ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

The magnitude of catheter-associated urinary tract infection, biofilm production, and antibiotic resistance patterns from patients visited at Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia.

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A research thesis submitted to the department of Medical Laboratory Science, College of Health Sciences, Addis Ababa University, in Partial fulfilment of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and public health microbiology)

July, 2023

Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATED STUDY

This is to certify that the thesis prepared by Yalelet Yihunew, entitled.

The magnitude of catheter-associated urinary tract infection ,biofilm production, and antibiotic resistance patterns from patients visited at Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia and submitted in partial fulfilment of the requirement Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and public health microbiology speciality) complies with the regulation of the university and meet the accepted standard with respect to originality and quality sign by the examining committee

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Acknowledgement

First and foremost, I would like to express my thanks to Addis Ababa University's Department of Medical Laboratory Sciences and College of Health Sciences for allowing me to do the study for this research. And for providing me with excellent scientific inspiration as well as the opportunity to undertake research, which allows me to learn and contribute.

Second, I'd like to thank my advisors, Kassu Desta (MSc, Associated professor), Gebreab Teklebirhan (MSc, PhD candidate), and Asegedech Asmamaw (BSc, MSc), for their kindness and continuous support in this research work.

I want to express my gratitude to the Yekatit 12 Hospital Medical College Microbiology Unit for providing me with various tools, reagents, and antimicrobial medications for my research. I am also appreciative of the workers at Yekatit 12 Hospital Medical College for their cooperation in collecting specimens and completing questionnaires, as well as the laboratory and management staffs for their gracious reception and assistance. I also want to express my gratitude to the Ethiopian Health Institute for the help provided by the enzyme immunosorbent assay machine in assigning support staff and allowing usage of the apparatus.

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List of abbreviations

AICU	Adult intensive care unit
AST	Antimicrobial susceptibility testing
ATCC	American type culture collection
CAUTI	Catheter association urinary tract infection
CLSI	Clinical and laboratory standard institute
ELISA	Enzyme linked immune sorbent assay
ENT	Ear, Nose, Throat
EPS	Extracellular polymeric substance
HCI	Health care association infection
HCF	Hospital care facility
HIMS	Health information management system
JUMC	Jimma university medical college
MICU	Medical intensive care unit
MDR	Multi rug resistance
NHSN	National health system network
NICU	Neonatal intensive care unit
OD	Optical density
ODC	Cut- of optical density
PUCU	Paediatric intensive care unit
SOP	Standard of procedure
SICU	Surgical intensive care unit
SPSS	Statistical package for the social science
UTI	Urinary tract infection
WHO	World health organization
Y12HMC	Yekatit 12 hospital medical college

ABSTRACT

Background: Urinary tract infections refer to the presence of microbial pathogens within the urinary tract (urethra, bladder, ureters, or kidneys**).** Self-replicating microbial biofilm population that is polluted by indwelling medical equipment, such as catheters, is a major contributor to nosocomial infections. On the surfaces of indwelling medical devices, a complex bacterial colony known as a bacterial biofilm produces an extracellular polysaccharide matrix. Catheter-associated urinary tract infections are one of the most common hospital-acquired illnesses.

Objective: To determine the magnitude of catheter-associated urinary tract infection, biofilm producing bacteria, and their antibiotic resistance among patients urinary tract infection at Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia**.**

Methods: Across-sectional study was conducted to determine the magnitude of catheter-associated urinary tract infection, biofilm production, and antibiotic resistance. Convenient sampling technique was used to collect data on catheter-associated urinary tract infections, which were collected between December 2022 and May 2023 from catheter urine. The microbiology laboratory received the catheter urine samples right away for isolation and identification of pathogenic bacteria. The presumptive isolates of gram-positive and gram-negative bacteria were further identified using a variety of gram-staining and biochemical techniques. The disc diffusion method was used to evaluate the antimicrobial susceptibility pattern of the isolates, and the microtiter (96-well plate) method was used to screen for the formation of biofilms by measuring optical density at 630 nm using an enzyme linked immunosorbent assay reader using.

Results: The prevalence of bacterial pathogens was 54% among the 270 study participants suspected of urinary tract infections related to catheter use. *Pseudomonas spp.* 23 (15.8%) and *E.coli 41* (28%) were the two most common bacterial isolates. The most prevalent isolate of gram-positive bacteria was *S. aureus* 23 (15.8%). For gram-positive bacteria, cefotaxime and penicillin both demonstrated an increase in antibiotic resistance (75.6% and 71.7%, respectively). The overall percentage of isolates that developed biofilms was 61%, with *E. coli* (43.9%), *pseudomonas spp.* (69.6%), *CONs* (57.9%), and *Citrobacter spp*. (100%), all of which created robust biofilms.

Conclusion: Bacterial isolates were widely distributed among catheter-related infections. The majority of bacterial isolates were at least partially antibiotic-resistant.

Keywords: Biofilm formation, Microtiter plat assay, Drug susceptibility pattern, Urinary tract infection

1. INTRODUCTION

1.1. Background

Urinary tract infections (UTIs) are the infection of urethra (urethritis), bladder (cystitis), or kidney (pyelonephritis).Urinary tract infection (UTI) is among the most common bacterial infections acquired in the community and in hospitals(1). CAUTIs are one of the most common hospital-acquired infections because 15 to 25% of hospitalised patients require an indwelling urinary catheter for the duration of their hospital stay(2).Urinary tract infections (UTIs) are the main source of healthcare-associated infections, which have a direct effect on healthcare quality and safety(3).

Risk factors associated with developing UTI include age related changes to the genitourinary tract, comorbid conditions, and instrumentation required to manage bladder voiding and) prevalence also increases with advancing age, catheterization, sexual activity, menopause, and urinary obstruction problems(4).

Catheter-associated urinary tract infections (CA-UTI) pathogenic bacteria were selectively isolated, and their in vitro biofilm formation, generation, and monomeric sugar content of extracellular polymeric substance (EPS), as well as the effects of sugar, salt, pH, and temperature, were all determined(5).

The development of a biofilm occurs before a catheter-associated urinary tract infection (CA-UTI), which is linked to gender, age, diabetes status, the length of the catheterization, the bacteria present before the catheterization, and antibiotic therapy(6).

Biofilms are formed up of a varied bacterial colony contained in a polymeric matrix with high adhesion characteristics and a persistent phenotype. One of the most challenging issues of modern medicine is biofilms (8). Many attempts to cure diseases caused on by biofilms have failed since currently available antibiotics have been developed to fight free-floating bacteria (7).They enter the bladder and may migrate or colonize to form a biofilm. along the surface of the catheter's intra-or extra luminal parts (8).

Urinary tract infection producing bacteria Defence mechanisms against antibiotics involve the production Of antibiotic deactivating enzymes, such as the several classes of β-lactamases or aminoglycoside modifying

enzymes, changes in antibiotic targets, and reduction of Intracellular antibiotic concentration, either by limiting the entrance of the antibiotic or Facilitating its expulsion(9).

Gram-negative bacteria is increasing, especially the emergence of AmpC enzyme and plasmid-mediated AmpC enzyme, which leads to the wide spread of drug-resistant strains. The resistance of urinary tract infection bacteria to carbapenems is mainly caused by three mechanisms: fifirst, the production of carbapenemases; second, the loss or down regulation of high yield ESBLs or AmpC enzyme binding stomatal proteins, resulting in the decrease of sensitivity to carbapenems; third, the alteration of penicillin-binding protein (PBP), the target of carbapenems(10).

Antimicrobials that are effective against bacteria that grow in suspension typically do not work as well against microorganisms that develop in biofilms. The accumulation of the extracellular polymeric substance, which tends to allow only minimal or no penetration of the antimicrobial agents into the microorganisms and frequently fails to exert its effects when used on the microorganisms that grow in biofilms, is one of the mechanisms that have been implicated in this phenomenon(11). .additionally, extracellular polymeric substance (EPS) might dilute the antimicrobial concentration before it reaches the specific biofilm cells, lessening the antibiotics' effectiveness against bacteria(12). As a result of the slow development of microorganisms in biofilms, they develop resistance to antimicrobial drugs that favour aggressive microbial growth. Furthermore, slow development encourages biofilm-forming organisms to express low levels of antimicrobial binding proteins. The activation of numerous genetic components that enable microbes to modify their cell envelope, the molecular targets, and the susceptibility to particular antimicrobials are additional mechanisms of resistance in biofilms. Intrinsic resistance is another name for this resistance mechanism(13).

The other Mechanisms of biofilm-mediated antimicrobial resistance Similarly, Stewart reported three mechanisms of antimicrobial resistance in biofilms: i. slow penetration, in which antibiotics may not be able to move beyond the surface layers of the biofilm ii. Development of resistance phenotypes, which allow the bacteria growing in biofilms to differentiate into protected phenotypes in an altered microenvironment in which the antibiotic actions are antagonized as a result of zones of nutrient accumulation or waste accumulation An increased expression of the efflux pump, is another mechanism that decreases the susceptibility of biofilms to antibiotics(14).

1.2. Statement of the problem

According to the National healthcare Safety Network(NHSN)), Catheter -associated urinary tract infection (CAUTIs) affect 150 million people worldwide each year, and CAUTI is a potentially life-threatening HAI for LTC residents (1.8 million to 4.1 million infections annually, 412,000 deaths annually, and so on). HAIs in long-term care facilities can be costly, ranging from $38 million to $137 million annually for antimicrobial therapy. UTI is among the most common infections worldwide, with substantial morbidity, mortality, and economic burden (15).

The development of bacterial biofilms is presently recognized as one of the most relevant drivers of persistent infections, and constitutes a serious challenge for clinical microbiologists and clinicians being 100-1000 fold more resistant to antimicrobial agents than normal (planktonic) cells. Phenotypic and physiological change in biofilm, restricted penetration of antibiotic into biofilms and expression of resistance genes were some of the factor that provides a higher resistance to antimicrobial treatment because antimicrobials have been developed against planktonic ally grown bacteria .They are more prone to biofilm infections because their lowest concentration levels are higher. Several medical devices have been connected to biofilm infections. Indwelling medical devices are one of the various gadgets that might cause urinary tract infections(16).

In sub-Saharan Africa country Overall, the prevalence of UTI was 32.12% with Escherichia coli being the most commonly isolated bacteria accounting for 86.4%(17). catheter-associated urinary tract infection and biofilm production bacteria are resistant to many routinely used antibiotics in most developing countries like Ethiopia, it is a common practice that antibiotics is purchased without prescription order, which led to underuse, overuse and misuse of antibiotics.

Furthermore, due to the rise in bacterial antibiotic resistance, it is currently very difficult to properly treat a number of chronic conditions that are caused by biofilms. Data are limited in Ethiopia in particular the study area. Drug resistance level has to be revised or updated every time as there are changes in the global resistance rate of different time interval. Therefore, we planned this study is to update the profile of bacteria associated with catheter-associated urinary tract infection, their antibiotic susceptibility pattern, and the biofilm forming potential of the bacteria isolates at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia.

1.3. Significance of study

In this study, accurate diagnosis and awareness of these catheter-associated UTIs and biofilm-producing bacteria are required for the optimal patient care. This study also provided data on gram-positive and gram-negative bacteria, biofilm formation, prevalence of catheter-associated UTIs, and treatment resistance profiles.

The current study's objective is to close this information gap regarding catheter-associated biofilm production .Healthcare experts, important community figures, and an interested audience received the findings in a number of ways; most likely, they were used to help with infection prevention and control methods and provide institutionally based treatment guidelines for public health officials. These findings need to be looked at in more extensive studies, nevertheless, to clarify any catheter associated biofilm –producing bacteria and patient survival. Research on catheter-associated biofilm –producing bacteria and their antimicrobial resistance is crucial to bring to Ethiopian health professionals' attention and is the beginning of future studies.

2. LITERATURE REVIEW

Urinary trac infections (UTIs) are the second most prevalent disease category among hospitalised patients. Catheter-related urinary tract infections are very dangerous for patients and expensive to treat(18). Urinary tract instrumentation almost always causes the bulk of UTIs that need medical care. CAUTIs are short for catheter –associated urinary tract infections.

2.1. Biofilm formation and urinary tract infection

In Pakistan, a cross-sectional study was carried out between July 2015 and January 2017. Identify catheter-associated urinary tract infections caused by microorganisms in biofilms: The study used 1,070 catheterized urine samples to examine causal pathogens and antibiotic resistance .results showed that 73.4% of the isolates were biofilm-producing bacteria, with non-biofilm-producing bacteria making up 26.6% of the total. The majority of pathogens retrieved were discovered to be Escherichia coli (52.3%), while *E.cloacae* (87 %%) produced the most biofilms. There were significant relationships between biofilm, gender, and catheterization time(19).

