PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF UROPATHOGENIC *ESCHERICHIA COLI* FROM URINARY TRACT INFECTION PATIENTS IN SELECTED HEALTH FACILITIES OF ADDIS ABABA, ETHIOPIA

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Phenotypic and molecular characterization of uropathogenic *Escherichia coli* from urinary tract infection patients in selected health facilities of Addis Ababa, Ethiopia

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Table of Contents

Abbreviations/Acronyms

ABU: Asymptomatic bacteriuria
aer: Aerobactin
afa: Afa adhesin (afimbrial adhesin)
bp: base pair
CFU: Colony forming unit
CI: Confidence interval
CLSI: Clinical and Laboratory Standards Institute
cnf: Cytotoxic necrotizing factor
DNA: Deoxyribonucleic acid
DNTPs: Deoxynucleoside triphosphates
ESBL: Extended spectrum beta lactamase
fim H: Type 1 fimbriae
hly: Hemolysin
Kb: Kilobase
LPS: Lipopolysaccharide
MTA: Material Transfer Agreement
MDR: Multidrug resistance
MIU: Motility indole urea
MSU: Midstream urine
NaOH: Sodium Hydroxide
OPD: Outpatient department
OR: odds ratio
pap: P fimbriae (pyelonephritis associated pilli)
PCR: Polymerase chain reaction
rpm: Rotation per minute
sfa: S and FIC fimbriae
SOPs: Standard operating procedures
TSI: Triple Sugar Iron
UK: United Kingdom
UPEC: Uropathogenic *Escherichia coli*
USA: United States of America
UTI: Urinary tract infection
UV: Ultraviolet
WHO: World Health Organization

List of Figures

Figures **Page**

List of Tables

Tables **Page**

List of Appendices

Page

Abstract

Introduction*: Urinary tract infection (UTI) is major causes of morbidity and mortality worldwide. Uropathogenic Escherichia coli (E. coli) bacterium is responsible for majority of (50-80%) cases of UTI. Drug resistant E. coli is a significant threat to effective treatments. Uropathogenic E. coli strains derive from different phylogenetic groups and possess an arsenal of virulence factors that contribute to their ability to overcome different defense mechanisms and cause disease. Despite its public health importance, data regarding the molecular characteristics of uropathogenic E. coli is lacking in Ethiopia. Therefore, for proper treatment and management of UTI; it is essential to determine drug resistance patterns, phylogroup and virulence genes of E. coli.*

Objective*: To assess the phenotypic and molecular characteristics of uropathogenic E. coli as well as relationship of virulence genes and drug resistance patterns of uropathogenic E. coli among patients with UTI in selected health facilities of Addis Ababa, Ethiopia.*

Methods: *A cross sectional facility based study was conducted on 780 patients with urinary tract infections visiting Tikur Anbessa Specialized Hospital (N=580), Yekatit 12 Hospital (N=30) and Zewditu Memorial Hospital (N=170), Addis Ababa, Ethiopia from January 1, 2017 to October 9, 2017 using a well-designed questionnaire and microbiological investigations. Uropathogenic E. coli bacteria were isolated from urine samples using bacterial culture and conventional biochemical tests. Phenotypic drug resistance patterns and plasmid profile was determined using Kirby Bauer disc diffusion and plasmid analysis respectively. Identification of phylogroup and genes that encodes for virulence factors was done using multiplex polymerase chain reaction (PCR). Data was processed and analyzed with SPSS version 16.0 and EPI info version 3.4.1 softwares. P-value less than 0.05 were considered significant.*

Result*: Among 780 UTI patients, 200 uropathogenic E. coli bacteria were isolated. E. coli isolates had highest resistance (86.5%) to ampicillin followed by ceftazidime (84%), ceftriaxone (80.5%), tetracycline (80%), trimethoprim-sulfamethoxazole (68.5%) and cefotaxime (66%). Highest susceptibility to meropenem (100%) and imipenem (100%) were observed. E. coli isolates were susceptible to amikacin (97.5%), nitrofurantoin (95%), ciprofloxacin (85.5%), norfloxacin (85%), chloramphenicol (83.5%), gentamicin (80%) and nalidixic acid (79%). Multidrug resistance (MDR) was observed to most (96.5%) E. coli isolates. Plasmid analysis showed the presence of plasmid/s in 165 (82.5%) E. coli isolates. Majority of tested E. coli isolates had upto 10 plasmids, the size of which commonly 23kb. The most frequent E. coli virulence gene amplified was fim H 164 (82%), followed by aer 109 (54.5%), hly 103 (51.5%), pap 59 (29.5%), cnf 58 (29%), sfa 50 (25%) and afa 24 (12%). Significant association (p-0.014) between fim H and chloramphenicol drug resistance as well as between aer genes, and gentamicin, ampicillin and nitrofurantoin drug resistance (p-0.028, p-0.018 and p-0.023 respectively) was observed. There was significant association between pap gene and urine urgency (p-0.016); sfa, and dysuria and urine urgency (p-0.019 and p-0.043 respectively); hly and suprapubic pain (p-0.002); aer and suprapubic pain, flank pain and fever (p-0.017, p-0.040, p-0.029 respectively). Majority of E. coli isolates were phylogroup B2 60 (30%) followed by D 55 (27.5%), B1 48 (24%) and A 37 (18.5%).*

Conclusion: *The overall incidence of antimicrobial resistant (including MDR) E. coli in this study was high to commonly used antibiotics but none to carbapenems. The virulence genes encoding components of adhesins, iron acquisition systems, and toxins were highly prevalent. Majority of E. coli isolates were phylogroup B2 followed by D. Therefore, periodic monitoring of drug resistance patterns and targeting major virulence genes as potential vaccine candidates is essential for better management of UTI and further large scale studies should be conducted in this area.*

Key words: Uropathogenic *E. coli,* urinary tract infection, drug resistance, plasmid profile, virulence factors and phylogroup

CHAPTER ONE

Introduction

1.1 Background

Urinary tract infections (UTIs) are infections caused by the presence and growth of microorganisms anywhere in the urinary tract. It is usually due to bacteria from the digestive tract which climb the opening of the urethra and begin to multiply to cause infection (Rahimkhani *et al.*, 2008; Okonko *et al*., 2009). In contrast to men, women are more susceptible to UTIs, and this is mainly due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora (Haider *et al*., 2010).

Urinary tract infections are usually classified by the site of infection as bladder (cystitis), kidney (pyelonephritis) or prostate (prostatitis). They are asymptomatic or symptomatic. UTIs that occur in a normal genitourinary tract with no prior instrumentation are considered as "uncomplicated", whereas "complicated" infections are diagnosed in genitourinary tracts that have structural or functional abnormalities including instrumentation such as indwelling urethral catheters and are frequently asymptomatic (Gonzalez and Schaeffer, 1999).

Risk factors associated with UTI are obstruction i.e. blockages that makes it difficult to empty the bladder can cause UTI. Obstructions can be caused by an enlarged prostate, kidney stones, urinary schistosomiasis and certain forms of cancer; gender i.e. women are more likely to get UTIs. This is because of shorter and wider urethra. Although UTIs in men are less common, it can be more serious; during sexual activity the pressure on the urinary tract during the act can move bacteria from the colon into the bladder. Most women have bacteria in their urine after intercourse. However, the body usually can get rid of these pathogens within 24 hours. Bowel bacteria have properties that allow them to stick to the bladder; conditions such as diabetes mellitus, prolonged use of urinary catheter, loss of oestrogen during menopause or otherwise loss of estrogen alters the normal bacteria in the vagina increase the risk of UTI; spermicides can cause skin irritation in some women, hence increasing the risk of bacteria entering into the bladder (Lights and Boskey, 2012).

UTI annual global incidence is of almost 250 million (Ronald *et al*., 2001; Loh and Sivalingam, 2007; Gastmeir *et al*., 1998). UTI can be either symptomatic or asymptomatic. Patients with significant bacteriuria who have symptoms referable to the urinary tract are said to have symptomatic bacteriuria. Asymptomatic bacteriuria (ABU) is a condition characterized by presence of bacteria in two consecutive clear-voided urine specimens both yielding positive cultures ($\geq 10^5$ colony forming unit [CFU] per millilitre of urine) of the same uropathogen, in a patient without classical symptoms. *E. coli* is the major etiologic agent in causing UTI, accounting for up to 90% of cases. *Proteus* species, *Klebsiella* species, *Pseudomonas aeruginosa*, Group B *Streptococcus*, *Staphylococcus aureus* and *Staphylococcus saprophyticus* are recognized organisms to cause UTI (Loh and Sivalingam, 2007). *E. coli* strains isolated from the urinary tract are known as uropathogenic *E. coli* (UPEC). UPEC cause 80-90% of community-acquired urinary tract infections (UTI) and 50% of nosocomial UTI (Schwartz *et al*., 2013).

There is growing concern regarding antimicrobial resistance worldwide, particularly to uropathogenic *E. coli* which is the dominant causative agent of UTI (Chakupurakal *et al*., 2010; Gupta *et al*., 2001; Mordi and Erah, 2006). In Ethiopia, UTI and drug resistance to UPEC are a major problems (Gizachew *et al*., 2013; Beyene and Tsegaye, 2011; Biadglegne and Abera, 2009). Bacterial drug resistance can easily be transferable to other bacteria through bacterial plasmid. Clinical isolates of *E. coli* which showed multiple drug resistance possess multiple plasmids with different size (Umolu *et al*., 2006).

Virulence factors involved in UPEC infection include fimbrial and non-fimbrial adhesins, toxins, iron acquisition factors, lipopolysaccharide (LPS), and capsules, which facilitate the colonization of bacteria in the urinary tract and invasion into host cells. These virulent factors encoded in genes in a plasmid or chromosomal DNA which can be transmitted to other bacteria (Slavchev *et al*., 2009).

Uropathogenic *E. coli* strains mainly classified into four phylogroups: A, B1, B2 and D. These various phylogroups differ in their phenotypic and genotypic characteristics, their ecological niche and ability to cause disease (Clermont *et al*., 2013; Clermont *et al*., 2000).

Therefore, assessing the phenotypic drug resistance patterns, plasmid profile, virulence genes and phylogroups of uropathogenic *E. coli* are important for management and prevention of UTI.

1.2 Statement of the Problem

Urinary tract infections are one of the most common human infections and a major cause of morbidity and mortality worldwide (Rajni *et al*., 2008; Foxman, 2002). It has been estimated that globally symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually (Razak and Gurushantappa, 2012). In Africa, including Ethiopia urinary tract infections are the most common causes of morbidity and mortality (Biadglegne and Abera, 2009; Romanus and Eze, 2011; Mbanga and Mudzana, 2014). *E. coli* is the major etiologic agent in causing urinary tract infections, which accounts for up to 80% of cases (Schwartz *et al*., 2013). Serotypes of *E. coli* consistently associated with UTI are designated as uropathogenic *E. coli* (UPEC) (Schwartz *et al*., 2013; Raksha *et al*., 2003). UPEC are implicated in 70-90% of community acquired UTIs and 50% of nosocomial UTIs (Johnson and Russo, 2005).

Antibiotic resistance, including multidrug resistance (MDR), is a major problem in UPEC is increasing every year (Gibreel *et al*., 2012). The high antimicrobial resistance of UPEC significantly reduces the therapeutic options and increases the treatment costs and mortality rates (Momtaz *et al*., 2013; Beyene and Tsegaye, 2011; Biadglegne and Abera, 2009). Antimicrobial resistance is dynamic and needs to be monitored every time to choose empirical treatment for UTIs by the physicians. Thus, this research fills the gap on the current status of drug resistance patterns of UPEC in Addis Ababa, Ethiopia which is important for appropriate treatment of UTIs.

Uropathogenic *E. coli* strains possess an arsenal of virulence factors that contribute to their ability to overcome different defense mechanisms to cause disease. The virulence factors that are located in virulence genes include fimbriae, iron-acquisition systems, flagella and toxins. Virulence genes are located on transmissible genetic elements (plasmid) and/or on the chromosome (Farshad *et al*., 2012) so that non-pathogenic strains acquire new virulence factors from accessory DNA (Johnson *et al*., 2005). The majority of uropathogenic *E. coli* strains responsible for UTIs belong to phylogroup B2 or, to a lesser degree, to phylogroup D, whereas commensal isolates belong to phylogroups A and B1 (Munkhdelger *et al*., 2017; Kot *et al*., 2016). Thus, identifying the phylogroup and virulence factors of UPEC is important which may help for future vaccine targets and epidemiological purpose.

1.3 Literature review

1.3.1 Urinary Tract Infection

Urinary tract infections (UTIs) cause morbidity and mortality, with an estimated 40-50% of women experiencing at least one cystitis episode in their lifetime (Kaper *et al.*, 2004). Individuals with UTI will have a significant number ($\geq 10^5$ CFU per millilitre of urine) of pathogens in the urinary system. Pathogens may be present in the bladder (cystitis), kidneys (pyelonephritis), urine (bacteriuria) or prostate (prostatitis) (Marrs *et al.*, 2005). UTIs are classified as either lower (confined to the bladder) or upper (pyelonephritis), and as either uncomplicated or complicated. An uncomplicated UTI is the one occurring in a normal host who has no structural or functional abnormalities, is not pregnant, or who has not been instrumented (for example, with a catheter). All other UTIs are considered complicated (Foxman, 2010).

1.3.1.1 Epidemiology and Etiology of Urinary Tract Infection

Annual global incidence of UTI is of almost 250 million (Ronald *et al.*, 2001; Loh and Sivalingam, 2007). The distribution of symptomatic infection has a somewhat different shape: women aged 15-29 years have the highest frequency (approaching 20%). Among women aged 18 years and older, the estimated incidence was 12.6%; for men, this incidence was only 3% (Foxman, 2010). Almost all patients with an indwelling urinary catheter for 30 days or longer develop catheter-associated UTI, which accounts for approximately 40% of all hospital acquired infections (Foxman *et al.*, 2002). About 20% of all UTIs cases occur in men (Griebling, 2005). Annually, UTI is the cause of approximately 8 million physician visits, which costs the United States (USA) health care system approximately 1.6 billion dollars (Cai *et al.*, 2013). A 2007-2008 surveillance study conducted at 34% of hospitals in the Netherlands found that 1.2% of patients had a catheter related UTI and 1.7% had a symptomatic UTI. 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 (Foxman, 2010). In Ethiopia, urinary tract infection causes significant morbidity and mortality (Beyene and Tsegaye, 2011; Biadglegne and Abera, 2009; Moges *et al.*, 2002).

E. coli is the major etiologic agent in causing UTI, accounting for up to 90% of cases. *Proteus* species, *Klebsiella* species, *Pseudomonas aeruginosa*, *Enterobacter* species, Group B *Streptococcus*, *Staphylococcus aureus, Staphylococcus saprophyticus*, *Staphylococcus haemolyticus Enterococci*, *Gardnella vaginalis* and *Ureaplasma urealyticum* are recognized organisms to cause UTI (Loh and Sivalingam, 2007; Cheesbrough, 2006).

1.3.1.2 Pathogenesis and Clinical Manifestation of Urinary Tract Infection

Urinary tract infection is presence of microorganisms somewhere in urinary tract and when the urine contains 10^5 organisms or more per millilitre. Infection of the bladder is called cystitis. It causes frequency, dysuria (pain on passing urine), suprapubic pain, sometimes haematuria and usually pyuria (increased number of pus cells in urine). The term acute urethral syndrome, (dysuria-pyuria) is used to describe acute cystitis accompanied by pyuria but in which no bacteria are detected by routine culture. Infection of the kidney is called pyelonephritis. It causes loin pain, pyuria, rigors, fever, and often bacteraemia (Cheesbrough, 2006; Mohajeri *et al*., 2014; Flores-Mireles *et al*., 2015).

UPEC is a causative agent in the vast majority of UTIs, including cystitis and pyelonephritis, and infectious complications, which may result in acute renal failure in healthy individuals as well as in renal transplant patients. UPEC expresses a multitude of virulence factors to break the inertia of the mucosal barrier. In response to the breach by UPEC into the normally sterile urinary tract, host inflammatory responses are triggered leading to cytokine production, neutrophil influx, and the exfoliation of infected bladder epithelial cells. Several signaling pathways activated during UPEC infection, including the pathways known to activate the innate immune response, humoral immunity and cell mediated immunity (Bien *et al*., 2012).

Uropathogens have specialized characteristics, such as the production of adhesins, siderophores and toxins that enable them to colonize and invade the urinary tract to cause urinary tract infections (Foxman, 2002). By multiplying and overcoming host immune surveillance, the uropathogens can subsequently ascend to the kidneys, again attaching via adhesins or pili to colonize the renal epithelium and then producing tissue-damaging toxins. Consequently, the uropathogens are able to cross the tubular epithelial barrier to access the blood stream, initiating bacteraemia (Flores-Mireles *et al*., 2015).

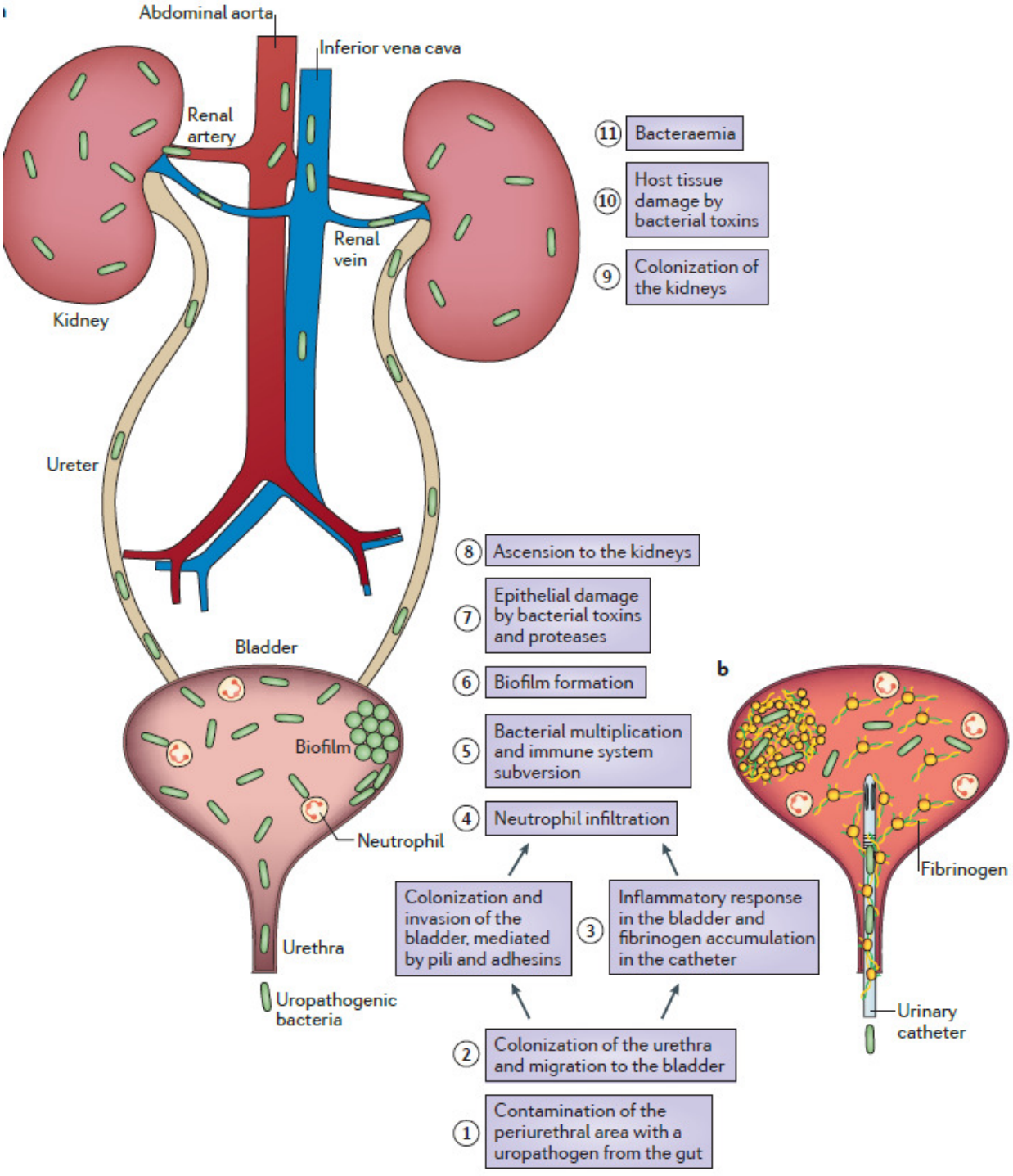

Fig 1: Pathogenesis of urinary tract infection (Flores-Mireles *et al.*, 2015)

1.3.1.3 Diagnosis and Treatment of Urinary Tract Infection

UTIs are defined by the presence of a significant level of bacteria in the urine (i.e. bacteriuria). A pure culture of $\geq 10^5$ CFU per milliliter of urine is indicative of UTI. Microscopic hematuria, leukocyte esterase test and nitrite tests are also suggestive tests for UTI (Cheesbrough, 2006). Clinically UTI is diagnosed by typical symptoms of the disease, such as the presence of at least two of the following complaints: dysuria, urgency, frequency, incontinence, suprapubic pain, flank pain or cost vertebral angle tenderness, fever ($\geq 38^{o}C$) and chills (Mohajeri *et al*., 2014; Yun *et al*., 2015; Cheesbrough, 2006; Usein *et al*., 2001).

Currently, antibiotics such as trimethoprim-sulfamethoxazole, ciprofloxacin and ampicillin are the most commonly recommended therapeutics for UTIs. However, increasing rates of antibiotic resistance and high recurrence rates threaten to greatly enhance the burden that these common infections place on society (Flores-Mireles *et al*., 2015). Although generally self limiting, treatment of UTIs with antibiotics leads to a more rapid resolution of symptoms and is more likely to clear bacteriuria, but also selects for resistant uropathogens and commensal bacteria and adversely affects the gut and vaginal microbiota. As uropathogens are increasingly becoming resistant to currently available antibiotics, it may be time to explore alternative strategies for managing UTI (Foxman, 2010).

1.3.2 Uropathogenic *Escherichia coli*

E. coli is Gram negative, facultative anaerobic bacteria which is the most common causative agent (50-80%) of UTI (Zhanel *et al*., 2006; Cheesbrough, 2006). Serotypes of *E. coli* consistently associated with UTI are designated as uropathogenic *E. coli* (Raksha and Srinivasa, 2003). UPEC are implicated in 70-90% of community acquired UTIs and 50% of nosocomial UTIs (Johnson and Russo, 2005). UPEC strains are often classified on the basis of O (somatic), K (capsular polysaccharide), and H (flagellar) antigens. There is a high frequency of the antigens O1, O2, O4, O6, O7, O8, O16, O18, O25, and O75 among UPEC strains, while specific K and H antigens display a less distinct pattern (Bidet *et al*., 2007).

1.3.2.1 Drug Resistance and Plasmid Profile of Uropathogenic *E. coli*

The presence of drug resistant *E. coli* including multidrug resistance (MDR) and extended spectrum beta lactamase (ESBL) producing *E. coli*, in clinical isolate has been documented as a very serious problem and a significant threat to quick survival of patients in the hospital, high economic burden, lost of hours in life's activities and high treatment failure (Igwe *et al*., 2014). Drug resistance to UPEC has been reported to commonly used antibiotics such as ampicillin, amoxicillin-clavulanic acid, norfloxacin, cefuroxime, ceftriaxone and co-trimoxazole (Derakhshandeh *et al*., 2015; Niranjan and Malini, 2014; Ranjini *et al*., 2015).

Treatment of UTIs cases is often started empirically and therapy is based on the information determined from the antimicrobial resistance pattern of the urinary pathogens (Abdu *et al*., 2018). However, a large proportion of uncontrolled antibiotic usage has contributed to the emergence of resistant bacterial infections (Grude *et al*., 2001; Kripke, 2005). ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem) (CDC, 2012). These bacterial enzymes have capacity to inactivate practically all cephalosporins (Turner, 2005). There is a large reservoir of resistant genes, in bacterial genomes and in extra-chromosomal pieces of DNA (plasmids) that encode different mechanisms of drug resistance. The transmission of antibiotic resistance is attributed to plasmids (Soulsby, 2005).

Although conventional antimicrobial susceptibility testing methods are useful methods for detecting resistance profiles and for selecting potentially useful therapeutic agents, they are insensitive tools for tracing the spread of individual strains within a hospital or region. Molecular methods like plasmid profiling helps to track bacterial strains and contribute to the evaluation of nosocomial infection outbreaks, recurrent infection and clonal dissemination of specific pathogens (Sader *et al*., 1995). They are also used as a means of providing additional information, to detect and evaluate the mode of dissemination of MDR pathogens. Plasmid analysis has also proved a useful method for differentiating bacterial isolates. The number and size of the plasmids present is used as the basis for strain identification. This strain typing technique has been used successfully for analysis of outbreaks of nosocomial infections and

community acquired infections caused by a variety of species of Gram negative rods (Khadgi *et al*., 2013).

