ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Bacterial and Fungal profile, antibacterial drug susceptibility pattern, and associated factors of isolates recovered from kidney transplant recipients' urine samples at Saint Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia.

By: Lemlem Tesfaye (BSc.)

Advisor: Adane Bitew (MSc, PhD)

Collaborator: Mr. Elias Seyoum (BSc, MSc)

Mr. Dejenie Shiferaw (MSc, PhD candidates)

A research thesis submitted to the Department of Medical Laboratory Sciences College of Health Science Addis Ababa University in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology)

September, 2021

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Lemlem Tesfaye, entitled: Bacterial and Fungal profile, antibacterial drug susceptibility pattern, and associated factors of isolates recovered from kidney transplant recipients' urine samples at Saint Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner_____ Signature _____ Date _____

Internal Examiner_____ Signature _____ Date _____

Advisor_____ Signature _____ Date _____

_____

Chairman of the Department or Graduate Program

Acknowledgements

My acknowledgement first goes to almighty GOD for his endless guidance in my life.

My special gratitude goes to my family who were always on my side in every aspect of my life. I also would like to thank all my friends who had been on side during the development of proposal to the final thesis report.

I would like to express my heart full gratitude to my advisor Dr. Adane Bitew for their constructive suggestion and comments to the end of this thesis. My sincere thanks also go to Mr Elias Seyoum who has provided me valuable information to prepare this study. My sincere thanks also go to Mr. Dejenie Shiferaw for assisting me in all aspects of data analysis.

I am grateful to all staffs of SPHMMC kidney transplant center laboratory technologists, nurses. I am very grateful thanks to the Ethiopian Public Health Institute, Clinical Bacteriology and Mycology Reference Laboratory staffs for their encouragement, supporting in technical aspect and allowed me to use available facilities.

I would like to extend my appreciation to all study participants who volunteered to participate in the study.

Finally, I would like to thank Medical Laboratory Sciences, College of Health Science, Addis Ababa University, for giving me the opportunity chance to do this research thesis.

Table of Contents

List of table

List of figures

LIST OF ABBREVIATIONS

AAU----------Addis Ababa University

AOR----------Adjusted Odds Ratio

AST----------Antibiotic Susceptibility Test

ATCC ----------American Type Culture Collection

CFU----------Colony Forming Units

CKD----------Chronic Kidney Disease

CLSI----------Clinical and Laboratory Standards Institute

CONS----------Coagulase Negative Staphylococcus

COR----------Crude Odds Ratio

EPHI ----------Ethiopian Public Health Institute

ESRD----------End-Stage Renal Disease

GFR----------Glomerular Filtration Rate

IFI----------Invasive fungal infections

MDR----------Multidrug resistance

MHA----------Muller Hinton Agar

RRT----------Renal Replacement Therapy
RTx. ----------Renal Transplantation
SPHMMC----------Saint Paul Hospital Millennium Medical College
UTIs----------Urinary Tract Infections
VUR----------Vesico Urethral Reflux

Abstract

Background*:* Urinary tract infections (UTIs) are the most infections in renal transplant recipients and it has serious consequences in several countries including Ethiopia.

Objective*:* To determine the bacterial and fungal profile, antibacterial drug susceptibility patterns and risk factors for urinary tract infections caused by bacteria and fungal etiological agents from urine of kidney transplanted patients at SPHMMC Addis Ababa, Ethiopia. **Method**: A hospital-based cross-sectional study was conducted from November 2020 to April 2021. A structured questionnaire was used to collect data on socio-demographic factors and clinical conditions. Blood, MacConkey, and brain heart infusion agar were used to culture all urine samples. Gram stain, biochemical tests, and chromagar for yeast were used to characterize all culture positives were done according to the standard technique. All bacterial isolates were tested for antibiotic susceptibility using the Kirby-Bauer method. The possible risk factors were assessed using simple and multivariate logistic regressions.

Results*:* 63 pathogenic microorganisms were found in 207 clinical specimens. Gram negative bacteria accounted for 63.5 %, Gram positive bacteria for 23.8% and fungal isolates represented 12.69 %. The most common bacteria identified in urinary tract infections (UTIs) were *E. coli*, *Klebsiella pneumoniae*, and *Coagulase negative staphylococcus*. *Candidia krusei and Candidia albicans* was among fungal isolates. Multidrug resistances were observed among 82% of Gram-negative bacteria. The highest rates of antimicrobial resistance were observed in Aminoglycosides and 3rd generation cephalosporins. Carbapenem, Nitrofurantoin, Nalidixic acid, Amikacin and Fluoroquinolones were the most effective antibiotic to treat UTIs.

Conclusion: *Candidia albicans* and *Candidia krusie* funguria were shown to be prevalent UTIs in people who had their kidneys transplanted, according to the study. Bacterial isolates showed resistance to commonly used antibiotics. These data are essential for identifying future potential prevention strategies against UTI in kidney transplant recipients, as well as the causal agent, related factor and antibiotic resistance pattern.

Key words: Urinary tract infection, Bacterial and fungal profile, Antibacterial susceptibility patterns, Risk factor Addis Ababa, Ethiopia.

1. Introduction

1.1 Background

Urinary tract infections (UTIs) are commonest infection characterized by the presence of clinically associated bacteria or fungi in the urine. These infections can be lower tract infections that occur within the bladder, urethra, prostate gland and epididymis or upper tract infections such as Pyelonephritis and these infections may affect several organs. Most fungal species have been commonly originates from the gastrointestinal tract and primarily affect the bladder and kidneys. These infections usually occur mainly in patient at high risk, immunosuppressive therapy and those immune-compromised hosts because of post kidney transplantation. Since, urinary tract infection (UTI) that occurs in post renal transplantation recipients remains a main cause of morbidity and mortality in renal transplant recipients. Therefore, early detection and appropriate treatment is both the role of physician and clinical microbiology laboratories [1-3].

Post-renal transplantation urinary tract infections are a common public health problem globally and in developing countries which continues to be a serious problem that needs immediate attention and treatments [4]. In Turkey, the mortality rate due to infection has ranged from 50 to 5% within last 20 years, it is considered as a severe risk factor for worse outcomes in kidney transplantation [5]. In adult renal transplant recipients, UTI are associated with a higher risk of both graft loss and patient death. Advanced age, female gender, pre-transplantation UTI, prolonged period of hemodialysis, diabetes mellitus, prolonged postoperative bladder catheterization, and immunosuppression, are the most risk factors. About 74% UTIs occur during the first year after kidney transplantation and about 81.9% occur, mostly within the first 3 months after surgery [6].

As many several studies highlighted, the most common pathogens isolated in Urinary tract infections among transplant recipients are *Escherichia coli Enterococcus spp.*, *Staphylococcus*, and *Klebsiella spp.* [7]. Microorganisms that are caused by fungi were regarded as relatively insignificant causes of infection. However, in recent studies, these microorganisms began to be recognized as important causes of disease related to the increased nosocomial cause of fungal UTIs in kidney transplant recipients.

Candida species are the most prevalent organisms among fungal urinary tract infections in persons who have undergone renal transplantation. Although these infections are commonly asymptomatic, they can disseminate infection, this cause pyelonephritis and attack other organs. Invasive fungal infections (IFI) constitute an important cause of morbidity and mortality among renal transplantation patients [8]. Invasive aspergillosis Cryptococcosis, non-Aspergillus molds, and endemic fungi. However, organisms vary with several factors such as type of healthcare facility, type of catheter used, immune status of the host, underlying complications, level of preventive undertaken and initial antimicrobial therapy [9].

The numerous risk factors that lead to UTIs following renal transplantation are host factors, anatomical, allograft, the widespread use of broad-spectrum antibiotics, underlying reason for kidney transplantation, pre transplant urological abnormalities, untreated or partially treated transplant UTIs, diabetes and hypertension. Among these factors diabetes mellitus strongly associated with UTIs that are caused by fungi [10].

A review of literatures clearly illustrates that the insufficiency of information from developing countries and periodic analysis of the results is coupled to roaring outcomes in urinary organ transplants. Furthermore, incidence of infections with nosocomial origin with multiple antibiotic resistances among transplant recipients has been emerging. Increased nosocomial bacterial resistance has emerged not only for Gram-positive bacteria but also for Gram-negative bacteria [10, 11].

Urine cultures are necessary for identification of UTIs and for antimicrobial susceptibility testing to guides the appropriate antibiotic for the treatment of UTI in renal transplant patients. Successful recovery of microorganisms from the urine by the laboratory depends on types of collection, collection container, specimen collection method, urine volume, and timing of urine cultures, use of supportive documents and report according to the recent guide lines [12].

Given, Ethiopia is one of the developing countries, the prevalence of UTI and the distribution bacterial and fungal etiological agents are poorly known. Although, this infections are usually asymptomatic and they can lead to pyelonephritis and disseminated infection among post transplant recipients. Against this back ground, the purpose of this study was to determine the

prevalence of UTIs, its causative agents, and bacterial antimicrobial resistance pattern isolated from renal transplant recipients in a transplant center in Ethiopia.

1.2. Statement of the Problem

Urinary tract infection (UTI) is the most important infection and major cause of morbidity and hospitalization in kidney transplanted patients. The infection significantly regarding given their association with poorer graft and patient survival, and conjointly accumulated hospitalization prices. The recurrent nature of the matter necessitates the utilization of multiple courses of antibiotic therapy, which aren't only costly but may result in microbial resistance and contribute to erratic levels of immunosuppression. Repeated infections can also cause graft inflammation and fibrosis [13].

Several recent studies have shown the conditions that predisposing an individual to UTIs vary with host and underlying diseases. Additional risk factors contributing to UTI post-transplant include immunosuppression and graft dysfunction or rejection [14]. Among the adult, UTI after transplantation has been associated with increased morbidity that appears to be related to the time of occurrence of the infection after transplantation [15].The use of antimicrobial had to be a major risk factor for *Candida* infection, probably because over growth of *Candida* species occurs in the absence of bacterial flora that compete for nutrients [16-18].

In Ethiopia, there is a limited data regarding UTIs in renal transplanted patients as well as bacterial and/or fungal pathogens. Our study differed from the previous study in that it included important bacteria and fungi that cause UTIs in kidney transplant recipients. Therefore, this research proposed to address the current status of fungal and bacterial profile, trend of antibacterial susceptibility pattern and associated factors of urinary tract infections among kidney transplanted recipients at SPHMMC.

1.3. Significance of the study

- Helps to update and understand the common microbial pathogens and their antimicrobial resistance status of kidney transplant recipient patients
- Provide physicians with current information on the level of antimicrobial resistance that helps them to choose suitable drugs for treatment.
- Will also help policy makers and concerned bodies to pay attention on the prevention and control of microbial infection among transplant recipient patients.
- Can serve as additional baseline data for further study.

2. Literature Review

2.1 Urinary Tract Infection (UTI)

Urinary tract infections (UTIs) are a common health concern which most Infectious disease physicians experience while dealing with renal transplant patients. In the studies of investigators reported in different countries, UTI is the most prevalence type of infection among transplant recipients, it is considered as the most important risk factor for weak graft function, mortality, and morbidity [20, 21].

According to a retrospective cohort study of 28,942 Medicare primary renal transplant recipients, the United States Renal Data System (USRDS) found that the cumulative incidence of UTI at the 3rd year was 60% for women and 47% for men. Most findings which were performed on UTI show that infection was more prevalent among females than males due to different factors such as pathogenicity and host factors [22].