A cross-sectional study comprising patients hospitalised in Pakistan was done from February 1 to July 1, 2021. The study covered a total of 100 individuals, of whom 82(82%) samples tested positively for bacterial growth and 18(18%) samples did not. The most common pathogen discovered out of the 82 strains was *E. coli* (46), which was followed by *Klebsiella spp*. (17%(,*pseudomonas*(17%),proteus(4%) and *S.aureus*(2%)(20)**.**

A Cross -sectional observational study entitled "The prevalence of uropathogens in catheter-associated urinary tract infections and their antimicrobial susceptibility" was carried out in Bangladesh from January 2018 to December 2018. Out of 252 examined samples, 74 (61.66%) of urinary catheterized samples and 73 (55.3%) of non-urinary catheterized samples both contained organisms that had been isolated from catheterized urine samples .In both catheterized and non-catheterized patients, Escherichia coli was the most frequently isolated bacterium (60.27, 50), followed by *Klebsiella spp*. (21.91, 27.07), Pseudomonas spp. (9.58%, 12.21%), *Acineobacter spp.* (1.36%, 4.05), and *S.aureus* (4.1%, 2.7%, and 2.7%), *CONs.* A total of 74 bacterial isolates, including 33 (44.59%) of those from catheterized patients and 19 (26.02%) of those from non-catheterized patients, produced biofilms *E.coli* formed the most biofilms in both isolates (21)**.**

In India, a prospective study was conducted from September 2017 to February 2018. Study of the bacterial isolates responsible for CAUTI biofilm production. 484 hospitalised patients in various wards who had been catheterized for the urine for longer than two days were included in this prospective investigation. Utilising a tissue culture plate, biofilm was discovered. 134 of the 484 catheterized CAUTI cases involved patients. *E. coli* was the most common bacterium isolated, followed by *Acineobacter spp.*(33%), *Klebsiella spp.*(28%), *P.aeruginosa* (16%), and *E.faecalis* (25%)(22).

The other prospective observational experiment took place in India from April 1, 2015, to March 31, 2016, and it lasted a full year. Bacteriology Profile and Antibiotic Sensitivity Pattern of Catheter-Associated Urinary Tract Infection in a Tertiary Care Hospital Bacterial growth was found on culture in 146 samples. CAUTI, with a prevalence of 27.70% overall, was more prevalent in women than in men. In total, 53 (36.30%) males and 93 (63.69%) females had CAUTI, with a p value of 0.00)(23)**.**

A Prospective Clinical Study on Catheter-Associated Urinary Tract Infections (CAUTI) and Antibiotic Sensitivity Pattern, Twenty of the 400 catheterized individuals in a prospective study over a year in India had CAUTI. Overall, the incidence was 5%. The rate of catheterization was higher among male patients than female patients. The most prevalent uropathogens associated with CAUTI were *E. coli* and Klebsiella pneumonia, which were followed by *P. aeruginosa* (20%) and *Acineobacter spp.* (20%)(24).

Another study has showed that during a period of 10 months, 350 isolates from 585 samples were treated to biofilm in Saudi Arabia in 2020. Non-biofilm-forming organisms made up 64 % of the isolates, whereas biofilm-forming microorganisms made up 36%. The most prevalence biofilm makers were *E. coli* (24%), *Klebsiella* (19%), *E. faecalis*, *S. aureus* (3%), *P. aeruginosa* (18%), and *Citrobacter* (17%)(25).

A cross-sectional study was conducted from January 2019 to January 2020 in Yemen and showed that among 227 patients with indwelling urinary catheters suffering from CAUTI, *E.coli* was the most common uropathogenic in catheter-associated UTIs (CAUTI), accounting for 46.3% of cases, followed by *K. pneumonia* (18.5%), *P. mirabilis* (7%), *CONs*(5.7%), *S. aureus* (4.8%), and *E. faecalis* (1.3%), and biofilm-producing bacteria were found in 49.3% of the samples (21.1% as high producers and 28.2% as moderate producers). *E. coli* developed the most biofilms (60%)), followed by *Pneumonia* (57.1%) and *P.aeruginosa* (37%)). Patients over 65 years old pre-UTI, lengthy catheterization duration, and diabetes were all related to biofilm-producing bacteria (26)**.**

Another cross-sectional study was performed on medical ward patients in Nigeria from April to June 2017. Out of the 50 urine samples that were gathered and examined, growth was seen in 27 (54%) of them, and 28 isolates were found. *P.aeruginosa* was the least prevalence (21.4%), followed by S.aureus (42.9%) and *K. pneumonia* (42.9%) in catheterized medical ward patients(27)**.**

They say that a cross-sectional study is now being performed in Nigeria. Antibacterial Susceptibility Pattern of Isolates from Inpatients with Urinary Tract Infection and Biofilm Detection The most prevalent bacterial species, representing 45.8% of all isolates, was *Klebsiella spp*. 22.5% of the isolates were identified as biofilm formers using the tissue culture plate method (28)**.**

Another cross-sectional study was carried out in Egypt between October 2017 and September 2018. Out of 600 catheterized patients, the study revealed 105 CAUTI patients. 17.5% of people had CAUTIs overall. 130 of the 138 organisms that were identified from the tips of 105 urinary catheters were bacteria. The majority of isolated organisms are *K. pneumonia* (27.54%), *P. aeruginosa* (23.19%), *E. coli* (21.73%), *Proteus spp*. (18.84%), *S. aureus* (2.17%), and *enterococci* (0.74%). Biofilm-dependent CAUTI was 82.85% common (87 cases out of 105 cases). The most frequent creator of biofilms was *K. pneumonia* (86.44%), followed by *E. coli* (83.33%). Three kinds of biofilm producers were identified: weak biofilm producers (47.69%), moderate biofilm producers (22.31%), and strong biofilm producers (9.23%)(29).

A cross-sectional study was conducted from February to August in Ethiopia. The magnitude of biofilm formation and antimicrobial resistance in 143 studies involved individuals with urinary catheters. Participants were enrolled in the study one at a time. Urine samples from catheterized patients were collected, processed, and identified using the standard bacterial isolation and identification procedures. Uropathogenic bacteria were monitored for growth in in vitro biofilms using microliter plates. The samples contained 60 bacterial strains, and urinary catheters were present in 57 of the 54 inpatients (94.7%) who had monomicrobial infections. The remaining six bacterial strains were donated by three study participants, each of whom contributed two bacterial isolates. The majority of the isolates were from gram-negative bacteria. *E. coli* was the first bacterium to emerge. Biofilms are formed by about 80% of bacterial isolates(30).

An institution-based, cross-sectional examination on catheter-associated urinary tract infection in Ethiopia was carried out from March to December 2019 by researchers at Arba Minch Ethiopia. A total

of 39/231 symptomatic CAUTI cases (or 16.8%) were recorded. Catheter insertion in the surgical ward, extended (>7 days) catheterization, and diabetes mellitus were all independent predictors of). *E. coli* (17/42; 40.5%), *Klebsiella spp*. (9/42; 21.4%), and *Enterococcus spp*.(5/42; 11.9%) were the three most prevalent bacterial isolates(31).

2.2. Antibiotic resistance

According to a prospective study on biofilm-producing microorganisms done between July 2016 and June 2017in Bangladesh.400 patients with probable CAUTI had their urine samples taken which were later processed microbiologically and tested for antibiotic sensitivity? When treated to routinely used antimicrobials such as Augmentin, Gentamycin, Ceftriaxone, Ciprofloxacin, and Cortimoxazole, Enterobacteriaceae demonstrated high resistance while remaining sensitive to meropenem(32).

According to a prospective study on biofilm-producing bacteria in India, they have much greater levels of antibiotic resistance than non-producers. The fact that 64% of isolates of multidrug-resistant (MDR) bacteria developed biofilms is indication that there is a strong association between biofilm development and multidrug resistance. Statistics demonstrated a connection between MDR infection and antibiotic use lasting longer than five days. ($p<0.027$). MDR was growing more widespread among biofilm producers, which made it more difficult to control CAUTI. A range of typical management practises are not very effective at managing biofilm producers(33)**.**

A prospective study was conducted. Between July 2014 and January in Nepal, 2015, 90% and 93.2% of *E. coli* were extremely resistant to ampicillin and amikacin, respectively, in paediatric cases of uropathogens. 23 (35.93%) of the 64 isolates were determined to be multidrug resistant(34).

In another, 779 urine cultures that were obtained in Somalia between January 2019 and December 2021 were retrospectively examined. 47% of the cultures included MDR uropathogens. Older patients, hospitalisations in intensive care units, and concurrent comorbidities were all related with higher rates of CA-UTI caused by multidrug-resistant uropathogens. Patients with MDR uropathogens experienced lengthy inpatient stays, with 49% remaining more than two weeks. Acineobacter spp. (88.5%), followed by *P.aeruginosa* (68%), exhibited the highest MDR trend. *Acineobacter spp*. and *K. pneumonia* were associated with prolonged hospital stays (>2 w at 73.1 and 69%, respectively). Ceftriaxone (85.7%),

meropenem (54.3%), ciprofloxacin (58.5%), and amikacin (27%) have the highest rates of antimicrobial resistance(35).

Between June 2018 and March 2019, researchers in Ghana conducted a cross-sectional investigation on the microbiological makeup, patterns of antibiotic resistance, and biofilm bacteria isolated from catheterized patients. The pathogens in biofilms were more ciprofloxacin-resistant than the pathogens in urine when they were isolated from both samples of bacteria. Compared to *E. coli* and *S. aureus*, *K. pneumonia* from both samples exhibited higher levels of antibiotic resistance(36).

A cross-sectional study of catheter-associated urinary tract infections in adult intensive care units at a specific tertiary hospital was carried out in Addis Ababa, Ethiopia, from October 2020 to September 2021. Numerous gram-negative bacterial isolates were found to be resistant to ceftriaxone 36 (94.7%), ampicillin 21 (91.3%), amikacin (16.0%), and meropenem (20.0%), according to the results. 52 (65.7%) of the 79 bacterial infections were antibiotic-resistant across the board. 37 (71.0%) and 15 (29%) of the microorganisms were gram-negative, respectively. Thirteen isolates of *Acineobacter,* (100%)of *Klebsiella spp.*, and (100%) of *E. coli* isolates were all multi-antibiotic resistant. 55.56% of the isolates of *Enterococcus* were antibiotic-resistant(37).

3. OBJECTIVES

3.1. General objective

To determine the magnitude of catheter-associated urinary tract infection, biofilm producing bacteria, and their antibiotic resistance among patients urinary tract infection at Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia.

3.2. Specific objective

To determine the magnitude of urinary tract infection associated with urinary catheter

To determine the magnitude of catheter-associated biofilm producing bacteria

To determine antibiotic resistance pattern

4. MATERIALS AND METHODS

4.1. Study Area

The location of this study was Yekatit 12 Hospital Medical College, which is close to the main campus of Addis Ababa University and the Ethiopian capital city of Addis Ababa. The Addis Ababa municipal Administrative Health Bureau over sees the hospital, which is a teaching hospital. It is situated in Addis Ababa city of government, the Arada Sub-city. The hospital opened its doors in 1915 with a capacity of 25 beds and 37 medical staff members. The hospital currently employs about 1024 health professionals, 453 administrative staff members, has about 400 beds, and offers a variety of medical services to almost 4 million people, according to hospital data.

Also According to hospital data that was gathered from the facility, the city's Administrative Health Bureau at Addis Ababa Pioneer Hospital is currently the only one in the city that offers care for the large number of neonatal and paediatric patients. The clinic also accepts referral cases from various regional states in Ethiopia and offers additional services like, psychiatry, burns, ear, nose, and throat (ENT), etc. According to the most recent health information management system (HIMS), the medical intensive care unit (MICU) and surgical intensive care unit (SICU) have a combined 42 beds, with a total of 160 monthly admissions between the paediatric intensive care unit (PICU),the neonatal intensive care unit(NICU) and the adult intensive care unit)AICU). In addition, the microbiology laboratory performs drug susceptibility testing and culture testing. Culture and sensitivity tests for urinary catheter patients are obtained from hospital clients.

4.2. Study design

A hospital –bas cross sectional study design was conducted to determine to the magnitude of catheter-associated urinary tract infection, biofilm-producing bacteria and their antibiotic resistance among patients with urinary tract infection at Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia.