The common use of empirical antibiotics has contributed to the rise of multidrug-resistant organisms, reducing treatment options and increasing costs (Davenport *et al*., 2017). Antibiotic resistance genes are located on mobile genetic elements and their distribution expedites their presence within communities. Thus, a high degree of resistance to various classes of antibiotics has been observed among UPEC strains, resulting in great concern for the treatment of UTIs (Rijavec *et al*., 2006). In contrast with WHO recommendations for UTI treatment with trimethoprim-sulfamethoxazole, many studies have proposed trimethoprim-sulfamethoxazole as an inappropriate antibiotic for the treatment of UTI (Abass *et al*., 2014; Farshad *et al*., 2012; Ramos *et al*., 2011).

According to different studies conducted in Ethiopia, UPEC strains showed drug resistance to different classes of antibiotics among UTI patients. A study conducted in Bahirdar, Ethiopia showed that UPEC isolates were resistant to ampicillin (83.1%), gentamicin (33.8%), tetracycline (66.2%), chloramphenicol (57.1%), trimethoprim-sulfamethoxazole (45.5%), norfloxacin (9%) and nitrofurantoin (7.8%). MDR was observed in 93.1% of UPEC isolates (Biadglegne and Abera, 2009). A study conducted in Hawassa, Ethiopia by Gizachew *et al*. (2013), *E. coli* isolates showed 100% resistance for ampicillin and 81.25% for trimethoprim-sulfamethoxazole followed by 43.8% resistance for both ciprofloxacin and chloramphenicol; whereas 96.87% *E. coli* susceptibility was observed for nitrofurantoin followed by ceftriaxone (84.4%), cefotaxime (81.3%) and gentamicin (75.0%). Similar study conducted in Jimma, Ethiopia indicated resistance to ampicillin (100%), tetracycline (28.6%), trimethoprim-sulfamethoxazole (28.6%) and ciprofloxacin (14.3%) by UPEC isolates (Beyene and Tsegaye, 2011).

In Africa, different studies showed UPEC strains developed drug resistance among UTI patients. A study conducted in Nigeria indicated that antibiotic susceptibility rates for UPEC were; doxycycline (31%), ampicillin (5%), ceftazidime (99.1%), cefotaxime (95.6%), amoxicillin/clavulanic acid (82.9%), cefuroxime (89%), imipenem (99%), ciprofloxacin (65.4%), sulphamethoxazole/trimethoprim (6.4%), nitrofurantoin (96.5%), gentamicin (72%), and

kanamycin (93.7%). Resistance of UPEC bacteria to these drugs especially to ampicillin and sulphamethoxazole/trimethoprim which are the most common oral drugs used in general practice calls for serious concern and therefore empirical treatment of urinary tract infections with these drugs should be avoided (Romanus and Eze, 2011). Similar study conducted in Nigeria, UPEC accounted for 150 (62.50%) of 240 urine isolates. Low susceptibility was observed against ampicillin 36%, ciprofloxacin 46% and norfloxacin 48%. More than 50% were susceptible to levofloxacin and streptomycin 54% each, cefuroxime and co-trimoxazole 52% each, while high susceptibility was for nitrofurantoin 78%, chloramphenicol 70% and gentamicin 64% (Abdu *et al*., 2018).

According to a study conducted in Egypt, drug resistance was reported against ampicillin 89%, trimethoprim-sulfamethoxazole 74%, nalidixic acid 66%, norfloxacin 63%, gentamicin 57%, ceftazidime 40%, amikacin 11%, nitrofurantoin 11%, meropenem 0% and imipenem 0% in UPEC isolates (Alabsi *et al*., 2014). Similar study conducted in Tunisia showed that presence of drug resistance against ampicillin (72.1%), cefotaxime (17.4%), nalidixic acid (41.8%), ciprofloxacin (38.8%), amikacin (17.4%), gentamicin (23.9%), tetracycline (63.7%) and cotrimoxazole (40.8%). All strains were susceptible to imipenem (Ferjani *et al*., 2014). Akingbade *et al*. (2014) from Nigeria reported that UPEC isolates were resistant to ampicillin (90.8%), erythromycin (75.8%), cotrimoxazole (70.0%), streptomycin (70.0%) and tetracycline (68.3%). Clinical isolates of *E. coli* which showed multiple drug resistance were also found to harbour plasmids with molecular sizes ranging from 2kb to 6.5kb and a maximum 26kb. Similar study in Nigeria also reported that *E. coli* isolates with high multi-drug resistance profiles were found to possess multiple plasmids with large sizes in the range of 6.557-23.130kb (Umolu *et al*., 2006).

In Europe, different studies investigated drug resistance to UPEC isolates. A study conducted in Spain, UPEC isolates showed drug resistance against ampicillin 63%, tetracycline 35%, trim-ethoprim-sulfamethoxazole 28%, amoxicillin-clavulanic acid 13%, gentamicin 4%, ciprofloxacin 3%, amikacin 1%, ceftazidime 0% and imipenem 0% (Guiral *et al*., 2015). Similar study conducted in Israel, UPEC isolates showed drug resistance against ampicillin 92%, trim-ethoprim-sulfamethoxazole 49% and ciprofloxacin 6% (Johnson *et al*., 2005). Another study done in Czech Republic, drug resistance was found toward ampicillin 43.4%, piperacillin 31.7%,

tetracycline 15.9%, trimethoprim-sulfamethoxazole 11.7%, but no drug resistance to amikacin and meropenem (Koreň *et al*., 2013).

In Asia, many scholars have reported the presence of drug resistant UPEC isolates among UTI patients and drug resistant UPEC isolates carried plasmids. A study conducted in Iraq showed the presence of drug resistance against tetracycline 83.3%, ampicillin 92.6%, amoxicillin-clavulanic acid 90%, cefotaxime 78%, nalidixic acid 78%, trimethoprim-sulfamethoxazole 73.3%, ceftriaxone 71.3%, gentamicin 70.7%, ciprofloxacin 52.6%, amikacin 46%, norfloxacin 48.6%, chloramphenicol 30% and imipenem 4.7% in UPEC isolates (Merza and Jubrael, 2015). Similar study conducted in Iran showed that drug sensitivities of the UPEC isolates were 19.8% (ampicillin), 24% (trimethoprim-sulfamethoxazole), 29.2% (tetracycline), 75.5% (naldixic acid), 80.4% (cefixime), 84.6% (gentaimcin), 91.4% (ciprofloxacin), 96.8% (nitrofurantoin), 96.8% (amikacin) and 100% (imipenem). Seventy nine percent (79%) of UPEC isolates harbored plasmids with average of 5.5 plasmids (range: 1-10) in each strain (Farshad *et al*., 2012). Another study conducted in Iran showed that 74% of UPEC isolated were MDR. High resistance was observed against ampicillin (81.3%), nalidixic acid (71.3%) and cotrimoxazole (61.3%) respectively. Fifty six percent (56%) of UPEC isolates showed extended spectrum beta lactamase production (Neamati *et al*., 2015).

Plasmid profiling of UPEC isolates in Nepal showed that all the isolates, except one, contained at least one plasmid. The isolates had from one to five plasmids. Five had two plasmids. Three had three plasmids. One had four, and one had five plasmids. Plasmid of size approximately 23 kb was common in all isolates. Only one isolate out of 25 isolates contained plasmid sized bigger than 23 kb (Khadgi *et al*., 2013). According to a study conducted in Mongolia, the most common antibiotic resistance was to cephalotin (85.1%), ampicillin (78.4%) and the least to nitrofurantoin (5.4%) and imipenem (2%). In total, 93.9% of isolates were MDR (Munkhdelger *et al*., 2017).

Scholars in India reported that UPEC isolates were resistant to different classes of antibiotics and carry plasmid DNA. According to Niranjan and Malini (Niranjan and Malini, 2014), 76.51% of UPEC isolates were MDR. The UPEC isolates showed high levels of resistance to ampicillin 88.4%, amoxicillin-clavulanic acid 74.4%, norfloxacin 74.2%, cefuroxime 72.2%, ceftriaxone 71.4% and co-trimoxazole 64.2%. The isolates were sensitive to amikacin 82.6%, piperacillin-

tazobactum 78.2%, nitrofurantoin 82.1% and imipenem 98.9%. Mukherjee *et al*. (2013), reported that a high level of resistance to UPEC isolates was observed against ampicillin 97.5%, nalidixic acid and cefelexin 95%, amoxicillin 92.5%, cotrimoxazole 82.5% and ciprofloxacin 80%. Forty five percent (45%) of UPEC isolates were ESBL producers. Ranjini *et al*. (2015), reported that 82.6% and 39.66% of UPEC isolates were MDR and ESBL producers respectively. High degree of resistance was observed to amoxicillin 93.2% and amoxicillin-clavulanic acid 90.5%. Similar study conducted in India reported that clinical isolates of *E. coli* were known to harbour plasmids of different molecular size ranging from 2-3kb to 6.5kb and maximum 26kb (Jan *et al*., 2009).

In South America, UPEC strains showed drug resistance to different classes of antibiotics. A study conducted in Mexico indicated that UPEC isolates were resistance to ampicillin (83.7%), norfloxacin (60.6%), nalidixic acid (56.4%), trimethoprim/sulfamethoxazole (56.4%), ciprofloxacin (55.5%), piperacillin (53.8%), gentamicin (23.9%), amoxicillin/clavulanic acid (19.6%), ceftriaxone (10.2%), ceftazidime (8.5%), cefuroxime (14.5%), nitrofurantoin (5.1%), amikacin (1.7%) and meropenem (0.85%). Thirty percent (30%) of UPEC isolates were MDR (Molina-López *et al*., 2011). Another study conducted in Mexico showed that presence of drug resistance against ampicillin 97.4%, cefotaxime 72.7%, trimethoprim-sulfamethoxazole 66%, ceftriaxone 48.9%, gentamicin 50%, amikacin 14.4%, nitrofurantoin 44.8% and chloramphenicol 25.8% in UPEC isolates (Paniagua-Contreras *et al*., 2015). Similar study conducted in Brazil, UPEC isolates showed drug resistance against ampicillin 51%, trimethoprim-sulfamethoxazole 44%, nalidixic acid 21%, gentamicin 16%, ciprofloxacin 13%, cephalothin 4%, cefoxitin 1%, amoxicillin-clavulanic acid 1%, ceftriaxone, 1% and imipenem 0.5% (Oliveira *et al*., 2011).

Understanding antibiotic resistance patterns and molecular characterization of plasmids and other genetic elements is also epidemiologically useful. Antibiotic susceptibility is reported to be dynamic in bacteria, and it differs according to time and environment (Umolu *et al*., 2006). Comparing plasmid profile is a useful method to assess the possible relatedness of individual clinical isolates of a particular bacterial species for epidemiological studies. Therefore, there is a need for periodic screening of common bacterial pathogens to determine their antibiotic susceptibility profile in different communities (Umolu *et al*., 2006; Horcajada *et al*., 2005). Thus, the aim of this study was to determine the phenotypic antimicrobial resistance patterns and

profiling of plasmid DNA of uropathogenic *E. coli* which contribute for proper treatment of urinary tract infections.

1.3.2.2 Virulence Factors of Uropathogenic *E. coli*

Uropathogenic *E. coli* harbor a number of virulence and fitness factors enabling the bacterium to resist and overcome different defense mechanisms. There is no particular factor, which allows the identification of UPEC among the commensal faecal flora apart from the ability to enter the urinary tract and cause an infection. Many of potential virulence or fitness factors occur moreover with high redundancy. Fimbriae are inevitable for adherence to and invasion into the host cells; the type 1 pilus is an established virulence factor in UPEC and indispensable for successful infection of the urinary tract. Flagella and toxins promote bacterial dissemination, while different iron-acquisition systems allow bacterial survival in the iron-limited environment of the urinary tract. The immune response to UPEC is primarily mediated by toll-like receptors recognizing lipopolysaccharide, flagella and other structures on the bacterial surface. UPEC have the capacity to subvert this immune response of the host by means of actively impacting on pro-inflammatory signalling pathways, or by physical masking of immunogenic structures. The large repertoire of bacterial virulence and fitness factors in combination with host-related differences results in a complex interaction between host and pathogen in the urinary tract (Flores-Mireles *et al*., 2015; Lüthje and Brauner, 2014; Totsika *et al*., 2012).

UPEC strains possess an arsenal of virulence factors that contribute to their ability to cause disease, including adhesins (e.g. type 1 and P fimbriae, genes like *papA* /pyelonephritis associated gene/, *pap*C, *pap*EF, *pap*GI, *pap*GII, *pap*GIII, *sfa* /Adhesin S /, *fim*H /fimbriae H/, *afa* /afimbrial adhesions I/, *bma*E), toxins (e.g. hemolysin, genes like *hly*A /hemolysin A/, *cdt*B) and iron-acquisition systems that utilise siderophores (e.g. enterobactin, salmochelin, aerobactin, genes like *fyu*A, *iut*A/aerobactin siderophore receptor/, *feo*B). Some of UPEC virulence genes includes toxins (*hly*A /hemolysin/ and *cnf*1 /cytotoxic necrotizing factor/), adhesions (*pap*A /pyelonephritis associated gene/, *afa* /afimbrial adhesins/ and *fim*H /type 1 fimbria/), iron transport system (*sit*A, *aer*), flagella and capsule [Fig 2] (Yun *et al*., 2014; Totsika *et al*., 2012; Wiles *et al*., 2008; Usein *et al*., 2001).

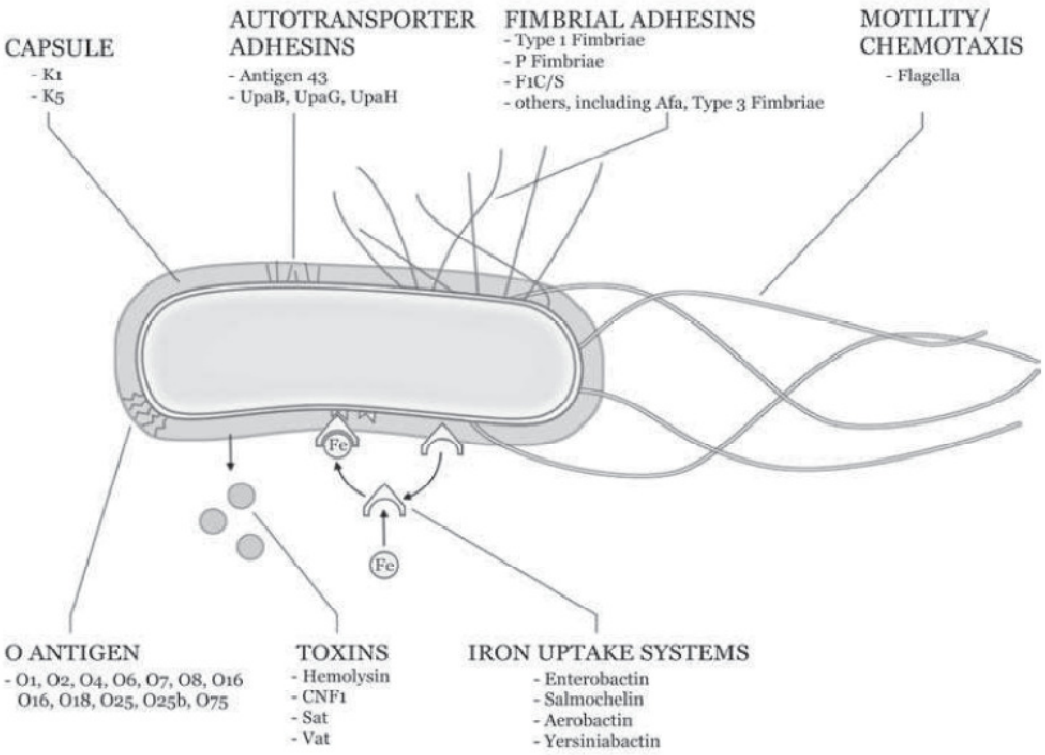

Fig 2: Major virulence factors of uropathogenic *E. coli* (Totsika *et al*., 2012)

Several virulence determinants contribute to the pathogenicity of *E. coli* in UTI. They are the product of different genes, which can be detected by PCR method. However, there is always the possibility of mutation at the level of the corresponding gene, leading to the absence of its detection. Therefore, a positive PCR shows the presence of the virulence gene, but a negative PCR does not point to the absence of the corresponding operon (Tarchouna *et al*., 2013).

Virulence factors are specific traits enabling *E. coli* to overcome host immune system and cause various diseases (Ejrnaes, 2011). Virulence genes are located on transmissible genetic elements (plasmid) and/or in particular regions on the chromosome that are called pathogenicity islands (Farshad *et al*., 2012). Pathogenicity islands are associated with the genome of pathogenic strains and led to coordinate horizontal transfer of virulence genes between strains of one species or even related species (Johnson and Stell, 2000). The generally accepted hypothesis today is that UPEC evolved from non-pathogenic strains by acquiring new virulence factors from accessory DNA horizontal transfer located at the chromosomal or plasmid level (Johnson *et al*., 2005).

In Africa, different scholars investigated UPEC isolates for the prevalence of virulence genes among UTI patients. A study conducted in Tunisia showed that virulence genes were detected in 91% UPEC isolates with the prevalence of genes coding for fimbrial adhesive systems was 68% for *fim*H, 41% for *pap*, and 34% for *sfa/foc*(S and FIC fimbriae). The *hly* and *cnf* genes coding for toxins were amplified in 19% and 3% of strains, respectively. A prevalence of 52% was found for the *aer* gene (Tarchouna *et al*., 2013). Similar study conducted in Egypt, virulence genes *pap* 54%, *sfa* 46%, *aer* 51% and *cnf* 17% were detected in UPEC isolates (Alabsi *et al*., 2014). Another study conducted in Zimbabwe showed that the *iut*A /aerobactin siderophore receptor/ (35%), *fim*H /type 1 fimbrial adhesin/ (32.5%), *vat* /vacuolating autottransporter toxin/ (17.5%), *sit*A /SitABCD system/ (17.5%), *sit*D (15%), *hyl*F /putative avian hemolysin/ (12.5%), *pst*B /pstSCAB system/ (10%) and *frz* /frz operon/ (7.5%) genes were detected in UPEC isolates (Mbanga and Mudzana, 2014).

In Europe, different studies investigated UPEC isolates for the prevalence of virulent genes among UTI patients. A study conducted in Czech Republic, Out of 210 tested urine samples, 140 (66.7 %) samples were found to be positive for *E. coli* and detected the following virulence genes: *aer* 71.7%, *pap* 67.6%, *sfa* 53.8%, *afa* 2.8%, *hly* 41.4%, and *cnf* 37.9% (Koreň *et al*., 2013). Similar study conducted in Romania, a total of 78 *E. coli* strains isolated from adults with UTI were screened by PCR. The following virulence genes were detected *fimH* 86 %, *pap* 36%, *sfa/foc* 23%, *afa* 14%, *hly* 23%, *cnf* 13% and *aer* 54% (Usein *et al*., 2001). A study conducted in Denmark, UPEC isolates carried the following virulence genes: *fim H* 98%, *pap* 47%, *sfa/foc* 46%, *afa* 4%, *hly* 43% and *cnf* 39% (Ejrnæs *et al*., 2011). Another study conducted in Poland, the prevalence of virulence genes in UPEC isolates were *fim H* 92.5%, *pap* 38.7%, *sfa/foc* 53.8%, *afa* 4.6%, *hly* 18.5%, *aer* 52.6% and *cnf* 12% (Kot *et al*., 2016). A study conducted in Spain, the virulence genes detected in UPEC isolates were *pap* 56%, *sfa* 29%, *hly* 42% and *cnf* 27% (Guiral *et al*., 2015).

In Asia, many studies have been done to assess the prevalence of virulence genes of UPEC among UTI patients. Studies conducted in Iran reported different virulence genes from UPEC isolates. Derakhshandeh *et al*, (2015) reported that the detected frequencies of the virulence factor genes from uropathogenic *E. coli* determined using PCR were *fim*H (34.1%), *pap*A (9.4%), *hly*D (21.2%), *cnf*-1 (3.5%), *sit*A (15.3%), and *tsh* (27.1%) but 41.1% of the isolated

E. coli strains did not encode any of these virulence genes. Similar study conducted in Iran showed that virulence genes of UPEC were detected in 84% isolates with *tra*T gene 74% and the *pap* gene 16.6% (Neamati *et al*., 2015). Karimian *et al*. (2012) showed that the presence of *cnf*1, *hly*A, *pap*, *iro*N, *afa*, *iuc*, *iha*, *omp*T/Episomal outer membrane protease/ and *irp*2 virulence genes were 50.4%, 50.4%, 50.4%, 42.27%, 8.13%, 10.56%, 17.88%, 4.87% and 11.38%, respectively; while Arabi *et al*. (2012) indicated that the *fim* and *sfa* fimbriae genes were observed in 2.7% of isolates, separately. Another study conducted in Iran, the prevalence of the virulence factors of UPEC for fimbriae type 1 (*fimH* gene), pyelonephritis associated pili (*pap* gene), S-family adhesions (*sfa* gene), hemolysin (*hly* gene) and aerobactin (*aer* gene), was 73%, 46%, 32%, 47%, 57%, respectively (Jalali *et al*., 2015). Similar study conducted in Iran showed UPEC virulence genes frequencies of *pap* 20.5%, *afa* 8.3% and *sfa* 21.5% were observed (Mohajeri *et al*., 2014).

According to a study conducted in Korea showed that the frequently identified virulence genes were gene that encodes adhesion; *fim*H 96.9%, gene that encodes for capsule synthesis; *kpsMTII* /Capsule-protein transport of polysaccharide/ 84.4%, gene that encodes siderophores; *feo*B 67.2%, *iut*A 53.1% and *fyu*A 45.3%, and gene that encodes adhesion *pap*A 45.3% (Yun *et al*., 2014). Another study conducted in China, *fim H* 87.4% was the most prevalent virulence gene among *E. coli* isolates, followed by *pap* 24.7%, *sfa* 8.1% and *afa* 10.1%. With regard to toxins, *hly* 11.6% was more prevalent than *cnf* 7.1%, and the prevalence of *aer* was 65.7% (Wang *et al*., 2014). Similar study conducted in Mongolia, higher prevalence of *fim H* (89.9%), *fyuA* (70.3%), *traT* (66.2%), *iutA* (62.2%), *kpsMTII* (58.8%), and *aer* (56.1%) genes were found in UPEC, indicating a putative role of adhesins, iron acquisition systems, and protectins that are main cause of UTIs (Munkhdelger *et al*., 2017). A study conducted in Iraq showed that presence of virulence gene *cnf* 42.7%, *hyl* 40.6%, *sfa* 22.7% and *afa* 18% in UPEC isolates (Merza and Jubrael, 2015). A study conducted in Thailand, showed the presence of virulence genes *pap* 16%, *cnf* 16%, *hlyA* 16%, *sfa* 9.5% and *afa* 8.3% (Themphachana *et al*., 2015).

In South America, scholars reported that UPEC isolates carries different virulence genes. A study conducted in Brazil showed that 90% UPEC showed at least one of the virulence genes, the prevalence being *traT* 76%, *aer* 41%, *sfa* 26%, *pap* 25%, *cnf1* 18%, *afa* 6% and *hly* 5% (Oliveira *et al*., 2011). Similar study done in Mexico, the most frequently occurring virulence

genes among UPEC strains included *kpsMT* 92.2%, *usp* 87.1%, *fim* 61.3%, *set* 36%, *astA* 33.5% and *pap* 24.7% (Paniagua-Contreras *et al*., 2015).

Virulence factors, such as fimbrial and afimbrial adhesins, toxins and siderophores provide means for *E. coli* strains to disrupt the host health equilibrium and cause infection, and there is variation of virulence factors in the strains of UPEC. Thus, knowing virulence factors of *E. coli* responsible for UTIs is important for proper management of UTI (Donnenberg and Welch, 1996). Identification of virulence factors can be useful for diagnosis and therapeutic strategies (Kawamura-Sato *et al*., 2010). The above literatures reveal that virulence genes of UPEC isolate varies geographically. Thus, the aim of this study was to determine the prevalence of the genes that encodes for virulence factors of uropathogenic *E. coli* which contribute for proper management of urinary tract infection.

