization, additional diagnostic tests, therapeutic use of antibiotics and sometimes additional surgery (34). Costs associated with SSI could have three broad components: direct medical costs, the indirect costs related to productivity and non-medical costs, and intangible costs related to diminished quality of life (35). The distribution of these costs between the three components is not known but a popular argument is that the costs of infections diagnosed during hospital admission are only the tip of the iceberg and that a great burden of cost occurs after discharge which is hidden from policy makers (36). As most cases of healthcare-acquired SSI appear after discharge from the hospital (37). However, most researchers perform their analysis from the hospital perspective only to provide evidence that hospitals can see economic benefits through investment in infection control programs. The presents study also tried to determine the cost associated with SSI from the hospital perspective by considering the costs for hospital bed, laboratory tests, drugs, injection of drugs and wound care.

According to a study in Iran to assess excess costs associated with common healthcare associated infections in an Iranian cardiac surgical unit from June 2006 to July 2007; the costs associated with surgical site infection was more than double for hospital bed and paraclinical tests, where as it was three fold for medications as compared to the costs for patients with no SSI (38). The annual California hospital HAI report showed that the mean length of hospital stay of patients with and without SSI was 8.4 and 3.5 days respectively. This report also showed the average cost needed for patients with SSI is almost a double of those without SSI (39). Another study

performed in Switzerland between 2000 and 2001 showed that SSI caused an average additional cost of 11040 US dollar per patient, and an additional total LOS of 18.7 days. A similar study in UK between 1997 and 2001 revealed that SSI caused an average additional cost of 5714 US dollars per patient. This study also showed that the LOS for patients with and without SSI was 25 and 9.6 days respectively (34). Literatures in relation to cost associated with SSI are scarce in Ethiopia, as well as Africa and other developing countries.

3. Hypothesis

1. There is no difference in the distribution of SSIs between patients with and without the different factors which are supposed to have a predisposing effect for the development of SSI.

2. Distribution of the cost and length of hospital stay is the same between patients with and without SSI.

4. Objectives

4.1. General objective

To assess the incidence of surgical site infection, predisposing factors and associated costs at Dessie Referral Hospital, Dessie, Ethiopia.

4.2. Specific objectives

- To determine the rate of surgical site infection in DRH, Dessie, Ethiopia.
- To identify the aerobic bacterial etiology of SSI in DRH, Dessie, Ethiopia.
- To describe the antibiotic susceptibility pattern of the isolated bacteria from SSI in DRH, Dessie, Ethiopia.
- To identify the associated predisposing factors for SSI in DRH, Dessie, Ethiopia.
- To determine the cost associated with SSI in DRH, Dessie, Ethiopia.

5. Methodology

5.1. Study design

A hospital based cross sectional study was conducted at DRH.

5.2. Study period

The study was conducted from July 22 – October 25, 2016.

5.3. Study area

The study was conducted in DRH which is found in Dessie, South Wollo Zone, Amhara National Regional State, Ethiopia. Dessie is located 401 KMs North East of the capital, Addis Ababa. In Dessie, there are two government and three private hospitals and eight health centers and several private higher clinics with different specialties. DRH is the only referral hospital in Wollo province serving for about 8 million people including the neighboring regions. The hospital has more than 200 beds offering different specialized services including: the Pediatrics, Surgery, Obstetrics and Gynecology, Orthopedics and Internal Medicine. On average about ten major operations are performed per day.

5.4. Source population

All patients who underwent surgery during the study period were considered as a source population.

5.5. Study population

All patients from obstetrics and gynecology and general surgical wards who underwent clean and clean contaminated surgeries and willing to participate during the study period were taken as a study population.

5.6. Sample size

The sample size was determined according to the CDC procedure associated manual, 2015 which stated "the requirement to perform SSI surveillance is to follow at least one NHSN operative procedure category for at least one month" (1). Based on this, about 10 procedure categories were followed for three months and 338 patients from the obstetrics and gynecology and general surgical wards that fulfilled the inclusion criteria during the study period were included to this study.

5.7. Sampling Technique

A consecutive convenient sampling technique was employed to select the study subjects.

5.8. Inclusion criteria

Patients admitted in obstetrics and gynecology and general surgical wards for surgery and underwent clean or clean contaminated surgeries, and/or those who were willing to give informed consent to participate in this study were included.

5.9. Exclusion criteria

Patients with; infection occurring 30 days after the operation if no implant is in place, Infection on episiotomy, contaminated wounds, Procedures in which healthy skin was not incised such as opening abscess, pediatrics below the age of 15 years and orthopedic surgeries were excluded from this study.

5.10. Study variables

5.10.1. Dependent variables

Surgical site infection, bacterial isolate, drug susceptibility pattern, cost related to SSI

5.10.2. Independent variables

Age, sex, residence, diabetic status, hypertension status, steroid intake, wound contamination level, nature of the procedure, preoperative prophylaxis, duration of

surgery, preoperative hospital stay, postoperative hospital stay, drugs prescribed after surgery, laboratory tests requested, number of injections, number of wound cares, presence of drains and ASA score.

5.11. Data collection and processing

5.11.1. Patient data collection

Pre structured and tested questionnaires were used to extract data from the patients' case notes. The information included was; socio demographic data, existing chronic disease (such as diabetes mellitus), past medical history, current drug use such as steroid, length of preoperative hospital stay, duration of the operation, antimicrobial prophylaxis, postoperative hospital stay, postoperative antibiotic given, and laboratory tests ordered.

5.11.2. Specimen collection

The specimens were collected aseptically on the first day when patients presented with clinical evidence of infection (purulent drainage from incision or drain) before the wound was cleaned with antiseptic. SSI cases were defined by surgical and obstetrics and gynecology residents who had given adequate information regarding CDC`s SSI criteria. The samples were collected by experienced nurses, from the depth of the wound with strict aseptic precautions with the help of sterile cotton swab sticks moistened with sterile saline for bacteriological examination. Two culture swabs from each sample were obtained, one for the direct smear study and the other for aerobic culture which was immediately sent to the Dessie regional laboratory in a separate sterile test tube for investigation (9).

5.11.3. Laboratory investigation

On day 1, direct microscopic examination was done by gram stain to look for pus cells and the bacteria. The first swab was used for making smear by rolling the swab stick on a clean glass slide, which then was alcohol fixed and stained by Gram stain following the standard operating procedure (SOP). The Gram stained smear was examined under the microscope and the bacteria was broadly classified into cocci and bacilli, gram positive or gram negative. This report was

then correlated with the growth in the culture plates after 24-48 hours. The second swab was inoculated on sheep blood agar and MacConkey agar as well as Manitol salt agar and incubated at 37 ^{0}C for 24-48 hours under aerobic conditions. The culture Media were prepared by following the manufacturer's instruction as well as inputs from SOPs (9).

On day 2, identification of the growth was performed by studying the morphology of the colonies, smear from pure colonies were prepared and stained with gram stain and microscopic study performed to aid the identification process. When there was a mixed growth, colonies of different morphological characteristics sub cultured on to appropriate Media in order to obtain a pure colony. The isolated bacteria were further microbiologically identified by using the relevant biochemical tests. The antibiotic susceptibility was performed by using the Kirby-Bauer disc diffusion method on Mueller Hinton agar using antibiotics according to CLSI guideline (CLSI document M100-S24) (40). The Muller Hinton agar was enriched with 5% sheep blood in the case of fastidious bacteria like streptococcus species.

Several isolated colonies of similar morphology were taken carefully and suspended in sterile nutrient broth and/or sterile normal saline. The suspension was then gently agitated to get a uniform suspension and turbidity of the suspension matched with McFarland 0.5% Barium Sulphate opacity standard. A sterile swab was dipped into the suspension of the isolate, squeezed against the side of the tube to remove excess fluid and spreaded over the Mueller Hinton agar plate following the standard operating procedure. Sensitivity discs for appropriate drugs were placed onto the media and incubated at 37°C for 24hrs (9).

On the day 3, the final identification of the bacteria was made by taking into account of the various biochemical tests. Antibiotic susceptibility patterns were reported by measuring the zone of inhibition with a millimeter scale (9). The antibiotic disc were reported as susceptible, intermediate, susceptible-dose dependent (SDD) and resistant, based on the criteria provided by CLSI document M100-S24 (40).

Identification of gram positive cocci was performed by using the following tests; Catalase test, Coagulase test, Salt tolerance test, and PYR-test (9). Whereas the identification of the gram negative bacilli was performed by using the following test; Oxidase test, Kligler iron agar test (KIA), Indole test, Urease test, Citrate utilization test, Lysine decarboxylase test (LDC), and Motility test (9).

Methicillin resistance for *Staphylococcus aureus* and *CoNS* was determined by Disc diffusion test using cefoxitin (30µg) disc on Mueller Hinton Agar. Plates were incubated and maintained at 33-35°C for 24 hours. Results were interpreted according to CLSI guidelines i.e. for *S. aureus and CoNS*, zone diameters ≤ 21mm and ≤ 24 mm respectively were considered resistant for Methicillin (40). ESBLs production was screened by using Ceftriaxone (30µg) disc on Mueller-Hinton Agar and isolates with zone diameters ≤ 25 mm were considered as positive for ESBL screening according to the CLSI guidelines and confirmed by double disc approximation method (40,41). Discs containing ceftazidime (30µg) and cefotaxime (30µg) were placed 20mm center to center to the amoxicillin/clavulanate (20/10µg) disc. The plate was then incubated at 37ºC for 18-20hours. An enhanced zone of inhibition toward the amoxicillin/clavulanate (20/10µg) disc was considered as positive for ESBL production (41). Multidrug resistant (MDR) bacteria were identified based on the ECDC definition in which a bacterium is classified as MDR when the isolate is non-susceptible to at least 1 agent in three or more antimicrobial categories (42).

For gram positive organisms susceptibility was tested against penicillin (10 unit), ampicillin (10µg), ceftriaxone (30µg), vancomycin (30µg), gentamycin (10µg), erythromycin (15µg), tetracycline (30µg), ciprofloxacin (5µg), clindamycin (2µg), triomethoprim/sulfamethaxazole (1.25/23.75µg), tetracycline (30µg), cefoxitin (30µg), cefepime (30µg) and chloramphenicol (30µg). Gram negative organisms were tested against ampicillin (10µg), amoxicillin/clavulanate (20/10µg), ceftriaxone (30µg), ceftazidime (30µg), gentamycin (10µg), ciprofloxacin (5µg), triomethoprim/sulfamethaxazole (1.25/23.75µg), (30µg), piperaciline (100 µg), cefepime (30µg), cefoxitin (30µg) and amikacin (30µg) (40).

Figure 5.1. Double disk approximation method for ESBL detection (photo taken during the study)

5.11.4. LOS and cost data

For completeness of the cost data the medical record of each of the study subjects was inspected twice; the first one was immediately after the surgery, to obtain most of the information regarding the type of the operation done, date of admission, past medical history and the like, and the second one was at the time of discharge, and this time information concerning; the date of discharge, all the antibiotics and the anti-pains given, all the laboratory tests requested, and the number of wound cares done to each patient were carefully extracted. In the case of readmission, LOS and cost due to readmission was aggregated with those of the original admission to form a single patient episode.