4.3. Study period

Data was collected from urinary tract catheterized patients attending Yekatit 12 hospital medical colleges between December, 2022 and May, 2023.

4.4. Population

4.4.1. Source population

All patients with UTI attending Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia, were part of the study's population.

4.4.2. Study population

The study included patients with urinary tract catheterization who attended Yekatit 12 Hospital medical colleges and who completed every requirement for eligibility.

4.5. Inclusion and Exclusion criteria

4.5.1. Inclusion

All urinary tract infections identified in individuals having urinary tract catheterization were included in the study.

4.5.2. Exclusion criteria

- Unwilling participants throughout the time of sample or study collection
- Catheter-related perforation (hole) in the urethra or bladder
- Contaminating other liquids, such as blood

4.6. Study variable

4.6.1. Dependent variable

- Biofilm producing bacteria
- Antimicrobial susceptibility patterns
- Urinary catheter associated urinary tract infection

4.6.2. Independent variable

- Social-demographic data, including information on gender, age, education, and other variables
- Admission wards /sources of admission
- Co -morbidity/underline disease
- Previous UTI and catheter
- Duration of catheterization
- Reasons for hospitalization

4.7. Sample size determination and Sampling technique

4.7.1. Sample size determination

The sample size was calculated via the single proportion percentage formula shown below: The percentage of bacteria that produce biofilms was (p) = 80%, according to a prior study conducted at the Jimma University Medical Centre in Southwest Ethiopia(30).

$$n = (z\ \alpha/2)2 * p\ (1\text{-}p)\ d2$$

Where n=size of sample

Z= 95% Confidence level ($z\ \alpha/2=1.96$)

P = 80% Prevalence (p = 0.8)

Marginal error is 5% (d=0.05), hence d=

$$n = \frac{(1.96)\ 2 * 0.8\ (1\text{-}0.8)}{(0.05)2} = 245$$

The ultimate sample size was 270 after a 10% non-respondent rate was added.

4.7.2. Sample technique

The recruitment of study participants was done through convenient sampling until the required sample size was reached.

4.8. **Measurement and data collection**

4.8.1. Data collection procedure

Important medical information was gathered from the patient's medical records, which was confirmed by a face-to-face interview, including socio-demographics, comorbidities, the ward, length of stay, and duration of catheterization. Sample collectors were trained how to collect urine samples aseptically from catheterized patients. The sample collector was given clean urine sample collection cups. The procedure used to collect urine samples involved clamping the catheter for a short period of time, storing the urine in the bladder, and then releasing the clamp to collect the urine sample, Because of the possibility of contamination, catheterized urine is collected in this manner. The urine sample was examined using standard microbiological procedures. Sample delivery, storage, and quality were under the main investigator's control, and samples were sent immediately for processing to the microbiological laboratory(38).

4.8.2. Laboratory analysis for urine sample collection

4.8.2.1. Catheter Urine Culture identification Procedures

Collection of catheter urine samples and immediately inoculated onto a sheep blood agar plate (BAP) and MacConkey (MAC) (all media are from Oxoid Ltd. & Co., Basingstoke, UK).Using a calibrated inoculating loop in Hampshire, England, the transfer capacity of 0.001 culture media that were incubated for 24 to 48 hours in an aerobic condition at 37 °C was determined. Gram-staining, , Indole, Oxidase, manitol fermentation, decarboxylation on lysine iron agar, kligler iron agar ,coagulase, catalase oxidase, citrate utilisation, lactose fermentation, production of hydrogen sulphide, urease test, motility, and other assays were used to identify bacteria isolates in the culture medium using their distinctive characteristics (40 , 41) .

4.8.2.2. **Antimicrobial Susceptibility Testing**

All materials, equipment, and procedures were initially kept current. Bacterial after-identification on blood agar and MacConkey agar plates denotes the use of the Kirby-Bauer disc diffusion technique on Muller-Hinton agar (Oxoid Ltd., Hampshire, England) to test only monomicrobial isolates for antimicrobial susceptibility, and the comparing of the zone of inhibition to the reference value using

clinical and laboratory standard institute (CLSI) criteria (42) (42). Bacteria that display resistance to three or more antimicrobial medications from various groups are said to exhibit multiple drug resistance (MDR)(41).

Loop full of bacteria cells was collected from a pure culture and put into a tube with 4-5 ml of ordinary saline before being gently stirred until it produced a uniform solution. The inoculum quantity was then calibrated by adjusting the suspension's turbidity to a density of McFarland 0.5 standards. A sterile cotton swab that had been dipped into the suspension was then used to gently rotate the excess off of the tube. Using the swab, the bacteria were equally distributed around the Mueller-Hinton agar (Oxoid, UK) surface. Sterile needle or forceps were used to apply an antibiotic disc to the surface of Mueller-Hinton agar. After distributing the antibiotic disc on the Mueller-Hinton agar, the plates were inverted and incubated for 24 hours at 37 oC in an aerobic condition. The isolates have been classified as sensitive, moderate, and resistant according to (CLSI) based on the diameters of the zones for inhibition encircling the discs.

Aminoglycosides (amikacin, tobramycin, gentamycin), penicillin (ampicillin, amoxicillin), beta-lactamases (clavulanicacid), the cephalosporin group (ceftriaxone, ceftazidime), and sulphonamides (trimethoprim, sulfamethoxazol) are among the antibiotics used to treat gram-negative bacteria.

Drug list for gram-positive bacteria: - penicillin (ampicillin, amoxicillin, penicillin G), beta-lactamases (clavulanicacid), the cephalosporin group (ceftriaxone, ceftazidime, cefuroxime, cefoxitin, Cefotaxime), sulphonamides (trimethoprim, sulfamethoxazol), Aminoglycosides (amikacin, gentamycin), , glycopeptide (Vancomycin)and tetracycline .

4.8.2.3. The microtiter plate method Biofilm Production Assay Protocol

There are a variety of biofilm detection techniques, including Congo agar medium, the tube method, and the tissue culture method; however, due to a lack of resources, this research study used microtiter plating in the ELISA reader method to find bacteria that produce biofilms. The MacConkey, BAP, and performance were all assessed using the control strains in accordance with the biofilm detection standard operating procedure. Managing materials, tools, and procedures correctly was also crucial. A tube containing sterile trypton the following antibiotic discs were used to treat gram-negative bacteria that were identified. Soy broth (TSB) with 1% glucose was inoculated with about 100 colonies of bacteria obtained from a fresh agar plate and cultured at 37 oC for 24 hours to test for biofilm formation. For the biofilm assay, the overnight culture was diluted into fresh media by a factor of 1:100. A sterile flat-bottom microtiter plate with 96 wells was then filled with 200 microliter of the diluted sample and incubated at 37 °C for 48 h. The bacteria suspension in each well was delicately removed after incubation. To get rid of free-floating "planktonic" microorganisms, the well was cleansed three times with phosphate-buffering saline solution with a PH of 87.3. 99% methanol was used to fix the plates and 220 microliters of crystal violet (CV) (0.1% w/v) was used to stain plates. Let dry for 10–15 minutes at room temperature. To get rid of unbound CV dye, wells were cleaned three times with phosphate buffered saline. A micro plate reader operating at a wavelength of 630nm was used to measure the optical density (OD) of the solubilized biofilm staining. Each strain's experiment was carried out in triplicate, and average data was computed, and the average values were calculated(42).

The mean values of OD obtained for blank tests were subtracted from the mean values of OD obtained for each test strain in order to modify the background staining of the micro plate for the classification of adherence. Based on the OD value acquired for each test strain in the previous inquiry, the biofilm development of the isolates was separated into four classes to correct the background staining of the micro plate. The isolates' biofilm forms were then divided into the following four groups in accordance with the findings of the previous study: non-adherent (OD<ODc), weak adherent (ODc<OD<2xODc), moderately adherent (2xODc <OD4xODc), highly adherent (4xODc<OD), and with ODc: the absorbance cut-off value (ODc) was determined by adding the values of the negative controls(43)

4.8.2.4. Quality Control

Prior to the experiment, patients who were catheterized completed standardised questionnaires to assure the quality of the data in terms of correctness, precision, completeness, and clarity. The collected data is double-verified for accuracy and consistency. Providing data collectors with the necessary training also helped confirm the quality of the data. The effectiveness and sterility of culture media were investigated; sterility was determined by incubating culture media overnight at 35–37 oC without an inoculation sample. Physical changes were looked for in cracks, including an excessive amount of moisture, colour, dehydration, contamination, and the date of expiration. The temperature of the refrigerator and incubator was observed.

The Ethiopian Public Health Institute laboratory provided standard reference strains of *S.aureus* (ATCC-25923), *E. coli* (ATCC-25922), and *P. aeruginosa* (ATCC-27853), which were used as quality control cultures and antimicrobial susceptibility tests. For in vitro biofilm evaluation techniques, a control strain of *S. epidermidis* ATCC12228 (a biofilm conformer or negative control) and *E. facealis* ATCC29212 (a biofilm-forming or positive control) was also employed.

4.9. Data collection quality assurance

4.9.1. Pre-analytical

The pre-analytical phase was based on accepted practises for specimen collection, lab delivery, sample preparation, specimen receipt in the lab, sample preparation for testing or culturing, and so on, all of which can be sources of error. More attention needs to be placed on the pre-analytical stage in order to decrease these errors. The pre-analytical process must be viewed as a whole, from test ordering to specimen delivery processing. Also By properly completing the standard data collection form, the pre-analytical phase undoubtedly satisfied the standard, and other pertinent information about the study samples was ensured. The primary investigator's job was to verify the accuracy of the obtained data and keep track of it during data collection and data entry.

4.9.2. Analytical phase

Process the specimen as quickly as possible after receiving it. Ensure that all media and supplies used have undergone the necessary quality assurance checks. All of the retrieved data, which was comprised of test results and questionnaire responses, is examined for legibility, completeness, consistency, and storage in a safe place. Data cleansing and cross-checking were completed. Missing data was retrieved from the questionnaire and laboratory records during data cleaning and cross-checking.

4.9.3. Post-analytical

The recording, interpretation, communication with the ordering physician, and filing of the report are all parts of the post-analytical step. The lead researcher will review the collected data each day to ensure its accuracy and completeness. Cross-checking was done during data entry to make sure the right information was entered

4.10. Data analysis and interpretation

The data are coded and entered into the statistical package for social science (SPSS) software version 26 for analysis. The crude ratio was estimated with a 95% confidence interval using descriptive statistics and logistic regression. Using the chi-squared test, the odds ratio with a 95% confidence interval was utilised to identify probable important factors among the numerous variables. Statistical significance was defined as a 0.05 p-value or higher.

4.11. Operational Definition

UTI: is defined as the presence of symptoms and signs suggestive of UTI and a urine culture containing one species of bacteria and 10 x 105 cfu/ml of viable bacteria.

CAUTI: is a UTI that develops in someone who has a urinary catheter in place or who had one removed within the preceding 48 hours.

Bacterial biofilms: are clusters of bacteria that attach to surfaces and/or to each other and are embedded in a self-produced matrix.

Multi-drug resistance: (MDR Bacteria that become resistant to at least one agent from three or more different antimicrobial classes are referred to be multi-drug resistant (MDR) bacteria.

4.12. **Ethical considerations**

The Addis Ababa public health research and emergency management core, as well as the departmental research and ethics review committee of the Department of Medical Laboratory Sciences at Addis Ababa University, received ethical clearance. Permission has been granted for the Yekatit 12 Hospital Medical College. Parents or guardians of study participants were informed of the study's objectives and protocol at the study location. In order to participate in the study, participants had to fill out a consent form. We received explicit written consent. Official approvals from every department were obtained. Additionally, the clinical samples gathered throughout the study period were exclusively used to accomplish the objectives of the tests. For participants who were under significant bacterial isolation, the information was conveyed as quickly as feasible to the accountable doctor.

4.13. Dissemination of the result

The Department of Medical Laboratory Sciences, School of Allied Health Sciences, and College of Health Sciences of Addis Ababa University received the study's results. The Addis Ababa City Administrative Health Bureau and the Yekatit 12 Hospital Medical College were made aware of the results. The research was also presented at multiple meetings, and a peer-reviewed journal received the report for possible publication.

5. RESULTS

5.1. Socio-demographic and clinical characteristic

In the present study, out of 270 urinary catheterized patients, 108 (40%) males and 162 (60%) women formed the study's 1.2.025 sex distribution ratio. Participants in the study ranged in age from 18 to 84. The majority of participants (67.4%) live in urban, and 113 of the participants, or 41.9% of the study population, are illiterate. 207 out of 270 respondents, or 76.7%, reported having a monthly income of greater than 5000 Ethiopian birr(table 1).

