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Bacterial Urinary Tract Infection among Adult Renal Transplant Recipients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

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List of abbreviations

ABU	Asymptomatic Bacteriuria
AGP	Acute Graft Pyelonephritis
AMR	Antimicrobial Resistance
APN	Acute Pyelonephritis
AST	Antibiotic Susceptibility Testing
ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standard Institute
DJ	Double J stent
ESBL	Extended Spectrum Beta Lactamases
ESRD	End Stage Renal Disease
GFR	Glomerular Filtration Rate
IDSA	Infectious Diseases Society of America
RTXs	Renal Transplant Recipients
SPHMMC	St. Paul's Hospital Millennium Medical College
SPSS	Statistical Package for Social Science
UTI	Urinary Tract Infection

Abstract

Background: Although significant advances have been made in surgical techniques and immunosuppression for renal transplantation, urinary tract infections continue to be a major public health problem globally. Post-transplantation urinary tract infection complications including asymptomatic bacteriuria, cystitis, and pyelonephritis are the most common form of bacterial infection following renal transplantation. It could result in graft function impairment and death.

Objectives: The study aimed to investigate the prevalence of bacterial isolates that cause urinary tract infections, assess antibiotic susceptibility pattern among symptomatic and asymptomatic renal transplant recipients attending for health care at St. Paul's Hospital Millennium Medical College.

Materials and Methods: A hospital-based cross-sectional study was carried out from December 2017 to August 2018 among 74 renal transplant recipients. The sociodemographic characteristics were collected using structured questionnaires. A first morning voided clean-catch mid-stream urine specimens were collected and 0.001ml inoculated onto a blood and MacConkey agar plates following the standard bacteriological protocols. It was incubated aerobically at 35–37°C for 18–24 hours. Cultural characteristics and series of biochemical tests were used for identification.

Results: Significant bacteriuria was found in 11/74 (14.9%), 95%CI =8.2-24.7) of the patients. The prevalence among females 6/32(18.75%) was higher than males 5/42(11.9%) without significant association (COR=2.09, 95%CI=1.04-8.45, P=0.253). UTI was higher in the age group between 35–49 years old (19.3%. Age was statistically significant and stronger independent associated risk factor with crude odds ratio=3.67, 95%CI=2.89-20.07 and 0.003, respectively. The most prevalent bacteria isolates were *Escherichia coli* 2(18.2%), *Staphylococcus aureus* 2(18.2%), *Acinetobacter spp*. 2(18.2%), *Enterococcus spp*. 2(18.2%), Coagulase-negative *Staphylococci 2*(18.2%)] followed by *Portus mirabilis* 1(9.1%).The majority (80%) of Gram-negative bacteria were resistant to ciprofloxacin, chloramphenicol, and trimethoprim/sulfamethoxazole. Multi drug resistance pattern was shown in 82% of the isolates.

Conclusion and Recommendations: The overall prevalence of UTI in the study was low with a prevalence of 14.9%. Better to establish routine urine cultures especially in the first 6-12 months.

Keywords: Kidney Transplantation, Urinary Tract Infection, Urine Culture, Antimicrobial Susceptibility Testing, St. Paul's Hospital Millennium Medical College.

Chapter I: Introduction

1.1. Introduction

Healthy person's urinary tract is being protected against infections by several nonimmune and immune mechanisms that are not fully functional in kidney transplant patients. Urinary tract infection (UTI), a mainly bacterial origin, is a common infectious complication in kidney transplant recipients with the global incidence that may up to reach 80% of all infections (Gozdowska *et al*., 2016). Post-transplantation UTI complications including asymptomatic bacteriuria, cystitis, and pyelonephritis are the most common form of bacterial infection following renal transplantation; which could result in graft loss. These can occur at any time after transplantation but with the highest incidence in the first 3-6 months after transplantation (Parasuraman and Julian, 2013).

In kidney transplant recipients, most episodes of UTI occur during the first 6 months after the transplant, the first month being the main period of events. During the first month, asymptomatic bacteriuria (ABU) occurs in 22%-71% of the patients and symptomatic UTI in 12%-34%. Despite improved surgical techniques, antimicrobial prophylaxis, new immunosuppressive therapies, and hygiene measures in the management of transplant patients, infectious complications remain a major cause of morbidity and mortality in solid organ transplantation patients and urinary tract infections are one of the most common infectious complications among renal allograft recipients (Fiorentino *et al*., 2019). The overall incidence of UTI in solid organ transplant recipients (SOT) was reported about 0.23 episodes/1000 days of transplant. This incidence varies significantly depending on the type of transplanted organ. Kidney recipients have the highest risk of developing UTI, with an incidence of 0.45 episodes/1000 days of transplant and a frequency of (7.3%), followed by kidney-pancreas 0.22/1000 (5%), heart 0.07/1000 (2.2%), liver 0.06/1000 (1.6%) and lung 0.02/1000 (0.7%). In the meantime, other authors described an incidence that ranged from 4% to 75% in renal allograft recipients (Fontser *et al*., 2017; Vidal *et al*., 2012).

It was indicated that 25% - 47% of renal recipients have at least one symptomatic UTI. Reported UTI discrepancy may be explained by the heterogeneity at establishing the definition of UTI and its clinical manifestations like asymptomatic bacteriuria (ABU), acute pyelonephritis (APN), lower UTI and urosepsis (Origuen *et al*., 2017). Others include differences in diagnostic criteria like frequency of routine urine culture testing, different follow-up times, different surgical techniques strategies,

diversity in the use of antimicrobial prophylaxis, the immunosuppression regimens employed and the study design (Khosravi *et al*., 2014).

While kidneys are the most frequently transplanted organs and renal transplantation is the preferred method for treating patients with end-stage renal disease (ESRD); post-transplantation urinary tract infection (UTI) is still a source of morbidity and graft failure, which reported worldwide. Renal transplant recipients develop UTIs more frequently than the general population (Säemann and Hörl, 2008). It is noteworthy that bacterial species leading to UTIs in renal transplant recipients are similar to those causing UTIs in the general population but UTI management in renal transplant recipients is undoubtedly more complex compared with the general population (Goldman JD and Julian K, 2019).

Several factors may contribute to kidney allograft loss. The frequency of UTIs depends on many factors such as age, female gender of renal transplant recipients, underlying co-morbidities (concomitant diseases), immunosuppressive protocol or the follow-up period. Now a day, kidney transplantation remains a well- established and treatment of choice for selected end-stage renal disease (ESRD) aimed patients to extending their survival and improving their quality of life while benefiting from the reduction in the mortality associated with long-term dialysis (Fiorentino *et al*., 2019). This is because ESRD is a progressive, debilitating chronic illness whereby kidneys are no longer capable of adequately removing fluids and wastes from the body or maintaining the proper level of certain kidney regulated chemicals in the bloodstream (Chuang *et al*., 2005*)*. Although prophylactic antimicrobial medications which commonly prescribed in the first 6-12 months after kidney transplantation, infections are a common complication in the early post-transplant period. Infections clearly contribute to post-transplant morbidity, mortality and costs, but estimates of these impacts in contemporary, national samples are lacking (Ariza *et al*., 2014).

UTIs among transplant recipients are more likely to be clinically asymptomatic compared to patients not on immunosuppressive therapy and are often associated with serious morbidity and even death. Post-transplantation UTI owing renal failure remains a serious cause of mortality and morbidity worldwide; with incidences being higher (Julien and Daniel, 2014). The prevalence especially in resource-limited settings like our country, Ethiopia, is implicated by a recent study done in Yemen that have shown that the incidence of bacterial UTI raises to 33.3% in a cohort of 150 renal transplant recipients resulting in a grave impact to the allograft outcome and survival (Gondos *et al*.,2015).

1.2. Statement of the problem

UTI is the most common infective complication following renal transplantation with the global incidence that may reach up to 80% of all infections. The infection can precipitate graft dysfunction, sepsis and death. The risk of post-transplantation UTI is multifactorial and determined by host, organism and anatomical factors that are interrelated to each other (Pelle *et al*., 2007). Asymptomatic bacteriuria and urinary tract infection are common complications after kidney transplantation (Deepa *et al*., 2017). In the population, if urinary tract infection occurred in the first six months post procedure, it carries a grave impact on both graft and patient survival in addition to infectious complications and bacteremia (Yacoub and Akl, 2011). Renal transplant recipients with urinary tract infection are often clinically asymptomatic due to immunosuppression. UTI may progress to acute pyelonephritis, bacteremia and the full-blown picture of urosepsis. The bacteremia- associated mortality of kidney allograft recipients is around 11% during the first- month post-transplant. On the other hand, if treating asymptomatic bacteriuria would not be of any benefit, it may impose a burden of potential side effects as well as the excessive costs on these patients (Origuen *et al*., 2017).

The absence of symptoms in patients with asymptomatic bacteriuria could reflect characteristics specific to the pathogen, the host or both. Some bacteria need a specific mechanism to cause symptomatic disease, such as attachment of bacteria via fimbriae adhesions, while some others with reduced capability for fimbriae expression appear to have the capacity for relatively rapid growth that allows them to cause asymptomatic bacteriuria (Kumar *et al*., 2018). Several studies declared that Gram-negative bacterial infections account for more than 70% of UTI and *E. coli* is the most common clinical isolate not only in the general population but also after kidney transplantation in many abundant documents. Enterobacteriaceae, *Enterococci*, *Staphylococci* and *Pseudomonas* are common pathogens (Khosravi *et al*., 2014). Several isolates from renal allograft recipients have been shown to be resistant to trimethoprim and sulfamethoxazole, which raises the question of the effectiveness of using these medications prophylactically (Yuan *et al*., 2018). The prevalence of UTIs may vary widely with age, sex and the presence of genitourinary abnormalities (Gondos *et al*., 2015). In women, the prevalence of asymptomatic bacteriuria among healthy women increases with advancing age from about 1% among schoolgirls to >20% among women over 80 years residing in the community. On the converse, in men, asymptomatic bacteriuria is rare among healthy young men, but prevalence

increases with advancing age to reach about 6% at 60 years of age and to 15% of men over 75 years of age. (Origuen *et al*., 2017).

Patients with indwelling urinary devices and on hemodialysis are at increased risk of having asymptomatic bacteriuria among renal transplants. Short-term indwelling urethral catheters acquire bacteriuria at the rate of two to seven percent per day; this risk reaches 100% in patients with long term indwelling urethral catheters and patients with the permanent ureteric stent. Patients undergoing hemodialysis have a prevalence of asymptomatic bacteriuria of 28% (Hollyer Ian and Michael G Ison, 2018). According to some reports, 25% to 50% of elderly women and 15%–40% of elderly men in long-term care facilities have bacteriuria. Asymptomatic bacteriuria in renal-transplant recipients in the early post-transplantation period is a serious disease (Origuen *et al*., 2017).

The effect of bacteriuria on transplant recipients can divide into its effect on morbidity and mortality caused by the infection; and second, potential effects of infection on developing rejection and its clinical course. Thus, treatment should be started with parenteral antibiotic therapy and be continued until the culture is negative. Many studies have not reported a significant association between asymptomatic bacteriuria and graft survival (De Souza and Olsburgh, 2008). These results might be biased by the fact that transplant recipients with poor graft survival could suffer from urinary infections due to urologic abnormalities. Advances in the clinical management of kidney transplant recipients have been yielding substantial improvements in short-term allograft survival and mediated in part by the reduction in the incidence of acute rejection (Vidal *et al*., 2012). Due to the high prevalence of urinary infection within the first post-transplant months and its correlation with worse patient and graft outcome, most centers use anti-microbial prophylaxis therapy shortly after the procedure despite the lack of a uniform period of treatment. While the others argue, there is no consensus about the period of therapy and it is not clear yet whether or not to treat every episode of asymptomatic bacteriuria after the first months in RTXs (Nicolle *et al*., 2005).

In Ethiopia, there is no published data assessing the prevalence UTI and local microbial patterns along with their drug sensitivity pattern in kidney transplant recipients which have a paramount importance to design either institutional based therapeutic guidelines for public health policymakers or to establish smooth communication between the attending physician and clinical microbiologist. This is particularly significant to establish rigorous infection preventions and control strategies in kidney transplant recipients aimed to mitigate hospital and community acquired infections.

1.3. Literature review

1.3.1. Definition of Post Renal Transplant UTI

Urinary tract infection can be defined as the microbial invasion of any of the tissues of the urinary tract extending from the renal cortex to the urethral meatus that encompasses the organs to collect and store urine and release it from the body like kidneys, ureters, bladder, urethra and accessory structures. It is usually due to bacteria from the digestive tract, which can ascend to the opening of the urethra and begin to multiply to cause infection (Flores-Mireles *et al*., 2015). In similar occasion, post renal transplant UTI is the most common infective complication following renal transplantation with the global incidence that may reach up to 80% of all infections in solid organ transplantation. These infections can precipitate graft dysfunction, sepsis, and death. Depending on the onset of illness, it could be categorized as early or late UTI (Abbott *et al*., 2004). Asymptomatic bacteriuria and symptomatic UTI are common complications after kidney transplantation. If UTI occurred in the first six months' post procedure, it carries a grave impact on both graft and patient survival in addition to infectious complications and bacteremia. That is why renal transplant recipients with UTI are often clinically asymptomatic as consequence of immunosuppressant regimens (Kotagiri *et al*.,2017; Memikoğlu *et al*., 2007).

UTI can be either symptomatic, asymptomatic, complicated, uncomplicated, relapsing or recurrent. Patients with significant bacteriuria and have at least two symptoms referable to UTI (dysuria, urgency, frequency, suprapubic pain, flank pain or costovertebral angle tenderness, fever ($\geq 38\ 0^{C}$) and chills ($\leq 36\ 0^{C}$) are said to be symptomatic. Asymptomatic bacteriuria (ABU) is a condition, characterized by the presence of bacteria in two consecutive clear-voided midstream urine specimens both yielding positive cultures ($\geq 10^{5}$ cfu /ml) of the same uropathogns in a patient without classical symptoms of UTI (Nicolle *et al*., 2005; Veroux *et al*., 2008).

1.3.2. Etiologic agents of Urinary Tract Infection

Organisms that cause UTI after renal transplantation can be bacteria, fungi, virus and parasite. However, bacterial origin account for the highest proportions of post -renal transplantation UTI (Tale 1.1). These bacterial origins of UTI may worsen both graft and patient survival leading to a significant proportion of mortality and morbidity in renal transplant recipients particularly those with acute pyelonephritis (APN); which is an independent risk factor for deterioration of graft function. UTI in

RTXs is often asymptomatic owing immune quell. Repeated screening for bacteriuria should be routinely done to treat them if necessary; It is thus mandatory that antibiotic policy is framed based on the antimicrobial susceptibility testing (Pelle *et al*., 2007; Helen, 2018). Cross sectional study of (n=50) among asymptomatic renal allograft recipients has shown that isolates of *E. coli, K. oxytoca* and *P. vulgaris* were most common etiologic agents. Gram-negative bacterial infections account for more than 70% of UTI and *E. coli* is the most common clinical isolate in patients with UTIs not only in the general population but also after kidney transplantation (Deepa *et al*., 2017; Fiorante *et al*., 2010; Shams *et al*., 2017).

Others like *Pseudomonas aeruginosa*, Coagulase-negative *Staphylococci* or *Enterobacter cloacae* frequently detected in the urine within the first 3–5 weeks following kidney transplantation, while *Enterococcus species* and *E. coli* predominantly detected during the first 6 to 12 weeks, respectively after surgery. These microorganisms are frequently found to be resistant to trimethoprim-sulfamethoxazole showing that *E. coli* in 84%, *E. cloacae* in 67%, coagulase- negative *Staphylococcus* in 86% and *Enterococcus* species in 46% of cases (Parasuraman R and Julian K, 2013). However, clinicians should be aware of the possibility of other microbial infections such as fungal, parasitic and viral pathogens (Shahid and Coleman, 2018). The MDR pathogens are still posing the major threat on RTXs (Yuan *et al*., 2018).