1.3.2.3 Association of Uropathogenic *E. coli* Virulence Genes with Antibiotic Resistance

According to a study conducted in Tunisia, virulence genes *fim H* (type 1 fimbriae adhesin), *afa* (afimbrial adhesin), *sfa/foc* (S/F1C fimbriae), *papG* (P fimbriae), *cnf1* (cytotoxic necrotizing factor 1), *sat* (secreted autotransporter toxin), *hly* (alpha hemolysin), *iutA* (aerobactin), *iroN* (catecholate siderophore receptor), *fyuA* (yersiniabactin receptor for ferric yersiniabactin uptake), *iha* (iron-regulated gene A homologue adhesin), *kpsMTII* (group 2 capsule), *ompT* (outer membrane protease T), *malX* (pathogenic island marker), *traT* (serum resistance associated gene), and *usp* (uropathogenic specific protein) were significantly more frequent among ciprofloxacin and cotrimoxazole susceptible strains than those resistant to these antibiotics (Ferjani *et al*., 2014).

Alabsi *et al*. (2014), demonstrated that there was a statistically significant ($P = 0.013$) association between presence of the *pap* gene in UPEC isolates and resistance to gentamicin. However, presence of the pap gene was not significantly ($P > 0.05$) associated with resistance to β-lactam antibiotics (ampicillin, cefradine/cefalexin, cefuroxime, cefoperazone and ceftazidime), quinolones (nalidixic acid and norfloxacin), aminoglycosides (amikacin), nitrofurantoin or

trimethoprim-sulfamethoxazole. The *sfa*, *aer* and *cnf1* genes were not significantly ($P > 0.05$) associated with UPEC resistance to any of the tested antibiotics.

According to a study conducted in Mongolia, statistical analysis revealed the existence of the following associations: UPEC strains positive for *papC* and *papGII* genes were more resistant to ampicillin and trimethoprim-sulfamethoxazole; for *kpsMTII* gene were more resistant to ampicillin; for *traT* gene were more resistant to cefuroxime; for *usp* gene were more resistant to ciprofloxacin than the isolates negative for these genes. Resistance to gentamicin, ciprofloxacin, and cefuroxime were significantly associated with all the genes encoding siderophore *iutA*, aerobactin *aer* (Munkhdelger *et al*., 2017).

Asadi *et al*. (2014), reported that statistical analysis revealed the existence of the following associations: between *fim H* gene and resistance to ciprofloxacin ($P = 0.006$), nalidixic acid ($P = 0.025$), and cotrimoxazole ($P = 0.02$); between *ibeA* gene and amikacin ($P = 0.02$) and cotrimoxazole ($P = 0.02$); *afa* gene and gentamycin ($P = 0.05$).

Farshad *et al*. (2010) reported that the prevalence of all virulent genes was lower in resistant groups of UPEC strains but not statistically significant except for *pap* and *cnf1* with nalidixic acid (8% and 4.5% positive in resistant vs. 29.4% and 29.6% positive in susceptible groups, respectively, $P < 0.05$) was observed.

According to a study conducted in China, among the UPEC studied, cefotaxime, cefoxitin, chloramphenicol and nitrofurantoin resistant isolates showed a reduced prevalence of virulence traits compared with susceptible strains. A possible explanation for the lower incidence of virulence traits among the resistant strains is that: virulence factor genes, as well as resistance genes, can be carried on conjugative plasmids; therefore, it is possible that resistance encoding plasmids, owing to incompatibility, are outcompeting virulence factor encoding plasmids. A high incidence of plasmid encoding genes, such as *traT*, *sitA*, and *iutA* among UPEC strains isolated in China, support this hypothesis. The exact explanation for the lower incidence of virulence traits among resistant UPEC strains tested needs additional study (Zhao *et al*., 2009).

1.3.2.4 Phylogroup of Uropathogenic *E. coli*

E. coli strains derive from different phylogenetic groups; phylogenetic typing in four groups: A; B1; B2, and D (Clermont *et al*., 2000). The majority of strains responsible for extraintestinal infections, including urinary tract infections, belong to group B2 or, to a lesser degree, to group D, whereas commensal isolates belong to groups A and B1 (Munkhdelger *et al*., 2017; Kot *et al*., 2016; Johnson *et al*. 2005). The clinical significance of these observations suggested that a simple method of assigning isolates to a phylogroup would be of value. This led to the development and validation of a PCR assay to detect the genes *chuA* and *yjaA,* and an anonymous DNA fragment *TspE4.C2* found in *E. coli* isolates around the world (Clermont *et al*., 2000).

Uropathogenic *E. coli* strains could be assigned to one of the main phylogroups; A, B1, B2 or D based on the presence/absence of *chuA, yjaA*, and an anonymous DNA fragment *TspE4.C2.* Pathogenic *E. coli* strains derive mainly from the more virulent phylogenetic group B2 (Ejrnaes *et al*., 2011; Horcajada *et al*., 2005; Clermont *et al*., 2000).

Uropathogenic *E. coli* strains of the various phylogroups differ in their phenotypic and genotypic characteristics, their ecological niche, life history traits and ability to cause disease (Clermont *et al*., 2013; Alm *et al*., 2011; Clermont *et al*., 2000).

Phylogenetic studies have revealed that UPEC bacteria fall into four main groups (A, B1, B2, and D). The majority of strains responsible for extraintestinal infections, including urinary tract infections, belong to group B2 or, to a lesser degree, to group D, whereas commensal isolates belong to groups A and B1 (Munkhdelger *et al*., 2017; Kot *et al*., 2016; Johnson *et al*. 2005).

Many scholars reported that majority of UPEC phylogroup belonged to phylogroup B2 (Ferjani *et al*., 2014; Ejrnæs *et al*., 2011; Kot *et al*., 2016; Johnson *et al*., 2005; Lee *et al*., 2015; Munkhdelger *et al*., 2017; Molina-López *et al*., 2011).

In Africa, according to a study conducted in Tunisia by Ferjani *et al*. (2014), showed that uropathogenic *E. coli* isolated from UTI patients predominantly belonged to phylogenetic group B2 (57.7%) followed by phylogroups A (22.9%), B1 (9%) and D (10.4%).

In Europe, different scholars reported that majority of UPEC isolates belonged to phylogroup B2. A study conducted in Denmark by Ejrnæs *et al*. (2011), showed that phylogenetic group B2 was dominant (67%), followed by phylogenetic group D (13%), A (11%) and B1 (3%). Another study conducted in Poland showed that *E. coli* strains from patients with UTIs, 38.1% belonged to phylogenetic group B2, 35.3% to group D, 18.5% to group A, and 8.1% to group B1 (Kot *et al*., 2016). A study conducted in Israel, majority of *E. coli* isolates belonged to phylogroup B2 55%, followed by A 20%, D 19%, and B1 6% (Johnson *et al*., 2005).

In Asia, scholars reported that phylogroup B2 was the dominant phylogroup of UPEC isolates among UTI patients. A study conducted in Korea by Lee *et al*. (2015), 79.3% *E. coli* isolates belonged to phylogroup B2, 15.5% to phylogroup D, 3.4% to phylogroup A and 1.7% to phylogroup B1. According to a study conducted in Pakistan, phylogenetic group B2 50% was most predominant followed by groups A 19%, B1 19% and D 12% (Bashir *et al*., 2012). A study conducted in Mongolia, phylogenetics of UPEC isolates showed that phylogenetic group B2 was dominant 33.8%, followed by D 28.4%, A 19.6% and B1 18.2% (Munkhdelger *et al*., 2017).

According to a study conducted in Mexico, phylogenetics analysis showed that 36% of the studied UPEC isolates belonged to phylogroup B2, 28.7% to A, 27.8% to D, and 8.4% to phylogroup B1 (Molina-López *et al*., 2011).

Scholars from different parts of the world reported that phylogroup A and phylogroup D were the dominant UPEC phylogroups (Romanus and Eze, 2011; Adib *et al*., 2014; Derakhshandeh *et al*., 2013; Wang *et al*., 2014; Themphachana *et al*., 2015).

In Africa, according to a study conducted in Nigeria, the majority of uropathogenic *E. coli* strains belonged to clonal phylogroup A 85.7%, followed by phylogroup B 8.3% and phylogroup D 1.5% (Romanus and Eze, 2011).

In Asia, scholars reported that UPEC phylogroup A and D were the dominat phylogroups. According to a study conducted in Iran, *E. coli* isolates belonged to A 45.99%, B1 13.14%, B2 19.71% and D 21.16% phylogenetic groups (Adib *et al*., 2014). Similar study conducted in Iran showed that 65.9% *E. coli* isolates belonged to phylogenetic group A, 17.6% belonged to phylogenetic group B2, and 16.5% of the isolates were found to belong to group D (Derakhshandeh *et al*., 2013). According to a study conducted in China by Wang *et al*. (2014), *E. coli* isolates belonged to phylogroup D (42.4%) predominated, followed by B2 (29.8%), A (19.2%) and B1 (8.6%). According to a study conducted in Thailand, 57.5% UPEC isolates belonged to phylogenetic group D, 20% belonged to phylogenetic group B2, 11% to phylogenetic group B1, and 11.5% to phylogenetic group A (Themphachana *et al*., 2015).

Uropathogenic *E. coli* strains of the various phylogroups differ in their phenotypic and genotypic characteristics, their ecological niche, life history traits and ability to cause disease (Clermont *et al*., 2013; Alm *et al*., 2011; Clermont *et al*., 2000). The above literatures reveal that phylogroup of UPEC isolates differ geographically. Thus, the aim of this study was to determine the phylogroups of uropathogenic *E. coli* which contribute for proper management of UTI.

1.4 Significance of the Study

UTI is responsible for morbidity and mortality worldwide (Flores-Mireles *et al*., 2015; Foxman, 2010). *E. coli* is the most common cause of UTI (Loh and Sivalingam, 2007; Cheesbrough, 2006). Studies have shown that uropathogenic *E. coli* strains are believed to display a variety of virulence properties that help them to colonize host mucosal surface and circumvent host defense to allow invasion of the normally sterile urinary tract (Farshad *et al*., 2012; Yun *et al*., 2014; Totsika *et al*., 2012; Wiles *et al*., 2008). These virulent factors are encoded in plasmid or chromosome that can be transmitted to other avirulent bacteria and makes it virulent (Slavchev *et al*., 2009; Farshad *et al*., 2012). Uropathogenic *E. coli* are classified into different phylogroups based on phylogenetic classifications (Clermont *et al*., 2000). To the best of our knowledge, there is no information on phylogenetics and genes that encode virulence factors of uropathogenic *E. coli* in Ethiopia. So, knowing the phylogroup and virulence factors of *E. coli* responsible for UTI is important for proper management, prevention and control of urinary tract infection. Nowadays *E. coli* is resistant to many antibiotics (Gibreel *et al*., 2012; Beyene and Tsegaye, 2011; Biadglegne and Abera, 2009). Drug resistance rate of *E. coli* isolates may vary greatly in different geographical areas and with time. Continuous surveillance must be undertaken both nationally and locally in order to develop national and local guidelines for antibiotic treatment. Thus, determining the drug resistance patterns of uropathogenic *E. coli* is important for optimal treatment of life threatening urinary tract infections.

Study Hypothesis

- The drug resistance patterns of uropathogenic *E. coli* in our research area is different from other areas
- The distribution of genes that encode virulence factors of uropathogenic *E. coli* in our research area is different from other countries
- There is relationship between virulence genes and drug resistance patterns of uropathogenic *E. coli*
- The distribution of phylogroups of uropathogenic *E. coli* in our research area is different from other countries

CHAPTER TWO

Objectives of the Study

2.1 General Objective

- To assess the phenotypic and molecular characteristics of uropathogenic *E. coli* as well as relationship of virulence genes and drug resistance patterns of uropathogenic *E. coli* among urinary tract infection patients in selected health facilities of Addis Ababa, Ethiopia

2.2 Specific Objectives

- To determine the phenotypic drug resistance level of uropathogenic *E. coli* among urinary tract infection patients in selected health facilities of Addis Ababa, Ethiopia
- To analyze plasmid profile of uropathogenic *E. coli* among urinary tract infection patients in selected health facilities of Addis Ababa, Ethiopia
- To identify genes that encodes for virulence factors of uropathogenic *E. coli* among urinary tract infection patients in selected health facilities of Addis Ababa, Ethiopia
- To assess relationship between virulence genes and drug resistance patterns of uropathogenic *E. coli* among urinary tract infection patients
- To identify phylogroup of uropathogenic *E. coli* among urinary tract infection patients in selected health facilities of Addis Ababa, Ethiopia

CHAPTER THREE

Materials and Methods

3.1 Study Design, Area and Period

A facility (hospital) based cross sectional study was conducted in selected governmental hospitals of Addis Ababa, Ethiopia; Tikur Anbessa Specialized Hospital, Yekatit 12 Hospital and Zewditu Memorial Hospital. These governmental hospitals were selected because they have microbiology laboratories that perform culture and antimicrobial sensitivity testing. The study was conducted from January 1, 2017 to October 9, 2017. Tikur Anbessa Specialized Hospital had a bed capacity of 575. The professional profile of Tikur Anbessa Specialized Hospital includes 430 Doctors, 825 Nurses, 33 Laboratory technologists and 16 Laboratory technicians. There were 10-30 UTI cases per day in Tikur Anbessa Specialized Hospital. Zewditu Memorial Hospital had a bed capacity of 190. The professional profile of Zewditu Memorial Hospital includes 42 Doctors, 284 Nurses, 18 Laboratory technologists and 17 Laboratory technicians. There were 5-20 UTI cases per day in Zewditu Memorial Hospital. Yekatit 12 Hospital had a bed capacity of 316. The professional profile of Yekatit 12 Hospital includes 98 Doctors, 344 Nurses, 21 Laboratory technologists and 16 Laboratory technicians. There were 10-20 UTI cases per day in Yekatit 12 Hospital.

3.2 Study Population and Study Subjects

3.2.1 Study Population

All patients visiting Tikur Anbessa Specialized Hospital, Yekatit 12 Hospital and Zewditu memorial Hospital, Ethiopia during the time of data collection.

3.2.2 Study Subjects

All patients with UTIs in outpatient department (OPD) and in-patient of Tikur Anbessa Specialized Hospital, Yekatit 12 Hospital and Zewditu memorial Hospital, Ethiopia who came to laboratory for culture during the time of data collection who fulfills inclusion criteria.

3.3 Sample Size and Sampling Technique

3.3.1 Sample Size

The sample size of the study was determined using a formula for a single population proportion (Daniel, 1995):

$n = \left(Z\frac{\alpha}{2}\right)^2 \frac{P(1-P)}{d^2}$ Where:

P= the prevalence of *E. coli* bacteria among patients with urinary tract infection from research conducted in Gonder, Ethiopia was 46% (Moges *et al*., 2002).

Z=a standard score corresponding to 95% confidence level (1.96)

d=the margin of error (3.5%)

n=the required sample size

$n = (1.96)^2 \frac{0.46\ (1-0.46)}{(0.035)^2}$ $n = 780$

Accordingly, the calculated sample size was **780**.

3.3.2 Sampling Technique

Convenient sampling (Urine samples were collected following standard operating procedure until it reached the desired sample size and no proportional allocation was made among different hospitals).

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion Criteria

Patients diagnosed with urinary tract infections. Patients who had typical symptoms of the disease, such as the presence of at least two of the following complaints: dysuria, urgency, frequency, incontinence, suprapubic pain, flank pain or cost vertebral angle tenderness, fever ($\geq$38^{o}C) and chills (Mohajeri *et al*., 2014; Cheesbrough, 2006; Usein *et al*., 2001).

3.4.2 Exclusion Criteria

Children less than 1-year old age, those patients who were under antibiotic treatment and who were not willing to participate in this study were excluded.

3.5 Data Collection Procedures

Data collection tools were adapted after review of relevant literatures. Research participants were those patients coming to Tikur Anbessa Specialized Hospital, Zewditu Memorial Hospital and Yekatit 12 Hospital that were diagnosed with urinary tract infections and gave urine sample for microbiological investigation. Socio-demographic and clinical data were collected from the patient directly and from patient record respectively by data collectors. Urine sample proccessing and microbiological investigations were conducted without delay in the Microbiology laboratory of department of Microbiology, Immunology and Parasitology, Addis Ababa University. Molecular characterization of *E. coli* isolates was conducted in college of Human Medicine, Michigan State University, USA.

3.5.1 Questionnaire

Socio-demographic and clinical data were collected using a well-designed questionnaires.

3.5.2 Microbiological Investigations

Sample Collection and Proccessing

Mid-stream urine (MSU) samples (10-20 ml) were collected using sterile container (bottle). The bottle was then labeled with unique sample number, date and time of collection; then immediately delivered to Microbiology Laboratory of department of Microbiology, Immunology and Parasitology for further microbiological investigations. The urine samples were inoculated onto MacConkey agar (Oxoid, UK) and incubated at 37°C for 24 hours. The urine specimen was considered as positive for UTI if a single organism was cultured at a concentration of $\geq 10^5$ CFU per millilitre of urine (Abdu *et al*., 2018; Cheesbrough, 2006).

Isolation of *E. coli*

E. coli isolates were presumptively identified by colonial morphology on MacConkey agar (Oxoid, UK). Distinct colonies on MacConkey agar plates were further identified and confirmed by conventional biochemical tests. *E. coli* bacteria were recognized by the following: negative for urease, citrate utilization and hydrogen sulfide generation; and positive for motility, lactose fermentation, glucose fermentation and indole test (Wang *et al*., 2014; Bashir *et al*., 2012; Cheesbrough, 2006; Farmer, 1999). Motility indole urea (MIU) media was prepared and autoclaved, followed by inoculation of culture and incubation at 37°C for 24 hours. Development

of cherry red color on addition of Kovac's reagent would indicate a positive result for indole and spread of growth from inoculation area would indicate motility. Test tubes containing Simon's citrate agar was autoclaved and followed by inoculation of culture and incubation at 37°C for 24 hours. Change of color of the slant Simon's citrate agar from green to blue would indicate a positive result. Triple Sugar Iron (TSI) agar was prepared and autoclaved, followed by inoculation of culture and incubation at 37°C for 24 hours. Color change of the slop and butt from red to yellow color would indicate a positive result for glucose and lactose fermentation. Gram staining was done when it is necessary (Cheesbrough, 2006). *E. coli* isolates were stored in Brain heart infusion broth vial at -20°c and transported to USA for genotyping.

Antimicrobial Susceptibility Testing

In-vitro antimicrobial susceptibility testing of the bacterial isolates was performed by Kirby-Bauer disc diffusion method [see Annex VI] (Cheesbrough, 2006). The following antimicrobial agents were used with their respective concentration: trimethoprim-sulfamethoxazole (SXT) (1.25/23.75µg), ampicillin (AMP) (10µg), nalidixic acid (NA) (30µg), amoxicillin-clavulanate (AMC) (20/10µg), ceftazidime (CAZ) (30µg), tetracycline (TE) (30µg), cefotaxime (CTX) (30µg), ceftriaxone (CRO) (30µg), gentamicin (CN) (10µg), ciprofloxacin (CIP) (5µg), amikacin (AK) (30µg), norfloxacin (NOR) (10µg), nitrofurantoin (F) (300µg), meropenem (MEM) (10µg), imipenem (IM) (10µg) and chloramphenicol (C) (30µg) (Oxoid, UK). The antimicrobials were selected based on the local consideration of the commonly used antimicrobial agents to treat urinary tract infections and considering the recommended antimicrobial agents for *E. coli* by Clinical and Laboratory Standard Institute (CLSI). The antibiotic disks were firmly placed on sterile Mueller-Hinton Agar (Oxoid, UK) plates previously seeded with a 24 hours old culture of the isolate (10^6 CFU/ml of 0.5 McFarland Standard). The plates were incubated at 37°C for 24 hours and diameter of zones of inhibitions was measured using caliper and compared with the standard set by CLSI (CLSI, 2017). *E. coli* ATCC 25922 was used as reference strain. UPEC isolates resistant to two or more different classes of antibiotics were classified as multidrug-resistant (MDR).

Plasmid DNA Extraction

Plasmid DNA was extracted from *E. coli* isolates using the alkaline lysis method by using Wizard® Plus SV Minipreps DNA Purification Systems kit (Promega Corporation, USA). The

manufacturer's instruction was strictly followed (see Annex XII) (Promega Corporation, 2017). The alkaline lysis method makes plasmid DNA linearized before the gel electrophoresis (Kado and Liu, 1981).

Plasmid Analysis

Twenty microliters (20µl) of the extracted plasmid DNA was mixed with 5µl of 6x loading dye on parafilm and loaded on slots of 0.7% agarose gel electrophoresis stained with 10µl 10,000x GelRed. Electrophoresis was carried out for 4 hours at 100 Volt on TAE buffer system and the gel was imaged under UV light (E-gel Imager; life technologies, USA). Plasmid number/s was counted by counting the number of bands observed on the agarose gel. Standard DNA molecular weight markers (1kb DNA ladder [Invitrogen™] and Lamda (λ) DNA/HindIII marker [Promega Corporation, USA]) were used to estimate the plasmid size (see Annex XII) (Khadgi *et al*., 2013; Jan *et al*., 2009; Kado and Liu, 1981).

Bacterial DNA Extraction

Bacterial DNA extraction was performed using an alkaline heat lysis method. The protocols were adapted from Michigan State University, College of Human Medicine, Zhang's Lab (see Annex VIII). *E. coli* strains that were preserved at -20^{o}C after identification were resuspended and grown on LB agar at 37^{o}C overnight. Bacteria colonies were inoculated and suspended in 1.5ml centrifuge tubes containing 200µl of 1xPBS solution, and then 800µl of 0.05M NaOH added and mixed by vortexing. The sample/mixture was incubated at 60^{o}C for 45 minutes. After 45 minutes, 240µl 1M Tris-Cl was added to neutralize NaOH and centrifuged at 13,000rpm for 3 minutes. One thousand microliters of the supernatant were stored at -20^{o}C as a template DNA stock (He *et al*., 2016; Abiodun *et al*., 2014; Tarchouna *et al*., 2013).

Detection of Virulence Genes of Uropathogenic *E. coli*

The genetic determinants that were studied include those coding for type 1 fimbriae (*fim H*), pili associated with pyelonephritis (*pap*), S and F1C fimbriae (*sfa* and *foc*), afimbrial adhesins (*afa*), hemolysin (*hly*), cytotoxic necrotizing factor (*cnf*) and aerobactin (*aer*). The selection of these virulence genes was based on literatures. These virulence genes are the commonest and different studies investigated them (Munkhdelger *et al*., 2017; Lee *et al*., 2015; Kot *et al*., 2016; Tabasi *et al*., 2016; Paniagua-Contreras *et al*., 2015; Derakhshandeh *et al*., 2015; Neamati *et al*., 2015;

Merza and Jubrael, 2015; Themphachana *et al*., 2015; Mbanga and Mudzana, 2014; Wang *et al*., 2014; Yun *et al*., 2014; Annapurna *et al*., 2014; Alabsi *et al*., 2014; Tarchouna *et al*., 2013; Karimian *et al*., 2012; Oliveira *et al*., 2011; Zhao *et al*., 2009; Santo *et al*., 2006; Usein *et al*., 2001).

Specific primers were used to amplify sequences of the *fim H*, *pap*, *sfa/foc*, *afa*, *hly*, *cnf*, and *aer* operons. Details of primer sequences and predicted sizes of the amplified products are given in Table 1.

Table 1: Primers used for *E. coli* virulence genes (Tarchouna *et al*., 2013; Usein *et al*., 2001)

Virulence factor	**Target gene(s)**	**Primer Name**	**Primer Sequence (5'- 3')**	**Size of amplicon (bp)**
Type 1 fimbriae	*fimH*	fimH-f fimH-r	5'-AACAGCGATGATTTCCAGTTTGTGTG-3' 5'-ATTGCGTACCAGCATTAGCAATGTCC-3'	465
P fimbriae	*papC*	pap1 pap2	5'-GACGGCTGTACTGCAGGGTGTGGCG-3' 5'-ATATCCTTTCTGCAGGGATGCAATA-3'	328
S and FIC fimbriae	*sfa/focDE*^{h} *region*	sfa1 sfa2	5'-CTCCGGAGAACTGGGTGCATCTTAC-3' 5'-CGGAGGAGTAATTACAAACCTGGCA-3'	410
Afa adhesins	*afaC*^{c}	afa-f afa-r	5'-CGGCTTTTCTGCTGAACTGGCAGGC-3' 5'-CCGTCAGCCCCCACGGCAGACC-3'	672
Hemolysin	*hlyCA region*	hly s hly as	5'-AGATTCTTGGGCATGTATCCT-3' 5'-TTGCTTTGCAGACTGTAGTGT-3'	556
Cytotoxic necrotizing factor	*cnf*	cnf s cnf as	5'-TTATATAGTCGTCAAGATGGA-3' 5'-CACTAAGCTTTACAATATTGA-3'	693
Aerobactin	*iucC*	aer s aer as	5'-AAACCTGGCTTACGCAACTGT-3' 5'-ACCCGTCTGCAAATCATGGAT-3'	269

Detection of *fim H*, *pap* and *afa,* and *sfa/foc* and *aer* sequences were done by multiplex PCR (polymerase chain reaction) as described by Usein *et al*. (2001), and Tarchouna *et al*. (2013), while *hly* and *cnf* detection was done by single-plex PCR (Tarchouna *et al*., 2013; Usein *et al*., 2001). PCR amplification of bacterial DNA lysate was done in a total volume of 25µl containing 20µl of Platinum® PCR SuperMix (The mixture contains Mg^{++}, dNTPs and recombinant *Taq* DNA polymerase at concentrations sufficient to allow amplification during PCR), 1.5µl DNA

template and 1.5-2µl (30 pmol of each) of the primers [see Annex XI] (Tarchouna *et al*., 2013; Usein *et al*., 2001).