Bed cost and LOS; the date of admission and discharge was recorded for each of the study subjects and the length of hospital stay calculated. The average bed cost of the hospital (50 birr per day) was taken and multiplied by the total length of hospital stay for each of the study

subjects in order to obtain the bed cost. The mean and median bed cost and LOS of patients with SSI was compared with that of patients without SSI.

Laboratory cost; all the laboratory tests requested for each of the study participants were taken from their medical record at the time of discharge. The price of each of the laboratory tests were taken from the hospital laboratory department. The laboratory tests requested for each patient were converted to cost by using the cost of each of the laboratory tests and the mean and median cost expended for laboratory tests by patients with SSI was compared with that of patients without SSI.

Drug cost; all the antibiotics and anti-pains given to each of the study participants were taken from the medication administration form which is found in the medical record of patients at the time of discharge. The price of each drug was taken from the hospital pharmacy department. The drugs given to each of the study participants were converted to cost by using the cost of each drug and the mean and median cost expended for drugs by patients with SSI was compared with that of patients without SSI.

Injection cost; the number of injections given for each of the study participants were taken from the medication administration form at the time of discharge and multiplied by the injection charge (injection charge of the hospital (5 birr per syringe) was used) to obtain the total injection cost for each patient. The mean and median cost incurred for injection by patients with SSI was compared with patients without SSI.

Wound care cost; the number of wound cares performed for each patient was taken from the medical record of patients at the time of discharge and multiplied by the wound care charge (wound care charge of the hospital (15 birr per wound care) was used), to obtain the total wound care cost for each patient. The mean and median cost incurred for wound care by patients with SSI was compared with patients without SSI.

Total cost; finally costs for; hospital bed, laboratory tests, drugs, injections and wound cares were aggregated together to obtain the total cost. The mean and median total cost of patients with SSI was compared with that of patients without SSI. The original cost data collected in local Ethiopian birr was converted to US dollar for the sake of easing the comparison of the findings of this study with others. The currency conversion was done by taking the average of the exchange rate at the start and at the end of the data collection from the website of the national bank of Ethiopia. At the start of the data collection the exchange rate was 0.0456 and at the end it was 0.0454, and the average the two 0.0455 was taken to convert the cost in Ethiopian birr to US dollar.

The total hospital stay and the cost data was checked for normality of distribution by Kolmogorov-Smirnov test, and the distribution was found to be not normal. Hence non parametric tests like Mann-Whitney U and Kolmogorov-Smirnov Z tests were used to test significance of the difference in the total length of hospital stay and costs between patients with and without SSI. $P \leq 0.05$ was considered significant.

5.12. Quality control

The reliability of the laboratory test results was ensured by implementing Quality control (QC) measures throughout the whole processes of the laboratory work. All materials, equipment and procedures were adequately controlled. Aseptic techniques were followed in all the steps of specimen collection and inoculation onto culture media to minimize contamination. All the culture Media were prepared according to the manufacturer's instruction. Standard operating procedures for sample collection, transport, culture and susceptibility testing of the isolated organisms were followed to ensure procedural quality. International Control bacterial strains were used in controlling the tests carried out in this study. Quality of the culture Media, antibiotic discs, as well as personal performance was controlled by using reference strains such as: *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *P. aeruginosa* ATCC 27853.

5.13. Data analysis

All filled questionnaires were coded before entering into the computer. Data cleaning was done by using consistence checks. The data was analyzed by SPSS version 20 and the results were explained by frequency distribution in tables and different figures. Binary and Multiple Logistic regression analysis was used to determine the association between dependent and independent variables, P-value of less than 0.05 were considered significant. Non parametric tests were used to test the costs data.

5.14. Dissemination plan

This study up on completion is supposed to serve as a reference material for researchers, experts or policy makers so as to make an intervention. Hence the finalized document of this study will be submitted to Department of Medical Laboratory Science, College of Health Science, Addis Ababa University. So it can serve as a reference in the library. In addition, a copy of it will be given to Dessie Referral Hospital and Regional Laboratory. It will also be sent for publication in local and international journal.

5.15. Ethical considerations

Ethical clearance was obtained from ethical review committee of Addis Ababa University, College of Health Science, Department of Medical Laboratory Sciences. Written permission to conduct the study in Dessie Referral Hospital and Dessie Regional Laboratory was asked from administrators of both institutions. An informed consent was requested from the patients, parents/guardians for participation into the study. Information about the study was given to the participants to ensure that they have the information needed to make an informed consent. A complete description of the aims of the study and assurance of confidentiality for any information was given to all the patients or parents/guardians (in case of 15-17 years). Appropriate counseling and assurance of confidentiality was given to participants with worries and anxiety about the study. Laboratory results of the study participants were communicated with the attending physician, for use in guiding patients' management.

6. Results

6.1. Socio-demographic characteristic of the study participants

This study was conducted for a period of three months between July 22 and October 25, 2016. During this period a total of 338 patients who fulfilled the inclusion criteria were enrolled in to this study. As it is shown in table 6.1, majority of the study participants were females (74.3 %). More than half (61.2 %) of the surgeries were performed in Gynecology and Obstetrics ward where as the rest 38.8 % were in General surgery. Distribution of the study subjects based on residence was proportional, 51.5 % and 48.5 % for rural and urban respectively. Age of the study participants ranged 16-76 years, the mean age was 35.25 ± 15.07 years and the median age was 28 years. Majority of the study subjects (42.6 %) were in the age group 25-34 years, followed by 15-24 years which accounts for 21.9 % and the least (5.9 %) were in the age group 65 years and above.

6.2. Culture results

Table 6.2 shows culture results of the samples taken from the study participants. During the study period all patients who fulfilled the inclusion criterias were followed for the development of clinical sign and symptoms of SSI from the second day of operation till the time of discharge. Patients who developed infection after discharge were identified from outpatient clinic and a follow up health center (Dessie health center). Out of the 338 study subjects 49 (14.5 %) of them were diagnosed as SSI clinically and wound swabs were taken from these patients for bacteriological study. About 41(83.7 %) of the 49 wound swabs showed a bacterial growth. A single bacteria was isolated from 34 (82.9 %) of the samples with bacterial growth where as the rest 7 (17.1 %) of the samples showed a mixed bacterial growth. A total of 48 bacteria were isolated and more than half (56.25 %) of them were gram negative rods, the rest (43.75 %) were gram positive cocci.

Table 6.1. Socio demographic characteristics of the study participants in DRH, from July 22 – October 25, 2016.

Variables	No	%
Sex of the participant		
Male	87	25.7
Female	251	74.3
Total	338	100
Ward type		
General Surgery	131	38.8
Gynecology and Obstetrics	207	61.2
Total	338	100
Residence		
Rural	164	48.5
Urban	174	51.5
Total	338	100
Age in years		
15-24	74	21.9
25-34	144	42.6
35-44	34	10.1
45-54	28	8.3
55-64	38	11.2
≥65	20	5.9
Total	338	100
Mean ± SD	35.25 ± 15.07	
Median	28	
Range	16-76	

Table 6.2. Culture results of samples taken from the study participants suspected to develop SSI in DRH, July 22 – October 25, 2016.

	Number	Percent
Culture result N=49		
Growth	41	83.7
No growth	8	16.3
Single bacterial growth	34	82.9
Mixed bacterial growth	7	17.1
The bacteria isolated N=48		
Gram negative rods	27	56.25
Gram positive cocci	21	43.75

6.3. The rate and distribution of SSI

Table 6.3 shows the rate of SSI in different procedures. Cesarean Section (CSEC) was the most frequently performed procedure followed by Colon surgery (COLO) and Appendix surgery (APPY). The rate of SSI was variable from procedure to procedure and from ward to ward. The rate of SSI was 16.8 % and 9.2 % in general surgical ward and obstetrics and gynecology ward respectively. Among the procedures; the highest rate of SSI was found in Prostate surgery (PRST), followed by Small Bowl surgery (SB), Vaginal Hyterectomy (VHYS) and Exploratory Laparatomy (XLAP) with their respective rate of 22.2 %, 21.1 %, 20 % and 18.8 %. The overall rate of SSI was 12.13 %.

Table 6.3. Distribution of SSI by surgical procedure in DRH, July 22 – October 25, 2016

Surgical procedure	Total number of Procedure	Total number of SSI	Rate of SSI %
Appendix surgery (APPY)	28	3	10.70
Colon surgery (COLO)	34	6	17.60
Cesarean Section (CSEC)	166	12	7.20
Hernioraphy (HER)	15	1	6.70
Abdominal Hysterectomy (HYST)	27	5	18.50
Prostate surgery (PRST)	18	4	22.20
Small Bowl surgery (SB)	19	4	21.10
Vaginal Hysterectomy (VHYS)	10	2	20.00
Exploratory Laparatomy (XLAP)	16	3	18.80
OTHERS	5	1	20.00
TOTAL	338	41	12.13

6.4. Bacterial etiology of SSI

As it is shown in figure 6.1 the most predominant isolate among gram positive organisms was *S. aureus* 14 (66.67 %), followed by *CoNS* 4 (19.05 %), and *Streptococci* 2 (9.52 %). The organisms isolated as gram negative rods were *E. coli* 9 (33.33 %), *Klebsiella species* 7 (25.93 %), *C. freundii* 3 (11.11 %), and *Pseudomonas species*, *Acitenobacter species, M. morganii* 2 (7.41 %) each and *S. marcescens* and *P. mirabilis* 1(3.7 %) each.

