ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

Title: Molecular characterization and antimicrobial susceptibility profile of bacterial isolates from patients with surgical site infection in Ethiopia: A multicentre prospective cross-sectional study

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A PhD DISERTATION PRESENTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN MEDICAL MICROBIOLOGY

June 2024

Addis Ababa, Ethiopia

ACKNOWLEDGMENTS

I would like to express my heartfelt thanks to my supervisors and advisors for their unreserved support and guidance throughout the research process. Under your guidance I have accomplished this dissertation I want to acknowledge the generous contributions of the following individuals gratefully: My advisors, Dr. Tamrat Abebe and Dr. Adane Mihret, from Department of Microbiology, Immunology & Parasitology, School of Medicine, College of Health Sceinces Addis Ababa University, and co-advisors Drs Getachew Tesfaye Beyene, Berhanu Seyoum, Alemseged Abdissa, from Armauer Hansen Research Institute; and very special thanks for Prof. Gote Swedberg, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.

My gratitude to The Centre for Innovative Drug Development and Therapeutic Trials for Africa (CDT-Africa) and the head of CDT-Africa in Addis Ababa Professor Abebaw Fekadu for supporting the project cost, every aspect of my visa processing and accommodation during my stay in Sweden for laboratory works. Additionally, my gratitude to the International Science Program (ISP) for related administrative issues and accommodation during my stay in Sweden for laboratory work.

I would also like to express my sincere gratitude to my colleagues at Addis Ababa University, especially to Mr. Habtamu Biazen (who fixed my computer problem) and Dr. Melese Hailu, who helped me when I was in Sweden for laboratory work. The Ph.D. research was supported by the School of Graduate Studies, Addis Ababa University, The Centre for Innovative Drug Development and Therapeutic Trials for Africa (CDT-Africa), and the Armauer Hansen Research Institute. Lastly, I thank my family for supporting me passionately during the study period.

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ABBREVIATIONS

AAERC

AAERC	AHRI/ALERT Ethics Review Committee
AHRI	Armauer Hansen Research Institute
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Testing
ATCC	American Type of Culture Collection
CDC	Center for Disease Control and Prevention
CLSI	Clinical Laboratory Standards Institute
CoNS	Coagulase Negative Staphylococcous
DMIP	Department of Microbiology Immunology and Parasitology
DTCRH	Debre Tabor Teaching and Referral Hospital
ESBL	Extended-Spectrum Beta Lactamases
HUCSH	Hawassa University Comprhensive Specialized Hospital
JUMC	Jimma University Medical Center
MALDI-TOF MS	Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry
MDR	Multidrug Resistance
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-Resistant S. aureus
MSSA	Methicillin-Sensitive S. aureus
SCC*mec*	Staphylococcal Cassette Chromosome mec
SOP	Standard Operation Procedure
SSI	Surgical Site Infections
STATA	South Texas Art Therapy Association
TASH	Tikur Anbessa Specialized Hospital
USA	United States of America
VISA	Vancomycin-Intermediate *S. aureus*
VRSA	Vancomycin-Resistant *S. aureus*
VSSA	Vancomycin Sensitive *S. aureus*

ABSTRACT

Background: Globally, surgical site infections (SSI) are the most reported healthcare-associated infections. The most common pathogens associated with surgical wound infection are Gram-negative bacteria, and *Staphylococcus aureus*. Antibiotic resistance is increasing annually, and a major challenge in the management of SSI. Despite several reports on phenotypic examinations of bacterial isolates, there are few data on the molecular characterstics and antimicrobial resistance genes of bacteri isolated patients with SSI in Ethiopia.

Objective: The aim of this study was to isolate and determine the antimicrobial susceptibility pattern; genes associated with drug resistance and identify risk factors associated with surgical site infection in Ethiopia

Methods: Methods: A multicentre study was conducted among patients who underwent surgical procedures at four hospitals located in Northern (Debre Tabor), Southern (Hawassa), Southwest (Jimma), and Central (Addis Ababa) parts of Ethiopia. A total of 752 clinically suspected patients who developed surgical site infection were enrolled at each study sitein the study., and Sample for bacterial culture SSI discharge culture was collected from the infection site and inoculated on different culture media that supports the growth of Gram-positive and Gram-negative bacteria performed and positive cultures were characterized by colony characteristics, Gram stain, and conventional biochemical tests. Species identification each bacterial isolate was confirmed using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). An antimicrobial susceptibility test (AST) was done on Mueller-Hinton agar using the disk diffusion method. The phenotypic detection of MRSA was made using cefoxitin (30µg), Extended-spectrum beta-lactamases (ESBL) production was determined by combination disc-diffusion test (CDT) on MHA by using ceftazidime (30µg CAZ) and cefotaxime (30µg CTX) alone and with ceftazidime + clavulanic acid (20/10µg) CAZ/CLA) and cefotaxime + clavulanic acid (20/10µg CTX/CLA) and carbapenemase production was determined phenotypically by modified Hodge test (MHT) including impemene (10µg) test. The MRSA, ESBL and carbapenem resistance determinants were further studied determined by polymerase chain reaction (PCR) and Whole Genome Sequencing (WGS). Logistic regression analysis was used. A $p\text{-value} < 0.05$ was considered statistically significant. Data were analysed using STATA 16 software.

Results: Of the 752 specimens collected 493 (65.5%) had growth and 494 bacteria were isolated. Among these, 286 (58%) and 207 (42%) were Gram-negative and Gram-positive isolates, respectively. *Staphylococcus aureus* (153, 31%), *Escherichia coli* (102, 20.7%), *Klebsiella pneumoniae* (48, 9.8%) and *Acinetobacter species* (43, 8.7%) were the most common isolates. The isolates had high levels of resistance to most of the tested antibiotics and alarming resistance to ertapenem (32.9%), amikacin (24.3%), imipenem (20.3%), and meropenem (17.6%). Among the isolated bacteria 96.1%of *E. coli* and 95.9% *K. pneumoniae* were Multidrug-resistant (MDR). Of the 135 GNB encoded At least one ESBL and carbapenemase gene were detected from 57.8% and 37.8% of the isolates respectively. Interms of specific bacteria species highest level of at least one ESBL gene was detected from *Pseudomonas aeruginosa* and *E. cloacae* (83.3%) of followed by *K. pneumoniae* (66.7%), and *E. coli* (57.9%). Similarly the highest level of carbapenemase gene was detected among of *P. aeruginosa* and *E. cloacae* (100%) each, followed by *A. baumannii* (56.2%), and *K. pneumoniae* (50%). The most frequently detected ESBL and carbapenemase gene were $bla_{CTX\text{-}M\text{-}15}$ (56.4%) and bla_{OXA} (29.6%) respectively. Among the Gram-positive bacteria, *S. aureus* was isolated from 163 participants (21.6%), of which 24.5% were cefoxitin-resistance, and only 0.6% showed vancomycin resistance. The gene *mecA* was detected from 27.5% (11/40) of cefoxitin-resistant Staphylococci through PCR and WGS. Only 12.9% (4/31) carried the *mecA* gene (MRSA) among MALDI-TOF confirmed *S. aureus* (31/40), and no *vanA* or *vanB* genes were identified. Our analysis of risk factors associated with SSI indicated age ≥ 61 years, prolonged duration of hospital stays, history of previous antibiotics uses, history of smoking, emergency surgery, and duration of operation as important risk factors. Infection with Methicillin-resistant staphylococcal was indicated to increase with age ≥ 61 years, prolonged duration of hospital stays, and history of previous antibiotic use ($p < 0.05$). In addition, there was a significant association between ESBL producing GNB and previous history of antibiotics ($P = 0.03$). **Conclusion:** ESBL and carbapenemase producing GNB as well as *S. aureus* were the main pathogens responsible for SSIs in the different hospitals in Ethiopia. The co-existence of different ESBL genes and carbapenamase genes in a single bacterial pathogen reflects dissimnation of MDR. There was also ahigh prevalence of *mecA* carriage among Coagulase negative staphylococci (CoNS). As a multicenter study this implies SSI is a major problem in hospitals in Ethiopia

Keywords: Molecular epidemiology; surgical site infections aetiologies; ESBL; Carbapenemase producing; Multidrug resistance; Risk factors; Ethiopia

1. CHAPTER 1: INTRODUCTION

1.1. Background

Surgical site infections (SSIs) previously known as post-operative wound infections are major global problem in the surgery department related to surgery, leading to many serious surgical complications (Raza *et al.*, 2013). As defined by the Centers for Disease Control and Prevention (CDC), surgical site infections are infections that occur within 30 days after surgery with no implant, or up to one year after surgery in patients receiving implants or prosthetic material is implanted at surgery (Owens and Stoessel, 2008, Horan *et al*., 1992). Surgical site infection is a substantial cause of morbidity and mortality. About 3-5% of patients who undergo elective surgery develop SSIs (Magill *et al.*, 2012) and SSI is the third most frequently all reported cases of nosocomial infections, accounting for 14% to 17% of all hospital-acquired infections and about 38% surgical patients devlop SSI (Spagnolo *et al*., 2013). Primarily it is a result of bacterial contamination during or after surgical procedure but only a small portion of it progress to clinical infection. The bacterial contamination lead to SSI depends on: the dose, the virulence of the bacteria and the resistance of the patient (Mangram *et al*., 1999). In low- and middle-income countries SSI is the most frequent type of health care-associated infection, in some settings, up to one third of patients who are operated develop SSI (Mengesha *et al*., 2014a).

Most surgical site infections are hospital acquired and vary from one hospital to the other and are associated with complications, increasing the morbidity and mortality (Isibor *et al.*, 2008). Patients with SSI are twice as likely to die, 60% more likely to spend time in an intensive care unit (ICU), and more than five times more likely to be readmitted to the hospital after discharge (Organization). The source of SSI may be endogenous or exogenous microorganisms related to both host and perioperative factors and the incidence of infection depends on different factors including types of wounds (Spagnolo *et al.*, 2013). Study confirmed that the rate of SSIs increases along the spectrum for clean (2.1%), clean-contaminated (3.3%), contaminated (6.4%), and dirty wound types (7.1%) (Taye, 2005b). The overall SSI rate in Ethiopia was reported to be in a range of 14.8-20.6% in general surgical wards at different teaching hospitals (Mengesha *et al*., 2014a, Godebo *et al*., 2013, Halawi *et al*., 2018). Most common pathogens associated with surgical wound infection are *Staphylococcus aureus, Escherichia coli, Klebsiella* species,

Proteus species, *Pseudomonas aeruginosa, Citrobacter* species, *Acinetobacter* species and Coagulase negative staphylococci (CoNS) (Godebo *et al*., 2013, Biadglegne *et al*., 2009).

From Gram positive not only *S. aureus* but also coagulase-negative Staphylococci (CoNS), which currently are defined as more than 40 species, are frequently associated with opportunistic human infections. *S. epidermidis* and *S. haemolyticus* are the major species of CoNS frequently isolated from clinical specimens (Martínez-Meléndez *et al*., 2015). Furthermore, *Mammaliicoccus sciuri* (previously called *S. sciuri*) is part of the normal flora of goats and camels, and it is a rare opportunistic pathogen in humans (Beims *et al.*, 2016). *S. aureus* possesses a unique set of virulence factors, including toxins, enzymes, and metallophores, which enable it to survive extreme conditions, promote tissue colonization, cause systemic infection, and evade the host's immunity (Cheung *et al*., 2021). By utilizing metallophores, this bacterium can sequester metal ions from its environment (Ghssein and Ezzeddine, 2022). *S. aureus* infections have previously been treated with beta-lactams, including penicillin and, later, methicillin, as well as sulfonamides, tetracyclines, and macrolides (David and Daum, 2017). However, antibiotic-resistant strains of *S. aureus* have developed due to repeated exposure to antibiotics, leading to an increase in methicillin-resistant *S. aureus* (MRSA) infections globally. MRSA is one of the cause of surgical site infections, and it is a prevalent bacterium that frequently colonizes hospital environments and causes hospital-acquired infections (David and Daum, 2017) and community-acquired infections (Hiramatsu *et al.*, 2014). Globally, the prevalence of vancomycin resistance *S. aureus* (VRSA) was 16% in Africa, 1% in Europe, 3% in South America, 4% in North America, and 5% in Asia (Wu *et al*., 2021). A systematic review and meta-analysis revealed a highly variable prevalence of VRSA and MRSA in Ethiopian *S. aureus* isolates. The MRSA prevalence ranged from 8.3% to 77.3% (with a pooled prevalence of 32.5%) (Eshetie *et al*., 2016). In the same way, the prevalence of VRSA range from 5.1% to 44.3% (Anagaw *et al.*, 2013). These days, MRSA is considered as a serious threat to public health, and it is one of the pathogens that need to be treated with high priority.

The Gram-negative bacteria (GNB), particularly ESBL and carbapenemase-producing Enterobacteriaceae*, Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, showed increasing drug-resistance pattern and mechanisms (Shaikh *et al*., 2015a). SSIs is currently a world-wide problem due to rapid spread of resistant microbes that affected the effectiveness of

antimicrobials (Biadglegne *et al*., 2009). In hospitals, approximately 30% to 50% of antibiotics are prescribed for surgical prophylaxis and 30% to 90% of these antibiotics are improperly used. Antimicrobial drugs, especially β-lactams, are frequently utilized inappropriately in prophylaxis (Mulu *et al*., 2012a, Munckhof, 2005).

The ESBL and Carbapenemase producing bacteria are serious threat to the currently available new generation β-lactam antibiotics and contributed to the increase of antimicrobial resistance throughout the world (Shaikh *et al.*, 2015b). The condition is serious in developing countries due to irrational prescriptions of antimicrobial agents and lack of periodically updated empirical therapy list (Fadeyi *et al*., 2008).

This irrational/inappropriate overuse of antimicrobials increases the selection pressure, favouring the emergence of drug-resistant bacteria, making the choice of empirical therapy more difficult and expensive, and this is poseing a serious threat to public health, thus increasing the global risk of SSI (Ranjan *et al*., 2011, Li and Webster, 2018).

Hence, the use of data from clinical laboratories' including antibiotic susceptibility testing (AST) or solid epidemiological data from ongoing nosocomial infection surveillance is needed to minimize the problem (Fadeyi et al., 2008). The phenotypic characterization facilitates the identification of isolated organisms, screening their resistance profiles and for selecting potentially useful therapeutic agents, but they are insensitive tools for tracing the spread of individual strains within a hospital or region using routine laboratory tests (Geleta *et al*., 2024). Thus, PCR and sequencing are the commonly used tools for specific detection, confirmation and tracking spread of resiatant isolates. However, the molecular epidemiology of bacterial isolates with their drug resistance gene profile is not well documented in Ethiopia, and published reports are scarce. Furthermore, the majority of earlier reports focus on the phenotypic characteristics of the isolates. Therefore, in this study, in addition to standard microbiology technques we used the Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) technique for the confirmation of bacterial isolates and multiplex polymerase chain reaction (PCR) and whole genome sequencing (WGS) for the detection of ESBL, carbapenemase, *mecA, femA*, *vanA,* and *vanB* genes. Because of time limitations, the results of WGS were not annonated and included in this thesis. However, the data will be analyzed and published.

1.2. Statement of the problem

Surgical site infection is the most common complication following surgery with an incidence of between 14%-17%. Even though standard protocols of preoperative preparation and antibiotic prophylaxis were properly practiced, the rate of surgical site infection is an increasing problem worldwide (Spagnolo *et al.*, 2013). It is also the third most frequently reported cases of nosocomial infections, accounting all hospital-acquired infections and 38% among that of surgical patients (Spagnolo *et al*., 2013). In developing countries, especially in Sub-Saharan Africa, this figure is twice or three times higher than in developed countries. In general, in Africa, including Ethiopia, surgical site infections are the most common causes of morbidity, mortality and increased the cost of the treatment (Biadglegne *et al*., 2009, Allegranzi *et al*., 2018).

Multidrug resistance (MDR), is a major problem of bacterial pathogens isolated from SSI in hospitals and is increasing yearly (Organization, 2014, Zahran *et al*., 2017), therefore the emergence of MDR is becoming a challenge for health personnel to treat hospital-acquired infections empirically (Godebo *et al*., 2013). In developing countries, including Ethiopia, published reports on molecular detections of bacterial pathogens and their antibiotics resistance patterns of frequently causing SSIs are relatively scarce (Iskandar *et al*., 2019) compared to the developed parts of the world. Besides, virtually all earlier reports depend on phenotypic laboratory methods to characterize pathogenic bacteria and studies were done at single sites with small sample sizes (Abayneh *et al*., 2022, Misha *et al*., 2021a, Bitew Kifilie *et al*., 2018a). A recent systematic review and meta-analyses study by Birhanu Y *et al*. (Birhanu and Endalamaw, 2020) focused on the pooled prevalence of SSI and its aetiology in Ethiopia. Accroding to the report *S. aureus* was the most frequent bacteria identified, and SSI had a high national prevalence (Shiferaw *et al.*, 2020a). Also, the study had limitations, the papers included used only phenotypic laboratory methods and the result did not display antimicrobial resistance (AMR) data. Thus, there have always been ambiguities in the interpretation of the findings when using phenotypic bacterial identification methods.

The paucity of data regarding the the phenotypic and molecular chacracterstics of bacteriae associated with SSI that guides interventions for treatment, control and prevention of SSIs in Ethiopia is higly needed. .

1.3. Significance of the study

SSI has huge impacts on patients' lives, economic burdens, health care finance, increases length of hospitalization, leads to emergence of antibiotic-resistant microorganisms, increases the costs of post-discharge care, all these will increase the morbidity and mortality associated (Brook et al., 2013). The rapid spread of antimicrobial resistance in bacterial populations has made the management and treatment of SSI a serious challenge in clinical and surgical practice. These is shown to be associated with emergence of multiple drug resistance pathogens that complicated the selection of empirical prophylaxis and treatment of SSI when it develops (Mulu et al., 2012a). In developing countries, including Ethiopia, published reports on molecular detections of bacterial pathogens and their antibiotics resistance patterns of frequently causing SSIs are relatively scarce. To the best of our knowledge, many studies done on surgical site infections are phenotypic (Abayneh *et al.,* 2022, Misha *et al*., 2021a, Bitew Kifilie *et al*., 2018a). Phenotypic analysis is still the cornerstone however, it time consuming and with low sensitivity usually requiring several days. In addition, accurate identification of the pathogen at species level may not be possible in all cases because some similarities in the biochemical profiles of the pathogens. Thus, to guide the selection of prophylactic antimicrobials used in surgical wards and management of SSI it is important to undertake molecular characterization of bacteria isolated from SSI in different sites. This study was therefore undertaken in as a part of national surveillance to guide the development of national and local guidelines for antimicrobial resistant pathogenic bacteria as well as their antibiotic treatment in Ethiopia. Having such data would help to establish guidelines for the management prevention and control of SSIs. It also can serve as base line data for health facilities for rational use of antibiotics for prophylaxis, and treatment.

2. CHAPTER 2: LITERATURE REVIEW

2.1. Magnitude of surgical wound infection

Surgical site infection is the most common complication following surgery; worldwide. Even though standard protocols of preoperative preparation and antibiotic prophylaxis were properly practiced, the rate of surgical site infection is an increasing problem worldwide *(Spagnolo et al.*, 2013). In 2002 CDC have estimated that 234.2 million major surgical procedures are undertaken every year worldwide; more than 27 million surgical procedures are performed annually in the US (Weiser *et al*., 2008). Approximately 300,000 SSIs are reported every year and cause approximately 8,000 patient deaths (Ata *et al.*, 2010, Magill *et al*., 2012).

A recent study in United States of America (USA) approximately 30% of patients undergoing surgery will develop post-operative surgical site infections. Worldwide it has an incidence of between 2- 20% suggesting that SSI was the most common reasons for hospital admissions after surgery and a cause of an unplanned readmission (Merkow *et al.*, 2015). In western countries, the incidence of such infections is 15–20% of all cases, with an incidence of 2–15% in general surgery (2004).

In developing countries, especially in Sub-Saharan Africa this figure is twice or three times higher than in developed countries due to lack of national infection prevention and control (IPC) policies, a lack of IPC personnel, poor adherence to existing hospital-acquired infections (HAI) prevention and guidelines (Mengesha *et al.*, 2014a, Abubakar *et al*., 2022). For instance, the pooled prevalence of HAIs in Africa was 12.76% and SSI was the most common HAIs and accounted for 41.6% of all HAIs, followed by bloodstream infection and respiratory tract infections. The prevalence of HAIs differs between wards with the highest rate found in the ICU (25.2%–100%), followed by neonatal ICU/ward (7.0%–53.6%) and paediatric medical ward (2.7%–33.0%) (Abubakar *et al*., 2022). Many factors, including procedures, hospitals, surgeons, patients, and geographic regions, influence the prevalence and incidence rates of wound infections following surgery (Ameh *et al*., 2009). A study investigating SSI impact and risk factors in Sub-Saharan African countries was performed. The impact of SSI ranged from 6.8% to 26% with predominance in general surgery. The pooled incidence of SSI was 14.8% with significant heterogeneity according to the specialty and the method of nursing care. Most risk

factors were long duration of procedure and wound contamination class. Other factors included hospital environment, inadequate care practices and underlying disease (Ngah *et al*., 2016). A study conducted in Egypt among women delivered by caesarean section showed the incidence of SSI post-caesarean section was 5.34% (Gomaa *et al*., 2021).

A prospective study of SSI after caesarean section was conducted from January 2014 to December 2016 by the methodology of the American National Nosocomial Infection Surveillance System in Kuwait. The prevalence of SSI was 2.1%, and of 152 cases of SSI, the prevalence of infection was 46.7% in women ⩽30 years and 53.3% in women >30 years (P = 0.119). The emergency caesarean section and inappropriate antibiotic prophylaxis are risk factors for developing SSI (Alfouzan *et al*., 2019).

The SSI report from Tanzania and Sudan showed incidence of 19.4% and 25.2% respectively (Barie, 2002, Ahmed, 2012). A cross sectional study carried in Khartoum Teaching Hospital showed that, out of the total patients 10.9% with wound infection, the rate post-operative SSI was 8.9% and 15.4% for clean and clean-contaminated procedures respectively (Elbur *et al*., 2013). In another study in Southwestern Uganda, overall SSI incidence was 16.4%, superficial 5.9%, deep and organ space SSIs each 47% (Lubega *et al.*, 2017b). A prospective study of surgical wound infection has been conducted on 1754 surgical patients in Tikur Anbessa hospital. Seven hundred twenty-eight (41.5%) wounds were classified as clean, 674 (38.4%), clean contaminated, 241 (13.7%), contaminated and 111 (6.3%) dirty and infected wounds. The total rate of wound infection was 14.8%. For clean, clean-contaminated, contaminated, and unclean and infected wounds, the infection rates were, 8.0%, 14.8%, 22.0%, and 44.2% respectively. Surgery performed by consultant surgeons' overall wound infection rates range from 5.4% to 27.9%, while resident rates were 17.3% (Taye, 2005a).

In general, in Africa including Ethiopia, surgical site infections are the most common causes of morbidity, mortality and increase healthcare costs (Biadglegne *et al*., 2009, Allegranzi *et al*., 2018). In prospective cross-sectional study conducted on surgical wound infection among surgical patients at Tikur Anbessa Hospital, the infection rate was 10.3%, 13.6%, 22.7% and 36.8% for clean, clean-contaminated, contaminated and dirty wounds, respectively (Tekie, 2008). A cross-sectional study was conducted on surgical site infection patients at Felege Hiwot

Referral Hospital Bahirdar, Ethiopia. Of the 294 patients who underwent clean and clean-contaminated operations, 10.9% experienced were verified bacterial infection. The rate of nosocomial infections among clean and clean-contaminated operations was 3.3% and 12.8% respectively. Nosocomial surgical site and blood stream infection rate was 10.2% and 2.4% respectively (Mulu *et al.*, 2012a).

In a prospective study conducted in Ethiopia among patients with abdominal surgical wounds, pathogenic bacteria were isolated in 38.7% of patients but on clinical grounds alone wound infection rate was 21% (Laloto *et al*., 2017). This result emphasises the necessity of using laboratory methods to confirm the diagnosis of wounds that may be infected. There was no discernible difference in the infection incidence between emergency and elective procedures. Wound infection was significantly associated with the class of wound, with the highest prevalence being 64.1% for contaminated and dirty wounds (Laloto *et al*., 2017).

SSIs are classified as either incisional or organ/space infections based on the involved tissues or organs. Incisional infections are further classified as superficial or deep and differ in clinical severity (2014.). Over half of all SSI across all surgical categories were superficial incisional infections, which were more prevalent than deep incisional and organ/space infections.

Deep incisional and organ/space SSI shared a greater extra length of hospital stay and cost than superficial incisional SSI for all categories of surgery, except limb amputation (Vincent *et al.*, 2004). Currently, their prevalence has been used as a marker for the quality of surgeons and hospitals (Coello *et al*., 2005, Vincent *et al.*, 2004).

2.2. Microbiology of surgical site infections

Most of the time, the typical SSI bacteria are aerobic and facultatively anaerobic. The predominant were *Enterobactriaceae* such as *E. coli, Klebsiella, Enterobacter, Proteus species, followed by P. aeruginosa, Acinetobacter spp,* and Gram-positive bacteria like *S. aureus*, *Enterococcus* spp., coagulase negative Staphylococcus spp. and non-hemolytic streptococci. Most SSIs specimens were poly-microbial (Akhi *et al.*, 2015).

A study conducted on bacterial profile, antibacterial resistance pattern, and associated factors from women attending postnatal health service at University of Gondar Teaching Hospital showed that the bacterial growth was 90 (84.1%) out of 107 suspected SSI patients. *S. aureus* (41.6%), *E. coli* (19.8%), *K. pneumoniae* (13.9%), coagulase negative staphylococcus (12.9%), and *Entrobacter spp*. (4%) were the predominant pathogens (Bitew Kifilie *et al*., 2018b).

A similar study conducted in Mekelle out of the 128 wound swabs taken, 96/128 (75%) were culture positive aerobically, yielding 123 bacterial isolates. The predominant bacterial isolates were *S. aureus* 44 (35.77%), *Klebsiella* species 29 (22.76%) and coagulase negative staphylococci (CoNS) 18 (14.63%). No bacterial isolates were found to be sensitive to all antibiotics tested (Mengesha *et al.*, 2014a).

In a study done in Nepal, about 65% of the clinical specimens were positive for bacterial growth, and Gram-positive bacteria were (57.4%) the leading pathogens among pyogenic wound infections. *S. aureus* (412, 49.28%), *E. coli* (136, 16.27%), *Klebsiella* spp. (88, 10.53%), and *Pseudomonas* spp. (44, 5.26%) were isolated. Drug resistance was shown to be high in both Gram-positive (51.9%) and Gram-negative (48.7%) bacteria (Rijal *et al.*, 2017).

A study conducted in India on evaluation of microbiological profile and antibiogram of aerobic bacteria isolated from pus samples reported that twenty-one different bacterial isolates were obtained from one hundred pus samples. The most common bacteria were *S. aureus* (28.5%), which was followed by coagulase-negative Staphylococci (23.8%). The results of the antibiotics susceptibility testing illustrated that a majority of the isolated organisms were MDR. *S. aureus* showed highest sensitivity to antibiotics like linezolid (83.3%) and teicoplanin (50%). Among the five isolated strains of coagulase negative staphylococci (CONS), three of them were MDR and the other two showed sensitivity to antibiotics cefoperazone, co-trimoxazole and ticarcillin/clavulanic acid (20%) (Kumari *et al*., 2018).

A study was performed at Rafidia Hospital-Nablus Palestineon, between February and April 2016 on detection of bacterial pathogens in surgical site infections and their antibiotic sensitivity profile. According to the findings, there were 56.7%, 30%, 6.7%, 3.3%, and 3.3% of pathogens among surgical site infections for *E. coli, S. aureus*, *Klebsiella spp., Enterobacter spp.,* and *Acinatobacter spp.,* respectively (Adwan *et al.*, 2016).

Results from a study conducted at a tertiary hospital in Dare Selam, in Tanzania showed that the most frequently isolated pathogen was *P. aeruginosa* from post-operative wound infections. Eighty eight percent (88%) of the Gram-negative bacteria isolated were MDR, but all were sensitive to carbapenems. ESBLs production was detected in 92.3% of *E. coli* and 69% of *K. pneumoniae*. Forty four percent (44%) of the 18 *S. aureus* isolates obtained were MRSA (Manyahi, 2012).

A study was done on antimicrobial susceptibility pattern of bacterial isolates from pus samples at Kenyatta National Hospital, Kenya. *S. aureus* was the most frequent isolate (29.9%), followed by *Pseudomonas* spp. (13.7%), *E. coli* (12%), *Proteus* spp. (9.7%), *Klebsiella* spp. (7.5%), *Acinetobacter spp* (7.1%), *Citrobacter* (6%), *Enterococcus* (4.6%), *Enterobacter* (4.4%), CONS (3.9%), *S. pyogenes* (0.8%), *S. agalactiae* (0.2%) and *S. viridans* (0.2%) (Ratemo, 2014).

A prospective cohort study performed on the incidence and predictors of surgical site infection in Ethiopia showed that out of 129 abdominal surgical wounds from 129 patients, fifty (38.7%) showed growth on culture. *S. aureus* and *E. coli* were the leading aetiologic agents with rates of 28.8% and 27.1% of pathogenic isolates respectively (Laloto *et al.*, 2017). A hospital based cross-sectional study done on aerobic bacterial isolates from post-surgical wound and their antimicrobial susceptibility pattern in Hawassa showed that the isolation rate of aerobic bacteria was 138 (71.1%). *S. aureus* was the most frequent isolates (37.3%); followed by *E.coli* (25.4%) (Dessalegn *et al*., 2014b).

A similar study conducted on antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University Specialized Hospital in Jimma. In this investigation, 145 bacterial isolates with an isolation rate of 87.3% were found among 150 specimens. The predominant bacteria isolated from infected wounds were *S. aureus* 47 (32.4%) followed by *E. coli* 29 (20%), *Proteus* species 23 (16%), coagulase negative staphylococci 21 (14.5%), *K. pneumoniae* 14 (10%) and *P. aeruginosa* 11 (8%) (Mama *et al*., 2014).

Another prospective study on multidrug-resistant bacterial isolates from patients suspected for SSIs at Tikur Anbessa Hospital Addis Ababa, Ethiopia was done. The study showed that the

overall bacterial prevalence was 75.6% (n=149/197) and predominant bacterial isolates were *S. aureus* 33.3% (n=56/168) and *E. coli* 14.3% (n=24/168) (Feleke *et al*., 2018). In similar study in Tikur Anbessa Hospital on surgical wound infection among surgical patients operated from April to July 2006. *P. aeruginosa* was the third dominant isolates following *S. aureus* and coagulase negative staphylococci respectively (Tekie, 2008).

A hospital-based prospective study conducted on pattern of bacterial pathogens and their susceptibility isolated from surgical site infections at selected referral hospitals, in Addis Ababa, Ethiopia. From a total of 107 swabs collected, 84.1% were culture positive. Gram negative bacteria were 73.1%. *E. coli* 23.1% was the most common organism isolated followed by multidrug resistant Acinetobacter species 22.1%.

Similarly, a cross-sectional study was conducted on surgical site infection patients at Felege Hiwot Referral Hospital Bahirdar, Ethiopia. Out of the 42 bacterial pathogens that were detected, *S. aureus* accounted for the majority of isolates with 26.2%, followed by *E. coli* and coagulase negative staphylococcus species each 21.4% (Mulu *et al*., 2012a).

2.3. Pathogenesis and clinical manifestation of surgical site infection

A precondition SSIs is bacterial contamination. A number of parameters, including the pathogens' dosage and virulence as well as the host's defence mechanisms, will determine the likelihood of SSI development after exposure (Seidelman and Anderson, 2021).

Most of the source of SSIs pathogens is the endogenous flora of the patients during surgery. Pathogens that causes SSI are related to both host and perioperative factors, acquired either endogenously from the patients own flora present within the patients body exposed during surgery or exogenously sources of pathogens may be procedure-related from surgical instruments, operating room surfaces or the environment, the air, and personnel (Spagnolo *et al*., 2013).

SSI depends on inoculum size of the bacteria; the risk of SSIs increases if the surgical site is contaminated with more than 10^5 organisms per gram of tissue (Krizek and Robson, 1975). The sites which are heavily colonized with bacteria such as the bowel and the female genital tract

(10^6-10^7bacteria/ml) are at higher risk of developing SSI as large inoculums of bacteria may invade the wound during operation. However, the dose of contaminating microorganisms required to produce infection may be much lower when necrotic tissue, dead space and foreign materials are present at the site i.e., 10^2 staphylococci per gram of tissue introduced on silk sutures (Krizek and Robson, 1975).

Pore-forming toxins (PFTs) are the most common bacterial cytotoxic proteins and are required for virulence in a large number of important pathogens including *S. aureus, C. perfringens,* and *S. pyogenes.* The more virulent the bacterial contaminant, the greater the probability of infection, and production of toxins and other substances may also act at the site of colonization and play a role in invasion (Los *et al*., 2013). Endotoxin is produced by many Gram-negative bacteria and induces the release of cytokines. In turn, cytokines can trigger the systemic inflammatory response syndrome that sometimes leads to multiple system organ failure (Wilson *et al*., 2002, Opal, 2010).

Some bacterial surface components, particularly polysaccharide capsules, inhibit phagocytic clearance, which effectively masks the bacterial surface from recognition by phagocytic cells, a critical and early host defense response to microbial contamination (Opal, 2010). Certain strains of clostridia and streptococci produce potent exotoxins that disrupt cell membranes or alter cellular metabolism (Los *et al*., 2013). A variety of microorganisms, including Gram-positive bacteria such as coagulase-negative staphylococci, produce glycocalyx and an associated component called slime, which physically shields bacteria from phagocytes and resistance to antibiotics (Donlan and Costerton, 2002).

Surgical site infection can occur from first day onwards after an operation but usually happens between the fifth and tenth days after surgery. It may originate during the operation i.e. as a primary wound infection or may occur after the operation from sources in the ward or as a result of some complications. The infection can be characterized by pain, redness, oedema, tenderness, gaping, abscess or purulent discharge, occurrence of fever (> 38°C), or positive culture of fluid or tissue from the surgical site within 30 days of the operation (Pradhan and Agrawal, 2009).

2.4. Risk factors for surgical site colonization and infection

Study on surgical outcomes has mainly focused on the role of patient pathophysiological risk factors and on the skills of the individual surgeon but also dependent on the quality of care received throughout the patients stay in hospital and the performance of a considerable number of health professionals, all of whom are influenced by the environment in which they work (Vincent *et al*., 2004).

The risk of wound infection can be microbe-related risk factors, with *S. aureus* and *S. pyogenes* being particularly virulent; host-related risk factors**,** with obesity, an index of disease severity, old age, protein-calorie malnutrition, and probably diabetes, cancer, and systemic infection; and operation-related risk factors**,** including an extended hospital stay prior to surgery, the length of the procedure, tissue damage, inadequate haemostasis, and foreign material in the wound the last of which significantly raises the risk of a major infection, despite a relatively small bacterial inoculum (Howard and Lee, 1995).

Prolonged antibiotic prophylaxis and overuse of antibiotics are significantly associated with increased risk of antibiotics resistance (Llor and Bjerrum, 2014). The rate of wound infection was high and prophylactic antibiotics were irrationally used (Elbur *et al.*, 2013). In addition, prolonged operative time can increase the risk of exogenous contamination, one study showed that the likelihood of developing SSI increased by 24% for each additional hour of operation (Cheng *et al*., 2017).

2.5. Antimicrobial resistance and plasmid profile

The challenges to control the surgical site infection are lack of standardized criteria for diagnosis of infection and the emerging of antimicrobial resistance (AMR) among bacterial pathogens (Ranjan *et al*., 2011, Li and Webster, 2018). Antimicrobial resistance is a worldwide public health problem and a natural phenomenon, which has accelerated by the misuse and overuse of antibiotics in humans, animals and plants and affect all region and income levels globally, more affected with low-and middle-income countries (EclinicalMedicine, 2021). Increased antimicrobial resistance is the cause of severe infections, complications, longer hospital stays, increased mortality. Lack of rational use of antibiotics is associated with an increased risk of

adverse effects, and increased medicalization of self-limiting conditions (Llor and Bjerrum, 2014).

Knowing antibiotic resistance pattern and molecular characterization of plasmids is epidemiologically useful. Plasmid or (extrachromosomal DNA molecules) encode a range of genes for a variety of nonessential functions, including drug resistance and virulence factors (Fang *et al*., 2008). The prevalence of antimicrobial resistance varies greatly between different pathogens and also antimicrobial resistance pattern of bacteria isolates keep changing and evolving with time and place resulting in an increased morbidity, mortality and increase health-care costs (Isibor *et al.*, 2008). This may be due to the emergence of resistance pathogens to empirically prescribed antimicrobial agents and the increased numbers of surgical patients who are elderly and have a wide variety of chronic, debilitating, or immune-compromising underlying diseases. The un-updated empirical mode of treatment leads to a selection pressure for pathogens to develop antimicrobial resistance, which may result into non-healing of the surgical site infections and making the patient even more ill and occasionally causes death (Brook *et al*., 2013, Mulu *et al*., 2012a). In a study done in India, on isolation and identification of bacterial pathogens from wound of diabetic patients, 100% vancomycin resistance *S. aureus* was isolated. In this study *S. aureus* only showed sensitivity to gentamycin and tetracycline (Daniel *et al.*, 2013).

In a study performed in Kenya, Gram positive isolates from surgical wound infection were susceptible to vancomycin, levofloxacin, linezolid and teicoplanin. A majority Gram-negative isolates were most susceptible to levofloxacin, amikacin, imipenem, and meropenem. The majority of Gram-negative isolate resistance to cephalosporins, ampicillin, augmentin, cotrimoxazole, and doxycycline was observed (Ratemo, 2014).

A study finding in Palastine reported that *E. coli* isolates were resistant against Nalidixic acid (88.2%), trimethoprim/sulfamethoxazole (76.5%), tetracycline (70.6%), norfloxacin (64.7%), and ciprofloxacin (58.5%). *S. aureus* showed high resistance against Nalidixic acid (88.9%), Norfloxacin (77.8%), Amoxycillin/clavulanic acid (77.8%), Kanamycin (66.7%) and Ciprofloxacin (55.6%). Methicillin resistant *S. aureus* (MRSA) accounted for 33.3% of *S. aureus*

isolates. Resistances to 3 or more antibiotics were detected in 94.1% (16/17) and 77.8% (7/9) of *E. coli* and *S. aureus* isolates, respectively (Adwan *et al*., 2016).

A study was performed at Rafidia Hospital-Nablus on prevalence and molecular characterization of β-lactamases among pathogens isolated from surgical site infections. ß-lactamase genes were found in 83.3% of cases. There were 83.3%, 23.3, and 13.3% prevalences of ESBLs, MBLs, and AmpC ß-lactamases, respectively. The most prevalent gene was *blaTEM* (100%). The prevalence of AmpC genes was 11.8% for *E. coli*, 11.1% for *S. aureus*, 100% for Enterobacter and 0% for both *Klebsiellsa* spp. and *Acinatobacter* spp. MBL genes were detected only in 41.2% of *E. coli* isolates. The most prevalent gene among MBL producer *E. coli* was bla_{SPM} (85.7%). In addition, 30% of the tested isolates harbor at least another type of ß-lactamases (Adwan *et al*., 2016). On the other hand, a study condacted in Gaza, Acinetobacter isolate showed nearly complete resistance to cephalosporins (cefuroxime 98.2%, cefotaxime 93.2%, ceftriaxone 93.3%, ceftazidime 87.5%, and gentamicin 81.3%), whereas lower rates of resistance were shown in ciprofloxacin 69.7% and amikacin 68.3%. Doxycycline was the most effective antimicrobial medication, having the lowest risk of resistance (22.1%) (Elmanama, 2006).

A study performed in Tanzania showed that most of the Gram-negative bacteria isolated were multiple resistant to the antimicrobial agents tested, but all were sensitive to carbapenems, and eighty-eight percent (88%) of enteric Gram-negative rods were multi-drug resistant. ESBL production was detected in 92.3% of *E. coli* and 69% of *K. pneumoniae*. Forty-four percent (44%) of the 18 *S. aureus* isolates obtained were MRSA (Manyahi, 2012).

A study performed in Hawassa reported that single and multiple antimicrobial resistances were observed in 6.8% and 93.2 % of the isolates, respectively. No bacterial isolate was found to be sensitive to all antibiotics tested (Dessalegn *et al*., 2014b).

In a study carried out in Mekelle, *S. aureus* isolates showed the highest resistance to penicillin (100%), ampicillin (95.5%), ceftriaxone (81.8%), and vancomycin (65.2%), while the least resistance was exhibited to amoxicillin/clavulanic acid (30.3%). Klebsiella spp. was resistant to gentamicin (100%), chloramphenicol (87.5%), ceftriaxone (87.5%), and ciprofloxacin (62.5%). *E. coli was* resistant to ampicillin (100%), gentamicin (46.7%), chloramphenicol (40%),

ceftriaxone (40%), and ciprofloxacin (40%). Proteus spp. was resistant to ampicillin (100%), chloramphenicol (66.7%), gentamicin (33.3%), and ceftriaxone (33.3%). Pseudomonas spp. was resistant to gentamicin (50%), chloramphenicol (100%), amoxicillin/clavulanic acid (100%), ampicillin (100%), and ceftriaxone (100%). All proteus and pseudomonas species were susceptible to ciprofloxacin. Isolates of CoNS showed 100% resistance to vancomycin, ceftriaxone, ampicillin, and penicillin but were sensitive to chloramphenicol. Single and multiple antimicrobial resistance was observed in 6.8% and 93.2% of the isolates, respectively (Dessalegn *et al.*, 2014b).

A study conducted in Jimma, Ethiopia, on the prevalence and antibiotics susceptibility pattern of CTX-M type extended–spectrum beta-lactamases among clinical isolates of Gram-negative bacilli from the total ESBL gene-positive isolates, 95.8% carried $bla_{CTX\text{-}M}$ genes, with $bla_{CTX\text{-}M\text{-}15}$ being predominant (97.1% of CTX-M genes). The *blaCTX-M*-carrying *Enterobacteriaceae* (*n* = 64) isolates showed no resistance against imipenem and meropenem and a moderate resistance rate against tigecycline (14.1%), fosfomycin (10.9%), and amikacin (1.6%), suggesting the effectiveness of these antibiotics against most isolates. On the other hand, all the *blaCTX-M*-postive *Enterobacteriaceae* showed a multidrug-resistant (MDR) phenotype with remarkable co-resistances (non-susceptibility rates) to aminoglycosides (92.2%), fluoroquinolones (78.1%), and trimethoprim/sulfamethoxzole (92.2%) (Zeynudin et al., 2018a).

A study was performed at Tikur Anbessa on surgical wound infection among surgical patients. The minimum resistance rate was 25% for amikacin and imipenem and the highest resistance rate obtained was for chloramphenicol which was 100%. 87% of the isolates of *P. aeruginosa* were multidrug resistant (Tekie, 2008). A similar study done at Tikur Anbessa showed that all bacterial isolates the multidrug resistance level was 65.5%. The MDR levels of Gram-positive and Gram-negative bacteria were 55.3% and 73.9%, respectively. The overall MDR rate was 70.4% (Taye, 2005a). A study conducted in Addis Ababa referral hospitals reported that more than 75% of the Gram-negative isolates showed multiple antibiotic resistances. Pan-antibiotic resistance was noted among 34.8% *Acinetobacter species* and 12.5% *E. coli* (Dessie *et al*., 2016).

All isolates in post-operative wound samples showed a high frequency of resistance to ampicillin, penicillin, and tetracycline but were susceptible to gentamicin, norfloxacin,

ciprofloxacin, vancomycin, ceftriaxone and amikacin. The overall multiple drug resistance patterns were found to be 82.9% & 85% (Mama *et al*., 2014, Bitew Kifilie *et al*., 2018b, Mengesha *et al*., 2014a). Another study in Gondar, showed that nearly 100% of Gram-positive and 95.5% of Gram-negative bacterial isolates showed resistance against two or more antimicrobial drugs (Mulu *et al*., 2012a). It is obvious that pathogens will continue to develop resistance to different classes of antimicrobial agents through intrinsic and acquired mechanism (figure 3.1) (Petchiappan and Chatterji, 2017). Among Gram-negative pathogenic bacteria the most common mechanism resulting in resistance to beta-lactam antibiotics is the production of enzymes like β-lactamases (Shaikh *et al*., 2015b). The association of ESBLs and the detection of *TEM, SHV, OXA* and *CTX-M-type* enzymes have been investgated in many studies (Lina *et al*., 2014, Ghenea *et al*., 2022).

These extended-spectrum β-lactamases (ESBLs) are usually plasmid-encoded, located on different transferable genetic elements, and have the capacity to hydrolyze many antibiotics of other classes (sulfonamides, aminoglycosides, cephalosporins, and quinolones) which complicates the treatment of many hospitalized patients (Fang *et al*., 2008). According to their amino acid sequence homology ESBLs are classified into several groups. The most important β-lactamases are cephalosporinases like (AmpC), ESBL and the carbapenemases like metallo-β-lactamases (MBLs) (Shaikh *et al*., 2015b).

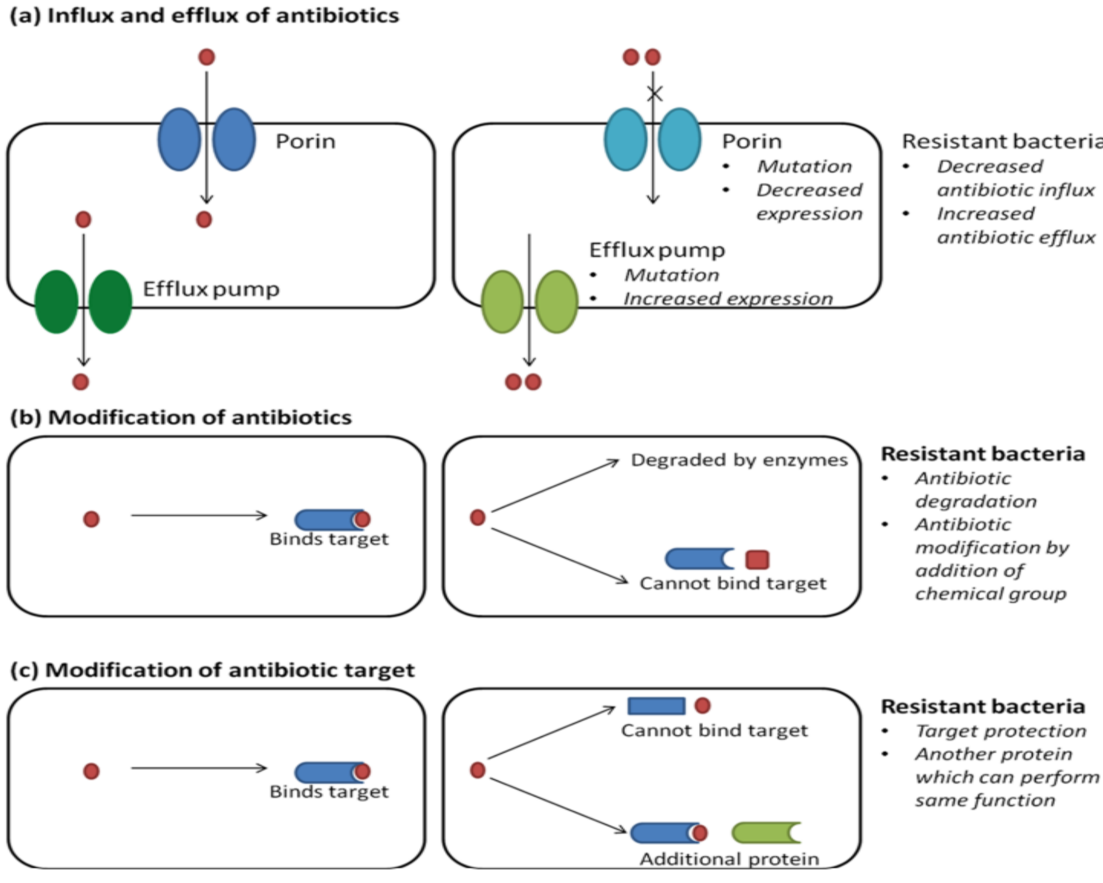


Figure 3.1. Mechanisms of antibiotics resistance

There are three main ways by which bacteria can increase their resistance: a) varying the efflux of antibiotics; b) modifying the antibiotics; or c) modifying their targets (Petchiappan and Chatterji, 2017).