The amplification was carried out in a multiplex PCR [T100™ Thermal cycler (BIO RAD) & PTC-200 Peltier Thermal cycler (MJ Research)]. Multiplex PCR for *fim H, afa* and *pap* conditions consisted of an initial denaturation at 94°C for 10 minutes, followed by 30 cycles of denaturation at 94°C for 2 minutes, annealing at 60°C for 30 seconds and 72°C for 1 minute, and final extension at 72°C for 10 minutes. Multiplex PCR for *sfa* and *aer* conditions consisted of an initial denaturation at 94°C for 10 minutes, followed by 30 cycles of denaturation at 94°C for 2 minutes, annealing at 55°C for 30 seconds and 72°C for 1 minute, and final extension at 72°C for 10 minutes. Single-plex PCR for *cnf* and *hly* conditions consisted of an initial denaturation at 94°C for 10 minutes, followed by 30 cycles of denaturation at 94°C for 2 minutes, annealing temperature for *cnf* and *hly* was 45°C and 50°C respectively and 72°C for 1 minute, and final extension at 72°C for 10 minutes. A 4.5µl aliquot of the PCR product was mixed with 6x blue loading dye on parafilm and loaded on 1.2% agarose gel electrophoresis stained with 10µL 10,000x GelRed. Electrophoresis was carried out for 120 minutes at 95 volt on TAE buffer system and the gel was imaged under UV light (E-gel Imager; life technologies, USA). Amplified DNA fragments of specific sizes were detected by UV-induced fluorescence and the size of the amplicons were estimated by comparing them with the 1 kb plus DNA ladder (Invitrogen™) included on the same gel [see Annex XI] (Tarchouna *et al*., 2013; Usein *et al*., 2001).

Strain J96 was used as positive control for *pap, sfa/foc, hly, cnf,* and *fim H* sequences and the strain K10 was used as positive control for *afa*. The positive control for *aer* was J96 and Cl_{1212} strains (Farshad *et al*., 2012; Le Bougue´nec *et al*., 2001; Johnson and Stell, 2000; Le Bouguénec *et al*., 1992; Bindereif and Neilands, 1985).

PCR for Phylogenetics of *E. coli*

PCR assay detect the genes *chuA* and *yjaA,* and an anonymous DNA fragment *TspE4.C2* found in *E. coli* isolates around the world (Clermont *et al*., 2000). Based on the presence/absence of these three fragments, and *E. coli* strain could be assigned to one of the main phylogroups; A, B1, B2

or D. All PCR reactions were carried out in a 25µl volume containing 20µl of 10X buffer (supplied with Taq polymerase), 2mM each dNTP, 2U of Taq polymerase (Invitrogen™ SuperMix); the amounts of primer used were 20pmol (2µl of each primers). PCR reactions (T100™ Thermal cycler, BIO RAD) were performed under the following conditions: denaturation 4 minutes at 94°C, 30 cycles of 5 seconds at 94°C and 20 seconds at 59°C, and a final extension step of 5 minutes at 72°C [see Annex X] (Clermont *et al*., 2013).

Table 2: Primers used for phylogenetics of *E. coli* (Clermont *et al*., 2013; Clermont *et al*., 2000)

Primer Name	Gene Target	Nucleotide Sequence	PCR Product (bp)
chuA.1b	*chuA*	5'-ATGGTACCGGACGAACCAAC-3'	288
chuA.2b		5'-TGCCGCCACTACCAAAGACA-3'	
yjaA.1b	*yjaA*	5'-CAAACGTGAAGTGTCAGGAG-3'	211
yjaA.2b		5'-AATGCGTTCCTCAACCTGTG-3'	
TspE4C2.1b	*TspE4.C2*	5'-CACTATTCGTAAGGTCATCC-3'	152
TspE4C2.2b		5'-AGTTTATCGCTGCGGGTCGC-3'	
AceK.f	*arpA*	5'-AACGCTATTCGCCAGCTTGC-3'	400
ArpA1.r		5'-TCTCCCCATACCGTACGCTA-3'	

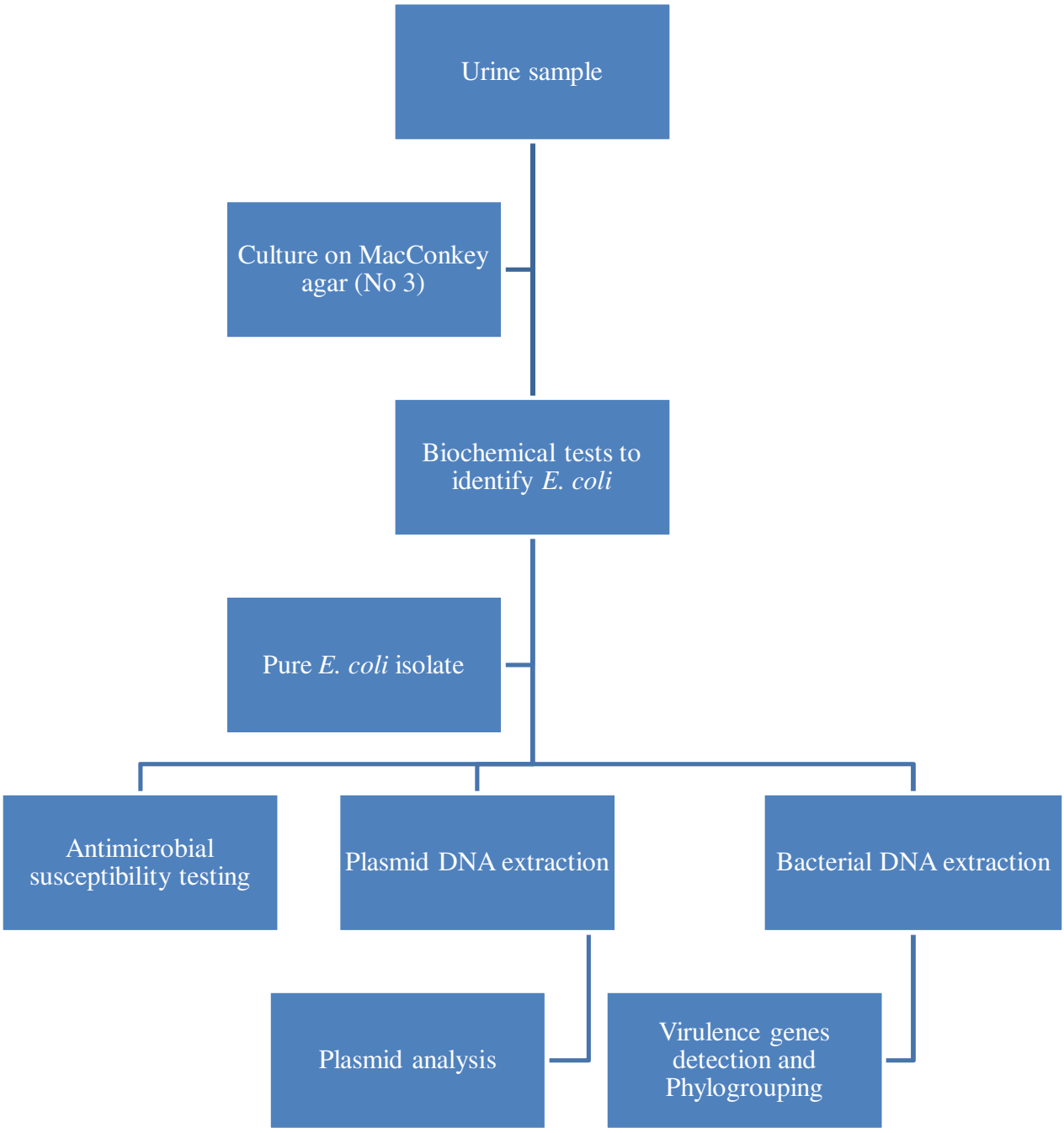

Fig 3: Lab investigation flow chart for uropathogenic *E. coli* isolates

3.6 Data Processing and Analysis

The data was checked for completeness, missing values, and coding of questionnaires, entered to Epi-info version 3.4.1 softwares, processed and analyzed using SPSS version 16.0. Regression and Chi-square test were performed. P-value <0.05 were considered as significant.

3.7 Quality Control

Training was given to data collectors. The English version of the questionnaire was translated to national language (Amharic). The reagents and materials were purchased from companies of known standard. Control strains were used during microbiological investigation of antimicrobial susceptibility testing, phylogenetics and virulence genes determination. Standard operating procedures (SOPs) were prepared and used throughout the procedures. Sterility of the culture media was checked frequently by incubating 10% of prepared culture media at 37^{o}C overnight and checked for growth.

3.8 Ethical Considerations

The research proposal was submitted to Departmental Research and Ethical Review Committee (DERC), Institutional Review Board (College of Health Sciences), and National research and ethical review committee (NRERC) to obtain ethical clearance. Official permission letter and/or ethical clearance letter were submitted to Tikur Anbessa Specialized Hospital, Yekatit 12 Hospital and Zewditu Memorial Hospital to conduct a study. Before starting data collection, the purpose of the study was explained to all study participants and written informed consent was obtained. Positive results (if microorganisms that indicate urinary tract infections were isolated from urine culture and results of antimicrobial susceptibility testing) of patients were communicated to their respective physicians for intervention of UTI. Confidentiality was maintained throughout the study. Participant's identification was kept anonymous; no names appear during the study and thereafter. The study subjects were explained that participation was voluntary and at any time if they do not want to participate, without any justification they could do so. Appropriate procedures were followed to transport *E. coli* isolates from Ethiopia to USA for molecular characterization of uropathogenic *E. coli* isolates. The Material Transfer Agreement (MTA) form was signed by the provider from Addis Ababa University and recipient from Michigan State University (see Annex V).

3.9 Operational definitions

Urinary tract infection (UTI): is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract and when a single organism is cultured at a concentration of $\geq 10^5$ CFU per millilitre of urine. Patients having the typical symptoms of the disease, such as the presence of at least two of the following complaints: dysuria, urgency, frequency, incontinence, suprapubic pain, flank pain or cost vertebral angle tenderness, fever ($\geq 38^oC$) and chills is considered as urinary tract infection patient (Mohajeri *et al*., 2014; Cheesbrough, 2006; Usein *et al*., 2001).

Plasmid: is an extra-chromosomal DNA.

Antibiotic: a drug, which is products of fungi and bacteria (Streptomycetes) that kills bacteria or inhibits their growth. Antibiotics are not effective against viruses (Also referred to as an antimicrobial).

Multidrug resistance (MDR): refers to resistance to two or more different classes of drugs by bacteria.

Virulence factors: are arsenals that are used by microorganisms to cause infection.

3.10 Plan for Dissemination and Utilization of the Study Findings

Final thesis paper will be submitted to Addis Ababa University. The results will be submitted to the concerned bodies. The final research paper will be presented to the scientific community. The study result will be published in peer reviewed journals.

CHAPTER FOUR

Results

4.1 Socio-demographic Status of Study Participants

A total of 780 patients with presumed urinary tract infection from Tikur Anbessa Specialized Hospital (N=580), Yekatit 12 Hospital (N=30) and Zewditu Memorial Hospital (N=170) were participated in this study. The mean age of study participants were 34 years (±14 SD). Of these, 265 (34%) were males and 515 (66%) were females with an overall female to male ratio of 2:1 (see Table 3).

Table 3: Socio-demographic status of study participants

Variables		Tikur Anbessa Specialized Hospital	Yekatit 12 Hospital	Zewditu Memorial Hospital	Total (percent)
Gender	Male	201	16	48	265 (34)
	Female	379	14	122	515(66)
Age	<18	59	1	11	71(9)
	18-25	90	5	37	132(17)
	26-45	312	14	105	431(55)
	46-65	106	7	14	127(16)
	>65	13	3	3	19(3)
Educational status	Illiterate	11	4	8	23(3)
	Read and write	81	8	14	103(13)
	Elementary school	116	0	19	135(18)
	High school	307	6	70	383(50)
	Above grade 12	62	12	51	125(16)
Marital status	Single	154	7	57	218(31)
	Married	317	23	96	436(63)
	Divorced	36	0	5	41(6)
Site of data collection	OPD	500	20	155	675 (86.5)
	Ward	80	10	15	105 (13.5)

OPD: Outpatient department

There was no significant association between gender, age, educational status and marital status, and *E. coli* isolation rate (p=0.392, 0.665, 0.532 and 0.466 respectively). There was no significant association between health facilities and site of data collections, and *E. coli* isolation rates (X^2=1.429 p-0.489 and X^2=0.067 p-0.796 respectively) [see Table 4].

Table 4: Association between *E. coli* isolation rates, and socio-demographic status and site of data collections

Variables		*E. coli*		X^2 (P-value)
		Present	**Absent**	
Gender	Male	63	202	0.734 (0.392)
	Female	137	378	
Age	<18	24	47	3.227(0.665)
	18-25	33	99	
	26-45	108	323	
	46-65	31	96	
	>65	4	15	
Educational status	Illiterate	9	14	4.121(0.532)
	Read and write	26	77	
	Elementary school	36	99	
	High school	100	283	
	Above grade 12	27	99	
Marital status	Single	50	168	1.526(0.466)
	Married	111	325	
	Divorced	13	28	
Health facilities	Tikur Anbessa Specialized Hospital	153	427	1.429(0.489)
	Yekatit 12 Hospital	9	21	
	Zewditu Memorial Hospital	38	132	
Site of data collection	OPD	172	503	0.067(0.796)
	In-patient	28	77	

OPD: Outpatient department

4.2 Clinical Data of Study Participants

The study participants had at least two of the following urologic symptoms and the majority of complaint was dysuria followed by urine urgency, urgency incontinence, flank pain, suprapubic pain, fever and chills (see Fig 4).

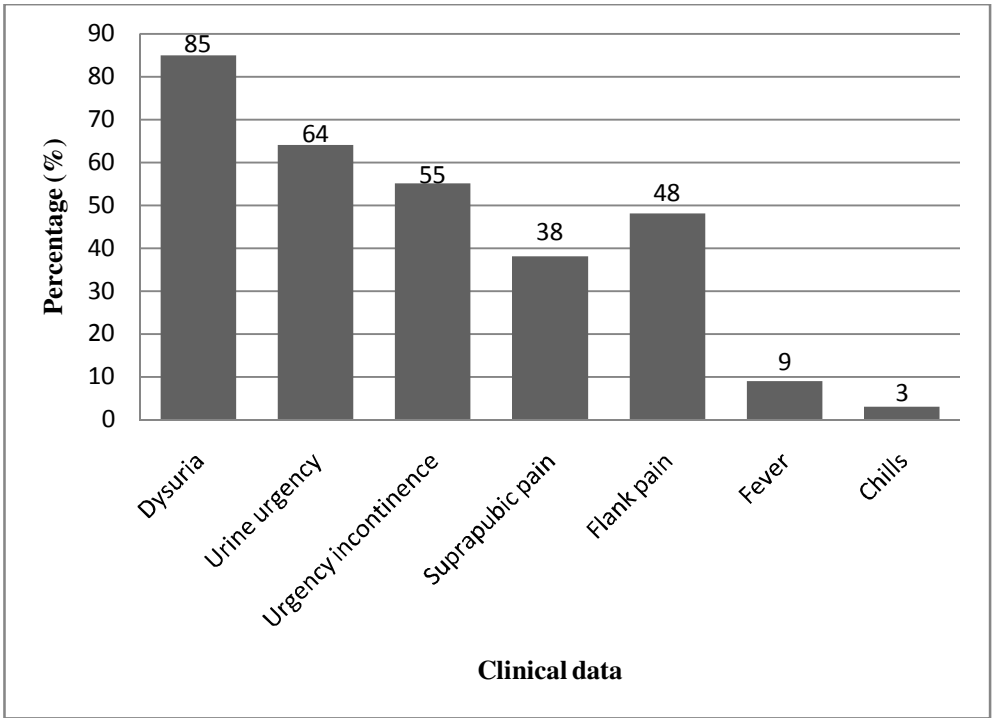

Fig 4: Clinical data of study participants

The most common urologic clinical manifestation combinations in this study were dysuria; urine urgency and urgency incontinence (see Table 5).

Table 5: Common combinations of clinical manifestations

Clinical data combinations	**Frequency**
Dysuria, urine urgency, urgency incontinence	176
Dysuria, urgency incontinence	100
Dysuria, urine urgency, flank pain	80
Dysuria, urine urgency	78
Dysuria, urgency incontinence, suprapubic pain	60
Urine urgency, urgency incontinence	54
Dysuria, suprapubic pain, flank pain	50
Dysuria, suprapubic pain	42

A total of 200 (25.6%) *E. coli* were isolated and there was significant association between *E. coli* isolation rate and urologic symptoms urine urgency, fever and chills (p= 0.002, 0.026, 0.033 respectively) [see Table 6].

Table 6: Association between *E. coli* isolation rate and clinical data

Clinical data		***E. coli***		**OR (95% C.I.)**	**P-value**
		Positive	**Negative**		
Dysuria	Present	175	489	1.303 (0.810, 2.095)	0.274
	Absent	25	91		
Urine urgency	Present	146	352	1.751 (1.229, 2.495)	0.002
	Absent	54	228		
Urgency incontinence	Present	122	309	1.372 (0.989, 1.904)	0.058
	Absent	78	271		
Suprapubic pain	Present	85	215	1.255 (0.905, 1.740)	0.173
	Absent	115	365		
Flank pain	Present	105	274	1.234 (0.895, 1.703)	0.199
	Absent	95	306		
Fever	Present	26	45	1.777 (1.064, 2.965)	0.026
	Absent	174	535		
Chills	Present	11	14	2.353 (1.050, 5.272)	0.033
	Absent	189	566		

4.3 Bacterial Isolations and Antimicrobial Susceptibility Patterns of *E. coli*

Urine sample of 780 study participants who had complaints of urologic symptoms of urinary tract infections were cultured and 200 (25.6%) *E. coli* isolates were identified by conventional biochemical tests.

Most *E. coli* isolates 153 (76.5%) were from Tikur Anbessa Specialized Hospital followed by Zewditu Memorial Hospital 38 (19%) [see Table 7].

Table 7: Distribution of uropathogenic *E. coli* isolates among different hospitals

Site of data collection	Frequency	Percentage
Tikur Anbessa Specialized Hospital	153	76.5
Zewditu Memorial Hospital	38	19
Yekatit 12 Hospital	9	4.5

Antimicrobial Susceptibility Patterns

The antimicrobial susceptibility patterns of 200 *E. coli* isolates which were subjected to similar testing procedure showed that *E. coli* isolates had highest resistance (86.5%) to ampicillin followed by ceftazidime (84%), ceftriaxone (80.5%), tetracycline (80%), trimethoprim-sulfamethoxazole (68.5%) and cefotaxime (66%). Highest susceptibility to meropenem (100%) and imipenem (100%) were observed. High susceptibility was observed to amikacin (97.5%), nitrofurantoin (95%), ciprofloxacin (85.5%), norfloxacin (85%), chloramphenicol (83.5%), gentamicin (80%) and nalidixic acid (79%) [see Table 8]. All *E. coli* isolates were resistant to one or more antibiotics.

Table 8: Antimicrobial susceptibility patterns of *E. coli* isolates

Antimicrobial agents	Number of resistance (%)	Number of intermediate (%)	Number of susceptible (%)
Ciprofloxacin	29 (14.5)	0	171 (85.5)
Norfloxacin	30 (15)	0	170 (85)
Nitrofurantoin	10 (5)	0	190 (95)
Trimethoprim-sulfamethoxazole	137 (68.5)	0	63 (31.5)
Tetracycline	160 (80)	0	40 (20)
Ceftriaxone	161 (80.5)	9 (4.5)	30 (15)
Ampicillin	173 (86.5)	6 (3)	21 (10.5)
Nalidixic acid	42 (21)	0	158 (79)
Amoxicillin-clavulanate	58 (29)	36 (18)	106 (53)
Ceftazidime	168 (84)	19 (9.5)	13 (6.5)
Cefotaxime	132 (66)	2 (1)	66 (33)
Amikacin	5 (2.5)	0	195 (97.5)
Meropenem	0	0	200 (100)
Imipenem	0	0	200 (100)
Chloramphenicol	33 (16.5)	0	167 (83.5)
Gentamicin	40 (20)	0	160 (80)

There was no drug resistance to amikacin in Zewditu Memorial Hospital and Yekatit 12 Hospital. Similarly, no drug resistance was observed to nitrofurantoin in Yekatit 12 Hospital (see Table 9).

Table 9: Distribution of drug resistance patterns among different hospitals

Antimicrobial agents	Frequency (percentage) of drug resistant isolates						
	Tikur Anbessa Specialized Hospital		Zewditu Memorial Hospital		Yekatit 12 Hospital		Total (percent)
	OPD	Ward	OPD	Ward	OPD	Ward	
Ciprofloxacin	18(62)	4(14)	5(17)	0	2(7)	0	29 (100)
Norfloxacin	19(63)	4(13)	5(17)	0	2(7)	0	30 (100)
Nitrofurantoin	7(70)	2(20)	1(10)	0	0	0	10 (100)
Trimethoprim-sulfamethoxazole	87(64)	16(12)	25(18)	2(1)	5(4)	2(1)	137 (100)
Tetracycline	106(66)	17(11)	28(18)	2(1)	5(3)	2(1)	160 (100)
Ceftriaxone	106(66)	17(11)	29(18)	1(1)	6(3)	2(1)	161 (100)
Ampicillin	113(65)	18(10)	31(18)	2(1)	6(4)	3(2)	173 (100)
Nalidixic acid	29(69)	5(12)	5(12)	2(5)	1(2)	0	42 (100)
Amoxicillin-clavulanate	39(67)	6(10)	8(14)	1(2)	3(5)	1(2)	58 (100)
Ceftazidime	111(66)	19(11)	27(16)	2(1)	6(4)	3(2)	168 (100)
Cefotaxime	87(66)	15(11)	21(16)	2(2)	5(3)	2(2)	132 (100)
Amikacin	4(80)	1(20)	0	0	0	0	5 (100)
Meropenem	0	0	0	0	0	0	0
Imipenem	0	0	0	0	0	0	0
Chloramphenicol	21(64)	3(9)	7(21)	1(3)	1(3)	0	33 (100)
Gentamicin	30(75)	6(15)	1(1.5)	1(1.5)	2(5)	0	40 (100)

Multidrug Resistance (MDR) Uropathogenic *E. coli*

Antimicrobial resistance profiles showed that majority (96.5%) of uropathogenic *E. coli* isolates were multidrug resistant (MDR).

The most common multidrug resistant (MDR) *E. coli* combinations found in this study was against 5 classes/subclasses of antibiotics (see Table 10).

Table 10: Common Multidrug resistance (MDR) uropathogenic *E. coli* combinations

Resistance pattern	No of resistant class of antibiotics	Number of isolates (n)			
		Tikur Anbessa Hospital	Zewditu Hospital	Yekatit 12 Hospital	Total (frequency)
CIP, NOR, SXT, CRO, AMP, TE, CAZ, CTX	5	13	5	2	20
SXT, CRO, AMP, AMC, TE, CAZ, CTX	5	6	4	0	10
SXT, CRO, AMP, TE, CAZ, CTX	4	38	8	4	50
SXT, AMP, TE, CAZ, CTX	4	11	4	2	17
CRO, AMP, TE, CAZ	3	23	6	3	32
SXT, CRO, TE, CTX	3	5	3	0	8
AMP, TE, CAZ	3	4	0	0	4

SXT: trimethoprim-sulfamethoxazole, AMP: ampicillin, AMC: amoxicillin-clavulanate, CAZ: ceftazidime, TE: tetracycline, CTX: cefotaxime, CRO: ceftriaxone, CIP: ciprofloxacin, NOR: norfloxacin

4.4 Plasmid Analysis of Uropathogenic *E. coli* Isolates

Plasmid analysis showed presence of plasmid/s in 165 (82.5%) uropathogenic *E. coli* isolates, but absent in rest 35 (17.5%). One kilo base plus (1kb plus) DNA ladder and Lamda/HindIII markers were used to determine the size of plasmids (see Fig 5). The maximum plasmid size that could be detected by Lamda/HindIII marker was 23kb.