Low-virulence bacteria that would not be pathogenic in immunocompetent hosts have implicated in post-transplantation UTI. It has suggested that immunosuppressant drugs can increase organism virulence; which facilitates bacterial urothelial adherence. Now a day, bacteria that become resistant to antimicrobial therapies are becoming problematic. It is likely that the organism and the strain that cause most post-transplantation UTIs vary between centers, depending on local immunosuppressive and antimicrobial protocols (Fiorentino *et al*., 2019; Papasotriou *et al*., 2011). The most common species isolated from urine samples included *E. coli* (42%), *Klebsiella pneumoniae* (15%) and *Enterococcus faecalis* 10% (Gozdowska *et al*., 2016). Concerning to late infections (i.e. occurring >6 months after kidney transplant), one study revealed that out of 102 isolates obtained, *E. coli* (n =47 [46%]) being the most isolate followed by *E. faecium* (n =10 [10%]), *K. pneumoniae* (n =9 [9%]), *E. faecalis* (n = 8 [8%]), and *P. aeruginosa* (n =6 [6%]) (Abbott *et al*., 2004). Rarer species included *Staphylococcus haemolyticus, Klebsiella oxytoca, Proteus mirabilis, Enterobacter cloacae, Staphylococcus epidermidis* (Kotagiri *et al*., 2017).

Table 1.1. Bacterial etiologic agents of post renal transplant urinary tract infections

UTI by types of pathogens (Helen, 2018)	
Types	**Common pathogens**
Un complicated UTI	*E. coli, S. saprophyticus, Enterococcus spp., K. Pneumoniae, P. mirabilis*
Complicated UTI	antibiotic-resistant *E. coli, P. aeruginosa, Acinetobacter baumannii, Enterococcus spp., Staphylococcus spp.*
CA-UTI	*P. mirabilis, Morganella morganii, Providencia stuartii, U. urealyticum.*
Recurrent UTI	*Enterobacter spp*., antibiotic-resistant *E. coli, Enterococcus spp., Staphylococcus spp. , P. mirabilis, K. pneumoniae*

CA-UTI = Catheter-Associated Urinary Tract Infection; **UTI** = Urinary Tract Infection

1.3.3. Epidemiology of Urinary Tract Infection

Urinary tract infections (UTIs) are a severe public health problem and caused by a range of pathogens; but most commonly by *E.coli, K.pneumoniae, P.mirabilis*, *E. faecalis* and *S.saprophyticus* (Figure 1.1). High recurrence rates and increasing antimicrobial resistance among uropathogns greatly increase the clinical and economic consequences (Voroux *et al*., 2008). UTIs are affecting 150 million people each year globally exceeding 1.4 million RTXs cases with 8% rising annual incidence (Naik *et al*., 2016). In 2007, in the United States alone, an estimated 10.5 million office visits for UTI symptoms (constituting 0.9% of all ambulatory visits) and 2-3 million emergency department visits. Currently, the societal costs of these infections including health care costs and time missed from work are approximately US $3.5 billion per year in the United States alone. UTIs are a significant cause of morbidity in infant boys, older men, and females of all ages (Flores-Mireles *et al*., 2015). Serious sequelae include frequent recurrences, pyelonephritis with sepsis, renal damage in young children, pre-term birth and complications caused by frequent antimicrobial use, such as high-level antibiotic resistance and *Clostridium difficile* colitis (Foxman, 2014; Valera et al., 2006).

A retrospective study to identify potential risk factors and microbiological profile (n=276) revealed that 67% were men with a mean age of 51years & at 12 months' post-transplantation 158 (57%) RTXs had no bacteriuria, 75 (27%) had asymptomatic bacteriuria, 21 (8%) had symptomatic UTIs without further complication and 22 (8%) with UTIs developed pyelonephritis or urosepsis. Most frequent pathogens identified were *E. faecalis* (35%) and *E. coli* (32%) and 36% of organisms were multidrug resistant (Kotagiri *et al*, 2017).

Post kidney transplantation UTI is common; affecting nearly half of RTXs in the first year after transplantation. Treatment of asymptomatic bacteriuria may be beneficial to prevent subsequent episodes of symptomatic UTIs, bacteremia, urosepsis, grave allograft and patient survival disregard of harmonized guidelines (Ak *et al*., 2013). Despite noticeable progress in surgical procedure and immunosuppression after kidney transplantation, UTI remains an important problem in these patients (Adamska *et al*., 2015).

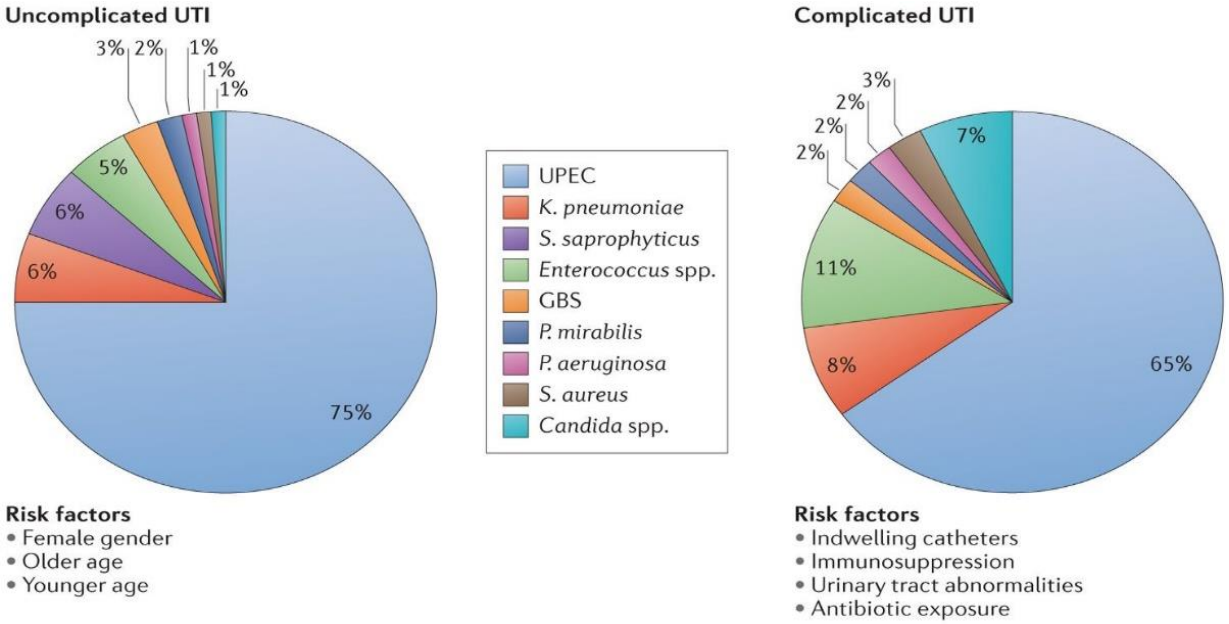

Figure 1.1. The epidemiology of Urinary Tract Infections (Flores-Mireles *et al.*, 2015)

According to a retrospective study including (146 males and 101 females) in Iran who were followed up for 1 year after transplantation, the finding indicated that UTI incidence was reported 22.7% among kidney transplant recipients and 41 patients had one UTI, 11 individuals had two UTIs, and four cases had three UTIs. The utmost frequent isolated microorganisms were *E. coli* (55.3%), coagulase-negative *Staphylococcus* (13%) and *Klebsiella spp.*(12.5%). Around 26.6% of infection episodes occurred during the 1st month after transplantation and mean time between transplantation and UTI occurrence was 79.5 ± 83.4 days. In the study, female kidney transplant patients have a statistically significant higher occurrence of UTIs versus male allograft recipients ($P < 0.005$) (Shams *et al*., 2017). The frequent kind of infection in kidney allograft recipients may have a varied range of 6%–86% but this significant variation in UTI reported rates might be due to local outbreaks, varying resistance

incidences, postoperative medical care, center-specific immunosuppressive therapy, hygienic states, and different diagnostic criteria (Shin *et al*., 2017).

Impact of UTI on short-term kidney graft outcome in Spain showed that of 867 patients who received a kidney transplant, 184 (21%) developed at least one episode of UTI, at a median of 18 days after transplantation. The prevalence of acute graft pyelonephritis (AGP) was 15%. The most frequent pathogens identified were *E. coli, Klebsiella* species, and *P. aeruginosa*, 37% of which considered multi-drug-resistant strains. Thirty-eight patients (4%) lost their grafts, 225 patients (26%) had graft function impairment and the 1-year mortality rate was 3%. Surgical re-intervention and the development of at least one episode of AGP were independently associated with 1-year graft function impairment. Moreover, the development of at least one episode of AGP was associated with graft loss at 1 year (Fontsere *et al*., 2017). Patients with AGP caused by a resistant strain had graft function impairment more frequently although this disparity did not reach statistical significance that was 53% vs. 36%, p =0.07 (Bodro *et al*., 2015; Ariza *et al*., 2014).

A nationwide assessment in the USA among a total of 1,044 renal transplant recipients representing a population estimate of 49,862 showed that UTI was most common in transplant recipients and prevalence was noted to be 28 and 65.9 cases per1000 for men and women respectively. UTI increased the likelihood of transplant complications (182% for men, 169% for women). Such infection also increased the length of stay by 87% among men and 74% among women. The study finally concludes UTI in renal transplant recipients was associated with prolonged length of stay, total charges and increased odds of transplant complications (Becerra *et al*., 2015). A single-center retrospective cohort study of (n=99) renal transplant patients followed for the first year after surgery in Brazil indicated that 320 (17.3%) tested positive for bacterial growth. Twenty-six (26%) patients developed UTI. The most frequent microorganisms isolated from patients with UTIs were *K. pneumoniae* (36%), with 33% of the strains resistant to carbapenems, followed by *E. coli* (20%) (Menegueti *et al*., 2015).

Another retrospective cohort observational study in Portugal among 127 patients showed that 53 patients (41.7%) presenting with at least one episode of UTI; 21 patients (16.5%) had recurrent urinary tract infection. Female gender was the only risk factor associated with the occurrence of urinary tract infections ($p < 0.001$, OR = 7.08, RR = 2.95) and recurrent urinary tract infections ($p < 0.001$, OR = 4.66, RR = 2.83). *E. coli* (51.6%), (15.5%) *K. pneumoniae* & *Enterobacter spp.* (9.9%) were the most

frequently identified pathogens. Urinary tract infections were the most important cause of hospital admissions (Bispo *et al*., 2014).

A retrospective study in Saudi Arabia among 27 adult renal transplant patients again dictated that the average age of the participants was 41.3±16.2 years, 16 - 73 years. Male RTXs were 51.9% (N = 14) and females were 48.1% (N = 13). Incidence of post-renal transplant UTI was 55.5% (N = 15) and gender (69.2% of the female RTXs developed UTI versus 30.8% of the males. *E. coli* was the most common pathogen (53.3%, N = 8) followed by *P. aeruginosa* (20%, N = 3). Similarly, most of the UTIs (73.3%) detected within one-month post-renal transplant and recurrent infection observed in 40.1% of the patients (Alkatheri, 2013).

A cross-sectional study in Iran showed that UTI diagnosed in 391 patients (33.56%). Gram-negative bacteria were the most prevalent isolated microorganisms with *E. coli* (43.53%), followed by *Enterobacter spp.* (35.37%) as the major organisms. Among Gram-positives, Coagulase-negative *Staphylococci* was isolated from 6.8% of cases. The rate of resistance to all tested antibiotics was highest in *Enterobacter spp*., however, the most common resistance was seen against cefixime, Cephalothin and cotrimoxazole in all tested Gram-negatives (Khosravi *et al*., 2014).

Another cross-sectional study that included 150 patients in Yemen showed that bacterial UTI found in 50 patients (33.3%). The prevalence among females 40.3% was higher than males 29%. UTI was higher in the age group between 41–50 years with a percentage of 28% and this result was statistically significant. The most prevalent bacteria to cause UTI was *E. coli* represent 44%, followed by *S. saprophyticus* 34%. Amikacin was the most effective antibiotic against Gram-negative isolates while ciprofloxacin was the most effective antibiotic against S. *saprophyticus* (Gondos *et al*., 2015). Another retrospective study done in Libya among (n=112), UTI diagnosed in 33 patients (29.5%). The mean age of affected patients was 43±20 years with a range of 20-63 years. Most of the episodes (72%) occurred during the first 3 months after transplantation and 60.6% of affected patients had more than one episode of infection. A larger proportion of females were affected than males (40.8% versus 20.6% respectively, P=0.02). No significant difference was detected in the proportion of affected patients whether the donor was living unrelated or living related (32.3% versus 28.4% respectively, P=0.43).The commonest causative microorganism was *E.coli* (38.7%), followed by *Klebsiella* (25.8%), *Staphylococcus* (25.8%), and others (9.7%). The commonest drug used for treatment was

ciprofloxacin (51.6%), followed by amoxicillin-clavulanic acid (22.6%), meropenem (12.9%) and others 12.9% (Elkehili *et al*., 2010).

1.3.4. Risk factors associated with post- renal-transplant UTI

Many risk factors are associated with post-transplantation UTI that enhance incidences like in renal graft loss or dysfunctions. For example, in the female gender, the shorter urethra and relative proximity of the urethra to the anus contribute to an increased risk of UTI compared with men (Vavalo et al., 2013; Fiorentino *et al*., 2019). Generally, many risk factors for UTI in renal allograft recipients are similar to those in the general population; especially the increased risk of developing a UTI in females. Similarly, anatomical factors predisposing to UTI like urinary stasis reflux and stones are more prominent in renal transplant recipients. Female gender, advanced age, pre-transplant UTIs, prolonged dialysis period, immunosuppression, acute rejection episodes, impaired graft function, bladder catheter postoperatively, technical complications associated with ureteral anastomosis , intraoperative ureteral stents , surgical manipulation of the graft (allograft trauma), contaminated graft perfusion solution , diabetes mellitus, history of vesicoureteral reflux, history of polycystic kidney disease, cadaveric donor are independent risk factors (Papasotiriou *et al*.,2011). The etiology of UTI following renal transplantation can be examined in terms of factors that relate to the host, the graft, the anatomical features of the recipient and the infection-causing organism. However, these factors can overlap and interact with each other. This reflects multifactorial causality (Elkehili *et al*., 2010; Ndemera and Bhengu, 2017).

I. Host factors

Female gender, advanced age, pre-transplant UTIs, diabetes mellitus, prolonged dialysis before transplantation and net immunosuppression have been shown to raise the risk of UTI. Shorter urethra in females and relative proximity of the urethra to the perirectal area raise the risk of UTI compared to men. Chuang *et al*.(2005) has shown that 55% of the patients 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. Higher risk attributed to impaired mobility, poor hygiene in institutionalized individuals, reduced native immunity, a higher rate of urinary retention secondary to prostatism and bladder atrophy (Ciszek *et al*., 2006). Untreated or partially treated pre-transplant UTIs pose a risk of progression or reactivation after transplantation. Immunosuppression places the transplant recipient at risk of all types of infections, including UTI. The net state of immunosuppression is the result of a

complex interaction among multiple factors, including immunosuppressive therapy (drug, dose, and duration), underlying immune deficiency, autoimmune disease, functional immune deficits, neutropenia, lymphopenia, uremia, malnutrition, and infection with immunomodulation viruses (Ooms *et al*., 2017 ; Sadeghi *et al*., 2005).

II. Surgical factors

A urethral catheter, which is a routine placement, is likely related to early post-transplantation UTI even when sterile technique is used. In the general population, the risk of bacteriuria increases by 5% with each day that a catheter is in situ. This increased risk is likely to apply to transplant recipients. Prompt catheter removal has been associated with a drop in UTI rates. Ureteric stents inserted at the time of transplantation to prevent leakage from the vesicoureteral anastomosis are associated with a 1.5-times increased relative risk of UTI. Vesicoureteral reflux disease increased the relative risk for development of a UTI up to 3 Times. Re-transplantation quadruples the risk of UTI (Capocasale *et al*., 2014; Ooms *et al*., 2017; Fiorentino *et al*., 2019).