2.6. Research questions

- Is the rate of SSI same across the different study sites? What type of bacteria is responsible for SSI in the different sites?

What is the role of MDR bacteria in SSI in the study sites? Do the bacteria involved share similar AMR pattern across different sites?

2.7. Study Hypothesis

- ❖ The rate of surgical site infection is similar across the different study sites
- ❖ The bacteria associated with SSI share simailr AMR profile across the different sites due to high levels of resistant bacteria is increasing.
- ❖ The emergence and spread ESBLs and carbapenemase genes is increasing encoded among bacteria isolated from different sites

2.8. Objectives of the study

General objective

To determine the common bacterial etiologies and characterise antibiotic resistance phenotypes and genotypes in bacterial isolates causing surgical site infection in selected University Teaching Hospitals in Ethiopia

Specific objectives

- ➢ To determine bacterial pathogens with surgical site infections
- ➢ To determine antibiotic resistance pattern of bacterial pathogens isolated from patients with surgical site infections
- ➢ To detect antimicrobial resistance t genes from bacterial patogens isolated from patients with surgical site infections.
- ➢ To assess associated risk factors associated with development of SSI.

3. CHAPTER 3: MATERIALS AND METHODS

3.1. Study design and period

A hospital based cross-sectional multicenter study was conducted between July 2020 and August 2021.

3.2. Study area

The study was conducted at four selected sites in Northern, Central, Southern and Southwest Ethiopia. The study was conducted in the four purposively selected University Teaching Hospitals in Ethiopia, namely, Debre Tabor Comprehensive Specialized Hospital (DTCSH), Hawassa University Comprehensive Specialized Hospital (HUCSH), Jimma University Medical Center (JUMC), and Tikur Anbessa Specialized Hospital (TASH) (Figure. 3.2).

DTCSH is comprehensive specialized hospital in North Ethiopia that provides health services to over 5 million people located in Debre Tabor town of South Gondar Administrative Zone, Amhara Regional State. The hospital has over 400 beds and provides surgical, paediatrics, emergency, maternity, gynaecologic/obstetric, andother services. The hospital has clinical microbiology laboratory. In addition, the hospital serves as a teaching center.

HUCSH is a hospital with 400 beds in Hawassa City Southern Ethiopia and provides high quality service at both outpatient and inpatient level for about 20 million populations of the southern regions of the country. The HUCSH also serves as a training center for undergraduate and postgraduate medical students and Health Science trainees. The hospital provides surgical, paediatrics, emergency, maternity, gynaecologic/obstetric and other services. The hospital has clinical microbiology laboratory.

JUMC is one of the oldest public hospitals in the country located in the Oromia region, Jimma zone, Jimma City Southwest of Ethiopia with 800 bed capacities and a catchment population of over 15 million people. The hospital provides surgical, paediatrics, emergency, maternity, gynaecologic/obstetric and other services. The hospital has clinical microbiology laboratory.

TASH is the teaching hospital of Addis Ababa University located in Addis Ababa, the capital of Ethiopia and the largest specialized hospital in Ethiopia, with over 700 beds. It is also a facility where the entire country can receive specialised clinical services that are unavailable in other public or private facilities.

The TASH has 200 doctors, 379 nurses and 115 other health professionals dedicated to providing health care services. Patients are cared for in the hospital by the many departments, faculty members, and residents in the School of Medicine who are undergoing specialised training.

In their outpatient and inpatient units, the hospital offers a variety of surgery services. The hospitals have ISO 15189:2022 accredited microbiology laboratories that performs culture and antimicrobial sensitivity testing.

While three hospitals had established microbiology laboratories the DTCSH had started performing bacteriological culture and antimicrobial susceptibility testing at the time of this study. Therefore, with the help of Armauer Hansen Research Institute (AHRI), DTCSH and my home institutions Debre Tabor University we established a bacteriology laboratory, which was used for wound culture processing and antimicrobial susceptibility testing

Figure 3.2. Map of the geographic regions of the four referral hospitals selected for this study in Ethiopia adopted from P. Wight, 2020 (Wight, 2020).

3.3. Population

3.3.1. Source population

The source populations were patients who have visited and/or admitted in the four selected hospitals during the study period.

3.3.2. Study population

The study populations were patients who had surgery and clinically suspected for surgical site infection.

3.3.3. Inclusion and Exclusion criteria

- Inclusion criteria

All surgical patients, irrespective of age, operated during the study period and who developed sign and symptoms of surgical site infection with in 30 days or within 1 year if there is implant and who gave consent and/or assent to participate in the study.

- Exclusion criteria

All patients, who develop surgical site infections later than 30 days after the operation, infected burn wounds, and patients with infection of an episiotomy

3.4. Variables

3.4.1. Dependent variables

Magnitude of bacterial isolates, antimicrobial resistance pattern, resistance gene determination and risk factor associations

3.4.2. Independent variables

Age, sex, residence, current drug use such as steroid, smoking, length of preoperative hospital stays, duration of operation, timing and type of antimicrobial prophylaxis, presenting complains, history of previous use of antibiotics within one month, type and nature of surgery, surgical procedure performed and type of surgical site criteria.

3.5. Sample size determination and sampling technique

3.5.1. Patient recruitment and sample size calculation

A total of 752 clinically diagnosed cases of SSI from different wards were enrolled in the study. The sample size was calculated based on a single proportion sample size estimation formula ($n= \underline{Z^2 P (1 - P)} /d^2$) using a proportion of 20% (Mengesha et al., 2014b). As this was a multicentre study, to increasing the sample size a precision (d) of 0.03 was used, where Z stands for Z statistic with a level of confidence of 95%, and the Z value of 1.96. With a 10% non-response

rate, the total sample size came to 752. A convenient sampling technique was used to recruit study participants until the required sample size was achieved, and proportional allocation was made among different hospitals based on the patient flow across the four study sites.

Accordingly, a total of 905/752 patients were recruited and 172, 184, 193, and 203 sample was collected respectively from DTCSH, HUCSH, JUMC, and TASH

Sampling technique

Samples were collected from all patients who were clinically susupected for surgical site infection during the study period (wound swab) following standard operating procedure for sample collection.

3.6. Data collection

Professional nurses with experience in wound swab sample collection and microbiologists working in the bacteriology laboratory were data collectors. Data collectors were trained on the data collection tool and the socio-demographic and clinical data was collected using a pre-tested data collection form. Data collectors reviewed patient medical records used to extract additional patient data. The entire wound swab sample was transportted with modified Stuart's Transport Medium to bacteriology laboratories following standard protocols within 1hrs. The principal investigator periodically reviewed the collected data sheets for completeness and laboratories for following SOPs.

3.6.1. Sample collection, handling and transportation

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. Pus, pus aspirates, and wound swabs were collected aseptically with a sterile syringe with a needle or with sterile cotton-tipped swabs by trained personnel after cleaning around the infected site with 10% povidone iodine without contaminating with skin commensals, and the the wound clean with normal saline then all wound swabs were dipped into modified

Stuart's Transport Medium and immediately transported to the bacteriology laboratory for culture and drug susceptibility testing within 1 hour.

3.6.2. Bacteria identification

For aerobic bacterial isolation, the swab was used to make Gram stain smears and inoculated directly on blood agar plate (BAP) (Oxoid Ltd, UK), bile esculine agar, manitol salt agar (MSA) and MacConkey agar (McA). The blood and MacConkey agar plates were incubated aerobically at 37°C for 24-48 hours. All positive cultures were identified by their colony characteristic appearance on their respective media, Gram-staining reaction and confirmed by the pattern of biochemical reactions using the standard method (Cheesbrough, 2006). Members of the family *Enterobacteriaceae* were identified by indole production, H_2S production, citrate utilization, motility test, urease test, oxidase, carbohydrate utilization tests, Triple sugar iron (TSI), Lysine decarboxylase (LDC) and malonate. *Klebsiella ozaenae* was identified from *Klebsiella pneumoniae* using malonate biochemical test. *P. aeruginosa* was differentiated from other *Pseudomonas species* using *P. aeruginosa* Screen 80 tablet (Rosco, DK- 2630) cetrimied *P. aeruginosa* selective media.

For Gram-positive bacteria catalase, coagulase, DNase, a bacitracin and optochin susceptibility test was used, and bile esculin hydrolysis was used for *Enterococcus* spp. identification, (Figure 3.5).

MALDI-TOF MS analysis

All bacteria species identity was confirmed using MALDI-TOF MS at the Clinical Microbiology Department of Uppsala University Hospital Uppsala, Sweden. From fresh cultures, a single colony of bacteria was smeared onto a MALDI-TOF plate, and the sample was air-dried. Next, 1 µl of formic acid was added to each cell and air-dried, and then 1 µl of MALDI matrix solution was applied to the cells and air-dried before reading. MALDI-TOF identification was automatically scored by the system software between 1 and 3 points. All isolates with scores two and above were accepted, and all results below 1.7 and flagged red were rejected. Samples with scores 1.7–2 and flagged yellow were re-analysed.

3.6.3. Antimicrobial susceptibility testing:

The antibiotics susceptibility tests were performed on Muller-Hinton agar (Oxoid) by using the Kirby-Bauer disk diffusion technique according to CLSI 2021). Briefly using a sterile wire loop, 3-5 pure colonies were transferred to a tube containing 5 mL of sterile normal saline (0.85% NaCl) and gently mixed Standard inoculum density was adjusted to 0.5 McFarland units. The excess broth suspension was removed by tapping against the tube wall. The bacterial suspension was swabbed on the MHA surface using a sterile swab, and then antibiotic discs were placed with sterile forceps at least 24 mm apart from one another ((CLSI), 2021). All antibiotics disks were OXOID products (Oxoid Ltd, UK), and susceptibility of Gram-negative isolates was tested against: ampicillin (10μg), gentamicin (10μg), amikacin (30μg), ciprofloxacin (5μg), chloramphenicol (30μg), ceftazidime (30μg), cefotaxime (30μg), ceftriaxone (30μg), cefuroxime (30μg), cefepime (30μg), tetracycline (30μg), amoxycillin+Clavulanate (20/10μg), Trimethoprim-sulfamethoxazole (1.25/23.75μg), ampicillin-sulbactam (10/10μg), aztreonam (30 μg), meropenem (10μg), Imipenem (10μg), ertapenem (30μg). Gram-positive isolates were tested against penicillin (10units), ampicillin (10μg), vancomycin (30μg), erythromycin (15μg), ciprofloxacin (5μg), cefoxitin (30μg), clindamycin (30μg), erythromycin (15μg), doxycycline (30μg), chloramphenicol (30μg), gentamicin (10μg), and oxacillin (5 μg), tetracycline (30μg), ((CLSI), 2021). Following that, the plates were incubated at 37°C for 18-24 hours. Each zone of inhibition was measured to the nearest millimeter, and classified as sensitive, intermediate, or resistant using the standard technique ((CLSI), 2021). A bacteria is defined MDR if has acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

3.6.4. Screening of ESBL Producing isolates

Phenotypic detection of extended-spectrum Beta-lactamase production was confirmed by using the Combination Disk Test (CDT) and the decreased susceptibility to one of the following antimicrobials examined (ceftriaxone, ceftazidime or cefotaxime) according to the Clinical and Laboratory Standard Institute (CLSI, 2021) recommendations (Humphries et al., 2021). These breakpoints indicative of ESBL production were CAZ ≤ 22mm, CRO ≤ 25 mm, and CTX ≤ 27mm. In this test, a disk containing cephalosporin alone (cefotaxime 30μg, ceftriaxone 30 μg or ceftazidime 30μg) was placed in the opposite direction to a disk containing cephalosporin plus

clavulanic acid (20/10µg) with a distance of 15 mm apart on Muller-Hinton agar medium. The inoculated media was then incubated at 37°C for 18–24 hours. After incubation, zones of growth inhibition are measured to the nearest mm, a difference of >5 mm for a disk containing cephalosporin plus clavulanic acid compared to a disk containing cephalosporin alone was considered positive

3.6.5. Phenotypic screening tests for carbapenemase

Detection of Carbapenemase-producing organisms by Modified Hodge test: In this test, carbapenemase production by the clinical bacterial isolates was identified if the isolates were able to produce the enzyme and permit the growth of standard *E. coli* ATCC 25922 strains towards a carbapenem disk. The results were noted based on the observation of clover leaf-like indentation. Initially, 0.5 McFarland standard dilution of the standard strain *E. coli* ATCC 25922 was prepared in 5 ml of Mueller-Hinton broth (MHB). A 1:10 dilution of the culture was prepared by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of MHB broth. A lawn culture was made by streaking an aliquot amount of diluted standard culture on MHA plate and allowing it to dry for 5 minutes. An imipenem disk at a concentration of 10 µg was placed in the center of the MHA plate. Now a swab from each test culture sample was streaked from the border of the disc to the rim of the plate. Nearly four isolates were streaked on the same plate with one drug. The plates were incubated at 35°C for 24h and observation of clover leaf-like indentation was considered as positive as shown below (Figure 3.4).

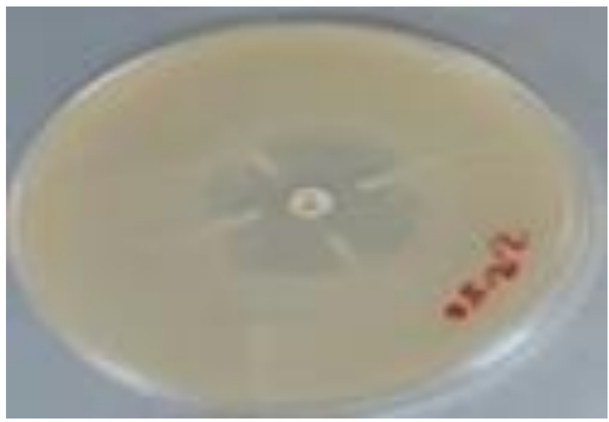


Figure 3.3. Postive Acinetobacter isolate with Modified Hodge Test (MHT) for IMP: imipenem

3.6.6. Detection of ESBL and carbapenemase genes by PCR

All of the positive ESBL (n = 40) and carbapenemase (n = 36) isolates according to phenotypic assays were further confirmed by PCR and sequencing. The genes investigated in this study were bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ for the detection of ESBL production. Furthermore, bla_{KPC} and bla_{NDM}-like enzymes were tested for carbapenenamase production.

The bacterial DNA was extracted by the boiling lysis method as previously described by El-Badawy et al (El-Badawy et al., 2017). Briefly, three to six fresh colonies of the bacteria were suspended in 100µl of DNase-free water in a sterile 1.5ml Eppendorf tube. The bacterial suspension was vortexed for 15 seconds and placed in a boiling water bath at 94°C for 10 minutes to lyse the bacterial cells. The lysed bacterial suspension was centrifuged at maximum speed (13,000 ×g) for 5 min. The supernatant, which contains total genomic DNA, was transferred to a new sterile tube using DNase-free tips. The quality and quantity of the extracted DNA were measured using Nanodrop (Thermo Scientific, US) and stored at -20°C.

Multiplex PCR was performed to detect bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ and bla_{KPC} and bla_{NDM} carbapenemase genes using specific primers (Dallenne et al., 2010) (Table 1). The PCR was performed with approximately 300ng template DNA, 0.2µM of each primer, and 7.5 µl of 2 x QIAGEN Multiplex PCR Master Mix (QIAGEN, Germany) in a final volume of 15µl. Amplification was performed in a thermocycler (Biometra, Germany) with cycling parameters including initial denaturation at 95°C for 15 minutes followed by 35 cycles each of denaturation at 94°C for 30 s, annealing at 58°C for 90 s, extension at 72°C for 90 s, and a final extension at 72°C for 10 minutes. The PCR products were visualized by electrophoresis in 1.5% agarose gel after staining with ethidium bromide. A 100bp ladder molecular weight marker (Promega, US) was used to measure the molecular weight of amplified products. The amplicon was visualized and its size was determined under UV trans-illuminator (Bio-Rad, US). The PCR products that tested positive were subjected to sequencing. PCR positive samples containing resistance genes were further analysed by WGS. (Laboratory flow chart figure 3.5)

3.6.7. DNA Extraction, Whole Genome Sequencing (WGS), and the Identification of Resistance Genes

DNA was extracted manually using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. DNA extraction was performed by taking 2–6 pure colonies that grew on cystine lactose electrolyte deficient agar at 37°C for 24 h aerobically. After the extraction, the DNA concentrations were measured with QubitTM3.0 (Thermo scientific, Waltham, MA, USA). All the extracted DNA samples were kept at −20°C until they were submitted and all the isolates were subjected to WGS at the *Science for Life Laboratory*, in Solna, Sweden. From each DNA sample, 20 µL was transferred into a 96-well WGS plate. Sequencing libraries were generated using Nextera XT (Illumina kits) and short-read sequencing was run on Illumina (HiSeq 2500) systems with a 150bp insert size paired end sequencing protocol at the Science for Life Laboratory. SPAdes (version 3.9) were used for the genome assembly.

With the assembled genomes, the acquired antimicrobial resistance genes were identified using the Res finder 4.1 web tools at the Center for Genomic Epidemiology http: ://www.genomicepidemiology.org/ (accessed on August 2023) using a threshold of 90% and 60% coverage. Each WGS run included quality control.

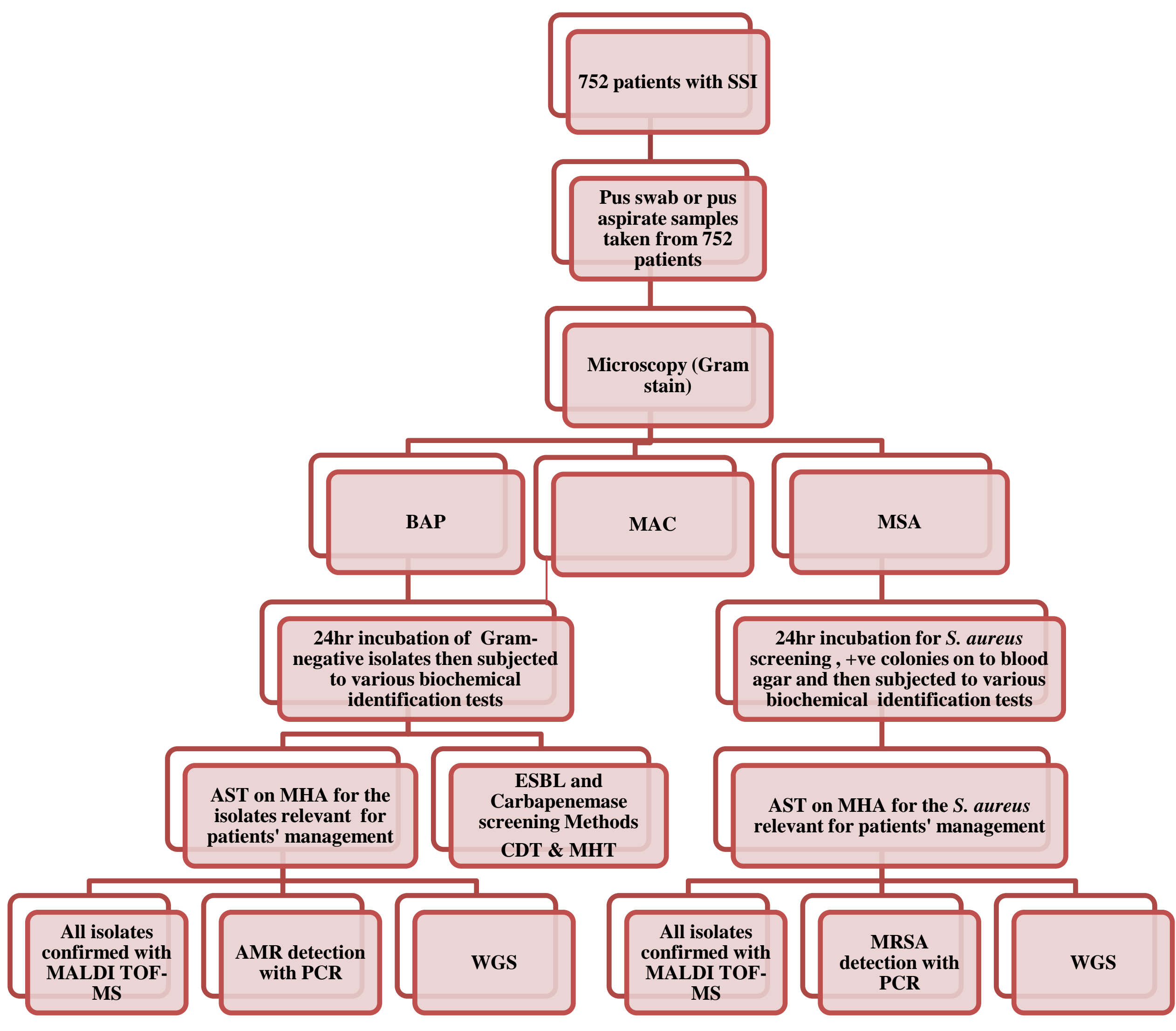


Figure 3.4. Laboratory investigation flow chart for surgical site infection isolates

3.7. Quality control

All specimens were collected according to the standard operating procedure (SOP). A double data entry method was used to ensure the accuracy of the data. The performance of all prepared media (BAP, MAC and MSA) was checked by inoculating control strains, *E. coli* (ATCC® 25922) and *S. aureus (*ATCC® 25923), for each new batch of agar plates ((CLSI), 2021). In addition, the sterility of culture media was checked by incubating 5% of the prepared media at 37 °C for 24–48 hrs. In addition, reagents for Gram-stain and biochemical tests were checked against control strains of *S. aureus* and *E. coli.* The 0.5 McFarland standards were used to standardize the inoculum density of the bacterial suspension for the susceptibility test. Each MALDI-TOF run also included quality control strains using *E. coli* (ATCC® 25922), and *S. aureus (*ATCC® 25923) (cefoxitin zone 21–29 mm) and *S. aureus* ATCC® 43300 (zone ≤ 21 mm) were used as control strains to determine the performance of the cefoxitin disk diffusion test. *S. aureus* ATCC® 29213 MIC of vancomycin broth value 0.5–2.0 µg/mL was used as a control strain to measure the performance of vancomycin ((CLSI), 2021). *K. pneumoniae* ATCC® 700603 used as control strain for screening of ESBL production (positive control), *K. pneumoniae* ATCC1 BAA-1705™ and *K. pneumoniae* ATCC1 BAA1706 were used as positive and negative controls respectively during MHT. During PCR analysis laboratory reference bla_{KPC} and bla_{NDM} genes were used as positive controls and *E. coli* ATCC1 25922 as a negative control. Before multiplexing, each pair of primers was verified using monoplex PCR.

3.8. Data analysis

The data were checked for completeness, missing values, and coding of questionnaires entered into Research Electronic Data Capture (RED-Cap) and exported to STATA version 16.0. Frequencies and cross-tabulations were used to summarize descriptive statistics (median, percentages or frequency). Associations of possible risk factors with SSIs were assessed using bivariate and multivariate logistic regression to study the effect of independent variables on the dependent variables. P-value less than 0.05 were considered statistically significant.

3.9. Ethical Considerations

The Department of Medical Microbiology, Immunology, and Parasitology (DMIP) and the AHRI/ALERT Research Ethics Committee (AAREC) reviewed and approved the study, and institutional review board (IRB) approval was obtained from Addis Ababa University's College of Health Sciences and AAREC, AAUMF03-008/2020. The study was also approved by AHRI/ALERT Ethics Review Committee (protocol number: P0/2919) of the Armauer Hansen Research Institute and National Ethical Review committee (Ref No. MoE//17 /246/767/23). Written permission letter was obtained from each study site before starting the data collection. The purpose and procedures of the study was explained to the study participants, participants' parents or guardians before recruitment to the study. Those study participants who gave written consent and those children whose parents or guardians gave assent were selected and enrolled in this study. Results obtained from all patients were communicated to attending physicians and all patients' information was kept confidentially by using identifier/code to prevent from unauthorized person.

3.10. Operational definition

Surgical site infection occurs near or at the incision site and/or deeper underlying tissue spaces and organs within 30 days of a surgical procedure performed (or up to 90 days for implanted prosthetics) (Borchardt and Tzizik, 2018).

Clean wound: where no inflammation is encountered and the respiratory, alimentary or genitourinary tracts were not entered.

Clean contaminated wound is where the respiratory, alimentary or genitourinary tracts were entered but without significant spillage.

Contaminated when acute inflammation is encountered, or there is visible contamination of the wound.

Dirty wound: wound in the presence of pus, where there is a previously perforated hollow viscous or compound/open injury more than four hours old (Garner, 1996).

Antibiotic: a drug that kills or inhibits the bacterial growth.

Multidrug resistance (MDR): acquired non-susceptibility to at least one agent in three or more antimicrobial categories

Elective surgery is a surgery that is scheduled in advance

Emergency surgery a surgery that is required to deal with an acute threat to life

CHAPTER 4: RESULTS

Bacterial etiologic agents among patients with surgical site infections

4.4.1. Socio-demographic characteristics and clinical data

The study participants age ranged from 3 days to 85 years with median of 28 years and 418 (55.6%) were males. Of the 752 study participants, 65.5% (493 /752) showed bacterial growth (Table 4.1). In the present study, a total of 752 patients from four different hospitals (172 from DTCSH, 184 from HUCSH, 193 from JUSTH, and 203 TASH) were enrolled for SSIs. DTCSH had the highest percentage of positive cultures (78.5%), followed by JUMC (65.3%), HUCSH (64.8%) and TASH (55.7%), (Table 4.1). Four hundred eighty-seven (64.8%) of patients had deep SSI, 724 (96.2%) of patients with clean or clean contaminated wounds dominated the wound class and 55.3% underwent surgeries lasting greater than an hour (Table 4.1).

Table 4.1. Socio-demographic characteristics and clinical data of patient's diagnosis with surgical site infection in Ethiopia between July 2020 and August 2021

Characteristic	Frequency (%)
Hospitals	
Sex	
Male	418 (55.6)
Female	334 (44.4)
Age in (year)	
≤ 18	159 (21.1)
19-40	419 (55.7)
41-60	130 (17.3)
≥ 61	44 (5.9)
DTCSH (n=172)	172(22.9)
Growth	135 (78.5)
No growth	37 (21.5)

HUCSH(n=184)	184 (24.5)
Growth	119 (64.8)
No growth	64 (34.8)
JUMC (n=193)	193 (25.7)
Growth	126 (65.3)
No growth	67 (34.7)
TASH (n=203)	203 (27)
Growth	113 (55.7)
No growth	90 (44.3)
Surgical site infection	
Superficial	265 (35.2)
Deep	487 (64.8)
Preoperative hospital stays	
≤ 7	298 (39.6)
>7	454 (60.4)
History of hospital admission	
Yes	133 (17.6)
No	619 (82.4)
Previous use of antibiotics	
Yes	388 (52.6)
No	364 (47.4)
Alcoholic	
Yes	67 (8.9)
No	685 (91.1)
Smoking	
Yes	448 (59.6)
No	304 (40.4)
Nature of surgery	
Elective	246(32.7)
Emergency	506(67.3)

Type of surgery	
Clean/Clean contaminated surgery	724(96.2)
Contaminated surgery	28(3.8)
Timing of surgical antimicrobial prophylaxis	
Before the operation	548(72.8)
During the operation	204(27.2)
Duration of operation	
≤ 1hr	336(44.7)
>1hrs	416(55.3)

Bivariate and multivariable regression analyses were used to see the relationship between the independent variables over the dependent variable. On bivariate regression analysis, age ≥ 61 (AOR = 2.83 (1.02-7.9); 95% CI; *p* = 0.18), SSI type (AOR = 0.99 (0.68-1.5); 95% CI; *p* = 0.97), preoperative hospital stays (AOR = 4.15 (2.9-6.1); 95% CI; *p* = 0.00), previous use of antibiotics (AOR = 2.83 (1.06-2.80); 95% CI; *p* = 0.03), smoking (AOR = 2.35 (1.44-3.83); 95% CI; *p* = 0.01), emergency surgery (AOR = 3.2 (2.2-4.8); 95% CI; *p* = 0.00), and duration of operation ≥1 hrs (AOR = 0.26(0.18-0.39); 95% CI; *p* = 0.00) had a statistically significant association with the occurrence of SSI. The type of surgery (wound), alcohol history and the timing of prophylactic antibiotics ≥1 h had no statistically significant association (Table 4.2). The result of the multivariate regression showed that ages ≥ 61 years, prolonged duration of hospital stay, history of previous antibiotics use, history of smoking, emergency surgery, and duration of operation were significant risk factors with (P-value 0.046, 0.000, 0.028, 0.001 and 0.000) respectively (Table 4.2).

Table 4.2. Bivariate and multivariable analysis to identify the association of patients' demographic and clinical characteristics with SSI culture growth in Ethiopia between July 2020 and August 2021

Characteristics	Bacterial growth n (%)		P-value	COR (95%CI)	AOR (95%CI)	P-value
	Growth	No growth				
Sex						
Male	309 (41.1)	109 (14.5)	0.194	0.81 (0.63-.1.12)	.94 (.64-1.37)	0.75
Female	233 (31)	101 (13.4)			1	
Age in (year)						
≤ 18	119 (15.8)	40 (5.3)	0.181	1.15 (0.94-1.42)	1.26 (.79-.03)	0.33
19-40	287 (38.2)	132 (18)			1	
41-60	98 (13)	32 (4.3)			1.26 (.75-2.12)	0.38
≥ 61	38 (5.1)	6 (0.8)			2.83 (1.02-7.9)	**0.046**
SSI						
Superficial	183 (24.3)	82 (11)			1	
Deep	359 (47)	128 (17)	0.15	1.27(0.92-1.76)	0.99 (.68-1.5)	0.97
Preoperative hospital stays						
≤ 7	145 (19.3)	124 (16)			1	
>7	397 (52.8)	86 (11)	0.00	3.91 (2.81-5.44)	4.15 (2.9-6.01)	**0.00**
Previous use of antibiotics						

Yes	326 (43.3)	72 (9.6)	0.000	2.641 (1.9-3.7)	2.83 (1.06-2.80)	**0.03**
No	216 (28.7)	138 (18)			1	
Alcoholic history						
Yes	54 (7.2)	13 (1.7)	0.093	1.71 (0.92-3.20)		
No	488 (64.9)	197 (26)				
Smoking history						
Yes	359 (47.7)	89 (11.8)	0.000	2.65 (1.92-3.66)	2.35 (1.44-3.83)	**0.001**
No	186 (24.7)	118 (16)			1	
Nature of surgery						
Elective	147 (19.5)	99 (13.2)			1	
Emergency	395 (52.5)	111 (5.5)	0.000	2.4 (1.73-3.32)	3.2 (2.2-4.8)	**0.00**
Type of surgery						
Clean/Clean contaminated surgery	518 (68.9)	206 (27)	0.09	1.58 (0.93-2.69)		
Contaminated surgery	24 (3.2)	4 (0.5)				
Timing of surgical antimicrobial prophylaxis						
Before the operation	150 (19.9)	58 (7.7)	0.984	0.99 (0.7-1.42)		
During the operation	392 (52.1)	152 (20)				
Duration of operation						
≤ 1hr	198 (27.7)	138 (17)			1	
>1hrs	344 (45.7)	72 (9.6)		1.33 (1.24-1.46)	1.26 (1.18-1.39)	**0.00**

4.4.2. Frequency and distribution of identified bacterial isolates

Among 752 wound discharge cultures performed, 493 (65.5%) yielded growth. Among these, 57.9% were Gram-negative and 42.1% were Gram-positive isolates. A total of 494 bacteria were isolated; *Staphylococcus aureus* (31%), *Escherichia coli* (20.7%), *Klebsiella pneumoniae* (9.8%) and *Acinetobacter species* (8.7%) were the most common (Figure.4.6).

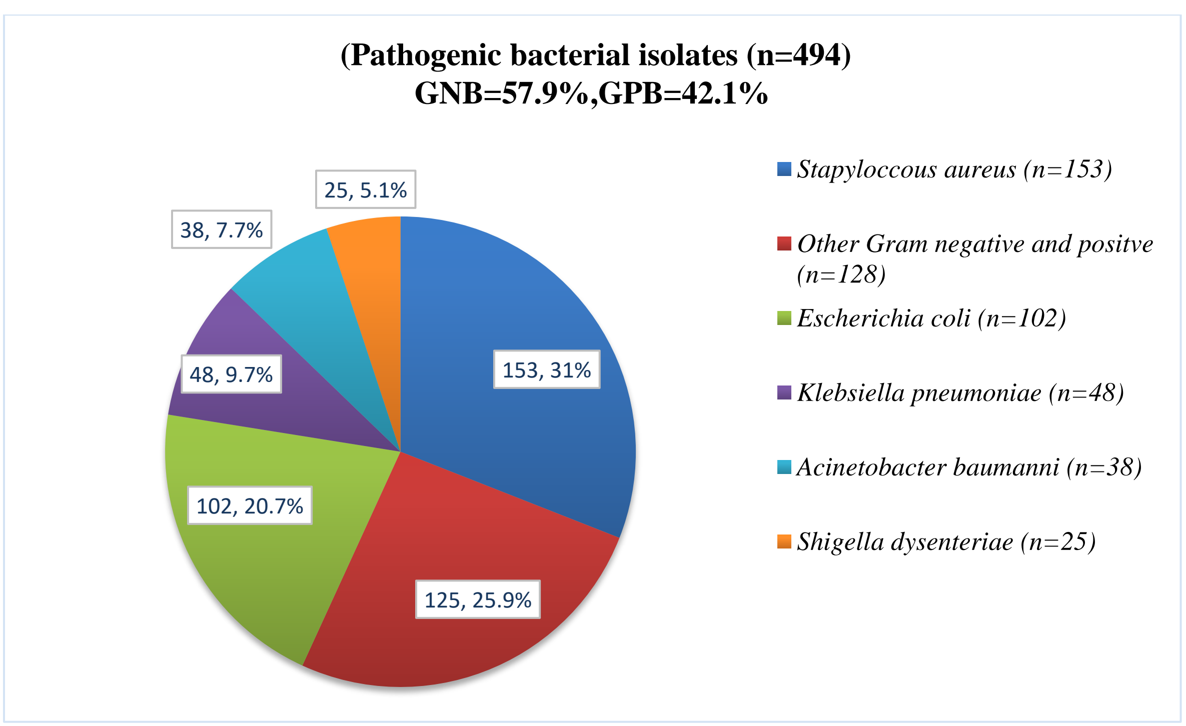
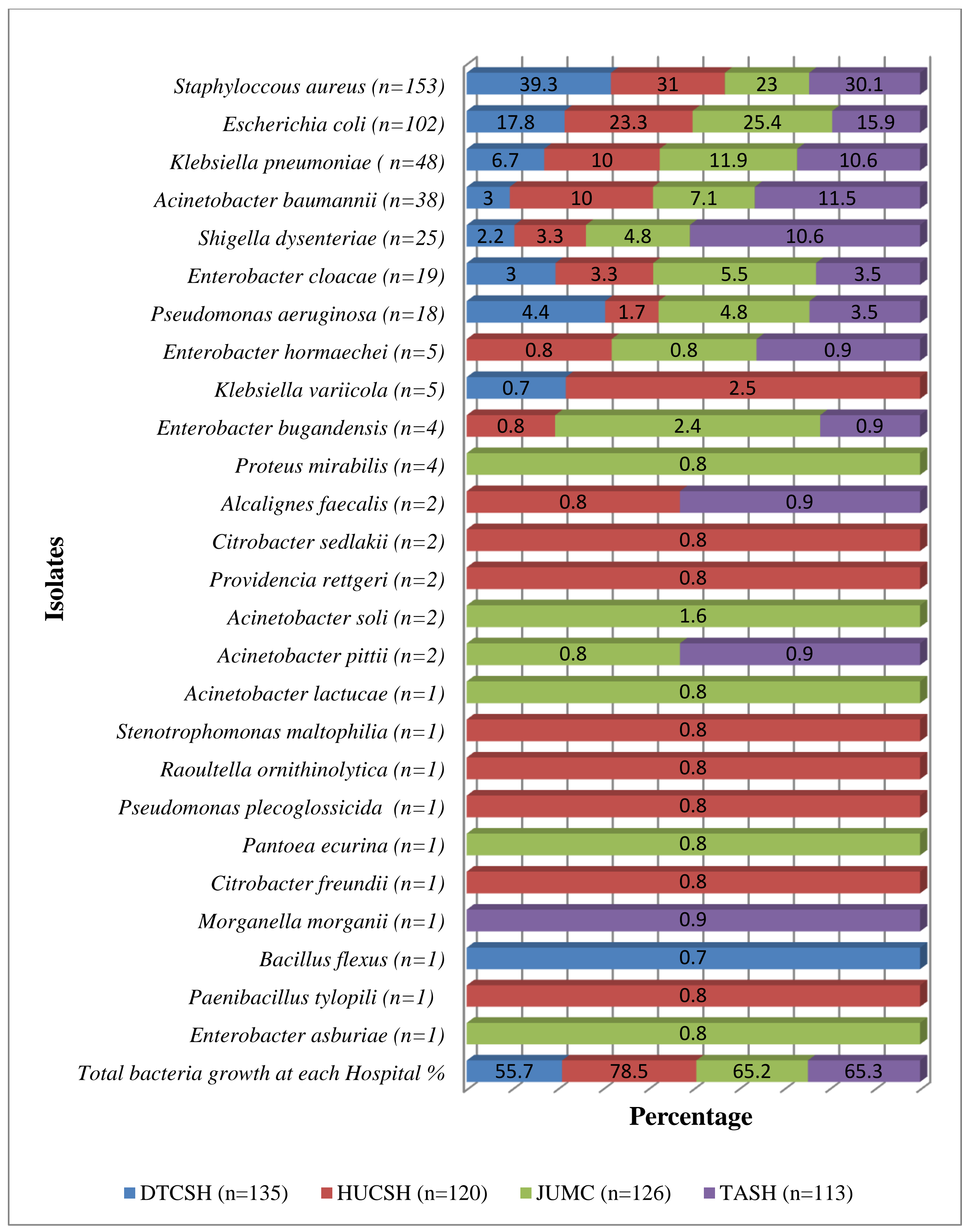


Figure 4.5. Frequency and distribution of bacteria isolated from patients investigated for surgical site infection at four different hospitals in Ethiopia, between July 2020 and August 2021.

Among the total of 494 isolates, 286 (57.9%) were Gram-negative and 208 (42.1%) were Gram-positive isolates. Of these bacteria, 2.6% (13/493) of cultures were a mixture of two colony types, while 2.4% (12/493) were commensals or contaminants and 480, (97.4%) showed single bacterial growth. Species of the mixed cultures were *Raoultella ornithinolytica, Paenibacillus tylopili, S. aureus* and coagulase negative staphylococci. Among the identified types of bacteria, *S. aureus* was the predominant one (153, 31%), followed by *E. coli* (102, 20.7%) and *Klebsiella pneumonia* (48, 9.8%) among SSIs. Other less frequently detected species were *Acinetobacter*

baumannii (38, 7.6%), *Enterobacter cloacae* (19, 5.1%), *Pseudomonas aeruginosa* (18, 3.7%), *Klebsiella variicola*, and *Enterobacter hormaeche* (1%) each.

Diverse species of *Acinetobacter, Enterobacter, Enterococcus, Staphylococcus, Aerococcus, Bacillus, Citrobacter,* and *Pseudomonas* were identified. While Gram-positives were found at all four hospitals (42.1%), they were mainly detected at DTCSH (40.4%), with 21.6%, 21.6%, and 16.3% isolated at TASH, HUCSH, and JUMC respectively. In addition, *Raoultella ornithinolytica, Stenotrophomonas maltophilia, Pantoea ecurina, Providencia rettgeri, Alcalignes faecalis, and Morganella morganii* were detected as rare bacterial pathogens (Figure 4.7A, 4.7B and 4.7C).



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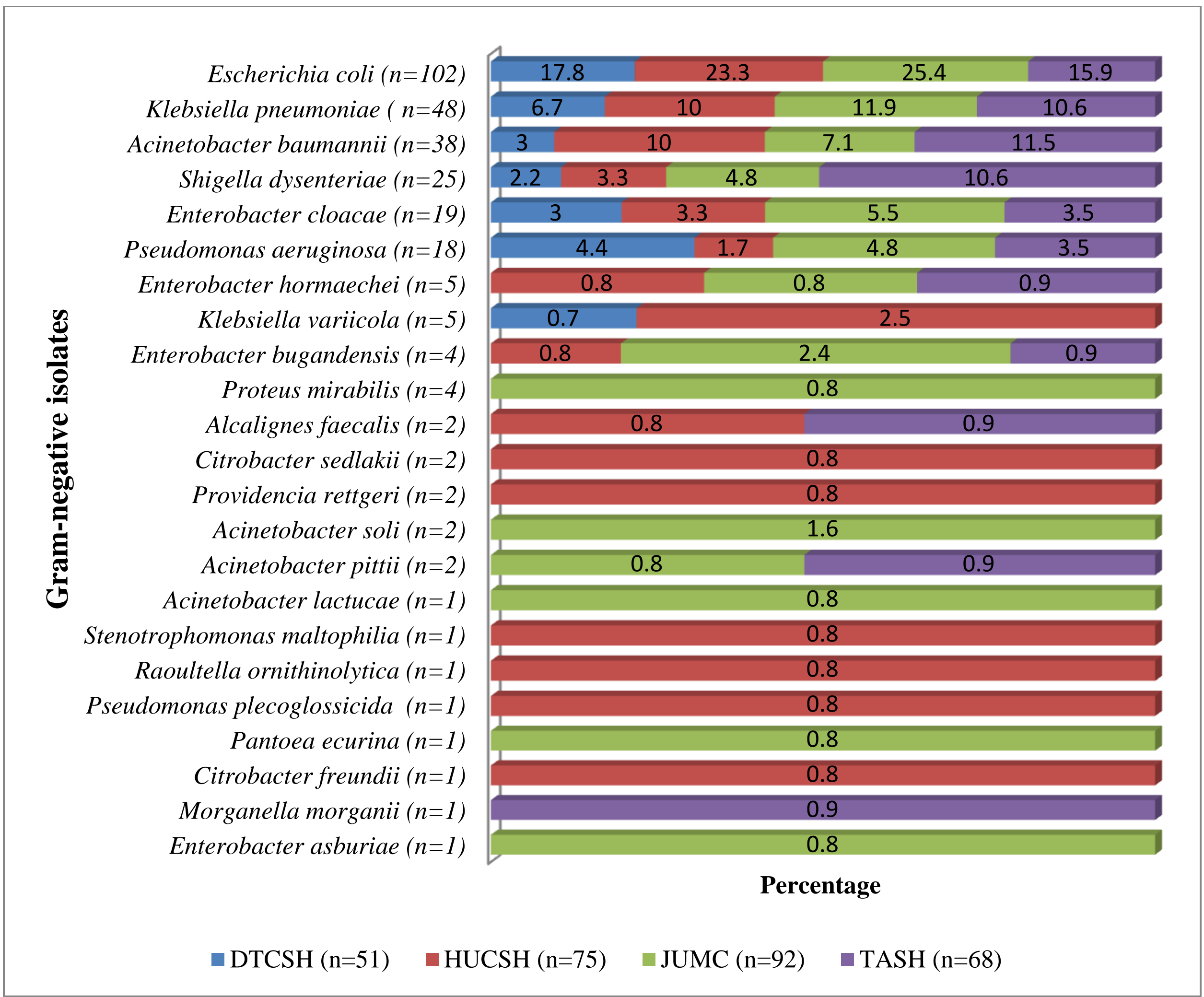

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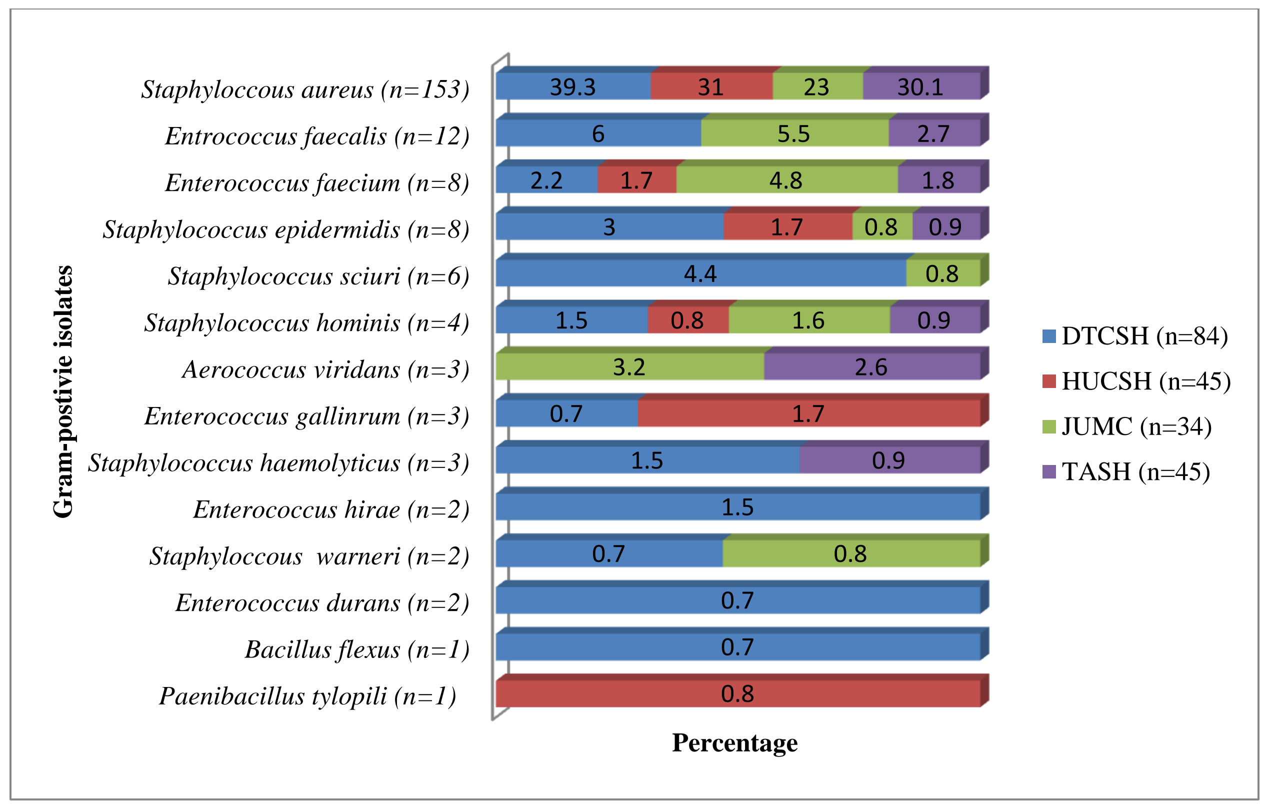


C

Figure 4.6. Frequency and distribution of bacterial isolates from the total number of bacteria isolated at each hospital in Ethiopia between July 2020 and August 2021

A; Total identified bacteria at each site, **B;** Gram-negative isolates and **C;** Gram-positive isolates. **DTCSH**; Debre Tabor Comprehensive Specialized Hospital; **HUCSH**: Hawassa University Comprehensive Specialized Hospital: **JUMC**; Jimma University Medical Center; **TASH**; Tikur Anbessa Specialized Hospital, n: number of bacterial isolates

4.4.3. Antibiotic resistance pattern of SSI bacterial isolates

The predominant isolate from Gram-positives, *S. aureus*, revealed a high level of resistance toward penicillin 90.1%, and ampicillin 76.5%, while 7.8%, 10.6%, and 12.4% of the isolates were resistant to clindamycin, chloramphenicol, and gentamicin respectively but 100% of *S.*

aureus were sensitive to vancomycin (Table 4.3). All isolates of *S. aureus* showed multiple drug resistance (resistance to two or more drugs).