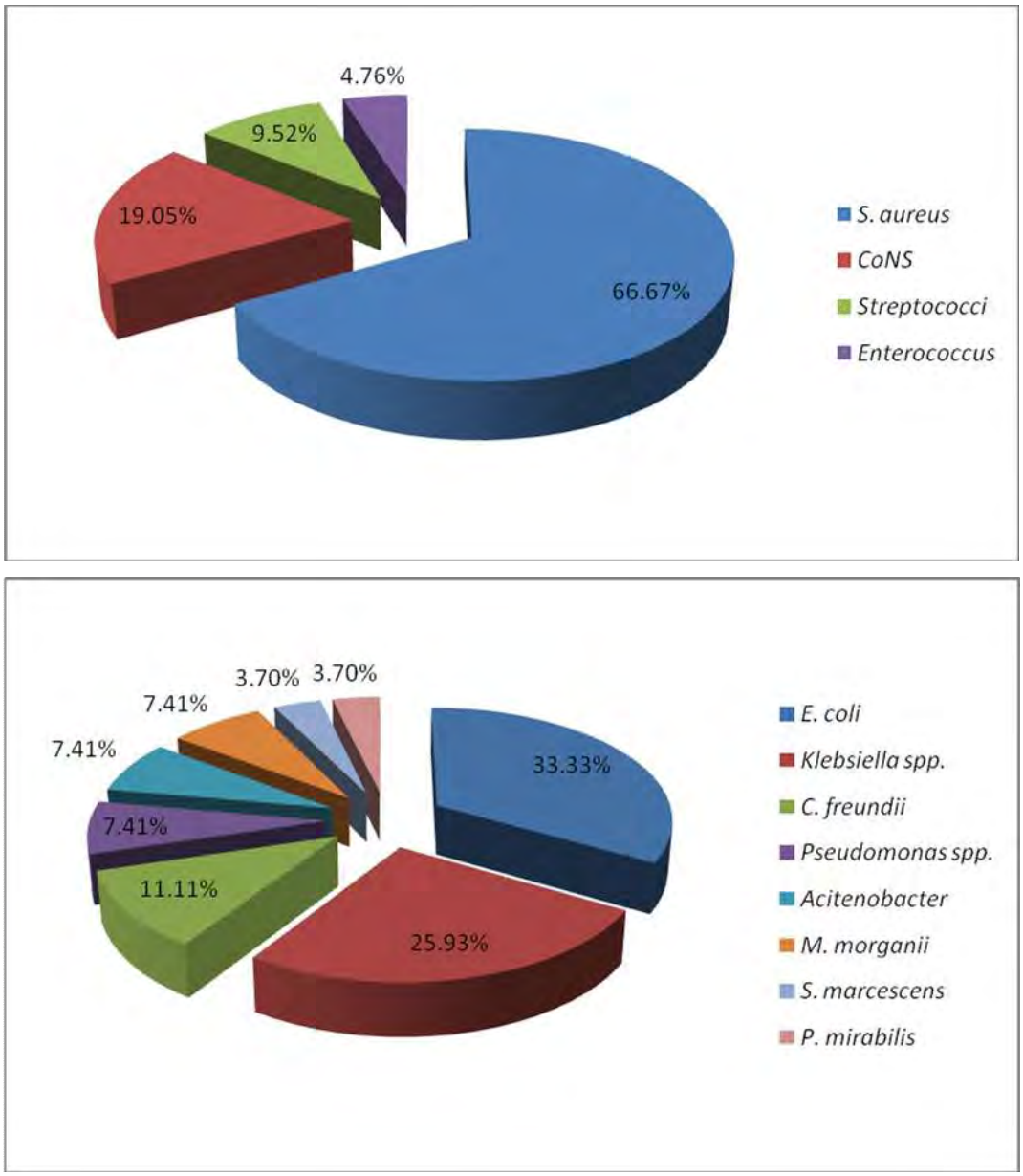


Figure 6.1. Distribution of pathogenic bacteria isolated from the study participants with SSI in DRH, July 22 – October 25, 2016.

Table 6.4 shows the frequency of pathogenic bacterial isolates in relation to type of operation. The majority of the isolates were from Cesarean section accounting for 15/48(31.25 %). Among these *S. aureus* was the most prevalent organism at 6/15(40 %) isolates followed by *Streptococci*, *Klebsiella species* and *C. freundii* 2/15 (13.3 %) each. *E. coli* was the most common organism

isolated from colon and Prostate surgery at 3/7 (42.9 %) and 2/5 (33.3 %) respectively. *S. aureus, CoNS, E. coli, Klebsiella species* and *M. morganii* (1 each) were the organisms isolated from post abdominal hysterectomy wounds.

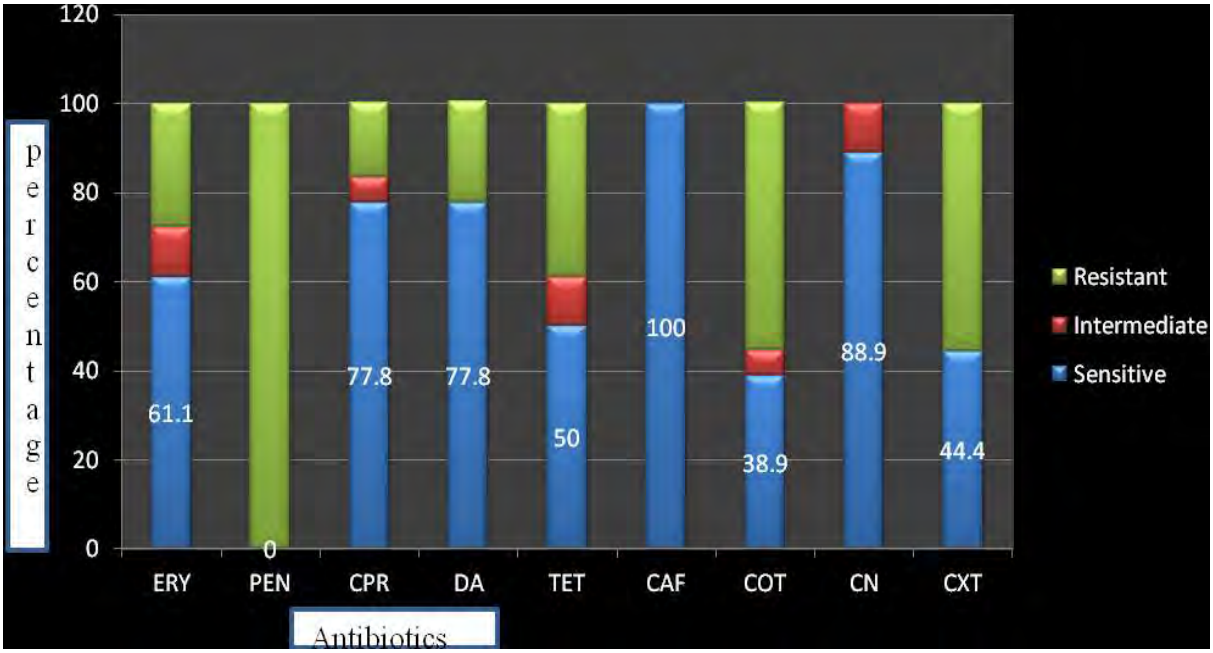
Table 6.4. Distribution of pathogenic bacterial isolate in relation to type of surgical procedure in DRH, July 22 – October 25, 2016.

	Surgical procedure									
Bacterial isolate	APPY N=4	COLO N=7	CSEC N=15	HER N=1	HYST N=5	PRST N=6	SB N=4	VHYS N=2	XLAP N=3	OTHERS N=1
S. aureus No (%)	1 (25)	1 (14.3)	6 (40)	1 (100)	1 (20)	1 (16.7)	1 (25)	1 (50)	1 (33.3)	0
CoNS No (%)	0	1 (14.3)	0	0	1 (20)	1 (16.7)	0	0	1 (33.3)	0
Enterococcus No (%)	0	0	0	0	0	1 (16.7)	0	0	0	0
Streptococci No (%)	0	0	2 (13.3)	0	0	0	0	0	0	0
E. coli No (%)	1 (25)	3 (42.9)	1 (6.7)	0	1 (20)	2 (33.3)	0	0	1 (33.3)	0
Pseudomonas spp. No (%)	0	1 (14.3)	0	0	0	0	0	0	0	1 (100)
P. mirabilis No (%)	0	0	0	0	0	0	0	1 (50)	0	0
Klebsiella spp. No (%)	2 (50)	1 (14.3)	2 (13.3)	0	1 (20)	0	1 (25)	0	0	0
C. freundii No (%)	0	0	2 (13.3)	0	0	0	1 (25)	0	0	0
S. marcescens No (%)	0	0	1 (6.7)	0	0	0	0	0	0	0
Acitenobacter No (%)	0	0	1 (6.7)	0	0	0	1 (25)	0	0	0
M. morganii No (%)	0	0	0	0	1 (20)	1 (16.7)	0	0	0	0

APPY=appendix surgery, COLO=colon surgery, CSEC= cesarean section, HER=hernioraphy, HYST=abdominal hysterectomy, PRST=prostate surgery, SB=small bowel surgery, VHYS=vaginal hysterectomy, XLAP=exploratory laparatomy

6.5. Antibiotic susceptibility pattern

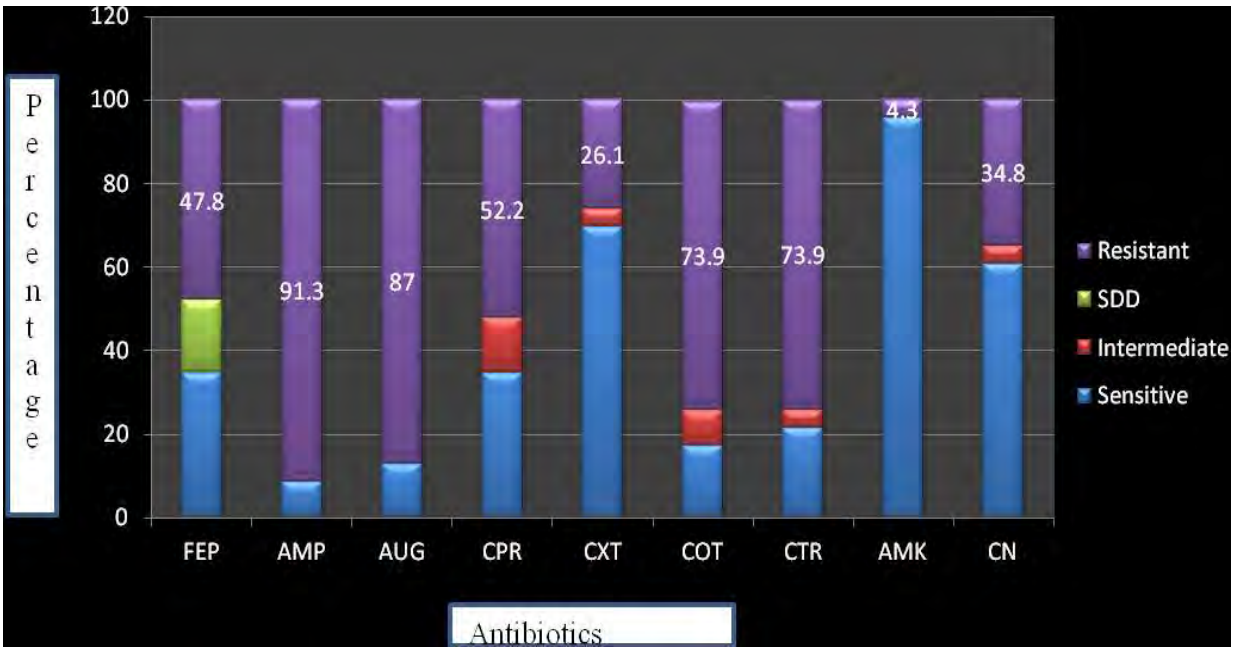
As presented in figure 6.2, 100 % of the *Staphylococcus species* were resistant to penicillin, whereas a moderate resistance was observed for the rest antibiotics tested including Cotrimoxazole (55.6 %), Cefoxitin (55.6 %) and Tetracycline (38.9 %). But all the *Staphylococcus species* were sensitive to Chloramphenicol and 77.8 % of them were also found to be sensitive for Ciprofloxacin and Clindamycin.



ERY=erythromycin, PEN=penicillin, CPR=ciprofloxacin, DA=clindamycin, TET=tetracycline, CAF=chloramphenicol, COT=cotrimoxazole, CN=gentamycin, CXT=cefoxitin

Figure 6.2. Antibiotic susceptibility patterns of *Staphylococcus species* isolated from SSI in DRH, July 25 – October 25 2016.

As summarized in figure 6.3, all *Enterobacteriaceae* isolates showed a high degree of resistance to multiple antimicrobial agents tested, 91.3 % for Ampicilin, 87 % for Augmentin and 73.9 % for Cotrimoxazole and Ceftriaxone. Moderate degree of resistance was observed for Ciprofloxacin, Cefepime and Gentamycin (52.2 %, 47.8 % and 34.8 % respectively). But 95.7 % of the *Enterobacteriaceae* isolates were found to be sensitive for Amikacin.