III. Allograft factors

Infected donor organ can turn out to be a source of infection. The infection may progress/reactivated. Transplantation of cadaveric kidney increases the incidence of UTI by about 20%. The use of organs from living donors leads to lower rates of UTI; because these kidneys subjected to shorter periods of cold ischemia and less-severe ischemic–reperfusion injury. Deceased-donor kidney recipients have more delayed graft dysfunction and acute rejection. Likely receive more immunosuppression making them more susceptible to infections (Fiorentino *et al*., 2019). In addition, Donor type (donor drive factors) several studies have suggested that the incidence of UTI is higher following cadaveric transplant. This is unlikely due to donor contamination or ischemia but rather due to shorter waiting times to allocate (warm schema time) for living donors and less intensive immunosuppression. Nowadays, there is a huge demand for living related donor, which remains exceptional in our center. Cadaveric donor protocols are not operating in our center. No matter of this, UTI prevalence is still getting high with vast clinical and economic implications (Lorenz and Cosio, 2010; Laura *et al*., 2018; Elkehili *et al*., 2010).

IV. Anatomical factors

Urinary stasis, reflux or stones raise the risk of UTI development. These features are more prominent in the renal transplant population. Stasis can develop in response to obstruction of the vesicoureteral junctions, bladder dysfunction or outflow obstruction and urethral disease (Shin *et al*., 2015). Reflux can affect both the native and the transplanted kidneys. Native kidneys, polycystic kidneys and ureteric stumps that remain after native nephrectomy can act as a reservoir for pathogens (Valera *et al*., 2006; Ciszek *et al*., 2006; Vavallo *et al*., 2013).

V. Microbial factors

The hierarchy of UTI pathogens in transplant recipients is close but not similar to the non-transplantation population. Bacterial pathogens from the majority causes with Gram-negative bacterial infections accounting for more than 70% of UTIs. Most common organisms have virulence factors that enable them to colonize and invade urothelium. E.g., *E.coli* expresses type 1 or P fimbriae, which increase the bacterium's pathogenicity in the urothelium. Low virulence bacteria that would not be pathogenic in immunocompetent have been implicated in post-transplantation UTI. Immunosuppressant drugs can increase organism virulence, which facilitates bacterial–urothelial adherence (Kumar *et al*., 2018; Lorenz and Cosio, 2010; Yuan *et al*., 2018).

1.3.5. The impact of UTI in renal transplant recipients

UTIs in kidney recipients are more likely to be clinically asymptomatic, as they do not mount the typical inflammatory response to infection because of immunosuppressive therapy. In fact, antimicrobials are the mainstays of treatment and should be accompanied by minimization of immunosuppression when possible (Pesce *et al*., 2019). The fact that use of long-term antimicrobial prophylaxis is controversial; as it might increase the likelihood of infective organisms becoming resistant to treatment. UTI in this group is often associated with acute pyelonephritis and rapidly developing bacteremia, potentially progressing to the full-blown picture of urosepsis, particularly during the early post-transplant period. Patients are at high risk for UTI in the first month post-transplant, where the bacteremia-associated mortality is high and require hospitalization (De Souza and Olsburgh, 2008).

A clinical study in Greece Papasotiriou *et al*. (2011) among 122 cohorts noted that small proportion (3–4%) of patients with chronic renal disease who develop acute pyelonephritis show unfavorable

long-term effects on renal function. However, the effect of UTI on graft survival in transplant patients remains controversial; so far, no consensus has been established as to whether the development of UTI in the solid organ recipient carries a higher mortality or graft loss, although a tendency to graft dysfunction has been suggested. The high rate of bacterial invasion among transplant recipients could be explained by different mechanisms; including the specific surgical and immunological trauma, the influence of early intense immunosuppression, and the requirement for urinary catheterization after the surgical procedure. Although early removal of urethral catheters, improved surgical techniques, and appropriate perioperative antibiotic prophylaxis regimens have reduced the incidence of UTI, it remains higher than in the general population. Some studies showed that approximately 25 % of patients had symptomatic UTIs during the first year after transplantation regardless of the immunosuppression protocol used. However, the fact that post-transplant UTIs are often asymptomatic suggests that the magnitude and implications of this problem are larger than is generally appreciated (AK *et al*., 2017; Laura *et al*., 2018; Yuan *et al.*, 2018).

A retrospective observational study on urinary tract infections and their impact on short-term graft outcome one year after transplantation among 867 patients indicated that the development of at least one episode of AGP was associated with graft loss at one year after transplantation. Patients with AGP caused by a resistant strain had graft function impairment more frequently, although this difference did not reach statistical significance (53% vs. 36%, p 0.07). Although AGP was significantly associated with graft function impairment and one-year post-transplantation graft loss, lower UTIs did not affect graft function (Bodro *et al*, 2015). Moreover, in contrast to the above finding, one study that analyzed a large cohort of kidney transplant recipients in the USA found that late UTI was significantly associated with an increased risk of subsequent death. Nevertheless, it is important to point out that some of such these studies might not use standardized definitions of UTI and that they did not differentiate between early and late infection making it difficult to generalize their conclusions in the absence of randomized controlled trials (Becerra *et al*., 2015).

Another study has shown that the average GFR at 3, 6,9,12 months after transplantation is slightly lower than month one and are statistically significant within the UTI group (Ooms *et al*., 2017). IL-8 levels in the urine of kidney transplant recipients diagnosed with asymptomatic bacteriuria are significantly higher than in sterile urine with comparable IL-6 levels. IL-8 excreted by urethral cells among other sources and co-responsible with IL-6 for the development of local inflammation, pyuria,

and symptoms of UTI (Sadeghi *et al*., 2005). Assessed the effect of UTI on graft function by evaluating several measures such estimated glomerular filtration rate and serum creatinine value. In 34% of transplant recipients (101 of 301) at least one UTI episode was diagnosed with 25% during the first post-transplantation year. Estimated glomerular filtration rate and creatinine level did not differ significantly between the UTI and non-UTI groups. When nuclear imaging was used to assess kidney function, a tendency toward deterioration of allograft function was observed in patients who developed at least one UTI episode after transplantation (P = .044). GPN is associated with acute renal injury and scarring (Ariza *et al*., 2014).

1.3.6. Pathogenesis and pathology of UTI

Urinary tract infections (UTIs) occur because of interactions between the uropathogns and host. Bacteria most commonly cause UTI from the patient's own intestinal flora that enters the urinary tract in a so-called retrograde fashion "ascending route" via of the urethra infection (Figure 1.2). Community-acquired UTIs usually result from a retrograde ascent of bacteria in the external urethral meatus and/or vaginal introits to the bladder. However, an infection may also occur via the blood or lymph with females at greater risk due to their anatomy like in proximity of the urethra to the vagina and rectum that allows fecal flora to colonize the ureteral area of women. After gaining entry to the bladder, uropathogns able to attach to the bladder wall and form a biofilm that resists the body's immune response (Fontser *et al*., 2017; Flores-Mireles *et al*., 2015).

1.3.7. Clinical presentations

Symptomatic post-transplantation UTI can present as acute cystitis or transplant pyelonephritis and/or native pyelonephritis. Typical features of these presentations of post-transplantation urinary tract infection include for lower urinary tract symptoms (cystitis) frequency, urgency, dysuria, hematuria and for upper urinary tract symptoms (pyelonephritis) involves fever/chills, flank pain and pain over graft(Table1.2). Immunosuppressive therapy can mask the clinical manifestations and signs of infection. An early intensive influence of anti-rejection medications leaves RTXs immune compromised and vulnerable to dozens of infection, increased health care costs due to prolonged hospitalizations (Markus, 2012; Fiorentino *et al*., 2019).

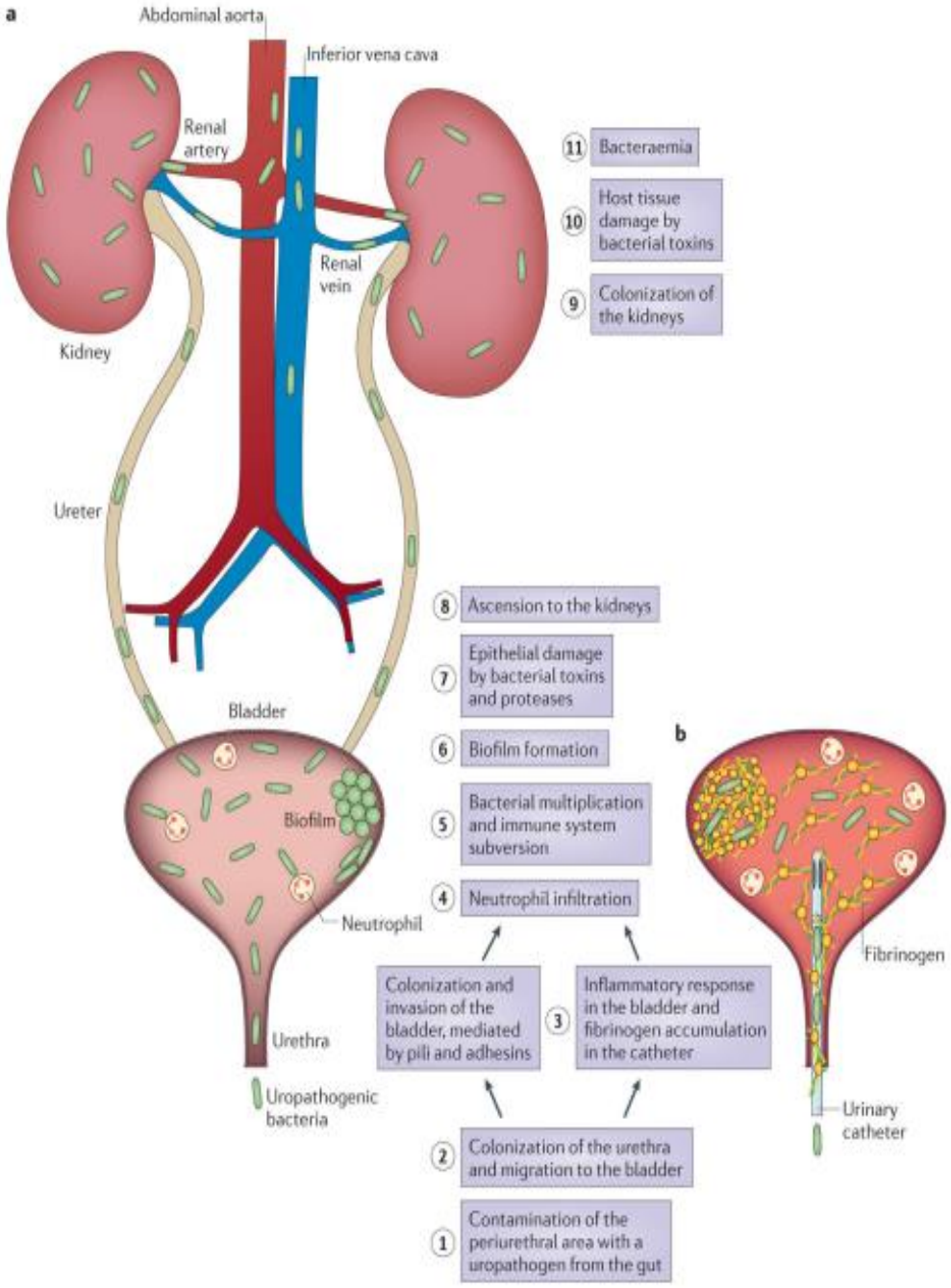

Figure 1.2. Pathogenesis of Urinary Tract Infections (Flores-Mireles *et al*., 2015)

1.3.8. Laboratory diagnostic tools

Typically, diagnosis of symptomatic UTI includes a quantitative count of bacteria ($\geq 10^5$) in an appropriately collected urine specimen in the presence of symptoms or signs of urinary infection

(Table 1.2). Not all organisms found in urine cultures are pathogens. For example, *Staphylococcus epidermidis* (except in the presence of ureteral stents), Lactobacillus, and *Gardnerella vaginalis* are unlikely to be pathogens. Urine cultures containing multiple organisms (i.e. "mixed flora") indicate that contamination has likely occurred. Other true pathogens may not grow well on routine culture media (e.g. unusual pathogens such as *Corynebacterium urealyticum* or *M. tuberculosis*) and specific culture media may need to be requested (Fiorentino *et al*., 2019;Nicolle *et al*., 2005). The usefulness of leukocyte esterase and nitrite screening by dipstick has not been demonstrated in renal transplant recipients. While pyuria (>10 WBC/HPF) does not necessarily confirm that the urinary tract is infected, the absence of pyuria should lead the clinician to question the diagnosis of a urinary tract infection (Leber, 2016). When a quantity of potential pathogen ≤ quantity of contaminating members of the normal microbiota or commensals, the urine specimen is considered not likely to be significant (Parasuraman and Julian, 2013).

Table 1.2. Laboratory diagnosis of post renal transplantation UTI

Diagnostic criteria for UTI (Parasuraman and Julian , 2013)		
Descriptions	**Clinical features**	**Laboratory investigations**
Acute uncomplicated UTI (cystitis in women)	Dysuria, urgency, frequency, suprapubic pain, no urinary symptoms in 4 weeks before this episode	>10 WBC/mm^3 $>10^3$ cfu/mL
Acute uncomplicated (pyelonephritis)	Fever, chills, flank/allograft pain	>10 WBC/mm^3 $>10^4$ cfu/mL.
Complicated UTI	Any combination of symptoms from categories 1 and 2 above	>10 WBC/mm^3 $>10^5$ cfu /mL in women $>10^4$ cfu/mL in men
Asymptomatic bacteriuria	No urinary symptoms	>10 WBC/mm^3 $>10^5$ cfu/mL
Recurrent UTI	At least 3 episodes /12 months or2/6	$>10^3$ cfu/mL

1.3.9. Treatment and prevention of UTI in kidney transplant recipients

I. Surgical Approach: Many causes of UTI are amenable to surgical management. Imaging techniques including ultrasound, computed tomography or magnetic resonance should be considered to identify these etiologies. Urodynamic and cystoscopy can also provide valuable information. The

most common structural urological abnormality leading to recurrent UTI is vesicoureteral reflux in the transplanted kidney. The most common open surgical strategies to correct vesicoureteral reflux include redoing ureteric implantation and ureter ureterostomy (Laura *et al*., 2018)

II. Management of post-transplant urinary indwelling catheter and ureteric stents: Prolonged use of indwelling urinary catheterization has been associated with increased risk of urinary tract infection and, thus, may favor the development of recurrent infections. While early removal of the indwelling urinary catheter is always desirable, this strategy is not always feasible in some RTXs. Other strategies such as chlorhexidine and antibiotic-coated stents to prevent biofilm formation are a promising approach. Nevertheless, further studies are needed before this approach can be implemented in clinical practice. Ureteric stents are placed in the transplanted ureter to prevent early ureteric stenosis. Early removal of ureteric stents following kidney transplantation may reduce the incidence of UTI and major urologic complications in the early stent periods (Hollyer Ian and Michael G Ison, 2018; Laura *et al*., 2018).

III. Monitoring for asymptomatic bacteriuria: This strategy is intended to prevent symptomatic UTI with early treatment of ABU. Monitoring by means of urine culture is indicated during the first 1 to 2 months post-transplantation when the presence of ureteric stents clearly increase the risk of upper UTI and recurrent UTI. The benefit of monitoring for ABU beyond the immediate post-transplantation period is still under debate. Persisting ABU is associated with increased risk of pyelonephritis. But, a randomized clinical trial didn't show a clear benefit of systematic screening and treatment ABU for the prevention of pyelonephritis (Origuen *et al*., 2017; Bodro *et al*.,2015).

IV. Antibiotic prophylaxis/treatment: There is no consensus or recommendations on the use of antibiotic prophylaxis in RTXs with especially recurrent UTI. Perioperative antibiotic prophylaxis (PAP) for routine renal transplantation surgery has been recommended; a single dose of a second-generation or third-generation cephalosporin before induction of anesthesia seems to provide wound and urinary tract protection as effectively as a prolonged course of antibiotics. In most centers , 6- 12 months of SXT therapy constitutes common practice in preventing opportunistic infections with *Pneumocystis jiroveci*, *Listeria monocytogenes*, *Nocardia asteroids*, and *Toxoplasma gondii* and to a lesser extent UTIs (Capocasale *et al*.,2014 ; De Souza and Olsburgh, 2008).