Table 4.3. Antimicrobial resistance pattern of Gram-positive bacteria isolated from patients diagnosed with surgical site infection in Ethiopia between July 2020 and August 2021.

Gram-positive isolates	**(%) of resistance to antimicrobial agents**													
	P	**AMP**	**E**	**TE**	**FOX**	**OXA**	**DOX**	**SXT**	**CPR**	**CN**	**CHL**	**DC**	**V**	**MDR %**
***S. aureus* (n=153)**	90.2	76.5	43.7	43	20	20	23.8	24.2	20	12.4	10.6	7.8	0	**100**
Other staphylococcus spp.														
***S. epidermidis* (n=8)**	75	75	37.5	62.5	12.5	12.5	25	25	25	25	12.5	12.5	0	73.9
***S. sciuri* (n=6)**	100	100	50	50	100	100	50	50	33	33	33	25	0	
***S. hominins* (n = 4)**	75	75	75	50	25	25	50	50	0	75	25	25	0	
***S. haemolyticus*(n = 3)**	100	67	67	67	33	33	67	33	0	67	67	67	33	
***S. warneri* (n=2)**	100	100	100	50	50	50	50	50	50	50	50	NA	0	
Total Staphylococcus (n=176)	**88.6**	**77.3**	**45.5**	**44.9**	**22.7**	**22.7**	**26.7**	**24.4**	**20.5**	**16.5**	**17.6**	**9.7**	**0**	**96.6**
***E. faecalis* (n=12)**	NA	66.7	66.7	75	NA	NA	NA	NA	NA	NA	66.7	NA	NA	
***E. faecium* (n=8):**	NA	75	100	75	NA	NA	NA	NA	NA	NA	75	NA	NA	

***E. gallinarum* (n=3)**	NA	100	NA	67	NA	NA	NA	33	NA	NA	67	NA	NA	
***E. hirae* (n=2)**	NA	50	50	50	NA	NA	NA	NA	NA	NA	50	NA	NA	
***E. durans* (n=2)**	NA	100	50	50	NA	NA	NA	NA	NA	NA	50	NA	NA	
***Total*(n=27)**	NA	**70.4**	**66.7**	**59.3**	NA	NA	NA	NA	NA	NA	**66.7**	NA	NA	
Other gram positives														
***A. viridans* (n=3)**	100	100	67	33	NA	NA	NA	67	100	67	67	NA	NA	
***B. flexus* (n=1)**	NA	100	100	100	NA	NA	NA	0	100	0	0	NA	NA	
***P. tylopili* (n=1)**	NA	100	100	100	NA	NA	NA	100	100	0	0	NA	NA	
Total (n=5)	**20**	**60**	**80**	**60**	**NA**	**NA**	**NA**	**80**	**100**	**40**	**40**	**NA**	**NA**	

P: Penicillin; AMP: Ampicillin; E: Erytromycin; TE: Tetracycline; FOX: Cefoxitin; OXA: Oxacillin; DOX: Doxycycline; SXT: Trimethoprim-Sulfamethoxazole; CPR: Ciprofloxacin; CN: Gentamicin; CHL: Chloramphenicol; DC: Clindamycin; V: Vancomycin; NA: not applicable, MDR: Multidrug resistance

S. aureus and non-S. *aureus* isolates showed 22.7% resistnce to Cefoxitin, which is a surrogate marker of methicillin. *Enterococcus* species showed 70.4% resistance to ampicillin and 66.7% to erythromycin. Table 4.3 shows the AMR pattern of Gram-positive bacteria.

The *Enterobacteriaceae* showed high resistance toward ampicillin (93.2%), ceftriaxone (90.5%), cefuroxime (88.7%), aztreonam (82.9%), ceftazidime (80.6%), cefepime (77%), ampicillin-sulbactam (76.1%), trimethoprim-sulfamethoxazole (77.5%), tetracycline (72.5%), and amoxicillin-clavulanic acid (71.2%) (Table 4.4).

Table 4.4. Antibiotics resistance of Gram-negative bacteria isolates among patients with surgical site infection in Ethiopia between July 2020 and August 2021.

Gram-negative isolates	**Resistance to antimicrobial agents (%)**																		
	AMP	**AMC**	**CHL**	**CRO**	**SXT**	**CN**	**AK**	**CXM**	**CTX**	**CPR**	**CAZ**	**FEP**	**IMP**	**MEM**	**ET**	**TE**	**SAM**	**ATM**	MDR %
E. coli (n=102)	94.1	68.6	41.2	99	71.5	57.8	10.8	73.5	89.2	73.5	79.4	76.5	11.8	9.8	24.5	70.6	72	64	96.1
K. pneumonia (n=48)	-	91.7	45.8	93.8	64.6	70.8	33.3	77.1	93.8	62.5	85.5	81.25	29.2	41.7	43.8	66.7	85.2	96.8	95.9
E. cloacae complex(n=29)	96.6	79.3	62.1	89.7	75.7	58.6	24.1	72.4	86.2	55.2	79.3	79.3	37.9	17.2	31	62.1	71.4	63.1	79.3
S. dysentriae(n=25)	100	28	32	92	64	28	16		84	44	76	76	20	20	20	48	66.9	66.5	82
K. variicola (n=5)	100	40	40	100	60	60	0	60	0	40	100	80	40	0	20	60	71.9	62.2	100
P. mirabilis(n=4)	100	25	25	75	50	50	0	75	25	50	50	75	0	0	0	50	66.1	65.2	100
Rare Enterobacteriaceae	88.8	88.8	44.4	77.8	44.4	66.7	22.2	77.8	77.8	66.7	77.8	66.7	33.3	11.1	22.2	55.5	45.1	38.7	100

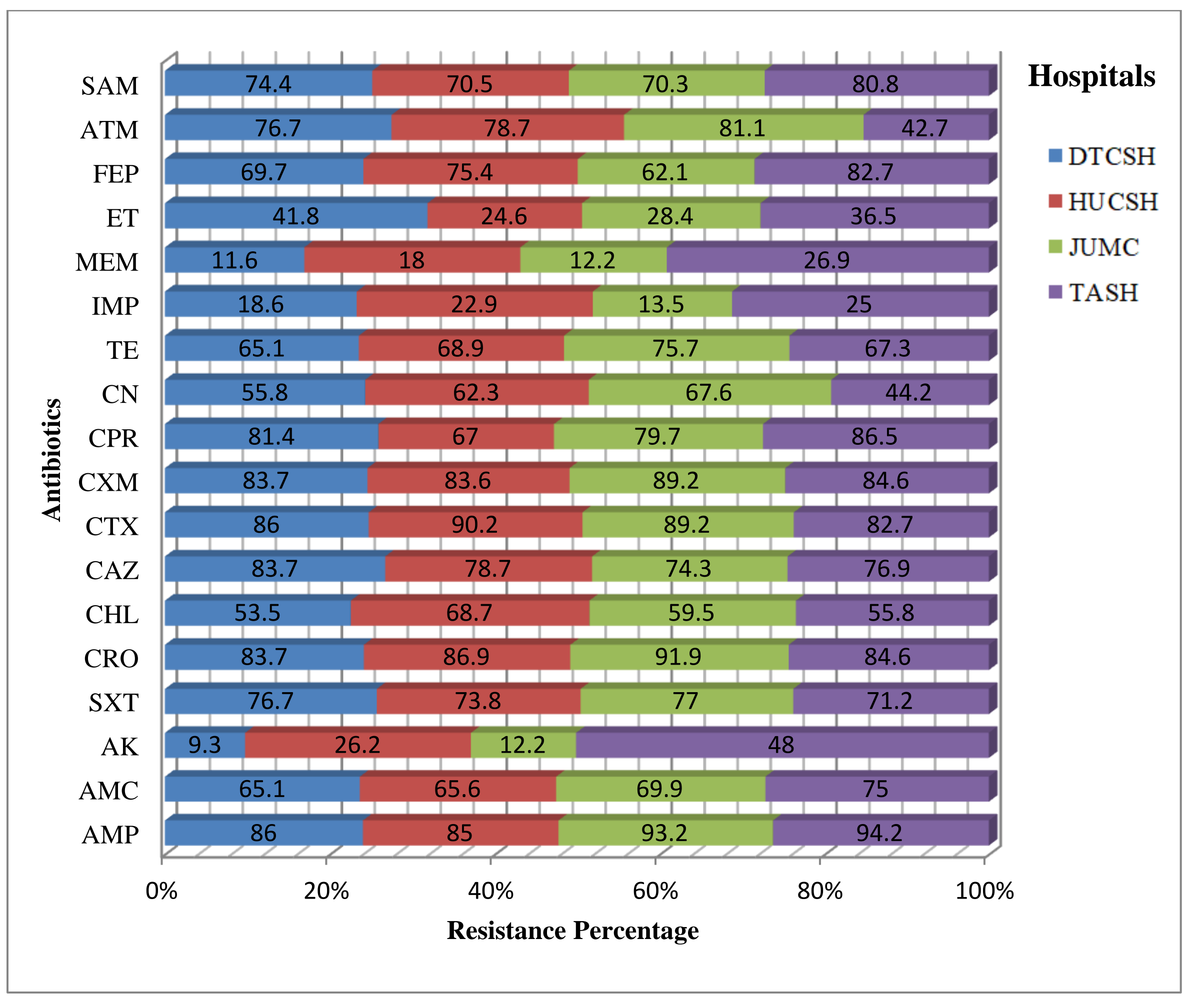
isolates(n=9)																			
Total Enterobacteriaceae isolates(n=222)	**93.2**	**71.2**	**62.2**	**90.5**	**77.5**	**60.1**	**24.3**	**88.7**	**90.5**	**81.1**	**80.6**	**77**	**20.3**	**17.6**	**32.9**	**72.5**	**76.1**	**82.9**	**93.3**
Acinetobacter species																			
A. baumammi(n=38)	-	-	-	94.7	81.4	86.8	42.1	-	95.3	73.7	89.5	84.2	65.9	84.2	92.1	-	63.1	-	95
Other Acinetobacter species(n=5)	-	-	-	95.3	20	60	0	-	95.3	40	60	60	20	40	100	-	40	-	60
Total Acinetobacter species(n=43)		**-**	**-**	**95.3**	**86**	**13.5**	**39.5**	**-**	**95.3**	**69.7**	**81.4**	**86**	**67.4**	**14.9**	**90.7**	**-**	**67.4**	**-**	**93**
Pseudomonas species																			
P. aeruginosa (n=18)	-	-	-	-	-	47.8	27.8	-	-	22.2	66.7	55.5	5.5	0	-	-	-	66.7	77.8
P.	-	-	-	-	-	0	0	-	-	100	100	100	0	0	10	-	-	100	100

plecoglossicida (n=1)														0				
Total pseudomonas species(n=19)	**-**	**-**	**-**	**-**	**-**	**47.3**	**21.1**	**-**	**-**	**26.3**	**68.4**	**57.9**	**5.3**	**0**	**-**	**-**	**-**	**68.4**	**73.8**

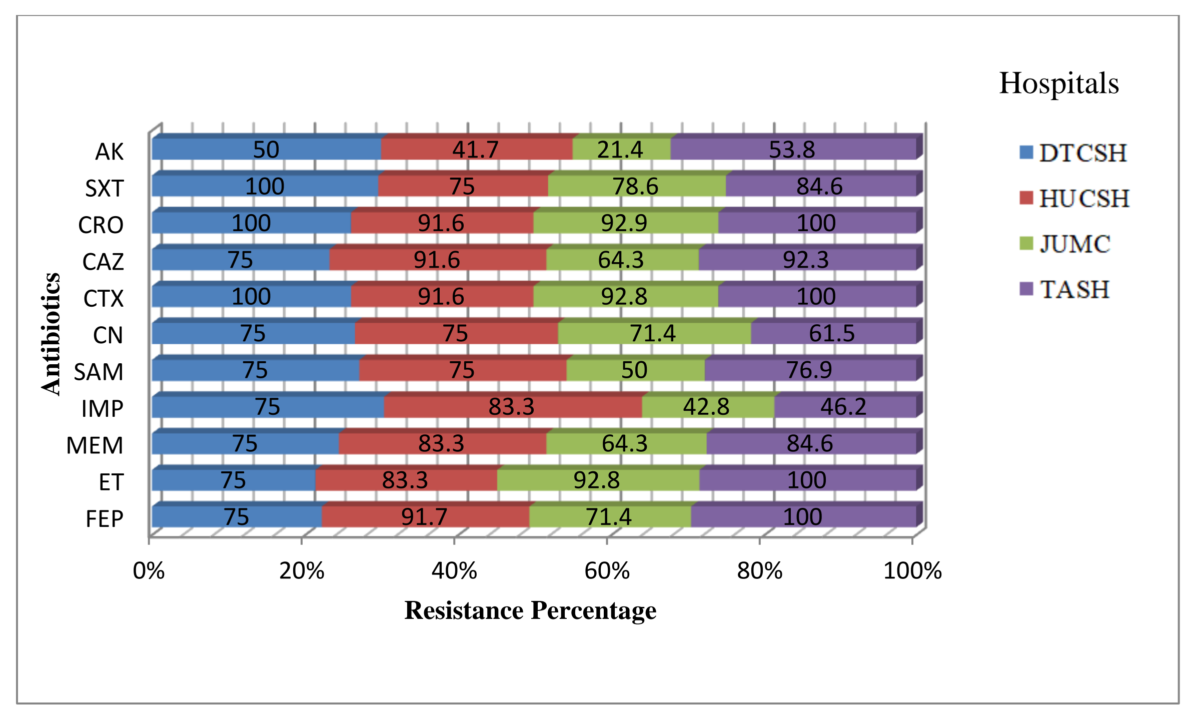
Key AMP: Ampicillin; AK: Amikacin; AC: Amoxicillin-Clavulanic Acid; ATM: Aztreonam; CHL: Chloramphenicol; CN: Gentamicin; CRO: Ceftriaxone; FEP: Cefepime SXT: Trimethoprim-Sulfamethoxazole; CPR: Ciprofloxacin; CXM: Cefuroxime; CTX: Cefotaxime; ET: Ertapenem; IPM: Imipenem; MEM: Meropenem; TE: Tetracycline

Lower resistance frequencies of *Enterobacteriaceae* were detected for amikacin (24.3%), imipenem (20.3%), meropenem (17.6%), while ertapenem (32.9%) was alarming (Table 4.4).

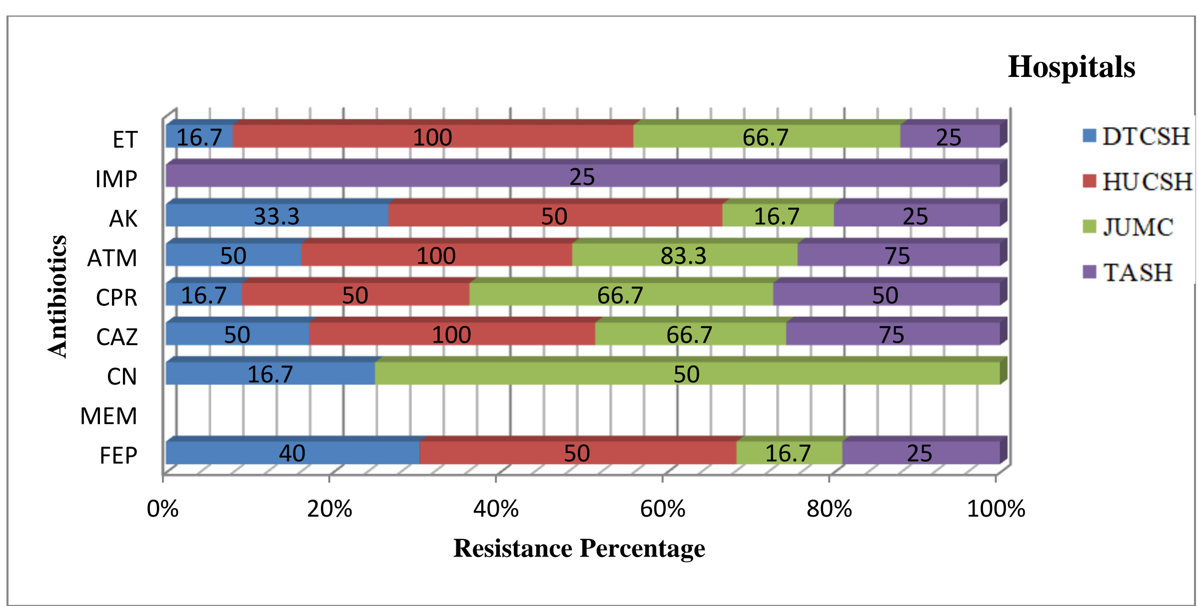
The resistance of *Enterobacteriaceae* to meropenem and imipenem was (11.6%, 18.6%), (18%, 22.9%), (12.2%, 13.5%) and (26.9%, 25%) at DTCSH, HUCSH, JUMC, and TASH, respectively (Fig.4.8).



A



B



C Figure 4.7. Magnitude of antibiotics resistance at four hospitals in Ethiopia

A, *Enterobacteriaceae* B, *Acinetobacter species* C, *Pseudomonas species*. The percentage represents the proportion of resistant isolates, out of the total number of isolates at all hospitals. AMP: ampicillin; AMC: amoxicillin/clavulanate; AK amikacin; SXT: trimethoprim-sulfamethoxazole; C: chloramphenicol; CAZ:

ceftazidime; CTX: cefotaxime; CRO: ceftriaxone; CXM: cefuroxime; CIP: ciprofloxacin; CN: gentamicin; TE: tetracycline; ATM: aztreonam; SAM: ampicillin-sulbactam; FEP: cefepime; IMP: Impemene; MEM: meropeneme; ET: Ertapeneme; DTCSH: Debre Tabor Comprehensive Specialized Hospital; HUCSH: Hawassa University Comprehensive Specialized Hospital JUMC: Jimma University Medical Center; TASH: Tikur Anbessa Specialized Hospital

The predominant isolate, *E. coli* (n=102) revealed a high level of resistance to ampicillin (94.6%), ceftriaxone (99%), cefotaxime (93.8), ceftazidime (79.4%), cefepime (77%), cefuroxime (73.5%), ampicillin-sulbactam (72%), trimethoprim-sulfamethoxazole (71.5%), tetracycline (70.6%), and low-level resistance to gentamicin (57.8%), chloramphenicol (41.2%), ertapenem (24.5%), imipenem (11.6%), amikacin (10.8%), meropenem (9.8%).

K. pneumoniae (n=48) were resistant to ampicillin (100%), ceftriaxone (100%), cefotaxime (93.8%), amoxicillin-clavulanic acid (91.7%), ceftazidime (88.5%), cefepime (81.2%), cefuroxime (77.1%), tetracycline (66.7%), ertapenem (43.8%), meropenem (41.7%), amikacin (33.3%), imipenem (29.2%). Amikacin and meropenem were 100% effective against all of the isolates of *Klebsiella variicola and Proteus mirabilis.* In the non-fermenter group, *A. baumannii* showed the highest resistance to cefotaxime (95.3%), ertapenem (92.1%), ceftazidime (89.5%), gentamicin (86.8%), cefepime (84.2%), meropenem (84.2%), and SXT (81.4%). In addition, *A. baumannii* has lower-level resistance to imipenem (65.9%) and ampicillin-sulbactam (63.1%) (Table 4.4). The resistance frequency of *Acinetobacter species* to meropenem at HUCSH, DTCSH, TASH and JUMC, was 83.3%, 75%, and 46.2%, 42.8%, respectively (Figure. 3B).

P. aeruginosa showed minimal resistance to ceftazidime (66.7%), cefepime (55.5%), gentamicin (47.8%), ciprofloxacin (22.2%), and amikacin (10.5%) (Table 4.4). In addition, 100% and 94.5% of Pseudomonas species were sensitive to meropenem and imipenem, respectively (Table 4.4, Figure. 3.8 C).

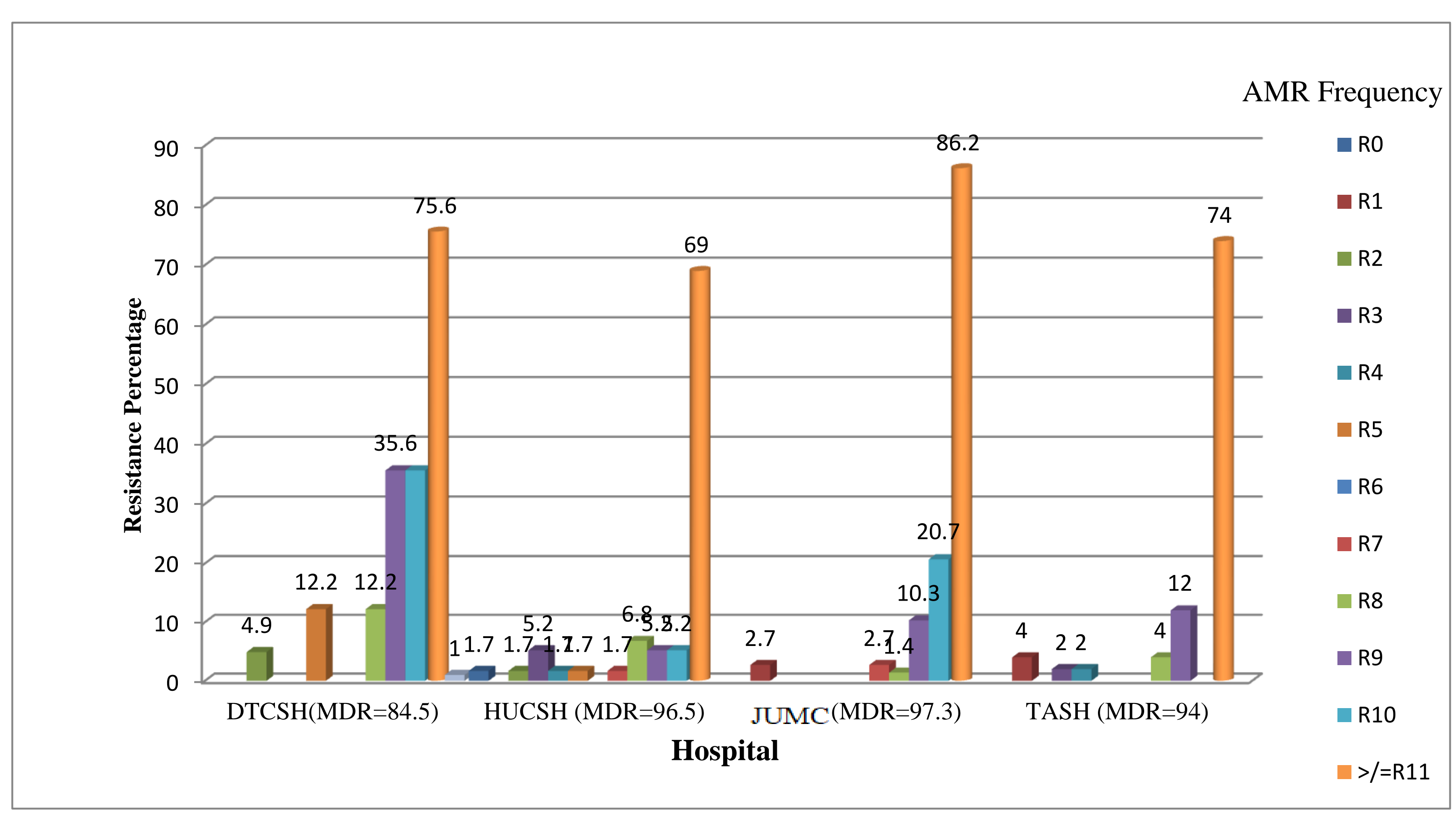
4.4.4. Multidrug resistance

The overall resistance at least one antimicrobial agent in three or more antibiotics (MDR) was observed in 100% of *S. aureus* (Table 4.3) and 93.3% *Enterobacteriaceae* (Table 4.4). For *Enterobacteriaceae*, the MDR frequency at DTCSH, HUCSH, JUMC, and TASH was 84.5%, 96.5%, 97.3%, and 94%, respectively (Fig. 3.6A). *E. coli, K. pneumoniae, E. cloacae, S.*

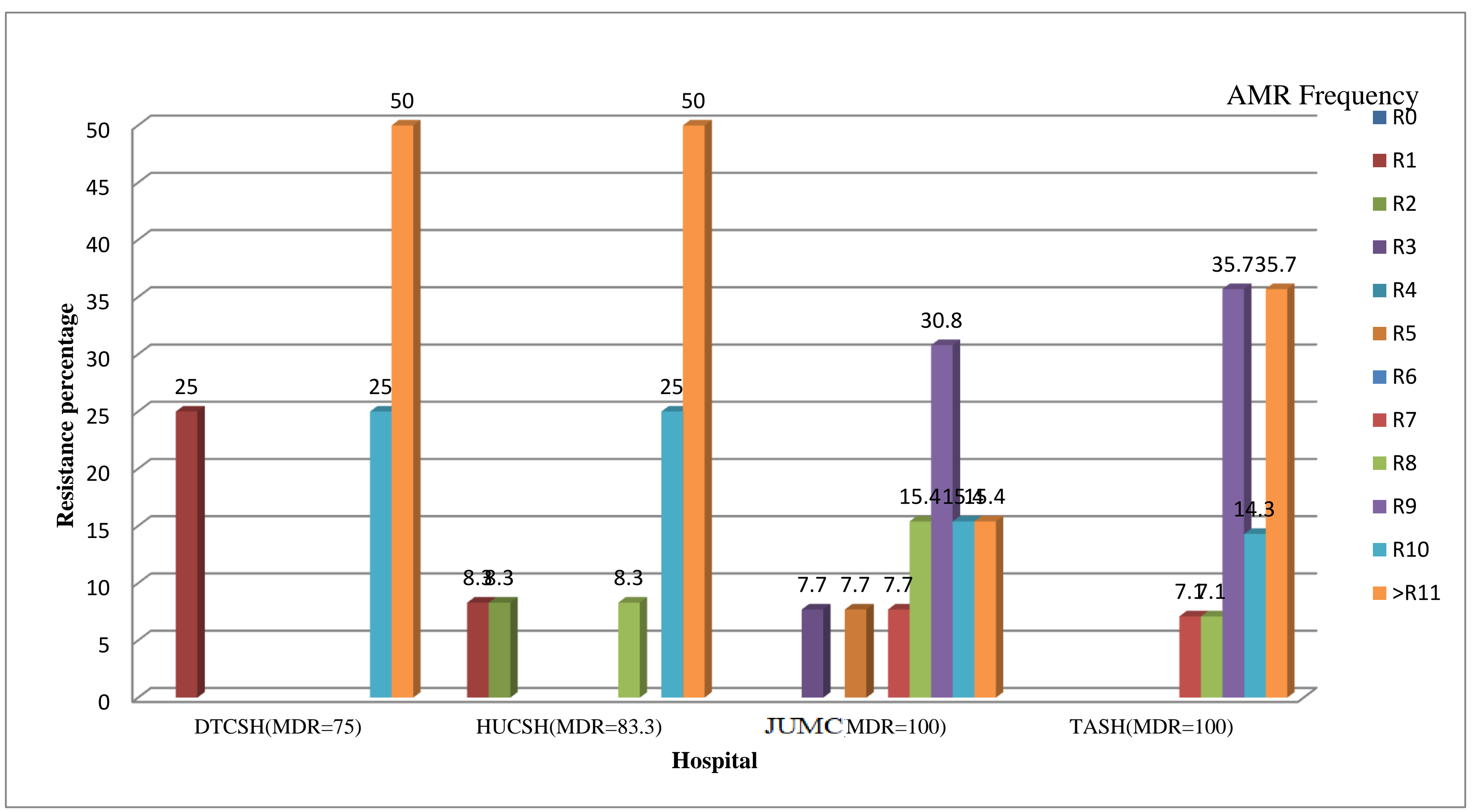
dysenteriae, K. variicola, and P. mirabilis showed an overall MDR frequency of 96.1%, 95.9%, 79.3%, 82%, 100%, and 100%, respectively. The overall MDR frequency of *A. baumannii* and *P. aeruginosa* was 95% and 77.8%, respectively (Table 4.4). The MDR frequency for *Acinetobacter species* was 75% at DTCSH, 83.3% at HUCSH, 100% at JUMC, and 100% at TASH (Figure. 4.9B).

On the other hand, MDR frequency for *Pseudomonas species* was 66.7%, 66.7%, 83.3% and 50% at DTCSH, HUCSH, and JUMC and TASH, respectively (Figure. 4.9 C).

A)



B)



C)

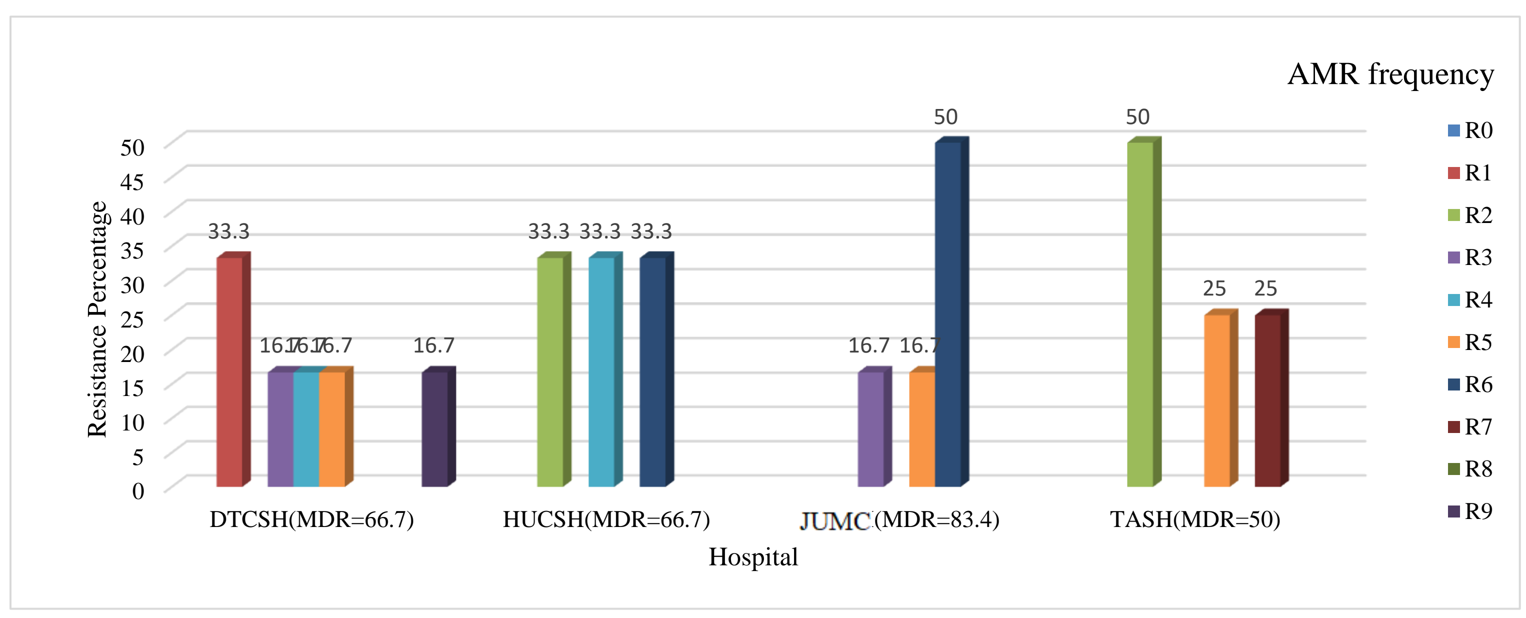


Figure 4.8. Frequency of multidrug resistance at four hospitals in Ethiopia between July 2020 and August 2021

A *Enterobacteriaceae* B *Acinetobacter species* C *Pseudomonas species*. Percentages represent the number of resistant isolates out of the total number of isolates at each hospital. Debre Tabor Comprehensive Specialized Hospital (DTCSH), HUCSH—Hawassa University Comprehensive Specialized Hospital, and Jimma University Medical Center (JUMC) and Tikur Anbessa Specialized Hospital (TASH); MDR—multidrug resistance; R-0, R-1, R-2… R **≥11** stand for resistance to 0, 1, 2, ..., ≥11 antibiotics

4.2. Molecular identification of MRSA

4.2.1. Socio-demographic characteristics and clinical data

The age of study participants with MRSA ranged from 8 days to 85 years, with a mean age (±standard deviation) of 35 ± 28.3 years and a median of 30 years, and 58.3% (95) were males. Fifty-nine (36.2%) of the participants received antimicrobial prophylaxis before the procedure, and 47.2% (63) underwent surgeries lasting longer than an hour (Table 4.6).

Table 4.5. Socio-demographic characteristics and clinical data of *S. aureus* among patients diagnosed with SSI at four different hospitals in Ethiopia between July and August 2021.

Variables/Characteristics		**Frequency of *S. aureus* (%)**
Gender	Male	95 (58.3)
	Female	68 (41.7)
Age in (years)	<=18	25 (20.9)
	19–40	77 (54)
	41–60	19 (13.5)
	≥61	42 (11.7)
Surgical site infection	Superficial	79 (48.5)
	Deep	84 (51.5)
Preoperative hospital stays	<7	77 (47.2)
	≥7	86 (52.8)
Previous use of antibiotics	Yes	79 (48.5)
	No	84 (51.5)
Smoking	Yes	16 (9.8)
	No	147 (90.1)
Alcoholic	Yes	48 (29.4)
	No	115 (70.6)
Nature of surgery	Emergency	55 (68.1)
	Elective	108 (31.9)
Type of surgery	Clean/Clean contaminated surgery	148 (90.8)
	Contaminated surgery	15 (9.2)
Timing of surgical antimicrobial prophylaxis	Before the operation	59 (36.2)
	During the operation	104 (63.8)
Duration of operation	<1 h	100 (52.8)
	≥1 h	63 (47.2)

Of all 752 participants, 5.3% (40/752) carried bacteria characterized as MRSA, while among isolates of *S. aureus*, the frequency of MRSA was 24.5% (Table 4.5). All methicillin-resistant isolates were also tested for vancomycin susceptibility. Except for one isolate (2.5%), all tested isolates were vancomycin sensitive (Table 4.5).

Table 4.6. Antibiotics resistance pattern of *S. aureus* isolates from patients diagnosed with surgical site infection at four different hospitals in Ethiopia July 2020 and August 2021.

Antibiotics	Methods	N (%), % = N/752	N (%), % = N/163	AST Results	Strain
Cefoxitin	I-Z ≥ 22 mm	123 (16.4)	123 (75.5)	S	MSSA
	≤21 mm	40 (5.3)	40 (24.5)	R	MRSA

Abbreviations: S, susceptible; R, resistant; I, intermediate; I-Z, inhibition zone; MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*; AST, antimicrobial susceptibility test.

The likelihood of MRSA SSI occurrence was about 3.7 times higher among patients aged ≥ 61 years (AOR = 3.73 (1.18–11.79)) compared to those aged ≤ 60. Similarly, the relative risk of MRSA SSI occurrence was about 1.9 times more likely among patients who had a hospital stay ≥ 7 days (AOR = 1.86 (0.69–5.31)). Also, those who had a history of antibiotic use had a 3.7 times higher risk of developing methicillin-resistant Staphylococci infections (AOR = 3.69 (1.06–2.80)) than methicillin-sensitive *S. aureus* (MSSA) SSI. The likelihood of SSI occurrence was about 3.16 times more likely among patients who had antimicrobial prophylaxis during the operation (AOR = 3.07 (1.10–9.39)) than those who had antimicrobial prophylaxis before the operation and all *p*-value < 0.05 (Table 4.7).

Table 4.7. Bivariate and multivariate analysis for identification of methicillin resistance Staphylococci predictors among patients diagnosed with surgical site infection at four hospitals in Ethiopia between July 2020 and August 2021.

Characteristics		Bacterial Growth		*p*-Value	COR (95%CI)	AOR (95%CI)	*p*-Value
		MRSA	**MSSA**				
Gender	Male	29 (17.8)	66 (40.5)	0.039	2.3 (1.04–5.0)	1.6 (0.6–4.5)	0.337
	Female	11 (6.7)	57 (35)			1	
Age in (years)	≤18	2 (1.2)	23 (14.1)	0.000	2.8 (1.9–4.154)	0.6 (0.10–3.1)	0.499
	19–40	11 (6.7)	66 (40.5)			1	
	41–60	2 (1.2)	17 (10.4)			1.6 (0.3–9.3)	0.628
	≥61	25 (15.3)	17 (10.4)			3.7 (1.18–11.8)	**0.025**
Preoperative hospital stays	≤7	13 (8)	64 (39.3)	0.034	2.3 (1.1–4.8)	1	
	>7	27 (16.7)	59 (36.2)			1.9 (0.7–5.3)	**0.000**
Previous use of antibiotics	Yes	26 (16)	53 (32.5)			3.7 (1.1–2.8)	**0.025**
	No	14 (8.9)	70 (42.9)	0.001	3.3 (1.7–7.6)	1	
History of alcohol intake	Yes	18 (11)	30 (18.4)			1.1 (0.13–8.7)	0.945
	No	22 (13.5)	93 (57.1)	0.015	2.5 (1.2–5.4)	1	
Nature of surgery	Elective	16 (9.8)	92 (56.4)			1	
	Emergency	24 (14.7)	31 (19)	0.000	4.5 (2.1–9.4)	2.0 (0.1–6.2)	0.00
Timing of surgical antimicrobial prophylaxis	Before the operation	7 (4.3)	57 (35)			1	
	After the	33 (20.2)	71(43.6)	0.006	3.5 (2.1–9.5)	3.1 (1.01–9.4)	0.05

	operation						
Duration of operation	≤1 h	19 (11.7)	81 (49.7)			1	
	>1 h	21 (12.9)	42 (25.8)	0.004	2.1 (1.3–4.4)	1.9 (0.6–5.7)	0.235

4.2.2. MALDI-TOF MS Identification of Methicillin-Resistant Staphylococcus Isolates

Of the 40 phenotypic MRSA bacterial isolates, MALDI-TOF MS only identified 77.5% (31/40) as *S. aureus*, while six were identified as *M. sciuri,* and the other three as *S. warneri, S. epidermidis,* and *S. haemolyticus* (Figure 4.10). The majority (70%) of methicillin-resistant Staphylococcus isolates were identified from Debre Tabor Comprehensive Specialized Hospital with 47.5% as *S. aureus*, 15% as *M. sciuri*, 2.5% as *S. warneri*, 2.5% as *S. epidermidis* and 2.5% as *S. haemolyticus* (Figure 4.11).

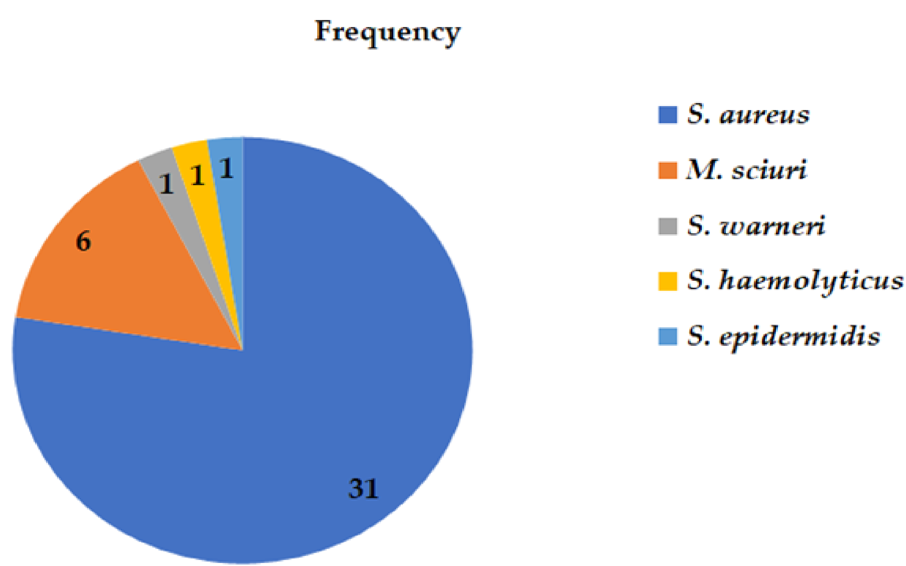


Figure 4.9. Frequency of methicillin- resistant Staphylococci isolates from patients diagnosed with surgical site infection at four different hospitals in Ethiopia using MALDI-TOF MS between July 2020 and August 2021.

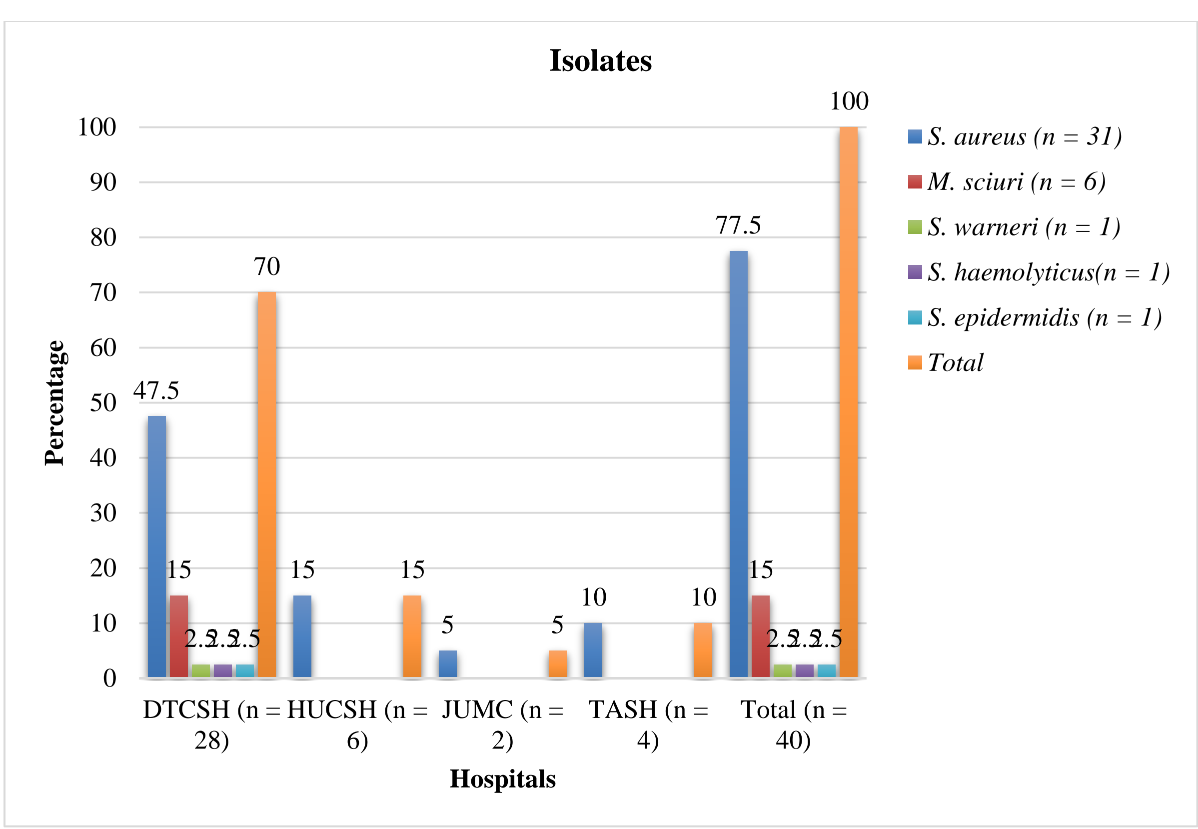


Figure 4.10. Frequencies of MALDI-TOF MS identification and distribution of phenotypic methicillin-resistant Staphylococci, and *M. sciuri* isolates at each hospital between July 2020 and August 2021

DTCSH; Debre Tabor Comprehensive Specialized Hospital; **HUCSH**: Hawassa University Comprehensive Specialized Hospital: **JUMC**; Jimma University Meical Center Hospital; **TASH**; Tikur Anbessa Specialized Hospital

4.4.3. PCR amplification of *mecA, femA, van A,* and *vanB*

Detection of *mecA, femA, van A,* and *vanB* was performed for all MRSA and methicillin-resistant Staphylococci other than *S. aureus* (MRSOSA). The PCR tests revealed that 27.5% (11/40) contained the *mecA* gene, 25% (10/40) were both *mecA*- and *femA*- positive, and 92.5% (37/40) showed the *femA* gene (Figure 4.12). On the other hand, from all eleven isolates that contained the *mecA* gene only four were *S. aureus*, whereas five were *M. sciuri*, one was *S. warneri,* and one was *S. haemolyticus,* respectively (Figure 4.12 and Table 4.8). Among *S. aureus* isolates,

only 12.9% (4/31) carried the *mecA* gene (MRSA), whereas 83.3% (5/6) of *M. sciuri* and both *S. warneri* and *S. haemolyticus* isolates carried *mecA* (Figure 4.12).