FEP=cefepime, AMP=ampiciline, AUG=augmentin, CPR=ciprofloxacin, CXT=cefoxitin, CTR=ceftriaxone, AMK=amikacin, CN=gentamycin

Figure 6.3 Antibiotic susceptibility patterns of *Enterobacteriaceae* isolated from SSI in DRH, July 22 – October 25, 2016

As summarized in Table 6.5, *Enterococcus* showed resistance for Erytromycin, penicillin and Chloramphenicol but sensitive for Ampiciline and Vancomycin. The *viridians streptococcus* was found to be sensitive for all the antibiotics tested whereas the *β hemolytic Streptococcus* was resistant for Erytromycin, penicillin and Cefepime. Both of the *Pseudomonas species* were resistant to Ceftazidime and Piperaciline but only one for Gentamycin and none of them for Amikacin. Both of the *Acinetobacter species* were resistant for all the antibiotics tested except Amikacin.

Table 6.5 Antibiotic resistance patterns of pathogenic bacterial isolates other than *Staphylococcus species* and *Enterobactericeae* from the study participants with SSI in DRH, July 22 – October 25, 2016

	Number resistant strains				
Antibiotics	*Enterococcus* N=1	*Viridans Streptococcus* N=1	*β hemolytic Streptococcus* N=1	*Pseudomonas Spp.* N=2	*Acinetobacter Spp.* N=2
ERY	1	–	1	–	–
PEN	1	–	1	–	–
CAF	1	–	–	–	–
AMP	0	–	–	–	–
VAN	0	0	0	–	–
DA	–	–	0	–	–
FEP	–	0	1	–	2
CRO	–	0	–	–	2
CAZ	–	–	–	2	2
PIP	–	–	–	2	2
AMK	–	–	–	0	0
CN	–	–	–	1	2
CPR	–	–	–	–	2

As it is shown in table 6.6, 88.9 % and 66.7 % of the gram negative and gram positive isolates respectively were multidrug resistant (MDR). Overall 79.2 % of the isolated bacteria were MDR.

Table 6.6. Frequency of multidrug resistant bacteria in gram positive and gram negative bacteria isolated from SSI in DRH, July 22 – October 25, 2016.

	Multidrug resistance (MDR)			
	Positive		**Negative**	
Group of the Isolate	Number	%	Number	%
Gram positive (N=21)	14	66.7	7	33.3
Gram negative (N=27)	24	88.9	3	11.1
Total (N=48)	38	79.2	10	20.8

As table 6.7 shows; 13, 10, and 7 of all the 48 isolates were resistant for five, three, and four different classes of antibiotics respectively. 6 of the 21 gram positive isolates and 12 of the 27 gram negative isolates were resistant for three and five different classes of antibiotics respectively.

Table 6.7. Distribution of gram positive and negative isolates based on their resistance for different number of classes of antibiotics in DRH, July 22 – October 25, 2016.

Group of the Isolate	Number of classes							
	Zero	One	Two	Three	Four	Five	Six	Seven
Gram positive N=21	1	2	4	6	3	1	2	2
Gram negative N=27	1	0	2	4	4	12	4	0
Total N=48	2	2	6	10	7	13	6	2

66.67 % of *E. coli* species and both of the *K. pneumonia species* were found to be *ESBL* producing strains. 6 (42.86 %) of the 14 *S. aureus species* were found to be *MRSA*, whereas all the 4 *coagulase negative staphylococcus (CoNS) species* were found to be *MRCoNS*.

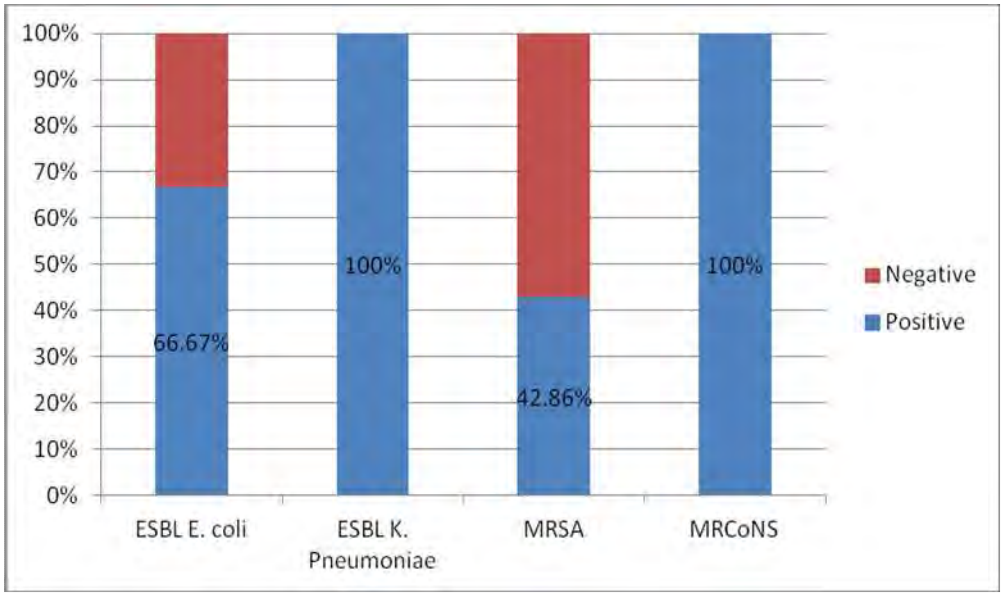


Figure 6.4. ESBLproduction and Methicillin resistance test results of the isolates from patients with SSI in DRH, July 22 – October 25, 2016.

6.6. Associated factors

Table 6.8 Logistic regression analysis of factors associated with SSI in DRH, July 22–October 25, 2016.

		Univariate analysis		Multivariate analysis	
Variables		**COR (95% CI)**	**P value**	**AOR (95% CI)**	**P value**
Age in years	15-24	1.78 (0.62-5.1)		2.1 (0.6-6.6)	
	25-34*				
	35-44	5.2 (1.8-15.2)	0.002	4.0 (1.2-13.8)	0.03
	45-54	2.04 (0.51-8.2)		0.8 (0.2-4.1)	
	55-64	6.9 (2.5-18.8)	0.001	2.2 (0.6-9.1)	
	≥ 65	4.2 (1.1-15.7	0.03	1.6 (0.3-8.2)	
sex	Male	1.4 (0.7-2.8)		0.6 (0.24-1.45)	
	Female*				
Residence	Rural	1.3 (0.66-2.4)		0.6 (0.3-1.4)	
	Urban*				
Preop. hospital stay	≤ 2 days*				
	> 2 days	2.24 (0.9-5.57)		0.6 (0.2-2.0)	
Diabetic Melitus	Yes	3.8 (1.67-8.8)	0.001	2.8 (0.6-12.4)	
	No*				
Steroid intake	Yes	2.1 (0.4-10.6)		1.5 (0.2-10.2)	
	No*				
Hypertension	Yes	2.23 (1.04-4.8)	0.04	5.5 (1.3-22.4)	0.018
	No*				
Drain	Yes	2.8 (0.85-9.3)		2.6 (0.5-13.8)	
	No*				
Wound class	Clean*				
	Clean contaminated	1.98 (0.25-15.46)		3.1 (0.3-34.4)	
Nature of surgery	Emergency*				
	Elective	3.7 (1.79-7.67)	0.001	3.2 (1.04-9.9)	0.043
ASA score	ASA-1*				
	ASA-2	3.4 (1.5-7.87)	0.004	2.2 (0.8-6.3)	
	ASA-3	10.17 (3.9-26.3)	0.001	8.6 (2.5-30.1)	0.001
Prophylaxis	Yes*				
	No	2.37 (0.9-6.3)		3.1 (0.9-11.0)	
Duration of surgery	0-60 minutes*				
	61-120 minutes	3.5 (1.76-7.04)	0.001	1.2 (0.5-3.2)	
	> 120 minutes	12.05 (2.3-63.8)	0.003	4.8 (0.6-37.8)	

* = reference category, only P values ≤ 0.05 are displayed in the table, AOR= adjusted odds ratio, COR= crude odds ratio, CI= confidence interval, preop. = preoperative

As it is presented above in table 6.8, diabetic mellitus, hypertension, elective surgery, ASA scores (2 and 3), duration of operation (61-120 minutes and > 120 minutes) and the age groups (35-44 years, 55-64 years and ≥ 65 years) showed a statistically significant association with SSI in the univariate logistic regression analysis. Whereas only hypertension, elective surgery, ASA-3 and the age group 35-44 years were found to be a statistically significant risk factors for SSI in the multivariate analysis.

6.7. LOS and Costs associated with SSI

On average a patient with SSI expended a total of 1393.61 Ethiopian birr, while it was only 537.8 Ethiopian birr for a patient without SSI. This means a patient with SSI expended a total of 855.81 more Ethiopian birr as compared to a patient without SSI. Similarly a patient with SSI spent an average of 4.77 more days in a hospital than a patient without SSI. In both cases the difference was statistically significant with a P value = 0.001.

Table 6.9. Costs (in Ethiopian birr) and lost days associated with SSI in DRH, July 22 – October 25, 2016.

	Infected patients	Uninfected patients	Excess cost/LOS	
Cost details	**Median, Mean ± SD**	**Median, Mean ± SD**	**Mean difference**	**P value**
Hospital bed cost	400, 457.32 ± 179.08	150, 219.19 ± 141.31	238.12	0.001
Laboratory cost	90, 111.46 ± 41.16	75, 84.92 ± 42.94	26.55	0.001
Drug cost	467, 487.88 ± 321.75	80, 155.22 ± 167.17	332.66	0.001
Injection cost	170, 162.93 ± 60.57	30, 54.10 ± 51.76	108.85	0.001
Wound care cost	165, 174.02 ± 68.99	0.00, 24.40 ± 36.76	149.63	0.001
Total cost	1335, 1393.61 ± 460.89	386, 537.80 ± 356.16	855.81	0.001
LOS (In days)	8, 9.15 ± 3.58	3, 4.38 ± 2.83	4.77	0.001

The cost data was also presented in US dollar for comparison purposes. As it is shown in table 6.10, a patient with SSI expended an average of 38.9 US dollar extra cost as compared to a patient without SSI.

6.10 costs (in US Dollar) associated with SSI in DRH, July 22 – October 25, 2016

Cost details	Infected patients Median, Mean ± SD	Uninfected patients Median, Mean ± SD	Excess cost/LOS Mean difference	P value
Hospital bed cost	18.2, 20.81 ± 8.15	6.8, 10.0 ± 6.4	10.81	0.001
Laboratory cost	4.1, 5.1 ± 1.9	3.4, 3.9 ± 1.95	1.2	0.001
Drug cost	21.2, 22.2 ± 14.64	3.6, 7.1 ± 7.6	15.1	0.001
Injection cost	7.7, 7.4 ± 2.76	1.4, 2.5 ± 2.35	4.9	0.001
Wound care cost	7.5, 7.9 ± 3.14	0.0, 1.1 ± 1.7	6.8	0.001
Total cost	60.7, 63.4 ± 21.0	17.6, 24.5 ± 16.2	38.9	0.001

7. Discussion

7.1. Incidence of SSI

Surgical Site Infection has been increased over the past few years. World Health Organization (WHO) documented that 66 % of establishing countries have no imprinted data related to the burden of SSI. Surveillance is an essential step to limit the rate of infection because it enlightens the magnitude of the problem and also facilitate the regulatory bodies to take valuable measures (43). In this study about 49 (14.5 %) of the patients had clinically suspected SSI. This rate of clinical SSI is slightly higher from a similar study done at Addis Ababa which reported 9.8 % (44), But the finding of this study was slightly lower than another study performed in Mekelle, Ethiopia which reported 20.98 % (20). This difference between studies could be due to the fact that the rate of SSIs varies widely between hospitals and between surgeons, suggesting that working practices play a critical role in the prevention of these infections (45).