V. Daily cranberries prophylaxis: The administration of cranberry extract appears to reduce the rates of UTI, particularly in women. Cranberry reduces adherence of *E.coli* to uroepithelial cells, and this effect is dose -dependent (De Souza and Olsburgh, 2008).

VI. Other strategies: like L-Methionine, topical estrogens,vaccines, probiotics and fecal microbiota transplantation (Laura et al., 2018 ; Za´rate and ME Nader-Macias,2006).

1.4. Significance of the study

The present study is expected to identify the local bacterial origins causing post-transplantation UTS along with their antimicrobial susceptibility patterns; identification of treatable risk factors that may lead to the reduction in the frequency of symptomatic UTIs in the study area. Antibiotic overuse could lead to the emergence of bacterial resistance and antimicrobial agents side effects. Thus, important costs are associated with the strategy of systematic screening and judicious treatment of post-kidney transplantation ABU such as the costs of urinalyses. This is a matter of concern in areas of limited health-care resources settings like Ethiopia. Urine analysis might be a non-invasive and cost-efficient method for monitoring renal transplants. Furthermore, the study will also serve as a piece of baseline information and a snapshot for those who are interested to conduct further studies on this area. The results will be disseminated via varieties of protocols to concerned bodies including health care providers, core community representatives and interested audience; probably it will aid in infection prevention and control strategies and an institutional based therapeutic guidance for public health policymakers. However, these outcomes ought to delineate by longitudinal studies to elucidate any grave effect on allograft and patient survival. The present study enable for empirical treatment of suspected bacterial infections in RTXs patients, the selection of antimicrobial agents based on local epidemiological data and on the patient's history of colonization or infection with antibiotic-resistant organisms.

1.5. Objectives of the study

General objective

- To determine the prevalence of urinary tract infection, antibiotic susceptibility testing and associated risk factors among adult renal transplant recipients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Specific objectives

- To determine the prevalence of bacterial urinary tract infection among adult kidney transplant recipients.
- To identify the bacterial etiologic agents causing urinary tract infections among adult renal transplant recipients.
- To perform antimicrobial susceptibility patterns of bacterial uropathogns identified from adult renal transplant recipients to selected antibacterial agents.
- To describe the risk factors associated with urinary tract infections among adult kidney transplant recipients.

Chapter II: Materials and Methods

2.1. Study Design, Period and Area

A hospital based cross-sectional study was conducted between December 2017 and August 2018. The study was conducted at St. Paul's Hospital Millennium Medical College at the National Kidney Transplantation Center. The college has more than 2800 clinical, academic, administrative and support staffs that provide medical specialty services to patients who are referred from all over the country; teaching medicine and nursing students and doing basic and applied researches. St. Paul's Hospital Millennium Medical College is the second largest hospital in Ethiopia. Now a day, the hospital has 700 beds and sees an average of 1200 emergency and outpatient clients daily and an annual average of 300,000 patients. It has a catchment population of more than 5 million. It provides healthcare and training to its students through its different biomedical and clinical departments.

2.2. Study participants

I. Study population and participants

All renal transplant recipients who were attending the follow-up clinic at St. Paul's Hospital Millennium Medical College at kidney transplantation center included.

II. Inclusion and Exclusion criteria

Inclusion criteria

All adult kidney recipients aged ≥18 years who came for their check-up to the renal transplantation center suspected for both asymptomatic and symptomatic bacteriuria were included.

Exclusion criteria

Any intake of antibiotics within the previous 7 days, Kidney recipient younger than 18 years, who declined to give consent, double organ transplants(Kidney-Pancreas, Liver-Kidney, kidney-Lung recipients) and previous surgery for urological reconstruction.

III. Sample size calculation

The sample size was estimated based on the 30% prevalence rate according to the study done in Libya by Elkehili et al. (2010) and calculated using the following single population proportion formula:

$$n = \frac{Nz^2Pq}{\{E^2 (N-1) + (z^2Pq)\}}$$

Where:

n = Minimum sample size

N=Total population of kidney recipients on follow up in our transplant clinic = 95 (i.e. N<10,000)

Z= Normal standard deviation 95% confidence interval (Z = 1.96)

P= Prevalence of the disease

q= 1 – Prevalence

E= Margin of error (0.05) then, replacing values the required sample size is finally=74.

Convenient sampling was employed to recruit study participants until the final sample size is fulfilled.

IV. Study variables

Independent variables

- The socio-demographic characteristics (gender, Age, level of education)
- Dialysis vintage, indwelling urethral catheter, DJ-stent, Donor status,
- Clinical sign and symptoms of UTI, Pre-transplantation UTI histories
- Comorbidity(Hypertensions ,DM, Stroke, Glomerulonephritis)
- Frequency of the previous treatments

Dependent variables

- UTI, Transplant complications (Graft loss, impairment or delayed function and infections)
- Bacterial isolates from urine culture, Antimicrobial susceptibility pattern

V. Operational definitions

Urinary tract infection: A pathological invasion of urothelium that results in an inflammatory response, which might present clinically but can be defined based on microscopy and positive urine culture (Parasuraman and Julian, 2013).

Asymptomatic bacteriuria: A symptom-free bacteria strain count $\geq 10^5$ cfu/mL in two consecutive midstream urine cultures >24 hours apart in women and a single sample $\geq 10^5$ cfu/mL in men or $\geq 10^2$ cfu/mL in a single specimen obtained through urethral catheterization (Origuen *et a*l.,2017 ; Nicolle *et al*.,2005).

Symptomatic UTI: Symptoms present (dysuria, urgency, frequency, suprapubic pain) in association with bacteria strain count $\geq 10^5$ cfu/mL in women or $\geq 10^4$ cfu/mL in men (Flores-Mireles *et al*., 2015).
Pyelonephritis: Symptoms of fever, chills, flank/allograft pain and bacteria strain count ≥105 cfu/mL in women or $\geq 10^4$ cfu/mL in men (Markus, 2012) (Table 2.1).

Recurrent UTI : Three or more UTI episodes in a 12- months period or at least two in 6- months period resulting from infection with different strains of microbes. In transplant recipients, recurrent UTI can include asymptomatic episodes (Laura *et al*., 2018) (Table 2.1).

Table 2.1. Laboratory diagnostic criteria of post renal transplantation UTI (Markus, 2012)

Type	Clinical appearance	Urine investigations
Acute uncomplicated lower UTI, cystitis (woman)	Dysuria, urgency, frequency	$>10^3$ cfu/mL >10WBC/mm^3
Acute, uncomplicated upper UTI: pyelonephritis	Fever, flank pain, no urologic abnormalities	$>10^4$ cfu/mL >10WBC/mm^3
Complicated UTI	Structural and functional abnormalities	>10 WBC/mm^3 $>10^5$ cfu/mL (female) $>10^4$ cfu/mL (male)
Asymptomatic bacteriuria(ABU)	No urologic symptoms	>10 WBC/mm^3 $>10^5$ cfu/mL in two urine samples > 24 hrs apart
Recurrent, uncomplicated UTI	Only female 3 episodes of uncomplicated UTI/1 year	$>10^3$ cfu/mL

2.3. Data Collection Methods

Informed consent was obtained from the study participants by attending physician and nurse (**Annex I and I**I). All relevant data concerning socio-demographic characteristics, related risk factors to UTI, clinical signs & symptoms of the study population were obtained using pre-designed structured questionnaires (**Annex III**). The questionnaires were pre-tested before the actual study was made for its completeness, simplicity and clarity on study subjects who were selected randomly based on the

objective of the study. Whereas data on the etiological agents were obtained using standard microbiological laboratory tests. Participants' essential data were also extracted from their medical record profiles upon which urine sample and other relevant data were collected and secured.

2.4. Sample Collection, Transport and Handling

Before urine sample collection, brief instruction on genital hygienic measures was given for each study participants. Freshly voided first morning clean-catch midstream urine samples (optimally 15-30 ml) were collected from all kidney recipients in wide-mouthed sterile plastic containers. Optionally, transferred to test tubes labeled with codes corresponding to the questionnaires. Later on, the collected urine specimens were subjected to microscopic examination and culture and sensitivity testing. The laboratory procedures performed at clinical Bacteriology and Mycology laboratory located at National Reference Laboratory of the Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. All the laboratory procedures performed using standard procedures (**Annex IV**).

2.5. Urine screening

Reagent strip test (Fortress Diagnostic Limited, United Kingdom) was used for detecting the presence of leukocyte esterase and nitrite in urine specimens. In a well-mixed, non-centrifuged urine specimen, the test strip was immersed for approximately two seconds. Excess urine was removed from the strip by wiping the edge of the urine container. Then the reagent areas were compared with the corresponding color chart on the container 60 seconds after immersion. Dipstick screening test and microscopy were the major components of urinalysis, which were useful for the rapid screening of UTI Goldman JD, Julian K. (2019) especially in resource-limited facilities like our country Ethiopia.

The biochemical reaction that was detected by the nitrite test was associated with members of the family of Enterobacteriaceae. Urinary pathogens, e.g. *E. coli, Proteus*, and *Klebsiella* species were able to reduce the nitrate normally present in urine in sufficient concentration. When first-morning urine is tested, about 80% to 90% of UTI caused by nitrate reducing pathogens could be detected. However, the test could be negative when the infection was caused by pathogens that do not reduce nitrates such as *E. faecalis, Pseudomonas, and Staphylococcus* species or when bacteria are too few in the urine. Another limitation to the test was that it requires testing a specimen of the first urine produced in the morning, as > 4 hours is required for bacteria to convert nitrate to nitrite at levels that

were reliably detectable. Occasionally the nitrite test could be negative because nitrate was lacking in the urine due to a person was on diet lacking vegetables (Leber, 2016).

2.6. Urine Microscopy

Regarding urine microscopy, urine samples were mixed properly in a container and five to ten ml of each well-mixed urine samples were centrifuged at 2500 rpm for 5 minutes. After centrifugation, the supernatant was discarded and a drop of two of the sediment was placed on the grease free slide, coverslip applied and examined under the microscope using the high-power field. Microscopy of urine from symptomatic patients could be of great diagnostic value. Gram stain is one of the most rapid, reliable, and inexpensive methods for estimating bacteriuria at > 10^5 cfu/ml and > 10 WBCs/hpf. However, because of its decreased sensitivity at < 10^5 cfu/ml, most investigators have recommended the Gram stain as a screen only for those patients with predictably high colony counts, mainly asymptomatic patients and those with presumed pyelonephritis. Although the Gram-stained smear of (0.01 m1) of well -mixed, uncentrifuged urine should be placed on a glass slide and accurate at >10^5 cfu/ml. Despite inexpensive, it is not the routine screening method in most laboratories because it is time-consuming and relatively insensitive (Leber, 2016; Nicolle *et al*., 2005).

2.7. Urine Culture and identification

Using calibrated wire loop (0.001ml) clean catch midstream urine samples were inoculated into MacConkey (MAC) and 5 % sheep blood agar plate (BAP) (Oxoid, UK). Cultures were incubated in aerobic atmosphere at 35-37ºC for 18-24 hrs. Colonies were counted to check the presence of significant bacteriuria. Colony count yielding bacterial growth of ≥10^5 cfu/ml of urine was considered significant bacteriuria. All positive cultures with significant bacteriuria were then subjected to test identification to species level by their colony characteristics and pattern of biochemical profiles using standard microbiological procedures. Enterobacteriaceae were identified by indole production, H2S production, lactose fermentation in TSI agar, citrate utilization, motility test, urease test, bile solubility, utilization of lysine in lysine decarboxylase (LDC agar). Oxidase test was also performed for non-fermenter Gram-negative rods. On the contrary, the Gram-positive bacteria were identified using catalase, coagulase and bile-esculin tests (Leber, 2016; Fiorentino *et al*.,2019).

2.8. Antibiotic Susceptibility Testing

The antimicrobial susceptibility testing of all identified isolates of urine samples was done based on the frequent prescription in our centers , market availability in conjunction to the criteria of the Clinical and Laboratory Standards Institute (CLSI, M100,2018) for the management of urinary tract infections in the study area. Selection of antibiotics based on the source of specimen and type of microorganisms was the second most important tool to come across AST testing. An operational manual designed for this purpose at Ethiopian Public health Institute (EPHI), clinical bacteriology and mycology section was used during AST testing. Briefly, from a pure culture, a loop full bacterial colony was taken and transferred to a tube containing 4-5 ml of normal saline and was mixed gently until it formed a homogenous suspension. Alternatively, the electronic vortex was used. The turbidity of the suspension was then adjusted to the density of a McFarland 0.5 standard in order to standardize the inoculum size.

A sterile cotton swab was then dipped into the suspension and the excess was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller-Hinton agar (Oxoid, UK). The inoculated plates were left at room temperature to dry for 3-5 minutes. With the aid of a sterile needle or forceps, the following concentration of antibiotic discs was laid on the surface of Mueller-Hinton agar.

The following antibiotic disks obtained from Oxoid were used against Gram-negative bacteria isolates: Amoxicillin-clavulanate acid (AMC=20+10µg), Cefepime (CFM=30µg), Ceftriaxone (CTR=30µg),Chloramphenicol(CHL=30µg),Ciprofloxacin(CIP=5µg),Gentamicin(CN=10µg),Meropenem (MER=10µg),Nitrofurantoin(NIT=300µg),Tetracycline(TET=30µg) and Trimethoprim-Sulfamethoxazole (SXT=1.25+23.75µg).

For Gram-positive bacterial isolates: Amoxicillin-clavulanate acid (AMC=20+10µg), Ceftriaxone (CTR=30µg),Chloramphenicol(CHL=30µg),Doxycycline(DC=30µg),Gentamicin(CNµg=10),Nitrofurantoin(NIT=300µg),Tetracycline(TET=30µg),Trimethoprim-Sulfamethoxazole (SXT=1.25+23.75µg),Vancomycin(VA=30µg),Penicillin(PEN=10µg),Clindamycin(CLN=2µg) and Erythromycin(ERY=15µg) were used for sensitivity testing. The plates were inverted and incubated in aerobic atmosphere at 37oC for 24 hours. Diameters of the zone of inhibition around the discs were measured using a ruler and the isolates were classified as sensitive, intermediate and resistant according to CLSI (M100, 2018).

Sensitive (S): Bacterial isolates inhibited by the usually achievable concentration of antimicrobial agent (CLSI, 28 edition, M100, 40-42, 2018).

Intermediate (I): Bacterial isolates with antimicrobial agent minimum inhibitory concentrations that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates (CLSI, 28 edition, M100, 40-42, 2018).

Resistant (R): Bacterial isolates uninhibited by the usually achievable concentrations of the agent with normal dosage (CLSI, 28 edition, M100, 40-42, 2018).

2.9. Data Quality Assurance

To assured the quality of the data for its accuracy, precision, completeness and clarity, pre-tested structured questionnaires were used on the patients who were attending the renal transplantation follow up clinic before the actual study was done. Collected data were checked for its completeness and internal consistency. Accordingly, modifications for pretest on both the questionnaires and the process of the study was made. Furthermore, the quality of the data was checked by giving adequate training for data collectors to ensure the data quality. Few corrective measures were taken for any gaps during any course of the study. The quality of culture media was tested for sterility and performance; sterility of culture media was checked by incubating overnight at 35-37 ºC without specimen inoculation. Any physical change like cracks, excess moisture, color, hemolysis, dehydration, and contamination was assessed and expiration date was checked. Standard reference strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853) were used for quality control throughout the study for culture and antimicrobial susceptibility test. For Gram staining reagents *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) was used. The temperature of incubator and refrigerator was monitored daily using a checklist designed for it.

2.10. Statistical analysis

All data were analyzed taking due to care for completeness, consistency, coding and sorting using SPSS version 20. Socio-demographic, clinical and laboratory data were sorted, cleaned, entered and analyzed. Tables and texts explained descriptive data. In all cases P-value, < 0.05 was taken as statistically significant. To assess associated risk factors for post-renal transplant UTI, bivariate and multivariate logistic regression risk factor analysis was done to calculate crude/adjusted odds ratio and 95%confidence interval.