Similar study conducted by Soemann and Horl in 2008 G.C, most of the UTIs (74%) occurred during the first year after kidney transplantation (81.9%), mostly within the first 3 months after surgery. The most common pathogens isolated in urine culture were *E. coli* 29%, *Enterococcus spp.* 24%, *Staphylococcus* 12% and *Klebsiella spp*. 10% [23]. Similar study in transplant center of Golestan Hospital, Ahvaz, Iran reported that *E. coli (43.53%)* and *Enterobacter spp*. (35.37%) as the major organism in renal transplant recipients [24]. In addition, in the study of Rivera-Sanchez in Mexico 2010 G.C, *E. coli* (31.5%), *Candida albicans* (21.0%) and *Enterococcus spp.* (10.5%) was identified [25].

According to the study by Barbouch in Saudi Arabia low prevalence of E. coli (18.4 %) and Klebsiella pneumonia (14.6 %) reported. [26]. Similar study conducted by Dantas, and Esezobor, *Enterobacter cloacae* and *Klebsiella spp*. was responsible for 30.4% and 40.0% of post-transplant UTIs, respectively [27, 28].

According to study by Seyyede at Mashada city in 2017G.C reported that, the most frequent isolated microorganisms from renal transplanted patients were *Escherichia coli* 55.3%,

coagulase-negative Staphylococcus, and *Klebsiella* spp. 26.6% and the high rate of candiduria (8.5%) of infection episodes occurred during the 1st month after transplantation [29].

According to study conducted by Shirazietal in 2005 G.C, In Iran showed Females are more affected than male the infection in females was 37% versus 32% in males with no observation of a significant variance between both sexes [30]. The risk of being female had a significant association with UTI in several studies, this may be due to anatomical differences in the urinary tract of males and females have been blamed for the higher incidence of UTIs in females [31].

The highest prevalence of UTI among renal transplant recipients was reported in Yemen (33.3%) [15].This result is nearly similar to that reported by Shirazietal in Iran with 33% [30]. Study conducted in Libya by Elkehili *et al;* showed high incidences of UTI among the kidney transplant recipients [32]. The high prevalence of UTI after kidney transplantation is because of the immunosuppressive drugs with the high dosage intake [33].

These results are in-line with previous reports. Several reports have found that *E. coli* is the most frequent causative pathogen of post-renal transplantation UTI. It has been suggested that the uropathogenic serotypes and adherence factors of *E. coli* contribute to allograft injury in KTRs [31].

Study's in Ethiopia at St. Paul hospital kidney transplant center, reported that the most prevalent bacteria isolates were Escherichia coli 2(18.2%), *Staphylococcus aureus* (18.2%), *Acinetobacter* spp. (18.2%), Enterococcus spp. (18.2%), Coagulase-negative Staphylococci (18.2%) followed by *Proteus mirabilis* 1(9.1%) [34]. Similarly to other researchers report, higher number of females was affected by post -renal transplantation UTI than males. This may be due to women are more susceptible to UTIs, which results from hormonal and immunological features [3, 28].

2.2. Predisposing factors for UTIs after KTx.

Kidney transplant recipients are more sensitive to infections caused by many risk factors. Various authors have suggested different potential UTI risk factors with UTI among KT patients, including female gender, old age, diabetes mellitus, immunosuppression, history of vesico urethral reflux (VUR), history of polycystic kidney disease and other risk factors related to the graft and the operation [25].

According to published studies, UTIs were found to be more frequent in a patient who was received deceased grafts compared with live grafts and female patients were more susceptible in general. Shorter urethra in females and relative proximity of the urethra to the perirectal area raise the risk of UTI compared to men [36].

Regarding the age of the recipient, several Authors have shown no correlation with UTIs. By contrast, several studies have identified older age as major risk factor for UTIs in transplant recipient. Kidney transplant in elderly subjects is often associated with a higher percentage of general infections than younger patients [37]

According to study conducted by Chuang report showed that 55% of the patient who were 65 years of age or older at kidney transplantation developed post-transplant UTIs compared to 30% of patients who were younger than 30 years [38]. Similar study conducted in Yemen UTI also increases among older patients (41–50years old) (28%) with significant association comparing with younger age groups [15]. Higher risk is attributed to impaired mobility, poor hygiene in institutionalized individuals, reduced native immunity, higher rate of urinary retention secondary to prostatism and bladder atrophy [39]

An ineffective cellular immunity and, probably, a lower tolerance to immunosuppression associated with comorbidities, such as diabetes, may contribute to the higher prevalence of bacterial infections found in older recipients.

Conceptual frame work

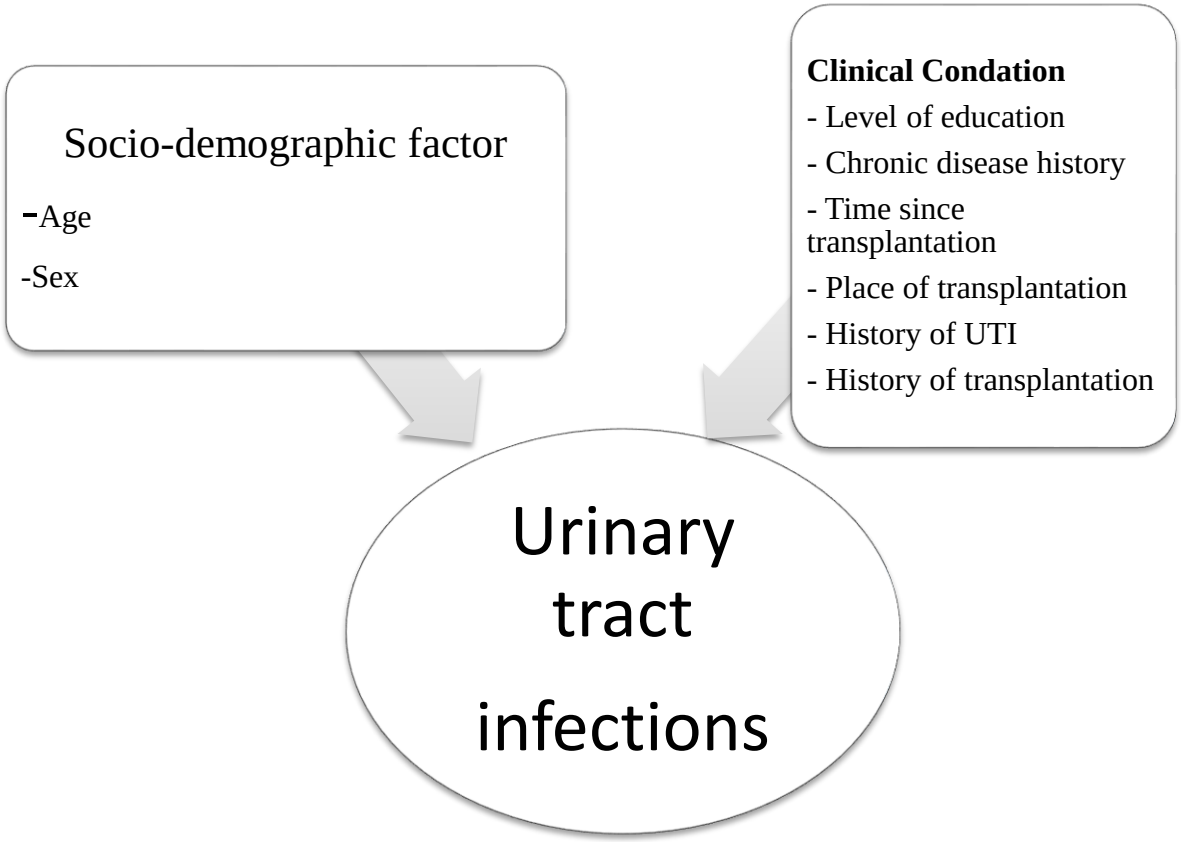

Figure 1- Conceptual frame work to show the relationship of various variables

3. Objectives

3.1. General Objective

- To determine Bacterial and Fungal profile, antibacterial drug susceptibility pattern, and associated factors of isolates recovered from kidney transplant recipients' urine samples at Saint Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia.

3.2. Specific objectives

- To determine bacterial and fungal profile among kidney transplanted recipients.
- To determine antimicrobial susceptibility patterns of the commonly isolated bacteria.
- To determine patient-specific risk factors associated with urinary tract infections in kidney transplant patients.

4. Materials and Methods

4.1.Study site, design and period

A cross-sectional was conducted at SPHMMC's kidney center from November 2020 to April 2021. SPHMMC is a one of the biggest referral teaching hospital, under the administration of Ministry of Health, located in Addis Ababa, Ethiopia. The hospital has 800 beds and give diagnostic and treatment services for about 370,000-400,000 patients per year. The kidney transplant center is the first and only living related kidney transplant program it was established in September 2015 in collaboration with the University of Michigan. Coordinated by the Ethiopian Federal Ministry of Health. In 2010, the Ethiopian government identified St. Paul's Hospital Millennium Medical College (SPHMMC) in the capital city, Addis Ababa as the future home of the transplant program. The kidney department gives service to new and follow-up patients and has 10 beds for kidney transplant candidate patients. Professionally, the unit has 2 nephrologists with palliative specialist, 5 general practitioners, 10 nurses and 4 laboratory technologists. SPHMMC offers the lowest cost for these services when compared to the private hospitals. The laboratory analysis was conducted on urine specimens in the department of clinical bacteriology and mycology reference laboratory at EPHI.

4.2. Population

4.2.1. Source Population

All kidney patients who were referred to SPHMMC kidney center for treatment.

4.2.2. Study subjects

All kidney recipients at kidney transplantation centers of SPHMMC who come to follow-up during the study period were included in the study.

4.3. Inclusion and Exclusion Criteria

4.3.1. Inclusion criteria

- Any kidney recipient and who were volunteering to give informed consent to participate in the study.

4.3.2. Exclusion Criteria`

- Patients with incomplete socio-demographic information.
- Patients who took UTI antibiotics for the last 48 hours.
- Duplicate samples from the same patients.

4.4. Study variables

4.4.1. Dependent variables

- Bacterial and fungal isolates.
- Antimicrobial susceptibility patterns.

4.4.2. The independent variables included

- Socio-demographics factor: age, sex, level of education
- Chronic disease history
- How long has it been since the transplant?
- Place of transplantation
- History of UTI
- History of transplantation

4.5. Measurement and Data collection

4.5.1. Sample size determination

Sample size was calculated using single population formula from prevalence value obtained from similar study conducted on bacterial profile and antimicrobial susceptibility pattern of urine culture isolates at SPHMMC. Using Fischer's formula with a maximum error of 5% (d) within a standard normal deviation of 1.96 for 95% confidence interval (CI) (Kiros T, etal.,2019).

Where, n- is the sample size,

$Z_{/2}$- for standard normal distribution at 95% confidence interval, is 1.96

p- Prevalence of microbial isolate 14.9% was taken to calculate the sample size.

d- Is margin of error assumed to be 5%.

$n = \frac{(Z_{/2})^2 * P(1-P)}{d^2} = \frac{(1.96)^2 * (0.149 * 0.851)}{(0.05)^2} = \frac{0.0025}{}$

= **195,** but 207 kidney transplant recipients were participated in our study.

4.5.2. Sampling method

Urine samples from study participants were collected using a convenient sampling technique.

4.6. Measurement and Data collection

4.6.1. Data collection procedure

Data were collected using pre structured questionnaire by the kidney transplant (OPD) nurses in order to gain information on socio-demographic factor and associated risk factors. The principal investigator took additional patient details from patient card. The questioner was prepared in English and translated to Amharic.