The culture positivity rate in this study was 41 out of 49 (83.7 %), and from these culture positive samples about 48 different bacteria were isolated. Hence the overall culture confirmed SSI rate was 41/338 (12.1 %). This rate of culture confirmed SSI in the present study was in agreement with studies performed in Bahirdar (10.9 %) and Mekellie (15.7 %), Ethiopia, as well as from other developing countries like two independent studies from India which reported 12.76 % and 17.2 % (10,20,30,46). Whereas it was higher than studies conducted in two European countries, Italy and Turkey which reported 2.6 % and 4.3 % respectively(7,25), this might be due to the fact that these studies were conducted at a national level and on large and diverse group of population as well as availability of advanced infection control practices including; advanced surgical techniques, improved operating room ventilation, sterilization methods, barriers, patient care and safety. As the poor state of infrastructure and equipment, unreliable supplies and quality of medications, shortcomings in organizational management and infection control, difficulties in the supply and training of personnel and severe under financing in the developing world, contribute to the difficulties in surgical safety and patient care (47).

The rate of SSI was different from procedure to procedure. The highest rate of SSI in the present study was seen in prostate surgery (22.2 %), followed by small bowel surgery (21.1 %). The least rate of SSI was seen in herniorapy (6.7 %). In this study the NHSN International Classification of disease-9- Clinical Modification (ICD-9-CM) codes for surgical procedure categories (1) were followed, but scarcity of similar studies with the same kind of procedure category codes and differences in surgical procedures performed in different studies posed a difficulty to compare the finding of this study with others`. However, the findings of this study is found to be similar with other studies based on the anatomical sites where the surgeries took place; abdominal area incised and either the gastrointestinal or the genitourinary tract entered being the most common areas of surgeries where the highest rate of SSIs were observed. One study in India showed that the highest rate of SSI was found in renal surgery, while rectal or colon surgeries showed the highest rate of SSI in studies conducted in Turkey, Switzerland and Italy (7,10,25,48). This could be explained by the fact that Surgeries which involved hollow viscera like the gastrointestinal and urogenital tract, exposes surrounding tissues to the microbial flora present in such areas (43).

7.2. Bacterial etiology of SSI

Collectively more than half of the pathogenic bacteria isolated from post surgical wound infection in the present study were *gram negative rods* (56.25 %). This finding was in line with other studies conducted in Bahirdar, Addis Ababa, Hawassa and Mekelle, Ethiopia as well as studies in Nigeria and two studies from India in which gram negatives accounted 52.2 %, 73 %, 59.3 %, 53 %, 68.8 %, 69.8 %, and 62.5 % respectively (14,20,30,44,49–51). In contrast to this study, studies conducted in Sudan and India reported *gram positive cocci* (55 % and 71.7 % respectively) as a predominant isolate over *gram negative rods* (6,52). Predominance of *gram negative rods* in this study can be justified by the fact that in recent years, there has been a growing number of post-operative wound infections due to *Gram negative organisms* as it was explained by Jnaneshwara KB et al. and Jeyaprakash M et al. (9,11).

In this study *S. aureus* was the predominant isolate (29.2 %), followed by *E. coli* (18.6 %). *S. aureus* was also the most predominant isolate in previous studies conducted elsewhere in Ethiopia like the studies in Bahirdar (26.2 %), Mekelle (34.2 %), and Hawassa (37.3 %), a similar finding was also found in a study from India (38.3 %) (6,20,30,51). But *E. coli* was reported as the most predominant isolate in a study conducted in Addis Ababa, Ethiopia and Nigeria which reported 23.1 % and 32.5 % respectively (14,44). Even though, a similarity was observed between the present study and most other previous studies mentioned above in that the predominant isolate was *S. aureus*, there was a big variation in the entire pattern of the organisms isolated between the present study and the previous studies. The possible reason for the variation in these studies could be attributed to differences in the populations investigated and diversity of surgical procedures performed. This can also be explained by the fact that the etiology of surgical wound infections could vary with geographical location; from country to country and from hospital to hospital even within the same region, as well as the type and location of surgery (3,11,18).

The bacterial profile in different surgical procedures was variable in this study. In the present study the bacteria isolated in appendix surgery were two *Klebsiella species* and *S. aureus* and *E. coli* (one each). Three independent previous studies also isolated these organisms in appendix surgery, even though, there was little variation in number between studies and additional isolates were reported (5,13,19). In this study *S. aureus* was the predominant isoate (six) in cesarean section. *S. aureus* was also the predominant isolate in cesarean section as it was reported by previous studies from Uganda and Nigeria but the pattern in the distribution of the entire isolates was different (5,19).

In abdominal hysterectomy the organisms isolated were *S. aureus, CoNS, E. coli, Klebsiella species* and *M. morganii* (one each). In contrast to this study *S. aureus* and *E. coli* were the only isolates detected in hysterectomy in one previous study conducted in Nigeria (5). *S. aureus, CoNS and E. coli* were the three organisms isolated from exploratory laparatomy. One study in Nigeria also showed that *S. aureus, CoNS and E. coli* as the isolates detected from exploratory laparatomy, even if, *S. aureus* was predominant over the two isolates and *Proteus species* was

also reported in addition to the aforementioned isolates (5). Scarcity of literatures on distribution of bacterial isolates based on different surgical procedures was encountered to compare the findings of this study with others. However, there was no perfect agreement in the pattern of distribution of the bacterial isolates in few of the procedures for which a literature was available for comparison. This difference could be attributed to differences in the study population, study area, surgical techniques, the antibiotics received, the anatomical site where the surgery was performed, the number and experience of the surgeons involved.

7.3. Antibiotic susceptibility pattern

According to the result of this study all the *Staphylococcus species* were resistant for penicillin. This finding concur with previous studies done in Hawassa, Ethiopia and India which also reported 100% resistance rate of *Staphylococcus species* for penicillin (49,51). But there was little variation in the resistance rate of *Staphylococcus species* for penicillin in studies conducted in Addis Ababa and Bahirdar, Ethiopia which reported 87 % and 60 % respectively (30,44). There was 100 % sensitivity for chloramphenicol by *Staphylococcus species* in the present study. This was similar with a previous study from India (50), but it was different from studies conducted in Bahirdar and Hawassa, Ethiopia and other African countries like Kenya and Nigeria which reported a sensitivity rate of 24%-73.3 % (5,30,51,53). Different degree of resistance (here isolates with intermediate susceptibility were considered as resistant) was found among *Staphylococcus species* for the rest antibiotics tested. The degree of resistance for each drug was variable between the present study and all the previous studies conducted in different parts of Ethiopia, Africa and India (5,20,30,44,46,49–51,53).

The current investigation documented high rate of methicillin resistance among Staphylococcus species (55.6 %). Of these six (42.86 %) of the *S. aureus* isolates in this study were found to be *MRSA* and the rest eight (57.14 %) were *methicillin sensitive S. aureus (MSSA)*. This finding concurs with a previous study from Debremarkos, Ethiopia which reported 49.3 % *MRSA* (54), and three independent previous studies conducted in India which reported 45 % - 50 % *MRSA*, but it was higher than studies conducted in Addis Ababa, Ethiopia and Uganda with the

respective rates of 10.5 % and 25 % (19,21,44,49,55). All the four *CoNS* in the present study were found to be *MRCoNS*. This was in a harmony with a previous study conducted in Addis Ababa which reported all the four CoNS isolates as *MRCoNS*, but it was much higher than that reported by Najam M et al. which reported 50 % (44,49).

The higher rate of methicilin resistance in this study could be due to indiscriminate use of multiple antibiotics, prolonged hospital stay, intravenous drug abuse, and carriage of *MRSA* in nose, hands and mobile phones of hospital staffs, patients and visitors (21,55,56). This time, mobile phones are extensively used by medical professionals, which may get contaminated when used carelessly in Intensive Care Unit (ICU) or surgical wards, hence they can act as a potential source of *MRSA* to patients and the community (56). One study in India showed that 71 different pathogenic bacteria were isolated from 75 Doctors` mobile phone swabs, of which 18 (25 %) were *S. aureus* and 15 (83 %) were *MRSA* (56).

In this study, the highest resistance (isolates with intermediate susceptibility were considered as resistant) exhibited by *Enterobacteriaceae* isolates was for ampiciline (91.3 %), followed by augmentine (87 %), cotrimoxazole (82.6 %) and ceftriaxone (78.3 %). Resistance of *Enterobacteriaceae* isolates for ampiciline in this study was in line with previous studies conducted in Bahirdar, Mekelle, Addis Ababa, and Hawassa, Ethiopia as well as Uganda which reported 88.2 % - 96.5 % (19,20,30,44,51). The resistance rate for augmentine in the present study was between the findings of previous studies from Mekelle, Ethiopia and India which reported 62 % and 98.7 % respectively (20,54). Resistance for cotrimoxazole by *Enterobacteriaceae* isolates in this study was in concord with a report from India (88.3 %), but it was slightly higher than the reports from Bahirdar (64.7 %) and Hawassa (58.6 %) (20,51,57). Ceftriaxone resistance by *Enterobacteria*ceae isolates in the present study was in a harmony with previous findings from Bahirdar (76.5 %), Mekelle (77.8 %) and Addis Ababa (75 %), Ethiopia (20,30,44).

This high rate of resistance for ampiciline, augmentine, cotrimoxazole and ceftriaxone in this study could be attributed to the fact that, they are relatively cheap and/or widely prescribed in

empirical treatment of various bacterial infections. This can be justified by the fact that; in this study ampicilline and ceftriaxone (alone or together with metronidazole) were the antibiotics given for 97.1 % of the patients who had received preoperative antibiotic prophylaxis. In this study only one (4.3 %) out of the 23 *Enterobacteriaceae* isolates showed resistance for amikacin. In contrast to this study, a study from India reported a 25.9 % resistance for amikacin (57).

Penicillin was among the first antibiotics (which is a beta-lactam) discovered and used extensively to treat bacterial infection for a long period of time. Extensive use of penicillin caused the bacteria to develop a mechanism of resistance by producing the enzyme beta-lactamase. To solve such problems researchers develop newer derivatives like cephalosporins, monobactams and carbapenems, but the bacteria continually evolved and changed existing beta-lactamase enzymes to break down these new compounds. The enzymes that can break down the newer derivatives are known as the extended spectrum beta-lactamases (ESBLs). ESBLs are usually plasmid mediated, most commonly found in *Klebsiella pneumoniae*, *Escherichia coli* and other Gram-negative bacilli (41). In the present study six out of nine (66.67 %) of the *E. coli* and both of the *K. pneumoniae* isolates were found to be ESBL producers. So far there are more than 340 different beta lactamases identified and the growth shoot shows no signs of slowing. Since ESBLs are harbingers of MDR and their distribution has been shown to differ from region to region, establishing a proper screening methods and a proper treatment protocol is unquestionable to limit their spread (58).