2.11. Ethical Considerations

Ethical approval was obtained from Department Ethics Research Committee (DERC) Department of Microbiology, Immunology, and Parasitology (DMIP), School of Medicine, College of Health Sciences, Addis Ababa University. Permission was obtained for the study after getting ethical clearance from DMIP. Subsequently, study participants were informed and consented about the objectives and benefits of the research and its findings preceding the data collection procedure.

Chapter III: Results

3.1. Socio-demographic characteristics of studied participants

A total of 74 study participants (38 with symptoms and signs of UTI and 36 without symptoms and signs of UTI) were included in the study. A majority, 42/74(56.8%) of them were males. The mean age was 41.55 years old with a standard deviation of 11.33 (41.55±11.33) and a median of 40.5. Meanwhile, males' mean age was 43.07±11.6 with a median of 45 while females' mean age was a little bit lower (39.56± 10.81and median of 37). Bacteriuric males were much younger than bacteriuric females (19 and 25 years old respectively). Majority of the study participants 31(41.9%) were within the age group of 35-49 followed by 18-34(29/74, 39.2%). Similarly, More than fifty percent of study participants were urban residents. Most of the subjects were government employees 26(35%) while the marital status indicated that 40.5% were married. Significant bacterial UTI was diagnosed in 11/74 (14.9%, 95%CI=8.2-24.7). Bivariate logistic regression to assess the associated risk factors with post-transplantation UTI has shown no significant association except to age ,crude odds ratio (COR)=3.67, 95%CI=2.89-20.07, P=0.003 as shown in Table 3.1.

Table 3.1. Sociodemographic characteristics of study participants with and without UTI, St Paul's Hospital Millennium medical college, Addis Ababa, Ethiopia

Variables	Total (%)	UTI no (%)	No UTI no (%)	Bivariate analysis		P-value
Gender				**COR**	**95%CI**	
Male	42(56.8)	5(11.9)	37(88.1)	0.848	0.57-11.31	0.419
Female	32(43.2)	6(18.75)	26(81)	2.09	1.04-8.45	0.253
Age						
18-34	29(39.2)	4(13.8)	25(86.2)	1.42	0.64-14.05	0.338
35-49	31(41.9)	6(19.3)	25(80.6)	3.67	2.89-20.07	0.003
50-64	10(13.5)	1(10)	9(90)	1	1	
Above 64	4(5.4)	0(0.0)	4(100)	0.88	0.58-3.27	0.444
Residence						
Urban	53(71.6)	9(17)	44(83)	N/A	N/A	
Rural	21(28.4)	2(9.5)	19(90.5)	0.18	0.06-2.06	0.531
Religion						
Orthodox	27(36.5)	5(18.5)	22(81.5)	N/A	N/A	
Muslim	26(35.1)	1(3.8)	25(96)	1	1	
Others	21(28.4)	5(23.8)	16(76.1)	1	1	
Occupation						
Housewife	13(17.6)	2(15.4)	11(84.6)	1.489	0.92-14.01	0.853
Merchant	21(28.4)	4(19)	17(81)	4.94	2.06-10.87	0.009
Government employee	26(35)	3(11.5)	23(88.5)	1	1	
Others	14(19)	2(14.3)	12(85.7)	1.08	0.96-20.17	0.951
Marital status						
Single	23(31)	6(26.1)	17(74)	5.64	0.73-13.22	0.222
Married	30(40.5)	4(13.3)	26(86.7)	1.724	1.081-6.82	0.391
Divorced	12(16.2)	1(8.3)	11(91.7)	1	1	
Widowed	5(6.8)	0(0.0)	5(100)	N/A	N/A	
Widower	4(5.4)	0(0.0)	4(100)	8.04	2.05-10.09	0.70
Educational level						
Student	11(14.9)	2(18.2)	10(91)	1	1	
Diploma	31(41)	5(16)	26(83.9)	2.872	0.81-5.06	0.579
Degree	13(17.6)	2(15)	11(84.6)	1	1	
Illiterate	15(20)	2(13)	13(86.7)	2.11	1.90-17.48	
Above degree	4(5.4)	0(0.0)	4(100)	N/A	N/A	
Income level(ETB)						
<5000	46(62.2)	10(21.7)	36(78.3)	0.51	0.48-8.27	0.293
>5000	28(37.8)	1(3.6)	27(96.4)	1	1	

3.2. Clinical characteristics of study participants

The dialysis vintage of the renal transplant recipients (RTXs) in our center revealed that 20 (27%) were within 6 months-1year. The mean dialysis vintage before transplantation was recorded as 9±1.5 months. (Minimum-maximum = 3-13 months). Simultaneously, average time since transplantation in months was 38.4±4.8 (Minimum – maximum =1-170 months). Its association with bacterial UTI was not statistically significant (P =0.08). All (100%) of the recipients perceived that renal transplantation was the preferred modality of renal replacement therapy to those who were living with end stage kidney disease (ESKD). In this study, both locally and abroad transplanted recipients were involved as shown in Table 3.3.

Table 3.2. Clinical characteristics of 74 renal transplant recipients diagnosed for post-renal transplantation UTI (December 2017-Augest 2018), SPHMMC

Characteristics	Response	Frequency(n)	Percent (%)
Mode of renal replacement therapy?	Transplantation	74	100
Year of transplantation	2008 E.C	21	28.4
	2009E.C	18	24.3
	2010 E.C	13	17.6
	Abroad since 2009G.C	22	29.7
Term of indwelling urethral catheter	Short (<30 days)	74	100
	Long (>30 days)	0	0
Insertion of double j stent	Yes	74	100
	No	0	0
Timing of urethral catheter removal	Early (≤3 days)	74	100
	Late (≥3days)	0	0
Timing of ureteric stent removal	Early (≤4 weeks)	74	100
	Late (≥4 weeks)	0	0
Donor's age	≤65 years old	74	100
	≥65 years old	0	0
Donor' type	Related living	74	100

In the present study, all (100%) of the study participants were much younger than 65 years old and related type of organ donation was exceptionally predominant in our transplantation clinic.

Table 3.3. Prevalence of UTI in related clinical variables of renal transplants recipients, SPHMMC

Variables	Total (%)	UTI no (%)	No UTI no (%)	Bivariate analysis		p-value
Dialysis vintage				**COR**	**95%CI**	
<6 months	16(21.6)	4(25)	12(75)	2.16	2.07-21.02	0.604
6 month-1 year	20(27.1)	2(10)	18(90)	2.71	0.92-15.04	0.992
>2 years	35(47.3)	5(14.3)	30(85.7)	0.68	0.34-7.02	0.231
No dialysis	3(4)	0(0.0)	3(100)	N/A	N/A	
Time since transplantation						
0-6 months	17(22.9)	3(17.6)	14(82.3)	2.29	0.42-2.96	0.391
7-12 months	19(25.7)	4(21)	15(79)	2.57	1.09-11.03	0.555
13-24 months	19(25.7)	2(10.5)	17(89.5)	0.71	0.54-6.38	0.08
>24 months	19(25.7)	2(10.5)	17(98.5)	1	1	
Pre- transplant UTI history						
Yes	5(6.8)	2(40)	3(60)	4.32	2.09-17.10	0.010
No	69(93.2)	9(13)	60(87)	0.51	0.26-2.11	0.997
Place of the transplantation						
Local	54(73)	9(16.7)	45(83.3)	4.01	0.18-19.06	0.481
Abroad	20(27)	2(10)	18(90)	0.89	0.69-8.81	0.671
Donor's gender						
Male	40(54.1)	5(12.5)	35(87.5)	1	1	
Female	34(45.9)	4(11.8)	30(88.2)	2.07	1.04-7.31	0.549
History of Catheterization						
Yes	5(6.8)	1(20)	4(80)	1.90	1.11-11.38	0.001
No	69(93.2)	10(14.5)	59(85.5)	0.53	0.21-0.98	0.941
Sex donor category						
Female to male	17(23)	2(11.8)	15(88.2)	0.23	0.10-1.93	0.234
Male to female	22(29.7)	5(22.7)	17(72.3)	1.09	1.07-13.03	0.306
Male to male	17(23)	2(11.8)	15(88.2)	N/A	N/A	
female to female	18(24.3)	2(11.1)	16(88.9)	1	1	

3.3. Prevalence of significant bacteriuria among renal transplant recipients

In the present study, significant bacteriuria was detected in 11/74 (14.9%) of the study subjects investigated for UTI as shown in Table 3.4. In the meantime, significant bacteriuria was detected in 3/38 (7.9%) and 8/36 (22%) of renal transplant recipients with symptoms and without symptoms of UTI, respectively (COR, 95%CI= 1.01(0.547-3.829 and P-value=0.847 respectively). The prevalence of UTI with respective to gender among renal transplant recipient revealed that male gender UTI was slightly inferior 5/42(11.9%) to female gender 6/32(18.75%) without association as shown Table 3.1.

Table 3.4. Significant bacteriuria from urine culture of renal transplant recipients

Significant bacteriuria	**Frequency(n)**		**Percent (%)**		
Yes	11		14.9		
No	63		85.1		
Total	74		100		
Renal transplant recipients	**Significant bacteriuria**				
	Yes	**No**	**Total**	**COR(95%CI)**	**P-value**
Asymptomatic no (%)	8(22.2)	28(77.8)	36(48.7)	1.01(0.547-3.829)	0.847
Symptomatic no (%)	3(7.9)	35(92.1)	38(51.3)		
Total no (%)	11(14.9)	63(85.1)	74(100)		

E.coli, P.mirabilis and *Acinetobacter spp.* were exclusively found in asymptomatic patients (Table 3.5).

Table 3.5. Bacterial species isolated from asymptomatic and symptomatic UTI among renal transplant recipients, SPHMMC, Addis Ababa, Ethiopia

Bacterial isolates	**Asymptomatic RTX no (%)**	**Symptomatic RTX no (%)**	**Total (%)**
Gram-negative	**5(62.5)**	**0(0.0)**	**5(45.4)**
E.coli	2(25)	0(0.0)	2(18.18)
Acinetobacter spp.	2(25)	0(0.0)	2(18.18)
P.mirabilis	1(12.5)	0(0.0)	1(9.1)
Gram-positive	**3(37.5)**	**3(99.9)**	**6(54.6)**
Enterococcus spp.	1(12.5)	1(33.3)	2(18.18)
CoNS	1(12.5)	1(33.3)	2(18.18)
S.aureus	1(12.5)	1(33.3)	2(18.18)
Total	**8(72.7)**	**3(27.3)**	**11(100)**

CoNS=Coagulase Negative *Staphylococci*

3.4. Bacterial etiologies

A total of 11 bacteria were isolated, out of these, 5 (45.4%) were Gram -negative bacteria and 6 (54.6%) were Gram-positive bacteria (P-value=0.741). Out of the 11 bacteria isolated, *E. coli*, *S.*

aureus, *Enterococcus spp*., CoNS and *Acinetobacter spp*. each of them accounted for 18.2%. The magnitude of early UTI (n=17patients, 3 isolates) vary significantly from late UTI occurring >6 months postoperative period (n=57 patients, 8 isolates). Indeed, over half of the infection episodes occurred during late post-operative procedure indicating late UTI episode occurrence seems higher than early UTI although no significant association as presented in table 3.3.

Table 3.6. Bacterial etiologic agents isolated from urine culture of renal transplants

Bacterial isolates	Frequency(n)	Percent (%)
Gram-Negative	**5**	**45.4**
E.coli	2	18.2
Acinetobacter spp.	2	18.2
P.mirabilis	1	9.1
Gram-positive	**6**	**54.6**
Enterococcus spp.	2	18.2
CoNS	2	18.2
S.aureus	2	18.2
Total	**11**	**100**

3.5. Antibiotic susceptibility data

The resistance pattern of Gram -negative bacteria against commercially available antibacterial drugs was superior to Gram-positive detected isolates.

I. Gram -positive bacteria

Antibiotic resistance pattern of Gram-positive bacteria (n=6) against 11 antibiotics is presented in Table 3.7. Coagulase- Negative *Staphylococci* (CoNS) and *S. aureus* were highly sensitive to clindamycin (100%). The overall resistance pattern of the Gram-positive bacteria showed that (83%) were resistant to ceftriaxone and amoxicillin-clavulanate acid followed by (67%) to chloramphenicol, nitrofurantoin, tetracycline and gentamycin. Overall, the rate of resistance ranges from 17%-83% against the 11 antibiotics tested for Gram- positive bacteria. Clindamycin was the most effective antibiotic (67%) among the tested groups. On the other hand, ceftriaxone was the least effective (0%) antibacterial regimen against Gram-positive isolates.

Table 3.7. Antibacterial susceptibility patterns of Gram-positive bacteria isolated from urine specimen of renal transplant recipients, Addis Ababa, Ethiopia

Urine Culture out put			Antibacterial agents tested										
			VA	**CTR**	**CHL**	**NIT**	**AMC**	**PEN**	**TET**	**SXT**	**ERY**	**CLN**	**CN**
Bacterial isolates	Total	Pattern	no(%)	no(%)	no(%)	no(%)	no (%)	no(%)	no(%)	no(%)	no (%)	no (%)	no (%)
S. aureus (n=2)	2	R I S	0(0) 2(100 0(0)	2(100) 0(0) 0(0)	2(100) 0(0) 0(0)	2(100) 0(0) 0(0)	1(50) 0(0) 1(50)	2(100 0(0) 0(0)	1(50) 0(0) 1(50	1(50) 0(0) 1(50)	0(0) 0(0) 2(100	0(0) 0(0) 2(100)	0(0) 0(0) 2(100)
CoNS (n=2)	2	R I s	1(50) 0(0) 1(50)	1(50) 1(50) 0(0)	2(100) 0(0) 0(0)	2(100) 0(0) 0(0)	2(100 0(0) 0(0)	0(0) 1(50) 1(50)	2(100 0(0) 0(0)	0(0) 1(50) 1(50)	0(0) 2(100 0(0)	0(0) 0(0) 2(100)	2(100) 0(0) 0(0)
Enterococcus spp.(n=2)	2	R I S	0(0) 2(100 0(0)	2(100) 0(0) 0(0)	0(0) 1(50) 1(50)	0(0) 0(0) 2(100)	2(100 0(0) 0(0)	1(50) 0(0) 1(50)	1(50) 0(0) 1(50)	2(100 0(0) 0(0)	2(100 0(0) 0(0)	2(100 0(0) 0(0)	2(100) 0(0) 0(0)
Total (n=6)	6	R I S	1(17) 4(67) 1(17)	5(83) 1(17) 0(0)	4(67) 1(17) 1(17)	4(67) 0(0) 2(33)	5(83) 0(0) 1(17)	3(50) 1(17) 2(33)	4(67) 0(0) 2(33)	3(50) 1(17) 2(33)	2(33) 2(33) 2(33)	2(33) 0(0) 4(67)	4(67) 0(0) 2(33)

Abbreviations: **R** = Resistant S = Sensitive **I** = Intermediate, **AMC**= Amoxicillin Clavulanate acid, **CTR**=Ceftriaxone, **CHL**=Chloramphenicol, **CLN**=Clindamycin, **ERY**=Erythromycin, **CN**=Gentamicin, **NIT**= Nitrofurantoin, **TET**=Tetracycline, **SXT**=Trimethoprim-Sulfamethoxazole, **VA**= Vancomycin, **PEN** =Penicillin

II. Gram –negative bacteria

Antibiotic resistance pattern of Gram-negative bacteria (n=5) against 11 antibiotics is depcited in

Table 3.8. The Gram-negative bacteria accounts for 45.4% post -renal transplantation UTI incidences of which 40% was *E.coli*. The most effective antibiotics against *E.coli* isolates were gentamycin (100%). Nevertheless, the least effective antibiotics against *E.coli* were ciprofloxacin, trimethoprim/sulfamethoxazole, nitrofurantoin, ceftriaxone, cefepime, and chloramphenicol held about (100%) resistance. Overall, the rate of resistance and sensitivity ranges from 0-100% and 0-60% respectively against the 11 antibiotics tested for Gram-negative bacteria. Unlike that within the same family of *Enterobacteriaceae,* ciprofloxacin, cefepime and ceftriaxone were the most effective

antibiotics against *P. mirabilis* (100%). Dissimilar to the Gram-positive isolates, gentamicin was the most effective antibiotic among the groups (100%) against the 11 antibiotics tested for Gram-negative bacteria.