4.6.2. Laboratory analysis

4.6.2.1. Sample collection and transportation

A 10 ml of Clean-catch midstream urine sample was collected using sterile labeled urine cup. The study participants were given appropriate instruction before providing urine samples. All collected specimen was transported within 2 hours to EPHI, clinical and mycology reference laboratory by trained sample porter using the cold chain box. Contaminated, delayed and repeat specimens from the same patient were excluded from the study.

4.6.2.2. Culture and identification

All urine samples were inoculated onto Blood Agar base (Oxoid, Basingstoke, Hampaire, UK) to which 10% sheep blood, MacConkey agar plates (Oxoid Ltd, UK) and Brain Heart Infusion Agar plates using a calibrated loop with a capacity of 1 µl (0.001 mL) in safety cabinet. All inoculated plates were incubated at 37 °C for 18–24 hr aerobically and inspected for the growth of bacteria and/or yeasts. All positive blood cultures were identified using their culture characteristics appearance on their respective media and Gram staining reaction. Bacterial isolates were confirmed by the pattern of standard biochemical reactions. Gram negative bacteria were identified by oxidase test, indole production, citrate utilization, motility, urease, oxidase, gas production, hydrogen sulfide production, carbohydrate fermentation, and lysine decarboxylases production tests. Yeasts were identified by colony morphology on brain heart infusion medium and gram stain. The CHROMagar Candida (bioM´erieux, France) was used to identify the species. To inoculate stored Candida species, we used HiCHrome Candida differential agar and incubated at 25-30°C for 48 hours. The species were identified based on the color of the colonies that were produced. *C. albicans* colonies are green, and *C.krusei* colonies are purple.

4.7.2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test was carried out by Kirby Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI) guidelines (30th edition). We were Muller Hinton agar (Oxoid, Basingstoke, England). Three to five pure colonies was picked from original culture plates and mix with test tube containing saline until it become equivalent to McFarland 0.5standards. Then the bacterial suspension was streaked in the medium by using cotton swap. After the inoculum is dried (3 to 5min) disks were placed on the agar using sterile forceps. Plates were incubated aerobic condition for 24hrs at 35^0C. After overnight incubation the zone diameter was measured with ruler undersurface of the petridish. The susceptibility and resistance was interpreted according to CLSI guidelines. Antibiotics used for disk diffusion test were Chloramphenicol (30µg), Cefotaxime (30µg), Cefazidime (30µg), Meropnem (10µg), Amoxicillin-Clavulanic acid (20/10 µg), Ceftraxone (30µg), Cefipime (30µg), Amikacin (30µg), Ciprofloxacin (5µg), Penicillin, Oxacllin (30µg) and Gentamycin (10µg). These antimicrobial agents were selected based on currently availability and frequently prescribed for the management of bacterial infections in Ethiopia [41].

4.8. Data Quality Assurance

The questionnaire was checked for its completeness and validity prior to the collection of data. The pretest of the questionnaire was performed before the beginning of the actual study to confirm the consistency of the study tool. The principal investigator checked data reliability and completeness throughout the data collection process. Pre-analytical, Analytical and post-analytical quality was assured throughout the process.

4.8.1. Quality control

All materials, equipment and procedures were properly monitored to ensure the quality of laboratory results. Pre-analytical, analytical and post analytical stages of quality assurance that are incorporated in standard operating procedures of the medical microbiology laboratory were strictly followed. The quality of culture media was tested for sterility and performance. Sterility of culture media was checked by incubating overnight at 35–37°C without specimen inoculation. Quality control was performed to check the quality of medium. Each new lots were quality controlled before use by testing of E. *coli* (ATCC 25922), S. *aureus* (ATCC 25923) and P. *aeruginosa* (ATCC 27853).To standardize the inoculums density of bacterial suspension 0.5 McFarland standards were used.

4.9. Data analysis and interpretation

Data was collected and analyzed for the frequency of different microbial isolates, antibacterial resistance pattern and associated risk factors. The findings were entered into EXCEL and analyzed using Statistical Product for Service Solution (SPSS) version 20. Tables and graphs were used to summarize clinical and socio-demographic characteristics.

Bivariate and multivariate logistic regression analyses were used to assess the risk factors of urinary tract infection. Odds ratio was calculated to assess degree of association between dependent and independent variables. P-value <0.05 was considered statistically significant for all cases.

4.10. Ethical consideration

The study was approved and ethical clearance was obtained from departmental research and ethical review committee (DRERC) of the medical laboratory sciences, school of allied health sciences, College of Health Sciences; Addis Ababa University(DERC committee's reference number: MLS/040/20). Permission letter also obtained from Institutional Review Board (IRB) of SPHMMC (reference number: PM23/233) and Addis Ababa Public health Research and Emergency Directorate for the study sites hospital to collect samples. The nature of the study was explained to the study participant. They were guaranteed to refuse to give sample when they have inconvenient during the study. All kidney recipient patients' data was used by giving them anonymous code. All significant finding was submmited to the responsible physician and treatments was given for the study participants. The information related with the patient result was kept confidential.

4.11. Dissemination of finding

The study result was disseminated to College of Health Sciences; Addis Ababa University, Federal Ministry of Health, SPHMMC, Addis Ababa public health research and emergency directorate, Ethiopian public health. The study will be presented on different workshop, meetings, stakeholders, conference and published on peer reviewed scientific journal.

Operational Definitions:

- UTI: Positive Urine culture $\geq 10^5$ CFU/Ml. and positive fungal culture
- **Asymptomatic: -** defined as isolation of microbial in quantitative counts $\geq 10^5$ CFU in a clean-catch voided urine specimen in the absence of any symptoms of lower or upper UTI or $<10^5$ CFU in patients treated with antibiotics or $\geq 10^3$ CFU in a single catheterized urine specimen, irrespective of the presence of leukocyturia.
- **Symptomatic: -** any symptom or sign
- **Multidrug Resistance** - a bacterium that is simultaneously resistant for three or more antimicrobials belonging to different antibiotic classes
- **History of UTI: -** is any history of infection pertaining to the urinary tract diagnosed by a physician.

5. Results

5.1.Socio-demographic characteristics

A total of 207 kidney transplanted recipients with UTIs were included in the study. Out of them, 121 (58.5%) were males and 86(41.5%) were females. The mean age of transplanted recipients participated in this study were 2.58±1.06 (SD) years. Socio-demographic characteristics and clinical diagnosis are summarized in table 5.1

Table1: Socio-demographic characteristics and clinical diagnosis of kidney transplanted recipients their culture results at SPHMMC from November to April 2021.

Gender	
Male	121(58.5%)
Female	86(41.5%)
Age category	
18–34	39 (18.8%)
35–49	61(29.5%)
50–64	54(26.1%)
Above 64	53(25.6%)
Educational level	
No formal education	17(8.2%)
Primary	35(16.9%)
High school	53(25.6%)
Collage/university	102(49.3)
Time of transplantation	
0–6months	18(8.7%)
7–12months	45(21.7%)
13–24months	80(38.6%)
>24months	64(30.9%)
History of UTI	
Yes	36(17.4)
No	171(82.6)
Place of the transplantation	
Inside	144(69.6%)
Outside	63(30.4%)
Clinical condition	
History of diabetic mellitus	65 (31.4)
History of hypertensive	24(11.6)
Both DM and hypertension	39 (18.8)
None	63 (30.4)
Other complication	16 (7.7)
	17

5.2. Prevalence of Bacterial and Fungal isolates

From 207 kidney recipient patients 63(30.46%) were found to be culture positive under aerobic cultural environments. Out of them, 15(23.8%) were gram positive bacteria, 40(63.49%) were gram negative bacteria and 8(12.69%) were fungal isolates. Among Gram-positive isolates *Coagulase negative staphylococcus* (66.6%) was the predominant bacteria followed by *Staphylococcus aureus* (26.6%). On the other hand, *Escherichia coli* was the predominate bacteria among Gram- negative bacterial isolates which accounted for (41.8%) followed by *Klebsiella spp* 18.2%. double infection identified in all fungal isolates with bacterial isolates. From the 55(26.6 %) bacterial and 8(3.84) fungal isolates, 15 (27.3%) were from males and 40 (72.7%) and all fungal isolates were from females. The most frequently isolate found among males were *E. coli* and *K. pneumonia* (26.7%), while *Escherichia coli* (47.5%) and *Coagulase negative Staphylococcus* (22.5%) (Figure1).

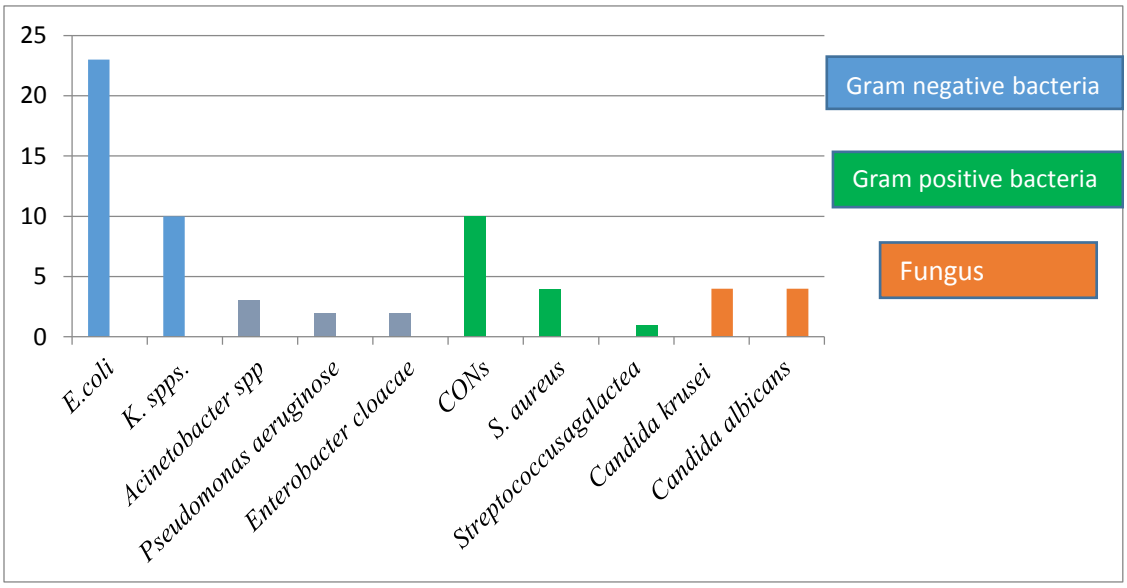

Figure1: Prevalence of microbial isolates from kidney transplanted recipients at SPHMMC during the study period.

5.3. Antibiotics resistance patterns

The current study was showed that in the overall resistance rate for different antibiotics tested. Gram positive bacteria exhibited high degree of resistance to Pencillin and Oxacillin. Out of the total ten *Coagulase negative staphylococcus* isolates 8(80%) were resistant to penicillin and 7(70%) to Oxacillin. Out of four *Staphylococcus aureus isolates* 3(75%) were resistant to Penicillin. On the other hand, *Streptococcus agalactae* were susceptible to most of the antibiotics tested. The antibiotics resistance pattern of gram positive bacteria isolated from urine specimens against 6 antibiotics is presented in Table 3.

Table 2: Antibiotic resistance patterns of gram positive bacteria isolated from urine cultures kidney transplanted recipients their culture results at SPHMMC from November to April 2021.