In this study only one *Enterococcus species* was isolated and it was resistant for erythromycin, penicillin and chloramphenicol but it was sensitive to ampicilin and vancomycin. Its resistance for erythromycin and penicillin was in agreement with previous reports from India (49,59). The chloramphenicol resistant was different from the study report from India in which only one of the six *Enterococcus* isolate was resistant for chloramphenicol (59). The sensitivity of *Enterococcus* to Ampicillin reported in this study was in contrast to previous studies from India which reported 100 % resistance for ampicilin (10,59). Vancomycin sensitivity by *Enterococcus species* was in a harmony with previous reports from India (10,57,59). The *Viridans Streptococcus* isolate in this study was sensitive for all the drugs tested (cefepime, ceftriaxone

and vancomycin). Similar to this study a previous report from Iran also reported that the *Virdans Streptococcus spp.* Showed 100 % sensitivity for all the antibiotics tested (60).

The two *Pseudomonas species* in the present study were resistant for ceftazidime and piperacilin, but sensitive to amikacin. Sensitivity for gentamycin by *Pseudomonas species* in this study was 50 %. The ceftazidime resistance in this study was in agreement with previous reports from Nepal and India which reported more than 80 % resistance (10,57,61), but it was higher than the studies from Addis Ababa, Ethiopia and Kenya with the respective reports of 33.3 % and 50 % (44,53). The resistance of *Pseudomonas species* for piperacilin in the present study was in agreement with a study from India (100 %) but it was higher than the report from Kenya (50 %) (50,53). 50 % resistance for gentamycin by *Pseudomonas species* was in concord with previous reports from Hawassa, Ethiopia and Kenya (50 % in both cases) (51,53), but it was lower than the 100 % resistance reported from India (50). Amikacin showed the best effectivity for *pseudomonas species* in this study, but previous studies from India and Nepal reported a low degree of resistance which was 20 % and 35 % respectively (50,61).

The two *Acinetobacter species* in this study were resistant for all the antibiotics tested (cefepime, ceftriaxone, ceftazidime, piperacilin, gentamycin, and ciprofloxacin) except amikacin. The sensitivity of *Acinetobacter species* for amikacin in the present study was different from three independent studies from India which reported 25 %, 50 % and 100 % resistance for amikacin (10,50,57). The least resistance for amikacin by all isolates tested in the present study could be due to unavailability of this drug around the study setting.

Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria both in the hospital and the community has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria (42). Spread of MDR bacteria is an accelerating global health security emergency because the pipeline for the development of new antibacterial drugs is virtually empty after the 1980s (62). In the present study around 80 % of all the isolates were found to be Multidrug Resistant (MDR). The MDR rate for *gram negative* and *gram positive* isolates was 88.9 % and

66.7 % respectively.

The overall MDR rate in this study was slightly lower than the previous reports from Bahirdar and Hawassa, Ethiopia which reported 97.6 % and 93.2 % respectively (30,51). The rate of MDR among *gram negative* isolates in this study was also relatively lower as compared to previous reports from Bahirdar and Addis Ababa, Ethiopia which reported 95.5 % and 97.4 % respectively (30,44). Although the rate of MDR in the present study was relatively lower than the reports from Bahirdar, Hawassa and Addis Ababa, Ethiopia due to differences in defining MDR (isolates not susceptible for at least two drugs were considered as MDR in the aforementioned previous studies), it was still alarmingly very high. And this coincide with the fact that the problem of MDR continues to grow especially in developing countries as a result of antimicrobial drugs overuse, over dosing, drugs prescription with improper susceptibility test, unethical drug promotion, self-medication and long duration of hospitalization (63).

7.4. Factors associated with SSI

Several different associated factors were found to increase the risk of developing SSI. In the present study the rate of SSI was higher in the age groups; 35-44 years, 55-64 and ≥ 65 years. A bivariate logistic regression analysis was conducted to determine the significance. According to this analysis the age group 55-64 years was found to be 6.9 times more at risk of developing SSI as compared to the reference group, with the P value=0.001. Similarly the age groups 35-44 and ≥ 65 years were 5.2 and 4.2 times more at risk of developing SSI with P values 0.002 and 0.03 respectively. This finding was comparable with a previous report from Bahirdar, Ethiopia and Italy which reported a statistically significant increased rate of SSI in the age groups ≥ 51and ≥ 65 years respectively (7,31).

Diabetic mellitus and hypertension were found to be statistically significant associated factors for SSI in the bivariate logistic regression analysis with the respective P values = 0.02 and 0.03. In agreement with this study diabetic mellitus was also reported as a statistically significant risk factor for SSI in a study conducted at Bahirdar, Ethiopia (31). The reason for the increased rate

of SSI among diabetic patients could be due to; hyperglycemic individuals have a decreased vascular circulation, which in turn reduces tissue perfusion and impairing cellular-level functions. The other thing could the reduced activity of the cellular immunity functions of chemotaxis, phagocytosis and killing by polymorphonuclear cells as well as monocytes/macrophages in hyperglycemic individuals (64).

Although both diabetic mellitus and hypertension were found as a statistically significant associated factors in the bivariate analysis, only hypertension was remained significantin the multivariate logistic regression analysis with P value = 0.018. Hypertension was also reported as a significant risk factor for SSI by Kikkeri N, et al (65). The role of hypertension in SSIs could be explained by the fact that; oxygen is important for cell metabolism, especially energy production by means of ATP, and is critical for nearly all wound healing processes. Hence, any condition that impairs circulation and oxygenation such as hypertension will inhibit the healing process (66).

A significantly increased rate of SSI was observed in elective surgery than emergency surgery with P value= 0.043. The relatively higher rate of SSI in elective surgery in this study was in contrast to a previous study done at Bahrdar, Ethiopia which reported a higher rate of SSI in emergency surgery than elective surgery, even if, the difference was not statistically significant (31). This discrepancy could be due to a large number of patients who undergone cesarean section in the present study. Most of the time cesarean section is performed as an emergency surgery but patients who undergone cesarean section discharged early in most of the cases. This early discharge could hide the problem and resulted in the low rate of SSI in emergency surgery in this study. The other reason could be; elective surgery patients, who may wait days or weeks before the actual date of surgery, are at the optimal risk of acquiring pathogenic bacteria from the hospital environment unless SSI prevention strategies are implemented strictly (64).

ASA-2 and ASA-3 were found to be statistically significant associated factors in the bivariate analysis with the respective P values = 0.004 and 0.001. This finding was in concord with previous studies from Bahirdar, Ethiopia and Kenya and Italy (7,31,53). But only ASA-3 was

remained significant in the multivariate analysis with P value = 0.001. Surgeries that took 61-120 minutes and above 120 minutes were found as a statistically significant associated factors in the bivariate analysis with P values = 0.001and 0.003 respectively. Similar to this study an increasing rate of SSI was reported as the duration of the operation increased by a previous study from Iraq (12). ASA score ≥ 3 and increased duration of the operation for a particular procedure were considered as a risk factor for SSI, as it is mentioned in the National Nosocomial Infection Surveillance (NNIS) Risk Index developed by CDC (64).

7.5. LOS and costs associated with SSI

The median and mean cost for hospital bed, laboratory tests, drugs, injection of drugs and wound care was found to be higher among patients with SSI than patients without SSI. The difference in all the cases was statistically significant (P value=0.001) based on the Mann-Whitney and Kolmogorov-Smirnov Z tests. The total median and mean cost in patients with SSI was 1335 birr and 1393.61 birr respectively and this was 386 birr and 537.8 birr respectively for patients without SSI. This means, on average an additional 855.81 birr extra cost was expended per patient as a result of SSI, in other words a patient with SSI paid 2.6 times higher average cost than a patient without SSI. Accurate comparison of the cost associated with SSI is usually difficult because of the difference in study designs and diverse cost evaluation methods (43). However, the finding of this study on the total cost associated with SSI was comparable with previous study reports from England and Germany which reported a more than two fold increase of the cost associated with SSI (23,67).

Regarding the total hospital stay, the median days spent in the hospital by patients, with and without SSI was 8 and 3 days respectively, and the average days spent in the hospital by patients with and without SSI was 9.15 and 4.38 days respectively. On average a patient with SSI spent 4.77 more days in the hospital as compared to a patient without SSI or the average days spent in the hospital by patients with SSI was 2.1 times higher than patients without SSI. The Mann-Whitney and Kolmogorov-Smirnov Z tests showed that this difference was statistically significant (P value=0.001). The result on the LOS in this study was comparable with previous

reports by Olsen MA et al. and Arefian H et al. which reported additional LOS of 4.9 and 6.5 days respectively (39,67).

The mean aggregate excess cost incurred by patients with SSI in US dollar was 38.9 in the present study. This finding was much lower than the reports from United Kingdom, Thailand and Spain which reported 5714, 4163 and 3859 US dollars respectively (34). The rational for this big difference could be due to Income (per capita GDP) differences between countries as in high income countries per capita health expenditure is over USD 3000 on average, while in resource poor countries it is only USD 30 per capita. The other possible reason for this great cost variation between countries could be differences in technological progress as developed countries could use advanced surgical procedures (which are expensive), newly innovated medical equipments and expensive and new generation of drugs (68).

Differences in health financing could also have its own impact on cost differences between countries; as per capita health expenditure is higher in countries with social health insurance as compared to countries that depend solely on general taxation (68). Since the health insurance system is in an infant stage in our country, most of the health care costs are covered by the out of pocket payments and especially in this case cost becomes the determining factor to seek medical care. Hence using advanced techniques, instruments and drugs and asking big prices will be difficult and this enforces the health care providers to look for cheaper alternatives. The other thing is government and external donors' subsidies in the health care provision could have its own role in lowering the health care costs.

8. Conclusion

The incidence of SSI in this study was 12.1 %. Collectively *gram negative rods* were the predominant organisms isolated from SSIs. However, *S. aureus* was the predominant isolate individually. The rate of ESBL production by *E. coli* and *K. pneumoniae* and methicilin resistance by *Staphylococcus species* was very high. Generally about 80% of all of the organisms isolated from SSIs were multidrug resistant. Hypertension, elective surgery, ASA-3 and the age group 35-44 years were found as statistically significant associated factors for SSI. On average, an additional 855.81 Ethiopian birr (or 38.9 US dollar) lost per patient due to SSI and patients with SSI spent 4.77 extra days in the hospital.

9. Recommendation

- DRH should undergo a regular surveillance to determine the rate of SSI, the causative agents and their antibiotic susceptibility.
- The hospital based data obtained from the regular surveillance should be fed back to the surgical team on a regular basis.
- DRH should have to review its current practice in relation to preventing SSIs.
- Dessie Referal hospital should use a microbiology laboratory service, to make the effort of preventing SSIs and limiting the spread of MDR evidence based.
- The use of penicillin, ampicillin, augmentin, cotrimoxazole and ceftriaxone to treat SSI should be limited.
- DRH should have to reduce the extra cost due to SSIs by strengthening its infection prevention practice.
- A nationwide survey concerning SSI (that includes both inpatient and post discharge) should be conducted by ministry of health to provide comprehensive information for policy makers.