Table 3.8. Antibacterial susceptibility patterns of Gram-negative bacteria isolated from urine specimen of renal transplant recipients, Addis Ababa, Ethiopia

Urine Culture out put			**Antibacterial agents tested**										
			CIP	**TET**	**CN**	**AMC**	**CHL**	**NIT**	**SXT**	**CPM**	**CTR**	**MEM**	**DC**
Bacterial isolates	Total	Pattern	no (%)	no (%	no(%)	no(%	no(%	no(%)	no(%	no(%	no(%	no(%)	no(%
E.coli (n=2)	2	R I S	2(100 0(0) 0(0)	1(50 0(0) 1(50)	0(0) 0(0) 2(100	1(50) 1(50) 0(0)	2(100 0(0) 0(0)	2(100 0(0) 0(0)	2(100 0(0) 0(0)	2(100 0(0) 0(0)	2(100 0(0) 0(0)	0(0) 2(100) 0(0)	1(50) 0(0) 1(50)
Acinetobacter spp.(n=2	2	R I S	2(100 0(0 0(0)	0(0) 0(0) 2(100	0(0) 0(0) 2(100	2(100 0(0 0(0	2(100 0(0) 0(0)	2(100 0(0) 0(0)	2(100 0(00) 1(50)	1(50) 0(0) 1(50)	2(100 0(0) 0(0)	1(50) 1(50) 0(0)	0(0) 0(0) 2(100
P. mirabilis (n=1)	1	R I S	0(0) 0(0) 1(100	1(100 0(0) 0(0)	0(0) 0(0) 1(10	0(0) 0(0 (100	0(0) 1(00) 0(0)	1(100 0(0) 0(0)	0(0) 1(100 0(0)	0(0) 0(0 1(100	0(0) 0(0) 1(100	0(0) 0(0) 1(100	1(100 0(0) 0(0)
Total (n=5)	5	R I S	4(80) 0(0) 1(20)	2(40) 0(0) 3(60)	0(0) 0(0) 5(100	3(60) 1(20) 1(20)	4(80) 1(20) 0(0)	5(100 0(0) 0(0)	4(80) 1(20) 0(0)	3(60) 0(0) 2(40)	4(80) 0(0) 1(20)	1(20) 3(60) 1(20)	2(40) 0(0) 3(60)

Abbreviations: **R** = Resistant, **S** = Sensitive, **I** = Intermediate, **AMC**= Amoxicillin-Clavulanate acid, **CFM**=Cefepime,**CTR**=Ceftriaxone,**CHL**=Chloramphenicol, **CIP**= Ciprofloxacin**, CN**=Gentamicin , **MER**=Meropenem, **NIT**=Nitrofurantoin, **TET**=Tetracycline, **SXT**=Trimethoprim-Sulfamethoxazole, **DC**=Doxycycline.

3.6. Multidrug resistance patterns of bacteria isolated from renal allografts

In the present study, Multi drug resistance (MDR = resistance in ≥ 3 drugs) was seen in 82 % of the isolates among diagnosed renal transplant recipients as shown in Table 3.9.

Table 3.9. Multidrug resistance patterns of bacteria isolated from renal allografts, SPHMMC

Bacterial isolates	Total (%)	Antibacterial patterns					
		R_0	R_1	R_2	R_3	R_4	$\geq R_5$
Gram-negative	**5(45.4)**	**0(0.0)**	**0(0.0)**	**0(0.0)**	**2(40)**	**1(20)**	**2(40)**
E.coli	2(40)	0(0.0)	0(0.0)	0(0.0)	1(50)	0(0.0)	1(50)
Acinetobacter spp.	2(40)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(50)	1(50)
P.mirabilis	1(20)	0(0.0)	0(0.0)	(0.0)	1(100)	0(0.0)	0(0.0)
Gram-positive	**6(54.6)**	**0(0.0)**	**1(16.7)**	**1(16.7)**	**0(0.0)**	**3(50)**	**1(16.7)**
S.aureus	2(33.33)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100)	0(0.0)
CoNS	2(33.33)	0(0.0)	1(50)	0(0.0)	0(0.0)	1(50)	0(0.0)
Enterococcus spp.	2(33.33)	0(0.0)	0(0.0)	1(50)	0(0.0)	0(0.0)	1(50)
Total	**11(100)**	**0(0.0)**	**1(9.1)**	**1(9.1)**	**2(18.2)**	**4(36.4)**	**3(27.3)**

R0-=No antibiotic resistance, R_1= Resistance to one, R_2=Resistance to two, R_3=Resistance to three, R_4-=Resistance to four, ≥ R_5=resistance to five and more drugs.

3.7. Risk factors associated with Post -renal transplantation UTI

The multivariate regression analysis to assess the risk factors with post-renal transplantation UTI is schemed in Table 3.10. In this regard, adjusted odds ratio (AOR) and 95% confidence interval (95%CI) and the p-values were measured as shown below.

Table 3.10. Multivariate risk factor analysis among post renal transplantation UTI, SPHMMC

Risk factor	Significant bacteriuria		AOR	95% CI	P-value
Age(years)	**Yes no (%)**	**No no (%)**			
18-34(n=29)	4(13.8)	25(86.2)	1	1	
35-49(n=31)	6(19.3)	25(80.6)	2.61	2.06–18.91	<0.001
50-64(n=10)	1(10)	9(90)	1.531	0.341-3.271	0.245
>64(n=4)	0(0.0)	4(100)	N/A	N/A	
Pre-transplant UTI history					
Yes(n=5)	2((40)	3(60)	3.48	2.12-9.39	0.02
No(n=69)	9(13)	60(87)	N/A	N/A	
History of previous catheterization					
Yes(n=5)	1(20)	4(80)	3.29	2.05–11.85	0.003
No(n=69)	10(14.5)	59(85.5)	1.84	0.27-15.38	0.195

AOR=Adjusted Odds Ratio, **95% CI** =95% Confidence Interval & **N/A**= Not Applicable.

Chapter IV: Discussion

Infections remain a major cause of morbidity and mortality in transplant recipients. Urinary tract infections (UTIs) mainly bacterial origin are a common infectious complication in solid organ transplantation (SOT), especially in kidney transplant recipients. Studies conducted in a variety of transplantation centers globally showed that the incidence of UTIs in these patients might reach 80% of all infections, with most of them developing in the first 6 months after transplantation. Impaired response of the immune system can be caused by multiple factors including the use of immunosuppressant agents, disturbed integrity of the natural barrier formed by the mucosa of the urinary tract, concomitant diseases, neutropenia, lymphopenia, metabolic disorders (diabetes mellitus), and nutrition disorders. Apart from decreased immunity due to immunosuppressant agents, additional problems may be posed by various preoperative urologic disorders, vesicoureteral reflux to the transplanted kidney as result of ureteral implantation technique, or instrumentation of the urinary tract like Foley catheter and double-J ureteral stent (Gozdowskaa *et al*., 2016; Vidal *et al*., 2015).

The present study revealed that the majority (56.8%) of study participants were males. However, a substantially higher number of females were affected by post renal transplantation UTI than males (18.75% versus 11.9% respectively) with no statistical significance association (crude odds ratio=2.09, 95%CI=1.04-8.45 & P=0.253). In a similar situation in harmony to the present study, a research paper by Kotagiri *et al*. (2017) in Australia has shown that a large number of females were affected (P=0.002). Another report that agrees to the present investigation by Shams *et al*.(2017) in Iran has shown female kidney transplant patients was a statistically significant higher occurrence of UTIs versus male allograft recipients (Females 16.2%, $P < 0.005$) claiming that shorter urethra and nearness of urethral opening to anus and vagina are causes of the higher prevalence than in male gender. Bispo *et al*. (2013) in the same way reported similar findings for a significant relationship with UTI ($p < 0.001$). The lack of significant association in the present study may be due to the fact that most of the patients are male or because of the immunosuppressive drugs with the high dosage that patients could have taken. In opposite to the present finding, from Yemen Gondos *et al*.(2015) , Saudi Arabia Alkatheri,(2013) and Portugal Bispo *et al*.(2014) higher female prevalence of UTI (female 40.3%, males 29%, female 69.2%,male 30.8% and female 68%, male 23% respectively) were reported with no statistically significant association. Importantly, Gozdowska *et al*. (2016) have thought that women are more susceptible to UTIs, which results from anatomical, hormonal,

immunological and behavioral features. Other studies by Sadegh *et al. (*2005) and Ciszek *et al*.(2006) in RTXs, men bacteriuric were associated with strong inflammatory response and stimulation in the urinary tract of cells producing pro-inflammatory cytokines such as IL-6, IL-8, and soluble IL-1 receptors1and 6, whereas in women, inflammatory response is inhibited.

In the present investigation, the rate of UTI incidence was higher in the age group of 35-49 years old (19.3%) comparing the younger age groups with a strong statistically significant association(P=0.003, COR and 95%CI=3.67,2.89-20.07 respectively). This is relatively in line with Gondos *et al*.(2015) in Yemen indicating that UTI was higher in the age group between 41–50 years with a percentage of 28% and a strong statistically significant association with UTI (P=0.010). In another way, inconsistence to the current report, another finding by Chuang *et al*.(2005) has shown that patients at 65 years or older developed post-transplant UTI with 55% compared to younger patients (38% of patients at <30 years old). The possible explanation for this disparity may be probably associated with the reason that older patients may be at higher risk due to inefficient voiding because of poor bladder contractility or outflow obstruction.

In the same way, Nicolle *et al*. (2005) reported that prevalence increases with advancing age to reach about (6%) at 60 years of age and to 15% in men over 75years of age. In the same paper, the prevalence of ABU was about 1% in schoolgirls but surges to>20% among women 80 years old residing in the community. However, the present result was incompatible with the previously published paper by Becerra *et al*. (2015) in the USA within the same age group (35-49, P=0.94). The high prevalence of UTI among older age group in the present study may be associated with a biological or behavioral predisposition of the host for uropathogenic strains and declined immunity with advanced age than relatively younger age groups. Although not sufficient, older patients have characterized with poor personal hygiene status that enhances contracting of several infections than younger. Upon advancing age, older persons capable of contracting co-infections owing diminish immunity. It is thought that impaired cellular immunity and immunosuppression tolerance are causes of old patients susceptibility to infection (Gondos *et al*., 2015). In addition, Kumar *et al.* (2016) stated that patients who receive allograft transplant especially orders are more susceptible to infections compared to youngers. This could be explained by accumulating evidence, which express direct association between post-transplantation extent immunosuppression therapy and infection occurrence risk. It is due to specific

type of drugs while high dosage intake is related to fewer acute rejections and more infections occurrence, such as UTI posing a great threat both on the graft outcome and on patient survival.

In the present study, the overall bacterial UTI was found 14.9% of the patients (95%CI=8.2-24.7). The present prevalence was quite smaller than the recent reports from different parts of the world ; Shams *et al*.(2017) in Iran , Becerra *et al*.(2015) in the USA, Menegueti *et al*.(2015) in Brazil, Elkehili *et al*.(2010) in Libya and Ooms *et al*.(2017) in Netherland reported as 22.7 %, 28 %, 26.2 %, 29.5% and 28%, respectively. Not surprisingly, the highest incidence of UTI among renal transplant recipients was also reported by Khosravi *et al*.(2014) in Iran, Gondos *et al*.(2015) in Yemen, Alkatheri, (2013) in Saudi Arabia that was 33.56 %, 33.5 % and 55.5% respectively. However, the current result was nearly similar to reported results from Portugal (16.5%) by Bispo *et al*.(2014) but much higher than the report by Kotagiri *et al*.(2017) in Australia (8%). The frequent kind of infection in kidney allograft recipients may have a varied range of 6%–86% globally (Säemann and Hörl, 2008). This significant variation in UTI reported rates might be due to local ascribe of outbreaks, varying resistance incidences, postoperative medical care, center-specific potent immunosuppressive therapy, hygiene status ,the lack of the robust definition of UTI in many clinical settings and study design. (Veroux *et al*., 2008; Vidal *et al*., 2015; Kotagiri *et al*., 2017).

In the present study, the prevalence of UTI in renal transplant recipient was strongly associated with the previous history of pre-transplantation urinary tract infection (P = 0.010, COR & 95%CI=4.32, 2.09-17.10 respectively) in bivariate risk factors analysis. This finding is compatible with Kumar *et al*. (2016) with P < 0.001 indicating the presence of pre-transplant UTI as a risk factor for post-transplant UTI. This might be due to the presence of resistant strains from those who had the previous history of UTI. In a similar way, the prevalence of urinary tract infection in RTXs was indicated by remarkable statistical association and predisposing factor with the previous history of catheterization which higher than those were who without the history of previous catheterization (P=0.001, COR and 95%CI=1.90, 1.11-11.38 respectively). This result agrees with different research works that reflected the risk of bacteriuria increase by 5% with each day that a catheter is in situ; an increased risk is likely to predispose transplant recipients to the higher risk of infections. Prompt catheter removal or replacement has been associated with a drop in UTI rates (Veroux *et al*.,2008; Chuang *et al*.,2005; Elkehili *et al*.,2010). In addition, this could be justified due to the long duration of catheterization,

frequent catheterization or contamination during inserting catheters. i.e. the risks of getting UTI probably depended on the rate and duration of ureteral catheterization.

Ooms *et al*. (2017) described that catheters increase the risk of UTI because they can serve as reservoirs for bacteria. These phenomena are common particularly in contracting of catheter-associated symptomatic UTI, recurrent and ABU in health care facilities. This was evidenced by Flores-Mireles *et al*.(2015) in that review having that catheter-associated UTIs (CAUTIs) are associated with increased morbidity and mortality, and are collectively the most common cause of secondary bloodstream infections. Consequently, it should be noted that urethral stent and Foley catheter should be implanted with cautions and whenever possible, it should be coated with antibiotics at either pre or post-operative procedure to mitigate the higher rate of UTI incidences globally. As speculated by Gozdowska *et al*.(2016) prophylactic ureteral stenting in kidney transplant recipients prevents complications like ureteric leak and obstruction; it is noteworthy that ureteral stents should be removed between 4 -6 weeks after kidney transplantation unless aggravating factors are expected.

In the contemporary study, the most prevalent bacteria isolates causing post –renal transplant UTI were *Escherichia coli* (18.18%), *Acinetobacter spp*.(18.18%), *P. mirabilis* (9.09%), *S. aureus* (18.18%,), *Enterococcus spp*. (18.18%), CoNS (18.18%). This result is incomparable with recently published research paper by Gozdowska *et al*.(2016); *E.coli* (42%) and *Enterococcus spp*. (10%). Similarly, the current finding dismilar to a retrospective study done by Kotagiri *et al*.(2017) that found *E.coli* (32%) and *Enterococcus spp*.(35%) which were responsible for post- renal transplantation UTI. In addition, another study unveils that *E. coli* (46%), *P. mirabilis* (26%), *S. aureus* (25.8%) and Coagulase-negative *Staphylococci* (6.8%) were etiologies of post-renal transplantation UTI which were relatively higher than the present result except to CoNS (Khosravi *et al*., 2014; Abbott *et al*, 2004). *Acinetobacte spp*. 6.7% (Wagenlehner and Naber, 2012) which is much lower than our result but analogous to current result, 20% (Kumar *et al*., 2016) was reported.

Another study by Ooms *et al*.(2017) among 115 UTI, *E.coli, P. mirabilis, Acinetobacter spp*.and *Enterococcus spp*. were reported as etiologic agents. Overall, many studies stated that the most common pathogen isolated from renal transplant recipients was *E. coli* that magnifies Gram-negative bacterial infection complications among RTXs accounting for more than 70% of UTI infections (Vidal *et al*.,2012; Khosravi *et al*.,2014). Abundant documents have been declared the possibility of coinfections. It seems fungal UTI may cause serious complications that influence both graft success

and patient survival in addition to viral and parasitic infections. It has expressed that fungi microorganisms are the cause of 3% of whole UTIs in RTXs. (Valera *et al*., 2006; Säemann and Hörl, 2008; Kumar *et al*., 2016; Fiorentino *et al*., 2019).