	Antibiotic						
Bacterial isolate		**GEN**	**OXA**	**PEN**	**SXT**	**CIP**	**CTX**
Coagulase Negative Staphylococcus (CONS) (n=10)	%R	3(30)	7(70)	8(80)	3(30)	0(0)	1(10)
S.agalactae (n=1)	%R	0(0)	0(0)	0(0)	1(100)	NT	0(0)
Staphylococcus aureus (n=4)	%R	1(25)	1(25)	3(75)	0(0)	0(0)	2(50)

Note: PEN: Penicillin OXA: Oxacllin GEN: Gentamycin CTX: Cefotaxime CIP: Ciprofloxacin SXT: Co-Trimoxazole R: Resistant NT: Not tested

Antimicrobial resistance levels of gram-negative organisms for the most commonly causing UTIs were relatively high. *Escherichia coli and Klebsiella Pneumoneia* exhibited high rate of resistance to most of the tested antibiotics. *Escherichia coli* showed highest resistance to Ampicillin 20(87.0%), followed by Tetracycline 17(73.9%) and Tobramycin 16(69.6%). In addition, its resistance level partially high to Cefotaxime 13(56.5%), for both Ceftazidime and Gentamycin 12(52.2%). Similarly, *Klebsiella Pneumoniae* also showed extremely resistance to Ampicillin 8(100%) and Gentamycin 6(75%). On the other hand, *Klebsiella oxytoca* isolates showed high level of resistance 1(100%) to Ampicillin and Amikacin. *Klebsiella ozenae* showed

resistance to Ampicillin, third generation cephalosporin, Aminoglycoside and Fluoroquinolones 1(100%).

From the member of non-enterobacterciae *Acintobacter* Spp. showed high resistance to Ampicillin 2(66.6%). On the other hand, *Pseudomonas* Spp. was showed low level of resistance to Aminoglycosides and Ciprofloxacin 1(50%).The majority of *Enterobactrecia* was susceptible to Meropnem. However, *Klebsiella Pneumoniae* 3(37.5%) and *Escherichia coli* 1(4.3%) showed little resistance to Meropnem (table 3).

Table 3: Antibiotics resistance pattern of gram negative and positive bacteria isolated from urine culture of renal transplants at SPHMMC from November to April 2021

Bacterial isolate	**Antibiotics**											
	GEN	**MEM**	**TOB**	**CIP**	**AMK**	**CFP**	**CAZ**	**TET**	**AMP**	**CTX**	**NIT**	**NA**
***Escherichia coli* (n=23)**	12(52.2)	1(4.3)	16(69.6)	9(39.1)	5(21.7)	11(47.8)	12(52.2)	17(73.9)	20(87.0)	13(56.5)	3(13.0)	5(21.7)
***Klebsiella pneumoniae* (n=8)**	6(75.0)	3(37.5)	6(75.0)	2(25)	3(37.5)	3(37.5)	4(50)	5(62.5)	8(100)	4(50)	2(25.0)	2(25.0)
***Acinetobacter spp* (n=3)**	2(66.6)	0(0)	2(66.6)	1(33.3)	0(0)	2(66.6)	0(0)	2(66.6)	2(66.6)	1(33.3)	NT	NT
***Klebsillea oxytoca* (n=1)**	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)
***Klebsillea ozaenae* (n=1)**	1(100)	0(0)	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)
Pseudomonas spp (n=2)	1(50)	0(0)	1(50)	1(50)	1(50)	0(0)	0(0)	1(50)	NT	1(50)	NT	NT
***Enterobacter cloacae* (n=2)**	2(100)	0(0)	1(50)	2(100)	2(100)	1(50)	1(50)	2(100)	2(100)	1(50)	0(0)	0(0)

Note: GEN: Gentamycin MEM: Meropnem TOB: Tobramycin CIP: Ciprofloxacin AMK: Amikacin CFP: Cefipime CAZ: Ceftazidime TET: Tetracycline AMP: Ampicilin CTX: Cefotaxim NIT: Nitrofurantoin NA: Nalidixic Acid R: Resistant NT: Not tested

5.4.Multidrug resistance pattern of bacterial isolates

Multi Drug Resistance **(**MDR) is a bacterium that is simultaneously resistant for three or more antimicrobials belonging to different antibiotic classes. In our study, multi drug resistance level was 82%. The most common MDR gram negative bacterial isolates were *Eschceria coli, Klebsiella pneumoniae, Acinitobacte*r spp, *Enterobacter cloacae, Pseudomonas* Spp. and

Klebsiella ozenae. Klebsiella oxytoca found to be non MDR for the antibiotics as described by the table 4.

Table 4: Multidrug resistance patterns of gram negative bacterial isolates from urine samples of patients of renal transplants at SPHMMC from November to April 2021

Bacterial Isolate	Ro	R1	R2	R3	R4	R5	R6	R7	Total MDR
E.coli(N=23)	0(0)	2(8.7)	2(8.7)	0(0)	4(17.4)	2(8.7)	3(13.0)	10(43.5)	**19(82.6)**
K. pneumoneia(N=8)	0(0)	0(0)	1(12.5)	0(0)	2(25)	0(0)	1(12.5)	4(50)	**7(87.5)**
***K.* oxytoca (n=1)**	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	**0(0)**
***K.ozaenae* (n=1)**	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	**1(100)**
Pseudomeunos spp(N=2)	0(0)	0(0)	1(50)	0(0)	0(0)	0(0)	1(50)	0(0)	**1(50)**
E.cloacae(N=2)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	1(50)	0(0)	**2(100)**
Acinitobacter spp(N=3)	0(0)	0(0)	0(0)	1(33.3)	0(0)	0(0)	0(0)	2(66.6)	**3(100)**
Total(N=40)	**0(0)**	**2(5)**	**5(12.5)**	**1(2.5)**	**6(15)**	**3(7.5)**	**7(17.5)**	**16(40)**	**33(82)**

Note: Ro: resistance to no antibiotics, R1-resistant for 1 antibiotic, R2- resistant for 2 antibiotics, R3- resistant for 3 antibiotics, R4- resistant for 4 antibiotics, R5- resistant for 5 antibiotics, R6 resistant for 6 antibiotics, R7 resistant for 7 antibiotics; ≥R3: resistance to 3 or more antibiotics from different classes (MDR)6.145(3.092 −12.214)

5.5. Predisposal Risk Factors Associated with Urinary Tract Infections

The prevalence of bacterial infection rate was higher in female 40(72.7%) than male 15(27.3%). The occurrence of bacterial infections in female was 6.145 times higher than males [COR (95%CI) 6.145(3.092, 12.214)]. Majority of these participants was asymptomatic. The infection rate was also relatively high in adult (50-64 age group) 44.4% followed by elderly(>64) 22.6%, age group 35–49 21.3% and within 18-34 age group 16.4% who share the lowest proportion of UTI patients.

In bivariate logistic regression analysis; gender, the presence of additional comorbidities such as having diabetes mellitus (40%), hypertension (16.4%) and hypertensive with DM were (18.2%),

this was significantly associated with bacterial and fungal infection in kidney transplanted recipients as described by the table 5.6

Variables	**Categories**	**Bacterial infection**				
		Culture Positive (%)	**COR(95%CI)**	***p-value***	***AOR*(95%CI)**	***p-value***
Gender	Male	15(27.3)	6.145(3.092 –12.214)	< 0.001	8.444(3.710-19.219)	< 0.001
	Female	40(72.7)				
Age category	18–34	6 (15.4)	.621(.211 - 1.833)	.388	.433(.114-1.636)	.217
	35–49	13 (21.3)	.925(.381 - 2.250)	.864	.862(.281-2.643)	.795
	50–64	24 (44.4)	2.733(1.183 - 6.318)	.019	2.318(.778-6.907)	.131
	Above 64	12 (22.6)				
Educational level	No formal education	8 (14.5)	2.889(1.004 - 8.310)	.049	.920(.226-3.749)	.907
	Primary	15 (27.3)	2.437(1.083- 5.484)	.031	2.585(.861-7.756)	.090
	High school	8 (14.5)	.578(.240 - 1.393)	.222	.427(.146-1.252)	.121

	Collage/university	24 (43.6)				
Time of transplantation	0–6months	4(7.3)	.857(.335 - 4.060)	.809	.538(.056-5.207)	.593
	7–12months	15(27.3)	1.500(.288 - 1.543)	.344	1.297(.247-6.802)	.759
	13–24months	20(36.4)	1.000(.468 - 2.136)	1.000	1.099(.422-2.862)	.846
	>24months	16(29.1)				
Place of the transplantation	Inside	36(65.5)	.772(.400 - 1.489)	.440	.480(.107-2.157)	.339
	Outside	19(34.5)				
History of UTI	Yes	16(29.1)	2.708(1.281 – 5.722)	0.009	1.958(.797-4.809)	.143
	No	39(70.9)				
Disease history	History of diabetic mellitus	22(40)	2.712(1.160 - 6.337)	.021	3.366(1.199-1.199)	.021
	History of hypertensive	9(16.4)	3.180(1.093-9.248)	.034	6.617(1.703-25.705)	.006
	Both DM and hypertension	10(18.2)	1.828(.681 - 4.901)	.231	1.945(.591-6.408)	.274
	Other complication	4(7.3)	1.767(.473 - 6.600)	.397	4.222(.821-21.713)	.085
	None	10(18.2)				

Review from the study participant files revealed occurrence of urinary tract infections previously in 36 recipients 17.4%. Fifteen were males and Twenty-one were females. There were 63 counts of bacterial and fungal isolates from the participant's records. 36.4% of the UTIs occurred within

one up to two years of transplantation. These were 1.00 times exposure than those of above 2 years since transplanted. There were no significant associations between places of transplantation

Table 5: Risk factor associated with microbial cause of urinary tract infections among kidney transplanted patients at SPHMMC

6. Discussion

UTIs are the most common infection following kidney transplantation that leads to greater complication. The recurrent nature of the problem necessitates the use of multiple courses of antibiotic therapy, which are not only costly but can result in microbial resistance and contribute to erratic levels of immunosuppressant. Repeated infections may also lead to graft inflammation and fibrosis [8].Our study focused on prevalence of UTI, its causative agents, and bacterial antimicrobial resistance pattern isolated from renal transplant. This study differing from other studies, we identified *candida* Spp, in which is still not routinely being carried out at kidney transplant centers in Ethiopia.

The prevalence of UTIs in the present study was 30.46%, this finding was higher than previous study conducted in Ethiopia 14.9% [34], Portugal 16.5% [42] and in Australia 8% [43].The differences in UTI reported rates may be caused by local ascribe of outbreaks, center-specific potent immunosuppressive therapy, lack of the robust definition of UTI and study designs in many clinical settings [44]. However, the current result was lower than reported in Yemen 33% [11], in Iran 33.56% [15], in Saudi Arabia 53.69% [28] and 63% in Japan [45].

In the present study the majority (58.5%) of study participants were males. However, higher number of Female recipients was reported to be at a greater risk for development of UTI than males 72.7%, and have correlation between prevalence of UTI and the gender of the recipient. It is consistence with previous findings by Kotagiri et al., [43] in Australia, Shams et al., [15] in Iran and Bispo et al., [42] in Portugal. Additionally, several studies reported that the risk of being female had a significant association with UTI than males [38, 6, 46]. The anatomical such as shorter urethra and relative proximity of the urethra to the perirectum, hormonal, immunological and behavioral features may be contributed to an increased risk UTI in women than men [47].

From our study, we determined the frequency of UTIs was greater among adult (44.4%) than younger patients (15.4%). This finding is comparable with a study by Chuang et al. where patients 65 years developed post kidney transplant UTI with 55% compared to younger patients [38].

An ineffective cellular immunity and, probably, a lower tolerance to immunosuppression associated with comorbidities, such as diabetes, hypertension and other complications may contribute to the higher prevalence of bacterial infections found in older recipients by lowering patient's immunity [48].