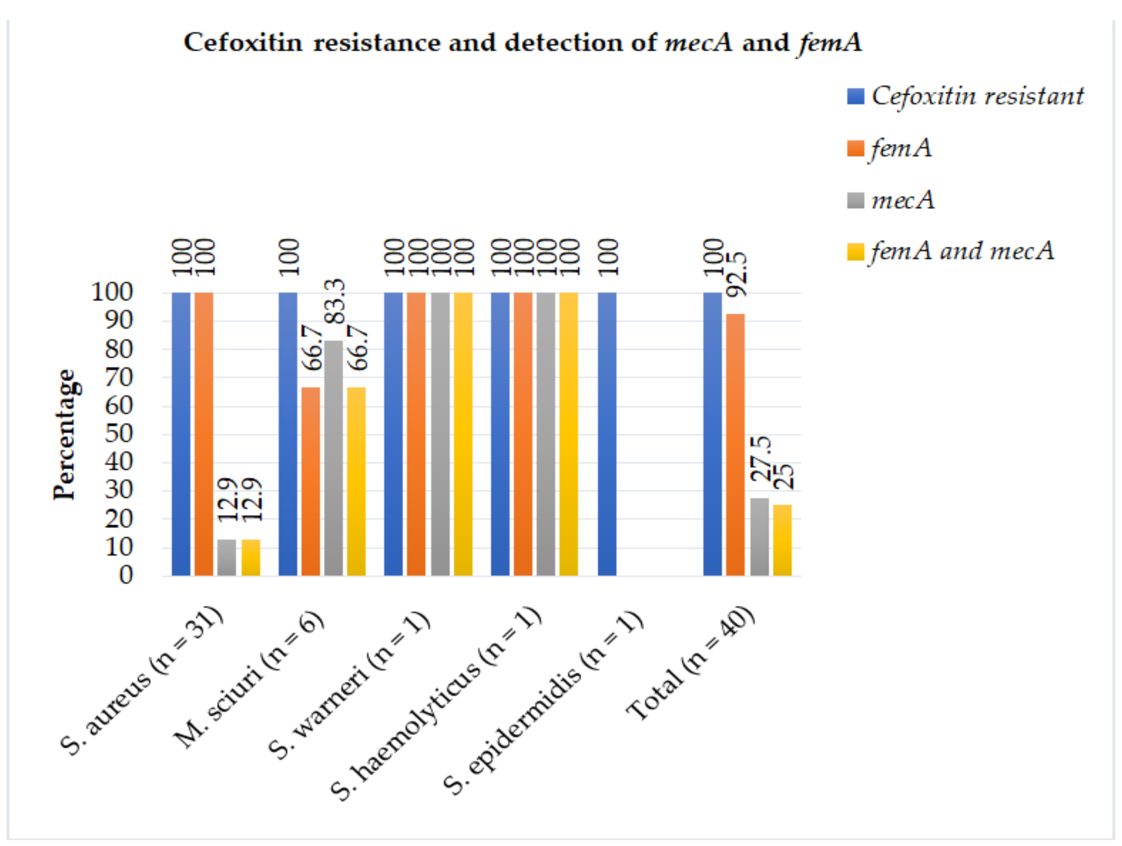
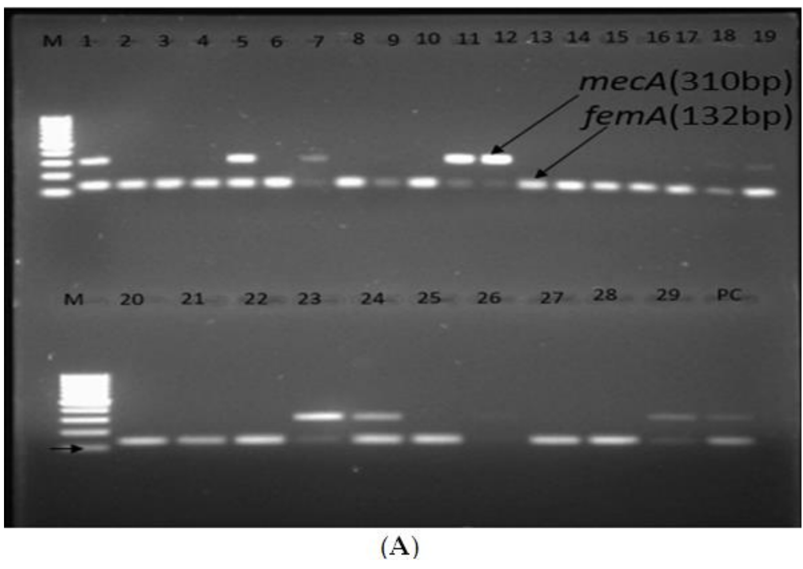
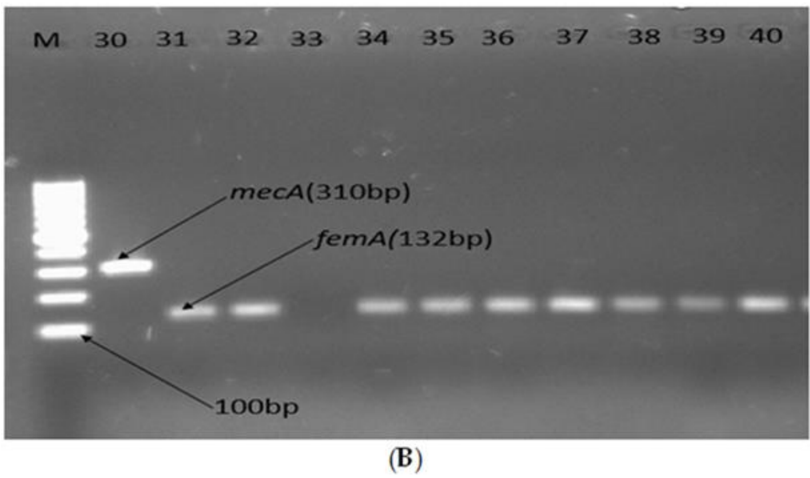


Figure 4.11. Frequency and distribution of cefoxitin resistance and PCR-confirmed genes among each Staphyloccocci isolate in patient diagnosed with surgical site infection, between July 2020 and August 2021.



(A)



(B)

Figure 4.12. (A and B) Agarose gel electrophoresis showing bands of *femA* and *mecA* genes of methicillin-resistant Staphylococci strains from patients' daignosed with surgical site infection at four different hospitals in Ethiopia, between July 2020 and August 2021

Lane M1:100bp molecular weight ladder; lane PC: positive control; lanes 1-40 are tested isolates, and positively amplified *femA* and *mecA* as indicated by 132bp and 310bp PCR amplicons, respectively.

The 11 isolates that contained the *mecA* gene, as shown in Figure 4.13A and B, were *S. aureus* (lanes 1, 5, 18, and 19), *M. sciuri* (lanes 7, 23, 24, 29, and 31), *S. hemolyticus* (lane 11), and *S.*

warneri (lane 12). These were analyzed for *vanA* and *vanB*, and none of these isolates showed *vanA* or *vanB* in the gel electrophoresis

Table 4.8. Presentation of the cefoxitin and vancomycin resistance patterns of *mecA* carrying Staphylococci, and the distribution of *femA* and van genes among patients diagnosed with SSI at four different hospitals in Ethiopia between 2020 and 2021.

Lane (*mecA*pos)	MALDI-TOF MS	Study Site	Cefoxitin	Vancomycin	*femA*	*mecA*	*vanA* and *vanB*
1	*S. aureus*	DTCSH	R	S	Pos	Pos	Neg
5	*S. aureus*	JUMC	R	S	Pos	Pos	Neg
7	*M. sciuri*	DTCSH	R	S	Pos	Pos	Neg
11	*S. haemolyticus*	DTCSH	R	R	Pos	Pos	Neg
12	*S. warneri*	DTCSH	R	S	Pos	Pos	Neg
18	*S. aureus*	TASH	R	S	Pos	Pos	Neg
19	*S. aureus*	HUCSH	R	S	Pos	Pos	Neg
23	*M. sciuri*	DTCSH	R	S	Pos	Pos	Neg
24	*M. sciuri*	DTCSH	R	S	Pos	Pos	Neg
29	*M. sciuri*	DTCSH	R	S	Pos	Pos	Neg
31	*M. sciuri*	DTCSH	R	S	Neg	Pos	Neg

Most of the isolates carrying both the *mecA* and *femA* gene were reported from Debre Tabor (Figure 4.14). At Debre Tabor Comprehensive Specialized Hospital, 72.7% of *mecA*-positive, 70% of cefoxitin-resistant, and 67.7% of *femA*-positive Staphylococci were discovered (Figure 4.14).

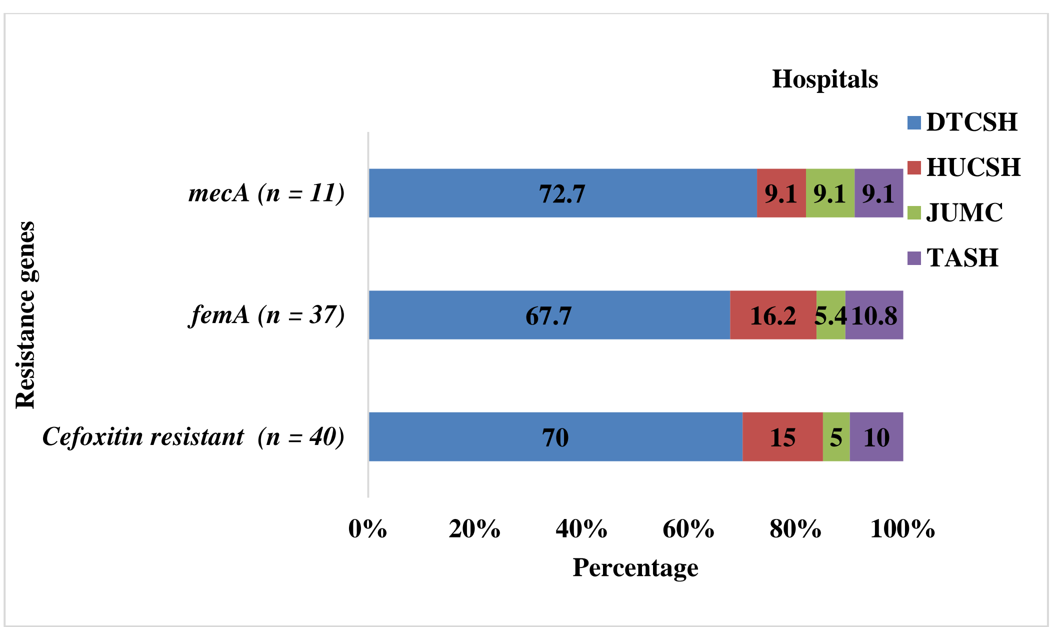


Figure 4.13: Frequency and distribution of cefoxitin-resistant isolates and *mecA,* and *femA* genes from the total number of Staphylococci and *M. sciuri* isolates at each hospital, between July 2020 and August 2021

DTCSH; Debre Tabor Comprehensive Specialized Hospital; **HUCSH**: Hawassa University Comprehensive Specialized Hospital: **JUMC**; Jimma University Medical Center; **TASH**; Tikur Anbessa Specialized Hospital

4.3. Molecular Epidemiology of Extended-Spectrum Beta-Lactamase Producing Gram-negative bacteria

4.3.1. Socio-demographic caracterstics of Extended-Spectrum Beta-Lactamase Producing Gram-negative bacteria

In the present study, a total of 752 patients investigated for surgical site infection and 286 were Gram-negative bacteria of these 135 were subjected to WGS. The male participants were 54.8% while the females were 45.2% (Table 4.9). The number of patients from Hawassa Comprehensive Specialized Hospital was 26, and the numbers from Debre Tabor Comprehensive Specialized Hospital, Jimma University Specialized Hospital, and Tikur Anbessa Specialized Hospital were 29, 40 and 40, respectively (Table 4.9).

Table 4.9. Socio-demographic data of the patients investigated for surgical site infection at four different hospitals in Ethiopia.

Variables	Characteristics	Total N (%)
Sex	Male	74 (54.8)
	Female	61 (45.2)
Age (in years)	<= 18	34 (25.2)
	19-40	62 (45.9)
	41-60	29 (21.5)
	≥61	10 (7.4)
Hospital	HUCSH	26 (19.3)
	DTCSH	29 (21.5)
	JUMC	40 (29.6)
	TASH	40 (29.6)
Preoperative hospital stays	≤ 7	57 (42.2)
	>7	78 (57.8)
Previous use of antibiotics	Yes	78 (57.8)
	No	57 (42.2)
Nature of surgery	Elective	39 (28.8)

	Emergency	96 (71.1)
Duration of operation	≤ 1hr	57 (42.2)
	>1hrs	78 (57.8)

4.3.2. Frequencies and Distributions of GNB at different Hospitals

From the 752 patients, wound cultures were performed at each study site, and a total of 286 GNB isolated from all the study sites and among them 135 were subjected for whole genome sequencing (WGS) (Table 4.10).

Table 4.10. Frequency and distribution of GNB isolated from the patients investigated for SSI and subjected for whole genome sequence in four Ethiopian hospitals

Gram- negative bacteria	DTCSH n (%)	HUCSH n (%)	JUMC n (%)	TASH n (%)
Escherichia coli (n=39)	11 (28.2)	9 (23.1)	10 (25.6)	9 (23.1)
Acinetobacter baumannii (n=38)	4 (10.5)	12 (31.6)	9 (23.7)	13 (34.2)
Klebsiella pneumonia (n=24)	7 (29.2)	2 (8.3)	6 (25)	9 (37.5)
Enterobacter cloacae (n=6)	1 (16.7)	1 (16.7)	2 (33.3)	2 (33.3)
Shigella disentriae (n=6)	2 (33.3)	-	1 (16.7)	3 (50)
Pseudomonas aeruginosa (n=6)	1 (16.7)	1 (16.7)	2 (33.3)	2 (33.3)
Enterobacter hormaechei (n=4)	-	1 (25)	3 (75)	-
Acinetobacter pittii (n=2)	-	-	1 (50)	1 (50)
Acinetobacter soli (n=2)	-	-	2 (100)	-
Citrobacter 1sedlakii(n=1)	-	1 (100)	-	-
Acinetobacter lactucae (n=1)	-	-	1 (100)	-
Pantoea ecurina (n=1)	-	-	1 (100)	-
Alcalignes faecalis (n=1)	-	1 (100)	-	-
Pseudomonas plecoglossicida (n=1)	-	1 (100)	-	-

Morganella morganii (n=1)	-	-	-	1 (100)
Enterobacter asburiae (n=1)	-	-	1 (100)	-
Enterobacter bugandensis (n=1)	-	-	-	1 (100)
Total (n =135)	**26 (19.3)**	**29 (21.5)**	**39 (28.9)**	**41 30.4)**

4.3.3. Detection of *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$

Among all Gram-negative bacteria, *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ genes were detected in 50.8%, 40.1% and 8% of the isolates at least once (Figure 4.15). Among the GNB isolated at each hospital, the detection of *bla*$_{CTX-M}$ at DTCSH, HUCSH, JUMC, and TASH was 21.1%, 19.7%, 39.4%, and 19.7% respectively. The *bla*$_{TEM}$ gene was detected in 30.9% of samples at JUMC and 25.5% of the samples at DTCSH. At TASH, blaSHV (54.5%) detection was higher while none was detected at HUCSH (Figure 4.15).

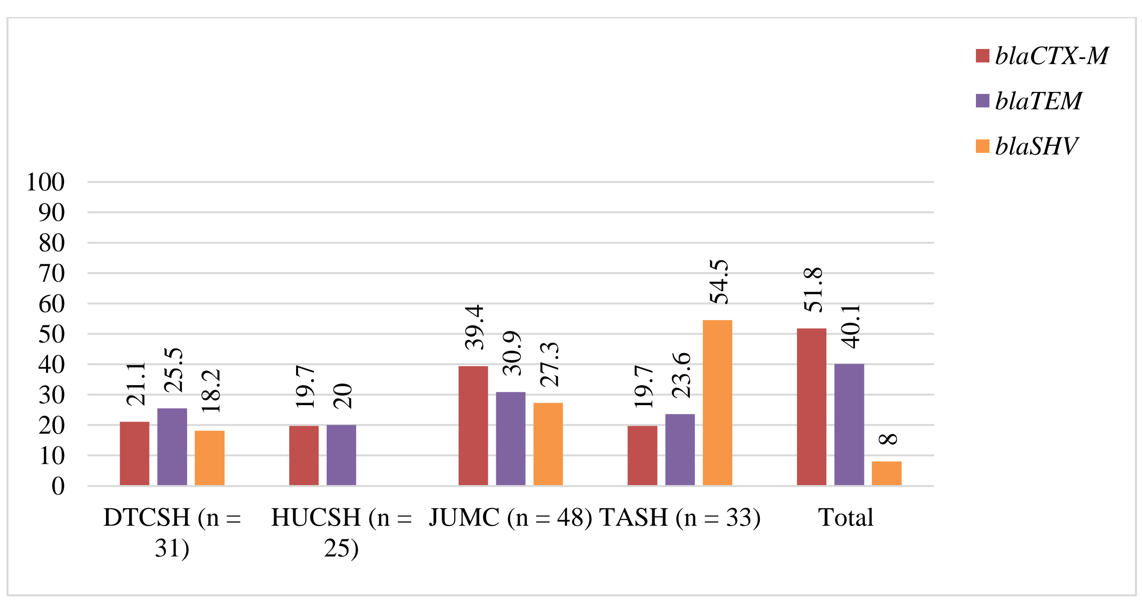


Figure 4.14. Frequency of *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ families detected at least once from GNB subjected to WGS per study site. DTCSH: Debre Tabor Comprehensive Specialized Hospital, HUCSH: Hawassa University Comprehensive Specialized Hospital, JUMC: Jimma University Medical Center, and TASH: Tikur Anbessa Specialized Hospital**.**

E. coli was the most frequent isolate and harbored high frequencies of bla_{CTX-M} (52.2%), bla_{TEM} (40.3%) and bla_{SHV} (8.2%) genes (Figure 3.16). Similarly, most GNB were found to have bla_{CTX-M} and bla_{TEM} gene families with different detection rates between the strains. The detection of blaSHV in *E. cloacae* (42.9%), *K. pneumoniae* (20.8%) and *S. dysenteriae* (16.7%) was high, while there was low detection of bla_{SHV} among *E. coli* (5.1%). No bla_{CTX-M}, bla_{TEM} and bla_{SHV} genes were detected in the rare isolates of *Alcalignes faecalis, Acinetobacter soli, Pseudomonas plecoglossicida, Morganella morganii, Enterobacter asburiae and Enterobacter bugandensis* (Figure 4.16).

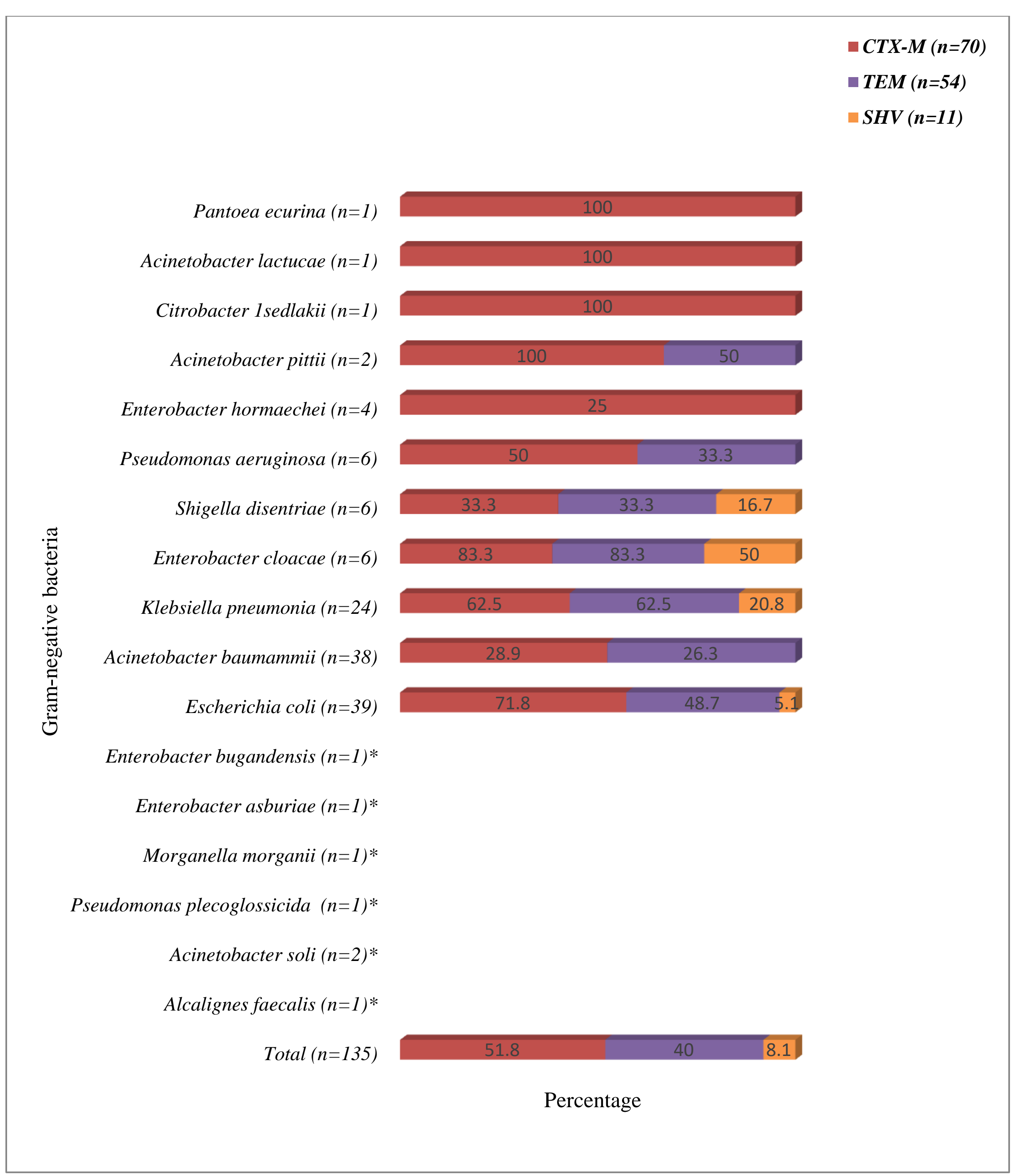
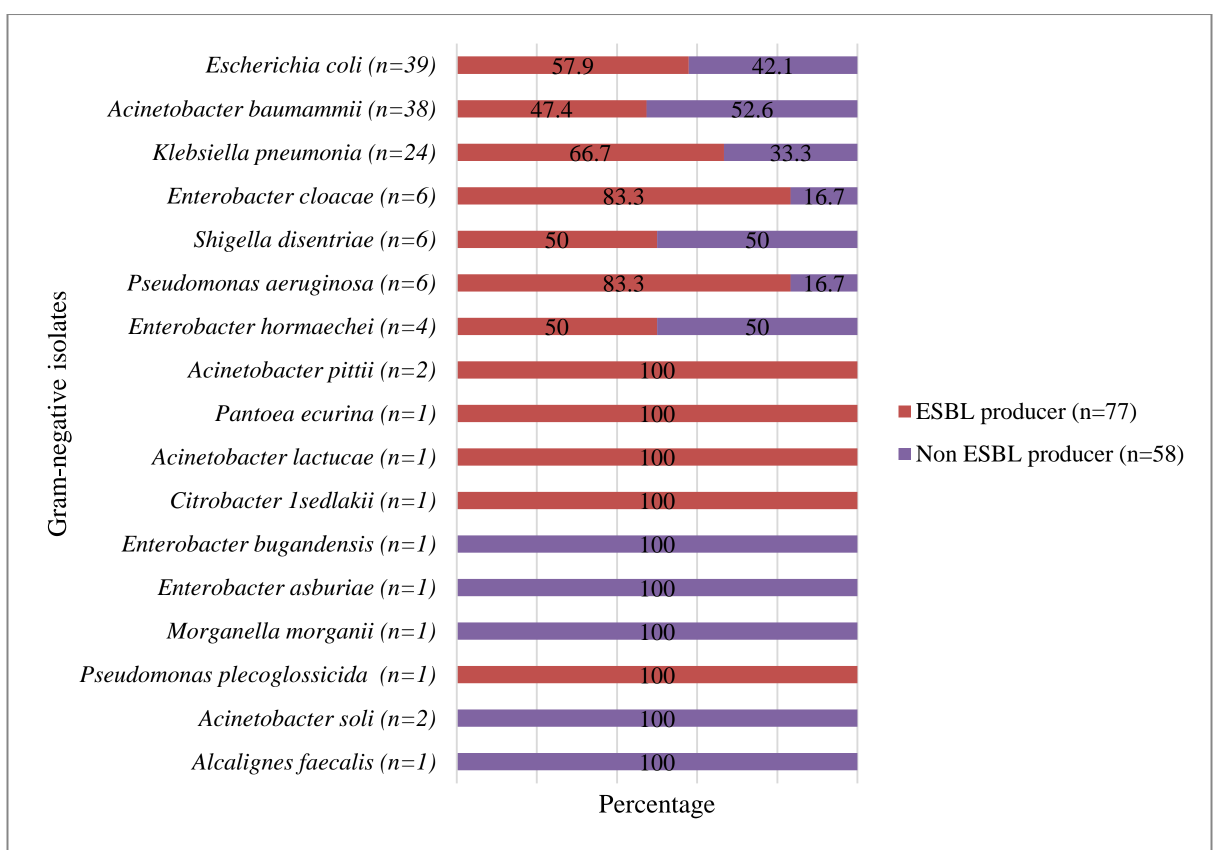


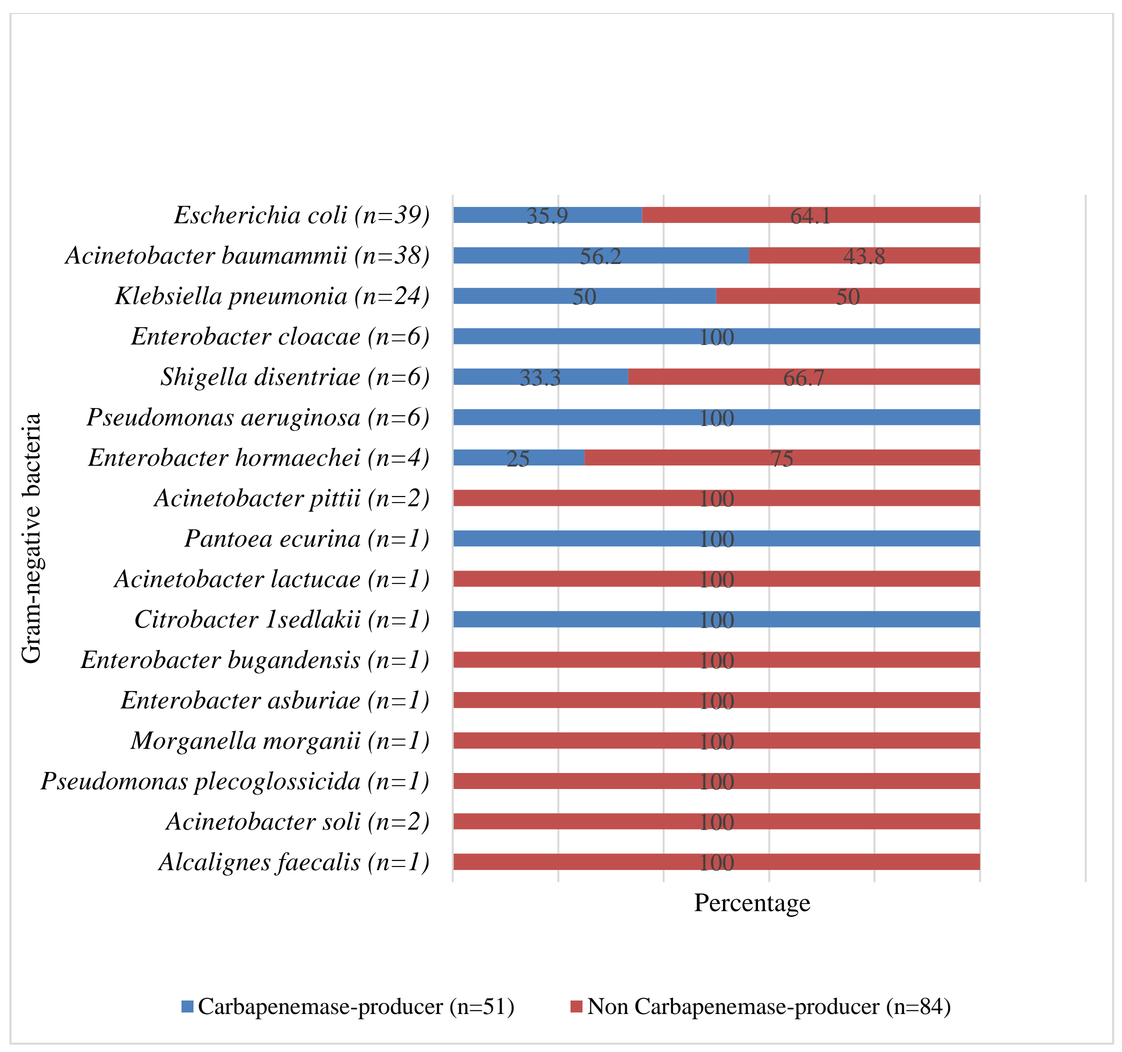
Figure 4.15. Frequency and distribution of Gram-negative bacteria that encoded *bla* $_{CTX-M}$, *bla* $_{TEM}$ and *bla* $_{SHV}$ genes detected using the whole genome sequence. * No *bla* $_{CTX-M}$, *bla* $_{TEM}$ and *bla* $_{SHV}$ detected.

4.3.4. Molecular Epidemiology of ESBL Producing Gram-negative bacteria

Of 135 GNB subjected to WGS, 57.8% encoded at least one ESBL gene. At least one ESBL gene was detected among 83.3% of *Pseudomonas aeruginosa,* 83.3% of *E. cloacae,* and 66.7% *Klebsiella pneumoniae*, 57.9% *E. coli* and 42.1% of *Acinetobacter baumannii* (Figure 4.17 A). In addition, 37.8% encoded at least one carbapenemase gene. Of these at least one carbapenemase gene was detected among 100% of *Pseudomonas aeruginosa,* and *E. cloacae* each, 56.2% *A. baumannii*, 50% *K. pneumoniae*, and 35.9% of *E. coli* (Figure 4.17B).



A



B

Figure 4.16. Frequency and distribution of Gram-negative bacteria that are ESBL (A) and carbapenemase producers and non-producers (B)

The frequencies of ESBL producing GNB detected at DTCSH, HUCSH, JUMC and TASH were 21.5%, 19.3%, 29.6% and 29.6%, respectively. Among the patients who showed wound culture positivity for GNB, the possible risk factors for the increased ESBL producing GNB were assessed, and the multivariate analysis showed statistically significant association only between the previous use of antibiotics (AOR = 27 (3-250); 95% CI; *p-value* = 0.003) independent variables and frequencies of ESBL producing GNB (Table 4.11).

Table 4.11. Frequency of Gram-negative bacteria that harbored at least one ESBL gene in relation to patient characteristics

Variables	Characte-ristics	Total	ESBL gene		CRO (95% CL)	P-value	AOR (95% CL)	P-value
		N (%)	Positive n(%)	Negative n(%)				
Sex	Male	74 (54.8)	35 (47.3)	39 (52.7)	.63(.31-1.3)	0.2	0.616 (0.29-1.31)	0.207
	Female	61 (45.2)	39 (63.9)	22 (36.1)				
Age (in years)	<= 18	34 (25.2)	17 (72.7)	17(27.3)	1			
	19-40	62 (45.9)	26 (78.9)	36 (21.1)	1.38(0.59-3.21)	0.4		
	41-60	29 (21.5)	10 (88.9)	19 (11.1)	1.9(0.69-5.26)	0.2		
	≥61	10 (7.4)	6 (60)	4 (40)	1.5 (0.39-6.28)	0.5		
Hospital	DTCSH	29 (21.5)	14 (48.3)	15 (51.7)				
	HUCSH	26 (19.3)	16 (61.5)	10 (38.5)				
	JUMC	40 (29.6)	31 (77.5)	9 (22.5)				
	TASH	40 (29.6)	17 (42.5)	23 (57.5)				
Preoperative hospital stays	≤ 7	57 (42.2)	43 (75.4)	14 (24.5)	1			
	>7	78 (57.8)	69 (88.5)	9 (11.5)	2.49 (.99-6.26)	0.01	0.223(0.27-1.92)	0.175
Previous use of antibiotics	Yes	78 (57.8)	74 (74.9)	4 (5.1)	8.54 (2.70-26.98)	0.00	27 (3.0-250)	**0.003**
	No	57 (42.2)	39 (68.4)	18 (31.8)	1			
Nature of surgery	Elective	39 (28.8)	25 (64.1)	14 (35.9)	0.96 (.32-1.49)	0.3		
	Emergency	96 (71.1)	53 (55.2)	43 (44.8)				
Duration of operation	≤ 1hr	57 (42.2)	27 (47.4)	30 (52.6)	1.1 (.55 -2.21)	0.8		
	>1hrs	78 (57.8)	45 (57.7)	33 (42.3)				

4.3.5. ESBL Genes

Several variants of bla_{CTX-M} were detected among the GNB sequenced from all the study sites (Table 4.12). The most frequent gene was $bla_{CTX-M-15}$ with an overall detection rate of 44.4%. The frequency of $bla_{CTX-M-15}$ at HUCSH, DTCSH, TASH, and JUMC was 15%, 23.3%, 20% and 41.7%, respectively. On the other hand, in addition to $bla_{CTX-M-15}$, three other bla_{CTX-M} variants were found at HUCSH while $bla_{CTX-M14b}$ found in all hospital sites (Table 4.12). *E. cloacae* (66.7%) were the most common $bla_{CTX-M-15}$ producer followed by *K. pneumoniae* (62.5%). On the other hand, *E. cloacae*, *K. pneumoniae, A. baumannii,* and *E. coli* harbored *blaTEM-1B* with the frequencies of 66.7%, 50%, 23.7% and 16% (Figure 4.18).

The detection of $bla_{CTX-M-15}$ from, *E. coli, S. disentriae,* and *A. baumannii* was 56.4%, 33.3%, and 21%, respectively. The detection of $bla_{SHV-187}$ from *E. cloacae* was 50% followed by 20.8% *K. pneumonia* (Figure 4.18). The $bla_{SHV-55,114,142,\ 64,184,186,194\ and\ 210}$ was another ESBL gene detected at 4.2 % frequency from a single *K. pneumoniae* at TASH only. The bla_{ACT-15} and bla_{ACT-16}, bla_{GES-11}, bla_{PAO} and bla_{CMY-72} were the other rare ESBL genes detected (Table 4.12).

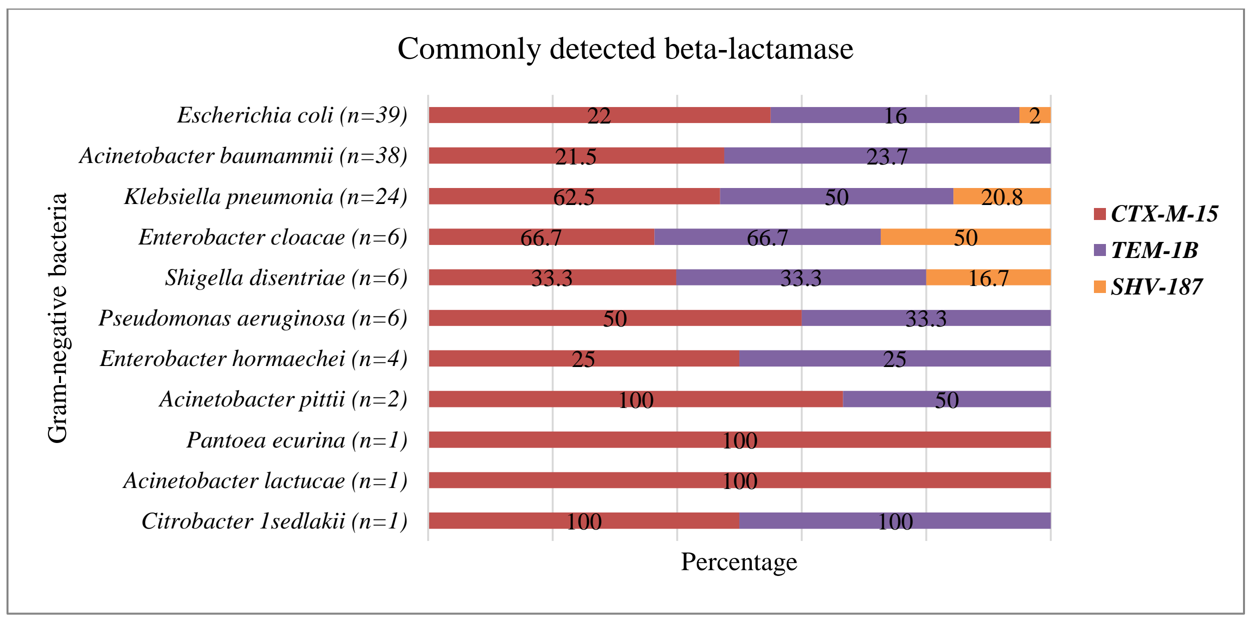


Figure 4.17. Frequency and distribution of $bla_{SHV-187}$, $bla_{CTX-M-15}$ and bla_{TEM-1B} that were detected frequently among Gram-negative bacteria

Table 4.12. **Frequency and distribution of ESBL-encoding genes detected at four Ethiopian hospitals**

Gene		**Hospitals**				**Gram-negative isolates harboring ESBL genes**											
ESBL Genes	**Total**	**DTCSH n (%)**	**HUCSH n (%)**	**JUMC n (%)**	**TASH n (%)**	***E. coli* n (%)**	***A. baumannii* n (%)**	***K. Pneumoniae* n (%)**	***E. Cloacae* n (%)**	***S. disentriae* n (%)**	***P. aeruginosa* n (%)**	***E. hormaechei* n (%)**	***A. pittii* n (%)**	***P. ecurina* n (%)**	***A. lactucae* n (%)**	***C. 1sedlakii* n (%)**	***P. plecoglossicida* n (%)**
$bla_{CTX-M-15}$	60 (44.4)	14 (23.3)	9 (15)	25 (41.7)	12 (20)	22 (56.4)	8 (21)	15 (62.5)	4(66.7)	2 (33.3)	3 (50)	1 (25)	2 (100)	1 (100)	1 (100)	1 (100)	
$bla_{CTX-M-14b}$	8(5.9)	1 (12.5)	3 (37.5)	3 (37.5)	1 (12.5)	4 (10.3)	2 (5.3)	1 (4.2)	1 (16.7)								
$bla_{CTX-M-2}$	2(1.5)		1 (50)		1 (50)	1 (2.6)	1(2.6)										
$bla_{CTX-M-27}$	1(0.7)		1(100)			1 (2.6)											
$bla_{TEM-169}$	3(2.1)		1 (33.3)		2 (66.7)	1 (2.6)	1 (2.6)	1 (4.2)									
bla_{SHV-55}	1(0.7)				1(100)			1 (4.2)									
$bla_{SHV-114}$	1(0.7)				1(100)			1 (4.2)									
$bla_{SHV-142}$	1(0.7)				1(100)			1 (4.2)									
$bla_{SHV-164}$	1(0.7)				1(100)			1 (4.2)									
$bla_{SHV-184}$	1(0.7)				1(100)			1 (4.2)									
$bla_{SHV-186}$	1(0.7)				1(100)			1 (4.2)									
$bla_{SHV-194}$	1(0.7)				1(100)			1 (4.2)									
$bla_{SHV-210}$	1(0.7)				1(100)			1 (4.2)									
bla_{CMY-72}	2(2.1)	1 (50)	1 (50)			1 (2.6)											1 (100)
bla_{ACT-15}	2(2.1)	1 (50)		1 (50)		1 (2.6)									1 (100)		
bla_{ACT-16}	2(2.1)			2 (100)			1 (2.6)					1 (25)					
bla_{PAO}	3(2.2)			2 (66.7)	1(33.3)						2 (33.3)			1 (100)			
bla_{GES-11}	4 (3)	1 (25)	2 (50)		1 (25)	1 (2.6)	2 (5.3)	1 (4.2)									

4.3.6. Carbapenemase-producing Genes

Several variants of carbapenemase producing genes were detected among the GNB sequenced from all the study sites (Table 4.13). The most frequent gene was bla_{OXA-1} with an overall detection rate of 29.6%. The frequency of bla_{OXA-1} at TASH, HUCSH, DTCSH, and JUMC was 15%, 17.5%, 27.5% and 40%, respectively. On the other hand, in addition to bla_{OXA-1}, seven other bla_{OXA} variants including $bla_{OXA-23,}$ $bla_{OXA-58,}$ $bla_{OXA-66,}$ $bla_{OXA-69,}$ and $bla_{OXA-180,}$ were detected. From all these variants except $bla_{OXA-180}$ was detected only from TASH while bla_{OXA-23} was detected from all hospital sites (Table 4.13). Among the isolates *E. cloacae* (66.7%) were the most common bla_{OXA-1} gene carrier followed by *K. pneumoniae* (45.8%) and *A. baumannii* (21%). The second predominant carbapenemase genes were bla_{NDM} carried 16 (11.9%) of GNB isolates and *E. cloacae*, *A. baumannii, K. pneumoniae,* and *E. coli* harbored bla_{NDM} with the frequencies of 33.3%, 31.6%, 4.1% and 1.2% respectively (Table 4.13). Among the variants of bla_{NDM} gene, the $bla_{\mathrm{NDM-1}}$ was the common (75%, 12/16) variant detected and it was detected from *A. baumannii* (Table 4.13).

Table 4.13. Frequency and distribution of carbapenemase-encoding genes detected at four Ethiopian hospitals.

CARB Genes	Total	**Hospitals**				Gram-negative isolates harboring carbapenemase-producing genes											
		DTCSH n (%)	HUCSH n (%)	JUMC n (%)	TASH n (%)	*E. coli* n (%)	*A. baumannii* n (%)	*K. Pneumoniae* n (%)	*E. Cloacae* n (%)	*S. dysentriae* n (%)	*P. aeruginosa* n (%)	*E. Hormae-chei* n (%)	*A. pittii* n (%)	*P. ecurina* n (%)	*A. lactucae* n (%)	*C. Isedlakii* n (%)	*P. plecoglossicida* n (%)
bla_{OXA-1}	40 (29.6)	11 (27.5)	7 (17.5)	16 (40)	6 (15)	13(33.3)	8 (21)	11 (45.8)	4(66.7)	2 (33.3)	2(33.3)	1 (2.5)		1 (100)		1 (100)	
bla_{OXA-23}	8 (5.9)	1 (12.5)	2 (25)	2(25)	3 (37.5)		8(100)										
bla_{OXA-58}	2 (1.5)		2 (50)		1 (50)		2(100)										
bla_{OXA-66}	1 (0.7)		2(33.3)	2 (33.3)	2 (33.3)		6(100)										
bla_{OXA-69}	9 (6.7)	1 (11)	5 (56)		3 (33)		8 (100)										
$bla_{OXA-180}$	2 (1.5)		2(100)				2 (100)										
$bla_{OXA-396}$	6 (4.4)	1(16.7)		4 (66.7)	1(16.7)		1 (2.6)				4(66.7)			1 (100)			
$bla_{OXA-494}$	6 (4.4)	1(16.7)		4(66.7)	1(16.7)		1 (2.6)				4(66.7)			1 (100)			
bla_{NDM-1}	12 (9)	1 (8.3)	4 (33.3)	2 (16.6)	5 (41.7)		11 (28.9)		1 (16.7)								
bla_{NDM-5}	4 (3)	1 (25)	2 (50)		1 (25)	1(2.3)	1 (2.6)	1 (4.2)	1 (16.7)								

4.3.7. Non-ESBL β-Lactamase Variants of *blaTEM* and *blaSHV*

The bla_{TEM} gene was detected 52 times across the four hospitals and of the bla_{TEM} gene variants bla_{TEM-1B} variant was the most common (90.4%, 47/52). There is not much difference in the deteaction rate of these genes among the different hospitals as shown in Table 3.14. Among the bla_{SHV} variants that are not ESBL, the most commonly detected was $bla_{SHV-187}$ (8.1%) that was identified at TASH (54.5%) at JUMC (27.3%), and at DTCSH (18.1%). This variant was not detected from HUCSH. The other bla_{SHV} variant was only detected from TASH. The common pathogen that hebor *blaSHV-187* were *E. cloacae* (50%) followed by *K. pneumoniae* (20.8%), while $bla_{SHV-187}$ was rare among *E. coli* (5.1%) (Table 4.14).

Table 4.14. Frequency and distribution of bla_{TEM} and bla_{SHV} genes and their variants detected from the four Ethiopian hospitals.

Gene variants	Genes	Hospitals n %				
		Total	DTCSH	HUCSH	JUMC	TASH
TEM variants detected	bla_{TEM-1B}	47 (34.8)	11(23.4)	9 (19.1)	17 (36.2)	10 (21.3)
	bla_{TEM-33}	3 (2.1)	1 (33.3)	1 (33.3)		1 (33.3)
	$bla_{TEM-219}$	1 (0.7)	1 (100)			
	bla_{TEM-1C}	1 (0.7)	1 (100)			
SHV variants detected	$bla_{SHV-187}$	11 (8.1)	2 (18.1)	-	3 (27.3)	6 (54.5)
	bla_{SHV-82}	1(0.7)				1(100)
	$bla_{SHV-139}$	1(0.7)				1(100)
	$bla_{SHV-163}$	1(0.7)				1(100)
	$bla_{SHV-182}$	1(0.7)				1(100)
	$bla_{SHV-187}$	1(0.7)				1(100)

4.3.8. Co-Occurrence of Multiple ESBL Genes

Multiple ESBL genes co-occurring or one ESBL gene co-occurring with additional bla_{TEM} and bla_{SHV} variations were found in various Gram-negatives (Table 3.14). It was discovered that GNB carrying the $bla_{CTX-M-15}$ gene also harboured non-ESBL variations of bla_{TEM} and bla_{SHV}, as

well as multiple more ESBL genes. The co-occurrence of $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1B}$ (n = 21) was the most frequent gene combination followed by $bla_{CTX\text{-}M\text{-}14B}$ and $bla_{TEM\text{-}1B}$. While the 3 combinations of $bla_{SHV\text{-}187}$ * $bla_{TEM\text{-}1B}$ * $bla_{CTX\text{-}M\text{-}15}$ were detected among 6 GNB, the 4 *CTX-M-15 * TEM-1B *ACT-15*CMY72* gene combination was detected from 1 *E. coli* at DTCSH (Table 4.15).

Table 4.15. Co-occurrence of multiple ESBL genes and ESBL genes with other common non-ESBL variants of bla_{TEM} and bla_{SHV}

Combinations of genes	Total	DTCSH	HUSH	JUMC	TASH
	n	n	n	n	n
CTX-M-15*TEM-1B	21	5	4	8	4
CTX-M-15*ACT15	1			1	
CTX-M-2* CTX-M-27	1		1		
CTX-M-15*PAO	1			1	
CTX-M-14b*TEM1B	2	1	2		1
CTX-M-15*TEM1C	1	1			
$bla_{SHV\text{-}187*}$ ***CTX-M-15***	2			1	1
TEM1B*VAN	1	1			
TEM-1B *CTX-M-15*ACT-15	2	1		1	
TEM-1B *CTX-M-15*ACT-16	2			2	
TEM-33 *TEM-169*CTX-M-15	2		1		1
SHV-187*SHV-106*CTX-M-15	1	1			
SHV-187 *TEM-219*CTX-M-15	1	1			
$bla_{SHV\text{-}187*}$ ***TEM-1B*CTX-M-15***	6	2		2	2
TEM-1B *CTX-M-15*CTX-M-14b	4	1		2	
TEM-1B *CTX-M-15*ACT-15*CMY6	1	1			
SHV-55*SHV-82*SHV-114*SHV-139*SHV-142*SHV-184*SHV-186*SHV-194*SHV-210	1				1

4.4. The emergence of pan-drug resistant *Acinetobacter baumannii*

4.4.1. Socio-Demographic Characteristics of Study Participants

Acinetobacter species were identified from 43 patients. The median age of these patients was 30 years (with range from 5 days–70 years) and a total of 21 (48.8%) were females (Table 4.16). Most of the bacteria were isolated from surgical wards (21/43) and orthopedics wards (10/43) of the hospitals as shown in Table 4.16.