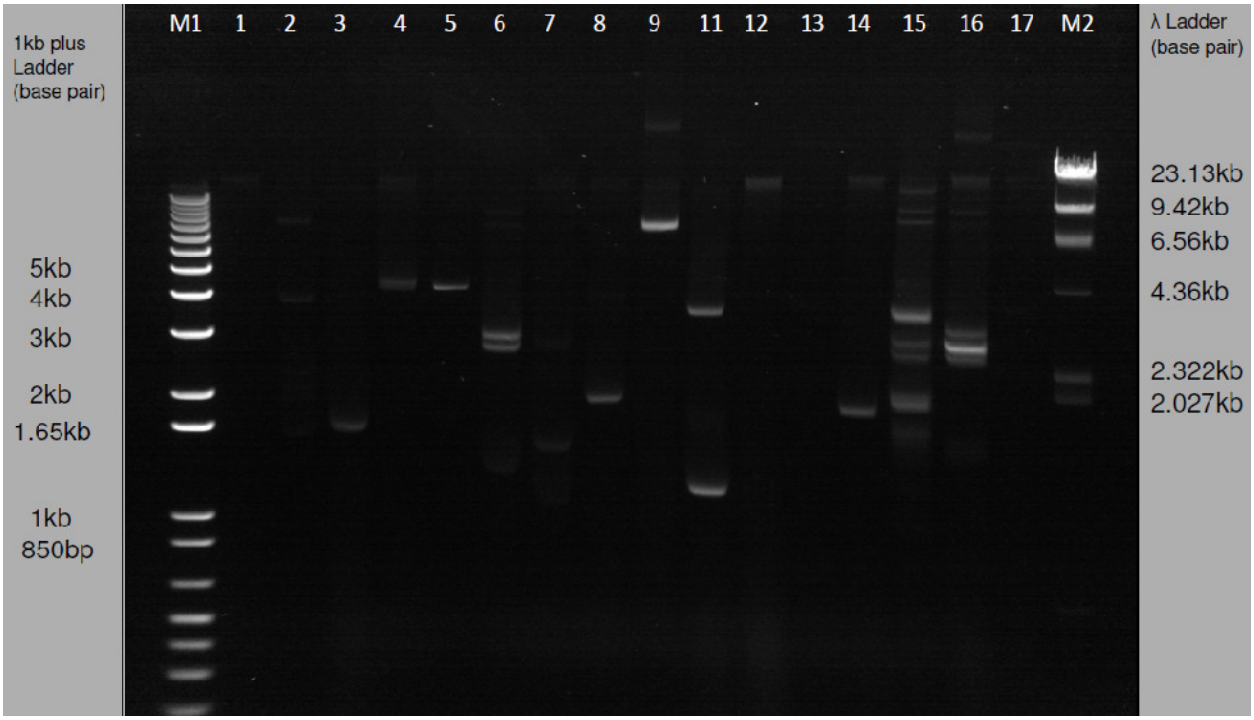

Fig 5: Representative 0.7% agarose gel electrophoresis of uropathogenic *E. coli* plasmids isolated from urinary tract infection patients. Lane M1, 1-kb plus DNA ladder; lane 1-16, plasmids of *E. coli* isolates from sample 1-17; Lane M2, Lamda/HindIII marker.

Table 11: Distribution of plasmid/s among different hospitals

Site of data collection	**Presence of plasmid/s**		**Total (percent)**
	Yes	**No**	
Tikur Anbessa Specialized Hospital	128	25	153 (76.5)
Zewditu Memorial Hospital	28	10	19 (19)
Yekatit 12 Hospital	9	0	9 (4.5)

Majority of *E. coli* isolates 88 (44.5%) carried 1 to 2 plasmids and the maximum number of plasmids carried by *E. coli* isolates was 10 (see Fig 6).

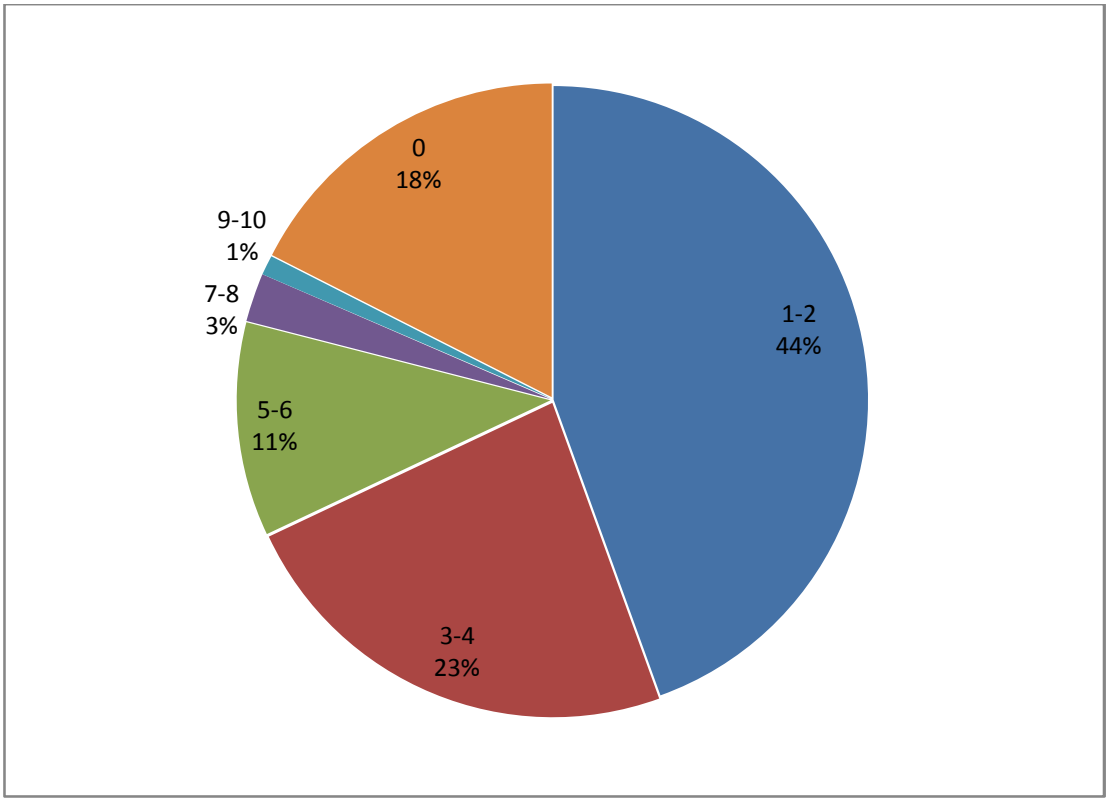

Fig 6: Number of plasmid/s carried by uropathogenic *E. coli* isolates

The plasmid size carried by *E. coli* isolates varies from 0.5kb to >23kb. Majority 220 (49.2%) of plasmid size ranges 1.6kb to 5kb. Many plasmids 148 (33%) had a size of 23kb. Some plasmids 20 (4.5%) were large in size (>23kb) [see Fig 7].

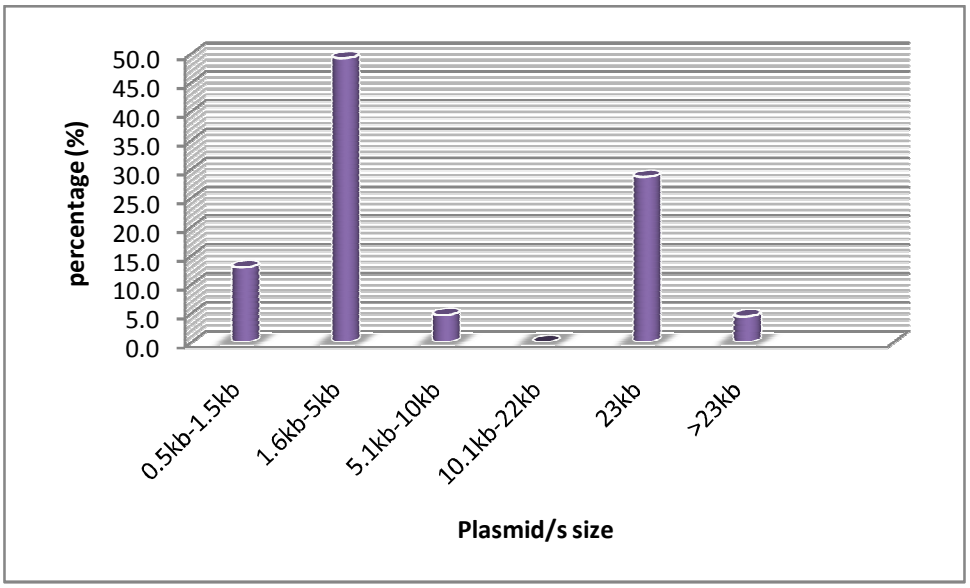

Fig 7: Plasmid/s size carried by *E. coli* isolates

In this study, we found no significant association between drug resistance and presence of plasmids (see Table 12).

Table 12: Association between presence of plasmids and drug resistance

Drug resistance		Plasmids		OR (95% C.I.)	P-value
		Positive	Negative		
Ciprofloxacin	Present	25	4	1.384 (0.449, 4.262)	0.570
	Absent	140	31		
Norfloxacin	Present	26	4	1.450 (0.472, 4.453)	0.515
	Absent	139	31		
Nitrofurantoin	Present	8	2	0.841 (0.171, 4.141)	0.688
	Absent	157	33		
Trimethoprim-sulfamethoxazole	Present	114	23	1.166 (0.539, 2.524)	0.696
	Absent	51	12		
Ceftriaxone	Present	133	28	1.039 (0.417, 2.591)	0.934
	Absent	32	7		
Ampicillin	Present	143	30	1.083 (0.380, 3.089)	0.792
	Absent	22	5		
Nalidixic acid	Present	36	6	1.349 (0.520, 3.500)	0.537
	Absent	129	29		
Tetracycline	Present	132	27	1.185 (0.493, 2.847)	0.704
	Absent	33	8		
Amoxicillin-clavulanate	Present	49	9	1.220 (0.533, 2.794)	0.637
	Absent	116	26		
Ceftazidime	Present	137	31	0.631 (0.206, 1.931)	0.417
	Absent	28	4		
Cefotaxime	Present	107	25	0.738 (0.332, 1.642)	0.455
	Absent	58	10		
Amikacin	Present	5	0	0.821 (0.768, 0.876)	0.589
	Absent	160	35		
Chloramphenicol	Present	26	7	0.748 (0.296, 1.893)	0.539
	Absent	139	28		
Gentamicin	Present	32	8	0.812 (0.337, 1.954)	0.642
	Absent	133	27		

4.5 Virulence Genes of Uropathogenic *E. coli* Isolates

Virulence genes were amplified and detected successfully in 198 (99%) *E. coli* isolates.

The most frequent *E. coli* virulence gene was *fim H* 164 (82%), followed by *aer* 109 (54.5%), *hly* 103 (51.5%), *pap* 59 (29.5%), *cnf* 58 (29%), *sfa* 50 (25%) and *afa* 24 (12%) [see Fig 8].

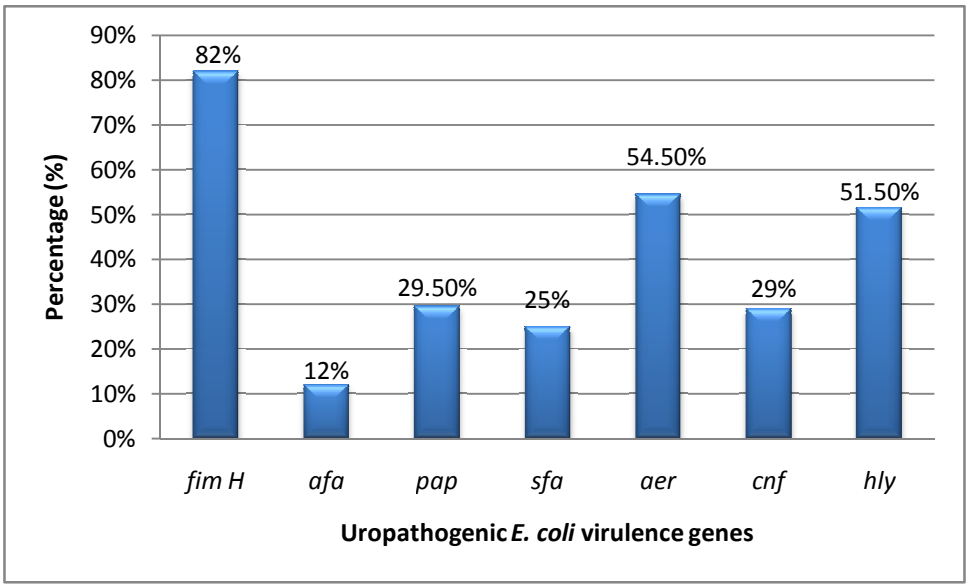

Fig 8: Virulence genes of uropathogenic *E. coli*

The most common co-occurance (combination) of virulence gene observed in this study was 24 *fim H* and *aer* followed by 17 *fim H, aer* and *hly* (see Table 13). Uropathogenic *E. coli* isolates that carried only a single virulence gene includes 18 *fim H*, 5 *hly*, 1 *cnf* and 1 *afa*.

Table 13: Common co-occurence (combination) of virulence genes of uropathogenic *E. coli*

Virulence gene combinations	**Frequency**
fim H, aer	24
fim H, aer, hly	17
fim H, pap, sfa	13
fim H, sfa, aer	11
fim H, sfa, cnf	11
fim H, pap	10
fim H, pap, hly	9
fim H, pap, aer, hly	8
fim H, cnf	8

The virulence genes *fim H* (456 bp), *afa* (672 bp), *pap* (328 bp), *sfa* (410 bp), *aer* (269 bp), *cnf* (693 bp) and *hly* (556 bp) were successfully amplified. One kilobase plus (1kb plus) DNA ladder was used to determine the base pair size (see Fig 9).

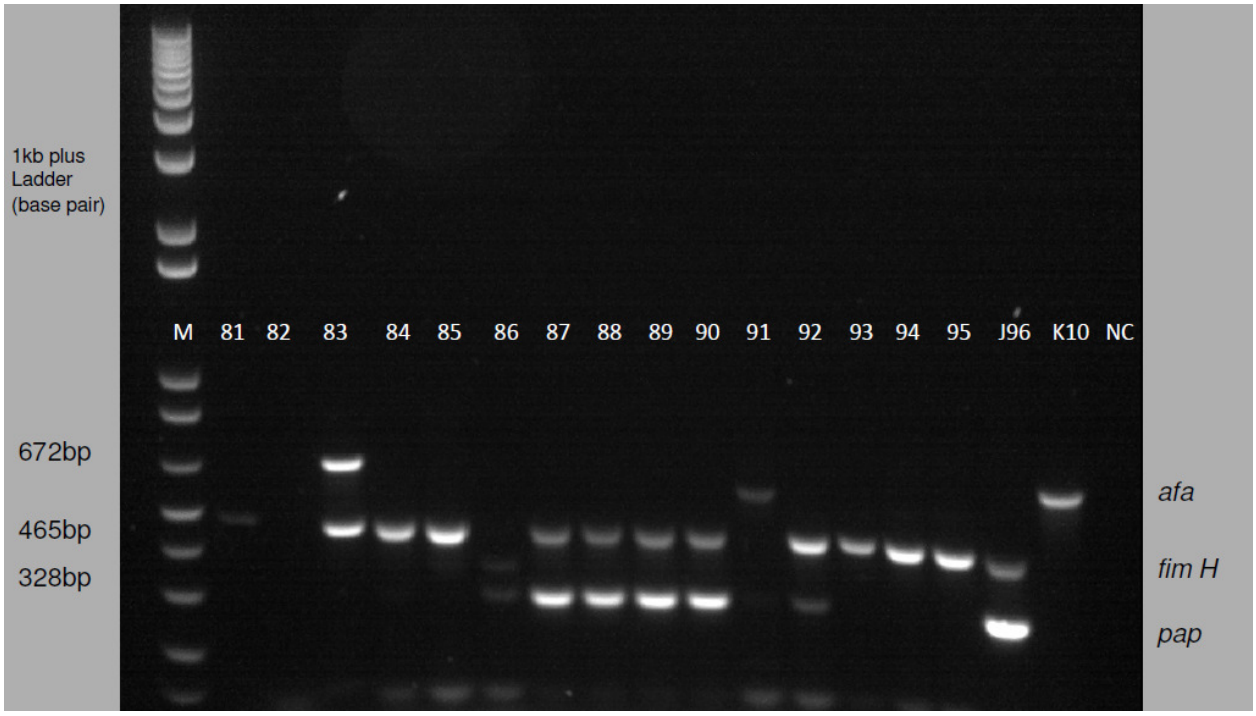

Fig 9: Representative 1.2% agarose gel electrophoresis of uropathogenic *E. coli* virulence genes isolated from urinary tract infection patients. Lane M, 1-kb plus ladder; lane 1-15, amplified *E. coli* PCR products (sample 81-95) with the following virulence genes, *pap* (328 bp), *fim H* (456 bp) and *afa* (672bp); lane 16, strain J96 positive control; lane 17, strain K10 positive control and lane 20, distilled water as negative control.

There was significant association between *pap* gene and urine urgency (*p-0.016*); *sfa* and dysuria and urine urgency (*p*-0.019 and *p*-0.043 respectively); *hly* and suprapubic pain (*p*-0.002); *aer* and suprapubic pain, flank pain and fever (*p*-0.017, *p*-0.040, *p*-0.029 respectively) [see Table 15].
No aerobactin (*aer*) gene was detected in Yekatit 12 hospital (see Table 14)

Table 14: Distribution of virulence genes among different hospitals

Virulence genes	**Tikur Anbessa specialized hospital**	**Zewditu memorial hospital**	**Yekatit 12 hospital**
fim H	121 (73.8%)	35 (21.3%)	8 (4.9%)
afa	15 (62.5%)	8 (33.3%)	1 (4.2%)
pap	44(74.6%)	11 (18.6%)	4 (6.8%)
sfa	21 (42%)	24(48%)	5 (10%)
aer	104 (95.4%)	5 (4.6%)	0
cnf	30 (51.7%)	22 (37.9%)	6 (10.4%)
hly	85 (82.5%)	16 (15.5%)	2 (2%)

Table 15: Association between virulence genes of *E. coli* and clinical data of urinary tract infections

Clinical data		Virulence genes															
		fim H				*afa*				*pap*				*sfa*			
		Present	Absent	OR (95%CI)	P-value	Present	Absent	OR (95%CI)	P-value	Present	Absent	OR (95%CI)	P-value	Present	Absent	OR (95%CI)	P-value
Dysuria	Present	146	29	1.958 (0.750, 5.112)	0.170	20	155	0.677 (0.211, 2.174)	0.512	51	124	0.874 (0.355, 2.153)	0.770	39	136	0.365 (0.153, 0.868)	0.019
	Absent	18	7			4	21			8	17			11	14		
Urine urgency	Present	116	30	0.483 (0.189, 1.236)	0.123	15	131	0.573 (0.234, 1.398)	0.217	50	96	2.604 (1.178, 5.756)	0.016	42	104	2.322 (1.011, 5.336)	0.043
	Absent	48	6			9	45			9	45			8	46		
Urgency incontinence	Present	95	27	0.459 (0.203, 1.037)	0.057	12	110	0.600 (0.255, 1.413)	0.239	36	86	1.001 (0.537, 1.867)	0.997	31	91	1.058 (0.548, 2.043)	0.867
	Absent	69	9			12	66			23	55			19	59		
Suprapubic pain	Present	73	12	1.604 (0.752, 3.425)	0.219	9	76	0.789 (0.328, 1.901)	0.597	20	65	0.600 (0.319, 1.129)	0.111	23	62	1.209 (0.635, 2.302)	0.563
	Absent	91	24			15	100			39	76			27	88		
Flank pain	Present	88	17	1.294 (0.628, 2.666)	0.484	10	95	0.609 (0.257, 1.445)	0.257	28	77	0.751 (0.408, 1.380)	0.356	31	74	1.676 (0.871, 3.225)	0.120
	Absent	76	19			14	81			31	64			19	76		
Fever	Present	22	4	1.239 (0.399, 3.846)	0.479	3	23	0.950 (0.262, 3.441)	0.619	8	18	1.072 (0.438, 2.622)	0.879	6	20	0.886 (0.335, 2.348)	0.808
	Absent	142	32			21	153			51	123			44	130		
Chills	Present	10	1	2.273 (0.282, 18.341)	0.693	1	10	0.722 (0.088, 5.902)	0.611	4	7	1.392 (0.392, 4.947)	0.735	3	8	1.133 (0.289, 4.447)	0.549
	Absent	154	35			23	166			55	134			47	142		

Table 15: Association between virulence genes of *E. coli* and clinical data (continued…)

Clinical data		Virulence genes											
		aer				*cnf*				*hly*			
		Present	Absent	OR (95%CI)	P-value	Present	Absent	OR (95%CI)	P-value	Present	Absent	OR (95%CI)	P-value
Dysuria	Present	96	79	1.122(0.485, 2.596)	0.788	48	127	0.567(0.238, 1.348)	0.195	92	83	1.411(0.607, 3.280)	0.422
	Absent	13	12			10	15			11	14		
Urine urgency	Present	78	68	0.851(0.453, 1.598)	0.616	46	100	1.610(0.776, 3.342)	0.199	79	67	1.474(0.787, 2.761)	0.225
	Absent	31	23			12	42			24	30		
Urgency incontinence	Present	71	51	1.465(0.828, 2.595)	0.189	38	84	1.312(0.694, 2.479)	0.403	65	57	1.200(0.680, 2.120)	0.529
	Absent	38	40			20	58			38	40		
Suprapubic pain	Present	38	47	0.501(0.284, 0.885)	0.017	28	57	1.392(0.753, 2.574)	0.291	33	52	0.408(0.230, 0.725)	0.002
	Absent	71	44			30	85			70	45		
Flank pain	Present	50	55	0.555(0.315, 0.975)	0.040	33	72	1.283(0.694, 2.374)	0.426	53	52	0.917(0.526, 1.599)	0.761
	Absent	59	36			25	70			50	45		
Fever	Present	9	17	0.392(0.165, 0.928)	0.029	11	15	1.982(0.850, 4.622)	0.109	13	13	0.933(0.409, 2.128)	0.870
	Absent	100	74			47	127			90	84		
Chills	Present	4	7	0.457(0.129, 1.614)	0.214	3	8	0.914(0.234, 3.572)	0.600	4	7	0.519(0.147, 1.834)	0.301
	Absent	105	84			55	134			99	90		

4.6 Relationship between Virulence Genes and Drug Resistance of *E. coli* Isolates

In this study, we found significant association (x^2=6.00 *p*-0.014) between *fim H* and chloramphenicol drug resistance. Similarly, significant association between *aer* genes, and gentamicin, ampicillin and nitrofurantoin drug resistance (*p*-0.028, *p*-0.018 and *p*-0.023 respectively) was observed. There was no significant association between the rest antibiotics against uropathogenic *E. coli* and virulence genes of uropathogenic *E. coli* (see Table 16).