10. Limitation of the study

- Due to limited laboratory facilities and experience fungi and anaerobic bacteria, which could act as a potential cause of SSI were not studied.
- Failure to incorporate all the SSIs that could occur after hospital discharge, due to the difficulty of getting patients after discharge.
- Failure to perform combined disk method for ESBL production test and failure to perform genotypic test for MRSA due to unavailability of resources.

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Annex I informed consent form

ID No……

Consent form to participate in the study, on Incidence of SSI, predisposing factors and associated costs, at DRH.

Hello, my name is _____, I am a postgraduate student at Addis Ababa University, undergoing research on Aerobic bacterial profile of surgical site infection with their antibiotic susceptibility pattern, predisposing factors and associated costs, in DRH.

What Participation Involves:

If you agree, you will be interviewed using questionnaire, detailed information on socio demographic characteristics, past medical history and physical examination will be requested. A pus swab will be taken from the site of surgical incision.

Confidentiality:

Any information you provide and your laboratory result will be kept secret in order to maintain confidentiality.

Risks:

You will face no harm because of joining this study. Sometimes, a minimal pain may occur during swabs taking.

Benefits:

If you agree to take part in this study, will benefit by knowing the result of culture and sensitivity pattern of a collected specimen, and whenever there are culture positive results appropriate medications will be prescribed and you will be advised accordingly. Participating in this study is completely your choice. You can stop participating in this study at any time, even if you have already given your consent.

Signature:

Do you agree?

Participant agrees …… Participant does NOT agree ……..

I, _____ have read the contents in this form. My questions have been answered. I agree to participate in this study.

Signature of participant (care taker) _____

Signature of research assistant _____

Date of signed consent _____

አባሪ-1 ስለጥናቱ መግለጫ እና የፍቃደኝነት መጠየቂያ ቅጽ

የተሳታፊ መለያ ቁጥር_____

ከቀዶ ጥገና በሁዋላ የሚከሰት ምርቀዛ የመከሰት ምጣኔ፣ ለቁስሉ ማመርቀዝ ምክኒያት ሊሆኑ የሚችሉ አጋላጭ ሁኔታዎች እንዲሁም ሊያስከትለው የሚችለውን ወጪ በደሴ ሪፈራል ሆስፒታል በሚካሔደው ጥናት ላይ ለመሳተፍ የፍቃደኝነተ መጠየቂያ ቅጽ፡፡

ጤና ይስጥልኝ ስሜ _____ ይባላል፤ በአዲስ አበባ ዩኒቨርሲቲ የሁለተኛ ዲግሪ ተማሪ ስሆን ከላይ በተመለከተው ርዕስ ዙሪያ ጥናት በማካሔድ ላይ ነኝ፡፡

በጥናቱ መሳተፍ የሚያካትታቸው ሁኔታዎች
በጥናቱ ለመሳተፍ ከተስማሙ፤ ሠፋ ያለ መረጃ በስነ-ህዝባዊ ባህሪያት እና የቀድሞ የጤና ታሪክ በተመለከተ ቃለመጠይቅ የሚደረግልዎት ሲሆን፣ የፊዚካላዊ ምርመራ እና ቀዶጥገና ከተሰራበት አካባቢ የማመርቀዝ ሁኔታ ከታዮ ከቀስሉ ላይ ናሙና የሚወሰድ ይሆናል፡፡

ሚስጥራዊነት
ማንኛውም እርሶ የሚሰጡት መረጃ ከላቦራቶሪ የሚገኘው ውጤት በጥብቅ ሚስጢር የሚያዝ መሆኑን ላረጋግጥልዎት እዎዳለሁ፡፡

ጉዳቶች
በዚህ ጥናት በመሳተፍዎ የሚደርስብዎት ምንም አይነት ጉዳት የለም፤ አልፎ አልፎ ናሙናው በሚወሰድበት ጊዜ እጅግ በጣም አነስተኛ ህመም ሊኖር ይችላል፡፡

ጥቅሞች
በዚህ ጥናት ለመሳተፍ ካተስማሙ ከፍተኛ ጥቅም የሚያገኙ ይሆናል ይኸውም የላቦራቶሪውን ውጤት (የተገኘውን ባክተሪያ እና የባክተሪያውን መድሀኒት ጋር የመላመድ ሁኔታ) ለርስዎም ለሚከታተልዎት ህኪምም እንድታውቁት ስለሚደረግ፤ የሚደረግልዎት ህክምናም በውጤቱ መሰረት በተሻለ ሁኔታ ሊሰጥዎት ይችላል፡፡ በዚህ ጥናት ላይ መሳተፍ ሙሉ በሙሉ የርስዎ ምርጫ ነው፡፡ በማንኛም ጊዜ በጥናቱ ላይ ያለዎትን ተሳትፎ ሊያቋርጡ ይችላሉ፤ ምንም እንኳን ፍቃደኝነትዎን ገልጸው የነበረ ቢሆንም፡፡

ፊርማ
ተሳታፊው/ዋ ተስማምቷል/ታለች---------- ተሳታፊው/ዋ አልተስማማም/ችም ----------

እኔ ስሜ _____ የተባልኩ በዚህ ቅጽ የተመለከተውን መረጃ ያነበብኩና ጥያቄዎቸ ሙሉ በሙሉ የተመለሱ በመሆኑ በጥናቱ ለመሳተፍ ተስማምቻለሁ፡፡

የተሳታፊው (ተንከባካቢው) ፊርማ _____

የጥናቱ ረዳት ፊርማ _____

ቀን _____

Annex IIQUESTIONAIRE

TITLE: **Incidence of SSI, predisposing factors and associated costs at DRH, Dessie, Ethiopia.**

Serial number……..

Date of interview ……Registration number ……

1. Surgical department in which patient admitted or attending
   (a) General & pediatrics surgery (b) gynecology/obstetrics
2. Age……..
3. Sex……...
4. Address……
5. Date of Admission……....
6. Date of surgery…….
7. Date of discharge……
8. Preoperative hospital stay: 1) <= 2 days 2) >2 days
9. Past medical history
   (1) DM (a) yes (b) no
   (2) Prolonged Streroid usage (a) yes (b) no
   (3) Hypertension (a) yes (b) no
10. Presence of added drain (a) yes (b) no
11. Surgical procedure performed _____
12. Type of surgery:
    (1) Clean surgery (2) Clean contaminated surgery (3) Contaminated surgery (4) Dirty surgery
13. Nature of surgery (1) Emergency surgery (2) Elective surgery
14. Patient general condition before surgery;
    (a) ASA-1 (b) ASA-2 (c) ASA-3 (d) ASA-4 (e) ASA-5
15. Antibiotic prophylaxis: (1) yes (2) no

16. If yes, type of the antibiotic prophylaxis given

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

17. Duration of operation in minutes (1) 0-30 (2) 31-60 (3) 61-120 (4) >120

18. Laboratory tests requested

    a. _____

    b. _____

    c. _____

    d. _____

19. Postoperative antibiotics given with their respective quantity

    a. _____

    b. _____

    c. _____

    d. _____

Laboratory results record sheet

1. Gram staining result (bacterial morphology, staining reaction and WBC/pus) ……

   ……..……..

2. Organisms isolated (1) ……..

   2) ……..

3. Sensitivity pattern of isolated organisms

a. Isolated organism _____

Drugs										
Diameter										
Interpretation										

አባሪ-II የመረጃ መሰብሰቢያ መጠይቅ

የጥናቱ ርዕስ፡- ከቀዶ ጥገና በሁዋላ የሚከሰት ምርቀዛ የመከሰት ምጣኔ፣ ለቁስሉ ማመርቀዝ ምክኒያት ሊሆኑ የሚችሉ አጋላጭ ሁኔታዎች እንዲሁም ሊያስከትለው የሚችለውን ወጪ በደሴ ሪፈራል ሆስፒታል፡፡

የተሳታፊ መለያ ቁጥር _____

ቃለ መጠይቁ የተካሔደበት ቀን_____የመዝገብ ቁጥር _____

1. ተሳታፊው ለቀዶ ህክምናው የተኛበት ክፍል

   ሀ) አጠቃላይ እና የህጻናት ቀዶ ጥገና ክፍል ለ) የማህጸንና ጽንስ ክፍል

2. እድሜ _____
3. ጾታ _____
4. አድራሻ _____
5. ወደ ሆስፒታሉ የገቡበት ቀን _____
6. ቀዶ ጥገና የተካሔደበት ቀን _____
7. ከሆስፒታል የወጡበት ቀን _____
8. ከቀዶ ህክማና በፊት በሆስፒታል የቆዩበት ቀን

   ሀ) ከሁለት ቀናት ያነሰ ለ) ከ ሁለት ቀናት በላይ

9. የቀድሞ የጤና ሁኔታ

   9.1 ስኳር ሀ) አዎ ለ) የለም

   9.2 ለረዥም ጊዜ ስቴሮይድ የመጠቀም ሁኔታ ሀ) አዎ ለ) የለም

   9.3 የደም ግፊት ሀ) አዎ ለ) የለም

10. የተጨመረ ማንጣፈፊያ ቱቦ (ድሬን) ሀ) አዎ ለ) የለም
11. የተሰራልዎ የቀዶ ጥገና አይነት _____
12. የቀዶ ጥገናው የብክለት መጠን

    ሀ) ክሊን ቀዶ ጥገና ለ) ክሊን ኮንታሚኔትድ ቀዶ ጥገና

    ሐ) ኮንታሚኔትድ ቀዶ ጥገና መ) ደርቲ ቀዶ ጥገና

13. የቀዶ ጥገና ሁኔታ ሀ) አስቸኳይ ቀዶ ጥገና ለ) የታቀደ ቀዶ ጥገና
14. የበሽተኛው አጠቃላይ የጤና ሁኔታ ለቀዶ ጥጋና በሚገባበት ጊዜ

    ሀ) አሶአ-1 ለ) አሶአ-2 ሐ) አሶአ-3

    መ) አሶአ-4 ሰ) አሶአ-5

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

16. ለጥያቄ ቁጥር 15 መልሱ አዎ ከሆነ የተሰጠው የመድሀኒት አይነት

    ሀ) ሴፍትሪያግዞን ለ) ጀንታማይሲን

ሐ) ሜትሮኒዳዞል መ) ሌላ ከሆነ ይጠቀስ _____

17. ቀዶ ጥገናው የወሰደው ጊዜ በደቂቃ

ሀ) 0-30 ለ) 31-60 ሐ) 61-120 መ) >120

18. የተደረጉ የላቦራቶሪ ምርመራዎች

ሀ) _____

ለ) _____

ሐ) _____

መ) _____

19. ከቀዶ ጥገና በሁዋላ የተሰጡ መድሀኒቶች አይነት እና ብዛት

ሀ) _____

ለ) _____

ሐ) _____

መ) _____

የላብራቶሪ ውጤት መመዝገቢያ ቅጽ

1. የግራም ስቴን ምርመራ ዉጤት(የባክተሪው ቅርጽ፣ አቀማመጥ፣ የወሰደው ቀለም እና የነጭ የደም ሴሎች ብዛት)

_____

_____

2. በካልቸር ውስጥ ያደጉ ባክተሪያዎች

2.1 _____

2.2 _____

3. የተገኙት ባክተሪያዎች መድሀኒትን የመላመድ ሁኔታ

ሀ) የተገኘው ባክተሪያ _____

መድሀኒቶች									
ዲያሜትር									
አንድምታ									

Annex III Biochemical tests

Catalase test

Purpose: this test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as 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 into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

Method

1. Pour 2–3 ml of the hydrogen peroxide solution into a test tube.
2. Using a sterile wooden stick or a glass rod (*not* a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution.