4.4.2. MALDI-TOF MS identification of *Acinetobacter species*

According to MALDI-TOF MS, a total of 43 *Acinetobacter species* isolated were identified from patients who were admitted to the selected hospitals in Ethiopia. From the total isolates, 38 (88.4%) were *A. baumannii*, 2 (4.7%) were *A. pittii* and *A. soli* and 1 (2.3%) were *A. lactucae* each (Table 4.16).

Table 4.16. Socio-demographic characteristics of study participants, and magnitude of isolated *Acinetobacter species* from SSI Patients at four Hospitals, Ethiopia between July 2020 and August 2021

Variables	**Characteristics**	**Total**	**Beta-lactamase gene**		**P -value**
		N (%)	Positive n (%)	Negative n (%)	
Sex	Male	22 (51.2)	19 (86.4)	3 (13.6)	0.63
	Female	21 (48.8)	16 (76.2)	5 (23.8)	
Age (in years)	<= 18	11 (25.6)	8 (72.7)	3 (27.3)	0.37
	19-40	19 (44.2)	15 (78.9)	4 (21.1)	
	41-60	9 (20.9)	8 (88.9)	1 (11.1)	
	≥61	4 (9.3)	4 (100)	0 (0)	
Ward	Pediatrics /NICU	6 (14)	5 (83.3)	1(16.7)	0.2
	ICU	2 (4.7)	1 (50)	1 (50)	
	Surgical	24(48.9)	19 (79.2)	5 (20.8)	0.28

	Orthopedics	11(23.3)	10 (90.9)	1 (9.1)	0.36
Acinetobacter species	*A. baumannii*	38 (88.4)	32 (84.2)	6 (15.8)	
	A. pittii	2 (4.7)	2 (100)	0 (0)	
	A. soli	2 (4.7)	0 (0)	2 (100)	
	A. lactucae	1 (2.3)	1 (100)	0 (0)	

n: number of Acinetobacter species; ICUs: Intensive Care Unit; NICU: neonatal intensive care unit

The majority of the *A. baumannii* isolates were identified from TASU (14/38; 36.8%) followed by and JUMC (13/38; 34.2%), while different species of Acinetobacter were detected from JUMC and TASH (Figure 4.19).

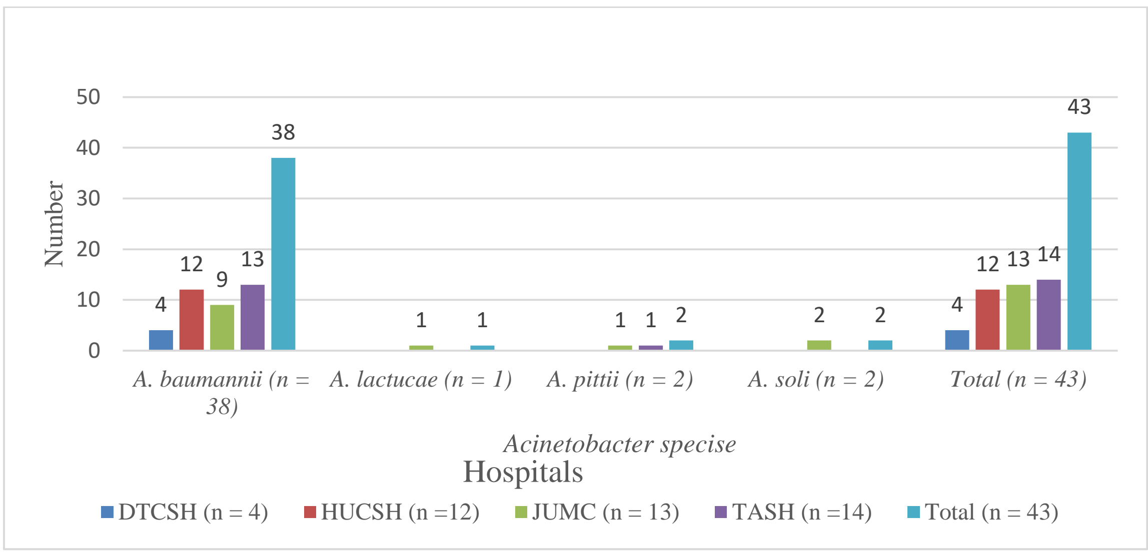


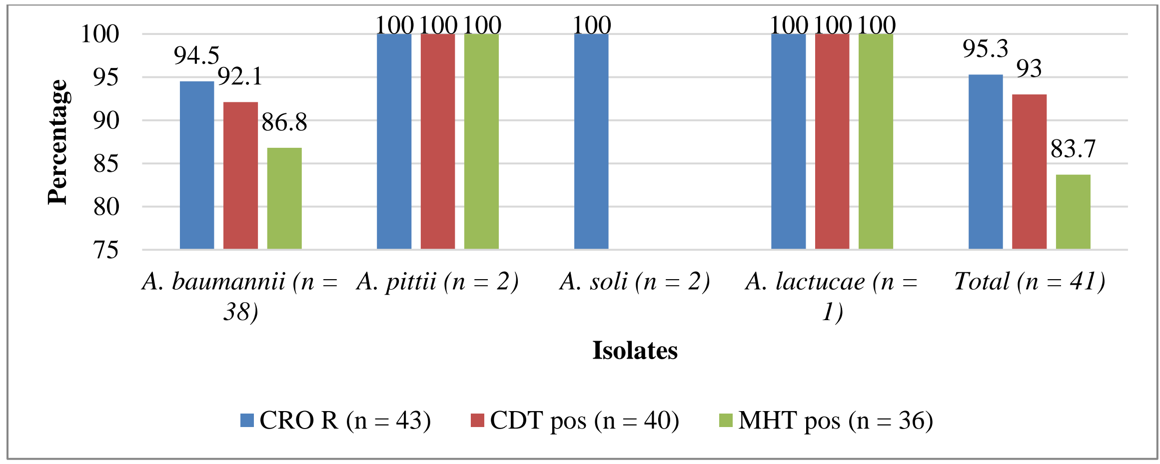
Figure 4.18. Frequency and distribution of *Acinetobacter species* isolates at each hospital in Ethiopia, between July 2020 and August 2021.

DTCSH; Debre Tabor Comprehensive Specialized Hospital; **HUCSH**: Hawassa University Comprehensive Specialized Hospital: **JUMC**; Jimma University Teaching Specialized Hospital; **TASH**; Tikur Anbessa Specialized Hospital, n: number of *Acinetobacter species*

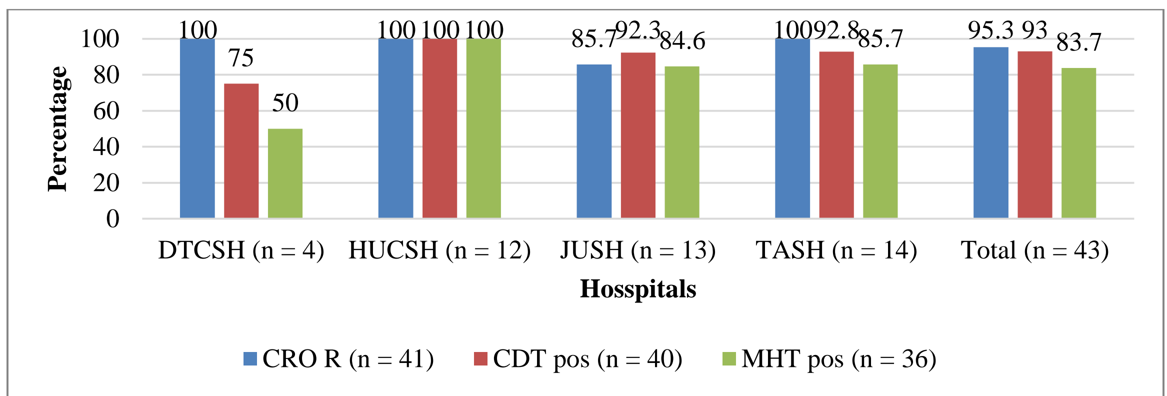
4.4.3. Prevalence of ESBL and Carbapenemase Producing Acinetobacter species

From the total 43 of *Acinetobacter species*, 95.3% (41/43) were resistant to ceftriaxone, 93% (40/43), and 83.7% (36/43) were confirmed for ESBL production by combined disk-diffusion (CDT), and carbapenemase production by modified hodge test (MHT), respectively (Figure 4.20A).

The highest frequency of ESBL and carbapenemase production was reported from HUCSH with 100% scored for ceftriaxone resistance, CDT and MHT positivity. Second was Tikur Anbessa Specialized Hospital with 100%, 92.8%, and 85.7% (Figure 4.20B).



A



B

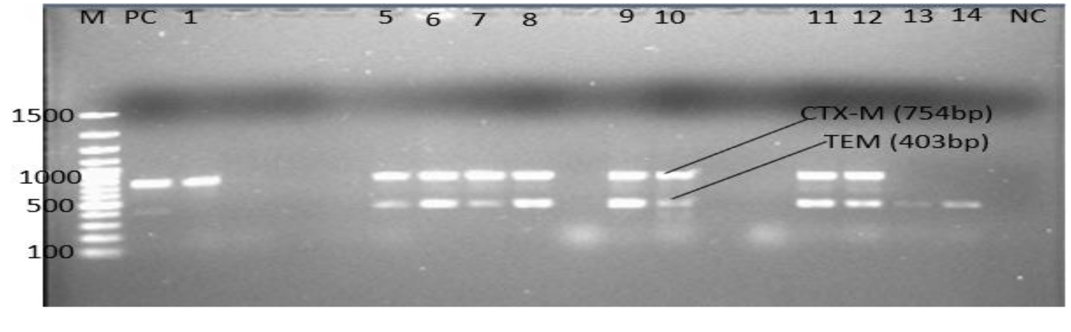
Figure 4.19. A and B. Frequency and distribution of beta-lactamase production among *Acinetobacter species* from patients investigated for surgical site infection at four different hospitals in Ethiopia, between July 2020 and August 2021**.**

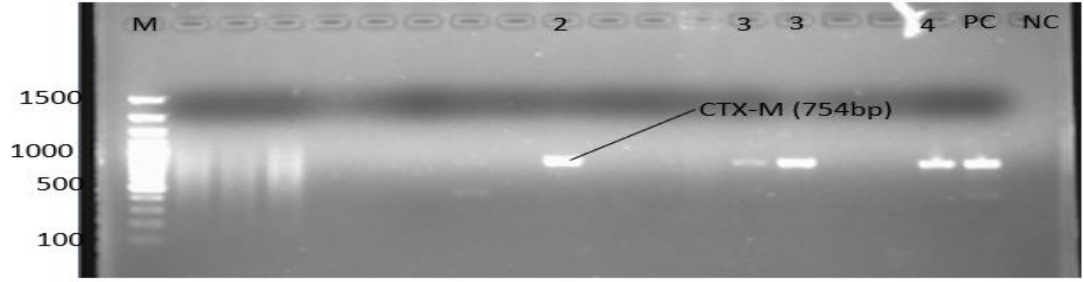
DTCSH; Debre Tabor Comprehensive Specialized Hospital; **HUCSH**: Hawassa University Comprehensive Specialized Hospital: **JUMC**; Jimma University Teaching Specialized Hospital; **TASH**; Tikur Anbessa Specialized Hospital, n: number of bacterial isolates. Abbreviations: CRO R, Ceftriaxone resistance; CDT, combination disc diffusion test; MHT, modified hodge test.

4.4.4. Detection of bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{KPC} and bla_{NDM} carbapenemase genes with PCR

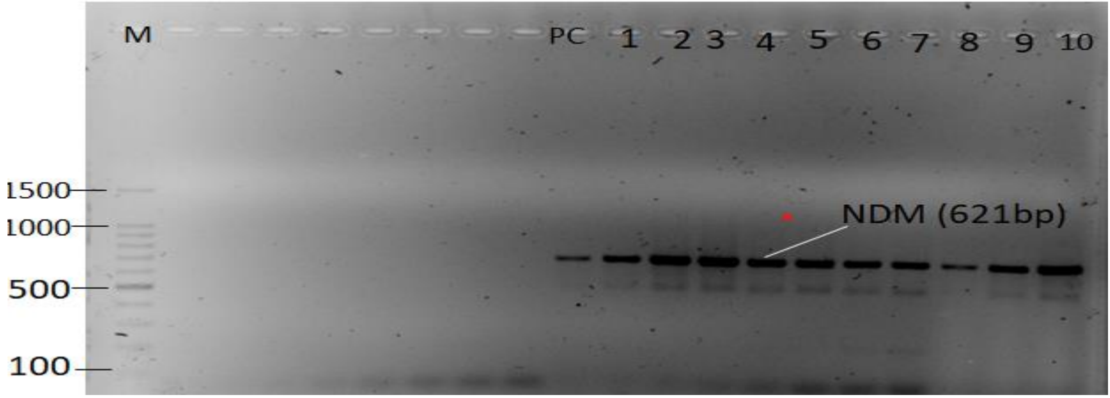
Of the 40 ESBL and carbapenemase-producing *Acinetobacter species* isolated detected with combined disk-diffusion (CDT) 12 (42.5%) were confirmed for one or more ESBL production genes by multiplex PCR. The bla_{TEM}, and $bla_{CTX\text{-}M}$ genes were detected in 10 and 12 isolates respectively, while bla_{SHV} was not detected.

Of the 40 MDR and ESBL-producing *Acinetobacter species* isolates only 12 (30%) *A. baumannii* isolates were positive carbapenemase genes in the multiplex PCR. The bla_{NDM} gene was detected in 12 isolates (only one isolate carried $bla_{\text{CTX-M}}$ and $bla_{\text{TEM-1}}$ genes) while the bla_{KPC} gene was not detected. Figure 4.21. A and B shows the gel image of the $bla_{\text{CTX-M}}$ (754bp), bla_{TEM} (403bp) and bla_{NDM} (621 bp) genes.





A



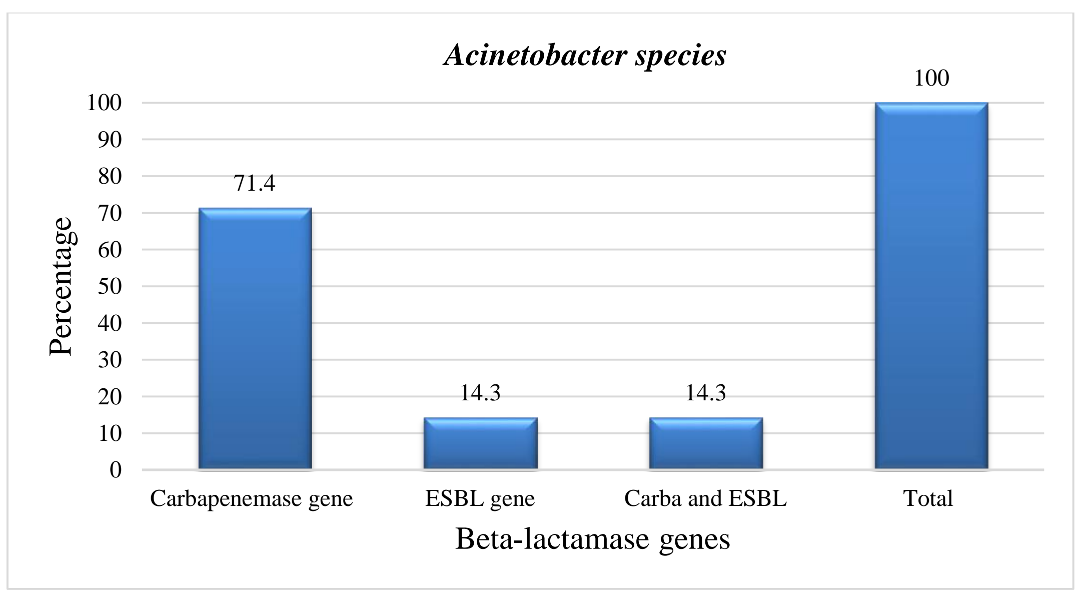
B

Figure 4.20. Gel image of the *bla*$_{CTX-M}$ (754 bp), *bla*$_{TEM}$ (403 bp) (A) and *bla*$_{NDM}$ (621 bp) genes (B). Lane M: 100bp DNA ladder, PC: Positive control, Lanes 1–14: Acinetobacter isolates, NC: Negative control.

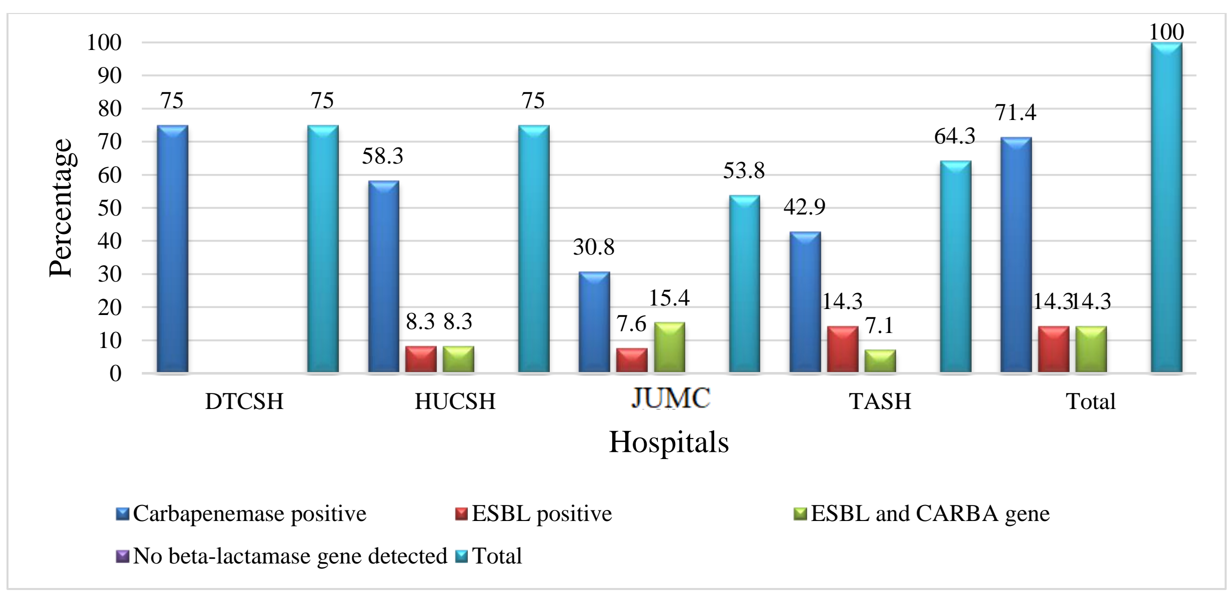
4.4.5. Detection of beta-lactamase genes

One or more beta-lactamase genes were found in 100% of *Acinetobacter species,* 71.4% had only carbapenemase, 14.3% had both carbapenemase genes and ESBL genes, and 14.3% had only ESBL genes (Fig 4.22A). In *A. baumannii*, 96.4% of the isolates harbored one or more beta-lactamase genes, and 74.1%, 11.1% and 14.8% of the isolates harbored only carbapenemase genes, only ESBL genes, and carbapenemase and ESBL genes respectively.

Among the isolates from each hospital, the total detection of one or more beta-lactamase genes were 53.8%, 64.3%, 75%, and 75% at JUMC, TASH, DTCSH, and HUCSH respectively (Figure 5B). In addition, the carbapenemase gene detection at DTCSH, HUCSH, TASH, and JUMC was 75%, 66.7%, 35.7%, and 30.8% respectively (Figure 4.22B).



A



B

Figure 4.21. Frequency (A) and distribution (B) of beta-lactamase genes from the total number of Acinetobacter species at each hospital in Ethiopia between July 2020 and August 2021

DTCSH; Debre Tabor Comprehensive Specialized Hospital; **HUCSH**: Hawassa University Comprehensive Specialized Hospital: **JUMC**; Jimma University Teaching Specialized Hospital; **TASH**; Tikur Anbessa Specialized Hospital.

4.4.6. Carbapenemase and ESBL produce genes detected by whole genome sequencing.

Out of the 40 *Acinetobacter* species that were ESBL producing as confirmed by phenotypic analysis using combination disk test (CDT), we selected 28 (27 *A. baumannii* and one *A. lactucae*) isolates and were subjected to WGS analysis for. The WGS confirmed only, 14.3% (4/28) harbored one or more ESBL genes, 71.4% (20/28) had one or more carbapenemase genes, and all (100%, 28/28) harbored d beta-lactamase genes (Table 4.17).

As shown in Table 4.16 among ESBL genes, $bla_{\text{CTX-M}}$ was the most detected genes from six isolates, five of these were $bla_{\text{CTX-M-15}}$, and one blaCTX$_{\text{-M-65}}$ (Table 4.17).

The $bla_{\text{CTX-M}}$ was detected from six isolates; two from *A. baumannii* isolates concurrently harbored $bla_{\text{OXA-1}}$ with one or more ESBL genes such as $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-65}}$, and $bla_{\text{ ACT-16}}$.

Additionally, *bla*$_{CTX-M-15}$ was carried by one *A. lactucae* concurrently with *bla*$_{ACT-15}$ genes (Table 4.17).

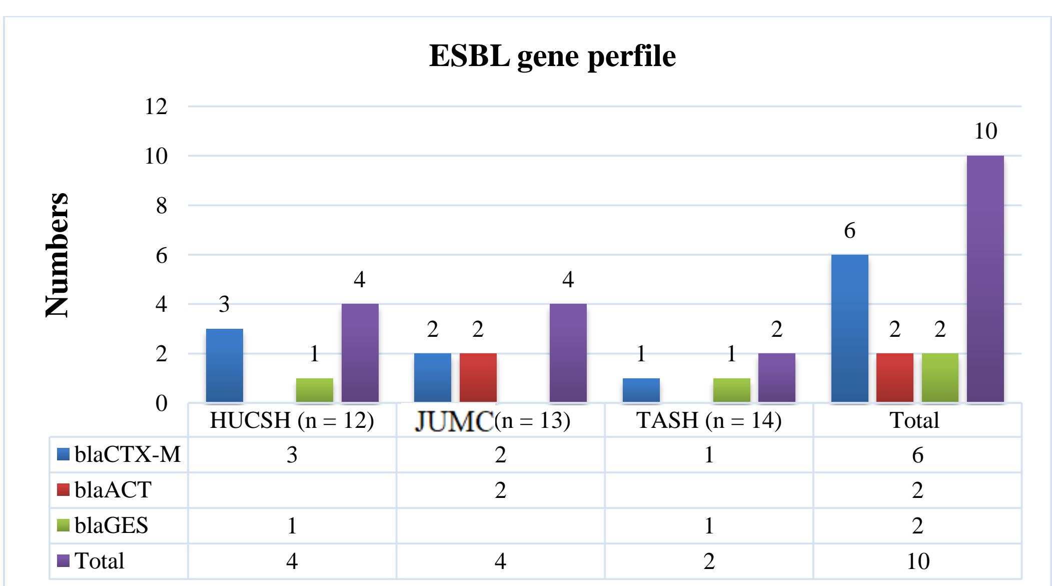
Table 4.17. Frequency and distribution of beta-lactamase gene detected among Acinetobacter species at each Ethiopian Hospital, between July 2020 and August 2021.

Isolates	*ESBL gene (n = 4/28) =14.3%*	*Carbapenamase gene (n=20/28) = 71.4%*	*ESBL and CARBA genes (n=4/28) =14.3%*	*DTCSH (n = 4)*	*HUCSH (n = 12)*	*JUMC (n = 13)*	*TASH (n = 14)*
A. baumannii (n = 4)		bla_{OXA-69}			3		1
A. lactucae (n = 1)	$bla_{CTX-M-15}$, bla_{ACT-15}					1	
A. baumannii (n = 2)	bla_{TEM-1B}, $bla_{CTX-M-15}$				1		1
A. baumannii (n = 1)	bla_{TEM-1B}, $bla_{CTX-M-15}$						1
A. baumannii (n = 1)			bla_{OXA-1}, $bla_{CTX-M-65}$			1	
A. baumannii (n = 2)			bla_{OXA-69}, $blaGES_{-11}$		1		1
A. baumannii (n = 1)		bla_{OXA-23}, $bla_{OXA-203}$		1			
A. baumannii (n = 1)		$bla_{OXA-396}$, $bla_{OXA-409}$				1	
A. baumannii (n = 3)		bla_{OXA-58}, $blaOXA_{-180}$, $blaNDM_{-1}$			2		1
A. baumannii (n = 3)		bla_{OXA-23}, $blaOXA_{-66}$, $blaNDM_{-1}$			1	1	1
A. baumannii (n = 4)		bla_{OXA-23}, $blaOXA_{-66}$, $blaNDM_{-1}$, $blaADC_{-25}$			1	1	2
A. baumannii (n = 1)			bla_{OXA-1}, bla_{TEM-1B}, $bla_{CTX-M-15}$, $blaACT_{-16}$			1	

A. baumannii (n = 2)		bla_{OXA-69}, $bla_{CARBA-5}$, $bla_{CARBA-16}$, $bla_{CARBA-49}$		1		1	
A. baumannii (n = 2)		bla_{OXA-69}, *blaNDM*$_{-1}$, *blaCARBA*$_{-5}$, $bla_{CARBA-16}$, *blaCARBA*$_{-49}$		1			1
Total (n = 28)				**3 (75%)**	**9 (75%)**	**7 (53.8%)**	**9 (64.3%)**

The bla_{TEM} alleles were detected in five isolates, from four *A. baumannii,* three isolates carried only $bla_{CTX-M-15}$ and the remaining one concurrently carried one or more ESBL or carbapenemase genes (Table 4.17).

Among the *Acinetobacter species* isolated at each hospital, the detection of bla_{CTX-M} at JUMC, TASH, and HUCSH was two, one, and one respectively while the bla_{CTX-M} allele was not detected at DTCSH. The total ESBL genes among hospitals JUMC, TASH, and HUCSH were six (50%), three (25%), and two (16.7%), respectively. At TASH bla_{TEM-1} were detected 2 (50%) concurrently with $bla_{CTX-M-15}$, moreover at JUMC and HUCSH were detected 1 (25%) at each hospital. At HUCSH and TASH, bla_{OXA-69} was detected at 1 (25%) concurrently with bla_{GES} at each hospital (Figure 4.22 and Table 4.16)



	HUCSH (n = 12)	JUMC(n = 13)	TASH (n = 14)	Total
blaCTX-M	3	2	1	6
blaACT		2		2
blaGES	1		1	2
Total	4	4	2	10

Figure 4.22. Frequency and distribution of ESBL gene detected at each Hospital in Ethiopia between July 2020 and August 2021.

DTCSH; Debre Tabor Comprehensive Specialized Hospital; **HUCSH**: Hawassa University Comprehensive Specialized Hospital: **JUMC**; Jimma University Teaching Specialized Hospital; **TASH**; Tikur Anbessa Specialized Hospital

The predominant carbapenemase genes of *bla*$_{OXA}$-type were detected in 24 (85.7%) of carbapenem resistant *A. baumannii.* Twenty isolates (20/24) carried only one or more carbapenemase genes; four of the isolates carried *bla*$_{OXA-69}$ gene and 16 isolates showed co-carriage of *bla*$_{OXA}$-type carbapenemases genes, including the combination of *bla*$_{OXA-23}$, *bla*$_{OXA-203}$ in one isolate, *bla*$_{OXA-396}$, *bla*$_{OXA-494}$ in one isolate, *bla*$_{OXA-58}$, *bla*$_{OXA-180,}$ *bla*$_{NDM-1}$ in three isolates, *bla*$_{OXA-23}$ and *bla*$_{OXA-66}$ and *bla*$_{NDM-1}$ in three isolates, *bla*$_{OXA-23}$ and *bla*$_{OXA-66}$, *bla*$_{NDM-1}$ and *bla*$_{ADC-25}$ in four isolates, *bla*$_{OXA-69}$, *bla*$_{CARBA-5}$, *bla*$_{CARBA-16}$, *bla*$_{CARBA-49}$ in two isolate, *bla*$_{OXA-69,}$ *bla* $_{NDM-5}$, *bla*$_{CARBA-5}$, *bla*$_{CARBA-16}$, *bla*$_{CARBA-49}$ in one isolate (Table 4.17).

The other carbapenemase genes in four *A. baumannii* included *bla*$_{OXA}$-type combined with one ESBL gene, and one *A. baumannii* harbored *bla*$_{TEM-1}$, *bla*$_{CTX-M-15}$ and *bla*$_{ACT-16}$ (Table 4.17).

The second predominant carbapenemase genes were *bla*$_{NDM}$ alleles carried in twelve *A. baumannii* 42.9% (six isolates also carried different alleles of *bla*$_{OXA}$ and four isolates carried concurrently *bla*$_{ADC-25}$, and two isolates concurrently harborded with *bla*$_{CARBA-5}$, *bla*$_{CARBA-16}$, *bla*$_{CARBA-49}$. The most frequent *bla*$_{NDM}$ alleles was *bla*$_{NDM-1}$ detected from twelve *A. baumannii* isolates (Table 4.17).

CHAPTER 5: DISCUSSION

In surgically treated patients, post-operative surgical site infection (SSI) is still one of the leading causes of morbidity and mortality, and it increases the cost of health care due to repeated readmission and long stay in the hospital. However, if appropriate startegies are in place SSI can be prevented. Among these startegies appropriate dosing, timing, and choice prophylactic antibiotic is one. In order to advice surgical team in Ethiopia it is crucial to identify the bacterial pathogens and choose an antibiotic that is efficient against the organism (Forrester *et al.*, 2022, Berríos-Torres *et al.*, 2017). This study, therefore, selected four big hospitals interms of their patient burden and/or volume of surgery among the public hopsitals in the different parts of Ethiopia.

In the current finding, a total of 494 different bacterial isolates were identified, and the culture-positive rate at each hospital was 78.5%, 65.3%, 65.2%, 55.7%, at DTCSH, JUMC, HUCSH, and TASH respectively. The overall culture positivity rate of SSI patients in this study is 65.5%. The estimated prevalence of SSI in Africa is 47.3% according to a systemic review and meta-analysis. The rate of HAI in East Africa is higher than the other African regions (Melariri *et al.*, 2024). The development of SSI is affected by a number of factors status of including immune status of the patient, presence of foreign body, degree of wound contamination, and use of prophylactic antibiotic. The current culture positivity rate is comparable with previous studies conducted in Jimma Ethiopia (71.7%) (Misha *et al.*, 2021a), India (68%) (Vasundhara Devi *et al.*, 2017) and Bangladesh (61.8%) (Khanam *et al.*, 2018), but lower than reports from Tikur Anbessa (75.6%) (Asres et al., 2017), Gondar (83.9%) (Mohammed *et al.*, 2017), and elsewhere (82%) (Mohammed *et al.*, 2013). The magnitude of SSI infection in these hospitals in Ethiopia is alarming and requires serious attention in this era where AMR is assumed to be the global threat.

Most SSIs are bacterial and the most common bacterial pathogens involved are MRSA, MSSA, coagulase-negative staphylococci, and enterococci (Hidron *et al.*, 2008). In this study however, we detected more gram-negative bacteria than gram positive (57.9% vs 42.1%). There wasn't much difference between the different hospitals in the detection rate of GNB. The GNB causing

SSI are mostly sourced from hospital environments and this may be related to poor hospital set-up, hygiene, lack of proper patient treatment followup, high number of patient visitors, and contamination from external environment (Ali and Al-Jaff, 2021). Similar to other study, the predmonnat GNB isolated were *E. coli*, *K. pneumoniae* and *A. baumannii* (Barry, 2021).

In line with other investigations, the predominant Gram-positive bacteria isolated were Staphylococcus (176/494) of this *S. aureus* was one of the most frequently identified followed by Entrococci. *S. aureus* account for 24% of SSI in Africa from systemic review and meta-analysis (Monk *et al*., 2024). However, there isn't much difference in the isolation rate of *S. aureus* by the different hospitals. As shown in the data there was wrong identification of some Staphylococcus as *S. aureus* using phenotypic analysis.

When it comes to the rate of bacterial isolation in this study, S. *aureus* was the highest followed by *E. coli, K. pneumoniae*, *A. baumannii, Shigella dysenteri, Entrobacter cloacae, and Pseudomonas aeroginasa.* This is in agreement to other studies in the region Africa at least for the top five isolates (Bunduki *et al.*, 2024).

Rare bacterial isolate identified as etiology of surgical site infections in this study include *R. ornithinolytica*, *S. maltophilia, A. faecalis,* and *P. tylopili*. Such newly emerging bacteria-causing infections in SSI patients may result in future challenges These species, along with *K. variicola* and *P. ecurina*, have never before been identified in patients in Ethiopia who were being evaluated for SSI. The isolation of these new SSI etiologies emphasizes the necessity of institution-based diagnostic and intervention practices. In this study, 40 MRSA bacterial isolates have been identified phenotypically. When this bacterial were analyzed with, MALDI-TOF MS only 77.5% (31/40) were identified as *S. aureus*, while six were identified as *M. sciuri,* and the other three as *S. warneri, S. epidermidis,* and *S. haemolyticus*. *S. aureus* revealed a high level of resistance to penicillin (88.6%) and ampicillin (77.3%), which was comparable to study in Turkey (Szaniawski and Spivak, 2019). *Mammaliicoccus sciuri* is a common inhabitant of mammalian skin with commensal life style but carrier of virulence and resistant genes that can be transmitted to *S. aureus* (Van der Veken *et al*., 2022).

The proportion of MRSA among the *S. aureus* isolates based on cefoxitin disk diffusions was 24.5% (40/163). MRSA was reported to be associated with 58% of SSI in Africa (Monk *et al*., 2024). A previous study conducted at Indian Hospital reported 21.7% of SSI is associated with MRSA similar to our finding (Kownhar *et al*., 2008). On the other hand, the finding showed higher frequency than earlier studies in Ethiopia from Dessie (9.8%) (Tsige *et al*., 2020) and Debre Markos (13.2%) (Kahsay *et al*., 2014), but it was below the national pooled prevalence estimate of Ethiopia (32.5%) (Eshetie *et al.*, 2016), Addis Ababa (68.4%) (Tadesse *et al*., 2018), Arba Minch (82.3%) (Mama *et al*., 2019), and Nigeria (44%) (Ibadin *et al*., 2017). Variations in MRSA prevalence across countries are influenced by demographics, antibiotic prescription policies, infection prevention and control strategies, staff and elderly hygiene education, healthcare system structure, and MRSA diagnostic facilities (Kavanagh, 2019, Al-Orphaly *et al*., 2021). The *femA* gene was detected in all *S. aureus* isolates, except two cefoxitin-resistant strains (6.7%). This finding was comparable with a study from China (Kobayashi *et al*., 1994a). Additionally, in the present study, the *femA* gene was found in *S. haemolyticus*, *S. warneri*, and 83.3% of *M. sciuri* cefoxitin-resistant strains. On the other hand, neither *mecA* nor *femA* were detected in *S. epidermidis* (Kobayashi *et al*., 1994a). The primers used should be specific to *S. aureus*; therefore, it is somewhat surprising that the two *S. aureus* lack the gene and that several other non-aureus isolate carry the gene. The explanation could be mutational changes in *S. aureus* and gene transfer to other species. All of these isolates have been sent for whole genome sequencing, and this matter will be analyzed further when the results are ready.

In the present study, the likelihood of methicillin-resistant staphylococcus SSI increased among patients aged ≥ 61 years. Similar finding has been reported in Brazil (Almeida *et al*., 2014). Previous study have suggested that patients on antibiotics, who had a previous wound infection, and with a hospital stay > 72 h showed an association with MRSA infection (Tefera *et a*l., 2021). Similarly, to our finding, previous use of antibiotics and preoperative hospital stays > 7 days demonstrated an association with methicillin-resistant Staphylococci for SSI. Unlike our study, a report by X. Yang et al. (Yang *et al*., 2021) showed that long, invasive procedures used in the ICU, such as tracheal intubation and ventilator usage, along with patients with cerebral infarction and other embolisms increase the likelihood of developing MRSA colonization and further infections.

From all methicillin-resistant Staphylococci, the *mecA* gene was carried by 27.5% of the isolates. This finding was comparable with a study from Nigeria, where 30.5% of the isolates carried the *mecA* gene (Ibadin *et al*., 2017). In the present study, 12.9% of the *S. aureus* carried the *mecA* gene, which is lower than studies reported in Ethiopia (20%) (Moges *et al.*, 2023), Nigeria (38%) (Ibadin *et al*., 2017), Bangladesh (25%) (Zahan *et al*., 2009), and Iran (45.1%) (Pournajaf *et al*., 2014). It should be noted that the majority of isolates exhibiting the *mecA* gene were discovered in Debre Tabor. Eight (72.7%) of the ten *mecA*-positive isolates were detected at Debre Tabor Hospital. This might be poor socio-economic status, personal demographics, antibiotic prescription practice, and infection control practices, which are associated with increased MRSA infection rates (Kavanagh, 2019, Al-Orphaly *et al*., 2021).

It is interesting that a significantly higher proportion of CoNS isolates harbour methicillin resistance genes, where 83.3% of *M. sciuri* and 50% of *S. haemolyticus* carried the *mecA* gene. This is in agreement with early reports that CoNS were the most common species in nosocomial infections and exhibit higher antibiotic resistance rates than *S. aureus.* This may be explained by the high prevalence of methicillin resistance linked with staphylococcal cassette chromosome (SCCmec) elements in CoNS (Garza-Gonzalez *et al*., 2010), and they are considered a major reservoir of SCCmec (Zong *et al.*, 2011). For instance, Berglund et al. described the likely transfer of a type V SCCmec from methicillin-resistant *S. haemolyticus* to MSSA, thus transforming into MRSA (Vestergaard *et al.*, 2019, Berglund and Söderquist, 2008). Another study revealed that the *mecA* homologue in *M. sciuri* may be an evolutionary precursor to MRSA pathogenic strains, highlighting the main routes of antibiotic resistance gene transfer (Wu *et al*., 2001). Furthermore, the report demonstrated that MSSA become MRSA by acquiring SCCmec from *S. epidermidis* through horizontal transfer (Bloemendaal *et al.*, 2010). These accounts suggest that horizontal interspecies transfer of mobile genetic elements could be a crucial element for MRSA global dissemination (Berglund and Söderquist, 2008, Miragaia, 2018, Wu *et al*., 2001).

The absence of the *mecA* and *vanA* genes in the MRSA and VRSA samples does not imply the absence of resistance, as resistance may be due to other mutations or cassette-containing resistance genes (Sun *et al*., 2014). Globally, resistant staphylococcal isolates lacking the *mecA* gene show the possibility for additional mechanisms to compete with *mecA* in the establishment

of MRSA (Zong, 2013, Elhassan *et al*., 2015). From those tested for vancomycin resistance, one isolate had a minimum inhibitory concentration of vancomycin greater than 8µg/mL, and it was identified as a vancomycin-resistant Staphylococcus. This result was consistent with Pournajaf et al.'s (Pournajaf *et al*., 2014) finding that vancomycin resistance was 2.5%, and this figure was lower than that from a systematic review from Ethiopia, where the pooled prevalence of VRSA was 5.3% (Eshetie *et al*., 2016), as well as the findings from Debre Markos (14.1%) (Tefera *et al*., 2021) and elsewhere (29.4%) (Alani *et al*., 2017). This difference might be due to the difference in the methodology, study site, and sample size. In the current study, the method was phenotypic, prospective cross-sectional study among all SSI patients. While the latter was on a systematic review and the other estimated only from single site surgery cases. MRSA's resistance against beta-lactams and methicillin is further complicated by its ability to develop resistance to vancomycin through accidental transmission of the *vanA* gene from Enterococcal strains (McGuinness *et al*., 2017). Vancomycin is a glycopeptide antibiotic that prevents the formation of the peptidoglycan layer by binding to the peptide precursor. Antibiotic overuse leads to bacterial resistance, thus prompting the search for new antimicrobial strategies (Ezzeddine and Ghssein, 2023). Genomics can identify antibiotic targets, and live non-multiplying bacteria can be targeted for new antibacterial, potentially resulting in new antibacterial that shorten therapy microorganisms, reduce adverse effects, and potentially reduce antibacterial resistance (Coates and Hu, 2007). Preclinical research explores metal uptake via bacterial metallophores (Ezzeddine and Ghssein, 2023). Bacteriophages have been demonstrated to be antibacterial in animals that are susceptible to certain infectious diseases (Coates and Hu, 2007).

When we look at the antimicrobial susceptibility pattern of the specific bacterial isolates, *A. baumannii* has been reported worldwide and has become a significant health problem due to the limited options for antibiotic treatment (Tickler *et al.,* 2023) Carbapenem-resistant *Acinetobacter species*, a critical priority for the World Health Organization, require urgent new antibiotics (Tacconelli *et al*., 2018). In the current study, *A. baumannii* showed the highest level to resistance cefotaxime (95.3%), ceftazidime (89.5%), gentamicin (86.8%), cefepime (84.2%), and trimethoprim-sulfamethoxazole (81.4%). Many studies have found that these organisms have a high-level resistance to the most commonly used antibiotics (Badia *et al.*, 2017a, Tena *et al*.,

2015, Patterson et al., 2020). In addition, *A. baumannii* also showed remarkably high resistance to ertapenem (92.1%), meropenem (84.2%), and imipenem (65.9%). Amikacin and meropenem were 100% effective against all of the isolates of *P. mirabilis* and *K. variicola* in our study. However, studies done in Hawassa (Dessalegn *et al*., 2014a), and elsewhere (Awoke *et al*., 2019) showed that ciprofloxacin were effective against *Proteus* and *Pseudomonas* isolates. The differences might be the rational use of antibiotics, and the fact that the cost of the drugs may be higher relative to others, so people do not take these drugs for self-medication in the study area (de With *et al*., 2016).

Carbapenem resistance among *Enterobacteriaceae* was 17.6%, 20.3%, and 32.9% to meropenem, imipenem, and ertapenem, respectively. Effective treatment options for *Enterobacteriaceae* were limited to amikacin, meropenem, and imipenem. The most frequent isolate was *E. coli,* which showed the highest resistance to ampicillin, ceftriaxone, cefotaxime, ceftazidime, cefepime, cefuroxime, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, and tetracycline. Similar studies were conducted in Ethiopia (Shiferaw *et al*., 2020b) and Iraq (Ali and Al-Jaff, 2021). This might be due to the indiscriminate use of antibiotics in both hospitals (Ranjan *et al*., 2011, Li and Webster, 2018). An alarming level of carbapenem-resistant *E. coli* to ertapenem (24.5%), imipenem (11.8%), and meropenem (9.8%) was detected; however, it was lower than in another study (Mora-Guzman *et al*., 2020). The cause of the higher rates compared to other settings may be irrational use or misuses of antibiotics. The discrepancy of antibiotics resistance across sites includes differences in the rational use of antibiotics (Ma *et al*., 2021).

In this study, multidrug resistance (MDR) was observed in 93.4% of Gram-negative bacteria isolated. A number of the bacteria isolated from SSIs exhbitted a concerning level of resistance against the antibiotics similar to findings from elsewhere (Ranjan *et al*., 2010). The frequency of isolating MDR *Enterobacteriaceae* across the hospitals was similar and very high (>90%) and calls for the implementation of context appropriate infection prevention and control strategies across the hospitals in the country. In addition, the MDR frequency for *Acinetobacter species* was 100% at JUMC. This finding was in line with a study conducted at TASH. While this finding was higher than studies conducted at DTCSH, and HUCSH. On the other hand, MDR frequency for *Pseudomonas species* was 83.3% at JUMC. This finding was higher than studies performed at DTCSH (66.7%), HUCSH (66.7%), and TASH (50%).

The detection of MDR bacteria in this study is in parallel with confirmation of the ESBL production as proved by phenotypic analysis using combined disk diffusion test that was found to be 93%. This result is higher than studies conducted by Kaur 27.5% (Kaur and Singh, 2018b) and Chaudhry 46.0% (Chaudhary and Payasi, 2012). In addition, the carbapenemase production rate as determined by the modified Hodge test was also 83.7% which support the identification of high rates of MDR bacteria in this study. This study was similar to the study conducted in Iran 86% (Fallah *et al*., 2014). While, the result is lower than the study conducted in Morocco 96.6% (Kabbaj et al., 2013), and higher than a study conducted in India 44.8% (Kaur and Singh, 2018b). These variations could be due to different antibiotic use, careful selection of antibiotics in their hospital settings, and infection control measures (Onduru *et al*., 2021).

Infections caused by ESBL-producing Gram-negative bacteria are increasing at an alarming rate and have become a serious public health threat worldwide. In our study, among the total ESBL genes 50% were detected at JUMC. This finding was higher than studies conducted at TASH and HUCSH. While no ESBL gene detected at DTCSH. The current study showed the molecular epidemiology of ESBL producing GNB among patients investigated for SSI at four referral hospitals located in the Amhara region, Addis Ababa, southern region and Oromia region of Ethiopia. Of all the GNB subjected to WGS, 57.8% encoded at least one ESBL gene. Similarly the previous reports from Nigeria 58% (Shitta *et al.*, 2021) and Gaza also showed 59% occurrence of ESBL (El Aila *et al*., 2023); On the other hand, this study, higher than the previous systematic reports of pooled prevalence of ESBL-producing GNB in Ethiopia was 50% *(Tufa et al*., 2020), and a lower detection rate was reported when it compared with study conducted in Jimma 63.4% (Zeynudin *et al.*, 2018b). These results, variation, revealed that the prevalence of ESBL gene types can vary between geographical regions, hospital, group of patients and type of patients and over use of antibiotics.

Among the GNB isolated at each hospital, the detection of $bla_{CTX\text{-}M}$ at HUCSH was 39.4%. This result was higher than the study conducted in DTCSH (21.1%), JUMC (19.7%), and TASH (19.7%). Among the four referral hospitals, $bla_{CTX\text{-}M}$ was the most frequently detected ESBL family (50.8%). This finding is comparable with study conducted in Nigeria reported 48% (Shitta *et al*., 2021), and lower than study performed in Jimma 95.8% (Zeynudin *et al*., 2018b); however, a very low detection rate were reported in the Nepal (Shrestha *et al*., 2022) and Saudi

Arbia 33% (Ibrahim *et al.*, 2021a). The variations might be due to genetic diversity, detection techniques across study sites, horizontal gene transfer, and selective pressure.

The *bla*$_{CTX\text{-}M\text{-}15}$ (44.4%) was the most abundant ESBL gene detected among *bla*$_{CTX\text{-}M}$. These findings were comparable with different studies across the globe (Zeynudin *et al*., 2018b, El Aila *et al*., 2023, Castanheira *et al*., 2021, Ibrahim et al., 2021a). The *bla*$_{CTX\text{-}M\text{-}15}$ detected from *E. cloacae* (66.7%), *K. pneumonia* (62.5%), *E. coli* (56.4%), *P. aeruginosa* (50%), and other ESBL producing Gram-negative bacteria. This finding suggests that other GNB have acquired the *CTX-M* genes. Similar findings have been reported in Jimma, Ethiopia (Zeynudin *et al*., 2018b). Due to the rising incidence of MDR SSI and the scarcity of available antibiotics for treatment, the rise in bacteria generating ESBL enzymes in SSI patients is cause for worrying. More importantly, the high level of ESBL producing strains among SSI causing GNB in low-income countries is a major public health problem, due to the limited laboratory services and therapeutic options available. The emergence of ESBLs has been made possible by the presence of ESBL-encoding genes on plasmids and within transposons and insertion sequences (Castanheira *et al.*, 2021).