In the current study, the rate of antimicrobial resistance and sensitivity to commonly tested antibiotics among the Gram-negative bacteria ranges from 0-100% and 0-60% respectively. The most effective antibiotics against the Gram-negative isolates was reported gentamycin (100%). However, the least effective antibiotics against *E.coli* were ciprofloxacin, trimethoprim/sulfamethoxazole and cefepime with resistance rate of 100%. Two isolates of *E.coli* showed intermediate resistance to meropenem. This could be probably arming phenomena to the emergence of multidrug-resistant strains that jeopardized clinicians only on the few choices of antimicrobial agents to treat their patients in the study area. The present finding was discordant with a retrospective study in Libya by Elkehili *et al*.(2010) that remarked the commonest drug used for treatment against Gram-negative particularly *E.coli* was ciprofloxacin (51.6%), followed by amoxicillin-clavulanic acid (22.6%), meropenem (12.9%) and others (12.9%). Resembling the present finding, Ooms *et al*.(2017) noticed that 24% of tested Enterobacteriaceae were resistant to ciprofloxacin and 86% to trimethoprim/sulfamethoxazole; came with concluding remarks that antibiotic prophylaxis using these two antibiotics is not effective. This justifies that the bacterial antibiotic prophylaxis selection should have adhered to conventional urinary culture so that prophylaxis should be tailored based on appropriate antibiogram batteries indeed clinicians await too.

Another cross-sectional study incompatible to the present investigation by Gondos *et al*.(2015) in Yemen has shown that amikacin was the most effective antibiotic against all Gram-negative bacilli and ciprofloxacin and doxycycline were the most effective antibiotics against Gram-positive cocci. In contrast, nalidixic acid and ampicillin were the least effective antibiotics in the scenarios. These disparities with present findings may be due to the lack of access or local market availability of antibiotics like naldixic acid and ampicillin were not tested in our center in the present study. The high resistance for the antibiotic in the present study indicates that the infection could happen after the kidney transplantation for enough long periods and not the result of using catheters or ureteric stent. Similarly, Elkehili *et al*. (2010) in Libya had clarified high resistance among the Gram-negative particularly to ciprofloxacin, which agrees to with the present result in that the least effective antibiotic was ciprofloxacin. In the same way, a study Menegueti *et al*.(2015) in Brazil revealed that out of 33%

resistant strains of *E.coli*, 20 % of them were resistant to meropenem but, 100% of them were with intermediate resistance in the current result.

Any discrepancy toward susceptibility results and different isolates in UTI varied from one country to another and from one researcher to another. This probably depends on the therapy regimen in those countries and the public understanding of antibiotic misuse/abuse, overuse from one side, besides the use of standard antibiotic susceptibility testing technique by researchers from another side & the extensive empirical nature of that center. Prolonged pre or post-operative prophylaxis against UTI incidence may favor the evolution of multidrug-resistant strains pathogens in addition to acute graft rejection or acute pyelonephritis (Yuan *et al.*, 2018; Gondos *et al*., 2015; Shahid and Coleman, 2018).

In the present study, the multivariate logistic regression has shown that 35-49 age groups (P = <0.001, adjusted odds ratio = 2.61, 95%CI = 2.06-18.19), the previous history of pre-transplantation UTI (P = 0.02, adjusted odds ratio = 3.48, 95%CI = 2.12-9.38) and the previous history of catheterization (P=0.003, adjusted odds ratio = 3.29, 95%CI = 2.05-11.85) were associated risk factor. Discordant to the present finding, Ooms *et al*.(2017) unveiled that older age groups(>65 years old) were the risk factors for post renal transplantation UTI(P=<0.001, AOR=3.58,95%CI=2.16-5.91). Non-comparable to the present study, Bodro *et al*.(2015) showed that older age groups was not a risk factor(AOR=1, 95%CI=0.9-1.1 and P-value=0.5) for post renal transplantation UTI. This mismatch may be due to study design on one side and the impact of potent immunosuppressive drugs, empirical antimicrobial prophylaxes on the other sides that altogether fosters antimicrobial selective pressure. In line to the present report Bispo *et al*. (2014) declared that the previous history of pre-transplant UTI was a stronger associated risk factor (P=0.004, AOR=7.73, 95%CI=1.90-31.36) for post- transplant UTI.

UTIs, mainly bacterial, are a common infectious complication and remain one of the risk factors for graft loss and patient death in kidney recipients. Unfortunately, Extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae is associated with UTIs and recurrent UTIs, which may cause subsequent impaired renal function or even graft loss among kidney recipients. Multiple drug resistant/extensively drug resistant (MDR/XDR) Gram-negative UTI represent a growing threat to kidney transplant recipients. However, MDR/XDR Gram-negative bacteria, a growing threat to transplant populations, have not been well studied in kidney recipients with UTIs since the interim standard definition of MDR/XDR was proposed (Yuan *et al*.,2018; Magiorakos *et al.*, 2012).

Another important finding of our study was the high percentage of multi drug-resistant strains. MDR was seen in 82% of the isolated bacteria. This is similar to other current study done by Yuan *et al*.(2018) in China which claimed that 81(86.4%) of the organisms were MDR. In the contrary, Gozdowska *et al*. (2016) from Poland reported that about one-fifth of RTX infections are multidrug resistant (37%, n=107). Furthermore, Bodro *et al*. (2015) stated that among the isolates, 37% of which were considered multidrug-resistant strains, which is much lower than our finding. Other findings by Adamska *et al*. (2015) from Poland and Kotagiri *et al*. (2017) from Australia reported relatively much smaller (47%, 37% respectively) MDR prevalence. Thus, It seems that current results may have been influenced by this high percentage of infections caused by MDR organisms. Indeed several studies speculated that an increase of the incidence of infections caused by MDR pathogens in solid organ transplant especially renal transplants recipients resulted in tremendous deleterious effect with focus in worse outcomes than infections caused by their antibiotic-susceptible counterparts despite lacking confirmation (Vidal *et al*., 2012).

The disparity in the present study might be due to potent immunosuppression protocols and selective antibiotic pressure that cumulatively enhance the emergence of antibiotic resistant strains. The frequency of multidrug-resistant organisms has been increasing throughout the world. Frequency is especially prevalent in RTXs reported that about two-thirds of organisms contributing to UTIs being multidrug resistant (Valera *et al*., 2006) which in turn agrees with our present result. This indicates that MDR was found to be very high in the present study. Several prior studies demonstrated that MDR isolates were responsible for up to 69.1% of all organisms leading to symptomatic UTIs in kidney recipients (Kotagiri *et al*., 2017; Origüen *et al*., 2016).

Therefore, the reasons for this alarming phenomenon might be inappropriate and incorrect administration of antimicrobial agents in empiric therapies and lack of appropriate infection control strategies, which can cause a shift to increase the prevalence of resistant organism in the community. In addition to this, Kotagiri *et al*. (2017) noted antibiotic resistance is a significant threat to RTXs because it increases health care costs, prolongs hospital stays, and can result in treatment failure, increased morbidity and mortality. Multi-drug resistant bacteria are may correlated with prophylactic antibiotics and immunosuppression due to together favors antimicrobial selective pressure.

Strengths and limitations of the study

(A). Strengths

- ✓ The study identified the bacterial etiologic agents causing infections in RTXs.
- ✓ To best of our knowledge, it is the first kind of assessment in post kidney transplantation UTI and associated risk factors in Ethiopia.
- ✓ It offered preliminary information for clinician in their empirical therapy.

(B). Limitations of the study

- Lack of MIC tests to determine the susceptibility of all isolates of *staphylococci* to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolate.
- Certain factors like comorbidities or concomitant infections were not included in the study.

Conclusion and Recommendations

Conclusion

In conclusion, the overall prevalence of UTI in our population was relatively low with a prevalence of 14.9%. Majority of the UTIs were asymptomatic. A higher percentage of females were involved. Among the Gram-negative bacteria that caused UTIs, *P. mirabilis* was noted to be the least isolated. The mean dialysis vintage of the patients were 9±1.5 months. The previous history of UTI & catheterizing of the patients was statistical and significantly associated with significant bacteriuria. The Gram-negative bacteria isolates were featured with a higher level of resistance than Gram-positive isolates. The MDR isolates in the present study accounts 82% for urinary isolates. Among the socio demographic data, age was associated risk factor for post renal transplantation UTI.

Recommendations

Based on the finding of the present study, the following recommendations were made:-

(1). It is better to establish routine urine cultures especially in the first 6-12 months after kidney transplantation for recipients on follow up.

(2). Evolution of MDR isolates on RTXs is rising. This would inform a better empirical therapy as the clinicians await culture results to endorse judicious treatment.

(3). Rigorous infection preventions and control strategies should be employed to mitigate both hospital and community-acquired UTI in the study area.

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Annexes

Annex I: Study participant information sheet (English version)

Good morning/afternoon. My name is Teklehaimanot Kiros. I am a medical microbiology M.Sc student from Addis Ababa University, School of Medicine, Black Lion specialized Hospital. I am going to carry out a study entitled under Urinary tract infection among renal transplant recipients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. This is to inform you that you are cordially invited to participate in the study. Your voluntary participation in this study is important. Ahead of this, please read carefully the points below.

Title of the project: Bacterial urinary tract infection among adult renal transplant recipients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Principal Investigator: Teklehaimanot Kiros

Purpose: We have planned to conduct a study with the objective of determining the distribution of bacterial pathogens in asymptomatic and symptomatic bacterial UTI among adult renal transplant recipients and their antimicrobial susceptibility patterns. It is important to know the type of organisms, their pattern of antimicrobial susceptibility and associated risk factors of UTI among adult renal transplant recipients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. Finally, the result of the study is helpful in the appropriate management of urinary tract infection.

Procedures: Your participation is solely voluntary in this study, which would require your response to an interview, to be physically examined and to give the urine sample for laboratory examination.

Risks associated: -There is no any anticipated risk by participating in this study.

Benefits: If there is any positive finding upon laboratory examination, the result will be reported to your physician for your appropriate treatment and management.

Confidentiality: Any information that is collected at any course of the study about you will be kept private and in a secured place.

Thank you very much!

Investigator's name: Teklehaimanot kiros Signature _____ Date _____

Address: Mobile: 0932-055335 or E- mail: tkt0932@gmail.com

በጥናቱ ለሚሳተፉ ግለሰቦች የመረጃ መጠየቂያ እና መቀበያ ፎርም

ጤና ይስጥልኝ እንደምን አደርክህ/ሽ። ስሜ ተ/ኃይማኖት ኪሮስ እባላለሁ:፡ በኢ.ኢ.ዩ. ጠናሳይንስ ኮለጅ የማክሮባሎጂ የማስተርስ ድግሪ ተማሪ ነኝ፡፡ በአሁኑ ሰአት **የኩላሊት እና የሽንት ቱቦ ህመም በኩላሊት ንቅለት ተከላ ታካሚዎች ላይ ጥናት እያካሄድኩ ነው** ፡፡ የኩላሊት እና የሽንት ቱቦ ኢንፌክሽን አምቸጪ ባክቲሪያ በህሙማን ላይ የተለያዩ ችግሮች ሲያመጡ ይታያል፡፡ ይህ ጥናት በቅዱስ ጳውሎስ ሆስፒታል ሚሊኒየም መዲካል ኮሌጅ የኩላሊትንቅለተከላ ማከል ይካሕዳል፡፡ በመሆኑም የሽንት ቱቦ ኢንፌክሽን ምልክት የሚያሳዩትን እና የማያሳዩትን በየትኛዉ ባክቲሪያ እንደተጠቁ መለየት እና ባክቲሪያዉ በየትኛዉ መድሀኒት ሊጠፋ 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 የኩሊት እና የሽንት ቱቦ ኢንፌክሽን (ህመም) በኩላሊ ንቅለት ተከላ 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 የኩላሊት እና የሽንት ቱቦ ኢንፌክሽን ህመም ካለቦት ወይም የምርመራ ውጤቱ ህክምና የሚያስፈልገው ከሆነ ተጨማሪ ምርመራ እና ህክምና እንዲያገኙ የረድተውታል፡፡በተጨማሪም ከጥናቱ በሚገኘው እውቀት በኩሊሉተ እና በሽንት ቱቦ ኢንፌክሽን ባክቴሪያ አማካኝነት የሚመጣውን በሽታ በተሻለ ደረጃ ለመቆጣጠርና ለበሽታው ትክክለኛውን ፀረ ባክቴሪያ ለመምረጥ ለ ሀኪሞች ይረዳቸዋል፡፡

በጥናቱ መሳተፍ እሚያስከፍለዉ ክፍያ ይኖራል? ሁለንም ዓይነት ለጥናቱ የሚያስፈልጉ ምርመራዎች በነፃ የሚሰሩ ሲሆን የህክምናና/የሆስፒታሉ ወጪዎች በሆስፒታለ 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፡፡ስlዚህ 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 አስመሌክቶ ጥያቄ ካለዎት፡፡ ወይም የጥናቱ የመጨረሻ ዉጤት ምን እንደሆነ ለማዉቅ ከፈለጉ በሚከተለዉ አድራሻ ሉያገኙን ይችለሉ፡

ጊዜወትን መሰዋት አድርገው ስለተባበሩኝ ከልብ አመሰግናለሁ!

ተ/ኃይማኖት ኪሮስ

ስልክ ቁጥር : 09-32-05-53-35/ወይም ኢ-ሚይል-tkt0932@gmail.com

Annex II: Consent form (English version)

I certify that nature, purpose, the potential benefits and possible risks associated with participation in this research proposal so that I have explained to the above individual and any questions about this information will be answered. Having got an explanation about the nature and purpose of this study, the procedures, the potential benefits and risks associated with participating in this study. I, the undersigned confirm that I am voluntarily agreed to participate in the study by appending my signature and give consent to participate in the study with a clear understanding of the objectives and conditions of the study. I_____ hereby give my consent for giving the requested information and urine specimen because the proposal has been explained to me in the language I can understand.

Participant's name _____ Participant's signature _____Date_____

Researcher's name _____ Researcher's signature_____ Date_____

የስምምነት መጠየቂያ ቅጽ

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_____ የተመራማሪውፊርማ_____ ቀን_____

Annex III: Questionnaires: general instruction

These questioners will be filled by the researcher or by the health care provider at the renal transplantation center while the sample is collecting from RTXs during the study period. Dear all, you are not expected to write your name. The process will be made by coding. Dear all please mark **"X"** or √ on the space provided.