Important: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur.

Results

Active bubbling . . . . . Positive catalase test

No bubbles . . . . . Negative catalase test (69).

Coagulase test:

Purpose: this test is used to differentiate *Staphylococcus aureus* which produces the enzyme **coagulase**, from S. epidermidis and S. saprophyticus which do not produce coagulase.

Principle:

Coagulase causes plasma to coagulate (**clot**) by converting the plasma fibrinogen to fibrin. Two types of coagulase are produced by most strains of S. aureus:

- **Free coagulase**: converts fibrinogen to fibrin by activating a *coagualse-reacting factor* present in plasma. It is detected by clotting in the ***tube test***
- **Bound coagulase** (clumping factor): converts fibrinogen directly to fibrin without requiring a *coagulase-reacting factor*. It can be detected by clumping of bacterial cells in the ***rapid slide test***.

Method for slide test (bound coaglulase):

1. Add one drop of either human plasma or latex reagent on to paper slide
2. Using a plastic or wooden stick, take part of test colony to the slide
3. Mix well and look for clumping within 10 seconds.

Results:

- **Clumping or clots formed (plasma coagulated)** within 10 seconds; Coagulase produced (**coagulase positive**) the organism is **S. aureus**.
- **No clumpimg** within 10 seconds; No coagulase produced (**coagulase negative**) the organism may be (**other staphylococci** S. epidermidis or S. saprophyticus) (69).

PYR test

PYR is a chromogenic substrate (L-pyrrolidonyl-β-naphthylamide, or PYR) which when hydrolyzed by PYRase (L-pyrroglutamyl-peptide hydrolase) produces a red color upon the addition of a specific reagent. PYR is a substrate that is hydrolyzed by 100% of the enterococci and group A streptococci but not by any other streptococcal species. Two to 4 drops of a buffer reagent is applied to the PYR test strip circle. The strip is then inoculated with 3–5 colonies of the organism and incubated at room temperature for 2 min. Two drops of a second reagent is applied to the to the test strip circle. An intense red color develops immediately around the colonies in the presence of hydrolyzed PYR. The PYR test is negative if no color, an orange

color or a weak pink-color develops. Staphylococci may cause a positive PYR reaction (70).

Salt tolerance test

Purpose: Trypticase soy broth is a general-purpose medium for the cultivation of both fastidious and nonfastidious organisms. With the addition of 6.5% sodium chloride, the medium can be used to differentiate between salt-tolerant and salt-intolerant organisms. It is especially useful for distinguishing *Enterococcus* spp., which are salt-tolerant, from non-enterococcal group D streptococci, such as *S. bovis* and *S. equinus* (70).

Principle: Inoculate the tube containing 6.5% sodium chloride with the organism and incubate at 35◦C in non-CO_2 for 24–48 h. A visible growth (turbidity) is considered positive and no growth is considered negative. If the medium is inoculated too heavily, the inoculum may be interpreted as growth, resulting in a false-positive reaction (70).

Oxidase test

Purpose: this test is a biochemical reaction that assays for the presence of cytochrome oxidase, an enzyme sometimes called indophenol oxidase.

Principle

In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colorless reagent becomes an oxidized colored product.

Method

There are many method variations to the oxidase test. These include, but are not limited to, the filter paper test, filter paper spot test, direct plate method, and test tube method.

Filter Paper Test Method

1. Soak a small piece of filter paper in 1% Kovács oxidase reagent and let dry.
2. Use a loop and pick a well-isolated colony from a fresh (18-24-hour culture) bacterial plate and rub onto treated filter paper.
3. Observe for color changes.
4. Microorganisms are oxidase positive when the color changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the color does not change or it takes longer than 2 minutes.

Oxidase positive bacteria : Pseudomonas, Vibrio cholera
Oxidase negative bacteria: E. coli, Klebsiell, Salmonella (69).

Indole test

Purpose: this test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. It is used as part of the IMViC (indole, MR-Vp Citrate) procedures, a battery of tests designed to distinguish among members of the family Enterobacteriaceae.

Method

1. Inoculate the tube of tryptone broth with a small amount of a pure culture.
2. Incubate at 37°C for 24 to 48 hours.
3. To test for indole production, add 5 drops of Kovác's reagent directly to the tube.
4. A positive indole test is indicated by the formation of a pink to red color (“cherry-red ring”) in the reagent layer on top of the medium within seconds of adding the reagent.
5. If a culture is indole negative, the reagent layer will remain yellow or be slightly cloudy.

Indole positive bacteria : *E. coli, Vibrio cholera*
Indole negative bacteria : *Klebsiella, Salmonella, Shigella spp* (69).

Citrate Test

Purpose: this test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the color change of a pH indicator. The citrate test is often part of a battery of tests used to identify gram-negative pathogens and environmental isolates.

Method

1. Use a fresh (16 to 18 hours) pure culture as an inoculation source.
2. Pick a single isolated colony and lightly streak the surface of the slant. A needle is the preferred sampling tool in order to limit the amount of cell material transferred to the agar slant.

Important: Avoid using liquid cultures as the inoculum source. Citrate utilization requires oxygen and thus screw caps, if used, should be placed loosely on the tube.

3. Incubate at 35^{o}C (± 2^{o}C) for 18 to 48 hours.
4. Some organisms may require up to 7 days of incubation due to their limited rate of growth on citrate medium.

Citrate positive: growth will be visible on the slant surface and the medium will be an intense Prussian blue. The alkaline carbonates and bicarbonates produced as by-products of citrate catabolism raise the pH of the medium to above 7.6, causing the bromothymol blue to change from the original green color to blue.

Citrate negative: trace or no growth will be visible. No color change will occur; the medium will remain the deep forest green color of the uninoculated agar. Only bacteria that can utilize citrate as the sole carbon and energy source will be able to grow on the Simmons citrate medium, thus a citrate-negative test culture will be virtually indistinguishable from an uninoculated slant.

Citrate positive bacteria: Klebsiella spp.

Citrate negative bacteria: E. coli (69).

Urease test

Purpose: this test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive *bacteria* from other *Enterobacteriaceae*.

Method

1. Use Christensen's Urea Agar
2. Use a heavy inoculum from an 18 to 24 hour pure culture to streak the entire slant surface. Do not stab the butt as it will serve as a color control.
3. Incubate tubes with loosened caps at 35^{o}C. Observe the slant for a color change at 6 hours, 24 hours, and every day for up to 6 days.
4. Urease production is indicated by a bright pink (fuchsia) color on the slant that may extend into the butt.

Note that any degree of pink is considered as a positive reaction. Prolonged incubation may result in a false-positive test due to hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea should be used. Rapidly urease-positive organisms (e.g., *Proteus* spp., *Morganella morganii*, and some *Providencia stuartii* strains) will produce a strong positive reaction within 1 to 6 hours of incubation. Delayed-positive organisms (e.g., *Klebsiella* or *Enterobacter*) will typically produce a weak positive reaction on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days). The culture medium will remain a yellowish color if the organism is urease negative.

Urease positive bacteria : Proteus spp., *Morganella morganii*
Urease negative bacteria : E. coli (69).

Kligler Iron Agar (KIA)

Purpose: KIA is a medium that differentiates Gram-negative bacilli on the basis of the ability to ferment carbohydrates and liberate hydrogen sulfide (H_2S). The medium contains 1 part glucose to 10 parts of lactose. Phenol red serves as an indicator to detect pH change, and ferrous sulfate detects the formation of H_2S. If the organism ferments glucose, the butt and slant of the agar will become acidic and turn yellow. If the organism ferments lactose, the slant will remain acidic (yellow). If the organism is unable to ferment lactose, the slant will revert to alkaline (red) when the glucose is used up and alkaline amines are produced in the oxidative decarboxylation of peptides (derived from protein in the medium) near the surface of the agar. Organisms unable to ferment glucose will not change the pH of the medium or will produce alkaline products, and the KIA tube will remain red. Blackening of the medium indicates H_2S production. Gas production is indicated by splits or cracks in the butt of the agar. Gas may also push the agar up the tube.

- If acid slant–acid butt (yellow–yellow): glucose and lactose fermented.
- If alkaline slant–acid butt (red–yellow): glucose fermented only.
- If alkaline slant–alkaline butt (red–red): glucose not fermented.
- The presence of black precipitate (butt) indicates hydrogen sulfide production, and presence of splits or cracks with air bubbles indicates gas production (70).

Early readings may result in false acid–acid results, and delayed readings may result in false alkaline–alkaline results. Copious amounts of H_2S may mask the glucose reaction. If this occurs, glucose has been fermented even if it is not observable (70).

Lysine Iron Agar (LIA)

Lysine iron agar is a differential medium used for the identification of enteric bacilli based on their ability to decarboxylate or deaminate lysine and produce hydrogen sulfide. Dextrose serves as a source of fermentable carbohydrate. The pH indicator, bromcresol purple, is changed to a yellow color at or below pH 5.2 and is purple at or above pH 6.8. Ferric ammonium citrate and sodium thio sulfate are indicators of hydrogen sulfide formation. Lysine serves as the substrate

for detecting the enzymes lysine decarboxylase and lysine deaminase. Using a sterile inoculating needle, stab the butt of the LIA slant twice then streak back and forth along the surface of the agar with the organism. Incubate at 35◦C ± 2◦C in non-CO2 for 18 to 24 hour. Alkaline (purple) reaction in the butt indicates lysine decarboxylation; red slant indicates lysine deamination, and black precipitate indicates H2S production. H2S may not be detected in this medium by organisms that are negative for lysine decarboxylase activity because acid production in the butt may suppress H2S formation. For this reason, H2S producing *Proteus species* do not blacken this medium (70).

Annex IV Manufacturers of Culture Media and Antibiotic discs

Media	Manufacturer	Antiviotics	Manufacturer
Simone citrate agar	Hi media	Amikacin	AB Tech
Motility media	TM media	Ampiciline	Oxoid
Brain heart infussion	Oxoid	Augmentin	AB Tech
Lysine Iron agar	TM media	Bacitracin	Oxoid
Muller Hinton agar	CONDA	Chloramphenicol	Oxoid
Manitol salt agar	Oxoid	Ceftazidime	Oxoid
Urea agar	TM media	Cotrimoxazole	AB Tech
Nutrient broth	Oxoid	Ciprofloxacin	AB Tech
Mackonkey agar	Oxoid	Ceftriaxone	AB Tech
Kligler iron agar	Uni-chem	Cefotaxime	Oxoid
Blood agar base	TM media	Clindamycin	Oxoid
NaCl	TM media	Erythromycin	AB Tech
		Cefepime	Oxoid
		Cefoxitin	Oxoid
		Gentamycin	AB Tech
		Novobiocin	AB Tech
		Penicillin	AB Tech
		Piperaciline	AB Tech
		Tetracycline	Oxoid
		Vancomycin	AB Tech

DECLARATION

I, Abdurrahman Ali, declare that this research paper is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award. Any material utilized for this study was duly acknowledged.

Signature_____ Date_____

Cell phone number_____

This work is presented for review under the supervision of;

Advisor name_____

Signature_____

Date_____