The *bla*$_{CTX\text{-}M\text{-}15}$ was very abundant at JUMC (41.7%), DTCSH (23.3%), and TASH (20%) while it was detected at a comparatively lower rate at HUCSH (15%). The detection of high levels of *bla*$_{CTX\text{-}M}$ at JUMC may possibly be explained because the hospital is the main destination of patients referred from all Oromia regions which is the largest region in Ethiopia. Although the dissemination of *bla*$_{CTX\text{-}M\text{-}15}$ was followed by *bla*$_{CTX\text{-}M\text{-}14B}$ in all hospitals, *bla*$_{CTX\text{-}M\text{-}2}$, *bla*$_{CTX\text{-}M\text{-}27}$ and *bla*$_{CTX\text{-}M\text{-}169}$ were detected only at DTCSH (northern). Today the *bla*$_{CTX\text{-}M}$ variant dominating worldwide, followed by *CTX-M-14*, and *CTX-M-27* is emerging in certain parts of the world (Castanheira *et al*., 2021).

Farthermore, in this result, *bla*$_{TEM}$ (40%) was also detected in abundance. This result is comparable with study performed in Nigeria was 30.9% (Shitta *et al*., 2021). On the other hand, the detection of *bla*$_{SHV}$ (8.1%) showed similarities with a study conducted in Burkina Faso were reported 5.9% of the *bla*$_{SHV}$ genes and Saudi Arbia 2.1% (Ibrahim *et al*., 2021a) while this finding is lower than study conducted in Asella, Ethiopia 27.3% (Tufa *et al.*, 2022). The ESBL variants of *bla*$_{SHV}$ detected in this study were *bla*$_{SHV\text{-}106}$. A similar result *bla*$_{SHV\text{-}106}$ detection rate was reported in Portugal (Mendonça *et al*., 2009).

More than one ESBL resistance gene in a single isolate increase the difficulty of treating with different antibiotic drugs (Castanheira *et al*., 2021). In this study, the co-existence of two different ESBL genes was frequently detected in a single isolate, similar to a study conducted in Saudi Arabia (Ibrahim et al., 2021b). Similarly in this finding also the majority of bacteria that encoded ESBL also carried other beta-lactamase genes, and multiple ESBL genes were found in multiple cases GNB. The $bla_{CTX\text{-}M\text{-}15}$ gene co-occurred with several other ESBL genes and non-ESBL variants of bla_{TEM} and bla_{SHV}. The co-occurrence of $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1B}$ was the most frequently detected gene combination, followed by the $bla_{SHV\text{-}187}$*TEM-1B*CTX-M-15* combination and *TEM-1B*CTX-M-15*CTX-M-14b*. The findings of this co-occurrence of multiple ESBL genes were in agreement with the other studies (Ibrahim *et al.*, 2021b) demonstrated the spread of GNB harbouring numerous ESBL genes in the study areas. In this finding several variants of carbapenemase producing genes were detected among the GNB sequenced from all study sites. The frequency of $bla_{OXA\text{-}1}$ at JUMC was 40%. This finding was higher than studies performed at TASH, HUCSH, and DTCSH, 15%, 17.5%, and 27.5%, respectively. On the other hand, the second predominant carbapenemase genes were bla_{NDM} alleles carried in twelve isolates 42.9%. Of this 41.7% of the bla_{NDM} were detected at TASH. This finding was higher than the other study sites. The variation of the resistant gene among study sites might be due to several factors, including the use of different antibiotics, environmental conditions, and the presence of various bacterial populations.

A. baumannii has been reported worldwide and has become a significant health problem (Tickler *et al*., 2023) especially Carbapenem-resistant *Acinetobacter species*, a critical priority for the World Health Organization, require urgent new antibiotics (Tacconelli *et al*., 2018). In the present study, the most common ESBL genes detected were $bla_{\mathrm{CTX\text{-}M}}$ from 6 isolates 21.4%. This data is comparable with the previous study reported from Saudi Arabia which was 20% (Ibrahim *et al*., 2021b) and lower than study reported in Nigeria (25%) (Uyanga *et al*.). On the other hand in our study, the bla_{TEM} gene was detected in 14.3% of the isolates which is lower than the study conducted in Saudi Arabia 70% (Ibrahim *et al.*, 2021b). On the other hand, this finding revealed that the bla_{SHV} was not detected in any of the *Acinetobacter species.* The result is similar to studies conducted in Iran (Khurshid *et al.*, 2020). However, the bla_{SHV} gene was common in *A. baumannii* isolated in Iraq 25% (Al-Thahab, 2013). These variations could be due to different

antibiotic use, and difference in study settings (Onduru *et al.*, 2021). In this finding of 43 isolates of *Acintobacter species* 85.7% were carbapenem-resistant, which is similar with a study conducted in Pakistan (89.1%) (Khurshid *et al.*, 2020). In *A. baumannii*, carbapenem resistance is frequently linked to the existence of metallo-β-lactamases (MBL) such as bla_{NDM-1} elsewhere in the world (Krizova *et al*., 2012, Kaur and Singh, 2018a). Similarly in our study, the bla_{NDM-1} gene was detected in 25.6% (11/43) *A. baumannii* isolates. In addition, our study is similar to the previous studies conducted in Libya (Mathlouthi *et al*., 2016), and Algeria (Bakour et al., 2014). On the other hand the bla_{NDM-1} gene from the total two was detected in *A. baumannii* isolates, from Jimma Hospital. This study was comparable with the first bla_{NDM} reported from Jimma Hospital (Pritsch *et al*., 2017). The predominant carbapenemase gene was bla_{OXA} type 58.1% mainly (bla_{OXA-23}, and bla_{OXA-69}) followed by metallo-β-lactamase bla_{NDM} 27.5%, genes. This study was comparable with the previous study conducted in Ethiopia (Adbaru *et al.*, 2023). More than one ESBL resistance gene in a single isolate increase the difficulty of treating with different antibiotic drugs (Castanheira *et al*., 2021)**.** In this study, the co-existence of two different ESBL genes was frequently detected in a single isolate, similar to a study conducted in Saudi Arabia (Ibrahim *et al.*, 2021b). The present study revealed that the co-existence of two or more carbapenemase encoding genes in a single isolate was 53.6% (15/28). This finding is higher than the study conducted in Jimma, Ethiopia (Adbaru *et al*., 2023) and lower than the study conducted in Tunisia 82% (Hammami *et al*., 2011). This varation might be genetic diversity, antibiotic usage, infection control practice and healthcare infrastructure. Many isolates also carried one or more other carbapenemase genes together with ESBL genes showing dissemination of multidrug-resistant (MDR) *A. baumannii* in Teaching and referral Hospitals in Ethiopia. The finding gives an alarming sign towards *A. baumannii* carrying both metallo-beta-lactamases and ESBL production genes conferring resistance to carbapenems and cephalosporins respectively. This combination of resistance genes can limit therapeutic options (Tickler *et al*., 2023). Early detection, strict adherence to infection control procedures and antimicrobial policy are the best lines of defence against *A. baumannii*. Moreover, the widespread distribution of NDM-1metallo-β-lactamase necessitates special consideration because the enzyme confers resistance to a wide spectrum of beta-lactam antibiotics on the bacteria, and their genetic makeup exhibits remarkable adaptability and mobility. Serious public health problems could arise from the spread of such

plasmids across many clinically significant bacterial species, especially GNB *A. baumannii*, in hospital settings (Karthikeyan *et al*., 2010).

Likewise, the bla_{OXA-23} gene is one of the common causes of resistance conferring high level of resistance and was detected in 8 isolates (18.6%). This figure is higher than the study conducted from Chain which is 4.5% but lower than studies conducted from Libya 29 strains (80.6%) (Mathlouthi *et al.*, 2016) and Pakistan (97.8%) (Khurshid *et al*., 2020). The prevalence of this gene may vary in the geographic area and the type of *Acinetobacter species.*

As far as risk factors associated with the occurrence of SSI are concerned, in the present study, the likelihood of SSI occurrences among patients aged ≥ 61 years increased by a factor of 2.8. Similar findings have been conducted in Ethiopia (Awoke *et al.*, 2019, Biadglegne *et al*., 2009, Mulu *et al*., 2012b) and elsewhere (Narula *et al*., 2020). This might be due to a weakened immune response to infectious agents and poor nutritional status (Ayala *et al*., 2021). Patients who had a longer duration of hospital stay developed SSIs 4.1 times more frequently (P = 0.000) than those who had shorter time. This finding was in agreement with many studies in Ethiopia (Lubega *et al*., 2017a, Awoke *et al*., 2019, Shiferaw *et al*., 2020b) and elsewhere (Narula *et al.*, 2020). This is a notable finding because it is associated with additional costs in a country with a staggering economy and healthcare system (Badia *et al*., 2017b). Similarly, the present study demonstrated that with previous use of antibiotics, patients had a 2.8 times higher chance of developing SSI than with non-previous use of antibiotics (Misha *et al*., 2021b). This could be because broad-spectrum antibiotics have a high risk of causing super-infection of resistant strains due to selective pressure (Ranjan *et al*., 2011, Li and Webster, 2018). The type of surgery was also statistically associated with SSI in the present study. Undergoing emergency surgery showed approximately 3.24 times higher chances of acquiring SSIs when compared to elective surgery (P = 0.000), which complies with related studies (Misha *et al*., 2021b).

The risk of developing SSI with smoking histories was found to be 2.35 times more than in those who did not have smoking history. There was a significant association between smoking patients' history and SSI (P = 0.001). This finding was in agreement with smoking history were independent predictors of SSIs in multivariate logistic regression analysis (Costa *et al*., 2018). Smoking weakens immunity and increases the risk of SSI (Jiang *et al.*, 2020).

5. CHAPTER 6: CONCLUSIONS AND RECOMMONDATIONS

6.1. Conclusions

This multicenter study identified frequent and diverse Gram-negative and Gram-positive SSI etiologies without significant variation in primary etiologies between hospitals. Isolation of various newly emerging bacterial strains in all sites showed the growing epidemiology and diversity of SSI etiologies. *E. coli* and *S. aureus* were the leading Gram-negative and Gram-positive isolates causing SSI, respectively. High level antimicrobial resistance was detected with varying frequency between hospitals. Gram-positive isolates revealed sensitivity to vancomycin and clindamycin, whereas, among Gram-negative isolates, amikacin, imipenem, and meropenem were the most active antibiotics. Ceftriaxone resistance was very high among patient who took it as treatment, and prophylaxis and developed SSIs. The finding of high levels of carbapenem resistance, especially towards ertapenem, is alarming.

A study identified *mecA*-positive Staphylococci species, including MRSA, but no VRSA was detected among these MRSA. What is more captivating in this study is a significantly high prevalence of *mecA* carriage among CoNS. In terms of geographic distribution, most of *mecA*-gene-positive Staphylococci was detected in DTCSH, with significant variations between hospitals, suggesting that strategies to control methicillin-resistant Staphylococci should be tailored to specific hospitals and the infection was linked to factors like older age, hospital stay, antibiotic history, and prophylaxis.

A high genotypic frequency of ESBL and carbapenemase producing GNB among SSI patients was detected. Numerous ESBL and carbapenemase gene variants were detected, with $bla_{CTX\text{-}M\text{-}15}$ and $bla_{OXA\text{-}1}$ being the most frequently occurring ESBL and carbapenemase producing genes. Multiple combinations of ESBL genes were detected among GNB. Inaddtion our results suggest the existence of different species of *Acinetobacter species* including *A. baumannii, A. pittii, A. soli* and *A. lactucae* in th**e** hospital settings. High level beta-lactamase genes were detected among *A. baumannii*. In addition, the co-existence of two different ESBL genes and high-level co-existence of two or more different carbapenemase genes was frequently detected in a single bacterial pathogen.

6.2. Recommendations:

a. Routine culture and AST needs to be done whenever SSIs are suspectedb. Effective antimicrobial stewardship

c. Establish strict guidelines for antibiotics prescriptions in the treatment and prevention of SSIs.

d. Limit ceftriaxone, a third-generation cephalosporin, in surgical prophylaxis and treatment.

e. Hence, to prevent the emergence and spread of MDR SSI, Prompt prevention and control measures for MRSA-high-risk populations, recommend effective antimicrobial stewardship and antibiotic treatment based on AST of the pathogens. At the national level, regular surveillance and monitoring of antimicrobial resistance patterns are indispensable. This includes the careful monitoring of the antibiotics used as prophylaxis and empiric treatment by the concerned bodies.

f. Diagnostic enhasment, like molecular detection

STRENGTHS AND LIMITATION

The strength of this study was multicentred, enrollment of all age groups, a reasonably large sample size and re-characterizing bacteria using MALDI TOF-MS an advanced bacterial identification method, and WGS for resistant genes determination. The limitations were unable to investigate anaerobic bacterial and fungal agents due to limited laboratory resources at the hospitals.

7. CHAPTER 7: REFERENCES

2004. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. *Am J Infect Control,* 32**,** 470-85.

2014. WHO. SUSP project in African surgical departments. (CLSI), C. A. L. S. I. 2021. Performans standards for Antimicrobial Susceptablity Testing *Wayne,PA: Clinical and Laboratory Standared Institiute* CLSI supplement M100.30th ed.

ABAYNEH, M., ASNAKE, M., MULETA, D. & SIMIENEH, A. 2022. Assessment of Bacterial Profiles and Antimicrobial Susceptibility Pattern of Isolates Among Patients Diagnosed with Surgical Site Infections at Mizan-Tepi University Teaching Hospital, Southwest Ethiopia: A Prospective Observational Cohort Study. *Infect Drug Resist,* 15**,** 1807-1819.

ABUBAKAR, U., AMIR, O. & RODRÍGUEZ-BAÑO, J. 2022. Healthcare-associated infections in Africa: a systematic review and meta-analysis of point prevalence studies. *Journal of pharmaceutical policy and practice,* 15**,** 99.

ADBARU, M. G., GUDINA, E. K., KROIDL, A. & WIESER, A. 2023. 2158. An outbreak of carbapenem-resistant Acinetobacter baumannii at a tertiary hospital in Ethiopia. *Open Forum Infectious Diseases,* 10**,** ofad500.1781.

ADWAN, G., ABU HASAN, N., SABRA, I., SABRA, D., AL-BUTMAH, S., ODEH, S., ABD ALBAKE, Z. & BADRAN, H. 2016. Detection of bacterial pathogens in surgical site infections and their antibiotic sensitivity profile. *Int J Med Res Health Sci,* 5**,** 75-82.

AHMED, M. I. 2012. Prevalence of nosocomial wound infection among postoperative patients and antibiotics patterns at teaching hospital in Sudan. *North American journal of medical sciences,* 4**,** 29-34.

AKHI, M. T., GHOTASLOU, R., BEHESHTIROUY, S., ASGHARZADEH, M., PIRZADEH, T., ASGHARI, B., ALIZADEH, N., TOLOUE OSTADGAVAHI, A., SORAYAEI SOMESARAEI, V. & MEMAR, M. Y. 2015. Antibiotic Susceptibility Pattern of Aerobic and Anaerobic Bacteria Isolated From Surgical Site Infection of Hospitalized Patients. *Jundishapur J Microbiol,* 8**,** e20309.

AL-ORPHALY, M., HADI, H. A., ELTAYEB, F. K., AL-HAIL, H., SAMUEL, B. G., SULTAN, A. A. & SKARIAH, S. 2021. Epidemiology of multidrug-resistant Pseudomonas aeruginosa in the Middle East and North Africa Region. *Msphere,* 6**,** e00202-21.

AL-THAHAB, A. A. 2013. Molecular detection of extended-spectrum beta-lactamases in clinical isolates of Acinetobacter baumannii. *J Biol Agric Healthc,* 3**,** 32-9.

ALANI, H. A., HASSAWI, D. S. & FLAYIH, M. T. 2017. Patterns of antibiotic resistance in Staphylococcus aureus isolates and detection the heteroresistance to vancomycin by population analysis method. *JUAPS,* 11**,** 26-33.

ALFOUZAN, W., AL FADHLI, M., ABDO, N., ALALI, W. & DHAR, R. 2019. Surgical site infection following cesarean section in a general hospital in Kuwait: trends and risk factors. *Epidemiol Infect,* 147**,** e287.

ALI, K. M. & AL-JAFF, B. M. A. 2021. Source and antibiotic susceptibility of gram-negative bacteria causing superficial incisional surgical site infections. *International Journal of Surgery Open,* 30**,** 100318.

ALLEGRANZI, B., AIKEN, A. M., KUBILAY, N. Z., NTHUMBA, P., BARASA, J., OKUMU, G., MUGARURA, R., ELOBU, A., JOMBWE, J. & MAIMBO, M. 2018. A multimodal infection control and patient safety intervention to reduce surgical site infections in Africa: a multicentre, before–after, cohort study. *The Lancet infectious diseases,* 18**,** 507-515.

ALMEIDA, G. C., DOS SANTOS, M. M., LIMA, N. G., CIDRAL, T. A., MELO, M. C. & LIMA, K. C. 2014. Prevalence and factors associated with wound colonization by Staphylococcus spp. and Staphylococcus aureus in hospitalized patients in inland northeastern Brazil: a cross-sectional study. *BMC Infect Dis,* 14**,** 328.

AMEH, E. A., MSHELBWALA, P. M., NASIR, A. A., LUKONG, C. S., JABO, B. A., ANUMAH, M. A. & NMADU, P. T. 2009. Surgical site infection in children: prospective analysis of the burden and risk factors in a sub-Saharan African setting. *Surgical infections,* 10**,** 105-109.

ANAGAW, B., SHIFERAW, Y., ANAGAW, B., BIADGLEGNE, F., MOGES, F., KASSU, A., UNAKAL, C. & MULU, A. 2013. Frequency of methicillin-resistant Staphylococcus aureus isolates from clinical specimens in Gondar University Hospital, Northwest Ethiopia. *Asian Journal of Medical Sciences,* 5**,** 59-64.

ASRES, G. S., LEGESE, M. H. & WOLDEAREGAY, G. M. 2017. Prevalence of multidrug resistant Bacteria in postoperative wound infections at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. *Archives of Medicine,* 9**,** 0-0.

ATA, A., LEE, J., BESTLE, S. L., DESEMONE, J. & STAIN, S. C. 2010. Postoperative hyperglycemia and surgical site infection in general surgery patients. *Archives of surgery,* 145**,** 858-864.

AWOKE, N., ARBA, A. & GIRMA, A. 2019. Magnitude of surgical site infection and its associated factors among patients who underwent a surgical procedure at Wolaita Sodo University Teaching and Referral Hospital, South Ethiopia. *PLoS One,* 14**,** e0226140.

AYALA, D., TOLOSSA, T., MARKOS, J. & YILMA, M. T. 2021. Magnitude and factors associated with surgical site infection among mothers underwent cesarean delivery in Nekemte town public hospitals, western Ethiopia. *PLOS ONE,* 16**,** e0250736.

BADIA, J. M., CASEY, A. L., PETROSILLO, N., HUDSON, P. M., MITCHELL, S. A. & CROSBY, C. 2017a. Impact of surgical site infection on healthcare costs and patient outcomes: a systematic review in six European countries. *Journal of Hospital Infection,* 96**,** 1-15.

BADIA, J. M., CASEY, A. L., PETROSILLO, N., HUDSON, P. M., MITCHELL, S. A. & CROSBY, C. 2017b. Impact of surgical site infection on healthcare costs and patient outcomes: a systematic review in six European countries. *J Hosp Infect,* 96**,** 1-15.

BAKOUR, S., TOUATI, A., BACHIRI, T., SAHLI, F., TIOUIT, D., NAIM, M., AZOUAOU, M. & ROLAIN, J. M. 2014. First report of 16S rRNA methylase ArmA-producing Acinetobacter baumannii and rapid spread of metallo-β-lactamase NDM-1 in Algerian hospitals. *J Infect Chemother,* 20**,** 696-701.

BARIE, P. S. 2002. Surgical site infections: epidemiology and prevention. *Surg Infect (Larchmt),* 3 Suppl 1**,** S9-21.

BARRY, C. L. 2021. Surgical wound infections. *Physician Assistant Clinics,* 6**,** 295-307.

BEIMS, H., OVERMANN, A., FULDE, M., STEINERT, M. & BERGMANN, S. 2016. Isolation of Staphylococcus sciuri from horse skin infection. *Open Veterinary Journal,* 6**,** 242-246.

BENDARY, M. M., SOLYMAN, S. M., AZAB, M. M., MAHMOUD, N. F. & HANORA, A. M. 2016. Genetic diversity of multidrug resistant Staphylococcus aureus isolated from clinical and non clinical samples in Egypt. *Cellular and Molecular Biology,* 62**,** 55-61.

BERGLUND, C. & SÖDERQUIST, B. 2008. The origin of a methicillin-resistant Staphylococcus aureus isolate at a neonatal ward in Sweden—possible horizontal transfer of a staphylococcal cassette chromosome mec between methicillin-resistant Staphylococcus haemolyticus and Staphylococcus aureus. *Clinical Microbiology and Infection,* 14**,** 1048-1056.

BERRÍOS-TORRES, S. I., UMSCHEID, C. A., BRATZLER, D. W., LEAS, B., STONE, E. C., KELZ, R. R., REINKE, C. E., MORGAN, S., SOLOMKIN, J. S., MAZUSKI, J. E., DELLINGER, E. P., ITANI, K. M. F., BERBARI, E. F., SEGRETI, J., PARVIZI, J., BLANCHARD, J., ALLEN, G., KLUYTMANS, J., DONLAN, R. & SCHECTER, W. P. 2017. Centers for Disease Control and Prevention Guideline for the Prevention of Surgical Site Infection, 2017. *JAMA Surg,* 152**,** 784-791.

BIADGLEGNE, F., ABERA, B., ALEM, A. & ANAGAW, B. 2009. Bacterial isolates from wound infection and their antimicrobial susceptibility pattern in Felege Hiwot referral Hospital North West Ethiopia. *Ethiopian Journal of Health Sciences,* 19.

BIRHANU, Y. & ENDALAMAW, A. 2020. Surgical site infection and pathogens in Ethiopia: a systematic review and meta-analysis. *Patient Saf Surg,* 14**,** 7.

BITEW KIFILIE, A., DAGNEW, M., TEGENIE, B., YESHITELA, B., HOWE, R. & ABATE, E. 2018a. Bacterial Profile, Antibacterial Resistance Pattern, and Associated Factors from Women Attending Postnatal Health Service at University of Gondar Teaching Hospital, Northwest Ethiopia. *Int J Microbiol,* 2018**,** 3165391.

BITEW KIFILIE, A., DAGNEW, M., TEGENIE, B., YESHITELA, B., HOWE, R. & ABATE, E. 2018b. Bacterial Profile, Antibacterial Resistance Pattern, and Associated Factors from Women Attending Postnatal Health Service at University of Gondar Teaching Hospital, Northwest Ethiopia. *International journal of microbiology,* 2018.

BLOEMENDAAL, A. L. A., BROUWER, E. C. & FLUIT, A. C. 2010. Methicillin resistance transfer from Staphylocccus epidermidis to methicillin-susceptible Staphylococcus aureus in a patient during antibiotic therapy. *PloS one,* 5**,** e11841.

BORCHARDT, R. A. & TZIZIK, D. 2018. Update on surgical site infections: The new CDC guidelines. *Jaapa,* 31**,** 52-54.

BROOK, I., WEXLER, H. M. & GOLDSTEIN, E. J. 2013. Antianaerobic antimicrobials: spectrum and susceptibility testing. *Clin Microbiol Rev,* 26**,** 526-46.

BUNDUKI, G. K., MASOAMPHAMBE, E., FOX, T., MUSAYA, J., MUSICHA, P. & FEASEY, N. 2024. Prevalence, risk factors, and antimicrobial resistance of endemic healthcare-associated infections in Africa: a systematic review and meta-analysis. *BMC Infectious Diseases,* 24**,** 1-18.

CASTANHEIRA, M., SIMNER, P. J. & BRADFORD, P. A. 2021. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. *JAC Antimicrob Resist,* 3**,** dlab092.

CHAUDHARY, M. & PAYASI, A. 2012. Molecular characterization and antimicrobial susceptibility study of Acinetobacter baumannii clinical isolates from Middle East, African and Indian patients. *J Proteomics Bioinform,* 5**,** 265-9.

CHEESBROUGH, M. 2006. Biochemical tests to identify bacteria *District Laboratory Practice in Tropical Countries* 2nded ed. United States of America, New York: Cambridge University Press.

CHENG, H., CHEN, B. P.-H., SOLEAS, I. M., FERKO, N. C., CAMERON, C. G. & HINOUL, P. 2017. Prolonged Operative Duration Increases Risk of Surgical Site Infections: A Systematic Review. *Surgical infections,* 18**,** 722-735.

CHEUNG, G. Y. C., BAE, J. S. & OTTO, M. 2021. Pathogenicity and virulence of Staphylococcus aureus. *Virulence,* 12**,** 547-569.

COATES, A. R. & HU, Y. 2007. Novel approaches to developing new antibiotics for bacterial infections. *Br J Pharmacol,* 152**,** 1147-54.

COELLO, R., CHARLETT, A., WILSON, J., WARD, V., PEARSON, A. & BORRIELLO, P. 2005. Adverse impact of surgical site infections in English hospitals. *J Hosp Infect,* 60**,** 93-103.

COSTA, T., MEDEIROS, P. & SALLES, M. 2018. Smoking increases the risk of surgical site infection after hydrocelectomy in adults: A retrospective cohort study in Brazil. *The Journal of Infection in Developing Countries,* 11**,** 950.

DALLENNE, C., DA COSTA, A., DECRÉ, D., FAVIER, C. & ARLET, G. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy,* 65**,** 490-495.

DANIEL, S. J. C., GOWTHAMI, E. & SOWMIYA, S. 2013. Isolation and identification of bacterial pathogens from wounds of diabetic patients. *International journal of current microbiology and applied sciences,* 2**,** 72-77.

DAVID, M. Z. & DAUM, R. S. 2017. Treatment of Staphylococcus aureus Infections. *Curr Top Microbiol Immunol,* 409**,** 325-383.

DE WITH, K., ALLERBERGER, F., AMANN, S., APFALTER, P., BRODT, H. R., ECKMANNS, T., FELLHAUER, M., GEISS, H. K., JANATA, O., KRAUSE, R., LEMMEN, S., MEYER, E., MITTERMAYER, H., PORSCHE, U., PRESTERL, E., REUTER, S., SINHA, B., STRAUß, R., WECHSLER-FÖRDÖS, A., WENISCH, C. & KERN, W. V. 2016. Strategies to enhance rational use of antibiotics in hospital: a guideline by the German Society for Infectious Diseases. *Infection,* 44**,** 395-439.

DESSALEGN, L., SHIMELIS, T., TADESSE, E. & GEBRE-SELASSIE, S. 2014a. Aerobic bacterial isolates from post-surgical wound and their antimicrobial susceptibility pattern: a hospital based cross-sectional study. *J Med Res,* 3**,** 18-23.

DESSALEGN, L., SHIMELIS, T., TADESSE, E. & GEBRE-SELASSIE, S. 2014b. Aerobic bacterial isolates from post-surgical wound and their antimicrobial susceptibility pattern: a hospital based cross-sectional study. *J Med Res,* 3**,** 18-22.

DESSIE, W., MULUGETA, G., FENTAW, S., MIHRET, A., HASSEN, M. & ABEBE, E. 2016. Pattern of bacterial pathogens and their susceptibility isolated from surgical site infections at selected referral hospitals, Addis Ababa, Ethiopia. *International journal of microbiology,* 2016.

DONLAN, R. M. & COSTERTON, J. W. 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clinical microbiology reviews,* 15**,** 167-193.

ECLINICALMEDICINE 2021. Antimicrobial resistance: a top ten global public health threat. *EClinicalMedicine,* 41**,** 101221.

EL-BADAWY, M. F., TAWAKOL, W. M., EL-FAR, S. W., MAGHRABI, I. A., AL-GHAMDI, S. A., MANSY, M. S., ASHOUR, M. S. & SHOHAYEB, M. M. 2017. Molecular

Identification of Aminoglycoside-Modifying Enzymes and Plasmid-Mediated Quinolone Resistance Genes among Klebsiella pneumoniae Clinical Isolates Recovered from Egyptian Patients. *Int J Microbiol,* 2017, 8050432.

EL AILA, N. A., AL LAHAM, N. A. & AYESH, B. M. 2023. Prevalence of extended spectrum beta lactamase and molecular detection of blaTEM, blaSHV and blaCTX-M genotypes among Gram negative bacilli isolates from pediatric patient population in Gaza strip. *BMC Infectious Diseases,* 23, 1-10.

ELBUR, A. I., M A, Y., EL-SAYED, A. S. A. & ABDEL-RAHMAN, M. E. 2013. Prophylactic antibiotics and wound infection. *Journal of clinical and diagnostic research : JCDR,* 7, 2747-2751.

ELHASSAN, M. M., OZBAK, H. A., HEMEG, H. A., ELMEKKI, M. A. & AHMED, L. M. 2015. Absence of the <i>mec</i>A Gene in Methicillin Resistant <i>Staphylococcus aureus</i> Isolated from Different Clinical Specimens in Shendi City, Sudan. *BioMed Research International,* 2015, 895860.

ELMANAMA, A. A. 2006. Antimicrobial resistance of Acinetobacter spp. isolated from pus specimens from Al-Shifa hospital, Gaza, Palestine. *Journal of Al-Aqsa University,* 10.

EMAMIE, A., ZOLFAGHARI, P., ZAREI, A. & GHORBANI, M. 2023. Prevalence and antibiotic resistance of ESKAPE pathogens isolated from patients with bacteremia in Tehran, Iran. *Indian Journal of Medical Specialities,* 14, 97.

ESHETIE, S., TAREKEGN, F., MOGES, F., AMSALU, A., BIRHAN, W. & HURUY, K. 2016. Methicillin resistant Staphylococcus aureus in Ethiopia: a meta-analysis. *BMC infectious diseases,* 16, 1-9.

EZZEDDINE, Z. & GHSSEIN, G. 2023. Towards new antibiotics classes targeting bacterial metallophores. *Microb Pathog,* 182, 106221.

FADEYI, A., ADIGUN, I. & RAHMAN, G. 2008. Bacteriological pattern of wound swab isolates in patients with chronic leg ulcer. *International Journal of Health Research,* 1.

FALLAH, F., NOORI, M., HASHEMI, A., GOUDARZI, H., KARIMI, A., ERFANIMANESH, S. & ALIMEHR, S. 2014. Prevalence of bla NDM, bla PER, bla VEB, bla IMP, and bla VIM Genes among Acinetobacter baumannii Isolated from Two Hospitals of Tehran, Iran. *Scientifica (Cairo),* 2014, 245162.

FANG, H., ATAKER, F., HEDIN, G. & DORNBUSCH, K. 2008. Molecular epidemiology of extended-spectrum β-lactamases among Escherichia coli isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. *Journal of Clinical Microbiology,* 46, 707-712.

FELEKE, T., ESHETIE, S., DAGNEW, M., ENDRIS, M., ABEBE, W., TIRUNEH, M. & MOGES, F. 2018. Multidrug-resistant bacterial isolates from patients suspected of nosocomial infections at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. *BMC research notes,* 11, 602-602.

FORRESTER, J. D., MAGGIO, P. M. & TENNAKOON, L. 2022. Cost of Health Care-Associated Infections in the United States. *J Patient Saf,* 18, e477-e479.

GARNER, J. S. 1996. Hospital Infection Control Practice Advisory Committee. Guideline for isolation precaution in hospitals. *Infect Control Hosp Epidemiol,* 17, 53-80.

GARZA-GONZALEZ, E., MORFIN-OTERO, R., LLACA-DIAZ, J. M. & RODRIGUEZ-NORIEGA, E. 2010. Staphylococcal cassette chromosome mec (SCCmec) in methicillin-resistant coagulase-negative staphylococci. A review and the experience in a tertiary-care setting. *Epidemiology & Infection,* 138, 645-654.

GELETA, D., ABEBE, G., ALEMU, B., WORKNEH, N. & BEYENE, G. 2024. Mechanisms of Bacterial Drug Resistance with Special Emphasis on Phenotypic and Molecular Characterization of Extended Spectrum Beta-lactamase. *New Microbiologica,* 47**,** 1-14.

GHENEA, A. E., ZLATIAN, O. M., CRISTEA, O. M., UNGUREANU, A., MITITELU, R. R., BALASOIU, A. T., VASILE, C. M., SALAN, A. I., ILIUTA, D., POPESCU, M., UDRIȘTOIU, A. L. & BALASOIU, M. 2022. TEM,CTX-M,SHV Genes in ESBL-Producing Escherichia coli and Klebsiella pneumoniae Isolated from Clinical Samples in a County Clinical Emergency Hospital Romania-Predominance of CTX-M-15. *Antibiotics (Basel),* 11.

GHSSEIN, G. & EZZEDDINE, Z. 2022. The Key Element Role of Metallophores in the Pathogenicity and Virulence of Staphylococcus aureus: A Review. *Biology (Basel),* 11.

GODEBO, G., KIBRU, G. & TASSEW, H. 2013. Multidrug-resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. *Ann Clin Microbiol Antimicrob,* 12**,** 17.

GOMAA, K., ABDELRAHEIM, A. R., EL GELANY, S., KHALIFA, E. M., YOUSEF, A. M. & HASSAN, H. 2021. Incidence, risk factors and management of post cesarean section surgical site infection (SSI) in a tertiary hospital in Egypt: a five year retrospective study. *BMC Pregnancy and Childbirth,* 21**,** 634.

HALAWI, E., ASSEFA, T. & HUSSEN, S. 2018. Pattern of antibiotics use, incidence and predictors of surgical site infections in a Tertiary Care Teaching Hospital. *BMC research notes,* 11**,** 538-538.

HAMMAMI, S., GHOZZI, R., SAIDANI, M. & BEN REDJEB, S. 2011. Carbapenem-resistant Acinetobacter baumannii producing the carbapenemase OXA-23 in Tunisia. *Tunis Med,* 89**,** 638-43.

HIDRON, A. I., EDWARDS, J. R., PATEL, J., HORAN, T. C., SIEVERT, D. M., POLLOCK, D. A. & FRIDKIN, S. K. 2008. Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infection Control & Hospital Epidemiology,* 29**,** 996-1011.

HIRAMATSU, K., KATAYAMA, Y., MATSUO, M., SASAKI, T., MORIMOTO, Y., SEKIGUCHI, A. & BABA, T. 2014. Multi-drug-resistant Staphylococcus aureus and future chemotherapy. *J Infect Chemother,* 20**,** 593-601.

HORAN, T. C., GAYNES, R. P., MARTONE, W. J., JARVIS, W. R. & EMORI, T. G. 1992. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections. *Infection Control & Hospital Epidemiology,* 13**,** 606-608.

HOWARD, R. & LEE, J. 1995. Surgical wound infections: epidemiology, surveillance, and clinical management. *Surgical Infectious Diseases***,** 401-412.

HUMPHRIES, R., BOBENCHIK, A. M., HINDLER, J. A. & SCHUETZ, A. N. 2021. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100, 31st Edition. *J Clin Microbiol,* 59**,** e0021321.

IBADIN, E. E., ENABULELE, I. O. & MUINAH, F. 2017. Prevalence of mecA gene among staphylococci from clinical samples of a tertiary hospital in Benin City, Nigeria. *Afr Health Sci,* 17**,** 1000-1010.

IBRAHIM, M. E., ALGAK, T. B., ABBAS, M. & ELAMIN, B. K. 2021a. Emergence of bla (TEM), bla (CTX-M), bla (SHV) and bla (OXA) genes in multidrug-resistant Enterobacteriaceae and Acinetobacter baumannii in Saudi Arabia. *Exp Ther Med,* 22**,** 1450.

IBRAHIM, M. E., ALGAK, T. B., ABBAS, M. & ELAMIN, B. K. 2021b. Emergence of bla TEM, bla CTX-M, bla SHV and bla OXA genes in multidrug-resistant Enterobacteriaceae and Acinetobacter baumannii in Saudi Arabia. *Experimental and Therapeutic Medicine,* 22**,** 1-11.

ISIBOR, J. O., OSENI, A., EYAUFE, A. & TURAY, A. 2008. Incidence of aerobic bacteria and Candida albicans in post-operative wound infections. *African journal of microbiology research,* 2**,** 288-291.

ISKANDAR, K., SARTELLI, M., TABBAL, M., ANSALONI, L., BAIOCCHI, G. L., CATENA, F., COCCOLINI, F., HAQUE, M., LABRICCIOSA, F. M., MOGHABGHAB, A., PAGANI, L., HANNA, P. A., ROQUES, C., SALAMEH, P. & MOLINIER, L. 2019. Highlighting the gaps in quantifying the economic burden of surgical site infections associated with antimicrobial-resistant bacteria. *World Journal of Emergency Surgery,* 14**,** 50.

JIANG, C., CHEN, Q. & XIE, M. 2020. Smoking increases the risk of infectious diseases: A narrative review. *Tob Induc Dis,* 18**,** 60.

KABBAJ, H., SEFFAR, M., BELEFQUIH, B., AKKA, D., HANDOR, N., AMOR, M. & ALAOUI, A. E. 2013. Prevalence of metallo-β-lactamases producing Acinetobacter baumannii in a Moroccan hospital. *International Scholarly Research Notices,* 2013.

KAHSAY, A., MIHRET, A., ABEBE, T. & ANDUALEM, T. 2014. Isolation and antimicrobial susceptibility pattern of Staphylococcus aureus in patients with surgical site infection at Debre Markos Referral Hospital, Amhara Region, Ethiopia. *Arch Public Health,* 72**,** 16.

KARTHIKEYAN, K., THIRUNARAYAN, M. A. & KRISHNAN, P. 2010. Coexistence of bla OXA-23 with bla NDM-1 and armA in clinical isolates of Acinetobacter baumannii from India. *Journal of antimicrobial chemotherapy,* 65**,** 2253-2254.

KAUR, A. & SINGH, S. 2018a. Prevalence of Extended Spectrum Betalactamase (ESBL) and Metallobetalactamase (MBL) Producing Pseudomonas aeruginosa and Acinetobacter baumannii Isolated from Various Clinical Samples. *J Pathog,* 2018**,** 6845985.

KAUR, A. & SINGH, S. 2018b. Prevalence of Extended Spectrum Betalactamase (ESBL) and Metallobetalactamase (MBL) Producing<i> Pseudomonas aeruginosa</i> and<i> Acinetobacter baumannii</i> Isolated from Various Clinical Samples. *Journal of Pathogens,* 2018**,** 6845985.

KAVANAGH, K. T. 2019. Control of MSSA and MRSA in the United States: protocols, policies, risk adjustment and excuses. *Antimicrobial Resistance & Infection Control,* 8**,** 1-8.

KHANAM, R. A., ISLAM, M. R., SHARIF, A., PARVEEN, R., SHARMIN, I. & YUSUF, M. A. 2018. Bacteriological profiles of pus with antimicrobial sensitivity pattern at a teaching hospital in Dhaka City. *Bangladesh J,* 5.

KHURSHID, M., RASOOL, M. H., ASHFAQ, U. A., ASLAM, B., WASEEM, M., XU, Q., ZHANG, X., GUO, Q. & WANG, M. 2020. Dissemination of bla(OXA-23)-harbouring carbapenem-resistant Acinetobacter baumannii clones in Pakistan. *J Glob Antimicrob Resist,* 21**,** 357-362.

KOBAYASHI, N., WU, H., KOJIMA, K., TANIGUCHI, K., URASAWA, S., UEHARA, N., OMIZU, Y., KISHI, Y., YAGIHASHI, A. & KUROKAWA, I. 1994a. Detection of mecA, femA, and femB genes in clinical strains of staphylococci using polymerase chain reaction. *Epidemiology & Infection,* 113**,** 259-266.

KOBAYASHI, N., WU, H., KOJIMA, K., TANIGUCHI, K., URASAWA, S., UEHARA, N., OMIZU, Y., KISHI, Y., YAGIHASHI, A. & KUROKAWA, I. 1994b. Detection of mecA, femA, and femB genes in clinical strains of staphylococci using polymerase chain reaction. *Epidemiol Infect,* 113**,** 259-66.

KOWNHAR, H., SHANKAR, E. M., VIGNESH, R., SEKAR, R., VELU, V. & RAO, U. A. 2008. High isolation rate of Staphylococcus aureus from surgical site infections in an Indian hospital. *J Antimicrob Chemother,* 61**,** 758-60.

KRIZEK, T. J. & ROBSON, M. C. 1975. Evolution of quantitative bacteriology in wound management. *The American Journal of Surgery,* 130**,** 579-584.

KRIZOVA, L., BONNIN, R. A., NORDMANN, P., NEMEC, A. & POIREL, L. 2012. Characterization of a multidrug-resistant Acinetobacter baumannii strain carrying the blaNDM-1 and blaOXA-23 carbapenemase genes from the Czech Republic. *J Antimicrob Chemother,* 67**,** 1550-2.

KUMARI, P. H. P., RANI, P. U. & VIJAYALAKSHMI, P. 2018. Evaluation of microbiological profile and antibiogram of aerobic bacteria isolated from pus samples. *Journal of Medical & Allied Sciences,* 8**,** 26-35.

LALOTO, T. L., GEMEDA, D. H. & ABDELLA, S. H. 2017. Incidence and predictors of surgical site infection in Ethiopia: prospective cohort. *BMC infectious diseases,* 17**,** 119.

LI, B. & WEBSTER, T. J. 2018. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society,* 36**,** 22-32.

LINA, T. T., KHAJANCHI, B. K., AZMI, I. J., ISLAM, M. A., MAHMOOD, B., AKTER, M., BANIK, A., ALIM, R., NAVARRO, A., PEREZ, G., CRAVIOTO, A. & TALUKDER, K. A. 2014. Phenotypic and molecular characterization of extended-spectrum beta-lactamase-producing Escherichia coli in Bangladesh. *PloS one,* 9**,** e108735-e108735.

LLOR, C. & BJERRUM, L. 2014. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. *Therapeutic advances in drug safety,* 5**,** 229-241.

LOS, F. C. O., RANDIS, T. M., AROIAN, R. V. & RATNER, A. J. 2013. Role of pore-forming toxins in bacterial infectious diseases. *Microbiology and molecular biology reviews : MMBR,* 77**,** 173-207.

LUBEGA, A., JOEL, B. & JUSTINA LUCY, N. 2017a. Incidence and etiology of surgical site infections among emergency postoperative patients in mbarara regional referral hospital, South Western Uganda. *Surgery research and practice,* 2017.

LUBEGA, A., JOEL, B. & JUSTINA LUCY, N. 2017b. Incidence and Etiology of Surgical Site Infections among Emergency Postoperative Patients in Mbarara Regional Referral Hospital, South Western Uganda. *Surgery research and practice,* 2017**,** 6365172-6365172.

MA, F., XU, S., TANG, Z., LI, Z. & ZHANG, L. 2021. Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans. *Biosafety and Health,* 3**,** 32-38.

MAGILL, S. S., HELLINGER, W., COHEN, J., KAY, R., BAILEY, C., BOLAND, B., CAREY, D., DE GUZMAN, J., DOMINGUEZ, K. & EDWARDS, J. 2012. Prevalence of healthcare-associated infections in acute care hospitals in Jacksonville, Florida. *Infection Control & Hospital Epidemiology,* 33**,** 283-291.

MAMA, M., ABDISSA, A. & SEWUNET, T. 2014. Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University Specialized Hospital, South-West Ethiopia. *Annals of clinical microbiology and antimicrobials,* 13**,** 14.

MAMA, M., AKLILU, A., MISGNA, K., TADESSE, M. & ALEMAYEHU, E. 2019. Methicillin-and inducible clindamycin-resistant Staphylococcus aureus among patients with wound infection attending Arba Minch Hospital, South Ethiopia. *International journal of microbiology,* 2019.

MANGRAM, A. J., HORAN, T. C., PEARSON, M. L., SILVER, L. C. & JARVIS, W. R. 1999. Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. *Infect Control Hosp Epidemiol,* 20**,** 250-78; quiz 279-80.

MANYAHI, J. 2012. *Bacteriological spectrum of post operative wound infections and their antibiogram in a Tertiary Hospital, Dar Es Salaam, Tanzania.* Muhimbili University of Health and Allied Sciences.

MARTÍNEZ-MELÉNDEZ, A., MORFÍN-OTERO, R., VILLARREAL-TREVIÑO, L., GONZÁLEZ-GONZÁLEZ, G., LLACA-DÍAZ, J., RODRÍGUEZ-NORIEGA, E., CAMACHO-ORTÍZ, A. & GARZA-GONZÁLEZ, E. 2015. Staphylococcal cassette chromosome mec (SCCmec) in coagulase negative staphylococci. *Medicina universitaria,* 17**,** 229-233.

MATHLOUTHI, N., EL SALABI, A. A., JOMÀA-JEMILI, M. B., BAKOUR, S., AL-BAYSSARI, C., ZORGANI, A. A., KRAIEMA, A., ELAHMER, O., OKDAH, L. & ROLAIN, J.-M. 2016. Early detection of metallo-β-lactamase NDM-1-and OXA-23 carbapenemase-producing Acinetobacter baumannii in Libyan hospitals. *International journal of antimicrobial agents,* 48**,** 46-50.

MCGUINNESS, W. A., MALACHOWA, N. & DELEO, F. R. 2017. Vancomycin Resistance in Staphylococcus aureus *Yale J Biol Med,* 90**,** 269-281.

MELARIRI, H., FREERCKS, R., VAN DER MERWE, E., HAM-BALOYI, W. T., OYEDELE, O., MURPHY, R. A., CLAASEN, C., ETUSIM, P. E., ACHEBE, M. O., OFFIAH, S. & MELARIRI, P. E. 2024. The burden of hospital-acquired infections (HAI) in sub-Saharan Africa: a systematic review and meta-analysis. *eClinicalMedicine,* 71.

MENDONÇA, N., FERREIRA, E., LOURO, D., PARTICIPANTS, A. & CANIÇA, M. 2009. Molecular epidemiology and antimicrobial susceptibility of extended-and broad-spectrum β-lactamase-producing Klebsiella pneumoniae isolated in Portugal. *International journal of antimicrobial agents,* 34**,** 29-37.

MENGESHA, R. E., KASA, B. G.-S., SARAVANAN, M., BERHE, D. F. & WASIHUN, A. G. 2014a. Aerobic bacteria in post surgical wound infections and pattern of their antimicrobial susceptibility in Ayder Teaching and Referral Hospital, Mekelle, Ethiopia. *BMC research notes,* 7**,** 575.

MENGESHA, R. E., KASA, B. G., SARAVANAN, M., BERHE, D. F. & WASIHUN, A. G. 2014b. Aerobic bacteria in post surgical wound infections and pattern of their antimicrobial susceptibility in Ayder Teaching and Referral Hospital, Mekelle, Ethiopia. *BMC Res Notes,* 7**,** 575.