Table 1.Socio-demographic characteristics among catheter associated urinary tract infection patient at Yekatit 12 hospital medical college, Addis Abeba, Ethiopia. From December, 2022to May, 2023

Variable	Frequency (N=270)	Total (%)	Positivity UTI%(N=146)	P-value
Gender				
Male	108	40%	59(40.4%)	0.483
Female	162	60%	87(59.6%)	
Age				
18-34	27	10	16(11%)	0.879
35-49	54	20	32(21.9%)	
50-64	108	40	60(41.1%)	
>64	81	30	38(26%)	
Residence				
Urban	182	67.4	95(65.1%)	0.12
Rural	88	32.6	51(34.9%	
Educational level				
Illustrate	111	41.9	59(40.4%)	0.284
Diploma	106	38.5	60(41.1%)	
Degree	53	19.6	27(18.5%)	
Income level				
1000-5000	63	23.3	39(26.7%)	0.498
>5000	207	76.7	107(73,3%)	

The study involved 270 participants in all, 230(85.2%) of whom had UTI symptoms and signs and 40(14.8%) of whom did not. Urogenital abnormalities (26.7%) were the most common diagnosis, followed by appendicitis or bowel obstruction. the underlying disease, hypertension (23.3%), and followed by neurological disorders (22.2%) and diabetes (20.4%). The majority of medical wards' initial admission In addition, endocrine, nutritional, and metabolic illnesses, several infectious diseases, and genitourinary tract infections were the most frequent causes of hospitalisation. Clinical features that are more typical include fever and dysuria (table 2).

Table 2. Clinical characteristic of catheter associated urinary tract infection (December 2022-May 2023), Y12HMC

Variable		Frequency (N=270 (%))	
		Yes	No
Length of hospital stay/day	1-7	82(30.4)	188(69.6)
	8-14	185(68.5)	85(31.5)
	15-21	3(1.1)	267(98.9)
Duration of catheterization/day	<4	54(20)	216(80)
	4-6	135(50)	135(50)
	>7	81(30)	189(70)
Source of admission	Emergency	13(4.8)	257(95.2)
	Medical	220 (81.5)	50(18.5)
	Surgical	30(11.1)	240(88.9)
	burn	7(2.6)	263(97.4)
Cause of hospitalization	Certain infectious diseases parasite diseases	34(12.6)	236(87.4)
	Endocrine, nutritional and metabolic diseases	61(22.6)	209(77.4)
	Injury, poison, external causes	26 (9.6)	244(90.4)
	Disease of the circulatory system	24(8.9)	246(91.1)
	Diseases of the respiratory system	23(8.50)	247(91.5)
	Diseases of the digestive system	25(9.3)	245(90.7)
	Diseases of the genitourinary system	31(11.5)	239(88.5)
	Disease of the blood and blood–forming organ	22(8.1)	248(91.9)
	illnesses of the neurological system, cancer,	24(8.9)	246(91.1)
Clinical Profile	Indication for catheterization	55(20.4)	215(79.6)
	History of previous catheterization	57(21.1)	213(78.9)
	Fever	37(16)	193(84)
	Dysuria	42(18.3)	188(81.7)
	Urgency	23(10)	207(90)
	Pelvic pain	26(11.3)	204(88.7)
	Frequency	10(4.3)	220(96.7)
	Blood in the urine	6(2.6)	224(97.4)
	Burring with urination	17(7.4)	213(92.6)
	Lower belly discomfort	12(5.2)	218(94.8)
	Vomiting	9(3.9)	221(96.1)
	nausea	16(7)	214(93)
	Shaking and chill	16(7)	214 (93)
	Back or side pain	3)1.3)	227(98.7)
	Flank pain	1(0.4)	229(99.4)
	Other specific----------	4(1.7)	2268.3)

5.2. Urinary tract infection with catheter association (CAUTI)

Out of the 270 urine samples obtained, 146 samples had an overall percentage of 54% (146/270). 101 (69.1%) of the 146 total isolates were gram-negative bacteria, while 45 (30.9%) were gram-positive bacteria (Table 3). With a prevalence of 41 (28%), *E. coli* is the predominant gram-negative bacteria, whereas S. aureus is the predominant gram-positive bacteria with a prevalence of 23 (15.8).CONS 19 (13%), *Pseudomonas spp*. 23 (15.8%), *Klebsiella spp.* 22 (15.1%), Citrobacter spp.3 (2.1%), *Acineobacter spp.* 6 (4.1%), *Proteus spp.* 6 (4.1%), and Enterococcus spp. 3 (2.1%) were among the gram-positive and gram-negative bacteria found in 146 positive urine catheter samples(figure 1).

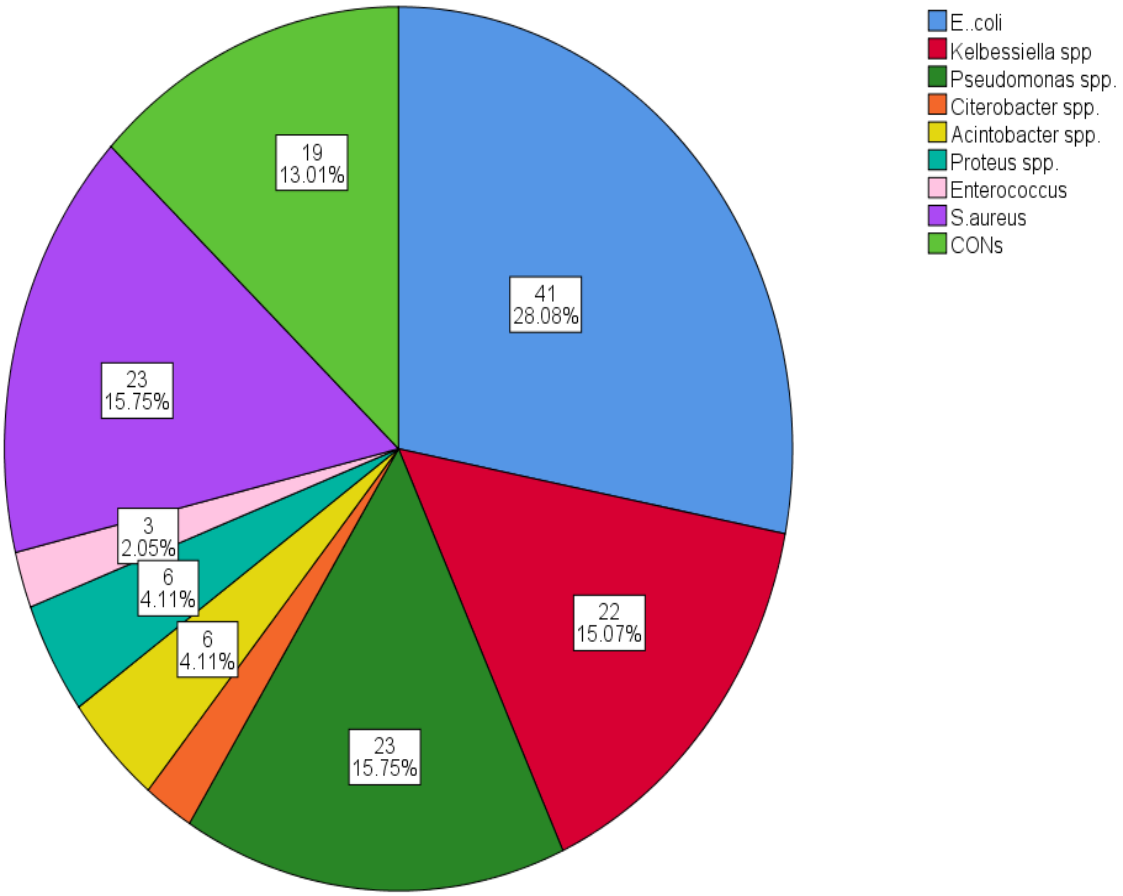

Figure 1.Distribution of uropathogenic bacteria isolates among urinary catheterized associated urinary tract infection patients at Yekatit 12 hospital medical colleges, Addis Ababa, Ethiopia, from December, 2022 to May, 2023

Patients with urogenital abnormalities had the highest prevalence of each of these bacteria, 48 (32.9%), according to catheter-associated bacteria. However, it was shown that those with neurological conditions (30.8%) had a higher incidence of catheter-associated bacteria, and that diabetes affects 39 (26.7%) persons. Additionally, 43 (29.6%) of the trial participants who received antibiotics displayed significant Bacteriuria (table 3).

Table 3. Clinical characteristic of urinary tract infection with catheter associated (December 2022-May 2023), Y12HMC

Variable	urinary tract infection with catheter -associated		Ch-square	
	Yes (%)	No (%)	$\chi 2$	p-vale
Primary diagnosis upon admission				
Urogenital abnormality	48(32.9)	98(67.1)		
Malignancy(urothelial carcinomas)	34(23.3)	112 (76.7)		
Leg or head injury	24(16.4)	122(83.6)	3.403	0.066
Appendicitis or bowel obstruction	32(21.9)	114(78.1)		
Chronic heart failure	28(19.2)	118(80.8)		
risk group				
Diabetes status	39(26.7)	107(73.3)		
Neurological disease	45(30.8)	101(69.2)		
Hypertension	44(30.1)	102(69.8)	8.22	0.004
Kidney or bladder stone	36(24.7)	110(75.3)		
Autoimmune disease	36(24.7)	110(75.3)		
Chronic kidney disease	25(17.1)	121(82.9)		
Enlarged prostate	35(24)	111(76)		
Antimicrobial drug receive	43(29.5)	103(70.5)		
Reason catheterization.				
Pre- or postoperative drainage	35(24)	111(76)		
Urine output measurement	33(22.6)	13(77.4)		
Incontinence	34(23.3)	112(76.7)	4.972	0.029
Urinary retention	36(24.7)	110(73.3		
Source of admission				
Medical	7(95.2)	139(95.2)		
Surgical	122(83.6)	24(16.4)		
Emergency	15(10.3)	131(89.7)	4.08	0.078
Burn	2(1.4)	144(98.6)		
Cause of hospitalization				
Certain infectious diseases	20(58.8)	14(41.2)		
Endocrine, nutritional and metabolic	36(59)	25(41)		
Injury, poison, external causes	15(57.7)	11(42.3)		
Disease of the circulatory system	8(33.3)	16(66.7)		
Diseases of the respiratory system	9(39.1)	14(60.9)		0.515
Diseases of the digestive system	16(64)	9(36)		
Diseases of the genitourinary system	20(64.5)	11(35.5)		
Disease of the blood	11(50)	11(50)		
illnesses of the neurological system,	11 (45.8)	13(54.2)		

5.3. Antimicrobial susceptibility bacteria isolates

This study evaluated the antimicrobial sensitivity of catheter-associated urinary tract infection and biofilm-forming bacteria using 15 antibiotics. Ampicillin and amoxicillin-clavulanic acid have resistance rates of 76% and 77.4%, respectively. Antibiotic resistance has been reported in gram-positive bacterial isolates. The most widely used drug resistance gram-positive bacteria was ciprofloxacin (95,6%), which was followed by ceftazidime and tobramycin (91,1%), meropenem (88.9%), and gentamycin (86.7%).Also, trimethoprim-sulfamethoxazole (SXT) (80%) and amoxicillin-clavulanic acid (82.2%). Additionally, these gram-positive isolates showed ampicillin resistance (80%), as well as cefotaxime and cefuroxime resistance (75.6% and 73.3%), respectively.

The trend of antibiotic resistance among gram-positive bacteria is shown in Table 5 (N = 45 versus 14 medications). Cefotaxime and Amikacilin were (34.8) and (43.5%) effective treat Staphylococcus aureus, respectively, and Amikacilin (41.4%) effective treat coagulase-negative staphylococcus. The range of the 14 drugs evaluated for resistance to gram-positive bacteria was between 28.9% and 95.6% overall (table 4).