Part 1: Socio-demographic characteristic questions

Hospital registration number/unique code: _____

Roll no	Characteristics	Alternatives	Skip	Remark
1	Age(years)	□ 18-34 □ 35-49 □50-64 □Above 64		
2	Gender	□Male □ Female		
3	Residence	□Urban □Rural		
4	Religion	□Orthodox □Muslim □Others		
5	Occupational status	□Housewife □Merchant Government employee □ Other		
6	Marital status	□Single □ Married □ Divorced Widowed □Widower		
7	Educational level	□ Illiterate □Student □ Diploma Degree □Above degree		
8	Income level (EBR)	□1000-5000 □>5000		

Part 2: Past medical history

R.no	Characteristics	Alternatives	Skip	Remark
1	Dialysis vintage	□<6 months □6 month-1year □>2years □No dialysis		
2	Year of transplantation	□ 2008 E.C □ 2009 E.C □2010 E.C □Abroad since 2009GC		
3	Renal replacement therapy	□Transplantation □Hemodialysis Peritoneal dialysis		
4	Term of indwelling urethral catheter	□Short □ Long		
5	Insertion of DJ stents	□Yes □ No		

6	Timing of urethra stent removal	□ Early (≤4 weeks) □Late (≥4weeks)		
7	Timing of urinary catheter removal	□Early (≤ 3 days) □ Late (≥ 3days)		
8	Donor's gender/donor category	□Female □Male		
9	Donor's age(years)	□≤65 □ ≥65		
10	Donor type	□Related living □Unrelated living Cadaveric (deceased)		
11	History of any pre-transplant UTI	□Yes □ No		
12	History of catheterization	□Yes □No		
13	Time since transplantation(months)	□0–6 □ 7–12 □13–24 □ >24		
14	Place of transplantation	□Local □Abroad		

Part 3: Current medical history

Physical examinations /History

R.no	Characteristics	Alternatives	Skip	Remark
1	Frequency:	□Yes □No		
2	Fever and chills	□Yes □No		
3	Dysuria	□Yes □No		
4	Hematuria	□Yes □No		
5	Urgency	□Yes □No		
6	Flank pain	□Yes □No		
7	Tender area over graft	□Yes □No		

መጠይቆች: አጠቃላይ መመሪያዎች

እነዚህ መጠይቆች በጥናቱ ወቅት በጥናት ተመራማሪው ወይም በጤና አጠባበቅ ሰጪው አካል በኩላሊትንቅለ ተከላ ማዕከል ይሞላሉ።በማናቸውም የትምህርት መስክ የተገኘ መረጃ የሚቀርበው ለጥናት ዓላማ ብቻ ነው። እና በምስጢር ተጠብቆ ይቆያል። በጥናቱ ውስጥ በጥሩ ሁኔታ እንድትሳተፉ እኔ በትህትና እጠይቃለሁ።ስምዎትን እና ሌሎች ተዛማጅ ነክ የሆኑ ማህበራዊ ጉዳዮችዎን መጻፍ አይጠበቅብዎትም። ሂደቱ በዲጂታል መልክ ይዘጋጅና ምስጢራዊነቱ የተጠበቀ ይሆናል።. ውድ ተሳታፊዎች እባክዎን X ምልክት ወይም (√)ያድርጉ።

ክፍል 1: የማህበረ-ሰነ-ሕዝብ-ሰነ-ባህሪ ጥያቄዎች

የሆስፒታል ምዝገባ ቁጥር / ልዩ ኮድ: _____

ተ.ቁ	ባህሪ	መልስ	ይለፈኝ	አስተየት
1	ዕድሜ	□18-34 □35-49 50-64 □ ከ 64 ዓመት በላይ		
2	ፆታ	□ወንድ □ ሴት		
3	የመኖሪያ ፈቃድ	□ከተማ □ ገጠር		
4	ሃይማኖት	□ኦርቶዶክሳዊ □ሙስሊም □ሌሎች		
5	የስራ ሁኔታ	□የቤት እመቤት □ ነጋዴ □የመንግሥት ሰራተኛ □ሌሎች		
6	የጋብቻ ሁኔታ	□ያላገባ □ያገባ □የፈታ □ባልዋ የሞተባት		
7	የትምህርት ደረጃ	□ምንም ያልተማረ □ተማሪው ዲፕሎማ □ ድግሪ □ በላይ		
8	የገቢ ደረጃ(ብር)	□1000-5000 □ >5000		

ክፍል 2: ያለፉ የህክምና ታሪክ

ተ.ቁ	ባህሪ	መልስ	ይለፈኝ	ኣስተየት
1	የመጥራት ጊዜ:	□<6 ወር □6 ወር -1 አመት □>2 አመት □የለም		
2	የቀዶ ጥገናው ቀን	□2008 ዓ. ም □ 2009 ዓ .ም □ 2010 ዓ ም □ ሌላ		
3	ፕሮቶኮል (ሎች)	□ንቅለ ተከላ □ሂሞዲላይሲስ □ የፒራቲክ ማጣሪያ		
4	የመተንፈሻ urethral ካቴተር ግዜ	□አጭር □ ረጅም		
5	ሁለት የጃን ጭረት ማስገባት:	□አዎ □ አይ		
6	የሽንት ንጣፍ ምጣኔን ማስወጣት	□የመጀመሪያ (≤ 3 ቀን) □ ዘግይቶ (≥ 3 ቀናት)		
7	የጃን ጭረት ማስወገጃ ቀጠሮ	□መጀመሪያ (≤4 ሳምንት) □ዘግይቷል (≥ 4ሳምንት)		
8	ለጋሽ ፆታ	□ ሴት □ወንድ		
9	ለጋሽ ዕድሜ	□65 ዓመት በታች □65 ዓመት በላይ		
10	የልዩ ዓይነት	□ተዛማጅ □ ያልተዛመዱ □ካዳቬሪክ (የሞተ)		
11	የ በፊት ቅድመ UTI ታሪክ	□አዎ □ አይ		
12	የ በፊት catheterization ታሪክ	□አዎ □ አይ		
13	የተተከለው ጊዜ(በወር)	□ከ 0 - 6 □7-12 □13-24 □> 24		
14	የኩላሊት ንቅለ ተከላ ቦታ	□አካባቢያዊ □ውጭ ሀገር		

ክፍል 3: ወቅታዊ የሕክምና ታሪክ

አካላዊ ፈተና / ታሪክ:

ተ.ቁ	ባህሪይ	መልስ	ይለፈኝ	አስተያየት
1	ድግግሞሽ	□አዎ □ አይ		
2	የሽንትምጥ/haematuria	□አዎ □ አይ		
3	አስቸኳይ ሁኔታ	□አዎ □ አይ		
4	ትኩሳትና ብርድ	□አዎ □ አይ		
5	ስሜቶች የወሊድ መወገቢያ ማዕዘን:	□አዎ □ አይ		

Annex IV: Laboratory standard operating procedures

(A). Urine culture

1. Principle

Urinary tract infections (UTI) are one of the most commonly encountered infectious diseases. Urine cultures account for the majority of the workload in the clinical microbiology laboratory. UTI is an illness that can occur from infancy through old age, in otherwise healthy persons, and in those who are immunocompromised or debilitated. Bacteriuria is considered by most clinicians to be a definitive marker of UTI. Urine is normally a sterile body fluid. However, unless it is collected properly, it can become contaminated with organisms from the perineum, urethra, or vagina. The patient should be given detailed instructions for proper specimen collection.

2. Specimen

Noninvasive urine specimens: Clean-catch or catheterized (indwelling). Urine specimens collected by invasive procedures: straight catheter, suprapubic aspiration, cystoscopy, nephrostomy. The specimen should be stored refrigerated if there is a delay in transport to the laboratory. Do not process the following specimens: 1) specimens delayed more than two hours without refrigeration or preservative; 2) 24-hour urine collection; 3) Foley catheter tips; 4) urine from the bag of a catheterized patient, and 5) duplicate specimens collected on the same day.

3. Materials

a. Media: Sheep blood agar, MacConkey agar

b. Sterile inoculating loop (1µl to deliver 0.001 ml and 10 µl to deliver 0.01 ml)

c. Bacterial identification reagents, kits, and susceptibility testing disks.

4. Quality Control (QC)

Process the specimen as soon after receipt as possible. If there is a delay in processing, place the specimen in the refrigerator. Verify that the patient name and identifiers on the specimen match those on the accompanying requisition. Ensure that all media and supplies used have passed the required QC and are used before their expiration date.

5. Safety Precautions

Standard safety precautions for the handling of patient specimens must be used when processing these specimens.

6. Procedure

Media inoculation and incubation: Use sterile loop calibrated to deliver 0.001 ml. Mix urine well. Hold the loop vertically and immerse it just below the surface of the urine. Deliver the loopful onto the plate. Make a straight line down the center of the plate and streak the urine by making a series of passes at 90^{o} angles through the inoculum. Use a calibrated loop to deliver 0.01 ml for the straight catheter, suprapubic, cystoscopy, and nephrostomy specimens.

Incubate overnight at 35^{o}C±2^{o}C. Incubate the plates and examine after 16 -24 hours. Isolate potential pathogens and identify using standard tests as per the laboratory protocol. Perform antimicrobial susceptibility testing (AST) on appropriate organisms. Do not identify resident urogenital flora to the genus or species level. If there is no growth at 24 hours, report as “No growth after 24 hours.” Reincubate plates for an additional 24 hours for the following: 1) tiny or scant colonies present that are barely discernible; 2) culture results do not correlate with clinical findings (e.g. the patient has sterile pyuria or symptoms without a positive culture); 3) specimen was collected by an invasive method.

7. Interpretation

Determine colony count of each organism morphotype. With a 0.001 ml loop (1 µl), one colony equals 1,000 CFU/ml. Interpretation is based on the method of collection and clinical condition:

- Asymptomatic patient; clean-catch or indwelling catheter specimen: report if growth is ≥ 100,000 colony forming unit (CFU)/ml of potential pathogen.
- Symptomatic ambulatory patient; clean-catch specimen: report if growth is ≥ 10,000 CFU/ml with one to two species of a potential pathogen. If >two species, urine is considered to be "contaminated," report: "mixed flora."
- Males; clean-catch specimen: report if growth is ≥1,000 CFU/ml of a potential pathogen.
- Specimens obtained by straight catheterization: report growth of ≥100 CFU/ml of any number of species of potential pathogens.
- All patient types, for specimens obtained by surgery or bladder aspiration: report growth any colony count of potential pathogens.
- All patient and specimen types: report any isolate of yeast.

Gram-negative bacilli account for the majority of UTIs, specifically *Eschericia coli, Proteus mirabilis, Klebsiella pnuemoniae, and Pseudomonas aeruginosa.* Among the Gram-positive cocci, *Enterococccus* spp., *Staphylococcus saprophyticus* and GBS (Group B *Streptococcus* or *Streptococcus agalactiae)*, are the major etiologic agents. *Staphylococcus aureus* is a rare causative agent of UTI and often represents infection in association with *S. aureus* bacteremia or urinary catheterization.

8. Reporting

a. Positive Cultures: Report colony count (CFU/ml), full identification and AST of the pathogen(s)

   Example: Greater than 100,000 CFU/ml *E. coli.*

b. Mixed cultures, report as Mixed Flora or contaminated

Example: “Greater than 100,000 CFU/ml mixed growth of Gram-positive and Gram-negative organisms. Culture result is suggestive of contamination. Please resubmit a new specimen.”

c. GBS should be reported from women in childbearing years and from known diabetics if ≥ 10,000 CFU/ml.

(B). Antimicrobial Susceptibility Test: Kirby Bauer Method

1.Principle

This procedure describes the standard technique used to determine the in-vitro susceptibility of aerobic nonfastidious organisms. Antimicrobial susceptibility testing (AST) should only be performed with pathogens for which well-standardized methods are available and pathogens whose resistance is known or suspected to be a clinical problem. AST should not be performed on normal flora or colonizing organisms. Kirby Bauer (KB) is a standardized procedure for performing AST by disk diffusion. A standardized inoculum of the bacteria is swabbed onto the surface of a Mueller Hinton agar (MHA) plate. Filter paper disks impregnated with antimicrobial agents are placed on the agar. After overnight incubation, the diameter of the zone of inhibition around each disk is measured. By referring to the standardized tables compiled by CLSI, a qualitative report of susceptible, intermediate or resistant can be obtained.

2. Materials

a. MHA, Tryptic Soy Broth (TSB), Normal Saline Solution (NSS)
b. Antimicrobial disks
c. 0.5 McFarland Standard
d. Sterile cotton swabs
e. Ruler or calliper

3. Specimen

The pure culture of the organisms from an 18-24 hour agar plate, preferably a nonselective medium like sheep blood agar.

4. Quality Control (QC)

Test QC strains by following routine procedure and record results in QC forms. Record lot number, and expiration dates of disks and agar. Compare to expected results based on current CLSI standards. Record any out of control result and proceed with the required corrective action.

5. Procedure

a. Bring agar plates and antibiotic disks to room temperature before use.

b. Prepare bacterial suspension.

The direct colony suspension method is the most convenient method for inoculum preparation. This method can be used for most organisms. Select 3 – 5 well-isolated colonies of the same morphologic type from an agar plate culture. Touch the top of each colony with a loop and transfer the growth into a tube containing 4 – 5 ml of TSB or NSS. Mix well and adjust turbidity with broth or NSS to match 0.5 McFarland standard.

The Growth method can be used alternatively and is sometimes preferable when colony growth is difficult to suspend directly and a smooth suspension cannot be made. It can be used for nonfastidious organisms (except Staphylococci) when fresh 24 hour colonies are not available. Select 3 – 5 well-isolated colonies of the same morphologic type from an agar plate culture. Touch the top of each colony with a loop and transfer the growth into a tube containing 4 – 5 ml of TSB. Incubate the broth culture at $35 \pm 2^{o}C$ until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually two to six hours). Mix well and adjust turbidity with TSB to match 0.5 McFarland standard.

c. Inoculate plate with a bacterial suspension

1. Within 15 minutes of adjusting turbidity, dip sterile cotton-tipped applicator swab into the inoculum and rotate against the wall of the tube to remove excess inoculum.

2. Swab entire surface of the agar plate three times, rotating plate approximately 60^{o} between streaking to ensure even distribution. As a final step, swab the rim of the agar.

3. Allow the inoculated plate to stand 3 -15 minutes (no longer than 15 minutes) before applying disks.

d. Apply antibiotic disks to agar surface using sterile forceps or dispenser.

Apply gentle pressure to ensure complete contact of the disk with agar.

Do not relocate a disk once it has made contact with agar surface. Instead, place a new disk in another location on the agar.

Place no more than 12 disks on 150 mm plate and no more than 5 disks on 100 mm plate.

The working supply of antibiotic disks should be stored in a refrigerator (2 – 8 ^{o}C) in a tightly-capped container with desiccant. Upon removal of the disks from the refrigerator, the package containing the cartridges should be left unopened at room temperature for approximately one hour to allow the temperature to equilibrate; this reduces the amount of condensation on the disks. If a disk dispenser is used, it should have a tight-fitting cover, be stored in the refrigerator, and be allowed to warm to room temperature before use.

e. Invert plate and incubate within 15 minutes of disk application. Incubate for 16 – 18 hours at 35 ± 2^{o}C in an ambient air incubator

6. Reading and Interpretation

Read plates only if lawn of growth is confluent. If individual colonies are apparent, the inoculum was too light and the test must be repeated.

a. Hold inverted plate a few inches above a black nonreflecting surface. Illuminate plate with reflected light.
b. Use ruler held on the back of the plate to measure the diameter of the zone of inhibition.
c. Measure the diameters of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the inhibited growth.
d. Measure the zones to the nearest millimeter (mm).
e. Refer to CLSI M100 tables for interpretation of zone sizes.

7. Result reporting

Report the organisms as either Susceptible (S), Intermediate (I), or Resistant (R) to the antimicobial agents that have been tested.

Annex V: Laboratory reporting formats (work sheets)

- **Urine specimen work-up (For Kidney transplant recipients only)**

			Urinalysis(UA)		
S.no	**Date**	**Patient's ID**	**Chemical**	**Microscopic**	**Remark**
1					
2					
3					

- **Culture work -up sheet**

Patient's ID:		**Specimen:**	**Receive date:**
Bacterial culture observations and work-up			
Date	**Observations &work-up**	**Performed by:**	
---/---/---			
Final culture report			
Comments			

- **AST results reporting**

Bacterial isolate	**Antibiotics tested**						
	patterns	**Tetracycline**	**Nitrofurantoin**	**Meropenem**	**Gentamicin**	**Ciprofloxacin**	**Ceftriaxone**
1	**S**						
2	**I**						
3	**R**						

Annex VI: Dummy tables

1. Socio-demographic characteristics of RTXs investigated for UTI.

Variable	UTI no (%)	No UTI no (%)	Total (%)	Bivariate analysis		P-value
				COR	95%CI	
1						
2						
3						

2. Association of variables with UTI among RTXs by multivariate logistic regression analysis

Risk factor	Significant bacteriuria		AOR	95% CI	P-value
	Yes no (%)	No no (%)			
1					
2					
Total					

3. Distribution of the causative agents of asymptomatic and symptomatic UTI among RTXs

Bacterial Isolates	UTI		
	Asymptomatic UTI No (%)	Symptomatic UTI No (%)	Total No (%)
1			
2			
Total			

Declaration

I, the undersigned, declare that this M. Sc Thesis is my original work and has not been presented for a degree in any of other University and all sources of materials used for the Thesis have been dully acknowledge.

Principal investigator:

Teklehaimanot Kiros (B.Sc) Signature _____ Date of Submission_____

Place of submission: Addis Ababa, Ethiopia

Supervisor:

Professor Daniel Asrat (MD, M.Sc, PhD) Signature _____ Date _____

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