In this study, diabetes mellitus was the major predisposing factor for post-transplant UTIs among these recipients with a percentage of 40%. The result was consistent with study done in Yemen, reported that half of the studied UTI patients were diabetic [15]. Additionaly, being a diabetic was a risk factor for UTI according to a reported by Khanna et. al. and AlRohani M. et.al. [49,50].

There is a wide variety of organisms that can cause post kidney transplant urinary tract infections studied by many researchers. The current study showed the predominance of Gram-negative bacteria over Gram-positive bacteria. It was similar to studies 65% by Elkihili, 53% by Chuang and previous findings done by different researchers (38, 39). From those isolates, The commonly isolated bacteria that cause UTIs in this study were reported as *Escheria coli, Klebsella pneumoneia* and *Coagulase negative staphylococcus.* This finding is more or less similar with previous studies from around the world [51-53].

Amongest the gram negative high isolation was observed as *Escheria coli,* it involved in 41.8% of the cases. This result was similar to several other studies paper where *Escherichia coli* were the most frequent etiology for UTI among kidney transplant patients [54-56]. The predominance of this bacterial pathogen may be due to the ability to produce or express type 1or pfimbriae, which enables the bacterium's pathogenicity in the urothelium. The second major causative isolate of UTI in our patients was *Klebsiella* Spp**.**18.2% which is slightly higher than the

prevalence reported by Barbouch et al. from Saudi Arabia 14.6% [28], 10% in the study of Chuang et al. in USA [38] but less than the prevalence reported by others [57].

Among the gram positive organsim causing UTIs in our study were *Coagulase negative staphylococcus*. Which was comparable to other previous studies in However, this finding disagrees with other studies [58, 59]. Difference in identification methods are known to influence the relative prevalence of bacteria which makes comparison difficult [57].

Bacterial etiologies of UTIs can show geographic variation and host factor may even vary over time within a population [31].

In the current study, Funguria has been confirmed in (12.69%) cases. All of them were developed mixed infection with *Coagulase negative staphylococcus*, *Enterobacter spp*. and *E. coli*. Our finding was higher than the incidence of candiduria reported by other studies [60,61]. We determined causative agent as *candida species* in all the cases. Since, the most fungal etiologic agent which cause UTIs in post kidney transplant recipients as *Candida albicans* and *Candida krusie* in our study which is similar with previous studies [62, 63]

According to other researchers, the incidence of infectious episodes is highly variable among the different studies; usually the incidence of infection is higher in the first month follow-up after transplantation and directly related to the dose of immunosuppression used. In the first months after kidney transplantation predominant nosocomial infections, especially those located in the urinary tract and surgical wound [64]. In or study, the occurrence of microorganism during the first six months after renal transplantation were 7.8% and this relatively disagree with previous study, but high prevalence 36.4% was recorded in the 13-24 months since transplanted. These variations may be due to high intake of immunosuppressant and also negligence of patients to take care of themselves from infectious organisms [64].

In the present study, the antimicrobial susceptibility pattern of gram negative bacteria reported high level of resistance to routinely used antimicrobials including Aminoglycosides and third generation cephalosporins. *E. coli* have shown high resistance to Ampicillin (87.0%), Tetracycline (73.9%) and Tobramycin (69.6%) despite of sensitive to Meropenem (95.6%) and

Nitrofurantoin (87%). This was supported by the previous study in Iran that a high resistance rate to antibiotics addressed by Khotaii et al. [65], in Poland [66] and in Turkey [67]. This high resistance results from misuse of these antibiotics with significant extent in private and health care setting, thus contribute for the development of drug resistance through selective pressure in kidney transplant patients.

In our study, *Klebsella* isolates have shown high resistance to folate pathway inhibitor and aminoglycosides. Third generation and fourth generation cephalosporins and Carbapenem also showed weak activity against this organism. This consistent with study in Mexico [27].These low resistances probably refer to the proper use of antibiotics used in this renal transplant center.

The rate of multi drug resistant strains were seen in 82% of bacteria isolates. Similarly to the current study done in China were reported 86.4% [68]. However, previous study reported by Gozdowska et al., [69] and Bodro et al., [70] were much lower than our finding (37%). This high multi drug resistance probably results in increases health care costs, prolongs hospital stays and can result in treatment failure to kidney transplants.

7. Strength and Limitation

7.1. Strength of the study

- Performing prevalence of fungal elements.
- Including all age groups.

7.2. Limitation of the study

- Anaerobic cultures were not available.
- Absences of few follow up patients during study period.

8. Conclusion

The effect of Urinary tract infection on post transplantation recipients was prevalent in the present study this may result in different complication. The frequency of Gram-negative bacteria that causes UTIs were higher compared to Gram-positive bacterial and fungal isolates in this study. The finding of this study showed that UTIs in KTR were predominantly caused by *E.coli,* Coagulase negative staphylococci and *Klebsiella species* respectively. *C. albicans* and *C.krusie* was common fungal UTIs in our study setting. Gram-negative bacteria showed resistance to most commonly used antibiotics such as Aminoglycosides and third generation cephalosporins. MDR was observed only in Gram negative bacterial isolates. Carbapenem, Nitrofurantoin, Nalidixic Acid, Amikacin and Fluoroquinolones were the most effective antibiotic against the gram negative and gram positive bacterial isolates.

9. Recommendations

- Active surveillance and hospital infection control policies should be applied on post kidney transplant UTIs to provide alternative treatment.
- Routine urine culture especially for female those post-transplant recipients on follow up at SPHMMC transplant center.
- Effective antibiotic policy practices can help in confronting the menace of antibiotic resistance to prevent the spread of multidrug resistant strains.
- Further study needs to be conducted.

10. References

1. Vidal E, Torre-Cisneros J, Blanes M, Montejo M, Cervera C, Aguado JM, et al. Bacterial urinary tract infection after solid organ transplantation in the RESITRA cohort. Transpl Infect Dis 2012; 14: 595-603.
2. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature REV Microbiol. 2015;13(5):269.
3. Dupont PJ, Psimenou E, Lord R, Buscombe JR, Hilson AJ, Sweny P: Late recurrent urinary tract infections may produce renal allograft scarring even in the absence of symptoms or vesicoureteric reflux. Transplantation 2007, 84:351-355.
4. Gozdowska J, Czerwińska M, Młynarczyk G, Kwiatkowski A, Chmura A, Durlik M. Urinary tract infections in kidney transplant recipients hospitalized at a transplantation and nephrology ward: 1-year follow-up. Transplant Proc. 2016;48(5):1580–9.
5. Ak O, Yildirim M, Kucuk HF, Gencer S, Demir T (2013) Infections in renal transplant patients: risk factors and infectious agents. Transplant Proc 45(3):944–948.
6. Pourmand G, Salem S, Mehrsai A, Taherimahmoudi M, Ebrahimi R, Pourmand MR. Infectious complications after kidney transplantation: a single-center experience. Transpl Infect Dis. 2007;9(4):302-9.)
7. Linares L, Cervera C, Cofán F, Ricart MJ, Esforzado N, et al. Epidemiology and outcomes of multiple antibi otic resistant bacterial infection in renal transplantation.*Transplant Proc* 2007; 39: 2222-2224.
8. Kauffman CA, Vazquez JA, Sobel JD, Prospective multicenter surveillance study of funguria in hospitalized patients. The National Institute for Allergy and Infectious Diseases (NIAID) Mycoses Study Group. Clin Infect Dis 2000;30:14–8.]
9. Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasivefungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis 2010;50: 1101–11.
10. Esezobor CI, Nourse P, Gajjar P. Urinary tract infectionfollowing kidney transplantation: frequency, risk factors and graft function. *Pediatr Nephrol* 2012; 27: 651-657.

11. Ciszek M, Paczek L, Bartlomiejczyk I et al. Urine cytokines profile in renal transplant patients with asymptomatic bacteriuria.Transplantation 2006; 81:1653 – 1657.
12. Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004Apr 15;38(8):1150-8.
13. de Souza R M, O lsburgh J . Urinary tract infection in the renal transplant patient. Nat Clin Pract 2008; 4 : 252– 264.
14. Ariza-Heredia EJ, Beam EN, Lesnick TG, Cosio FG, Kremers WK, Razonable RR. Impact of urinary tract infection on allograft function after kidney transplantation. Clin Transplant. 2014; 28:683–90.
15. Gondos AS, Al-Moyed KA, Al-Robasi AB, Al-Shamahy HA, Alyousefi NA. Urinary tract infection among renal transplant recipients in yemen.
16. Schmaldienst S, Dittrich E, Hörl WH. Urinary tract infections after renal transplantation. Curr Opin Urol. 2002 Mar;12(2):125-30.
17. Kauffman CA, Vazquez JA, Sobel JD, Prospective multicenter surveillance study of funguria in hospitalized patients. The National Institute for Allergy and Infectious Diseases (NIAID) Mycoses Study Group. Clin Infect Dis 2000;30:14–8.
18. Storfer SP, Medoff G, Fraser VJ, Powderly WG, Dunagan WC. Candiduria: retrospective review in hospitalized patients. Infect Dis Clin Pract 1994;3:23–9.
19. Harris AD, CastroJ, SheppardDC,CarmeliY,SamoreMH.Riskfactors for nosocomial candiduria due to Candida glabrata and Candida albicans. Clin Infect Dis 1999;29:926–8.
20. Elkehili I, Kekli A, Zaak A, Salem E. Urinary tract infection in renal transplant recipients. Arab Journal of NephrologyandTransplantation.2010;3(2):53–5.
21. Lofaro D, Vogelzang JL, van Stralen KJ, Jager KJ, Groothoff JW. Infection-related hospitalizations over 30 years of follow-up in patients starting renal replacement therapy at pediatric age. Pediatr Nephrol. 2016; 31:315–23.
22. Abbott KC, Swanson SJ, Richter ER, Bohen EM, Agodoa LY, Peters TG, Barbour G, Lipnick R, Cruess DF. Late urinary tract infection after renal transplantation in the United States. Am J Kidney Dis. 2004 Aug;44(2):353-62.
23. Säemann M, Hörl WH. Urinary tract infection in renal transplant recipients. Eur J Clin Invest 2008; 38 Suppl 2: 58-65.

24. Valera B, Gentil MA, Cabello V, Fijo J, Cordero E, 1.Cisneros JM. Epidemiology of urinary tract infections in renal transplant recipients. Transplant Proc 2006; 38: 2414-2415
25. Rivera-Sanchez R, Delgado-Ochoa D, Flores-Paz RR, García-Jiménez EE, Espinosa-Hernández R, et al.Prospective study of urinary tract infection surveillan ce after kidney transplantation. BMC Infect Dis 2010; 10: 245.
26. Barbouch 18. S, Cherif M, Ounissi M, Karoui C, Mzoughi S, Hamida FB, Abderrahim E, Bozouita A, Abdalla T, Kheder A. Urinary tract infections following renal transplantation: a single-center experience. Saudi J Kidney Dis Transpl 2012; 23: 1311-1314.
27. Dantas SR, Kuboyama RH, Mazzali M, Moretti ML. Nosocomial infections in renal transplant patients: risk factors and treatment implications associated with urinary tract and surgical site infections. J Hosp Infect 2006; 63: 117-123.
28. Esezobor C21. I, Nourse P, Gajjar P. Urinary tract infection following kidney transplantation: frequency, risk factors and graft function. Pediatr Nephrol 2012; 27: 651-657.
29. Shams SF, Eidgahi ES, Lotfi Z, Khaledi A, Shakeri S, Sheikhi M, et al. Urinary tract infections in kidney transplant 1st year after transplantation. J Res Med Sci. 2017;22(3):45–62.
30. Shirazi M, Ranjbar R, Hemati F, Sadeghifard N. Bacterial infections in renal transplant recipients. Iranian Journal of Public Health.2005; 34(3):62–6.
31. Sousa SR, Galante NZ, Barbosa DA, Pestana JOM. Incidência e fatores de risco para complicações infecciosas no primeiro ano após transplante renal. J Bras Nefrol. 2010; 32(1):77-84.
32. Shirazi M, Ranjbar R,Hemati F, Sadeghifard N. Bacterial infections in renal transplant recipients.IranianJournalofPublicHealth.2005; 34(3):62–6.
33. Dantas SR, Kuboyama RH, Mazzali M, Moretti ML. Nosocomial infections in renal transplant patients: risk factors and treatment implications associated with urinary tract and surgical site infections. J Hosp Infect 2006; 63: 117-123.