Table 4**.**Antimicrobial resistance pattern of Gram-positive bacteria isolated from urinary catheterized patients from December, 2022 to May, 2023

	Antimicrobial resistance (%)		
Antimicrobial	*S.aureus* (N=23)	CONs (N=19)	Enterococcus spp (N**=3)**
AMP	17(73.6%)	15(78.9%)	2(66.7%)
GE	18(78.3%)	13(68.4%)	
FOX	16(69.6%)	13(68.4%)	
CRO	17(73.9%)	12(63.2%)	
AMC	19(82.6%)	15 (71.9%)	2(66.6%)
CIP	17(73.9%)	13(68.4%)	2(66.7%)
AK	13(56.5%)	11(57.9%)	
P	17(73.9%)	11(64.7%)	2(66.7%))
SXT	17(73.9%)	12(63.2%)	2(66.7%))
TET	17(73.9%)	13(68.4%)	2(66.7%)
CTX	15(65.2%)	11(64.7%)	
CXM	5(68.2%)	11(64.7%)	
CAZ	17(73.9%)	13(68.4%)	
VAN			2(66.6**%)**

AMP; ampicillin, GN; gentamycin; CIP: Ciprofloxacin; AMC: amoxicillin-clavulanic acid: SXT; Trimethoprim/sulfamethoxazole, Ceftriaxone, TET, Tetracycline, CRO: Ceftriaxone, AK; Amikacilin; Penicillin, VAN; Vancomycin, CAZ; ceftazidime, FOX; cefoxitin, CXM: Cefuroxime, CTX; cefotaxime

The antimicrobial resistance pattern of gram-negative bacteria to 10 antibiotics is discussed in (Table 6) (n = 101). Among gram negative organisms, the most prevalent resistance medication was Ciprofloxacin (86.1%), followed by Ceftriaxone. (81.2%), Meropenem (78.2%), and Tobramycin (77.2%. Meropenem, an antibiotic, had the best results (44%), when used to treat isolated E. coli isolates. Ceftriaxone, amoxicillin-clavulanic acid, and ceftazidime had the lowest efficacy against E. coli (82.9, 78, and 78), respectively. The sensitivity and resistance ranges for the 10 antibiotics tested for gram-negative bacteria range commonly from 25.7% to 86.2%. Amikacilin was the most efficient antibiotic from the same Enterobacteriaceae family. Multidrug resistance was higher in biofilm producers than in non-producers. Amoxicillin-clavulanic acid, ceftazidime, Tetracycline, gentamicin, meropenem, and Amikacilin resistance rates for gram-negative and gram-positive biofilm producers were 78%, 91%, 84%, and 29.2%, respectively(table 5,(table 6)(table 7).

Table 5.Antimicrobial resistance pattern of gram-negative bacteria isolated from urinary catheterized patients.

Antimicrobial	anti-microbial resistance(%)gram-negative bacteria isolates					
	E.coli	*Klebsiella spp*. P*seudomonas spp*.		*Citrobacter spp*.	*Acineobacter spp.*	*proteus*
	(N=41)	(N=22)	(N=23)	(N=3)	(N=6)	(N=6)
AMP	R=24(58.7%)	7(31.8%)	17(73.9%)	1(33.3%)	3(50%)	2(33.3%)
AK	R=24(58.7%)	7(31.8%)	17(73.9%)	1(33.3%)	3(50%)	2(33.3%)
AMC	R=32(78%)	15(68.2%)	20(87%)	3(100%)	5(83.3%)	5(83.3%)
CIP	R=26(63.4%)	15(68.2%)	19 (82.6%)	3(100%)	4(66.7%)	5(83.3%)
GN	R=30(73.2%)	15(68.2%)	19 (82.6%)	3(100%)	4(66.7%)	5(83.3%)
CRO	R=34(82.9%)	16(72.7%)	19 (82.6%)	3(100%)	4(66.7%)	5(83.3%)
MEP	R=23(56%)	13(59%)	15 (65.2%)	2(66.7%)	4(66.7%)	4(66.7%)
CAZ	R=32(78%)	20(90.9%)	19 (82.6%)	3(100%)	5(83.3%)	5(83.3%)
TOB	R=34(82.9%)	18(81.8%)	18 (78.3%)	3(100%)	4(66.7%)	5(83.3%)
SXT	R=26 (65.9%)	6(72.7%)	16 (69.6%)	2(66.7%)	4(66.7%)	4(66.7%)

Table 6.Antimicrobial resistance patterns between prevalence of biofilm production and non-biofilm former

Biofilm producer, non-biofilm producer and Antimicrobial resistance pattern (%)				Chi-square	
Antimicrobial		Susceptibility			p-value
		Biofilm producer (N=89) (%)	Non –Biofilm producer (N=57) (%)	X^2	
AMP	Sensitive	15(16.9)	20(35.1)	6.388	0.012
	Resistance	74(83.1)	37(64.9)		
AMC	Sensitive	12(13.5)	21(36.8)	10.838	0.001
	Resistance	77(86.5)	36(63.2)		
AK	Sensitive	63 (70.8)	44(77.2)	0.728	0.395
	Resistance	26(29.2)	13(22.8)		
CIP	Sensitive	8(9)	8(14)	0.902	0.343
	Resistance	81(91)	49(86)		
GN	Sensitive	11(12.4)	20(35.1)	10.732	0.001
	Resistance	78(87.6)	37(64.9)		
CRO	Sensitive	12(12.4)	21(36.8)	10.838	0.001
	Resistance	77(86.5)	36(63.2)		
MEP	Sensitive	10(11.2)	7(29.8)	7.965	0.005
	Resistance	79(88.8)	40(70.2)		
CAZ	Sensitive	8(9)	15(26.3)	7.86)	0.005
	Resistance	81(91)	42(73.7)		
TOB	Sensitive	9(10.1)	18(31.6)	10.623	0.001
	Resistance	80(89.9)	39(68.4)		
CXM	Sensitive	6(21.4)	6(35.3)	1.040	0.313
	Resistance	19(78.6)	11(64.7)		
CTX	Sensitive	5(17.9)	6(35.3)	1.764	0.192
	Resistance	23(82.1)	11(64.7)		
P	Sensitive	5(16.7)	8(53.3)	6.544	0.011
	Resistance	25(83.3)	7(46.7)		
FOX	Sensitive	3(11.5)	9(47.4)	7.207	0.008
	Resistance	23(88.5)	10(52.6)		
SXT	Sensitive	17(19.1)	19(33.3)	3.788	0.052
	Resistance	72(89.9)	38(66.7)		
TET	Sensitive	4(16)	12(60)	9.388	0.002
	Resistance	21(84)	8(40)		

In the present study, multi-drug resistance (MD=resistance to _>3 drugs) was seen in 85.8% of the isolates among those diagnosed with catheter-associated urinary tract infection (table 7).

Table 7. Multi-drug resistance urinary catheterized associated urinary tract infection patient

Bacterial isolation	Total %	Antibiotics resistance					
		RO	R1	R2	R3	R4	≥R5
Gram positive	45(30.9)	0(0.0)	1(2.2)	6(13.33%)	4(8.9%)	7(15.6%)	27(60%)
S.aureus	23(15.8)	0(0.0)	(0.0)	3(13%)	1(4.3%)	5(21.7%)	14(60.9%)
CONs	19(13)	0(0.0)	1(5.3)	2(10.5)	2(10.5)	2(10.5)	12(63.2%)
Enterococcus spp.	3(2.1)	0(0.0)	0(0.0)	1(33.3)	1(33.3%)	0(0.0)	1(33.3%)
Gram negative	101(69.1)	0(0.0)	0(0.0)	11(10.9)	8(7.9%)	14(13.9%)	79(78.2%)
E.coli	41(28)	0(0.0)	0(0.0)	3(7.3)	3(7.3%)	6(14.6%)	30(73.2%)
Pseudomonas spp.	23(15.8)	0(0.0)	0(0.0)	2(8.7)	2(8.7%)	4(17.4%)	15(65.2%)
Klebsiella spp.	22(15.1)	0(0.0)	0(0.0)	3(13.6)	1(4.5)	3(13.6%)	15(68.2%)
Acineobacter spp.	6(4.1)	0(0.0)	0(0.0)	1(16.7)	1(16.6%)	1(16.7%)	2(33.3%)
Citrobacter spp.	3(2.1)	0(0.0)	0(0.0)	1(33.3)	(0.0)	1(33.3%)	1(33.3%)
Proteus spp.	6(4.1)	0(0.0)	0(0.0)	1(16.7)	1(16.7)	1(16.6%)	3(50%)
Tota1	146(100)	0(0.0)	1(0.7)	17(11.6)	10(6.7)	23(15.4)	93(63.7)

R0=No antibiotic resistance, R1= Resistance to one, R2=Resistance to two, R3=Resistance to three, R4-=Resistance to four, ≥ R5=resistance to five and more drugs.

5.4. The magnitude of biofilm formation

The presence of biofilm producers among bacteria that cause UTIs was assessed in the current study.89 (61%) of the 146 bacterial isolates tested positive for biofilm in vitro, while 57 (39%) strains tested negative biofilm. Strong, moderate, or weak biofilms are produced by Klebsiella spp., *S. aureus*, and Citrobacter spp. in proportions of 9 (40.9%), 8 (34.8%), and 2 (66.7%), respectively. The most common biofilm-producing bacteria were discovered to be *Enterococcus spp.* and Citrobacter spp. 3 (100%) and *S. aureus* 18 (78.3%), followed by *Pseudomonas spp*. 16 (69.6%), *Klebsiella spp*. (68.2%), CONs 11 (57.9%), *Acineobacter spp*. 3 (50%), and *Proteus spp*. 2 (33%).

The rates of bacterial biofilm formation were categorized into four: 30(33.7%) of them as strong former; 27(30.3%) as moderate; 32 (26.0%) as weak and 57(39%) as non-biofilm former. Gram-positive isolates made up 32 (36% of them), while Gram-negative isolates made up 57 (64%), with no statically significance between the two (p =0.6) (Table 9). all patient clinical traits, such as the primary admissions diagnosis, the presence of underlying disorders, persons with diabetes status, those who have taken

antibiotics, those who have undergone a medically necessary catheterization, and the length of Using bacterial isolate biofilm formation patterns, this study found no association between catheterization and length of hospital stay (most of the time, p >0.05). In catheter-associated urinary tract infection (CAUTI), a biofilm is created. This biofilm is linked to a number of risk factors, such as diabetes, autoimmune diseases, cancer, urogenital abnormalities, the length of catheterization, and bacteria in the urinary system before catheterization. In the current study, 23.6% of patients who had hypertension also had BFP, compared to 22.8% of BFNP participants. Diabetes was more prevalent (27%) in BFP patients than in (26.3 %(table 8)

Table 8.Biofilm producer bacteria isolates among urinary catheterized associated urinary tract patient

	Bacteria formation pattern biofilm formation				
Bacteria isolation	BFP	SBF	MBF	WBF	NBFP
Gram-negative	57(64%)	21(36.8)	16(28%)	20(355)	44(77.2%)
E.coli (N= 41)	18(43.9%)	2(4.9%)	6(14.6%)	10(24.6%)	23(56.1%)
Klebsiella spp. (N=22)	15(68.2%)	9(40.9%)	3(13.6%)	3(13.6%)	7(31.8%)
Pseudomonas spp. (N=23)	*16(69.6)*	*8(34.8%)*	*4(17.4%)*	*4(17.8%)*	*7(30.4%)*
Proteus spp. (N=6)	2(33.3%)	----------	2(33.3%)	-------	4(66.7)
Acineobacter spp. (N=3)	3(50%)	1(16.7%)	1(16.7%)	1(16.7%)	3(50%)
Citrobacter spp. 9N=11)	3 (100%)	1(33.3%)	-----	2(66.7%)	--------
Gram positive	32(36%)	9 (28%)	11(34.4%)	12(37.5%)	13(22.8%)
Cons (N=19)	11(57.9%)	4 (21.1%)	2 (10.5%)	5(26.3%)	8(42.1%)
S.aureus (N=23)	18 (78.3%	5(21.7%)	8 (34.8%)	5 (21.7%)	5(217%)
Enterococcus spp (N=3)	3(100%)	------	1(33.3%)	2(66.7%)	----------
Total	89 (61(%)	30 (33.7%)	27(30.3%)	32(36%)	57(39%)

BFP=Biofilm producer, NBFP=Non biofilm producer, SBP= strong biofilm production, MBP= moderate biofilm production n, WBP= weak biofilm production, NBP= non-biofilm production, Cons=coagulase negative staphylococcus, N=Number

6. DISCUSSION

Urinary catheterization is a typical procedure that patients get through flexible tubes at medical facilities. Because of this, catheter-associated UTIs are now known to be among the most hospital acquired infections around the globe. The bacteria can colonise and cling to the urinary tract due to its higher use in more situations and longer catheterizations .The bacteria involved in the procedure have the potential to infect the system and increase hospital stays, which would eventually place extra burden on the healthcare systems.(26).therefore, in order to determine the distribution catheter -associated bacteria pathogen, their capacity to form biofilms, and patterns of antimicrobial susceptibility among catheter visits at Yekatit 12 Hospital Medical College, the current investigation was carried out.