Table 16: Association between drug resistance and virulence genes of uropathogenic *E. coli*

Antibiotic susceptibility		Virulence genes															
		fim H				*afa*				*pap*				*sfa*			
		Present	Absent	X^2	P-value	Present	Absent	X^2	P-value	Present	Absent	X^2	P-value	Present	Absent	X^2	P-value
Ciprofloxacin	R	24	5	0.013	0.908	3	26	*	0.528	9	20	0.038	0.845	6	23	0.336	0.562
	S	140	31			21	150			50	121			44	127		
Norfloxacin	R	25	5	0.043	0.837	3	27	*	0.498	9	21	0.004	0.948	6	24	0.471	0.493
	S	139	31			21	149			50	120			44	126		
Nitrofurantoin	R	8	2	*	0.566	0	10	*	0.612	2	8	*	0.726	1	9	*	0.456
	S	156	34			24	166			57	133			49	141		
SXT	R	116	21	2.103	0.147	15	122	0.445	0.500	41	96	0.038	0.845	33	104	0.193	0.66
	S	48	15			9	54			18	45			17	46		
Ceftriaxone	R	130	31	0.881	0.348	21	140	*	0.582	51	110	1.882	0.17	41	120	0.096	0.757
	S	34	5			3	36			8	31			9	30		
Ampicillin	R	140	33	*	0.424	19	154	*	0.334	50	123	0.221	0.639	46	127	1.272	0.189
	S	24	3			5	22			9	18			4	23		
Nalidixic acid	R	38	4	2.558	0.108	4	38	0.309	0.578	15	27	0.987	0.320	11	31	0.040	0.841
	S	126	32			20	138			44	114			39	119		
Tetracycline	R	131	28	0.080	0.777	17	142	*	0.283	47	112	0.001	0.971	39	120	0.092	0.762
	S	33	8			7	34			12	29			11	30		
Amoxicillin Clavulanic	R	46	12	0.400	0.527	7	51	0.001	0.985	17	41	0.001	0.970	18	40	1.587	0.208
	S	118	24			17	125			42	100			32	110		
Ceftazidime	R	136	32	0.781	0.377	21	147	*	0.773	47	121	1.172	0.279	39	129	1.786	0.181
	S	28	4			3	29			12	20			11	21		
Cefotaxime	R	108	24	0.009	0.926	14	118	0.714	0.398	40	92	0.120	0.729	31	101	0.475	0.491
	S	56	12			10	58			19	49			19	49		
Amikacin	R	5	0	*	0.588	1	4	*	0.476	1	4	*	0.538	0	5	*	0.334
	S	159	36			23	172			58	137			50	145		
Chloramphenicol	R	32	1	6.000	0.014	5	28	0.372	0.542	7	26	1.305	0.235	7	26	0.302	0.582
	S	132	35			19	148			52	115			43	124		
Gentamicin	R	33	7	0.008	0.927	1	39	*	0.053	15	25	1.539	0.215	9	31	0.167	0.683
	S	131	29			23	137			44	116			41	119		

Table 16: Association between drug resistance and virulence genes of uropathogenic *E. coli* (continued…)

Antibiotic susceptibilty		Virulence genes											
		aer				*cnf*				*hly*			
		Present	Absent	X^2	P-value	Present	Absent	X^2	P-value	Present	Absent	X^2	P-value
Ciprofloxacin	R	18	11	0.784	0.376	10	19	0.498	0.482	11	18	2.500	0.114
	S	91	80			48	123			92	79		
Norfloxacin	R	19	11	1.111	0.292	10	20	0.322	0.570	12	18	1.869	0.172
	S	90	80			48	122			91	79		
Nitrofurantoin	R	9	1	*	0.023	2	8	*	0.520	7	3	*	0.333
	S	100	90			56	134			96	94		
SXT	R	72	65	0.664	0.415	44	93	2.052	0.152	66	71	1.925	0.165
	S	37	26			14	49			37	26		
Ceftriaxone	R	89	72	0.202	0.653	47	114	0.015	0.903	83	78	0.001	0.976
	S	20	19			11	28			20	19		
Ampicillin	R	100	73	5.640	0.018	52	121	0.696	0.404	90	83	0.140	0.708
	S	9	18			6	21			13	14		
Nalidixic acid	R	26	16	1.176	0.278	11	31	0.204	0.652	24	18	0.678	0.410
	S	83	75			47	111			79	79		
Tetracycline	R	87	72	0.015	0.903	47	112	0.118	0.731	83	76	0.153	0.696
	S	22	19			11	30			20	21		
AMC	R	35	23	1.125	0.289	12	46	2.740	0.098	31	27	0.124	0.725
	S	74	68			46	96			72	70		
Ceftazidime	R	92	76	0.029	0.865	50	118	0.296	0.586	87	81	0.034	0.853
	S	17	15			8	24			16	16		
Cefotaxime	R	74	58	0.381	0.537	42	90	1.498	0.221	66	66	0.350	0.554
	S	35	33			16	52			37	31		
Amikacin	R	1	4	*	0.179	0	5	*	0.324	1	4	*	0.201
	S	108	87			58	137			102	93		
Chloramphenicol	R	17	16	0.142	0.706	10	23	0.033	0.857	19	14	0.584	0.445
	S	92	75			48	119			84	83		
Gentamicin	R	28	12	4.844	0.028	7	33	3.212	0.073	25	15	2.422	0.12
	S	81	79			51	109			78	82		

* Fisher's Exact Test was used because expected count is less than 5; R: Resistance; S: Sensitive; AMC: Amoxacillin-clavulanate; SXT: Cotrimoxazole

4.7 Phylogenetic grouping of uropathogenic *E. coli*

The distribution of phylogenetic groups amongst *E. coli* isolates was determined by the following genes; *arp A* (400bp), *chu A* (288bp), *yja A* (211bp) and an anonymous DNA fragment that is found in *E. coli* worldwide; *TspE4C2* (152bp). One kilobase plus (1kb plus) DNA ladder was used to determine the base pair size (see Fig 10).

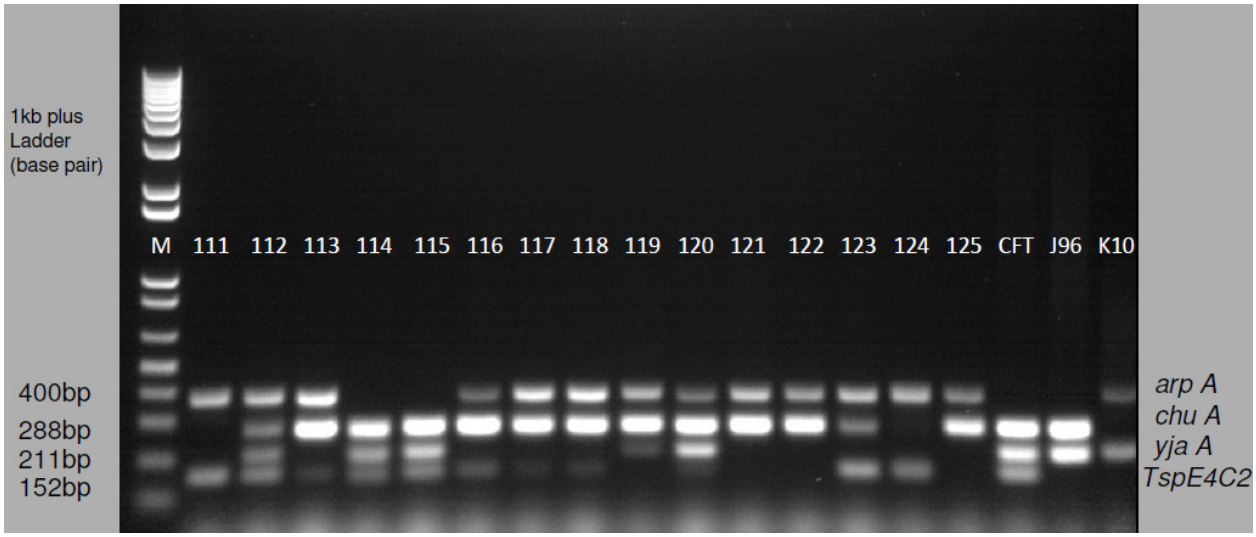

Fig 10: Representative 1.2% agarose gel electrophoresis of uropathogenic *E. coli* genes used to classify *E. coli* into different phylogroup. Lane M, 1-kb plus ladder; lane 1-15, amplified PCR products (sample 111-125) with the following *E. coli* phylogrouping genes; *arp A* (400bp), *chu A* (288bp), *yja A* (211bp) and *TspE4C2* (152bp); lane 16, strain CFT073 positive control; lane 17, strain J96 positive control and lane 20, strain K10 positive control.

Phylogenetic analysis indicated that majority of uropathogenic *E. coli* isolates were phylogroup B2 60 (30%) followed by phylogroup D 55 (27.5%), phylogroup B1 48 (24%) and phylogroup A 37 (18.5%) [see Fig 11].

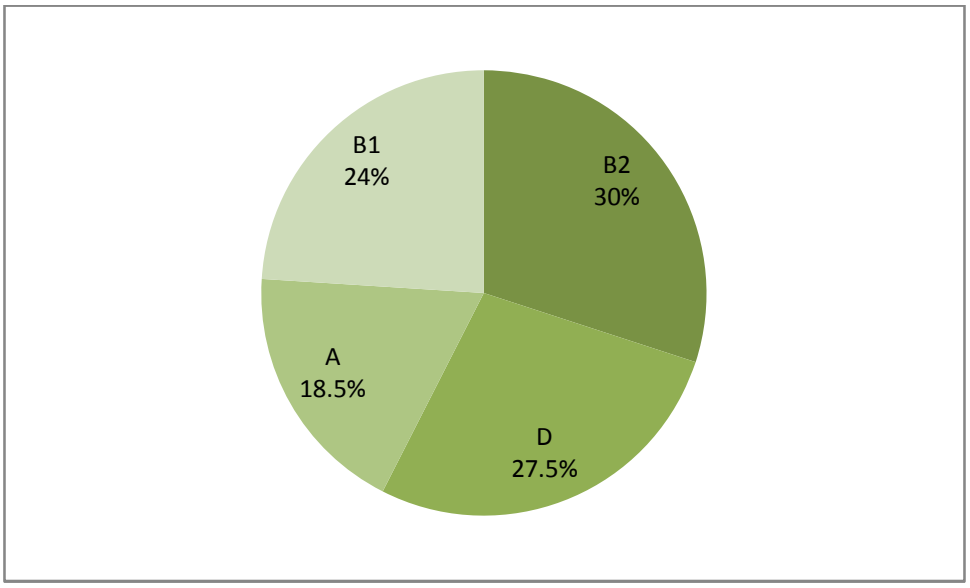

Fig 11: Phylogroup of uropathogenic *E. coli* isolates

Phylogroup B2 and B1 were the dominant phylogroup in Tikur Anbessa specialized hospital whereas phylogroup B2 and D were the dominant phylogroup in Zewditu memorial hospital (see Table 17).

Table 17: Distribution of phylogroup among different hospitals

Phylogroup	**Tikur Anbessa Specialized Hospital**	**Zewditu Memorial Hospital**	**Yekatit 12 Hospital**
A	27 (73%)	7 (18.9%)	3 (8.1%)
B1	44 (91.7%)	4 (8.3%)	0
B2	44(73.3%)	14 (23.4%)	2 (3.3%)
D	38 (69.1%)	13(23.6%)	4 (7.3%)

There was significant association between presence of plamids and phylogroup B2 and D (p-0.008 and p-0.019 respectively) [see Table 18].

Table 18: Association between phylogroup and presence of plasmids

Phylogroup		**Plasmids**		**OR (95% C.I.)**	**P-value**
		Positive	**Negative**		
A	Present	34	3	2.768 (0.799, 9.587)	0.096
	Absent	131	32		
B1	Present	37	11	0.631 (0.283, 1.406)	0.257
	Absent	128	24		
B2	Present	43	17	0.373 (0.177, 0.789)	0.008
	Absent	122	18		
D	Present	51	4	3.467 (1.163, 10.337)	0.019
	Absent	114	31		

There was significant association between *E. coli* phylogroup B2 and three virulence genes namely *afa*, *pap* and *sfa* (*p*-0.014, *p*-0.002, *p*-0.004 respectively). Similarly, there was significant association between *E. coli* phylogroup D and two virulence genes namely *fim H* and *pap* (*p*-0.043, *p*-0.019 respectively). There was significant association between *E. coli* phylogroup A and virulence genes *fim H* and *afa* (*p*-0.011, *p*-0.002 respectively). Phylogroup B1 had significant association with *pap* gene (*p*-0.001). The virulence factor that encodes *pap* gene had significant association with *E. coli* phylogroup B2, D and B1 (*p*-0.002, *p*-0.019, *p*-0.001 respectively) [see Table 19].

In this study, we found no significant association between *E. coli* phylogroup and drug resistance except phylogroup D has significant association with ceftazidime drug resistance (*p*-0.025) [see Table 20].

Table 19: Association between phylogroup and virulence genes of uropathogenic *E. coli*

Virulence genes		Phylogroup															
		A				B1				B2				D			
		Present	Absent	X^2	P-value	Present	Absent	X^2	P-value	Present	Absent	X^2	P-value	Present	Absent	X^2	P-value
fim H	Present	25	139	6.407	0.011	41	123	0.500	0.480	48	116	0.232	0.630	50	114	4.079	0.043
	Absent	12	24			7	29			12	24			55	31		
afa	Present	10	14	9.708	0.002	5	19	0.150	0.699	2	22	6.097	0.014	7	17	0.038	0.845
	Absent	27	149			43	133			58	118			48	128		
pap	Present	6	53	3.852	0.050	3	56	16.416	0.001	27	32	9.902	0.002	23	36	5.535	0.019
	Absent	31	110			45	96			33	108			32	109		
sfa	Present	7	43	0.895	0.344	9	41	1.316	0.251	23	27	8.127	0.004	11	39	1.011	0.315
	Absent	30	120			39	111			37	113			44	106		
aer	Present	25	84	3.126	0.077	22	87	1.913	0.167	38	71	2.697	0.101	24	85	3.610	0.057
	Absent	12	79			26	65			22	69			31	60		
cnf	Present	9	49	0.482	0.488	13	45	0.113	0.737	23	35	3.626	0.057	13	45	1.060	0.303
	Absent	28	114			35	107			37	105			42	100		
hly	Present	17	86	0.561	0.454	25	78	0.009	0.926	36	67	2.479	0.115	25	78	1.110	0.292
	Absent	20	77			23	74			24	73			30	67		

Table 20: Association between phylogroup and drug resistance of uropathogenic *E. coli*

Antibiotic susceptibility		Phylogroup															
		A				B1				B2				D			
		Present	Absent	X^2	P-value	Present	Absent	X^2	P-value	Present	Absent	X^2	P-value	Present	Absent	X^2	P-value
Ciprofloxacin	R	7	22	0.715	0.398	5	24	0.849	0.357	7	22	0.555	0.456	10	19	0.829	0.362
	S	30	141			43	128			53	118			45	126		
Norfloxacin	R	7	23	0.547	0.460	5	25	1.041	0.308	7	23	0.747	0.387	11	19	1.487	0.223
	S	30	140			43	127			53	117			44	126		
Nitrofurantoin	R	1	9	*	0.692	3	7	*	0.706	4	6	*	0.491	2	8	*	0.730
	S	36	154			45	145			56	134			53	137		
SXT	R	25	112	0.018	0.892	30	107	1.054	0.305	46	91	2.649	0.104	36	101	0.326	0.568
	S	12	51			18	45			14	49			19	44		
Ceftriaxone	R	30	131	0.010	0.921	38	123	0.072	0.789	50	111	0.438	0.508	43	118	0.260	0.610
	S	7	32			10	29			10	29			12	27		
Ampicillin	R	35	138	*	0.110	39	134	1.491	0.222	52	121	0.002	0.964	47	126	0.071	0.790
	S	2	25			9	18			8	19			8	19		
Nalidixic acid	R	4	38	2.841	0.092	10	32	0.001	0.974	17	25	2.778	0.096	11	31	0.046	0.831
	S	33	125			38	120			43	115			44	114		
Tetracycline	R	31	128	0.511	0.475	36	123	0.785	0.376	51	108	1.591	0.207	41	118	1.143	0.285
	S	6	35			12	29			9	32			14	27		
AMC	R	10	48	0.086	0.770	11	47	1.135	0.287	19	39	0.296	0.586	18	40	0.512	0.474
	S	27	115			37	105			41	101			37	105		
Ceftazidime	R	33	135	0.910	0.340	41	127	0.094	0.759	53	115	1.198	0.274	41	127	5.046	0.025
	S	4	28			7	25			7	25			14	18		
Cefotaxime	R	23	109	0.298	0.585	31	101	0.056	0.812	45	87	3.094	0.079	33	99	1.217	0.270
	S	14	54			17	51			15	53			22	46		
Amikacin	R	0	5	*	0.586	3	2	*	0.091	1	4	*	0.527	1	4	*	0.580
	S	37	158			45	150			59	136			54	141		
Chloramphenicol	R	3	30	2.321	0.128	9	24	0.232	0.630	9	24	0.140	0.708	12	21	1.557	0.212
	S	34	133			39	128			51	116			43	124		
Gentamicin	R	5	35	1.194	0.275	8	32	0.439	0.508	15	25	1.339	0.247	12	28	0.157	0.692
	S	32	128			40	120			45	115			43	117		

* Fisher's Exact Test was used because expected count is less than 5: R: Resistance; S: Sensitive; AMC: Amoxacillin-clavulanate; SXT: Cotrimoxazole

CHAPTER FIVE

Discussion

E. coli have been reported as the most common cause of urinary tract infections (Flores-Mireles *et al*., 2015; Foxman, 2010; Raksha *et al*., 2003) and this thesis focused on characterization of *E. coli*. The focus of this study was to assess the phenotypic drug resistance, plasmid profile, virulence genes and phylogroups of uropathogenic *E. coli* as well as relationship of virulence genes and drug resistance patterns of uropathogenic *E. coli* among urinary tract infection patients.

5.1 Socio-demographic Status of Study Participants

In this study higher proportion of urinary tract infections in females (66%) than in males (34%) were observed. Many scholars have reported similar findings (Gizachew *et al*., 2013; Abdu *et al*., 2018; Farshad *et al*., 2012; Ranjini *et al*., 2015; Wang *et al*., 2014; Jadhav *et al*., 2011; Kumar *et al*., 2013; Molina-López *et al*., 2011; Paniagua-Contreras *et al*., 2015). UTI is more common in females than in males because structurally the female urethra is less effective in preventing the bacterial entry for colonization i.e. the urethra is shorter and wider (Abdu *et al*., 2018; Lights and Boskey, 2012). Some other reasons could be absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora (Haider *et al*., 2010).

Highest incidence of urinary tract infections was observed in the age groups 26-45, and other studies reported similar findings (Gizachew *et al*., 2013; Abdu *et al*., 2018; Ranjini *et al*., 2015; Kumar *et al*., 2013). This could be due to the fact that this age group is sexually active. Sexual intercourse may access entry of bacteria in to bladder. The prevalence of UTI has a J-shaped distribution, with a higher frequency among the very young and a gradual increase with age in both men and women. Until the age of 60 years and older, the prevalence is significantly higher for women than men (Foxman, 2010; Johnson, 1991). The distribution of symptomatic urinary tract infection has a somewhat different shape: women aged 15-29 years have the highest frequency (Foxman, 2010; Foxman and Brown, 2003).

5.2 Clinical Data of Study Participants

The study participants had at least two of the following urologic symptoms: dysuria, urine urgency, urgency incontinence, flank pain, suprapubic pain, fever and chills. These findings are in agreement with studies conducted in Nigeria (Akingbade *et al*., 2014), Romania (Usein *et al*., 2001), Denmark (Ejrnaes, 2011), South Korea (Yun *et al*., 2015), Iran (Tabasi *et al*., 2016) and Brazil (Santo *et al*., 2006). These urologic symptoms are indicative of UTIs.

5.3 Bacterial Isolation

In this study, 200 (25.6%) *E. coli* isolates were identified, which is in agreement with studies conducted in Ethiopia 35.5% (Gizachew *et al*., 2013), Egypt 36% (Alabsi *et al*., 2014), India 25.5% (Anusha *et al*., 2015) and Iran 27.5% (Kazemnia *et al*., 2014), but lower than studies conducted in Nigeria 62.5% (Abdu *et al*., 2018), 100% (Romanus and Eze, 2011), Czech Republic 66.7% (Koreň *et al*., 2013), India 56.3% (Jan *et al*., 2009) and Mexico 60.4% (Paniagua-Contreras *et al*., 2015). These differences in isolation rate could be due to sample size and methodology differences. There was significant association between *E. coli* isolation rate and urologic symptoms urine urgency, fever and chills. This indicates that there is high possibility to find *E. coli* isolates when the patients manifest the above symptoms. But prior antibiotic usage by UTI patients will result in low isolation rate of bacteria using culture technique. In our study, there was no significant association between socio-demographic status and *E. coli* isolation rates, which is in agreement with a study conducted in Hawassa, Ethiopia (Gizachew *et al*., 2013).

5.4 Antimicrobial Resistance Patterns of Uropathogenic *E. coli*

The most frequent antimicrobial resistance found in this study was against ampicillin (86.5%), which is in agreement with studies conducted in Ethiopia 83.1% (Biadglegne and Abera, 2009), Egypt 89% (Alabsi *et al*., 2014), Nigeria 83% (Abiodun *et al*., 2014), India 85.5% (Kumar *et al*., 2013) and Mexico 83.7% (Molina-López *et al*., 2011). Resistance to tetracycline was observed in 80% of UPEC isolates, which is comparable to studies conducted in Ethiopia 66.2% (Biadglegne and Abera, 2009), Nigeria 75% (Mbanga and Mudzana, 2014) and Iran 70.8% (Farshad *et al*., 2012). Currently, ampicillin and tetracycline are not listed on national standard treatment

guidelines for treatment of UTIs in Ethiopia (FMHACA, 2014). The indiscriminate and improper use of these antibiotics in the past years may be the reason for high drug resistance.

Resistance to trimethoprim-sulfamethoxazole, also frequently used in the treatment of UTI, was found in 68.5% of UPEC isolates; other studies also found comparable frequencies of 70% in Nigeria (Akingbade *et al*., 2014), 74% in Egypt (Alabsi *et al*., 2014), 60.42% in Iran (Adib *et al*., 2014), 67.3% in India (Kumar *et al*., 2013) and 66% in Mexico (Paniagua-Contreras *et al*., 2015), but higher than studies conducted in Ethiopia 28.6% (Beyene and Tsegaye, 2011), 45.5% (Biadglegne and Abera, 2009) and South Korea 35.9% (Yun *et al*., 2015). In contrast with WHO recommendations for UTI treatment with trimethoprim-sulfamethoxazole, many studies have proposed trimethoprim-sulfamethoxazole as an inappropriate antibiotic for the treatment of UTI (Farshad *et al*., 2012; Ramos *et al*., 2011; Abass *et al*., 2014). Trimethoprim-sulfamethoxazole is recommended for treatment of UTIs in Ethiopia by national standard treatment guidelines (FMHACA, 2014). In Addis Ababa, self-medication and noncompliance with medication are common practice by the society and these could be the reason for high drug resistance to trimethoprim-sulfamethoxazole.

Resistance to ceftriaxone was observed in 80.5% of UPEC isolates, which is comparable to studies conducted in Nigeria 86% (Abiodun *et al*., 2014), India 81.8% (Anusha *et al*., 2015) and China 84.8% (Wang *et al*., 2014), but higher than studies conducted in Nigeria 23.3% (Akingbade *et al*., 2014) and Mexico 10.2% (Molina-López *et al*., 2011). Resistance to ceftazidime was found in 84% of UPEC isolates, which is in agreement with other studies in India 80% (Annapurna *et al*., 2014) and China 85.4% (Wang *et al*., 2014), but higher than studies conducted in Nigeria 15.8% (Akingbade *et al*., 2014), Egypt 40% (Alabsi *et al*., 2014) and Mexico 8.5% (Molina-López *et al*., 2011). Resistance to cefotaxime was observed in 66% of UPEC isolates, which is in agreement with studies conducted in Nigeria 68% (Abiodun *et al*., 2014), India 67.5% (Mukherjee *et al*., 2013), 66.66% (Annapurna *et al*., 2014) and Iraq 78% (Merza and Jubrael, 2015), but higher than studies conducted in Ethiopia 18.7% (Gizachew *et al*., 2013), Nigeria 4.4% (Romanus and Eze, 2011) and South Korea 7.8% (Yun *et al*., 2014). Ceftriaxone is recommended for treatment of severe pyelonephritis in Ethiopia by national standard treatment guidelines (FMHACA, 2014). Resistance to ceftriaxone could be due to transmission of resistant strains/isolates among hospitalized patients and noncompliance with medication.

Generally, differences in antibiotic resistance patterns could be due to variations in antibiotic prescribing habits among different countries; i.e. in some areas people buy antibiotics without prescription and use it. Addis Ababa is a capital city of Ethiopia where over-the-counter availability of antibiotics is high. The increase in antibiotic resistance observed in this study could be due to an irrational consumption of antibiotics, transmission of resistant isolates among people, self-medication, and noncompliance with medication.

No drug resistance was observed to meropenem and imipenem, which is in agreement with studies conducted in Egypt (Alabsi *et al*., 2014), Spain (Guiral *et al*., 2015), India (Shariff *et al*., 2013), South Korea (Yun *et al*., 2015), China (Wang *et al*., 2014) and Iran (Farshad *et al*., 2012). It would be prudent to restrict the use of carbapenems to cases of complicated UTI or those having sepsis or for patients admitted in the intensive care units as their injudicious use may lead to the spread of carbapenemases and further limit the antibiotic armamentarium (Ranjini *et al*., 2015). Carbapenems such as meropenem and imipenem are not in national treatment guidelines list for treatment of UTIs in Ethiopia (FMHACA, 2014). The use of such antibiotics by society is uncommon and this could be the reason for the absence of drug resistance to meropenem and imipenem. Therefore, these antibiotics can be used for treatment of severe UTIs.