34. Kiros T, Asrat D, Ayenew Z. Bacterial urinary tract infection among adult renal transplant recipients at St. Paul's hospital millennium medical college, Addis Ababa, Ethiopia Kiros et al. BMC Nephrology (2019) 20:289
35. Fiorentino M, Pesce F, Schena A, Simone S, Castellano G, Gesualdo L. Updates on urinary tract infections in kidney transplantation. J Nephrol. 2019:1–11.

36. Fiorante S, Lopez-Medrano F, Lizasoain M, Lalueza A, Juan RS, Andrés A, et al. Systematic screening and treatment of asymptomatic bacteriuria in renal transplant recipients. Kidney Int. 2010; 78(8):774–81.
37. Ariza-Heredia EJ, Beam EN, Lesnick TG, Cosio FG, Kremers WK, Razonable RR. Impact of urinary tract infection on allograft function after kidney transplantation. Clin Transplant. 2014; 28:683–90.
38. Chuang P, Parikh CR, Langone A. Urinary tract infections after renal transplantation: a retrospective review at two US transplant centers. Clin Transpl. 2005; 19(2):230–235.
39. Menegueti MG, Pereira MF, Bellissimo-Rodrigues F, Garcia TMP, Saber LTS, Nardim MEP, et al. Study of the risk factors related to acquisition of urinary tract infections in patients submitted to renal transplant. Rev Soc Bras Med Trop. 2015; 48(3):285.
40. Organization WHO guidelines on drawing blood: best practices in phlebotomy: World Health Organization; 2010.
41. Bauer A, Kirby W, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology. 1966;45(4_ts):493-6.

42. Bispo A, Fernandes M, Toscano C, Marques T, Machado D, Weigert A.Urinary tract infections in a cohort of kidney transplant recipients. Acta Medica Port.2014;27(3): 364–71.
43. Kotagiri P, Chembolli D, Ryan J, Hughes PD, Toussaint ND. Urinary tract infections in the first year post–kidney transplantation: potential benefits of treating asymptomatic bacteriuria. Transplant Proc. 2017;49(9):2070–5.

44. Shams SF, Eidgahi ES, Lotfi Z, Khaledi A, Shakeri S, Sheikhi M, et al. Urinary tract infections in kidney transplant 1st year after transplantation. J Res Med Sci. 2017;22(3):45–62.
45. Nizze H, Brockmöller S, Bast R, Schmitt E. Infectious causes of death in renal transplant patients. A clinical pathological autopsy study. Verh Dtsch Ges Pathol 1991; 75: 218-23.

46. Menegueti MG, Pereira MF, Bellissimo-Rodrigues F, Garcia TMP, Saber LTS, Nardim MEP, et al. Study of the risk factors related to acquisition of urinary tract infections in patients submitted to renal transplant. Rev Soc Bras Med Trop. 2015; 48(3):285.

47. Kumar A, Agarwal C, Hooda AK, Ojha A, Dhillon M, Hari Kumar KV. Profile of infections in renal transplant recipients from India. J Family Med Prim Care. 2016;5(3):611–4.)
48. Meier-Kriesche HU, Ojo A, Hanson J, Cibrik D, Lake K, Agodoa LY, Leichtman A, Kaplan B (2000) Increased immunosuppressive vulnerability in elderly renal transplant recipients. Transplantation 69(5):885–889)
49. Khanna P, Abraham G, MohamedAli A, Miriam P, Mathew M, Lalitha M, etal. Urinary tract infections in the era of newer immunosuppressant agents:A tertiary care center study. Saudi Journal of Kidney Diseases and Transplantation.2010;21(5):876)
50. AlRohani M. Causes of chronic renal failure at one center in Yemen. Saudi Journal of Kidney Diseases andTransplantation.2003;14(1):80.)
51. Memikoğlu KO, Keven K, Sengül S, Soypaçaci Z, Ertürk S, Erbay B: Urinary tract infections following renal transplantation: a single-center experience. Transplant Proc 2007, 39:3131-3134.
52. Nampoory MR, Johny KV, Costandy JN, Nair MP, Said T, Homoud H, AlMuzairai I, Samhan M, Al-Moussawi M: Infection related renal impairment: a major cause of acute allograft dysfunction. Exp Clin Transplant 2003, 1:60-64
53. Sharma KK, Ayyagiri A, Dhole TN, Prasad KN, Kishore J: Prevalence of infections in renal transplant recipients of north India. Indian J Pathol Microbiol 2007, 50:453-457. 15.

54. Memikoğlu KO, Keven K, Sengül S, Soypaçaci Z, Ertürk S, Erbay B: Urinary tract infections following renal transplantation: a single-center experience. Transplant Proc 2007, 39:3131-3134
55. Nampoory MR, Johny KV, Costandy JN, Nair MP, Said T, Homoud H, AlMuzairai I, Samhan M, Al-Moussawi M: Infection related renal impairment: a major cause of acute allograft dysfunction. Exp Clin Transplant 2003, 1:60-64.
56. Alangaden GJ, Thyagarajan R, Gruber SA, Morawski K, Garnick J, EI-Amm JM, West MS, Sillix DH, Chandrasekar PH, Haririan A: Clin Transplant. Infectious complications after kidney transplantation: current epidemiology and associated risk factors 2006, 20:401-409.
57. Sqalli TH, Laboudi A, Arrayhani M, Benamar L, Amar Y, Ouzeddoun N, Bayahia R, Rhou H. Urinary tract infections in renal allograft recipients from living related donors. Saudi J Kidney Dis Transpl. 2008 Jul;19(4):551-3.
58. Leigh DA: Bacteraemia in patients receiving human deceased renal transplants. J Clin Pathol 1971, 24:295-299, Sweny P: Infection and cancer following renal transplantation. Saudi J Kidney Dis Transplant 2006, 17:189199.
59. Moradi M, Abbasi M, Moradi A, Boskabadi A, Jalai A: Effect of antibiotic therapy on asymptomatic bacteriuria in kidney transplant recipients. Urol J 2005, 2(1):32-35.
60. Fraser VJ, Jones M, Dunkel J, Storfer S, Medoff G, Dunagan WC. Candidemia in a tertiary care hospital: epidemiology, risk factors, and predictors of mortality. Clin Infect Dis 1992;15:414–21.
61. Sobel JD, Kauffman CA, McKinsey D, Candiduria: arandomized, double-blind study of treatment with fluconazole and placebo. Clin Infect Dis 2000;30:19–24.
62. Marchetti O, Bille J, Fluckiger U, et al. Epidemiology of candidemia in Swiss tertiary care hospitals: secular trends, 1991–2000. Clin Infect Dis 2004;38:311–20. 12.
63. Fidel PL Jr, Vazquez JA, Sobel JD. Candida glabrata: review of epidemiology, pathogenesis, and clinical disease with comparison to C. albicans. Clin Microbiol Rev 1999;12:80–96.
64. Cherif M, Ounissi M, Karoui C, Mzoughi S, Hamida FB, Abderrahim E, Bozouita A, Abdalla T, Kheder A. Urinary tract infections following renal transplantation: a single-center experience. Saudi J Kidney Dis Transpl 2012; 23: 1311-1314.

65. Khotaii Q, Mamishi S, Saligeh RN. Antibiotic resistance of germs Isolated from urinary tract infection. Iran J Pediatr 2002; 12: 28-32.
66. Kamath N, John G, Neelakantan N, Acute graft pyelonephritis following renal transplantation. Transpl Infect Dis, 2006;8:140-147.
67. Azap Ö, Togan T, Yesilkaya A et al (2013). Antimicrobial susceptibilities of uropathogen Escherichia coli in renal transplant recipients: dramatic increase in ciprofloxacin resistance. Transplant Proc, 45(3):956-7.
68. Yuan X, Liu T, Wu D, Wan Q. Epidemiology, susceptibility, and risk factors for acquisition of MDR/XDR gram-negative bacteria among kidney transplant recipients with urinary tract infections. Infect Drug Resist. 2018;14(11):707–15.)
69. Gozdowska J, Czerwińska M, Młynarczyk G, Kwiatkowski A, Chmura A, Durlik M. Urinary tract infections in kidney transplant recipients hospitalized at a transplantation and nephrology ward: 1-year follow-up. Transplant Proc. 2016;48(5):1580–9.
70. Bodro M, Sanclemente G, Lipperheide I, Allali M, Marco F, Bosch J, et al. Impact of urinary tract infections on short-term kidney graft outcome. Clin Microbiol Infect. 2015;21(12):1104.

11. Annexes

Annex I: Laboratory Test Procedures

1. Specimen collection

Urine sample collection

The first urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most concentrated and therefore the most suitable for culture, microscopy, and biochemical analysis.

Procedure

1. Label the container with the date, the name and number of the patient, and the time of collection.
2. Give the patient a sterile, dry, wide-necked, leak proof container and request a 10–20 ml specimen.
    - ❖ Explain to the patient the need to collect the urine with as little contamination as possible, i.e. a clean-catch specimen.
    - **Female patients:** Wash the hands. Cleanse the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect the urine with the labia held apart.
    - **Male patients:** Wash the hands before collecting a specimen (middle of the urine flow).

2. Specimen transport and storage

Specimens should ideally be stored and transported in sealed plastic bags. Laboratory processing should occur as soon as possible after specimen collection. When immediate delivery to the laboratory is not possible, refrigerate the urine at 4–6 °C. When a delay in delivery of more than 2 hours is anticipated, add boric acid preservative to the urine.

3. Gram stain technique

Principle

Differences in Gram reaction between bacteria is due to differences in the permeability of the cell wall of Gram positive and Gram negative organisms during the staining process. Following staining with a triphenyl methane basic dye such as crystal violet and treatment with iodine, the dye–iodine complex is easily removed from the more permeable cell wall of Gram negative bacteria but not from the less permeable cell wall of Gram positive bacteria. Retention of crystal violet by Gram positive organisms may also be due in part to the more acidic protoplasm of these organisms binding to the basic dye (helped by the iodine).

Laboratory procedure for Gram staining technique

1. Labeling the slides clearly with the date and patient's name and number.
2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.
3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.
4. Fix the dried smear by using heat or alcohols (methanol).
5. Cover the fixed smear with crystal violet stain for 30-60 seconds.
6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.
7. Tip off all the water, and cover the smear with lugol's iodine for 30-60 seconds.
8. Wash off the iodine with clean water.
9. Decolorize rapidly (few seconds) with 3% acetone alcohol. Wash immediately with clean water.
10. Cover the smear with neutral red or safranine stain for 2 minutes.
11. Wash off the stain with clean water
12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.
13. Examine the smear microscopically, first with the 40 X objective to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Result

• Gram positive bacteria ----------dark purple

• Gram-negative bacteria ----------pale to dark red.