Depending on the people involved and the research region, the bacteria that cause UTIs may alter over time. But in almost all of cases, gram-negative bacteria were discovered to be frequent bacterial isolates. Similarly, it was shown that gram-negative bacteria accounted for 69.1% of the bacteria isolates in this study. Comparable findings come from other study.

E. coli (28%) was the most frequently isolated species of uropathogenic bacteria in this study, followed by S. aureus and pseudomonas sp. similar studies have been reported from other countries, including Ethiopia (31), Bangladesh (22), and India (24). By infecting the urethra and ascending into the bladder, these bacteria that predominate in the gut's natural flora might cause a urinary tract infection. Additionally, exposure to contaminated surfaces or equipment by hospital staff increases the risk of catheter-associated infection. Other studies carried out in Pakistan (21), India (23) reported that *p.aerugonisa* and *s.aureus* was the frequency bacteria isolates, in contrast to the finding that *E. coli* was the leading aetiology. This difference in distribution of bacteria isolate may be due to different study area duration of catheterization and sample size.

According to clinical profiles, the distribution of catheter-associated bacteria indicated that patients with urogenital anomalies had the highest prevalence of each of these bacteria, 48 (32.9%), with a chi-square value of 5.496 and a p value of 0.019. This has statistical significance because a p-value less than 0.05**.** On the other hand, people with neurological illnesses (30.8%) were observed to have a larger prevalence of catheter-associated bacteria (chi-square value = 8.22, p = 0.004). Diabetes affects 39 (26.7%), with a chi-square value of 13.602 and a p value of 0.00. Chi-squared = 6.896; p = 0.009. 43 (29.6%) of the trial participants who received antibiotics showed considerabl**e** bacteriuria. In comparison to patients who

had catheterization for less than eight days, individuals on catheterization for eight to fourteen days had a higher risk of catheter-associated bacteriuria 85(58.2%) (Chi-square = 15.66, p = 0.00)**.** As per the literature, diabetes is distinguished by varied degrees of insulin resistance, impaired insulin release, and increased synthesis of glucose. Patients with diabetes mellitus are at greater risk of infection, with the urinary system being the most frequent infection source**.**

Antibiotic resistance is one of the biggest and most urgent issues in global public health. The issue of bacterial drug resistance has been widely reported, especially in illnesses linked to healthcare, and it is now one of the health-security concerns (31). Pathogens linked to UTIs are no exception to the trend of rising drug resistance among bacteria. A treatment regimen's success or failure can be proved to be influenced by compliance and resistance. Knowing the local susceptibility profile status and the prevalence of antibiotic resistance are crucial guiding principles when choosing a treatment plan and are beneficial in fighting uropathogens. This study found that ciprofloxacin (86.1%), ceftazidime (81.2%), meropenem (78.2%), and amoxicillin-clavulanic acid (75.2%) were all highly resistant to gram-negative bacteria isolates. A similar study in Bangladesh (33) showed that amoxicillin-clavulanic acid, and ciprofloxacin had high resistance. A chromosomal gene mutation (intrinsic resistance) or the acquisition of a plasmid-mediated resistance determinant (extrinsic resistance) is two possible causes of the medication resistance seen among the uropathogens in our investigation. In contrast to extrinsic genes, which are acquired through horizontal transfer, intrinsic resistance mostly results from selection pressure (including abuse, overuse, and misuse of antibiotics in therapy). A shift in the resistance determinant's environment from chromosomal to plasmid mediated, which resulted in their enhanced expression and dissemination, actually makes the intrinsic mechanism more dangerous.

In the present investigation, ciprofloxacin (95.6%), ceftazidime and tobramycin (91.1%), and meropenem (88.9%) were the most highly resistant gram-positive bacteria, as were gentamycin (86.7%),amoxicillin-cavulanic acid (82.2%), and ampicillin (80%) were the resistance. In the same environment, similar observations were made in India. On the other hand, most of the gram-positive isolates were resistant to ceftriaxone (82.2%), cefoxitin (73.3%), and penicillin (71.7%). The resistance of these bacteria to Amikacilin (28.9%) and tetracycline (64.5%), however, was relatively lower. This may be in contrast to the earlier study from Nepal, which revealed stronger Amikacilin resistance (93.2%). Therefore, this research's findings in our area may not guarantee continued usage of these medications because the switch to increased resistance could occur quickly.

In this study, more bacteria that formed biofilms exhibited greater levels of amoxicillin-clavulanic acid antimicrobial resistance than bacteria that did not form biofilms (86.5% vs. 63.2%). A similar investigation that was conducted in numerous places found that biofilm formers had higher levels of amoxicillin-clavulanic acid resistance than non-biofilm formers (34). This could explain why bacteria that produce biofilms are more resistant to attack than bacteria that do not produce biofilms. The quorum-sensing-mediated adhesion of the bacteria to a surface or to one another, a change in gene expression that produces a phenotype distinct from the planktonic state, and an extracellular matrix made of host components and secreted by the bacterial products are just a few of the components that make up a biofilm. Additionally, this higher resistance may be attributed to the biofilm formers' improved efflux mechanism properties. Enhanced plasmid transfer, changed target genes, and metabolic pathways that promote antibiotic resistance may also be associated with it (35). The prior biofilm isolates were multidrug-resistant to at least three antimicrobials in almost 76% of cases. This alarming report recommends employing in vitro susceptibility tests and culture-based methods to help select antibiotics that work. Biofilm-producing bacteria are extremely resistant to or multi-drug-resistant (36). This might be as a result of the bacteria at the base of the biofilm having inactive metabolic processes and insufficient antibiotic concentrations reaching those areas of the biofilm, which together with active antibiotic degradation mechanisms prevent the drug from building up to an effective concentration. May also they display resistance to antibiotics by several means, including reduced penetration of antibiotics into biofilms, a lower growth rate, and the expression of resistance genes.

In the present research, 89 (61%) of the 146 isolates showed the potential to form biofilm that ranged from weak to strong. This can contribute to drug resistance development and play a significant role in pathologic processes. This finding is compatible with research undertaken in Pakistan, Saudi Arabia, and Yemen, with biofilm formation rates of 73.4%, 36%, and 49.3%, respectively. However comparison than others and this study lower biofilm formation rate was found in Saudi Arabia (36%) (26).Higher growth of biofilm rate was found in Egypt (82.85%) (30). the variation can be related to differences in geographical location, differences in study period with the study population and variety of origin of the strains. Biofilm generating bacteria are responsible for many persistent infections and are famously difficult to remove. It is a widely recognised harmful mechanism in most bacterial species.

According to this study, 57 (39%) of the 146 catheter-associated UTIs discovered during the current investigation were biofilm-negative. In addition to the typical uropathogenic bacteria, *E. coli* (43.9%),

Pseudomonas (69.6%), S. aureus (78.3%), Klebsiella (68.2%), Cons (57.9%), *Acineobacter* (50%), *Proteus* (33.3%), and *Enterococcus* and *Citrobacter* (100%) were biofilm producer. Prior investigations Egypt (30) and Yemen (27) showed that biofilm-producing bacteria comprised 82.85and 49.3% respectively of the isolates, whereas non-biofilm-forming species made up 17.15% and 50.7 of them. Also in Saudi Arabia(26) showed *E. coli* (24%), Klebsiella (19%), *E. fecalis* (8%), S. aureus (3%), *P.aeruginosa* (18%), and *Citrobacter* (17%) were the most prevalent biofilm producers (9%).

7. STRENGTH AND LIMITATION OF STUDY

7.1. Strength of study

The study identified the bacterial pathogens that cause infections and form biofilms on catheters. To the best of our knowledge, Ethiopia is the only country that has assessed the formation of biofilm for UTI and related risk factors. It gave beginning knowledge to clinicians who use empirical therapy

7.2. Limitation of study

Despite the fact that many researches have been conducted on CAUTI detection methods and biofilm detection, there is little information available about the frequency of CAUTI in our region. The minimum inhibitory concentration (MIC) approach is advised for antimicrobial susceptibility testing of Vancomycin, although this was left out due to financial restrictions.

8. CONCLUSION AND RECOMMENDATION

8.1. Conclusion

This study found that the prevalence of catheter-associated bacteriuria was 54% overall, with the most frequent isolates being E. coli and other Gram-negative bacteria, such as pseudomonas spp. More than 61% of the bacterial isolates in UTIs associated to catheters were from biofilms. The findings of this study also revealed that bacterial isolates were more likely to exhibit resistance to commonly prescribed antimicrobial medications. Amikacin seems to be the best option in terms of effectiveness against the majority of isolated Gram-positive and Gram-negative bacteria. These medications may be the first line of defence against UTIs connected to catheters. Medical practitioners need to be aware of the likelihood of uropathogens establishing biofilms among patients utilising urinary catheters

The majority of the bacterial isolates exhibited increased levels of antimicrobial drug resistance. As a result, the diagnosis and treatment of CAUTIs should be based on an understanding of the bacterial aetiology and patterns of antibiotic resistance. Antibiotic drugs for the empiric treatment of CAUTIs are selected by doctors with the support of periodic monitoring of antibiotic resistance patterns. Also Antibiotic resistance and biofilm growth must be regularly tracked in all UTI cases in order to provide patients with the best possible therapy. In order to provide patients with CAUTI with the best care possible, it is advised to regularly assess antibiotic resistance and, preferably, the ability of isolates to develop biofilm at regular intervals in one institution. This is a result of the peculiar and challenging management of CAUTI brought on by bacteria that develop biofilms.

8.2. Recommendation

According to the antibiotic sensitivity profile, Amikacilin is more successful than other drugs at treating catheter-associated biofilm-producing bacteria and urinary tract infections. To prevent the spread of multiple drug-resistant bacteria, it is important to identify the precise etiological agent and patterns of antibiotic resistance while treating catheter-associated urinary tract infections.

Additionally To maintain and increase the effective and reasonable use of antibiotics for the management of urinary tract infections, an updated local antibiotic policy and guide should be prepared or changed from the previous version and given to all relevant organisations.

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10. ANNEXES

Annex I: Information sheet (English version)

Greetings in the morning. My name is Yalelet Yihunew. I am an MSc student at Addis Ababa University College of health sciences and department of medical laboratory sciences, specializing in diagnostic and public health microbiology. I'll be conducting research on biofilm formation and antibiotic resistance in catheters associated linked to urinary tract infection at Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia. This is to let you know that you are cordially invited to take part in the research. It is critical that you participate in this study voluntarily. Before proceeding, please read the following points carefully.

Project title**:** The magnitude of catheter-associated urinary tract infection, biofilm producing, and antibiotic resistance patterns from patients visited at Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia.

Principal investigator:-Yalelet Yihunew (BSc, MSc candidate)

Purpose**:** We intend to perform a study to asses' bacterial pathogen biofilm production and catheter infection associated with urinary tract infection among catheter patients, as well as their antibiotic susceptibility patterns. at Yekatit 12 hospital medical college, Addis Ababa, Ethiopia. It's critical to understand the types of organisms, their antimicrobial susceptibility patterns, and related risk factors for UTI among catheter patients. Finally, the findings of the study are useful in the treatment of urinary tract infections and biofilm production bacteria, as well as to indicate best control ways.

Procedure**:**

You will be asked to complete an interview, undergo a physical examination, and give a urine sample fo r laboratory culture as part of this study, all of which are completely voluntary.

Risk of Association**:** There is minimal known risk associated with participating in this study

Benefit**:** Your doctor will be notified if a positive result is discovered on a laboratory culture media, such as bacterial growth or antibiotic resistance, so that they can handle the situation appropriately.

Confidentiality: Any information about you that is given during the interview for the purpose of study will be kept confidential and secure. Thank you very much!