High susceptibility of UPEC isolates to ciprofloxacin (85.5%), nitrofurantoin (95%) and norfloxacin (85%) were observed, which is comparable to other studies conducted in Ethiopia (Biadglegne and Abera, 2009; Beyene and Tsegaye, 2011), Egypt (Alabsi *et al*., 2014), Spain (Guiral *et al*., 2015) and Iran (Adib *et al*., 2014; Farshad *et al*., 2012; Momtaz *et al*., 2013). These outcomes, thus supports earlier report ascribed to Gupta *et al*. (2011) that nitrofurantoin would be a better choice for UTI empiric (treatment) therapy and it could be administered while awaiting the culture result. Fluoroquinolones such as ciprofloxacin and norfloxacin are widely used for empirical treatment of UTI, including the cases of upper urinary tract infections (Rocha *et al*., 2012). In Ethiopia, these antibiotics are currently recommended for treatment of urinary tract infections (FMHACA, 2014). Currently, these antibiotics should be continued to be used as drug of choice for treatment of UTIs. But antimicrobial resistance patterns should be monitored since there is a possibility of emergence of drug resistance in the society due to indiscriminate use of antibiotics habits by the society.

Aminoglycosides such as amikacin and gentamicin are the other group of antibiotics that have shown a good sensitivity profile. The draw backs of using them include requirement for parenteral administration and low safety profile especially among the elderly. This can be overcome by opting for single day dosing or by using in combination with other antimicrobial agents (Ranjini *et al*., 2015).

The overall incidence of antibiotic resistance of *E. coli* in this study was high and majority of the strains were MDR (96.5%). This result is comparable to studies conducted in Ethiopia 93.1% (Biadglegne and Abera, 2009), Mongolia 93.9% (Munkhdelger *et al*., 2017), India 92.5% (Mukherjee *et al*., 2013), 100% (Dash *et al*., 2012) and Mexico 97% (Paniagua-Contreras *et al*., 2015), but higher than studies conducted in South Korea 21.9% (Yun *et al*., 2015), India 50% (Jan *et al*., 2009) and Mexico 30.2% (Molina-López *et al*., 2011). MDR causes major consequences such as empirical therapy of *E. coli* infections as well as possible co-selection of antimicrobial resistance mediated by MDR plasmids (Farshad *et al*., 2012). The MDR organisms are generally acquired by a horizontal transmission from other patients, which may have been selected by antibiotic use. There is evidence that in many patients with *E. coli* UTIs, the uropathogenic clone colonizes the large intestine. Just like any other *E. coli* strain, the uropathogenic *E. coli* clones can be excreted in faeces and carried in water, through which they can be ingested by other people. Once they are ingested, they can colonize the large intestine, from where they can initiate urinary tract infections in the new host (Shariff *et al*., 2013).

In our study, the most common combinations of multidrug resistance (MDR) were against trimethoprim-sulfamethoxazole, ceftriaxone, ampicillin, tetracycline, ceftazidime and cefotaxime. A study conducted in Nigeria reported that the most common *E. coli* MDR combinations were cloxacillin, ampicillin, amoxicillin, streptomycin, erythromycin and tetracycline (Akingbade *et al*., 2014). According to Jan *et al.* (2009), the most common MRD combinations were ampicillin, nitrofurantoin, tetracycline, amoxacillin-clavulanate, gentamicin, amoxacillin and cefoxitin. According to Adib *et al*. (2014), among multidrug resistant patterns, cefazolin, nalidixic acid and cotrimoxazole as well as cefazolin and cotrimoxazole were the most prevalent patterns. High prevalence of multiple antibiotic resistance strains is a possible indication that very large population of bacterial isolates has been exposed to several antibiotics (Abdu *et al*., 2018).

Nowadays antimicrobial resistance is a challenge for successful treatment of UTIs due to lack of timely and accurate identification and determination of the antimicrobial susceptibility of uropathogens, indiscriminate use of antibiotics, resistance can easly be transmitted through plasmids and geographical variation in prescription habits of antibiotics (Mbanga and Mudzana, 2014; Rijavec *et al*., 2006; Stamm, 2002).

Indiscriminate use of antimicrobial agents by healthcare providers or by way of self-prescribing and over-the-counter availability is major risk factors for the development of high levels of antimicrobial resistance (Akingbade *et al*., 2014; Gibreel *et al*., 2012; Horcajada *et al*., 2005; Johnson *et al*., 2005; Mbanga and Mudzana, 2014). Some other factors contributing towards resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients, and the use of antibiotics as livestock food additives for growth promotion (Ranjini *et al*., 2015; Akingbade *et al*., 2014). Another important mechanism facilitating the increase in antimicrobial-resistant UTIs is the introduction and clonal expansion of competitive, resistant *E. coli* strains in the community (Nordstrom *et al.,* 2013).

To reduce the incidence of resistance, empirical antibiotic selection in treatment of UTI must be based on the knowledge of local prevalence of causative uropathogens and their respective antimicrobial sensitivities rather than on universal guidelines (Abdu *et al*., 2018). Therefore, proper use of antibiotics could be helpful to tackle antibiotic resistance; i.e. implementing good prescribing and dispensing practices as well as the patients should take the antibiotics for specified period (dates) and within a specified time interval as prescribed by the physician. In addition, most health facilities found in Ethiopia do not have laboratory services that perform antimicrobial susceptibility testing and this makes difficult to detect antimicrobial resistance. Thus, establishment of antimicrobial susceptibility testing laboratory facilities is important for screening of drug resistance and selection of emperic treatments of UTIs.

The above data analysis indicates that eventhough there is variation in phenotypic drug resistance pattern of uropathogenic *E. coli* among different area and countries, our findings are relatively similar or comparable to other areas.

5.5 Plasmid Analysis of Uropathogenic *E. coli*

In this study, plasmid size carried by *E. coli* isolates varies from 0.5kb to 23kb or more. Majority of uropathogenic *E. coli* isolates carried plasmid size of 1.6kb to 5kb. This result is comparable to studies conducted in Nigeria where clinical isolates of *E. coli* which showed multiple drug resistance were found to harbour plasmids with molecular sizes ranging from 2kb to 6.5-23kb and a maximum 26kb (Akingbade *et al*., 2014; Umolu *et al*., 2006). Similarly, a study conducted in India, clinical isolates of *E. coli* were known to harbour plasmids of different molecular size ranging from 2-3kb to 6.5kb and maximum 26kb (Jan *et al*., 2009). Another study conducted in Iran reported plasmid size range from 1kb to 33kb (Farshad *et al*., 2012). In a present study, the range of number of plasmid was 1-10, which is similar to a study conducted in Iran (Farshad *et al*., 2012). A study conducted in Nepal indicated that 23kb plasmid size was common, which is similar to our study (Khadgi *et al*., 2013).

In our study, there was no significant association between presence of plasmids and phenotypic drug resistance, which is in agreement with other studies (Farshad *et al*., 2012; Khadgi *et al*., 2013). Since antibiotic resistance genes are located on mobile genetic elements (like plasmids), their distribution expedites their presence within communities (Rijavec *et al*., 2006). Eventhough, in this study no significant association between presence of of plasmids and drug resistance, there is always a possibility that plasmid is a major means for transmission of drug resistance and responsible for increased antimicrobial resistance among UPEC strains (Soulsby, 2005).

5.6 Virulence Genes of Uropathogenic *E. coli*

Identification of virulence factors of UPEC are important for the pathogeneisis of UTI, severity of infection, targets for vaccine and drug development (Donnenberg and Welch, 1996; Flores-Mireles *et al*., 2015; Kawamura-Sato *et al*., 2010; Mike *et al*., 2016; Terlizzi *et al*., 2017).

5.6.1 Adhesins/ Fimbriae

In our study *fim H* adhesion gene, which is a major facilitator of UPEC entry into host cells, was the most common and present in 164 (82%) UPEC isolates, which is comparable to studies conducted in Romania 86% (Usein *et al*., 2001), Mongolia 89.9% (Munkhdelger *et al*., 2017), Iran 86.17% (Momtaz *et al*., 2013), 79.67% (Karimian *et al*., 2012) and China 87.4% (Wang *et al*., 2014). *Fim H* gene was the most common virulence gene in our study indicating that UPEC

uses *fim H* as a major virulence factor for colonization of urinary tract cells. Targeting *fim H* as vaccine candidate is important for prevention of UTI. The effectiveness of the FimC–FimH vaccine was shown to be due, in large part, to antibodies that block the function of *Fim H* in bladder colonization. Modifications of this vaccine are currently under development, with the aim of inducing greater immune stimulation (Flores-Mireles *et al*., 2015). In this study, we found no significant association between *fim H* gene and clinical symptoms of UTI (p>0.05).

Pyelonephritis associated pilli (*pap*) gene was found in 59 (29.5%) UPEC isolates, which is comparable to studies conducted in Romania 36% (Usein *et al*., 2001), Poland 38.7% (Kot *et al*., 2016), Iran 30.2% (Farshad *et al*., 2012), Brazil 32% (Santo *et al*., 2006) and Mexico 24.7% (Paniagua-Contreras *et al*., 2015), but lower than studies conducted in Egypt 54% (Alabsi *et al*., 2014), Czech Republic 67.6% (Koreň *et al*., 2013) and Iran 57% (Rahdar *et al*., 2015). In this study, there was significant association between presence of *pap* gene and urine urgency (*p-0.016*), which was commonly observed clinical symptom in most UTI patients. This indicates that UPEC uses *pap* genes as virulence factor to cause UTIs. P fimbria (*pap*) plays an important role in the pathogenesis of ascending UTIs and pyelonephritis in humans (Bien *et al*., 2012).

S and FIC fimbriae (*sfa* genes) were found in 50 (25%) UPEC isolates, which is similar to studies conducted in Tunisia 34% (Tarchouna *et al*., 2013), Romania 23% (Usein *et al*., 2001), Pakistan 27% (Bashir *et al*., 2012), Iran 21.5% (Mohajeri *et al*., 2014), 32% (Jalali *et al*., 2015) and Iraq 22.7% (Merza and Jubrael, 2015). However, it is lower than studies conducted in Denmark 46% (Ejrnæs *et al*., 2011), Czech Republic 53.8% (Koreň *et al*., 2013), Iran 81% (Rahdar *et al*., 2015) and South Korea 100% (Lee *et al*., 2015), and higher than studies conducted in Mongolia 8.8% (Munkhdelger *et al*., 2017) and China 8% (Wang *et al*., 2014). In our study, there was significant association between presence of *sfa* gene, and dysuria and urine urgency (*p*-0.019 and *p*-0.043 respectively). This indicates that *sfa* genes are important for pathogenesis of UPEC to cause UTI and responsible for clinical symptoms of UTIs. The S fimbriae may facilitate bacterial dissemination within host tissues and are often associated with *E. coli* strains that cause sepsis and ascending UTIs (Bien *et al*., 2012).

Afa adhesin (*afa* gene) was found in 24 (12%) UPEC isolates, which is similar to studies conducted in Romania 14% (Usein *et al*., 2001), Iraq 18% (Merza and Jubrael, 2015), Iran 12% (Rahdar *et al*., 2015), Brazil 11% (Santo *et al*., 2006) and Mexico 12.8% (Paniagua-Contreras *et*

al., 2015), but higher than a study conducted in Czech Republic 2.8% (Koreň *et al*., 2013). UPEC strains expressing Afa adhesins have a unique renal tissue tropism. Clinical and experimental findings suggest that *E. coli* strains with Afa adhesins have properties potentially favoring the establishment of chronic and/or recurrent infection (Bien *et al*. 2012; Le Bougue´nec *et al.,* 2001). In this study, we found no significant association between *afa* gene and clinical symptoms of UTI (p>0.05).

5.6.2 Toxins

In this study, 103 (51.5%) UPEC isolates had hemolysin (*hly*) gene, which is comparable to studies conducted in Denmark 43% (Ejrnæs *et al*., 2011), Czech Republic 41.4% (Koreň *et al*., 2013), Iran 50.4% (Momtaz *et al*., 2013; Karimian *et al*., 2012) and South Korea 62% (Lee *et al*., 2015), but higher than studies conducted in Zimbabwe 12.5% (Mbanga and Mudzana, 2014), Tunisia 19% (Tarchouna *et al*., 2013), Poland 18.5% (Kot *et al*., 2016), China 11.6% (Wang *et al*., 2014) and Mexico 15.4% (Paniagua-Contreras *et al*., 2015). In our study, hemolysin gene was significantly associated with suprapubic pain (*p*-0.002). This indicates that hemolysin may be responsible for clinical manifestation in UTI patients. Alpha-hemolysin encoded by *hlyA* is an extracellular cytolytic protein toxin that is produced by up to 50% of UPEC isolates. Alpha-hemolysin has been associated with clinical severity in UTI patients (Yun *et al*., 2014).

Alpha-hemolysin is associated with upper UTIs such as pyelonephritis. Approximately 50% of all cases of pyelonephritis, which leads to renal complications, are caused by *hlyA* (Bien *et al*., 2012). The UPEC pore-forming toxin *hlyA* has received attention as a potential vaccine target and was evaluated in a mouse model of pyelonephritis to assess protection against renal damage (Sivick and Mobley, 2010). Vaccination with *hlyA* reduced the incidence of renal scaring compared with controls; however, it did not protect against UPEC colonization of the kidneys (O'Hanley *et al*., 1991).

In our study, we found 58 (29%) UPEC isolates that had cytotoxic necrotizing factor 1 (*cnf1*) gene, which is similar with studies conducted in Czech Republic 37.9% (Koreň *et al*., 2013), Iran 22.9% (Farshad *et al*., 2012) and 36.5% (Tabasi *et al*., 2016) and Pakistan 20% (Bashir *et al*., 2012), but higher than studies conducted in Tunisia 3% (Tarchouna *et al*., 2013), Romania 13% (Usein *et al*., 2001) and Poland 12.1% (Kot *et al*., 2016). The cytotoxic necrotizing factor 1 (*cnf1*) is produced by one-third of all pyelonephritis strains and may also be involved in kidney

invasion. However, the detailed role of *cnf1* in invasion processes during pyelonephritis remains unclear and is a matter of debate. In vivo, *cnf1* may lead to bladder cell exfoliation and to enhanced bacterial access to underlying tissue (Bien *et al*., 2012). In this study, we found no significant association between *cnf1* gene and clinical symptoms of UTI ($p>0.05$).

5.6.3 Iron Acquisition System

In this study, we found 109 (54.5%) aerobactin (*aer*) gene, which is similar to studies conducted in Tunisia 52% (Tarchouna *et al*., 2013), Egypt 51% (Alabsi *et al*., 2014), Romania 54% (Usein *et al*., 2001), Poland 52.6% (Kot *et al*., 2016), Iran 57% (Jalali *et al*., 2015) and Turkey 51.6% (Uzun *et al*., 2015), but lower than studies conducted in South Korea 81% (Lee *et al*., 2015) and Iran 73.1% (Tabasi *et al*., 2016). There was significant association between *aer* gene and suprapubic pain, flank pain and fever (*p*-0.017, *p*-0.040, *p*-0.029 respectively). Thus, the high prevalence of aer gene in our study may be due to UPEC utilizes aerobactin virulence gene as a means of acquisition of iron and associated with clinical features of suprapubic pain, flank pain and fever which were observed in most UTI patients.

Iron acquisition systems have shown great promise as targets for vaccine development because uropathogens require a source of iron during colonization and persistence. Effective siderophore-based vaccines function in part by preventing cognate siderophore uptake, making this an exciting area of therapeutic development against UTIs (Flores-Mireles *et al*., 2015). The use of siderophore-protein conjugates was found to elicit immune responses targeted to bacterial siderophores and to successfully protect against UTI (Mike *et al*., 2016).

The virulence genes prevalence between our study and different studies abroad may vary according to the phylogenetic group, clinical conditions of host, geographical localization, sample size and methodological variation. Several virulence determinants contribute to the pathogenicity of uropathogenic *E. coli* in urinary tract infections. They are the product of different genes, which can be detected by PCR method (Kot *et al*., 2016; Tabasi *et al*., 2016; Lee *et al*., 2015; Momtaz *et al*., 2013; Usein *et al*., 2001). However, there is always the possibility of mutation at the level of the corresponding gene, leading to the absence of its detection. Therefore, a positive PCR shows the presence of the virulence gene, but a negative PCR does not point to the absence of the corresponding operon. However, this phenomenon remains scarce (Tarchouna *et al*., 2013).

The most common co-occurance (combination) of virulence genes observed in this study was 24 *fim H* and *aer* followed by 17 *fim H, aer* and *hly*. Other studies reported different combination of virulence gene combinations; where a study conducted by Rahdar *et al*. (2015), in Iran reported *fim*, *sfa* and *pap* as dominant combinations followed by *fim* and *sfa* combinations. Santo *et al*. (2006), in Brazil reported *hly* and *aer* as dominant combinations followed by *hly*, *aer* and *fim H* combinations. Tarchouna *et al*. (2013), in Tunisia reported *fim H* and *afa* as well as *fim H* and *pap* as dominant combinations. This variation in combinations of virulence genes by uropathogenic *E. coli* can be explained by UPEC uses different virulence factors for pathogenesis to cause infection.

The above data analysis indicates that the distribution of virulence genes that encodes virulence factors of uropathogenic *E. coli* in our research area is similar to other countries.

5.7 Relationship between Virulence Genes and Drug Resistance of Uropathogenic *E. coli* Isolates

In this study, we found significant association (x^2=6.00 *p*-0.014) between *fim H* and chloramphenicol drug resistance. The mechanism of association between virulence gene and drug resistance is unclear and needs to be investigated (Alabsi *et al*., 2014; Zhao *et al*., 2009). Similarly, significant association between *aer* genes, and gentamicin, ampicillin and nitrofurantoin drug resistance (x^2=4.84 *p*-0.028, x^2=5.64 *p*-0.018 and *p*-0.023 respectively) was observed. These findings are similar to a study conducted by Munkhdelger *et al.* (2017), resistance to gentamicin, ciprofloxacin, and cefuroxime were significantly associated with all the genes encoding aerobactin (*aer*), and UPEC strains positive for *papC* and *papGII* genes were more resistant to ampicillin and timethoprim-sulfamethoxazole. In another study by Asadi *et al*. (2014), stronger associations were found between *fim H* gene and resistance to ciprofloxacin, nalidixic acid, and cotrimoxazole, between *ibeA* gene and amikacin and cotrimoxazole, and between *afa* gene and gentamicin. A study conducted by Alabsi *et al.* (2014), revealed no significant association between the presence of any of the investigated genes and resistance of UPEC to the tested antibiotics despite the high prevalence of these genes, except for the *pap* gene in the case of gentamicin.

The above findings indicate that there are other virulence factors associated with UPEC resistance to commonly used antibiotics. These results are supported by other investigations indicating that other virulence factors could be the reason for UPEC resistance to different antibiotics (Alabsi *et al*., 2014; Idress *et al*., 2010; Zhao *et al*., 2009).

It appears that there is a correlation between drug resistance and reduced virulence. Although the mechanism of the correlation between drug resistance and reduced virulence remains unclear, it has been proposed that the loss of incompatible pathogenicity islands in high-resistance strains may contribute to this phenotype. However, this has not yet been proven (Wang *et al*., 2014).

The above data analysis indicates that there is relationship between some virulence genes and drug resistance of uropathogenic *E. coli*; it is very difficult to conclude on presence of strong association between virulence genes and drug resistance of uropathogenic *E. coli*.

5.8 Phylogroup of Uropathogenic *E. coli*

Extended phylogenetic analyses have shown that virulent extraintestinal *E. coli* strains belonged typically to phylogroup B2 and less often to phylogroup D (Lee *et al*., 2015; Iranpour *et al*., 2015; Basu *et al*., 2013; Ejrnaes, 2011). In our study, phylogroup B2 was the most common followed by phylogroup D. Our finding is in agreement with studies conducted in Denmark (Ejrnæs *et al*., 2011), Poland (Kot *et al*., 2016), China (Luo *et al*., 2012), South Korea (Lee *et al*., 2015), Pakistan (Bashir *et al*., 2012), Iran (Iranpour *et al*., 2015) and Mexico (Molina-López *et al*., 2011), where it was found that the majority of UPEC isolates belong to phylogenetic group B2. These findings are indicative of virulent strains of UPEC are common in our study area among UTI patients and measures needs to be taken to combat these virulence strains through designing and implementing appropriate prevention and control strategies.

In our study, the phylogenetic analysis indicated that majority of uropathogenic *E. coli* isolates were phylogroup B2 60 (30%) followed by phylogroup D 55 (27.5%), phylogroup B1 48 (24%) and phylogroup A 37 (18.5%), which is in agreement with a study conducted by Munkhdelger *et al* (2017), where B2 (33.8%) was dominant *E. coli* strains followed by D (28.4%) strains, A (19.6%) strains and B1 (18.2%) strains respectively. Similar study conducted by Kot *et al* (2016), showed that 38.1% *E. coli* strains belonged to phylogenetic group B2, 35.3% to group D, 18.5% to group A, and 8.1% to group B1.

Phylogenetic group A, associated with commensal strains, represented 18.5% of *E. coli* isolates, which is higher than studies conducted in South Korea 3.44% (Lee *et al*., 2015) and Iran 0.7% (Iranpour *et al*., 2015), but other studies reported phylogroup A was the dominant phylogroup (Romanus and Eze, 2011; Adib *et al*., 2014; Derakhshandeh *et al*., 2013; Grude *et al*., 2007) suggesting that the gastrointestinal tract may be the main reservoir of strains that may be able to colonize the urinary tract (Romanus and Eze, 2011). In some studies phylogroup D was the dominant *E. coli* strain (Gao *et al*., 2017; Themphachana *et al*., 2015; Wang *et al*., 2014). These different prevalences of the phylogenetic groups reported in different studies may be explained by the health status of the host, dietary and host genetic factors, environmental, social, and geographic conditions, or differences in sampling area and methods (Kazemnia *et al*., 2014; Derakhshandeh *et al*., 2013).

In our study, there was significant association between *E. coli* phylogroup B2 and three virulence genes namely *afa*, *pap*, and *sfa* (p-0.014, p-0.002, p-0.004 respectively). This finding is explained by the fact that *E. coli* strains belonging to phylogenetic group B2 contained a greater number of virulence factor genes than *E. coli* belonging to a non-B2 phylogenetic group as also reported by other studies on UPEC (Ferjani *et al*., 2014; Wang *et al*., 2014; Ejrnæs *et al*., 2011; Johnson *et al*., 2005). There was also significant association between *E. coli* phylogroup D and two virulence genes namely *fim H* and *pap* (p-0.043, p-0.019 respectively), which is in agreement with a study conducted in Thailand (Themphachana *et al*., 2015). These findings indicate that phylogroup B2 and D carries virulence genes that encode virulence factors which enables UPEC to colonize urinary tract and cause urinary tract infections.

In this study, we found no significant association between *E. coli* phylogroup and drug resistance except phylogroup D had significant association with ceftazidime drug resistance (p-0.025). It has been suggested that drug resistant uropathogenic *E. coli* isolates are concentrated in non-B2 phylogenetic groups (Adib *et al*., 2014; Ferjani *et al*., 2014). Piatti *et al*. (2008), has observed that phylogroup B2 being the frequent phylogenetic group, was significantly higher among susceptible strains than the resistant strains. Houdouin *et al*. (2006) proposed that the phylogroup A strains in the fecal flora could have developed from a greater exposure to antibiotics. Romanus and Eze (2011) reported that clonal phylogroup A accounts for 95% of urinary tract infection caused by *E. coli* strains that were resistant to ampicillin, sulphamethoxazole/trimethoprim,

tircacillin and doxycycline. The presence of majority of the strains in one clonal group could be possible as a consequence of increasing antimicrobial selection pressure.

The above data analysis indicates that the distribution of phylogroup of uropathogenic *E. coli* in our research area is similar to other countries.

Limitation of the tudy

This study did not investigate uropathogenic *E. coli* serogroup, Extended spectrum β-lactamase, all virulence genes and drug resistance genes due to lack of resources.

CHAPTER SIX

Conclusions and Recommendations

Conclusions

- High drug resistance to *E. coli* isolates were observed against trimethoprim-sulfamethoxazole, ampicillin, ceftriaxone, ceftazidime and cefotaxime.
- High susceptibility to amikacin, nitrofurantoin, ciprofloxacin, norfloxacin, chloramphenicol and gentamicin were observed.
- No drug resistance to meropenem and imipenem were observed.
- High multidrug resistance (MDR) uropathogenic *E. coli* was observed.
- Majority of uropathogenic *E. coli* isolates carried one or more plasmids.
- The virulence genes encoding components of adhesins, iron acquisition systems, and toxins were highly prevalent in uropathogenic *E. coli* isolates.
- There was significant association between some virulence genes and drug resistance of uropathogenic *E. coli* isolates.
- Majority of uropathogenic *E. coli* isolates were phylogroup B2 followed by D.