4. Culture and identification

A. Biochemical tests for gram positive bacteria

Catalase test

Catalase test to differentiate staphylococci which produce the enzyme catalase from streptococci which are non catalase producing.

Principle

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

Procedure

1. Pour 2-3 ml of 3% hydrogen peroxide to a test tube
2. Using a sterile wooden stick take the test organism & immerse into the H_2O_2solution.56
3. Look for immediate bubbling

Interpretation

Active bubbling . . . . . Positive catalase test

No bubbles . . . . . .Negative catalase test

Controls

Positive control: Staphylococcus aureus (ATCC 25923)

Negative control: S. pyogenes (ATCC 19615)

Coagulase test

This test is used to identify *S. aureus* which produces the enzyme coagulase from other commonly isolated staphylococci.

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

Procedure

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

Clumping within 10 sec . . . . . Positive

No clumping within 10 sec . . . . . Negative

Controls

Positive control: Staphylococcus aureus (ATCC 25923)

Negative control: Staphylococcus epidermidis (ATCC 12228)

Note: If slide test is negative proceed to Tube test method

Tube test method (free coagulase)

Procedure

1. Take three small test tubes and label (Test organism, Positive control and Negative control).

2. Pipette 0.2 ml of plasma into each tube.

3. Add 0.8 ml of the test broth culture to tube Test organism.

Add 0.8 ml of the S. aureus culture positive control

Add 0.8 ml of sterile broth to negative control

4. After mixing gently, incubate the three tubes at 35–37 ^{0C}. Examine for clotting after 1 hour. If no clotting has occurred, examine after 3 hours. If the test is still negative, leave the tube at room temperature overnight and examine again

Results

Clotting of tube content. . . . . Positive

No clotting or fibrin clot . . . . . Negative

B. Biochemical test for gram negative bacteria

Indole test:

Principle

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethyl aminobenzaldehyde. This reacts with the indole to produce a red colored compound. Kovac's reagent is recommended in preference to Ehrlich's reagent for the detection of indole from enterobacteria.

Method

1. Inoculate the test organism in a bijou bottle containing 3 ml of sterile tryptone water.
2. Incubate at 35–37 ^{O}C for up to 48 h.
3. Test for indole by adding 0.5 ml of Kovac's reagent. Shake gently. Examine for a red colour in the *surface layer* within 10 minutes.

Interpretation

Red surface layer . . . . . Positive indole test

No red surface layer . . . . . …...Negative indole test

Urease test (Christensen's (modified) urea broth):

Principle

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red.

Procedure

1. Inoculate heavily the test organism in a bijou bottle containing 3 ml sterile Christensen's modified urea broth.

2. Incubate at 35–37 ^{O}C for 3–12 h (preferably in water bath for a quicker result).

3. Look for a pink color in the medium.

Interpretation

Pink color . . . . . Positive urease test

No pink color . . . . . …. . . Negative urease test

Triple Sugar Iron (TSI) Agar Slant

Principle

TSI agar tests are used to determine whether gram negative bacilli utilize glucose and lactose or sucrose fermentative and produce hydrogen sulfide. It contains 1% lactose, 1% sucrose and0.1% glucose and peptone. Phenol red and ferrous sulphate serves as an indicator for acidification of medium and H_2S production.

Procedure

1.Using a sterile inoculating needle, stab the butt of the TSI agar slant twice then streak back and forth along the surface of the agar with the organism.

2. Incubate at 37^{O}C for 18 to 24 h.

3. Look for the color change and gas production.

Interpretation

If acid slant–acid butt (yellow–yellow): glucose and sucrose and/or lactose fermented.

If alkaline slant–acid butt (red–yellow): glucose fermented only.

If alkaline slant–alkaline butt (red–red): glucose not fermented.

The presence of black precipitate (butt) indicates hydrogen sulfide production, and

Presence of splits or cracks with air bubbles indicates gas production.

Citrate utilization test using Simmon's citrate agar

Principle The medium contains citrate as the sole source of carbon and inorganic ammonium salt as the sole source of nitrogen. Bacteria that can grow on this medium produce an enzyme, citrate-permease, capable of converting citrate to pyruvate. Pyruvate can then enter the organism's metabolic cycle for the production of energy. Growth is indicative of utilization of citrate, an intermediate metabolite in the Krebs cycle.

Procedure

1. Streak the slant back and forth with light inoculums picked from the center of a well isolated colony.

2. Incubate aerobically overnight at 35–37 ^{O}C for up to 4-7days.

3. Observe a color change from green to blue.

Interpretation

Blue. . . . . Positive citrate test

Green. . . . . Negative citrate test

Motility Test (using motility agars):

Principle Motility agar prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility examined after 35^{O}C for 24 hour. Motility indicated by the presence of diffuse growth (appearing as coloring of the medium) away

from the line of inoculation. But if the bacteria are non-motile, the growths of the bacteria along the stab, diffusion not occur.

Oxidase test

Principle

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be used When the organism is oxidase-producing, the phenylenediamine in the reagent oxidized to a deep purple colour.

Procedure

1. Place a piece of filter paper in a clean petridish and add 2 or 3 drops of freshly prepared oxidase reagent.
2. Using a piece of stick or glass rod (not an oxidized wire loop), remove a colony of the test organism and smear it on the filter paper.
3. Look for the development of a blue-purple colour within a few seconds.

Interpretation

Blue-purple color . . . . . Positive oxidase test (within 10 seconds)

No blue-purple color . . . ….. . Negative oxidase test (within 10 seconds)

5. Laboratory procedure for Antimicrobial sensitivity testing

Procedure for Performing the Disk Diffusion Test

I. Direct Colony Suspension Preparation

Prepare a saline suspension of the isolate from an overnight incubated agar plate (use a nonselective medium, such as blood agar) to obtain 0.5 McFarland turbidity (1.5 x 108cfu/ ml of the bacteria). For visual comparison, look through the suspension in transmitted light against a white background with contrasting black stripes.

II. Inoculating Test Plates

Fresh Müeller Hinton agar (MHA) plates used the same day or stored in a refrigerator (2-8ºC); if refrigerated, they should be wrapped in plastic to minimize evaporation. Just before use, if excess moisture is visible on the surface, plates should be placed in an incubator (35ºC) or, with lids a jar, in a laminar-flow hood at room temperature until the moisture evaporates (usually 10 to 30 minutes).

Müeller Hinton agar (MHA) plate inoculated within 15 minutes after the inoculum has been adjusted. A sterile cotton swab is dipped into the suspension, rotated several times, and gently pressed onto the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The swab will then be streaked over the entire surface to the agar plate three times, with the plate rotated approximately 60o each time to ensure even distribution of the inoculum. A final sweep of the swab made around the agar rim. The lid may be left partly open for 3 to 5 minutes but no longer than 15 minutes to allow any excess surface moisture to be absorbed before the drug-impregnated discs are applied. All significant isolates should have antimicrobial susceptibilities determined according to SOP MIC-001.

Procedure

Emulsify colonies of similar appearance in small volume of nutrient broth. Match the turbidity of the suspension against the turbidity standard which has a similar appearance to an overnight broth culture.

1. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).
2. Spread the inoculum evenly over the Muller-Hinton agar plate with the swab
3. Using a similar inoculation technique, inoculate an overnight broth culture of the Control organism evenly across the upper and lower third of the plate.
4. Using a sterile forceps or needle, place the antimicrobial disc on the inoculated plate
5. Incubate the plate aerobically at 35 ^{O}C for 18-24 hours.
6. Read the tests after checking that the bacterial growth of the test and control organism is neither too heavy nor too light.
7. Measure the radius of the inhibition zone. Interpret result based on the inhibition zone.

Sensitivity (S): Zone of radius is wider than, equal to, or not more than 3mm smaller than the control.

Intermediate (I): Zone radius is more than 3mm smaller than the control but not less than 3mm.

Resistant (R): No zone of inhibition or zone radius measure 2mm or less.

Annex II: Dummy Tables

3. Urine sample collection format

Patient.code	Age	Sex	Time of collection	Date of collection	Name& Signature

Annex III: Questionnaire

1. Questionnaire (English Version)

My name is Lemlem Tesfaye; I came from Addis Ababa University College of Health Sciences School of Medical laboratory. My study aim is investigating principally the status of bacterial urinary tract infections, antibiotic susceptibility pattern and assessment of associated risk factors. You are selected to be one of the participants in the study and you will be kindly requested to give right answer according to the questioner direction.

I. General data

Study number _____ Study date _____

Sex _____ Date of birth ____/____/_____

Highest educational attainment

1. No formal education _____
2. Primary school (1-8) _____
3. High school (9-10) _____
4. College / university degree _____

II. Past medical history

1. Etiology of kidney disease

Chronic glomerulonephritis _____

Diabetic nephropathy _____

Hypertensive renal disease _____

Obstructive urophathy _____

Polycystic kidney disease _____

Others _____

2. Date of starting dialysis /____/____/____
3. Date of transplantation /____/____/_____
4. Timing of urinary catheter removal ____ early (within 7 days) ____ late (> 7 days)

5. How many times have you been transplanted _____
6. Occurrence of UTI in the past transplantation Yes _____ No _____

III. Current Medical History

1. History :
   - Frequency: _____
   - Dysuria: _____
   - Urgency: _____
2. Examination :
   - Temp $\geq 38^0$C _____
   - Tender suprapubic _____
   - Tender area over graft_____
3. Current immunosuppressives and their dosage

Drug	Dosage	frequency
Prednisolone	_____/_____	
Tacrolimus	_____/_____	
Mycophenolate	_____/_____	
Sirolimus	_____/_____	
Others	_____/_____	

4. Have you receive any antibiotics in the last 1 months?

   Yes _____ No _____

   If yes, which one(s): _____

2. Questionnaire (Amharic Version)

ለምለም ተስፋዬ እባላለሁ፡፡ የመጣሁት ከአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ የትምህርት ክፍል ሲሆን የዚህ ጥናት ዋና ዓላማ የሽንት ባክቴሪያ እና የፈንገስ በሽታ አምጪ ተህዋስያን እና ህመሙን የሚያባብሱ ተዛማጅነት ያላቸውን ነገሮች ማወቅ ነው፡፡ እርስዎ ጥናቱ ውስጥ ከሚሳተፉት ውስጥ አንዱ እንዲሆኑ ተመርጠዋል ስለሆነም በጥያቄው አቅጣጫ መሰረት ትክክለኛውን መልስ እዲመልሱ በአክብሮት እንጠይቅዎታለን፡፡

የሽንት ቧንቧ ኢንፌክሽን በኩላሊት ንቅለተከላ ተቀባይ ላይ፣.