MERKOW, R. P., JU, M. H., CHUNG, J. W., HALL, B. L., COHEN, M. E., WILLIAMS, M. V., TSAI, T. C., KO, C. Y. & BILIMORIA, K. Y. 2015. Underlying reasons associated with hospital readmission following surgery in the United States. *Jama,* 313**,** 483-495.

MIRAGAIA, M. 2018. Factors Contributing to the Evolution of mecA-Mediated β-lactam Resistance in Staphylococci: Update and New Insights From Whole Genome Sequencing (WGS). *Front Microbiol,* 9**,** 2723.

MISHA, G., CHELKEBA, L. & MELAKU, T. 2021a. Bacterial profile and antimicrobial susceptibility patterns of isolates among patients diagnosed with surgical site infection at a tertiary teaching hospital in Ethiopia: a prospective cohort study. *Annals of Clinical Microbiology and Antimicrobials,* 20**,** 33.

MISHA, G., CHELKEBA, L. & MELAKU, T. 2021b. Incidence, risk factors and outcomes of surgical site infections among patients admitted to Jimma Medical Center, South West Ethiopia: Prospective cohort study. *Ann Med Surg (Lond),* 65**,** 102247.

MOGES, F., TAMIRU, T., AMARE, A., MENGISTU, G., ESHETIE, S., DAGNEW, M., FELEKE, T., GIZACHEW, M. & ABEBE, W. 2023. Prevalence of Methicillin-ResistantStaphylococcus aureus and Multidrug-Resistant Strains from Patients Attending the Referral Hospitals of Amhara Regional State, Ethiopia. *International Journal of Microbiology,* 2023.

MOHAMMED, A., ADESHINA, G. O. & IBRAHIM, Y. K. 2013. Incidence and antibiotic susceptibility pattern of bacterial isolates from wound infections in a tertiary hospital in Nigeria. *Tropical journal of pharmaceutical research,* 12**,** 617-621.

MOHAMMED, A., SEID, M. E., GEBRECHERKOS, T., TIRUNEH, M. & MOGES, F. 2017. Bacterial Isolates and Their Antimicrobial Susceptibility Patterns of Wound Infections among Inpatients and Outpatients Attending the University of Gondar Referral Hospital, Northwest Ethiopia. *Int J Microbiol,* 2017**,** 8953829.

MOHAMMED, Y., GADZAMA, G. B., ZAILANI, S. B. & ABODERIN, A. O. 2016. Characterization of Extended-Spectrum Beta-lactamase from Escherichia coli and Klebsiella Species from North Eastern Nigeria. *J Clin Diagn Res,* 10**,** Dc07-10.

MONK, E. J. M., JONES, T. P. W., BONGOMIN, F., KIBONE, W., NSUBUGA, Y., SSEWANTE, N., MULEYA, I., NSENGA, L., RAO, V. B. & VAN ZANDVOORT, K. 2024. Antimicrobial resistance in bacterial wound, skin, soft tissue and surgical site infections in Central, Eastern, Southern and Western Africa: A systematic review and meta-analysis. *PLOS Global Public Health,* 4**,** e0003077.

MORA-GUZMAN, I., RUBIO-PEREZ, I., GONZÁLEZ, R. M., GARCIA, D. D. & MARTIN-PEREZ, E. 2020. Surgical Site Infection by Carbapenemase-producing Enterobacteriaceae: A Challenge for Today's Surgeons. *Cirugía Española (English Edition),* 98**,** 342-349.

MULU, W., KIBRU, G., BEYENE, G. & DAMTIE, M. 2012a. Postoperative Nosocomial Infections and Antimicrobial Resistance Pattern of Bacteria Isolates among Patients Admitted at Felege Hiwot Referral Hospital, Bahirdar, Ethiopia. *Ethiopian journal of health sciences,* 22**,** 7-18.

MULU, W., KIBRU, G., BEYENE, G. & DAMTIE, M. 2012b. Postoperative Nosocomial Infections and Antimicrobial Resistance Pattern of Bacteria Isolates among Patients Admitted at Felege Hiwot Referral Hospital, Bahirdar, Ethiopia. *Ethiop J Health Sci,* 22**,** 7-18.

MUNCKHOF, W. 2005. Antibiotics for surgical prophylaxis. *Australian Prescriber,* 28.

NARULA, H., CHIKARA, G. & GUPTA, P. 2020. A prospective study on bacteriological profile and antibiogram of postoperative wound infections in a tertiary care hospital in Western Rajasthan. *J Family Med Prim Care,* 9**,** 1927-1934.

NGAH, J. E., BÉNET, T. & DJIBRILLA, Y. 2016. Incidence of surgical site infections in sub-Saharan Africa: systematic review and meta-analysis. *The Pan African medical journal,* 24**,** 171-171.

ONDURU, O. G., MKAKOSYA, R. S., ABOUD, S. & RUMISHA, S. F. 2021. Genetic Determinants of Resistance among ESBL-Producing <i>Enterobacteriaceae</i> in Community and Hospital Settings in East, Central, and Southern Africa: A Systematic Review and Meta-Analysis of Prevalence. *Canadian Journal of Infectious Diseases and Medical Microbiology,* 2021**,** 5153237.

OPAL, S. M. 2010. Endotoxins and other sepsis triggers. *Endotoxemia and Endotoxin Shock.* Karger Publishers.

ORGANIZATION, W. H. Global guidelines for the prevention of surgical site infection. Geneva: WHO; 2016.

ORGANIZATION, W. H. 2014. *Antimicrobial resistance: global report on surveillance*, World Health Organization.

OWENS, C. & STOESSEL, K. 2008. Surgical site infections: epidemiology, microbiology and prevention. *Journal of Hospital Infection,* 70**,** 3-10.

PATTERSON, S. B., MENDE, K., LI, P., LU, D., CARSON, M. L., MURRAY, C. K., TRIBBLE, D. R., BLYTH, D. M. & GROUP, I. T. 2020. Stenotrophomonas maltophilia infections: clinical characteristics in a military trauma population. *Diagnostic microbiology and infectious disease,* 96**,** 114953.

PEREZ-ROTH, E., CLAVERIE-MARTıN, F., VILLAR, J. & MENDEZ-ALVAREZ, S. 2001. Multiplex PCR for simultaneous identification of Staphylococcus aureus and detection of methicillin and mupirocin resistance. *Journal of clinical microbiology,* 39**,** 4037-4041.

PETCHIAPPAN, A. & CHATTERJI, D. 2017. Antibiotic Resistance: Current Perspectives. *ACS Omega,* 2**,** 7400-7409.

POIREL, L., WALSH, T. R., CUVILLIER, V. & NORDMANN, P. 2011. Multiplex PCR for detection of acquired carbapenemase genes. *Diagnostic microbiology and infectious disease,* 70**,** 119-123.

POURNAJAF, A., ARDEBILI, A., GOUDARZI, L., KHODABANDEH, M., NARIMANI, T. & ABBASZADEH, H. 2014. PCR-based identification of methicillin–resistant Staphylococcus aureus strains and their antibiotic resistance profiles. *Asian pacific journal of tropical biomedicine,* 4**,** S293-S297.

PRADHAN, G. B. & AGRAWAL, J. 2009. Comparative study of post operative wound infection following emergency lower segment caesarean section with and without the topical use of fusidic acid. *Nepal Med Coll J,* 11**,** 189-91.

PRITSCH, M., ZEYNUDIN, A., MESSERER, M., BAUMER, S., LIEGL, G., SCHUBERT, S., LÖSCHER, T., HOELSCHER, M., BELACHEW, T., RACHOW, A. & WIESER, A. 2017. First report on bla (NDM-1)-producing Acinetobacter baumannii in three clinical isolates from Ethiopia. *BMC Infect Dis,* 17**,** 180.

RANJAN, K. P., RANJAN, N., BANSAL, S. K. & ARORA, D. 2011. Prevalence of Pseudomonas aeruginosa in post-operative wound infection in a referral hospital in Haryana, India. *Journal of Laboratory Physicians,* 2**,** 129-32.

RANJAN, K. P., RANJAN, N., BANSAL, S. K. & ARORA, D. R. 2010. Prevalence of Pseudomonas aeruginosa in post-operative wound infection in a referral hospital in Haryana, India. *Journal of laboratory physicians,* 2**,** 074-077.

RATEMO, N. 2014. *Antimicrobial susceptibility pattern of acterial isolates from pus samples at Kenyatta National Hospital, Kenya.* MSc Dissertation. University of Nairobi.

RAZA, M. S., CHANDER, A. & RANABHAT, A. 2013. Antimicrobial susceptibility patterns of the bacterial isolates in post-operative wound infections in a tertiary care hospital, Kathmandu, Nepal. *Open Journal of Medical Microbiology,* 3**,** 159.

RIJAL, B. P., SATYAL, D. & PARAJULI, N. P. 2017. High Burden of Antimicrobial Resistance among Bacteria Causing Pyogenic Wound Infections at a Tertiary Care Hospital in Kathmandu, Nepal. *Journal of pathogens,* 2017.

SEIDELMAN, J. & ANDERSON, D. J. 2021. Surgical Site Infections. *Infect Dis Clin North Am,* 35**,** 901-929.

SHAIKH, S., FATIMA, J., SHAKIL, S., RIZVI, S. M. & KAMAL, M. A. 2015a. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi J Biol Sci,* 22**,** 90-101.

SHAIKH, S., FATIMA, J., SHAKIL, S., RIZVI, S. M. D. & KAMAL, M. A. 2015b. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi journal of biological sciences,* 22**,** 90-101.

SHIFERAW, W. S., AYNALEM, Y. A., AKALU, T. Y. & PETRUCKA, P. M. 2020a. Surgical site infection and its associated factors in Ethiopia: a systematic review and meta-analysis. *BMC surgery,* 20**,** 1-15.

SHIFERAW, W. S., AYNALEM, Y. A., AKALU, T. Y. & PETRUCKA, P. M. 2020b. Surgical site infection and its associated factors in Ethiopia: a systematic review and meta-analysis. *BMC surgery,* 20**,** 1-15.

SHITTA, G., MAKANJUOLA, O., ADEFIOYE, O. & OLOWE, O. A. 2021. Extended Spectrum Beta Lactamase (ESBL), bla(TEM),bla(SHV) and bla(CTX-M), Resistance Genes in Community and Healthcare Associated Gram Negative Bacteria from Osun State, Nigeria. *Infect Disord Drug Targets,* 21**,** 595-602.

SHRESTHA, A., ACHARYA, J., AMATYA, J., PAUDYAL, R. & RIJAL, N. 2022. Detection of Beta-Lactamases (ESBL and MBL) Producing Gram-Negative Pathogens in National Public Health Laboratory of Nepal. *Int J Microbiol,* 2022**,** 5474388.

SPAGNOLO, A., OTTRIA, G., AMICIZIA, D., PERDELLI, F. & CRISTINA, M. L. 2013. Operating theatre quality and prevention of surgical site infections. *Journal of preventive medicine and hygiene,* 54**,** 131.

SUN, J., DENG, Z. & YAN, A. 2014. Bacterial multidrug efflux pumps: mechanisms, physiology and pharmacological exploitations. *Biochemical and biophysical research communications,* 453**,** 254-267.

SZANIAWSKI, M. A. & SPIVAK, A. M. 2019. Recurrent Paenibacillus infection. *Oxford Medical Case Reports,* 2019**,** omz034.

TACCONELLI, E., CARRARA, E., SAVOLDI, A., HARBARTH, S., MENDELSON, M., MONNET, D. L., PULCINI, C., KAHLMETER, G., KLUYTMANS, J., CARMELI, Y., OUELLETTE, M., OUTTERSON, K., PATEL, J., CAVALERI, M., COX, E. M., HOUCHENS, C. R., GRAYSON, M. L., HANSEN, P., SINGH, N., THEURETZBACHER, U. & MAGRINI, N. 2018. Discovery, research, and development

of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis,* 18**,** 318-327.

TADESSE, S., ALEMAYEHU, H., TENNA, A., TADESSE, G., TESSEMA, T. S., SHIBESHI, W. & EGUALE, T. 2018. Antimicrobial resistance profile of Staphylococcus aureus isolated from patients with infection at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. *BMC Pharmacology and Toxicology,* 19**,** 1-8.

TAYE, M. 2005a. Wound infection in Tikur Anbessa hospital, surgical department. *Ethiopian medical journal,* 43**,** 167-174.

TAYE, M. 2005b. Wound infection in Tikur Anbessa hospital, surgical department. *Ethiop Med J,* 43**,** 167-74.

TEFERA, S., AWOKE, T. & MEKONNEN, D. 2021. Methicillin and vancomycin resistant Staphylococcus aureus and associated factors from surgical ward inpatients at Debre Markos Referral Hospital, Northwest Ethiopia. *Infection and Drug Resistance***,** 3053-3062.

TEKIE, K. 2008. Surgical wound infection in Tikur Anbessa hospital with special emphasis on Pseudomonas aeruginosa. *Unpublished MSc thesis in medical microbiology, Addis Ababa University, Medical Faculty, Ethiopia. http://etd. aau. edu. et/dspace/bitstream/123456789/2621/1/KASSAYE% 20TEKIE.. pdf (Accessed on January 31, 2014).*

TENA, D., FERNÁNDEZ, C. & LAGO, M. R. 2015. Alcaligenes faecalis: an unusual cause of skin and soft tissue infection. *Japanese Journal of Infectious Diseases,* 68**,** 128-130.

TICKLER, I. A., KAWA, D., OBRADOVICH, A. E., FANG, F. C., TENOVER, F. C. & HEALTHCARE ASSOCIATED INFECTIONS, C. 2023. Characterization of Carbapenemase-and ESBL-Producing Gram-Negative Bacilli Isolated from Patients with Urinary Tract and Bloodstream Infections. *Antibiotics,* 12**,** 1386.

TSIGE, Y., TADESSE, S., T, G. E., TEFERA, M. M., AMSALU, A., MENBERU, M. A. & GELAW, B. 2020. Prevalence of Methicillin-Resistant Staphylococcus aureus and Associated Risk Factors among Patients with Wound Infection at Referral Hospital, Northeast Ethiopia. *J Pathog,* 2020**,** 3168325.

TUFA, T. B., FUCHS, A., TUFA, T. B., STÖTTER, L., KAASCH, A. J., FELDT, T., HÄUSSINGER, D. & MACKENZIE, C. R. 2020. High rate of extended-spectrum beta-lactamase-producing gram-negative infections and associated mortality in Ethiopia: a systematic review and meta-analysis. *Antimicrobial Resistance & Infection Control,* 9**,** 1-10.

TUFA, T. B., MACKENZIE, C. R., ORTH, H. M., WIENEMANN, T., NORDMANN, T., ABDISSA, S., HURISSA, Z., SCHÖNFELD, A., BOSSELMANN, M., HÄUSSINGER, D., PFEFFER, K., LUEDDE, T., FUCHS, A. & FELDT, T. 2022. Prevalence and characterization of antimicrobial resistance among gram-negative bacteria isolated from febrile hospitalized patients in central Ethiopia. *Antimicrob Resist Infect Control,* 11**,** 8.

UYANGA, F. Z., EKUNDAYO, E. O. & NWANKWO, E. O. bla TEM, bla SHV and bla CTX-M-15 Extended Spectrum Beta-lactamase Produced by Acinetobacter baumanii, Enterobacter clocae and Proteus mirabilis from Pregnant Women in Three Secondary Health Care Facilities in South-south.

VAN DER VEKEN, D., HOLLANDERS, C., VERCE, M., MICHIELS, C., BALLET, S., WECKX, S. & LEROY, F. 2022. Genome-based characterization of a plasmid-associated

micrococcin P1 biosynthetic gene cluster and virulence factors in Mammaliicoccus sciuri IMDO-S72. *Applied and Environmental Microbiology,* 88**,** e02088-21.

VASUNDHARA DEVI, P., SREENIVASULU REDDY, P. & SHABNUM, M. 2017. Microbial profile and antibiotic susceptibility pattern of orthopedic infections in a tertiary care hospital: a study from South India. *Int J Med Sci Public Health,* 6**,** 838-841.

VESTERGAARD, M., FREES, D. & INGMER, H. 2019. Antibiotic Resistance and the MRSA Problem. *Microbiol Spectr,* 7.

VINCENT, C., MOORTHY, K., SARKER, S. K., CHANG, A. & DARZI, A. W. 2004. Systems approaches to surgical quality and safety: from concept to measurement. *Ann Surg,* 239**,** 475-82.

WEISER, T. G., REGENBOGEN, S. E., THOMPSON, K. D., HAYNES, A. B., LIPSITZ, S. R., BERRY, W. R. & GAWANDE, A. A. 2008. An estimation of the global volume of surgery: a modelling strategy based on available data. *Lancet,* 372**,** 139-44.

WIGHT, P. 2020. Threats to Ethiopia's fragile democratic transition. *conflict trends,* 2020**,** 3-11.

WILSON, J., SCHURR, M., LEBLANC, C., RAMAMURTHY, R., BUCHANAN, K. & NICKERSON, C. 2002. Mechanisms of bacterial pathogenicity. *Postgraduate medical journal,* 78**,** 216-224.

WU, Q., SABOKROO, N., WANG, Y., HASHEMIAN, M., KARAMOLLAHI, S. & KOUHSARI, E. 2021. Systematic review and meta-analysis of the epidemiology of vancomycin-resistance Staphylococcus aureus isolates. *Antimicrob Resist Infect Control,* 10**,** 101.

WU, S. W., DE LENCASTRE, H. & TOMASZ, A. 2001. Recruitment of the mecA gene homologue of Staphylococcus sciuri into a resistance determinant and expression of the resistant phenotype in Staphylococcus aureus. *J Bacteriol,* 183**,** 2417-24.

YANG, X., ZHAO, J., WANG, Y., WU, J., WANG, X., WANG, Y., ZHANG, Y. & LI, H. 2021. Molecular Epidemiology of Methicillin-Resistant Staphylococcus aureus in Hospitalized Patients in Eastern Heilongjiang Province, China. *Infect Drug Resist,* 14**,** 1635-1643.

ZAHAN, N. A., HOSSAIN, M. A., MUSA, A. K., SHAMSUZZAMAN, A. K., MAHAMUD, M. C., MAMUN, A. A., PAUL, S. K., AHMED, S., SUMONA, A. A., BEGUM, Z., ALAM, M., YUSUF, M. A. & UDDIN, M. S. 2009. PCR for mecA gene of methicillin resistant Staphylococcus aureus. *Mymensingh Med J,* 18**,** 21-6.

ZAHRAN, W. A., ZEIN-ELDEEN, A. A., HAMAM, S. S. & SABAL, M. S. E. 2017. Surgical site infections: Problem of multidrug-resistant bacteria. *Menoufia Medical Journal,* 30**,** 1005.

ZEYNUDIN, A., PRITSCH, M., SCHUBERT, S., MESSERER, M., LIEGL, G., HOELSCHER, M., BELACHEW, T. & WIESER, A. 2018a. Prevalence and antibiotic susceptibility pattern of CTX-M type extended-spectrum β-lactamases among clinical isolates of gram-negative bacilli in Jimma, Ethiopia. *BMC infectious diseases,* 18**,** 524-524.

ZEYNUDIN, A., PRITSCH, M., SCHUBERT, S., MESSERER, M., LIEGL, G., HOELSCHER, M., BELACHEW, T. & WIESER, A. 2018b. Prevalence and antibiotic susceptibility pattern of CTX-M type extended-spectrum β-lactamases among clinical isolates of gram-negative bacilli in Jimma, Ethiopia. *BMC Infect Dis,* 18**,** 524.

ZONG, Z. 2013. Characterization of a complex context containing mecA but lacking genes encoding cassette chromosome recombinases in Staphylococcus haemolyticus. *BMC Microbiology,* 13**,** 64.

ZONG, Z., PENG, C. & LÜ, X. 2011. Diversity of SCC mec elements in methicillin-resistant coagulase-negative staphylococci clinical isolates. *PloS one,* 6**,** e20191.

Annexes

Annex I. Participant Information sheet: English Version

Name of principal investigato: **Seble Worku (PhD candidate)**

Introduction:

Dear participant, you are invited to participate in the research study conducted by a PhD candidate, from Addis Ababa University. Your participation is absolutely based on voluntarily basis. The research team includes a principal investigator, Medical microbiologists, data/specimen collectors including surgeons and supervisors from AAU & AHRI.

Please much time as you need to read/listen the information sheet

Purpose of the research project:

We are asking you to take part in the study because surgical site infection is our country's major public health problems. Studies showed that the bacterial isolates, drug resistance pattern, resistant gene profile and risk factors because SSI varies from place to place. Even though few studies have been conducted to determine Molecular characterization of bacterial profile of surgical site infection and antimicrobial resistance patterns in Ethiopia: A multicentre prospective cross-sectional study that causes SSI in different sites of the country; in the study areas, there is a lack of updated information about bacterial isolates and antimicrobial sensitivity among SSI as well as there is no study on plasmid profile, genes that encodes drug resistance and risk factors in Ethiopia. The findings of this study may contribute on designing strategies which help to improve treatment and management of surgical site infection specifically

Procedure:

If you are willing to participate in this project, you need to understand and give your consent. The required clinical sample (swab/ needle aspiration or/and venous blood if physician suspect surgiery related sepsis) collected by experienced health personnel. Amount of blood volume required from patients for blood culture is 1ml-10ml it depends on age (age<1year-1ml., 2-5year,

2ml., 6-14year, 3-5ml., >15year,10ml). If the microbes obtained from the sample, resistance profile conducted but the result not reached to change their treatment.We also asked you some questions about yourself by using a set of questions

Risks and discomforts:

We do not expect that any harm happened to you because of joining this study. A minimal pain may occured during swabs/needle aspiration taking.

Benefits to participants:

If you agreed to take part in this study, the benefit by knowing the result of culture and sensitivity pattern of a collected specimen, and whenever there are culture positive results appropriate medications prescribed and advised accordingly

Cost: No payment will be requested from you as a fee to participate in the study

Payment/ compensation for participation

You not received any payment for your participation in this research study.

What Participation Involves: If you agree to join the study, you interviewed using questionnaire, detailed information on social demographic characteristics, past medical history and physical examination requested at a time. A wound swabs or needle aspiration taken from the wound site.

Confidentiality:

All information collected on questionnaires entered into computer with out your name but identification number assigned on it. The questionnaires handled with greater secrecy, and kept under lock and key, and not be revealed to anyone except the principal investigator and the physicians following you/ your child in order to maintain confidentiality.

In Case of Injury:

Anticipate beyond minimal risk associated with invasive procedure to collect specimen. However, if any physical injury resulting from participation in this research should occur, we provided with medical treatment according to the current standard of care in Ethiopia.

Alternative:

If you choose not to participate in the study, you continue to receive all services from the hospital laboratory.

Participation and withdrawal:

Taking part in this study is completely your choice. If you choosen not to participate in the study or if you decided to stop participating in the study you continued to receive all services that you would normally get from hospital. You can stop participating in this study at any time, even if you have already given your consent. Refusal to participate or withdraw from the study not involved penalty or loss of any benefits to which you are otherwise entitled.

Person to contact:

The project gets approval from AAU and AHRI IRB. Have you any question?

If you have any question you can contact the principal investigator and the institution, you may ask at any time you want:

Principal investigator: **Seble Worku** Cell phone +251-911782487

E-mail workuseble@ymail.comSignature:

Advisor: Dr. Tamrat Abebe Cell phone 0911447227 Dr. Adane Mihrte Cell phone +251-911408984

Institutional review board faculty of Medicine, Addis Ababa University,

Telephone 0118961396; E-mail: Chs.irb2aau.edu.et

Annex-IAmharic version of Information sheet

የመረጃቅጽ

ለጥናቱተሳታፊዎች /ወላጆች/አሳዳጊዎችየሚሰጥማብራሪያ

ርእስ፡በቀዶጥገናቁስልዉስጥየሚገኙባክቴሪያዎችንመለየት፣የሚያጋልጡቅድመሑኔታዎችንናየጸረ-ባክቴሪያመድሀኒትየመቋቋምባህሪያቸዉንሆስፒታልከሚመጡታካሚዎችላይለማዎቅነዉ፡፡

የተመራማሪውስም: **ሰብለወርቁ**

መግቢያ :

በመጀመሪያእርስዎበዚህጥናትዉስጥተሳታፊእንድሆኑሲጠየቁተሳታፊየሚሆኑትፈቃደኛከሆኑብቻነዉ፡፡ ይህንንጥናትየሚያካሂዱትሰዎችየተዋቀሩትበዋናተመራማሪ፣በጥናቱተቆጣጣሪዎችእናናሙናሰብሳቢዎችነዉ፡፡

ዓላማ

የዚህጥናትተዋናአላማበቀዶጥገናቁስልዉስጥየሚገኙኢንፌክሽንአምጪተህዋሲ (ባክቴሪያ) መለየት፣ የሚያጋልጡቅድመሑኔታዎችንናየጸረ-ባክቴሪያመድሀኒትየመቋቋምባህሪያቸዉንለማጥናትይቻልዘንድነዉ፡፡

ለጥናቱየሚያስፈልግናሙናአወሳሰድ

ይህንጥናትበሆስፒታልዉስጥለማካሄድየእርስዎንግለ-ታሪክመጠየቅናፈቃደኛነትዎንማግኘትአስፈላጊነዉ፡፡ የጥናቱንአላማናጥቅምበሚገባተረድተዉለመሳተፍፍላጎትከለዎት፣ ለምረመራየሚያስፈልገዉንናሙናይሰጣሉእናናሙናለሚዎስደዉሰዉለሚጠይቀዎትጥያቄተገቢዉንመልስይሰጣሉ፡፡

ከጥናቱጋርተያይዞየሚመጣጉዳት

በዚህጥናትዝርዝርኣሰራርሂደትውስጥአካላዊወይምአእምራዊጉዳትአይኖርም፡፡ ነገርግንናሙናዉበሚወሰድበትጊዜመጠነኛየሆነየህመምስሜትሊሰማወትይችላል፡፡ ይህየህመምስሜትምንምአይነትችግርአያመጣብዎትም፡፡

የጥናቱጥቅም፡

የምርምሩዉጤትበቀዶጥገናዉስጥየሚገኙአደገኛየሆኑተህዋሰያንንበማጥናትየመፈወስአቅምያላቸዉንመድሀኒቶችንለማወቅያስ ችላል፡፡እንደዚሁምበእርስዎቁስልላይእነዚህባክቴሪያዎችከተገኙአስፈላጊዉመድሀኒትእንድያገኙለሃኪሙይነገርሎታል፡፡

በተጨማሪምይህጥናትየመከላለልስራበሆስፒታልእናበህብረተሰቡዘንድእንዲኖርያግዛል፡፡
እንደዚሁምደግሞየላቦራቶሪአገልግሎትበሌለበትጤናተቋምትክክለኛመድሃኒትለመስጠትእንደአመላካችሆኖያገለግላል፡፡

ለመሳተፍክፍያ

የለዉም፡፡

ለተሳትፎየሚሰጥማካካሻ፡

ምንምአይነትየካሳክፍያየለዉም፡፡

የመረጃሚስጢራዊነት:

ሁሉምከተሳታፊዎችየሚሰበሰቡመረጃዎችበሚስጢርየሚያዙእናየሚጠበቁይሆናሉ፡፡
በማንኛውምምክኒያትተሳታፊዎችእነማንመሆናቸውንየሚያሳይበመጠይቁይሁንበሌላነገርአይኖርም፡፡
የተሰበሰቡመረጃዎችለሶስተኛወገንተላልፎአይሰጥም፡፡በተጨማሪምውጤቱተሰብስቦየሚያዘውበዋናአጥኚነው፡፡

ከጥናቱጋርተያይዞየሚመጣጉዳት

በዚህጥናትዝርዝርኣሰራርሂደትውስጥአካላዊወይምአእምራዊጉዳትአይኖርም፡፡
ነገርግንናሙናዉበሚወሰድበትጊዜመጠነኛየሆነየህመምስሜትሊሰማወትይችላል፡፡
ይህየህመምስሜትምንምአይነትችግርአያመጣብዎትም፡፡

አማራጭ:

ማንኛዉምበጥናቱመሳተፍፍላጎትየሌለዉከዋናዉየሆስፒታሉላቦራቶሪሄዶማሰራትይችላል፡፡

በፍቃደኝነትላይየተመሰርተተሳትፎ :

በጥናቱለመሳተፍሙሉበሙሉበተሳታፊዎችፍቃደኝነትየተመሰረትነው፡፡
ከዚህበተጨማሪየተሳታፊዎችመሳተፍእናአለመሳተፍበራሳቸዉብቻየሚወሰንእንጂየማንምጣልቃገቢነትአይኖረውም፡፡
እንደዚሁምደግሞተሳታፊዎችበማንኛውምግዜያለምንምቅጣትጥናቱንማቋረጥይችላሉ፡፡

ተጠሪ:

ጥያቄካለመጠየቅይችላሉ?

በማንኛዉምጊዜመጠየቅየሚፈልጉትጥያቄካለመጠየቅይችላሉ፡፡

ይህየጥናትእቅድበአዲስአበባዩኒቨርሲቲናበአርማወርሀንሰንየምርምርተቋምየጥናትተቆጣጣሪቦርድየፀደቀነዉ፡፡

ስለጥናቱጥያቄካለወትየተመራማሪአድራሻ : **ሰብለወርቁ**

ስልክ:+251911782487**ኢ-ሜል:**workuseble@ymail.com

- Institutional review board faculty of Medicine, Addis Ababa University,

  Telephone 0118961396; E-mail: Chs.irb2aau.edu.et

Information Sheet for Participants: Afan Oromo Version

MataDureeQorannoo: - Baakteeriyaa Madaa baqaqsanii yaaluu faalame keessatti argamuu fi miidhaa baakteriyaan qorichaan walbare fiduu danda'u

Maqaa qorattuu: - Sablee Warquu (Kadhimamtuu PhD)

- **Seensa**

Obbo/Aadde qorannoo kana irratti akka hirmaattaniif kan aferamtaniif qorannoo Kaadhimamtuu PhD Yuunivarsitii Finfinneetii dhufteen adeemsifamuuf. Hirmaannaan keessan fedhii keessan irratti kan hundaa'eedha.Gareen qorannochaa qorataa walii gala, Ogeessa maayikiroobaayooloogii,raga sassaabdota, ogeessota baqaqsanii yaaluu fi qindeessitoota yuunivarsitii Finfinnee fi Dhaabbata Qorannoo Armawar Hansen waliin wal ta'uudhaani.

- **Kaayyoo Qorannoo**

Dhibeen madaa baqaqsanii yaaluu faalameen dhufu dhukkuba lubbuu namaagalaafachuu danda'uufi yoo dafamee baramee fi wal'aaname rakkoo hamoo sababa dhukkuba Kanaan dhufan hambisuu yookaan hir'isuu fi wal'aanamanii yeroo gabaabaa keessatti fayyamuu danda'uudha.

Kaayyoon qorannoo kanaa baakteeriyaa madaa baqaqsanii yaaluu faalame fiduu danda'uu fi baakteeriyaa qorichaan wal bare dhibee kana fiduu dand'u adda baasuu fi qorannoo geggeessuu dha.

- **Adeemsa Hojii**

Qorannoo keessatti hirmnaachuun guutummaan guutuutti filannoo keessan.Saamuudni barbaachisu (maddarraa kan haqamu/lilmoodhaan kan waraabamu) ogeessamuuxannoo qabuun kan fuudhamuudha.Gaaffilee waa'ee keessan ibsu muraasas isin gaafanna.

- **Miidhaa fi mudannoo**

Qorannoo kana irratti waan hirmaattaniif miidhaan isin mudatu hin jiru. Wanti godhamu saamuuda qorannoo dhukkuba keessaaniif ta'an isinirraa funaanuu fi bu'aa isaanii galmeessuu qofa dha.

- **Faayidaa Hirmaattotaa**

Bu'aan qorannoo kanaa isin yaaluuf ni fayyada jedheen abdadha. Akkasumas, bu'aan qorannoo kanaa ogeessi gorsaa fi yaalii barbaachisaa akka isiniif kennaniif ni fayyada.

- **Baasii**

Hirmaannaa keessaniif qarshiin isinii kennamu hin jiru.

- **Onneessituu fi beenyaa**

Qoranno kana irraatti hirmaachuuf baasii isin baastan hin jiru. Qorannoo kanarratti qooda fudhachuuf yoo murteessitan gaaffilee waa'ee keessan ibsan muraasa, Seenaa dhukkuba kanaan dura isin qabdaniifi madaa keessan irraa saamuuda xiqqoo ni fudhatama

- **Icitummaa**

Iccita dhuunfaa keessanii ni kabajna; ragaa dhuunfaa keessaniis iccitaan ni qabna. Dhimmi dhuunfaa keessanii marti beekumsa keessaniin ala hin saaxilamu. Ragaan isin irraa funaanamu marti maqaa keessaniin osoo hin ta'in lakkoofsa iccitaa addaan galmeeffama. Ragaan isinirraa funaanamu marti hamma seeraan deeggamarumutti iccitaan ni qabama. Ragaan isinirraa funaanamus dhimma armaan olitti ibsame qofaaf oola. Bu'aan qorannoo kanaa qabiyyee saayinsummaa isaatiif maxxanfamuu ni dana'a. Garuu, eenyuumaan kessan bakka kam irrattiyyuu hin ibsamu.

- **Yoo miidhaan jiraate**

Qorannoo kanarratti hirmaachuudhaan miidhaan isinirra yookaan daa’ima keessanirra ga’u akka hin jirre isiniif mirkaneessuu feena.Garuu sababa qorannoo kanarratti hirmaattaniif miidhaan isirras ta’e daa’ima keessan irra ga’e yoo jiraate yaalii barbaachisaa isiniif goona.

❖ **Filannoo**

Qorannoo kanarratti hirmaachuuf yoo fedhii dhabdan taajijila laaboraatoorii hospitaalichi kennu hundumaa argachuuf mirga guutuu qabdu.

❖ **Mirga hirmaattotaa fi hirmaannaa addaan kutuu**

Hirmaannaan keessan fedhii keessan qofarratti waan hundaa’uuf diduuf mirga guutuu qabdu. Erga jalqabdaniis yeroo barbaaddanitti addaan kutuu ni dandessu. Murtoon keessan garuu tajaajila argachuu qabdan mara irratti dhiibbaaa hin fidu. Qorattoonni qo'annoo kanaa yeroo barbaadanitti akka isin hirmaannaa keessan addaan kuttan godhuu ni danda’u. Kunis kan ta’u yoo isin fedhii dhabdan fi adeemsa qoranichaa hordofuu diddan ta’a. Kunis miidhaa yookiin adabbii tokkollee isirraan hin ga’u.

❖ **Wal-qunnamtii**

Gaaffii kamuu yoo qabaattaniif, yaadni ifa hin taane yoo jiraate fi shakkii waa’ee qo'annoo yoo qabdan teessoo armaan gadiin quunnamuu ni dandeessu.

Qorattuu: - Sablee Warquu Bilbila: +251-911408984

E-mail: workuseble@ymail.com Mallatto:

Gorsaa: Dr Adaanee Mihrat Bilbila: +251-911408984

Waan mirga keessan ilaallatu yoo ta’e, hoogganaa boordii Gulaalaa fi Itiksii Yuuniversitii Finfinnee Lakk Bilbila 0118961396; Chs.irb2aau.edu.et

Annex II. Participant consent form

English version consent form (Age >18)

Date -----------------------

Serial no…………

I have been informed about the study entitled "Antibiotic Resistance Profiles of Bacterial Isolates from Surgical Site Infection in Ethiopia. The objective and application of the study were explained to me. I am also informed that all information collected from me will keep confidential. Moreover, I have also been well informed of my right to keep hold of, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care and hospital access. It is therefore with full understanding of the situations that I agreed to give the written consent to the researcher to use the specimen taken from my surgical site for investigation. In addition; the results will be reported timely to physician in charge for appropriate treatment and management of my case. Therefore I agree to participate on this study with full voluntarism.

Name of study participant--------------------------------Signature ------------------Date ---------

Name of witness ------------------- Signature---------------------Date-----------------

Name of data collector---------------------- Signature--------------------- Date----------------

Name of investigator ------------------------- Signature ---------------------- Date -----------

Place of data collection (Name of the Health facility)------------------

Thank you for your participation!

Consent Form (Amharic version) ቀን-----------

የስምምነት ቅጽ (ከ 18 አመትበላይለሆኑ)

መለያቁጥር---------

እኔተመራማሪውእንደገለጸልኝየጥናቱዋናአላማዉበቀዶጥገናቁስልዉስጥየሚገኙኢንፌክሽንአምጪተህዋሲ (ባክቴሪያ) መለየት፣
የሚያጋልጡቅድመሁኔታዎችንናየጸረ-
ባክቴሪያመድሀኒትየመቋቋምባህሪያቸዉንለማጥናትበሚካሄደውጥናትዉስጥእንድሳተፍፍቃደኛመሆኔንናአለመሆኔንተጠይቄአ
ለዉ፡፡
በርግጥምይህባክቴሪያመለየትእናመድሀኒትየተለማመደሆኖከተገኘእናባክቴሪያውኢንፌክሽንለማምጣትየሚጠቀምበትመሳሪያአ
ይነትከተለየይህምርምርበባክቴሪያአማካኝነትየሚመጣየቁስልዉስጥኢንፌክሽንለመከላከልየሚረዱመፍትሔዎችንለመቀየስየሚ
ረዳመሆኑንበቅድሚያተነግሮኛል፡፡
ለዚህጥናትወይምምርምርያገለግልዘንድበፈቃደኝነትላይየተመሰረተየቁስልናሙናእኔእንድሰጥእናየቁስልናሙናውእንዲመረመር
ተጠይቄአለሁ፡፡
በተወሰደውየቁስልናሙናውስጥየቁስልኢንፌክሽንአምጪተህዋስያንየተገኙእንደሆነእኔንለሚያክመውሐኪምውጤቱንእንዲያው
ቀውእንደሚደረግተነግሮኛል፡፡በዚህምመሰረትእኔለምርምሩየሚያገለግልየቁስልናሙናለመስጠትተስማምቼአለሁ፡፡
እኔከፈለኩኝማንኛውምውጤትበሚስጥርእንደሚያዝተነግሮኛል፡፡
በዚህምርምርወይምጥናትእኔምንምአይነትማበረታቻወይምየገንዘብጥቅምእንደማላገኝተነግሮኛል፡፡
ይህንንየስምምነትቅጽከመፈረሜበፊትእንዳስብበትበቂጊዜተሰጥቶኝየተስማማሁመሆኔንበፊርማዬለማረጋገጥእወዳለሁ፡፡

የተሳታፊውስም---ፊርማ-------------------ቀን---------------

የምስክርስም (ላልተማረየምርምሩተሳታፊ)------------------------------------ፊርማ-------------------ቀን----------

የመረጃሰብሳቢውስም---------------------------------------ፊርማ-------------------ቀን--------------

የተመራማሪውስም---ፊርማ--------------------ቀን-------------

መረጃውየሚሰበሰብበትቦታ (የጤናተቋሙ) ስም ---

በጥናትናምርምሩበመሳተፍዎበጣምእናመሰግንዎታለን!!

Afan Oromo Version Consent Form (Age >18)

T. Lakk______________ Guyyaa_____________

Qorannoo “Baakteeriyaa Madaa baqaqsanii yaaluu faalame keessatti argamuu fi miidhaa baakteriyaan qorichaan walbare jedhamu irratti akkan hirmaadhuuf afferameera. Kaayyoon qorannoo kanaa fi haala adeemsa qorannocha waliigalaa fi faayidaan isaa mara naaf ibsameera.Ragaa ani kennu maraa fi saamuudni laaboraatoorii narraa funaanamu akka iccita guutuun qabamus naaf ibsameera. Akkasumas, qorannocha irratti hirmaachuufi dhiisuun koo fedhii koo qofaan akka murteeffamuufi yeroo barbaadetti hirmaannaa koo akkan addaan kutuuf danda’u naaf ibsameera. Murtoon koo kunis hospitaalicha keessatti tajaajila an argadhu kan nan ittisnee fi rakkoo kamuu akka narraan hin geenyes hubadheera.

Anis kana hubadhee, haala jiru mara xiinxalee qorannicha irratti hirmaachuu fi madaa koo irraa saammuda barbaachisu kennuuf fedhii koo guutuun murteesseera. Firiin qorannoo kootiis yeroodhaan hakiima kootti naaf laatamee tajaajila barbaachisaa akkan argadhu natti himameera. Kanaafuu, qorannoo kana irratti fedha koon hirmachuu koofi waligala.

Maqaa hirmaata ___________________mallattoo ____________ Guyyaa_________________

Maqaa ragaa ____________________mallattoo ____________ Guyyaa____________________

Maqaa raga funaanaa ________________mallattoo ____________Guyyaa_________________

Maqaa Qorataa____________________mallattoo ____________ Guyyaa_________________

Bakka ragaan itti sassaabame (Maqaa Dhaabbata Fayyaa)________________________________

Hirmaannaa Keessaniif Galatoomaa!

Assent form, for age 12-17 years old study volunteers

I---------------being a person aged _______years hereby consent to in the intended research as explained and understoodby me.

I have understood the implications of risks and immediate benefits of the investigation (research) and I agreed the child to give the swab specimen.I understand that I have the right to withdraw from the research at any time, for any reasonwithout penalty or harm. In case of withdrawal, I understand that the researchers will continue totake care of me like any other patient.

All the above conditions have been explained to me in the language, which I can understand. Name of the patient: ____

Patient signature: ____

Name of Data collector: ____

Data collector signature: _______________________________________

Date: ____

Witness: _________________________ Date: __________________________

ለአቅመ አዳም ላለደረሱ እድሜያቸው ከ 12.17 የሆኑ ወጣቶች ብጥናቱ ተሳታፊ ለመሆን የሚያደርጉት አወንታዊ ማረጋገጫ ፊርማ

እኔ ____ እድሜየ_________________________________ የሆነ የውጣት በሚገባ እና በዝርዝር በተገለጠለኝ ጥናት ተሳታፊ እሆን ዘንድ ስምምነቴን ለ _______________________ እሰጣለሁ። በጥናቱ ውስጥ የተካተቱ ዝርዝር፤ አሰራሮች፤ አድጋዎች፤ ምርመራዎችና እግዛዎችን ሁሉ በሚገባ የተረዳሁ ሰለሆነ በጥናቱ ተሳታፊ እንድሆን ፈቅጃለሁ። በተጨማሪ በማንኛውም ድረጃ ከዚሁ ከተጠቀሰው ጥናት እንድውጣ ሙሉ መብት እንደሚኖረኝ ተገንዝቤአለሁ።

ይህንን ለማድረግ ስወሰን ምንም አይነት ቅጣት/ጉዳት እንደማይደርስ ተገቢ የሆኑ ህክምናዎች ሁሉ እንድሚደረጉልኝ በማመን ነው። እነዚህ የስምምንት መስረቶች ሁሉ በሚገባ በምረዳው ቃንቃ የተገለፖልኝ መሆኑን አረጋግጣለሁ።

የተሳታፊው ስም---ፊርማ------------------ቀን---------------

የምስክርስም (ላልተማረየምርምሩተሳታፊ)-----------------------------------ፊርማ------------------ቀን----------

የመረጃሰብሳቢውስም--ፊርማ------------------ቀን--------------

የተመራማሪውስም--ፊርማ------------------ቀን--------------

መረጃውየሚሰበሰብበትቦታ (የጤናተቋሙ) ስም --

በጥናትናምርምሩበመሳተፍዎበጣምእናመሰግንዎታለን!!

Afan Oromo version Assent form for age 12-17 years old study volunteers

Ani ______________________________________ kan jedhamu umriinkoo ______kan ta'e qorannoo kana irratti hirmaachuuf waliin gala.

Haala adeemsa qorannochaa waliigalaa fi faayidaa isaa mara naaf ibsamee madaa koo irraa saamuuda laboraatoorii qorannichaaf barbachisu kennuuf fedhii kootiin waliigala. Akkasumas, qorannocha irratti hirmaachuu fi dhiisuun koo fedhii koo qofaan akka murteeffamuu fi yeroon barbaadetti hirmaannaa koo akkan addaan kutuu danda'uu hubadheera. Murtoon koo kunis adabbii tokkollee akka narraan hin geenyee fi akka dhukkubsataa kamiittuu tajaajilli barbaachisu akka naaf kennamu naaf ibsameera.

Wantootni armaan olitti dubbataman kunneen afaan ani dubbadhuun/hubadhuun naaf ibsamaniiru.

Maqaa Dhukkusataa: ____

Mallattoo Dhukkubsataa: ____

Maqaa ragaa funaanaa: ____

Mallattoo ragaa funaanaa: ____

Guyyaa: ____

Ragaa: __________________________ Guyyaa: ___________________ ___________

Parental/guardian consentform age less than <1-12 years

I ________________________________being a person aged ≥ 18yrs and being the parent/Lawful guardian of_____________________________ hereby consent to _________________________________ in the intended research as explained and understood by me. I have understood the implications of risks and immediate benefits of the investigation (research) to ____________________. I have agreed my child to give the surgical site infection specimen. I understand that I have the right to withdraw my child from the research atany time, for any reason without penalty or harm. In case of withdraw; I understand that the researchers will continue to take care of like any other patient.

All the above conditions have been explained to me in the language, which I can understand.
Guardian's full name: ____
Guardian's signature: ____
Date: ____
Child's full name: ____
Person obtaining consent: ____
Signature of person obtaining consent: ____
Witness: __________________________ Date: __________________________

እድሜያቸው ለአቅመ አዳም ላለደረሱ የስምምነት ማረጋገጫ ፊርማ

እኔ ___________________________________ እድሜየ 18 እና ከ18 በላይ የሆነ ወጣት____________________________ በሚገባ እና በዝርዝር በተገለፀለኝ ጥናት ተሳታፊ ይሆን ዘንድ ስምምነቴን ለ _____________________ እሰጣለሁ። በጥናቱ ውስጥ የተካተቱ ዝርዝር፤ አሰራሮች፤ አድጋዎች፤ ምርመራዎችና እግዛዎችን ሁሉ በሚገባ የትረዳሁ ሰለሆነ በጥናቱ ተሳታፊ እንድሆን ፈቅጃለሁ። በተጨማሪ ወጣት ________________በማንኛውም ድረጃ ከዚሁ ከተጠቀሰው ጥናት እንዲወጣ/እንድትውጣ የማድረግ ሙሉ መብት እንደሚኖረኝ ተገንዝቤአለሁ።

ይህንን ለማድረግ ስወሰን በተሳታፊው ምንም አይነት ቅጣት/ጉዳት እንደማይደርስ ተገቢ የሆኑ ህክምናዎች ሁሉ እንድሚደረጉለት በማመን ነው። እነዚህ የስምምንት መስረቶች ሁሉ በሚገባ በምረዳው ቃንቃ የተገለፆልኝ መሆኑን አረጋግጣለሁ።

የወላጅ/ህጋዊ የአሳዳጊ ሙሉ ስም--ፊርማ-------------------ቀን-------

የምስክርስም ሙሉ ስም ------------------------------------ፊርማ-------------------ቀን----------

የመረጃሰብሳቢውስም--ፊርማ-------------------ቀን-------------

የተመራማሪውስም--ፊርማ-------------------ቀን-------------

መረጃው የሚሰበሰብበት ቦታ (የጤናተቋሙ) ስም --
በጥናትና **ምርምሩ** በመሳተፍዎ በጣም እናመሰግንዎታለን!!