The investigators: name is Yalelet Yihunew. Signature ----------date ----------

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Annex I: Information sheet (Amharic version)

በጥናቱ ለሚሳተፉ ግለሰቦች የመረጃ መጠየቂያ እና መቀበያ ፎርም ጤና ይስጥልኝ እንደምን አደርክህ/ሽ። ያለለት ይሁነዉ እባላለሁ፡፡ በአ.አ.ዩ. ጤና ሳይንስ ኮለጅ ፐፕሊክ እና ዲያግኖሰቲክ የማክሮባሎጂ የማስተርስ ድግሪ ተማሪ ነኝ፡፡ በአሁኑ የሽንት ቱቦ ህመም እና ሽንት መሽናት የማችሉ ሰዎችን የሽንት መሽኒያ የሚሆን መሳሪያ መጠቀም የሚፈጥረዉን ጉዳት ታካሚዎች ላይ ጥናት እያካሄድኩ ነው ፡የሽንት ቱቦ ህመም እና ሽንት መሽናት የማይችሉ ሰዎችን የሽንት መሽኒያ የሚሆን መሳሪያ መጠቀም የሚፈጥረዉን ኢንፌክሽን አምቸጪ ባክቲሪያ በህሙማን ላይ የተለያዩ ችግሮች ሲያመጡ ይታያል፡፡ ይህ ጥናት በየካቲት 12 ሆስፒታል መዲካል እየታከሙ ባሉ ህሙማን እያካሄድኩ በመሆኑም የሽንት ቱቦ ኢንፌክሽን ምልክት የሚያሳዩትን እና የማያሳዩትን በየትኛዉ ባክቲሪያ እንደተጠቁ መለየት እና ባክቲሪያዉ በየትኛዉ መድሀኒት ሊጠፉ እንደሚችል የሚያመለክት ሲሆን ይህ ደግሞ ሃኪሙን ህሙማንን በትክክል ለማከም የሚያግዝ ሲሆን በተጨማሪም ተያያዥነት ያላቸዉን ችግሮች ለማወቅ እና የመፍትሔ ዕርምጃ እንዲወሰድ ለማመልከትም ይረዳል፡፡

የጥናቱ ኣላማ ፡ የጥናቱ ዋናአላማ ፡የሽንት ቱቦ ህመም እና ሽንት መሽናት የማይችሉ ሰዎችን የሽንት መሽኒያ የሚሆን መሳሪያ መጠቀም የሚፈጥረዉን ታካሚዎች ምን ያህል እንደሆነ ለማውቅ ነው፡፡ እርስዎ በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ 15-30 ሚ.ሊ ወይም ግማሽ የቡና ስኒ የሚሆን የሽንት ናሙና ይሰጣሉ፡፡ በተጨማሪም ኣንድኣንድ ዉጤትዎን ከህክምና ካርድዎት ላይ እንወስዳለን፡፡

ስለ እኔ የሚያዙ መረጃዎች በሚስጥር ይጠበቃል? የሚሰጡት መረጃ ሚስጥራዊነቱ የተጠበቀ ነው፡፡ በስም አይጻፉም፤ የዚህ ኮድ መፍቻ በፋይሌ ተቆሌፎ የሚቀመጥ ሲሆን የተፈቀደሆት ሰው ብቻ ፋይለን ማየት ይችላል፡፡ ከዚህ ጥናት በሚወጡ ዘገባዎች ወይንም የህትመት ውጤቶች ላይ ስም ወይም ሌላ የእርስዎን ማንነት የሚገሌጽ መረጃ አይኖርም፡፡ ከምርመራ የሚገኘውም ውጤት ወይም ሌላ መረጃ ለሚመለከታቸው አካለት ለምሳሌ፤ እርስዎን የሚንከባከቡ የህክምና ባለሙያዎች እና ጥናቱን ለሚያካሄዱት ባለሙያዎች እንዲሁም ጥናቱ ስነምግባርን ጠብቆ ይከናወናል፤ዉጤቱ ተጨማሪ ምርመራ የሚያስፈልገዉ ከሆነ እና ህክምና ካሰፈለገው ሀኪሙ ዉጤቱ ይሰጠዋል፡፡

በጥናቱ መሳተፍ ምን ጥቅም ይኖረዋል? በጥናቱ በመሳተፈዎ ምንም አይነት ክፍያ አይጠየቁም ወይም የሚያገኙት ገንዘብ አይኖርም ነገር ግን የሽንት ቱቦ ኢንፌክሽን ህመም ካለቦት ወይም የምርመራ ውጤቱ ህክምና የሚያስፈልገው ከሆነ ተጨማሪ ምርመራ እና ህክምና እንዲያገኙ የረድተውታል፡፡በተጨማሪም ከጥናቱ በሚገኘው እውቀት ለሽንት መሽኒያ ተብሎ የተደርገዉ መሳሪያ እና በሽንት ቱቦ ኢንፌክሽን ባክቴሪያ አማካኝነት የሚመጣውን በሽታ በተሻለ ደረጃ ለመቆጣጠርና

ለበሽታው ትክክለኛውን ፀረ ባክቴሪያ ለመምረጥ ለሀኪሞች ይረዳቸዋል።በጥናቱ መሳተፍ እሚያስከፍለዉ ክፍያ ይኖራል? ሁለንም ዓይነት ለጥናቱ የሚያስፈልጉ ምርመራዎች በነፃ የሚሰሩ ሲሆን የህክምናና/የሆስፒታሉ ወጪዎች በሆስፒታል አሰራር መሰረት ምንም ክፍያ አይኖረዉም።ስለማበረታቻ (ማካካሻ)፡ በዚህ የዳሰሳ ጥናት ውስጥ ስለተሳተፉ ወይም እንዲሳተፉ ለማድረግ ምንም ዓይነት ማካካሻ ወይም ማበረታቻ አይሰጥም።በጥናቱ በመሳተፍ ፈቃደኛ አለመሆን ወይም መሳተፍ ከጀመሩ በኋላ ራስን የማግለል መብት: በጥናቱ የሚሳተፉት ፈቃደኛ ከሆኑ ብቻ ነው።ስዚህ መሳተፍ አለመሳተፍ ከጀመሩ በኃላ ማቋረጥ ወይም መመለስ የማይፈልጉት ጥያቄ ከሆነ ይለፈኝ ማለት ሙሉ መብትዎ ነው። በጥናቱ መሳተፍ ወይም አለመሳተፍ አገልግሎት ላይ ምንም አይነት ጥቅምም ሆነ ጉዳት አይኖረውም። ይህንን ጥናት አስመልክቶ ጥያቄ ካለዎት። ወይም የጥናቱ የመጨረሻ ዉጤት ምን እንደሆነ ለማዉቅ ከፈለጉ በሚከተለዉ አድራሻ ሉያገኙን ይችለሉ።

ጊዜወትን መሰዋት አድርገው ስለተባበሩኝ ከልብ አመሰግናለሁ

ያለለት ይሁነዉ

ቁጥር: 0921264295 /ወይም ኢ-ሚይል-yaleletyihunew@gmail.com

Annex III: consent form (English version)

I confirm that I have discussed the nature, purpose, potential benefits, and potential hazards of participating in this research proposal with the aforementioned individual, and that any queries regarding this information will be answered. After receiving an explanation of the study's nature and aim, the procedures, and the potential benefits and hazards of participation in the study, By signing below, I, the undersigned, acknowledge that I have voluntarily accepted to engage in the study and provide consent to participate in the study with a clear awareness of the study's aims and conditions.

Participate name ----------participate signature----------date----------

Researcher name ----------signature ----------date ----------

በዚህ ምርምር ውስጥ ከመሳተፍ በፊት ከጥናቱ ጋር የተያያዘው ለምሳሌ ስለ ጥናቱ ተፈጥሮ፣ዓላማ ፣ጥቅሞች፣ ሌሎችን እና ሊያስከትሉ የሚችሉ አደጋዎች ከላይ በተጠቀሰው ግለሰብ የተብራራልኝ ሲሆን ስለዚህ መረጃ ለሚነሱ ማንኛውም ጥያቄዎች መልስ ይሰጣቸዋል። በመሆኑም እኔ በፊርማዬ በመታገዝ በጥናቱ ውስጥ ለመሳተፍ በፈቃደኝነት መስማማቴን እና የጥናቱን አላማ እና ሁኔታ በግልፅ በመረዳት በጥናቱ ላይ ለመሳተፍ ፈቃድ ሰጥቻለሁ።ስለዚ እኔ /ተማሪ/አቶ/ወ.ሮ/ወ.ት/ዶር_____የተባልኩ በሽታ አምጪ የሆኑትና ባክቴሪያ የተባሉትን ረቂቅ ህዋሳት ለመመርመር በሚረዳው ምርምር፣ ለምርምሩ የሚያስፈልጉ መጠይቆችን ፣መረጃና የሽንት ናሙና ለመስጠት በሚገባኝ ቋንቋ የተብራራልኝ በመሆኑ በጥናቱ ለመሳተፍ በሙሉ ፍቃዴ የተስማማሁ መሆኔን በፊርማዬ አረጋግጣለሁ።

የተሳታፊዋ ስም_____ የተሳታፊዋ ፊርማ _____ ቀን_____

የተመራማሪው ስም_____ የተመራማሪው ፊርማ_____ ቀን_____

Annex IV. Questionnaires: general instruction

During the study period, these questions will be filled out by the researcher or a health care professional at the Yekatit 12 hospital while the sample is being collected from catheter patients. Please note that you do not need to write your name. Coding will be used to carry out the procedure**.**

- ✓ Please put an "X" or "√ "in the space provided.

Part 1: Question about socio-demographic characteristics

Registered hospital number (or unique code)

Roll No	Characteristic	Alternative
1	Age (year)	1.1-17 2. 18-34 3.35-49 4.50-64 5. Above 64
2	Gender	1. Male 2. Female
3	Residence	1. Urban 2. Rural
4	Religion	1. Orthodox Christianity 2. Muslim,3. Protestant Christianity 4.other other ----------
5	Occupation status	1. House wife, 2.merchant, 3. Government employee 4. other ---
6	Marital status	1. Married 2.single 3.divorced 4. widowed 5. widower
7	Educational level	1. Illiterate 2.Student 3.Diploma 4. Degree and above
8	Income level(EBR)	1. 1000-5000 2.>5000

Part 2: Primary diagnosis upon admission**.**

1	Characteristic	Alternatives
2	Urogenital abnormality	1.yes 2.no
3	Malignancy	1.Yes 2.No
4	Leg or head injury	1.Yes 2.No
5	Appendicitis or bowel obstruction	1.Yes 2.No
3.	Presence of underlying illness	1.Yes 2.No
1	*Diabetes status*	*1.yes2.yes*
2	*Hypertension (hpn)*	*1.Yes 2.No*
3	*Chronic heart failure*	*1.yes 2.no*
4	*Neurological disease*	*1.Yes 2.No*

5	*bladder stone*	*1.Yes 2.No*
6	*Chronic kidney disease(CKD)*	*1.yes 2.No*
7	*Autoimmune disease*	*1.Yes 2.No*
8	*Benign prostatic hyperplasia*	*1.Yes 2.No*
9	*Antimicrobial drug received*	*1.Yes 2.No*
4. Reason catheterization.		1.Yes 2.No
1	Pre- or postoperative drainage	1.Yes 2.No
2	Urine output measurement	1.Yes 2.No
3	Incontinence	1.Yes 2.No
4	Urinary retention	1.Yes 2.No
5	Keeping patient dry	1.yes 2.no
6	*Duration of catheterization/day*	*1.<4 days 2.4–6 days 3.≥7 days*
7	*Length of hospital stay/day*	*1. <10 days 2. ≥10 days*
	Source of admission	1.Emergency 2.Medical 3.Surgical 4.Gynaecology and obstetrics 5.Operation room 6.burn
	Cause of hospitalization	1.Certain infectious diseases and parasite diseases 2.Endocrine ,nutritional and metabolic diseases 3.Injury ,poison, external causes ,4.Disease of the circulatory system ,5.Diseases of the respiratory system 6.Diseases of the digestive system ,7.Diseases of the genitourinary system,8.Disease of the blood and blood–forming organ,9.Cancer, nervous system, other diseases
	Indication for catheterization	1.Yes 2.No
	History of previous catheterization	1.yes 2.no
	History of previous uti	1.yes 2.no
	Is the patient is asymptomatic	1.yes 2.no
	Is the patient symptomatic	1.yes 2.no
If you answered yes, choose a symptom from list below.		
	Fever	1.yes 2.no

	Dysuria	1.yes 2.no
	Urgency	1.yes 2.no
	pelvic pain	1.yes 2.no
	Frequency	1.yes 2.no
	blood in the urine	1.yes 2.no
	burring with urination	1.yes 2.no
	Lower belly discomfort	1.yes 2.no
	Nausea.	1.yes 2.no
	Vomiting	1.yes 2.no
	shaking and chill	1.yes 2.no
	Back or side pain	1.yes 2.no
	Flank pain	1.yes 2.no
	Supra pubic pain	1.yes 2.no
	Others ____ ____Specify	

Biofilm formation testing protocol

1. Growing a Biofilm

1. Inoculate minimum of colonies isolated from fresh agar plates in sterile trypticase soy broth
2. Incubate the inoculated trypticase soy broth at 37o C for 24 h.
3. Dilute the overnight culture 1:100 into fresh medium for biofilm assays.
4. Add 200 µL of the dilution per well in a 96 wells of flat bottom microtiter plate.
5. Incubate the microtiter plate for 48hrs at 37°C.