I. አጠቃላይ መረጃ

የጥናት ቁጥር _____ የጥናት ቀን _____

ጾታ _____ የትውልድ ቀን ____ / ____ / _____

I. ከፍተኛየትምህርትደረጃ

1. መደበኛትምህርትየለም _____
2 ፡የመጀመሪያደረጃትምህርት(1-8) _____
3. ሁለተኛደረጃትምህርት(9-10) _____
4. የኮሌጅ / የዩኒቨርሲቲዲግሪ _____

II. ያለፈውየህክምናታሪክ

1. የኩላሊትበሽታ

ሥርየሰደደየጨጓራቁስለት _____
የስኳርበሽታነርቭበሽታ _____
የደምግፊትየኩላሊትበሽታ _____
የሆድድርቀት _____

ፖ/ሊክቲስቲክየኩላሊትበሽታ _____

1. ዲያሊስስ የጀመሩትቀን / ___ / _____ / _____
2. ንቅለተከላ ያደረጉበት ቀን / _____ / _____ / _____
3. የሽንት ካቴተር ማስወገጃ ጊዜ _____ ቀደም ብሎ (በ 7 ቀናትውስጥ) _____ ዘግይተው (› 7 ቀናት)
4. የኩላሊት ምንጭ _____
5. ስንት ጊዜ ተተክለው ነበር _____
6. ባለፈው መተላለፊትየየሽንት ቧንቧኢንፌክሽን ድግግሞሽ አዎን _____ አይ _____

III. ወቅታዊየሕክምናታሪክ

1. ታሪክ

- ☐ ድግግሞሽ _____
- ☐ ዲስሌሲያ _____
- ☐ አስቸኳይ

2. ምርመራ

- የመፀትመጠን ≥38^{0C} _____
- ንቅለተከላ አከባቢ ቀስለት_____

3. የወቅቱየበሽታመከላከያመድሃኒቶችእናየእነሱመጠን

የአደንዛዥ ዕፅመጠንድግግሞሽ

- ☐ ፕሪንሲሎንሎን _____ _____
- ☐ ሳይኮለላይን _____ _____
- ☐ ታኮሎመስ _____ _____
- ☐ማዮህኖላት _____ _____
- ☐ _____ ሌሎች _____ _____

4. በአለፉት 1 ወሮችውስጥምንምአንቲባዮቲክአግኝተዋል?

አዎ _____ የለም _____

አዎክ ሆነ፣የትኛው _____

Annex IV: Participants' Information sheet

1. Participants' Information sheet in English Version

Title of the Research Project: Bacterial and Fungal profile, antibacterial drug susceptibility pattern, and associated factors of isolates recovered from kidney transplant recipients' urine samples at Saint Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Principal Investigator: Lemlem Tesfaye (BSc, MSc candidate)

Name of the Organization: Addis Ababa University, College of Health Sciences School of Medical laboratory.

Introduction

First of all I would like to thank your cooperation to participate in the research. You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams include principal investigator and advisors from Addis Ababa University School of Medical laboratory Sciences and from Ethiopian public health institute, clinical bacteriology and mycology department. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

The purpose of the study is to determine Bacterial and Fungal profile, antibacterial drug susceptibility pattern, and associated factors of isolates recovered from kidney transplant

recipients' urine samples at Saint Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Duration

The study was conducted among transplant recipients' urine samples at SPHMMC from November 4, to April 4, 2021.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study. Specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required clinical samples will be collected by trained professional. You are requested to give your consent to the sample collector. After consent, a sample will be taken from Vein puncture. Moreover, there will be structured questioner for additional information.

Potential risks and Discomfort

During collection of specimen from you, appropriate protection will be taken and all samples will be collected by trained health professionals. If anything happened, appropriate medical care will be provided to you.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related with your social interest but any information that is obtained in connection with this study will remain confidential.

Potential benefits to subjects and the society

You will not receive any payment for your participation in this research. However, based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial for management of urinary tract infections. Therefore, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and prevent your child's participation in the study. This decision will not affect you and your child's current or future medical care in the health facility. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result free. The information you give us is important to prevent and control the scope of the problem, we respectfully ask you to answer directly to the question.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisor for further information.

Lemlem Tesfaye Phone: 0913 386543 E-mail: lemlemtesfaye11@gmail.com

Dr. Adane bitew Phone: 0911 03 91 62 E-mail: adane.bitew@aau.edu.et

2. Participants' Information sheet in Amharic Version

የጥናቱ ርዕስ-

በቅዱስ ጳዉሎስ ሆስፒታል ውስጥ ከኩላሊት ከተተከሉ የኩላሊት ተቀባዮች የሽንት ናሙናዎች ላይ በሽታ የሚያመጡ ጀርሞችን መጠን ማወቅ ፣ የፀረ-ተህዋሲያን የመቋቋም ዘይቤ እና ህመሙን የሚያባብሱ ተዛማጅነት ያላቸውን ነገሮች ማወቅ ::

የዋናተመራማሪውስም- ለምለም ተስፋዬ

የተቋሙስም-

አዲስአበባዩኒቨርሲቲየድህረምረቃትምህርትቤት፤የጤናናሳይንስኮሌጅ፤የህክምናላቦራቶሪትምህርትክፍል

መግቢያ

በአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየሕክምናላቦራቶሪሳይንስት/ክፍልበማስተርስድግሪተማሪየመ መረቂያጥናትላይእዲሳተፉተጋብዘዋል፡፡ጥናቱንየሚያካሄደውከአዲስአበባዩኒቨርሲቲየህክምናላብራቶ ሪሳይንስዋናመርማሪእናበአንድየአዲስአበባዩኒቨርሲቲየጥናቱአማካሪነው፡፡እባክዎበዚህጥናትለመሳተ ፍከመስማማትዎበፊትከዚህቀጥሎየሚገኘዉንምንባብበጥሞናያንብቡናግልጽያልሆነልዎትንማንኛዉ ምሃሳብይጠይቁ፡፡

የጥናቱዓላማ- የጥናቱዓላማ በቅዱስ ጳዉሎስ ሆስፒታል ውስጥ ከኩላሊት ከተተከሉ የኩላሊት ተቀባዮች የሽንት ናሙናዎች ላይ በሽታ የሚያመጡ ጀርሞችን መጠን ማወቅ ፣ የፀረ-ተህዋሲያን የመቋቋም ዘይቤ እና ህመሙን የሚያባብሱ ተዛማጅነት ያላቸውን ነገሮች ማወቅ ነው፡፡

የጥናቱዘዴእናየሚጠበቅበዎትተሳትፎ-

በዚህጥናትለመሳተፍፈቃደኛከሆኑየጥናቱንዓላማመረዳትናስምምነትዎንመስጠትያስፈልግዎታል፡፡ ከእርስዎየተሰበሰቡናሙናዎችለምርምርዓላማጥቅምላይይውላሉ፣እናየናሙናዎውጤትእንደአስፈላ ጊነቱለሚመለከታቸውየባለሙያሰራተኞችናለስራዉአግባብነትላላቸዉሰዎችቢነገርየማይቃወሙመሆ ኑንመስማማትይጠበቅብዎታል፡፡የጥናቱንዓላማለማሳካትየሚያስፈልገው የሽንት ናሙና ነዉ፡፡ በተጨማሪም ስለ ርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይኖርብዎታል፡፡

ጥናቱ የሚያደር ሰው ችግር:

ናሙናበሚሰበሰብበትወቅትምንምአይነትየከፋችግርአያጋጥምዎትም፡፡ሆኖምግንናሙናዉንለመሰብሰ ብልምድያለዉባለሙያስለሚመደብናአስፈላጊዉየጥንቃቄእርምጃስለሚወሰድየህመምስሜትአይኖር ም፡፡ለዚህምተገቢውንህክምናያገኛሉ፡፡

የጥናቱሚስጢራዊነት-

ስለራስዎየሰጡትማንኛዉምመረጃናከተወሰደዉናሙናላይየተገኘዉየላቦራቶሪዉጤትየሚዉለዉለጥ

ናቱአላማብቻነዉ፡፡ይህንማህደርሊያገኙየሚችሉትየተወሰኑየጥናቱተባባሪሰዎችብቻናቸው፡፡ከዚያምበ
ላይስለእርስዎያለውንማንኛውንምመረጃየተለየየይለፍቃልባለውየኮምፒውተርየመረጃማህደርውስጥ
እንዲቀመጥይደረጋል፡፡ከማህበራዊፍላጎትዎጋርእንዲጠየቁየሚያስገድድምንምአይነትስጋትየለውም
፣ነገርግንከዚህጥናትጋርበተያያዘየተገኘውመረጃበምስጢርየተጠበቀነው፡፡

ጥናቱየሚያስገኘውጥቅም-

በዚህምርምርውስጥየሚያጉትምንምዓይነትየገንዘብእንዲሆምየተለየጥቅማጥቅምባይኖርምከጥናቱበ
ሚገኘውውጤትላይበመመርኮዝሕክምናይደረግልዎታል፡፡የእርስዎተሳትፎየእርስዎንናየወገንዎትን
የሽንትኢንፌክሽኖችንለማወቅናለመቆጣጠርከፍተኛጥቅምይኖረዋል፡፡ስለዚህበተዘዋዋሪሌሎችህመም
ተኞችንእናህብረተሰቡንበዚህመልኩእየተጠቀሙነው፡፡

በጥናቱያለመሳተፍወይምራስንከጥናቱየማግለልመብት-

የእርስዎበዚህጥናትላይየሚኖርዎትተሳትፎሙሉበሙሉበበጎፈቃደኝነትላይየተመሰረተነዉ፡፡በማንኛ
ውምሰዓትመውጣትእናልጅዎበጥናቱውስጥእንዳይሳተፍመከላከልይችላሉ፡፡በዚህጥናትዉስጥላለመ
ሳተፍወይምለመሳተፍከወሰኑበኃላለማቋረጥየሚወስኑቢሆንምእንኩዋበዚህ ሆስፒታል የሚሰጠዉ
ማንኛዉም አገልግሎት አይቋረጥም፡፡ ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም
አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለብዎት፡፡ የላብራቶሪ ምርመራ ውጤቱንም
በነጻ ማግኘት ይችላሉ፡፡ ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና
ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ
አክብሮት እንጠይቃለን፡፡

በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ
ማስቀመጥ ይጠበቅዎታል፡፡

ተጨማሪመረጃዎችንለማግኘትከፈለጉ

ይህንንጥናትበተመለከተወይምከዚህጥናትጋርበተዛመደመልኩስለሚያጋጥሙድንገተኛአደጋዎችወ
ይምጥያቄካለዎትበሚመለከተውአድራሻይጠቀሙ፡፡

ለምለም ተስፋዬ ስልክ: 0913 38 65 43 **ኢ-ሚይል**: lemlemtesfaye11@gmail.com

ዶ/ር አዳነ ቢተው ስልክ: 0911 03 91 62 **ኢ-ሚይል**: adane.bitew@aau.edu.et

Annex v: Informed consent/Ascent form

1. Informed consent form for adults (≥18 years) in English version

Code No…..……

I had been informed that the objective of this study is to determine the prevalence urinary tract infections, antimicrobial susceptibility profile and assess risk factors. The results of this study have an importance to treat me and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosing of urinary tract infections in Ethiopia. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include urine sample and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation results.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

For those who can't read the information

Advisor name……..

Signature ……

Date ……..

2. Informed consent form for adults (≥18 years) in Amharic version

የሚስጥር ቁጥር ----------

የጥናቱ አላማ የሽንት ናሙናዎች ላይ በሽታ የሚያመጡ ጀርሞችን መጠን ማወቅ ፣ የፀረ-ተህዋሲያንየመቋቋም ዘይቤ እና ህመሙን የሚያባብሱ ተዛማጅነት ያላቸውን ነገሮች ማወቅ እነደሆነ በቂ ገለጻ ተደርጎልኛል። ለጥናቱም የሽንት ናሙና እና መጠይቅን መመላት እንደሚያስፈልግ ተገልጾልኛል ። የጥናቱንምአላማዎችምተረድቻለሁ።በቃለመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደ ሚሆኑ ተነግሮኛል። በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለ መስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል።

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ። የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ።

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

ፊርማ---------- ቀን ----/---/----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ስም ----------

ፊርማ ……

ቀን----------

Declaration

I, the undersigned agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office.

M.Sc. candidate: Lemlem Tesfaye

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Dr Adane Bitw (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.