Afan Oromo Version Parental/guardian consent form age less than <1-12 years

Ani ___________________________________ kan jedhamu nama umriin koo 18 ol ta'eefi maatii/ guddisee mucaa_______________________kanan ta'e qorannoo kana irratti mucaakoo hirmaachisuuf walii galteekoo _________________________dhaaf nan kenna. Qorannoon kanarratti haala dhibeen kun itti qoratamuu fi wal'aanamu, akkasuma haala adeemsa qorannocha waliigalaa fi faayidaa isaa mara naaf ibsamee jira

Saamuuda laboraatoorii qorannichaaf barbachisu mucaan koo akka kennuuf fedhii kootiin waliigala. Akkasumas, mucaakoo qorannocha irratti hirmaachisuu fi dhiisisuun koo fedhii koo qofaan akka murteeffamuu fi yeroon barbaadetti hirmaannaa mucaa koo akkan addaan kutuu danda'uu fi murtoon koo kunis adabbii tokkollee akka mucaa koo irraan hin geenyee fi tajaajilli barbaachisaan mucaa koof akka godhamu naaf ibsameera.

Maqaa maatii: ____

Mallattoo maatii: ____

Maqaa Mucaa: ____

Maqaa nama walii galtee fudhatee: ____________________________________

Mallattoo nama walii galtee fudhatee: _________________________________

Ragaa: _______________________________ Guyyaa: ___________________

Annex III. Questionnaire

Title: Molecular characterization of bacterial profile of surgical site infection and antimicrobial resistance patterns in Ethiopia: A multicentre prospective cross-sectional study

Information's extracted from directly from the patients and patients' clinical case notes (patients' files)

Socio-demography data

Study site___________________________Serial number _____________________

Patient: Inpatient________________ or Outpatient_______________

Ward_________________________

Clinical diagnosis: ---------------------------

Surgical site infection criteria (underline): **Superficial, deep, organ space**

Date of interview ……………………….

1. Surgical department in which patient admitted or attending (a) ☐ General & pediatrics surgery (b) ☐ gynecology/obstetrics (c) ☐ orthopedics /trauma D) ☐ Others----------------

2. Age……………….. 3. Sex……………………... 4. Address…………………5. Date of Admission…………

6. Date of surgery………………………7. Date of discharge………………………

8. Preoperative hospital stay: 1) ☐ ≤ 3 days 2) ☐ 4-7 days 3) ☐ More than 7 days

9. Presenting complains: (1) ☐ Pain or swelling at the operation site (2) ☐ Gaping at the operation Site (3) ☐ Discharge from surgical site

10. Past medical history (1) ☐ DM (2) ☐ Prolonged steroid usage (3) ☐ Hypertension (4) ☐ boils

11. Past medical history of hospital admission (1) ☐ Yes (2) ☐ No

12. If yes, how many times (1) ☐ Once (2) ☐ Twice (3) ☐ Three (4) More than three

13. Last admission was (1) ☐ Within 6 months (2) ☐ Within a year (3) ☐ More than one year ago.

14. History of previous use of antibiotics within one month (1) ☐ Yes (2) ☐ No

15. If yes, what type of antibiotics (1) ☐ Gentamycin (2) ☐ Ceftriaxone (3) ☐ Ciprofloxacin (4) ☐ Metronidazole (5) ☐ Others ______________________________

16. For how long have been in such antibiotics (1) ☐ <=7 days (2) ☐ 8-15 days (3) >15 days

17. Family social history (1) Smoking, a) Yes ☐ b) No ☐ (2) Alcoholic, a) Yes ☐ b) No ☐

18. Preoperative diagnosis (1) ☐ Open fracture (2) ☐ Closed fracture (3) ☐ Obstructed labor (4) ☐ Peritonitis (5) ☐ Diabetic foot (6) ☐ Others __________________________

19. Surgical procedure performed (1) ☐ Caesarian section (2) ☐ Surgical debridement and External fixation (3) ☐ Open reduction internal fixation (ORIF) (4) ☐ Laparotomy (5) ☐ *Total Abdominal Hysterectomy* (TAH) (6) ☐ Amputation (7) ☐ Surgical debridement (8) ☐ Others___________________________

20. Type of surgery: (1) ☐ Clean surgery (2) ☐ Clean contaminated surgery (3) Contaminated ☐ surgery (4) ☐ Dirty surgery

21. Nature of surgery (1) ☐ Emergency surgery (2) ☐ Elective surgery

22. Preoperative hair removal (1) ☐ Previous night before surgery (2) ☐ Morning of surgery

23. Timing of surgical antimicrobial prophylaxis (1) ☐ Before the operation (2) ☐ During operation (3) ☐ After operation (4) ☐ Not initiated at all.

24. Type of surgical antibiotic prophylaxis given (1) ☐ Ceftriaxone (2) ☐ Gentamycin (3) ☐ Metronidazole (4) ☐ Others ____________________

25. Duration of operation in minutes (1) ☐ 0-60 (2) ☐1-120 (3) ☐20

Questioner (Amharic version)

ርእስ- በቀዶጥገናውስጥየሚገኙአደገኛተወስያንን(ባክቴሪያ) መለየት፤የሚያጋልጡቅድመሁኔታዎችንናየፀረ-ባክቴሪያ መድሃኒት መቃቃም ማወቅ

የጥናትቦታ ___________________________________

መለያቁጥር………..

ቀን……….

1.ቀዶ ጥገና የተደረገበት ክፍል ሀ). ጠቅላላናየሀፃናትቀዶጥገና ለ). ማህፀንና ፅንስ ቀዶ ጥገና ሐ). የአጥንትቀዶጥገና መ) ሌሎች___________________________

2.እድሜ…………………….

3. ፆታ………………………..

4. አድራሻ…………………..

5. ሆስፒታል የተኛበ ትቀን……………

6. ቀዶ ጥገና የተደረገበት ቀን……….

7. ከሆስፒታል የወጣበት ቀን………..

8. ከቀዶ ጥገና በፊት ሆስፒታል የቆዩት ቀን ብዛት 1) ☐ ≤3 ቀናት 2) ☐ ከ4-7 ቀናት 3) ☐ >7 ቀናት

9. ታካሚው አሁን ያላቸው ችግር 1) ☐

ቀዶ ጥገና ከተደረጉበት ቦታ ላይ እብጠት ወይም ህመም2) ☐

ቀዶ ጥገና ከተደረጉበት ቦታ ላይ ክፍት መፍጠር 3) ☐ ቀዶ ጥገና ከተደረጉበት ቦታ ላይ ፈሳሽ መፍጠር ☐

10. በፊት የነበረ የጤና ታሪክ 1) ☐ የስኳር ህመም 2) ☐ ለረጅም ጊዜ ስቴሮይድ መጠቀም 3) ☐ የደም ግፊት 4) መግል መቋጠር ☐

11. ከአሁን በፊት ሆስፒታል ተኝተው ታክመው ያውቃሉ 1) አዎ 2) ☐ የለም ☐

12. አዎ ከሆነ ስንት ጊዜ (1) ☐ አንድ ጊዜ (☐ ሁለት ጊዜ (3) ☐ ሶስት ጊዜ (4) ☐ ከሶስት ጊዜ በላይ

13. በቅርብ ጊዜ የተኙት መቼ ነበር (1) ☐ ባለ ፉት 6 ወራት (2) ☐ ባለፈው አንድ ዓመት (3) ☐ ከአንድ ዓመት በፊት ☐

14. በዚህ አንድ ወር ውስጥ የፀረ ተወህስያን (ባክቴርያ) መድሓኒት ተጥቅመው ያውቃሉ

(1) ☐ አዎ (2) ☐ የለም

15. አዎ ከሆነ ምን መድሃኒት (1) ጀንታማይሲን ☐ (2) ሴፍትሪያግዞን ☐ (3) ሲፕሮፍሎክሳሲን ☐

(4) ሜትሮኒዳዞል ☐ (5) ሌላካለይጠቀስ________________________________

16. እኒህን መድሃኒቶች ለምን ያህል ጊዜ ወሰዱ (1) ☐ ≤7 ቀናት (2) ☐ 8-15 ቀናት (3) ☐ ከዚያበላይ

17. የቤተሰብ ማህበራ ዊታሪክ (1) ☐ ማጨስ (2) ☐ አልኮል

18. ቅድመ ቀዶ ጥገና የምርመራ ውጤት (1) ☐ በአይን የሚታይ የአጥንት ስብራት (2) ☐ አይን የማይታይ

የአጥንት ስብራት (3) ☐ በወሊድ ጊዜ ልጁ ከዳሌ አጥንት ወደ ማህፀን አለመውረድ (4) ☐

የሆድ ዕቃ መቃጠል ስሜት (5) ☐ በስኳር ህመም ምክንያት የሚመጣ የእግር ችግር ☐ (6) ሌሎች ☐__________

19. የተደረገው ቀዶ ጥገና (1) በቀዶ ጥገና መውለድ ☐

በቀዶ ጥገና የሞተን ወይም የተጎዳን የሰውነት ክፍል ማስወገድና የአጥንት ስብራትን አጥንቱ ሳይከፈት መጠገን (2) ☐

አጥንቱ ተከፍቶ መጠገን (3) ☐ (4) የሆድ እቃን ለማግኘት የሚደረግ ቀዶ ጥገና ☐

5)የማህፀንና የዳሌ አጥንት ማውጣት ቀዶጥ ገና ☐ እግር ወይም እጅ መቆረጥ (6) ☐

በቀዶ ጥገና የሞተን ወይም የተጎዳን የሰውነት ክፍል ማስወገድ (7) ☐ 8) ሌሎች______

20. የቀዶ ጥገና አይነት: (1) ንጹህ የሆነ ቀዶ ጥገና (ቀዶ ጥገና የሚደረግበት ቦታ ላይ ምንም አይነት እብጠት፣የሰውነት መቆጣት፣መቅላት…የሌለውና አፍ ውስጥ፣መተንፈሻ አካል፣የሆድ እቃ፣የሽንት ቧንቧ ቀዶ ጥገናን ሳይጨምር ☐

(2) አፍ ውስጥ፣መተንፈሻ አካል፣የሆድ እቃ፣የሽንት ቧንቧ አካባቢ የሚደረግ ቀዶ ጥገና ☐

(3) ቁስልና እብጠት፣የሰውነት መቆጣት፣መቅላት…ያለው ነገር ግን ፈሳሽ የሌለው ቦታ ላይ የሚሰራ ቀዶ ጥገና ☐

(4) እብጠት፣የሰውነት መቆጣት፣መቅላት እንዲሁም መግል ያለው ቦታላይ የሚሰራ ቀዶጥ ገና ☐

21. የቀዶ ጥገና ሁኔታ (1) ☐ ድንገተ ኛቀዶ ጥገና (2) ☐ የግድ አፋጣኝ ያልሆነ ቀዶ ጥገና

22. ከቀዶ ጥገና በፊት ፀጉር መላጨት (1) ☐ ከቀዶ ጥገና በፊት ያለው ሌሊት (2) ☐ ጠዋት ከቀዶ ጥገና በፊት

23. የባክቴሪያ መከላከያ መድሃኒት የወሰዱት መቼነው (1) ☐ ከቀዶ ጥገና በፊት (2) ☐ ቀዶ ጥገና ጊዜ

(3) ☐ ከቀዶ ጥገና በኋላ (4) ☐ አልወሰዱም

24. ለባክቴሪያ መከላከያ የወሰዱት መድሃኒት (1) ☐ ሴፍትሪያግዞን (2) ☐ ጀንታማይሲን (3) ☐ ሜትሮኒዳዞል (4) ሌሎች____

25. ቀዶ ጥገናው የወሰደው ጊዜ በደቂቃ (1) ☐ 0-60 (2) ☐ 61-120 (3) ☐ >120

Annex VI. Laboratory results forms

1. Study site______________
2. Patient serial No__________Laboratory ID No. _____________________
3. SSI wound culture: A) Growth B) No growth
4. If there is growth, the type of isolated bacteria ____________________
5. Blood culture for sepsis diagnosis: A) Growth B) No growth

   If there is growth, the type of isolated bacteria ____________________

AST results of Gram-negative

Drugs	AMP:	AK	AC	ATM	CHL	CN	CRO	FEP	SXT	CPR	CXM	CTX	ET	IPM	MEM	TE
Diameter																
Interpretation																

AMP: Ampicillin; AK: Amikacin; AC: Amoxicillin-Clavulanic Acid; ATM: Aztreonam; CHL: Chloramphenicol; CN: Gentamicin; CRO: Ceftriaxone; FEP: Cefepime SXT: Trimethoprim-Sulfamethoxazole; CPR: Ciprofloxacin; CXM: Cefuroxime; CTX: Cefotaxime; ET: Ertapenem; IPM: Imipenem; MEM: Meropenem; TE: Tetracycline

AST results of Gram-positive

Drugs	P	AMP	E	TE	FOX	OXA	DOX	SXT	CPR	CN	CHL	DC	V
Diameter													
Interpretation													

P: Pencillin; AMP: Ampicillin; E: Erytromycin; TE: Tetracycline; FOX: Cefoxitin; OXA: Oxacillin; DOX: Doxycycline; SXT: Trimethoprim-Sulfamethoxazole; CPR: Ciprofloxacin; CN: Gentamicin; CHL: Chloramphenicol; DC: Clindamycin; V: Vancomycin;

Reported by: ________________________________ Date: _________________

Reviewed by: ____________________________________ Date: ________________

Annex V: Materials used in the study

- Blood agar base

-MacConkey agar

-Muller Hinton agar

- Amin’s transport media

-Dehydrated Trypto soy broth

-Triple sugar iron agar

-Simon's citrate agar

-Lysine decarboxylase agar

-Urea agar base

- Motility agar

- Plasma

-Kovac's reagent

-3% hydrogen per oxide

-Oxidase reagent test strip

-Sterile cotton wool

- Antibiotic disks

- Wire loop & straight wire

- Sprit lamp

- Petri dish

- Slide, matches

-Diethyl ether

- Reagent; crystal violate

-Gram's iodine

-Acetone alcohol, safranin

-Cotton, test tube, gauze, forceps

- McFarland standard

- Test tube rack

- Disposable glove, ruler

- Aluminum foil

- Immersion oil

- Microscope

- A balance and weighing papers

- A 1-liter Erlenmeyer flask, cotton plugged or screw capped

- A 1-liter graduated cylinder

-Water proof marker, pen, pencil, paper

- Normal saline (0.85% $^{w}/_{v}$)

- A 1-liter glass beaker

- Distilled water

Procedure:

A. To prepare the culture media

1. Read the label on a bottle of dehydrated agar media. It specifies the amount of dehydrated powder required to make 1 liter (1,000 ml) of medium. Calculate the amount needed for 1/2 liter and weigh out this quantity.
2. Place 500 ml of distilled water in an Erlenmeyer flask. Add the weighed, dehydrated agar while stirring with a glass rod to prevent lumping.
3. Set the flask on a tripod over an asbestos mat.
4. When the agar mixture is completely dissolved, remove the flask from the flame or hot plate, close it with the cotton plug or cap, and it has to be sterilized in the autoclave.
5. When the flask of sterilized agar is returned to you, allow it to cool to about 50°C (the agar should be warm and melted, but not too hot to handle in its flask). Remove the plug or cap with the little finger of your right hand and continue to hold it until you are sure it won't have to be returned to the flask. Quickly pour the melted, sterile agar into a series of petri dishes. The petri dish tops are lifted with the left hand and the bottoms are filled to about one-third capacity with melted agar.
6. Replace each petri dish top as the plate is poured. When the plates are cool (agar solidified), invert them to prevent condensing moisture from accumulating on the agar surfaces.
7. Place inverted agar plates in the 37°C incubator. They should be incubated for at least 24 hours to ensure they are sterile (free of contaminating bacteria) before you use.

B. Collection and processing of specimen from wound infection

1. The specimen will be collected by an experienced nurse and special care was taken to avoid contaminating the specimen with commensal organisms from the skin.
2. With sterile cotton tipped applicator stick moistened with normal saline collect sample from the infected site.

3. Label the sample as soon as possible with the patient code number
4. Inoculate in to BAP, MacConkey and MSA aseptically
5. Incubate the plate aerobically at 35-37 °Cfor 18-24 hours.
6. Examine and report the culture; look for colony characteristics and perform biochemical test.
7. Determine drug susceptibility pattern of the isolated organism

C. Gram stain procedures

1. Prepare a thin smear of the culture or specimen was observed.
2. Allow to air-dry and fix the smear.
3. Cover the fixed smear with crystal violet for 1 min.
4. Rinse with clean water and tip off all the water.
5. Cover the smear with Lugol's iodine for 1 min.
6. Wash off the iodine with clean water.
7. Add acetone-alcohol for 30 sec.
8. Wash the smear immediately with clean water.
9. Cover the smear with saffranin for 1-2 minutes.
10. Rinse with clean water.
11. Wipe the back of the slide and place in a draining rack for the smear to air-dry.
12. Examine microscopically, first with the 40x objective and then with the oil immersion objective for white cells, bacteria and other structures.

- Gram- positive bacteria -------------Dark purple

- Gram- negative bacteria ------------Pale to dark red.

D. Biochemical testing procedures

Specimen Collection, Isolation, and Identification of *S. aureus*

Wound swabs or aspirates were collected based on standard operation procedure (SOP). The identification of *S. aureus* strains was based on standard bacteriological procedures, including morphological, cultural, and biochemical characterization (Bendary et al., 2016). The specimens

were inoculated on blood agar plates (BAP) (Oxoid), and mannitol salt agar (MSA) (Oxoid), and then incubated at 35°C for 24 hours. The bacterial isolates were identified through Gram staining, catalase and coagulase tests. Gram-positive cocci, catalase-positive, slide coagulase-positive and golden yellow colonies on MSA were considered confirmatory tests for *S. aureus*

Procedure

1. Pour 2-3 ml of 3% hydrogen peroxide to a test tube
2. Using a sterile wooden stick take the test organism and immerse into the hydrogen peroxide solution
3. Look for immediate bubbling
4. Interpretation :Active bubbling--positive test and No release of bubbles-negative test

Coagulase test: This test is used to differentiate *S. aureus* from other *Staphylococcus* spp.

Procedure

1. Place a drop of physiological saline on two separate slides
2. Emulsify the test organism in each of the drop to make thick suspension
3. Add one drop of plasma to one of the suspensions and mix gently. Look for clumping of the organism within 10 seconds
4. Interpretation

Clumping within 10 seconds ------------------ *S. aureus*

No clumping within 10 seconds -------------other staphylococcus species

Identification of Gram negative bacteria: was based on their test result with a series of Biochemical tests.

Procedure

1. Prepare a suspension of the test organism with nutrient broth. 3-4 colony of test organism in 5 ml nutrient broth.
2. A loop full of the bacterial suspension is inoculated in to indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, manitol, urea agar and motility medium.
3. Incubate at 35-37 ^{O}c for 18-24 hours.
4. Look for color change (turbidity for motility) of the medium
5. Identify the test organism by considering the result of the six biochemical tests.

Annex VI: Bacterial Identification by MALDI-TOF MS Required materials.

MALDI disposable plate

MALDI Matrix prepared with acetonitrile and trifluoroacetic acid

Toothpick stick (Autoclaved) Pipette and 1µL tips (1µl)

Procedure:

1. Pick overnight grown single colony with sterile toothpick stick and apply a thin layer on to the spot plate

2. Overlay the spot with 1µl of the matrix and let it dry for 1min at room temperature

3. Ship the plate to Orebro for analyzing

4. Analyze the spots with MALDI-TOF MS

5. Record the results on to the excel template

E. Antimicrobial susceptibility testing

Procedure

1. Prepare a suspension of the test organism by emulsifying several colony of the organism in a small volume of nutrient broth
2. Much the turbidity of suspension with turbidity standard
3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).
4. Spread the inoculums evenly over the Muller-Hinton agar plate with the swab
5. Using a sterile forceps or needle, place the antimicrobial disk on the inoculated plate
6. Incubate the plate aerobically at 35-37°C for 18-24 hours
7. Read the test after checking that the bacterial growth is neither heavy nor light. Measure the radius of the inhibition zone.
8. Interpret the reaction of the test organism to each antibiotics used as sensitive, intermediate, or resistance as per the standard CLSI.

Sensitive – zone of radius is wider or equal to the control

Intermediate –zone of radius is more than three mm smaller than the control

Resistance – no zone of inhibition.

Gram-negative isolates were tested against ampicillin (10µg), gentamicin (10µg), amikacin (30µg), ciprofloxacin (5µg), chloramphenicol (30µg), ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg), cefuroxime (30µg), cefepime (30µg), tetracycline (30µg), amoxycillin+Clavulanate (20/10µg), Trimethoprim-sulfamethoxazole (1.25/23.75µg), ampicillin-sulbactam (10/10µg), aztreonam (30 µg), meropenem (10µg), Imipenem (10µg), ertapenem (30µg). Gram-positive isolates were tested against penicillin (10units), ampicillin (10µg), vancomycin (30µg), erythromycin (15µg), ciprofloxacin (5µg), cefoxitin (30µg), clindamycin (30µg), erythromycin (15µg), doxycycline (30µg), chloramphenicol (30µg), gentamicin (10µg), and oxacillin (5 µg), tetracycline (30µg), ((CLSI), 2021). Following that, the plates were incubated at 37°C for 18-24 hours. Each zone of inhibition was measured to the nearest millimeter, and classified as sensitive, intermediate, or resistant using the standard technique

((CLSI), 2021). MDR was a bacterium that was simultaneously resistant at least one drug in three or more categories.

Annex VII: DNA genomic lysate procedure

Procedure

- Turn on the UV light in the Biosafety cabinet for at least 10 minutes, then open the barrier, turn on the blower and allow the fan to run for 15 minutes before placing samples in. This ensures proper air flow. Sterilize surface with 70% ethanol.
- Remove isolates of interest from freezer or refrigerator and place in sterilized Biosafety Cabinet.
- Begin by turning on the incubation block and allowing it to warm to 60°C.

❖ Solution Preparation

- Prepare 50mL 1X PBS, pH 7.4 solution by adding 5mL 10X PBS, pH 7.4 into 45mL dH2O
- Prepare 50mL 1M NaOH by adding 1.998g NaOH in 50mL dH2O. Make fresh every time.
- Prepare 50mL 0.05M NaOH by adding 2.5mL of 1M NaOH to 48mL dH2O
- Prepare 50mL 1M Tris-Cl by adding 7.882g Tris-HCl to 50mL dH2O
- Label 1.5mL centrifuge tubes respective to the designated sample ID.
- Inoculate, using aseptic technique, a portion of the colonies and place in respective 1.5mL centrifuge tube.
- Add 200µL 1X PBS, pH 7.4 into each tube and mix vigorously by vortex until cells are suspended (*You can add this BEFORE you place your colony in. It may prevent the biofilm from sticking to the tube).*
- Add 800µL 0.05M NaOH and vortex.
- Place samples in 60°C water bath or incubator for 45 minutes (during this time it is suggested you begin labeling the new tubes for the supernatant)
- After 45 minutes add 240µL 1M Tris-Cl to neutralize the NaOH
- Centrifuge at 13,000rpm for 3 minutes at room temperature.
- Transfer 1000µL supernatant into a newly labeled 1.5mL centrifuge tube

- If some of the sample has not formed a pellet continue to centrifuge for another 3 minutes. Place DNA lysate at -20oC until use.

Annex VIII: Polymerase Chain Reaction

1. Subculture from frozen colony on Tryptic Soya Agar (TSA) and incubate overnight at 37°C 2.
2. Inoculate 3-4 colonies on Tryptic Soya Broth (TSB) and incubate overnight at 37°C 3.
3. Transfer 1mL of bacterial suspension to clean Eppendorf tube and extract DNA by Genelut bacterial DNA extraction kit (for E. coli) or Phenol- chloroform DNA extraction method (for Aeromonas)
4. Level PCR tube for the respective samples and calculate saple volume for DNA 80ng/µL
5. Master mix preparation

❖ For conventional PCR add:

• 2.5 µL 10X Taq buffer

• 0.5 µL dntps

• 1 µL primer mix (both forward and reverse)

• Template

• 0.125 µL Taq DNA polymerase and

• Nuclease free water until 25 µL (Total reaction volume)

❖ For m-PCR

• 25 µL 2x QIAGEN Multiplex PCR Master Mix

• 5 µL 10x primer mix, 2 µM each primer

- RNase-free water
- Template DNA
- Total reaction volume 50 µL

Run PCR with thermocycling conditions.

❖ For conventional PCR

- Initial denaturation for 30s at 95°C and then 35 cycles were applied individually as follows, 60seconds at 72°C (extension), and final extension for 10 minutes at 72°C.

❖ For m-PCR

- Apply first thermal cycles for 15 minutes at 94°C, and then 40 cycles individually as follows: 30 seconds at 94°C (denaturation), 90 seconds at 60°C (annealing temperature), 90 seconds at 72°C (extension), and final extension for 10 minutes at 72°C.

Agarose Gel Electrophoresis procedure

Objective: this was outline the procedure for both preparing the agarose (0.8%-1.5%) for gel electrophoresis as well as preparing the apparatus and UV light camera for imaging. Agarose gel electrophoresis is a process where current is passed through a matrix gel medium (agarose) surrounded by a conductive buffer (TAE) to separate DNA and nucleic acids by size or charge. TAE buffer for electrophoresis is commonly at 1X concentration made from either 10X or 50X concentrations, and is composed of Tris-acetate (pH 8.0) and EDTA which sequesters divalent cations. TBE buffer can be used, but TAE buffer is more efficient when double stranded DNA is involved.

Making the agarose is a rather simple procedure, but does require some time and attention if any spills are to be avoided. When casting the gel you will also use GelRed stain as an intercalating nucleic acid stain at 10,000X concentration. This is just as efficient as ethidium bromide without the health risks.

Procedure

- For a 1% agarose gel weigh 5g of agarose in a medium weigh boat. For a 1.5% agarose gel weigh 7.5g of agarose in a medium weigh boat. The final volume made will always be 500mL.
- Pour the agarose into the 600mL beaker or 1L Erlenmeyer flask
- Add 500mL TAE buffer to the beaker/flask

❖ **Using the microwave oven:**

- Place the beaker into the microwave and turn the timer to 8 minutes.
- It is important to watch the beaker constantly because it was begin to boil over when the boiling point is reached. When it begins to boil stop the microwave and allow the liquid to settle. If it looks clear, then remove and pour into marked container. If not close the microwave and continue the cook until clear.
- It is very important not to let the agarose boil over. It could ruin the microwave as well as losing a large volume of liquid agarose.

❖ **Setting up the electrophoresis apparatus**

- Using the large 20 lane electrophoresis apparatus make sure it has been cleaned before use.
- Take the gel cast and slightly water the rubber seals on the top and bottom before sliding it into place.
- Slowly and carefully push the gel cast so the walls of the cast are perpendicular to the walls of the running apparatus and a water-tight seal is created.
- Place the 20-lane combs in their holds with either the 1mm or 10mm lane side down. It doesn’t matter which you use, but the 1mm will fill more easily than the 10mm.
- If you haven’t already, place the 1.2% agarose (or whichever percent you will be using) in the microwave and bring to a boil

- Once the agarose has liquefied remove it from the microwave using the rubber insulated grips (red) sitting on top of the microwave. The bottle will be extremely hot!
- Pour 100mL of agarose (carefully) into the 125mL Erlenmeyer flask o Add 10µL 10,000X GelRed stain to the agarose in the flask and mix until it disappear
- IMPORTANT: let the agarose sit in the flask for 10-15 minutes and cool. If poured into the gel cast while hot it can melt the rubber seals, the combs or damage the apparatus itself. Wait until it has cooled to the point where you can keep your bare hand on it.
- Once the agarose has cooled pour it into the cast and wait for it to solidify. This can take up to 30 minutes. Prepare your samples in the meantime (next section).
- Once the agarose has solidified very carefully remove the lane combs and avoid cracking or breaking the gel. Lift the combs straight up.
- Remove the cast very carefully by picking it straight up and out of the apparatus. Once out position it so the bottom (part that does not have any lanes) is facing you and the electrodes are on the RIGHT.
- Since this apparatus is set up so the RED (cathode) was always be on the bottom.
- Once the cast in facing the correct direction pours the 1X TAE buffer into the apparatus until the gel is covered by at least a few centimeters. DO NOT follow the "Fill Line" mark. It is incorrect.

❖ **Preparing samples for electrophoresis**

- While the agarose is solidifying you can prepare your samples for electrophoresis.
- In order to follow where the samples are on the gel you was use 6X Blue/Orange loading dye. It is green in appearance.
- Add 1.5µL of 6X blue loading dye to each sample to be loaded and mix
- Place the samples back in 4°C until the gel has hardened.

❖ **Running the gel**

- Once the gel has solidified, the 6X loading dye has been added to each sample and the 1X TAE buffer has been added to the apparatus you may load the samples.
- You may load them in whichever order is best for the experiments you run, but loading them follows the same procedure. For easy reference use the gel electrophoresis label sheet. The 100bp ladder will ALWAYS be Lane 1 with 6 µL
- Each lane will have 4.5µL of sample
- The best way to go about loading each lane is to stand over the gel and very carefully and slowly dispense the samples into the gel. It is very important that to avoid shooting the sample out of the lane that you DO NOT push the pipette to its second stop. Push the sample into the lane and do not release the plunger until the tip is out of the buffer.
- Once all the samples have been loaded, carefully move the apparatus over to the power supply.
- Place the cover on (it can only go one way) and push the electrodes into their receivers.
- The red should be on the bottom, the black on top. ALWAYS RUN TO RED
- Plug the other end of the cables into their respective ports. Make sure the power supply is OFF when you do this as to avoid a short
- Once all the plugs are in place and the cover is secured turn the power supply on.
- The running voltage for best results is about 95V
- At this voltage in a 1.5% gel it will take about 90 minutes, but make sure to check every once in a while to make sure the samples don't run off.
- You should see bubbles coming from the bottom. If you don't there is a bad connection. Turn the power supply off, remove the connectors from the power supply, remove the lid and reconnect everything again. If no bubbles are seen again there is something wrong and the gel should not be run until the problem is discovered.

❖ **Visualizing the gel**

- As the gel is running you noticed the loading dye has separated into three colors; blue, orange and yellow. The yellow indicates 50bp, the blue indicates 100bp and the orange represents 500bp. Once the yellow has reached 2/3 the distances from the bottom of the gel you can turn off the power supply and remove the cables and cover.
- Have some paper towels ready and remove the gel cast (with the gel) from the apparatus.
- Make sure to drain the remaining TAE buffer back into the apparatus. No need making a mess.
- Place the cast on the paper towels and tilt it to collect any remaining buffer.
- Bring the cast and gel to the UV camera and turn the camera on by pressing the power button at the very top of the machine.The program not opened unless the camera is turned on!
- The camera is now on and you can place the gel on the UV light base by removing the top half of the machine (part with the camera is separate from the base)
- Place the gel sideways onto the UV light base so it fits perfectly. It was sideways when you image it, but that can be rotated later.
- Once the gel in on the base, replace the camera back into position and turn the UV light “ON” by pressing the light bulb on the base itself. This was turn the UV light on and an image appeared on the computer screen.
- The image might be dim, but on the bottom you can alter the sensitivity, brightness and aperture time to get the best picture you can.
- Simply increasing the sensitivity from “3” to “4” works the best.
- After you have imaged the gel and have the pictures you need turn the UV light base OFF
- Remove the gel from the base and wrap it in the paper towels it was brought there with and dispose of it in the biohazard waste basket.
- Use distilled water to clean the base with either paper towels
- Clean all the equipment used and place on the drying rack.
- Wipe down any surfaces with 70% ethanol before leaving.

Table 1. Primers used for detection of bla_{SHV}, bla_{TEM}, bla_{CTX-M}, bla_{KPC} and bla_{NDM}

Target gene	Primer name	CGCCTGTGTATTATCTCCCT	Size bp	References
bla_{SHV}	F	CGCCTGTGTATTATCTCCCT	293	(Mohammed *et al.*, 2016)
	R	CGAGTAGTCCACCAGATCCT		
bla_{TEM}	F	TTTCGTGTCGCCCTTATTCC	403	
	R	ATCGTTGTCAGAAGTAAGTTG		
bla_{CTX-M}	F	CGCTGTTGTTAGGAAGTGTG	754	
	R	GGCTGGGTGAAGTAAGTGAC		
bla_{KPC}	F	CGTCTAGTTCTGCTGTCTTG	798	(Poirel *et al.*, 2011)
	R	CTTGTCATCCTTGTTAGGCG		
bla_{NDM}	F	GGTTTGGCGATCTGGTTTTC	621	
	R	CGTCTAGTTCTGCTGTCTTG		

Table 1. Primers used in multiplex PCR for the detection of the *mecA*, *vanA, vanB* and *famA* genes July 2020 to August 2021

Target gene	Primer name	Primer sequence (5′-3′)	Size bp	References
mecA	MF	GTAGAAATGACTGAACGTCCGATAA	310	(Perez-Roth *et al.*, 2001)
	MR	CCAATTCCACATTGTTTCGGTCTAA		
vanA	VF	GGGAAAACGACAATTGC	732	(Emamie *et al.*, 2023)
	VR	GTACAATGCGGCCGTTA		
vanB	VF	ACCTACCCTGTCTTTGTGAA	300	
	VR	AATGTCTGCTGGAACGATA		
femA	FF	AAAAAAGCACATAACAAGCG	132	(Kobayashi *et al.*, 1994b)
	FR	GATAAAGAAGAAACCAGCAG		

Annex IX: **List of papers**

Peper one

Worku *et al. Ann Clin Microbiol Antimicrob* (2023) 22:96
https://doi.org/10.1186/s12941-023-00643-6

Annals of Clinical Microbiology and Antimicrobials

RESEARCH **Open Access**

Bacterial profile of surgical site infection and antimicrobial resistance patterns in Ethiopia: a multicentre prospective cross-sectional study

Seble Worku[1,2,3*], Tamrat Abebe[3], Ashenafi Alemu[2], Berhanu Seyoum[2], Göte Swedberg[4], Alemseged Abdissa[2], Adane Mihret[2,3] and Getachew Tesfaye Beyene[2]

Abstract

Background Globally, surgical site infections (SSI) are the most commonly reported healthcare-associated infections.

Methods A multicentre study was conducted among patients who underwent surgical procedures at four hospitals located in Northern (Debre Tabor), Southern (Hawassa), Southwest (Jimma), and Central (Tikur Anbessa) parts of Ethiopia. A total of 752 patients clinically studied for surgical site infection were enrolled. The number of patients from Debre Tabor, Hawassa, Jimma, and Tikur Anbessa, hospitals was 172, 184, 193, and 203, respectively. At each study site, SSI discharge culture was performed from all patients, and positive cultures were characterized by colony characteristics, Gram stain, and conventional biochemical tests. Each bacterial species was confirmed using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI TOF). An antimicrobial susceptibility test (AST) was done on Mueller–Hinton agar using the disk diffusion method. Logistic regression analysis was used to assess associations of dependent and independent variables. A p-value < 0.05 was considered statistically significant. Data were analysed using STATA 16 software.

Results Among 752 wound discharge cultures performed, 65.5% yielded growth. Among these, 57.9% and 42.1% were Gram-negative and Gram-positive isolates, respectively. In this study, a total of 494 bacteria were isolated; *Staphylococcus aureus* (31%), *Escherichia coli* (20.7%), and *Klebsiella pneumoniae* (9.8%) were the most common. Rare isolates (0.8% each) included *Raoultella ornithinolytica, Stenotrophomonas maltophilia, Alcalignes faecalis, Pantoea ecurina, Bacillus flexus, and Paenibacillus tylopili. Enterobacteriaceae* showed high levels of resistance to most of the tested antibiotics but lower levels of ertapenem (32.9%), amikacin (24.3%), imipenem (20.3%), and meropenem (17.6%) resistance. Multidrug-resistant (MDR) frequency of *Enterobacteriaceae* at Debre Tabor, Hawassa, Jimma, and Tikur Anbessa hospitals was 84.5%, 96.5%, 97.3%, and 94%, respectively. Ages ≥ 61 years (AOR = 2.83, 95% CI: 1.02–7.99; P 0.046), prolonged duration of hospital stay (AOR = 4.15, 95% CI: 2.87–6.01; P 0.000), history of previous antibiotics use (AOR = 2.83, 95% CI: 1.06–2.80; P 0.028), history of smoking (AOR = 2.35, 95% CI: 1.44–3.83; P 0.001), emergency surgery (AOR = 2.65, 95% CI: 1.92–3.66; P 0.000), and duration of operation (AOR = 0.27, 95% CI: 0.181–0.392; P 0.000) were significant risk factors.

Conclusion The most prevalent isolates from Gram-positive and Gram-negative bacteria across all hospitals were *S. aureus* and *E. coli*, respectively. Many newly emerging Gram-negative and Gram-positive bacteria were identified.

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Peper two

Article

Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* among Patients Diagnosed with Surgical Site Infection at Four Hospitals in Ethiopia

Seble Worku [1,2,3,*], Tamrat Abebe [1], Berhanu Seyoum [3], Ashenafi Alemu [3], Yidenek Shimelash [4], Marechign Yimer [3], Alemseged Abdissa [3], Getachew Tesfaye Beyene [3], Göte Swedberg [5] and Adane Mihret [1,3]

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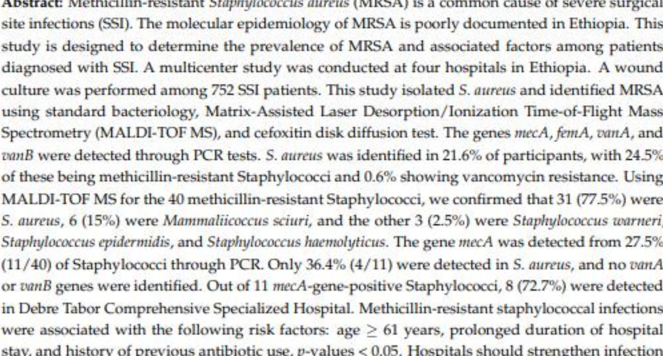

Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common cause of severe surgical site infections (SSI). The molecular epidemiology of MRSA is poorly documented in Ethiopia. This study is designed to determine the prevalence of MRSA and associated factors among patients diagnosed with SSI. A multicenter study was conducted at four hospitals in Ethiopia. A wound culture was performed among 752 SSI patients. This study isolated *S. aureus* and identified MRSA using standard bacteriology, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), and cefoxitin disk diffusion test. The genes *mecA*, *femA*, *vanA*, and *vanB* were detected through PCR tests. *S. aureus* was identified in 21.6% of participants, with 24.5% of these being methicillin-resistant Staphylococci and 0.6% showing vancomycin resistance. Using MALDI-TOF MS for the 40 methicillin-resistant Staphylococci, we confirmed that 31 (77.5%) were *S. aureus*, 6 (15%) were *Mammaliicoccus sciuri*, and the other 3 (2.5%) were *Staphylococcus warneri*, *Staphylococcus epidermidis*, and *Staphylococcus haemolyticus*. The gene *mecA* was detected from 27.5% (11/40) of Staphylococci through PCR. Only 36.4% (4/11) were detected in *S. aureus*, and no *vanA* or *vanB* genes were identified. Out of 11 *mecA*-gene-positive Staphylococci, 8 (72.7%) were detected in Debre Tabor Comprehensive Specialized Hospital. Methicillin-resistant staphylococcal infections were associated with the following risk factors: age $\geq$ 61 years, prolonged duration of hospital stay, and history of previous antibiotic use, *p*-values < 0.05. Hospitals should strengthen infection prevention and control strategies and start antimicrobial stewardship programs.

Keywords: surgical site infection; methicillin-resistant *Staphylococci*; molecular epidemiology; antimicrobial resistance; Ethiopia

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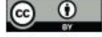
Citation: Worku, S.; Abebe, T.; Seyoum, B.; Alemu, A.; Shimelash, Y.; Yimer, M.; Abdissa, A.; Beyene, G.T.; Swedberg, G.; Mihret, A. Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* among Patients Diagnosed with Surgical Site Infection at Four Hospitals in Ethiopia. *Antibiotics* **2023**, *12*, 1681. https://doi.org/10.3390/antibiotics12121681

Academic Editor: Samantha Flores-Treviño

Received: 31 October 2023
Revised: 22 November 2023
Accepted: 23 November 2023
Published: 29 November 2023



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1. Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive coccus that causes significant infections worldwide, including bacteremia, endocarditis, osteomyelitis, and skin and soft tissue infections, due to its easy transmission and commensal nature [1]. Not only *S. aureus* but also coagulase-negative Staphylococci (CoNS), which currently are defined as more than 40 species, are frequently associated with opportunistic human infections.

Paper three

Worku *et al. BMC Infectious Diseases* (2024) 24:459
https://doi.org/10.1186/s12879-024-09362-5

BMC Infectious Diseases

RESEARCH Open Access

Molecular characterization of carbapenemase and extended spectrum beta-lactamase producing *Acinetobacter baumannii* isolates causing surgical site infections in Ethiopia

Seble Worku[1,2,3*], Tamrat Abebe[1], Berhanu Seyoum[3], Bikila Alemu[4], Gebrie Denkayehu[5], Tamrayehu Seyoum[3], Dawit Hailu Alemayehu[3], Alemseged Abdissa[3], Getachew Tesfaye Beyene[3], Adane Mihret[1,3] and Göte Swedberg[6]

Abstract

Background *Acinetobacter baumannii* is an opportunistic pathogen that can cause a variety of nosocomial infections in humans. This study aimed to molecularly characterize extended-spectrum beta-lactamase (ESBL) producing and carbapenem-resistant *Acinetobacter species* isolated from surgical site infections (SSI).

Methods A multicentre cross-sectional study was performed among SSI patients at four hospitals located in Northern, Southern, Southwest, and Central parts of Ethiopia. The isolates were identified by microbiological methods and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Antibiotic susceptibility was determined using disk diffusion. The presence of phenotypic ESBL and carbapenemase production was detected by employing standard microbiological tests, including combined disk diffusion (CDT). ESBL and carbapenem resistance determinants genes were studied by polymerase chain reaction (PCR) and sequencing.

Results A total of 8.7% *Acinetobacter species* were identified from 493 culture-positive isolates out of 752 SSI wounds. The species identified by MALDI-TOF MS were 88.4% *A. baumannii*, 4.7% *Acinetobacter pittii*, 4.7% *Acinetobacter soli*, and 2.3% *Acinetobacter lactucae*. Of all isolates 93% were positive for ESBL enzymes according to the CDT. Using whole genome sequencing 62.8% of the *A. baumannii* harbored one or more beta-lactamase genes, and 46.5% harbored one or more carbapenemase producing genes. The distribution of beta-lactamases among *Acinetobacter species* by hospitals was 53.8%, 64.3%, 75%, and 75% at JUSH, TASH, DTCSH, and HUCSH respectively. Among ESBL genes, bla_{CTX-M} alleles were detected in 21.4% of isolates; of these 83.3% were $bla_{CTX-M-15}$. The predominant carbapenemase gene of bla_{OXA} type was detected in 24 carbapenem-resistant *A. baumannii* followed by bla_{NDM} alleles carried in 12 *A. baumannii* with bla_{NDM-1} as the most common.

Conclusions The frequency of *Acinetobacter species* that produce metallobetalactamases (MBLs) and ESBLs that were found in this study is extremely scary and calls for strict infection prevention and control procedures in health facilities helps to set effective antibiotics stewardship.

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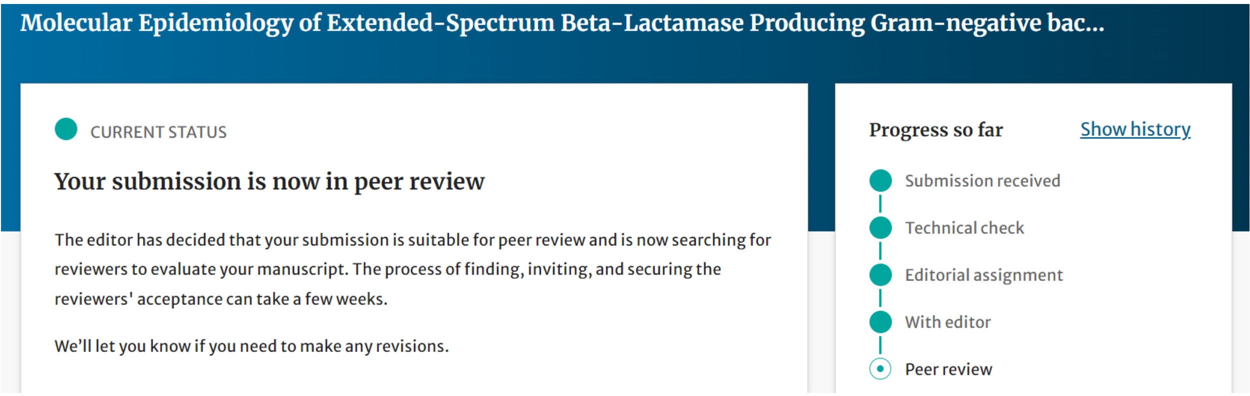
Paper four



Ministry of Science and Technology, Federal Democratic Republic of Ethiopia
Federal Health Research Ethics Review Committee

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Annex #: Material Transfer Agreement

This Material Transfer Agreement (MTA) has been prepared for use by **Armauer Hansen Research Institute (AHRI)** and **Uppsala University** in all transfer of research material (samples, derivatives, and specimens) related to the protocol ***Molecular epidemiology and characterization of antibiotic resistance profiles of bacterial isolates among patients with surgical site infection in Ethiopia: A multi-center prospective cross-sectional study***
Provider: **Seble Worku Kebede**

Recipient: Göte Swedberg

1. Provider agrees to transfer to recipient's designated (**Göte Swedberg**) the following research materials /specimen. *Molecular epidemiology and characterization of antibiotic resistance profiles of bacterial isolates among patients with surgical site infection in Ethiopia: A multi-center prospective cross-sectional study.*
   The research material will only be used for research purposes as described in the protocol by recipient's investigator in designated laboratory for the research project described below, under suitable containment conditions. This research material will not be used for commercial purposes such as screening, production or sale for which a commercialization license may be required. Recipient agrees to comply with all National and International guidelines rules and regulations applicable to the Research Project and the handling of the Research Material.

   a) Are the research materials of human origin?

Material Transfer Agreement
Signature page

For Recipient:

Recipient's Investigator: Göte Swedberg
Signature

Date 2022-11-17

Duly Authorized: Catharina Svensson
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Declaration

I, the undersigned declare that this PhD dissertation is my own work has not been presented for a degree in any other university and all sources of materials used for the thesis proposal have been dully acknowledged.

The candidate: **Seble Worku**

Signature: ___________________

Date of Submission: --------------- Addis Ababa, University

Advisors name	Signature	Date
Tamrat Abebe (PhD)	--------------	---------------
Adane Mihrte (DVM, M.Sc., PhD)	---------------	---------------
Gote Swedberg (PhD)	---------------	--------------
Getachew Tesfaye (PhD)	---------------	---------------
Brehanu Seyume (PhD)	---------------	---------------
Alemseged Abdissa (PhD)	----------------	----------------

Addis Ababa University School of medicine