2**. Staining the Biofilm**

1. After incubation, the microtiter plate content of each well will be removed by tapping the bottom plates using micropipete.
2. Wash the wells with 0.2 mL of phosphate buffer saline (pH 7.3) four times. This step helps remove unattached cells and media components that can be stained in the next step, and significantly lowers background staining.
3. Fix adherent organisms forming-biofilms in the microtiter plate with 99% methanol.

4. Add 220 µL of a 0.1% solution of crystal violet into each well to stain the biofilm formed on the surface of microtiter plate.

5. Incubate the microtiter plate at room temperature for 10-15 min.

6. Wash the plate 3-4 times with phosphate buffer saline

7. Turn the microtiter plate upside down and dry for a few hours or overnight.

3. Quantifying the Biofilm

1. Add 220µL of decoloring solution (95% ethanol alcohol) into each well of the microtiter plate to solubilize the CV.

2. Incubate the microtiter plate at room temperature for 10-15 minute. Don't allow to stay more time to prevent ethanol evaporation.

3. Transfer 200µL of the solubilized CV to a new flat bottomed microtiter plate.

4. Briefly mix the content of each well by blowing up and down using pipet.

5. Measure the absorption at 570nm to quantify the biofilm formed.

6. Make microtiter plate incubated with sterile TSB as negative control.

NB: For each isolate biofilm formation tests were carried out in triplicate and the results were averaged.

Annex V: Laboratory standard operating procedures

(A). Urine culture

1. Principle

Urinary catheter infection associated with infections of the urinary tract (UTI) is one of the most prevalent infectious disorders. The majority of the workload in the clinical microbiology laboratory is urine cultures .UTIs can affect people of all ages, from infancy to old age, and can affect those who are generally healthy as well as those who are immune compromised or debilitated. Most clinicians believe bacteriuria to be a reliable indicator of a urinary tract infection. For proper specimen collection, the patient should be given precise instructions

2. Specimen

Catheterization (indwelling) Straight catheters, suprapubic aspiration, and other invasive methods were used to collect urine samples. If there is a delay in transporting the specimen to the

Laboratory, it should be kept chilled. The following specimens should not be processed:

1) specimens not refrigerated or preserved for more than two hours; 2) Foley catheter tips; 3) urine fr om a catheterized patient's bag; and 4) duplicate specimens obtained on the same day.

3. Materials

A. appropriate medial/ Blood agar and MacConkey agar are examples of media.

b. Sterile inoculating loop (1 liter for 0.001 ml, 10 liters for 0.01 ml)

c. Reagents, kits, and susceptibility testing disks for bacterial identification**.**

4. Quality Control (QC)

Process the specimen as quickly as possible after receiving it. Place the specimen in the refrigerator if th ere is a delay in processing. Check that the patient's name and identifiers on the specimen correspond to those on the request. Ensure that all media and supplies used have undergone the necessary quality assurance checks and are used before their expiration dates.

5. Safety Precautions

When processing these specimens, standard safety procedures for handling patient specimens must be followed.

6. Procedure

For media injection and incubation, use a sterile loop that is calibrated to provide 0.001 ml. thoroughly combine the urine. While holding the loop vertically, place it slightly below the surface of the urine. Put the meat loop on the serving dish. Draw a straight line through the middle of the plate, and then make several 90 degree passes with the urine through the inoculum. Use a calibrated loop to provide 0.01 ml for the straight catheter, suprapubic, specimens. Incubate overnight at 35 o C to 2 o C. The plates should be incubated for 16 to 24 hours before being examined. Following laboratory protocol, isolate and identify potential pathogens using conventional testing. The right organisms should be used for antimicrobial susceptibility testing (AST). If there is no growth after 24 hours, report "No growth after."

For the following, incubate the plates for an additional day and a half: 1) The specimen was acquired via an invasive technique; 2) There are only a few tiny or hazy colonies present.

7. Interpretation

Ascertainthecolonycountforeach morphotype of an organism. 1,000 CFU/ml per colony in a loop of 0.00 1 ml (1 l). Data is interpreted using the collection method and clinical state:
Report a potential pathogen's growth if it reaches 100,000 colony forming units (CFU)/ml in an indwelli ng catheter specimen.Straight catheterization specimens reveal up to 100 CFU/ml of proliferation of any number of potential pathogen species.For samples taken after surgery or bladder aspiration, report grow th or any colony count of potential pathogens for all patient types.For all patients and specimen types, any yeast isolate should be recorded. Gram negative bacteria like E. coli, Proteus mirabilis, Klebsiella pneumonia, and Pseudomonas aeruginosa are responsible for the majority of UTIs. The most prevalent Gram positive cocci are Enterococcus species, Staphylococcus saprophytic .Although Staphylococcus aureus is a rare cause of urinary tract infections, they usually co-occur with urinary catheterization or S. aureus bacteraemia.

8. Reporting

a. Positive Cultures: Report the pathogen's colony count (CFU/ml), detailed identity, and AST (s) one example is E. coli with more than 100,000 CFU/ml.
b. If you have a mixture of cultures, report it as Mixed Flora or Contaminant.
"Mixed growth of gram-positive and gram-negative organisms greater than 100,000 CFU/ml." The outcome of the culture suggests contamination. Kindly provide a new specimen

(B). Antimicrobial Susceptibility Test: Kirby Bauer Method

1. Principle

The standard technique for determining the in-vitro susceptibility of aerobic non fastidious organisms is described in this procedure. Only pathogens for which well-standardized procedures are available and pathogens for which resistance is known or considered to be a clinical problem should be tested with antimicrobial susceptibility testing (AST). Normal flora and colonizing organisms should not be

subjected to AST. Kirby-Bauer (KB) is a method for performing AST by disk diffusion that is standardized. The bacteria are swabbed onto the surface of a Mueller Hinton agar (MHA) plate in a uniform inoculum. On the agar, antimicrobial-impregnated filter paper disks are inserted. The diameter of the zone of inhibition around each disk is measured after an overnight incubation period. A qualitative analysis can be performed by referring to CLSI's standardized.

2. Material

McFarland Standard of 0.50d for MHA, TSB, and NSS (Normal Saline Solution) (NSS) antibacterial discs, Ruler or calliper

3. Specimen

An 18–24 hour agar plate is used for the pure cultivation of the organisms, preferably using a non-selective medium like blood agar or Maconky agar.

4. Quality control

By incubating 5% of sheep blood overnight at 35–37 oC without specimen inoculation, the sterility of the blood will be examined on Blood Agar, MacConkey Agar, and MHA as a quality control. Known S. aureus (positive control) and S. pyogenes will be used to test the efficiency of the catalase reagent (hydrogen peroxide) (negative control). S. aureus, gram-positive bacteria, and E. coli, gram-negative bacteria will be utilized as quality control for gram-staining reagents. Any physical alterations, such as cracks, excessive moisture, color, haemolysis, dehydration, and so on, will be documented prior to the use of any reagents or culture media. The level of contamination and the expiration date will both be determined. The temperatures of the incubator and refrigerator will be monitored daily. P. aeruginosa (ATCC 25923), S. aureus (ATCC 25923), and E. coli (ATCC 25922)

.5. Procedure

A .agar plates and antibiotic disks should be used at room temperate.

B. creates a bacterial suspension.

The easiest way to prepare an inoculum is through direct colony suspension. Most creatures can be studied using this technique. Choose 3–5 colonies with the same morphologic types that are well-isolated from an agar plate culture. Transfer the growth into a tube holding 4-5 mL of TSB or NSS by lightly touching the top of each colony with a loop. Mix thoroughly and bring turbidity to the 0.5 McFarland level using broth or NSS.

When it is impossible to directly suspend colony growth and a smooth suspension cannot be achieved, the growth method can be utilized instead and is occasionally preferred. When fresh 24 hour colonies are not available, it can be utilized for non-fastidious organisms (with the exception of Staphylococci). Choose 3–5 colonies with the same morphologic type that is well-isolated from an agar plate culture. Each colony's top should be touched with a loop before the growth is transferred into a tube containing 4-5 mL of TSB. The broth culture should be incubated at 35 °C for two to six hours, or until the turbidity of the 0.5 McFarland standards is met or exceeded. Mix thoroughly and use TSB to get turbidity up to the 0.5 McFarland level.

c. The plate should be inoculated with a bacterial suspension.

1. After 15 minutes of turbidity adjustment, remove any extra inoculum by rotating a sterile cotton-tipped applicator swab against the tube wall.

2. Three times, wipe the whole surface of the agar plate, turning the plate by about 60 degrees in between swabs to ensure uniform distribution. Swab the rim of the agar as a last step.

3. Before applying disks, let the contaminated plate stand for 3 to 15 minutes (but no more than 15 minutes).

D. Using sterile forceps or dispensers, apply antibiotic disks to the agar surface.

To achieve complete contact of the disk with the agar, gently press down.

Once a disk has come into contact with an agar surface, it cannot be moved. Instead, put a fresh disk in a different spot on the agar.

Neither the 150 mm plate nor the 100 mm plate should include more than 12 or five disks, respectively.

The working stock of antibiotic disks should be kept in a tightly-capped container with desiccant in a refrigerator (2 to 8 o C). The container holding the cartridges needs to be left unopened at room temperature for roughly an hour after the disks are taken out of the fridge to allow the temperature to stabilize. By doing this, the quantity of condensation on the disks is reduced. When using a disk

dispenser, make sure that it has. 15 minutes after applying the disk, invert the plate and begin the incubation process. Incubate in an incubator with ambient air for 16–18 hours at 35 °C.

6**. Reading and interpretation**

a. Only if the growth grass is confluent should plates be read. Individual colonies will indicate a too-light inoculum and necessitate repeating the test.

A.Hold the plate inverted a few inches over a dark, non-reflective surface. Plate with reflected light illuminated.

B .Measure the diameter of the zone of inhibition using a ruler that is resting on the plate's back..

c. Calculate the disk's diameter as well as the diameter of the zones of total inhibition as determined by unaided vision. Ignore the slight growth of microscopic colonies at the edge of the inhibited growth that can only be seen with a magnifying glass.

d. Measure the zones to the nearest millimetre.

e. The zone sizes are explained in the CLSI M100 tables.

Result reporting

An Assessment Worksheet for Culture

Patients ID:	Specimen type:	Receive:
Bacterial culture evaluations and observations		
	Observation and work up	Performed by
Date ---------/----------/----------		
The final report		

Patients ID:	Specimen type:	Receive:
Bacterial culture evaluations and observations		
Date	Observation and work up	Performed by

Date --/--/		
The final report		

AST Result report

Indicate if the organisms are susceptible (S), intermediate or resistant (R) to the tested antimicrobial treatments**.**

Bacteria isolate	Antimicrobial susceptibility testing				
	Pattern	Sensitive	Intermediate	Resistance	Remark
1	Amikacin(AK)30 µg				
2	Ampicillin (AMP) 10µg				
3	ceftaroline				
4	Amoxicillin clavulanic acid(AMC) 20µg				
5	Trimethoprim-sulfamethoxazole				
6	cephalexin				
7	ciprofloxacin (CIP)5 µg),				
8	Gentamycin (CN) 10µg				
9	Ceftriaxone (CTR)30 µg				
10	Meropenem(MER) 10 µg				
11	Ceftazidime (CAZ) 30µg,				
14	Vancomycin				
15	Tobramycin (TOB) 10 µg				
16	Cefazoline				
17	cefepime (CFM) 30 µg				
18	cefuroxime 30 µg				
19	Cefotetan 30 µg				
20	Penicillin G				
21	tetracycline (TET) 30 µg,				

22	Cefoxitin (FOX) 30µg				
23	cefotaxime 30µg,				
24					
25					

Annex VI. Declaration

I, the undersigned, declare that this M.Sc. research is my original work, has not been presented for a degree at this or any other university, and that all sources of materials used for the research have been properly acknowledged.

M.Sc. candidate:

Yalelet Yihunew (B.Sc.)

Signature: _____

Date of submission: _____

This research was submitted with our approval as advisors.

Advisor: - Kassu Desta (M.Sc., Associated professor)

Signature:_____

Date _____

Advisor: Gebereabe (M.Sc., PhD candidate)

Signature:_____

Date:_____

Place: Addis Ababa, Ethiopia.

Advisor: Asegedech (B.Sc., M.Sc.)

Signature: _____

Date:_____

Place: Addis Ababa, Ethiopia.