Recommendations

Based on the above findings, the following recommendations are made:-

- It is important to periodically monitor the antibiotic resistance patterns to choose empirical treatments for better management of urinary tract infection, which has direct impact on the outcome of the patient.
- The judicious use of antibiotics and the proper implementation of an antibiotic policy in hospitals will be helpful in limiting the emergence of drug resistance.
- Health education on antibiotic use has to be given to the society; this may help in combating emergence of drug resistance against uropathogenic *E. coli* among UTI patients.
- Targeting the most common uropathogenic *E. coli* phylogroup and virulence genes for vaccine against urinary tract infections should be considered and further research has to be conducted in this aspect.
- Further large scale and comprehensive studies (national wide) including at community and hospital level on drug resistance and virulence genes of uropathogenic *E. coli* should be conducted.

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Annex I: Information sheet

Name of the principal investigator: Belayneh Regasa

Name of the organization: Addis Ababa University

Tel: +251 911 721624

Introduction:

This information sheet is prepared by group of researchers whose main aim is to assess the phenotypic and molecular characteristics of uropathogenic *Escherichia coli* among urinary tract infection patients in selected health facilities of Addis Ababa, Ethiopia; namely Tikur Anbessa Specialized Hospital, Yekatit 12 Hospital and Zewditu memorial Hospital. Moreover, it will indicate a true idea about the existence of drug resistant *Escherichia coli* and virulence factors associated with such infections in the study area. The investigators include a Medical Microbiology postgraduate student and academic advisors from Addis Ababa University, college of Health Sciences, Department of Microbiology, Immunology and Parasitology as well as an advisor from Michigan State University, United States of America.

Purpose:

Urinary tract infection is our country's major public health problems. Studies showed that the virulence factors, drug resistance pattern and plasmid profile of *Escherichia coli* bacteria that cause urinary tract infection varies from place to place. Even though few studies have been conducted to determine antimicrobial sensitivity pattern of bacterial pathogens that causes urinary tract infection in different communities of the country; in the study areas, there is a lack of understanding of the level of drug resistance of *Escherichia coli*, which is the commonest (50-80%) cause of urinary tract infection as well as there is no information on plasmid profile and genes that encodes for virulence factors of uropathogenic *Escherichia coli* in Ethiopia. The findings of this study may contribute on designing strategies which help to improve treatment and management of urinary tract infection specifically and the health status of our community in general.

Procedure:

You/your child are kindly invited to take part in this project which is aimed to assess the phenotypic and molecular characteristics of uropathogenic *Escherichia coli* among urinary tract infection patients in selected health facilities of Addis Ababa, Ethiopia. If you/your child are willing to participate in this study, you/your child need to understand and you/your child will be asked to sign the consent form. For laboratory examinations, you/your child will provide urine sample, in which it will be collected following standard protocol. The laboratory examination results will be kept confidential using coding system whereby no one will have access to your/your child laboratory result. If microorganisms indicating urinary tract infections are isolated using urine culture, it will only be communicated to the physicians attending you/your childfor further intervention for urinary tract infection. You/your child will also be asked some personal socio-demographic questions and the information you give kept confidential.

Risk and discomfort:

There is minimum discomfort and pain when urine sample is collected and during interview

Benefits:

If you/your child participate in this research and microorganisms that cause (indicate) urinary tract infections are isolated from urine culture, the physician attending you will be communicated for further intervention for urinary tract infection. In addition your/your child participation will help us to determine genes that encode for virulence factors, the phenotypic drug resistance level and plasmid profile of uropathogenic *Escherichia coli* among urinary tract infection patients in health facilities of Addis Ababa, which is an input to design control strategies of such infection.

Incentives:

You/your child will not be provided any incentives to take part in this research.

Confidentiality:

The information we collect from this research project will be kept confidential. Information about you/your child that will be collected from the study will be stored in a file, which will not have your name on it, but a code number assigned on it. It will be kept under lock and key, and it will not be revealed to anyone except the principal investigator and the physicians following you/ your child.

Right to refuse or withdraw:

You/your child have full right to refuse from participating in this research if you do not wish to participate. If you/your child wish to refuse to take part or withdraw from this study, you/your child will not be penalized or your/your child health service will not be compromised.

Whom to contact:

If you have any questions contact any of the following individuals and you may ask at any time you want:

1.Belayneh Regasa (MSc)- Addis Ababa University, College of Health Sciences, Department of Microbiology, Immunology and Parasitology, Addis Ababa. Tel: +251 911 721624

2. Dr. Tamrat Abebe (PhD)- Addis Ababa University, College of Health Sciences, Department of Microbiology, Immunology and Parasitology, Addis Ababa. Tel: +251 911 447227

3. Dr. Yimtubezenash Woldeamanuel (MD, PhD)- Addis Ababa University, College of Health Sciences, Institutional Review Board, Addis Ababa. Tel: +251 911 225832

Amharic version of Information sheet

የመረጃ ቅጽ

የተመራማሪው ስም፡- በላይነህ ረጋሳ
የድርጅቱ ስም፡- አዲስ አበባ ዩኒቨርሲቲ
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መግቢያ

ይህ መረጃ መስጠሰቢያ ቅጽ የተዘጋጀው በተመራማሪው ቡድን ሲሆን አላማችንም በአዲስ አበባ በሚገኙ የጤና ተቋማት ማለትም በጥቁር አንበሳ ሆስፒታል፤ በየካቲት 12 ሆስፒታል እና በዘውዲቱ መታሰቢያ ሆስፒታል የሽንት ቧንቧ ኢንፌክሽን የሚያመጣ ባክቴሪያ(ተህዋስያን) ወይም ኢኮላይ ተብሎ የሚጠራውን ባክቴሪያ ባህሪ ለማጥናት፤ተህዋስያኑ (ባክቴሪያው) መድሀኒት መለማመዱን እና በሽታው እንዲከሰት ባክቴሪያው የሚጠቀምበት መሳሪያ (virulence factors) አይነት ለመለየት ነው፡፡ በተጨማሪም ይህ ምርምር በእርግጥም የሽንት ቧንቧ ኢንፌክሽን የሚያመጣው ኢኮላይ ተብሎ የሚጠራው ባክቴሪያ መድሀኒት መለማመዱን እና በሽታው እንዲከሰት ባክቴሪያው የሚጠቀምበት መሳሪያ (virulence factors) አይነት ምርምር በሚካሔድበት አካባቢ መኖሩን ያረጋግጣል፡፡ የዚህ ምርምር ተመራማሪዎች የአዲስ አበባ ዩኒቨርሲቲ የድህረ ምረቃ ተማሪ፤ የአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ መምህር እና አሜሪካን የሚገኘው የሚሺጋን ዩኒቨርሲቲ መምህር ናቸው፡፡

አላማ

የዚህ ምርምር አላማ በአዲስ አበባ በሚገኙ የጤና ተቋማት ማለትም በጥቁር አንበሳ ሆስፒታል፤ በየካቲት 12 ሆስፒታል እና በዘውዲቱ መታሰቢያ ሆስፒታል የሽንት ቧንቧ ኢንፌክሽን የሚያመጣ ባክቴሪያ(ተህዋስያን) ወይም ኢኮላይ ተብሎ የሚጠራውን ባክቴሪያ መድሀኒት መለማመዱን፤ ባክቴሪያው በሽታው እንዲከሰት የሚጠቀምበት መሳሪያ (virulence factors) አይነት ለመለየት እንዲሁም የባክቴሪያውን ባህሪ ለማጥናት ነው፡፡ የሽንት ቧንቧ ኢንፌክሽን ለአገራችን ዋና የጤና ችግር ነው፡፡ የተለያዩ ጥናቶች እንደሚያመለክቱት ዋነኛ የሽንት ቧንቧ ኢንፌክሽን አምጪ ተህዋሲ ወይም ኢኮላይ ተብሎ የሚጠራው ባክቴሪያ መድሃኒት የመለማመድ፤ በሽታው እንዲከሰት የሚጠቀምበት መሳሪያ (virulence factors) አይነት እና የባክቴሪያው ባህሪ ከአከባቢ አካባቢ ይለያያል፡፡ ምንም እንኳን የተወሰኑ ምርምሮች በዚህ በሽታ ዙሪያ ቢካሔዱም ቅሉ በሽታውን የሚያመጣው ዋነኛ ተህዋሲ ወይም ኢኮላይ ተብሎ የሚጠራው ባክቴሪያ የመድሃኒት ልምምድ መጠን ምርምሩ በሚካሔድበት አካባቢ የመረጃ እጥረት አለ፡፡ እንዲሁም ኢኮላይ ተብሎ የሚጠራው ባክቴሪያ በሽታው እንዲከሰት የሚጠቀምበት መሳሪያ (virulence factors) በኢትዮጵያ ውስጥ ምንም አይነት መረጃ የለም። ስለዚህ ይህንን ምርምር ለማከናወን እና ከላይ የተጠቀሰውን አላማ ለማሳካት ያቀድን ሲሆን የምርምሩ ውጤትም በሽታውን የመከላከያ ዘዴዎችን ለመቀየስ እና የህብረተሰቡን ጤና ለማሻሻል ይረዳል፡፡

አካሔድ (ፕሮሲጀር)

እርስዎ/የእርስዎ ልጅ በዚህ ምርምር ውስጥ እንዲሳተፉ ተጋብዘዋል፡፡ እርስዎ/የእርስዎ ልጅ በዚህ ምርምር የሚሳተፉ ከሆነ በፍቃደኝነት ላይ የተመሰረተ የስምምነት ቅጽ ላይ መስማማታችሁን ለማመልከት መፈረም ይኖርባችኃል፡፡ በተጨማሪም አንዳንድ አስፈላጊ የግል መረጃዎችን እንድትሰጡ ትጠየቃላችሁ፤ ሚስጥራዊነቱም የተጠበቀ ይሆናል፡፡ ለላቦራቶሪ ምርመራ የሚሆን የሽንት ናሙና እንድትሰጡ የምትጠየቁ ሲሆን ናሙናዉም የሚሰበሰበው በአግባቡ ፕሮቶኮል ተከትሎ ነው፡፡

የላቦራቶሪ ውጤት ምስጢራዊነትም የተጠበቀ ይሆናል፡፡ በተወሰደው የሽንት ናሙና ውስጥ የሽንት ቧንቧ ኢንፌክሽን አምጪ ተህዋስያን የተገኙ እንደሆነ እርስዎን/የእርስዎ ልጅ በሚያክም ሐኪም አማካኝነት አስፈላጊውን ህክምና ያገኛሉ፡፡

ጉዳት እና አለመመቸት

የሽንት ናሙና ሲሰጡ እንዲሁም የተወሰኑ የግል መረጃ ስትሰጡ (ኢንተርቪው ሲደረጉ) የተወሰነ አለመመቸት ሊኖር ይችላል፡፡

ጥቅም

እርስዎ/የእርስዎ ልጅ በዚህ ምርምር ውስጥ ብትሳተፉ እና በተወሰደው የሽንት ናሙና ውስጥ የሽንት ቧንቧ ኢንፌክሽን አምጪ ተህዋስያን የተገኙ እንደሆነ እርስዎን/የእርስዎን ልጅ ለሚያክመው ሐኪም ውጤቱን እንዲያውቀው ይደረጋል፡፡ በተጨማሪም የእርሶ ተሳትፎ በአዲስ አበባ በሚገኙ የጤና ተቋማት ማለትም በጥቁር አንበሳ ሆስፒታል፤ በየካቲት 12 ሆስፒታል እና በዘውዲቱ መታሰቢያ ሆስፒታል የሽንት ቧንቧ ኢንፌክሽን የሚያመጣ ባክቴሪያ(ተህዋስያን) ወይም ኢኮላይ ተብሎ የሚጠራውን ባክቴሪያ ባህሪ ለማጥናት፤ ተህዋስያኑ (ባክቴሪያው) መድሀኒት መለማመዱን እና በሽታው እንዲከሰት ባክቴሪያው የሚጠቀምበት መሳሪያ (virulence factors) አይነት ለመለየት እና ለማጥናት ሚያስችለን ሲሆን ይህም ደግሞ በሽታውን የመከላከያ ዘዴዎችን ለመቀየስ ያስችለናል፡፡

ማካካሻ ወይም ማበረታቻ

እርስዎ/የእርስዎ ልጅ በዚህ ምርምር ውስጥ ብትሳተፉ ምንም አይነት ማካካሻ ወይም ማበረታቻ አታገኙም፡፡

ምስጢራዊነት

በዚህ ምርምር አማካኝነት የሚሰበሰቡ መረጃዎች ሁሉ ሚስጢራዊነት የተጠበቀ ነው፡፡ ከእርስዎ/ከእርስዎ ልጅ የሚሰበሰቡ መረጃዎች ሁሉ በፋይል ውስጥ የሚቀመጡ ሲሆን፤ የእርስዎ/የእርስዎ ልጅ ስም ግን በፋይሉ ላይ የማይጠቀስ ሆኖ መለያ (ኮድ) ግን ይሰጠዋል፡፡ መረጃው በቁልፍ የሚቆለፍ ሲሆን ከተመራማሪው እና እርስዎን/የእርስዎን ልጅ ከሚከታተለው ሐኪም በስተቀር ማንም ሰው መረጃውን አያውቀውም(አያገኘውም)፡፡ መረጃውም የሚውለው ለዚህ ምርምር አገልግሎት ብቻ ነው፡፡

በምርምሩ የመሳተፍ ወይም ያለመሳተፍ ነፃ ፍቃድ

እርስዎ/የእርስዎ ልጅ/ በምርምሩ ላይ አለመሳተፍ ከፈለጉ/ገ ያለመሳተፍ ሙሉ መብት አልዎት/ው፡፡ እርስዎ/የእርስዎ ልጅ በዚህ ምርምር ባይሳተፉ/ፍ ምንም አይነት የጤና አገልግሎት መጓደል አያጋጥምዎትም/ውም፡፡

ማንን ማናገር ይችላሉ?

ማንኛውንም አይነት ጥያቄ ለመጠየቅ ከፈለጉ ከዚህ በታች የተዘረዘሩትን ሰዎች ማግኘት እና ማናገር ይችላሉ፤

1. በላይነህ ረጋሳ- የማይክሮባዮሎጂ፤ ኢሚኖሎጂና ፓራሲቶሎጂ ትምህርት ክፍል፤ ጤና ሳይንስ ኮሌጅ፤ አዲስ አበበ ዩኒቨርሲቲ፤ አዲስ አበባ፡፡ ስልክ ቁጥር፡ +251 911721624
2. ዶ/ር ታምራት አበበ- የማይክሮባዮሎጂ፤ ኢሚኖሎጂና ፓራሲቶሎጂ ትምህርት ክፍል፤ ጤና ሳይንስ ኮሌጅ፤ አዲስ አበባ ዩኒቨርሲቲ፤ አዲስ አበባ፡፡ ስልክ ቁጥር፡ +251 911 447227
3. ዶ/ር ይምጡበዝናሽ ወልደአማኑኤል- አዲስ አበባ ዩኒቨርሲቲ፤ ጤና ሳይንስ ኮሌጅ፤ ኢንስቲቱሽናል ረቪው ቦርድ (አይ.አር.ቢ)፤ አዲስ አበባ፡፡ ስልክ ቁጥር፡ +251 911 225832

Annex II: Consent Form

English version consent form (Age ≥18)

Code No ----------

I have been informed about a study that plans to determine genes that encode for virulence factors, phenotypic drug resistance level and plasmid analysis of uropathogenic *Escherichia coli* among urinary tract infection patients in Tikur Anbessa Specialized Hospital, Yekatit 12 Hospital and Zewditu memorial Hospital entitled "Phenotypic and molecular characterization of uropathogenic *Escherichia coli* from urinary tract infection patients in selected health facilities of Addis Ababa, Ethiopia" which will help in understanding virulence factors, level of drug resistance and plasmid profile of uropathogenic *Escherichia coli* among urinary tract infection patients; and investigating the extent to which drug resistant uropathogenic *Escherichia coli* is a public health problem in the area. This study could contribute to in recommending the use of appropriate antibiotic and control measures that can minimize the transmission of the disease in the study area.

For the study I have been requested to give urine sample. I have been informed that following Lab investigation if microorganisms from urine that indicate urinary tract infections are isolated, it will only be communicated to the physicians attending me for further intervention for urinary tract infection. Based on this, I have agreed to participate in the study. The investigators also informed me that if I want all the laboratory results would be kept confidential. I have been also told that no incentive will be given by investigator. I have been given enough time to think over before I signed this informed consent. It is therefore, with full understanding of the situation that I gave my informed consent and cooperates at my will in the course of the conduct of the study.

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

Name of witness (for illiterate participant)----------Signature ----------Date ------

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

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

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

Thank you for your participation!

Consent Form (Amharic version)

የስምምነት ቅጽ (ከ 18 አመት በላይ ለሆኑ)

መለያ ቁጥር----------

እኔ ተመራማሪው እንደገለጸልኝ በሽንት ቧንቧ ኢንፌክሽን አምጪ ተህዋሲ (ባክቴሪያ) ወይም ኢኮላይ ተብሎ በሚጠራው ባክቴሪያ ላይ በሚካሄደው ጥናት ዉስጥ እንድሳተፍ ፍቃደኛ መሆኔንና አለመሆኔን ተጠይቄአለዉ፡፡ የጥናቱ ዋና አላማዉ በአዲሰ አበባ በሚገኙ የጤና ተቋማት ማለትም በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል፤ በየካቲት 12 ሆስፒታል እና በዘውዲቱ መታሰቢያ ሆስፒታል የሽንት ቧንቧ ኢንፌክሽን የሚያመጣ ባክቴሪያ(ተህዋስያን) ወይም ኢኮላይ ተብሎ የሚጠራውን ባክቴሪያ ባህሪ ለማጥናት፤ ተህዋስያኑ (ባክቴሪያው) መድሀኒት መለማመዱን እና በሽታው እንዲከሰት ባክቴሪያው የሚጠቀምበት መሳሪያ (virulence factors) አይነት ለመለየት እና ለማጥናት ነው፡፡ በርግጥም ይህ ባክቴሪያ መድሀኒት የተለማመደ ሆኖ ከተገኘ እና ባክቴሪያው የሽንት ቧንቧ ኢንፌክሽን ለማምጣት የሚጠቀምበት መሳሪያ (virulence factors) አይነት ከተለየይህ ምርምር በኢኮላይ ባክቴሪያ አማካኝነት የሚመጣ የሽንት ቧንቧ ኢንፌክሽን ለመከላከል የሚረዱ መፍትሔዎችን ለመቀየስ የሚረዳ መሆኑን በቅድሚያ ተነግሮኛል፡፡

ለዚህ ጥናት ወይም ምርምር ያገለግል ዘንድ በፈቃደኝነት ላይ የተመሰረተ የሽንት ናሙና እኔ እንድሰጥ እና የሽንት ናሙናው እንዲመረመር ተጠይቄአለሁ፡፡ በተወሰደው የሽንት ናሙና ውስጥ የሽንት ቧንቧ ኢንፌክሽን አምጪ ተህዋስያን የተገኙ እንደሆነ እኔን ለሚያክመው ሐኪም ውጤቱን እንዲያውቀው እንደሚደረግ ተነግሮኛል፡፡ በዚህም መሰረት እኔ ለምርምሩ የሚያገለግል የሽንት ናሙና ለመስጠት ተስማምቼአለሁ፡፡ እኔ ከፈለኩኝ ማንኛውም ውጤት በሚስጥር እንደሚያዝ ተነግሮኛል፡፡ በዚህ ምርምር ወይም ጥናት እኔ ምንም አይነት ማበረታቻ ወይም የገንዘብ ጥቅም እንደማላገኝ ተነግሮኛል፡፡ ይህንን የስምምነት ቅጽ ከመፈረሜ በፊት እንዳስብበት በቂ ጊዜ ተሰጥቶኝ የተስማማሁ መሆኔን በፊርማዬ ለማረጋገጥ እወዳለሁ፡፡

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

የምስክር ስም (ላልተማረ የምርምሩ ተሳታፊ)----------ፊርማ----------ቀን----------

የመረጃ ሰብሳቢው ስም----------ፊርማ----------ቀን----------

የተመራማሪው ስም----------ፊርማ----------ቀን----------

መረጃው የሚሰበሰብበት ቦታ (የጤና ተቋሙ) ስም ----------

በጥናትና ምርምሩ በመሳተፍዎ በጣም እናመሰግንዎታለን!!

Assent for 12 -17 years old study volunteers

I _____being a person aged _____years hereby consent to _____ in the intended research as explained and understood by me.

I have understood the implications of risks and immediate benefits of the investigation (research) and agreed to give the urine specimen.

I understand that I have the right to withdraw from the research at any time, for any reason without penalty or harm. In case of withdrawal, I understand that the researchers will continue to take care of me like any other patient.

All the above conditions have been explained to me in the language, which I can understand.

Name of the patient: _____

Patient's signature: _____

Name of Data collector: _____

Data collector signature: _____

Date: _____

Witness: _____ Date: _____

Consent form for age less than 18 years

I _____being a person aged ≥ 18yrs and being the parent/Lawful guardian of_____ hereby consent to _____ in the intended research as explained and understood by me.

I have understood the implications of risks and immediate benefits of the investigation (research) to _____. I have agreed_____to give the urine specimen.

I understand that I have the right to withdraw _____ from the research at any time, for any reason without penalty or harm. In case of withdrawal, I understand that the researchers will continue to take care of _____ like any other patient.

All the above conditions have been explained to me in the language, which I can understand.

Guardian's full name: _____

Guardian's signature: _____

Date: _____

Child's full name: _____

Person obtaining consent: _____

Signature of person obtaining consent: _____

Witness: _____ Date: _____

<u>የስምምነት ማረጋጋጫ ፊርማ /ለአቅመ ዓዳም ላልደረሱ/</u>

እኔ ---------- እድሜዬ ከ18 እና ከ18 በላይ የሆነ የወጣት ---------- ---------- ወላጅህጋዊ አሳዳጊ የሆንኩ ወጣት ---------- በሚገባ እና በዝርዝር በተገለፀልኝ ጥናት ተሳታፊ ይሆን ዘንድ ስምምነቴን ለ---------- እሰጣለሁ፡፡ በጥናቱ ውስጥ የተካተቱ ዝርዝር አሰራሮች፣ አደጋዎች፣ ምርመራዎችና እገዛዎችን ሁሉ በሚገባ የተረዳሁ ስለሆነ፣ በጥናቱ ተሳታፊ እንዲሆን ፈቅጃለሁ፡፡ በተጨማሪ፣ ወጣት----- ---------- በማንኛውም ደረጃ ከዚሁ ከተጠቀሰው ጥናት እንዲወጣ/እንድትወጣ የማድረግ ሙሉ መብት እንደሚኖረኝ ተገንዝቤአለሁ፡፡ ይህንን ለማድረግ ስወስን በወጣቱ(ቷ) ላይምንም ዓይነት ቅጣት/ጉዳት እንደማይደርስ ተገቢ የሆኑ ህክምናዎች ሁሉ ለወጣቱ(ቷ) እንደሚደረጉ በማመን ነው፡፡ እነዚህ የስምምነት መሰረቶች ሁሉ በሚገባ በምረዳው ቋንቋ የተገለጸልኝ መሆኑን በፊርማዬ አረጋግጣለሁ፡፡

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

የወጣቱ(ቷ) ሙሉ ስም ----------
የተመራማሪው ሙሉ ስም ----------
የምስክር ሙሉ ስም ---------- ፊርማ----------
ቀን ----------

<u>ለአቅመ ዓዳም ላልደረሱ እድሜያቸው ከ12-17 የሆኑ ወጣቶች በጥናቱ ተሳታፊ ለመሆን የሚያደርጉት አወንታዊ ማረጋጋጫ ፊርማ</u>

እኔ ---------- እድሜዬ ----------የሆነ የወጣት በሚገባ እና በዝርዝር በተገለፀልኝ ጥናት ተሳታፊ እሆን ዘንድ ስምምነቴን ለ---------- እሰጣለሁ፡፡ በጥናቱ ውስጥ የተካተቱ ዝርዝር አሰራሮች፣ አደጋዎች፣ ምርመራዎችና እገዛዎችን ሁሉ በሚገባ የተረዳሁ ስለሆነ፣ በጥናቱ ተሳታፊ እንድሆን ፈቅጃለሁ፡፡ በተጨማሪ፣ በማንኛውም ደረጃ ከዚሁ ከተጠቀሰው ጥናት እንድወጣ ሙሉ መብት እንደሚኖረኝ ተገንዝቤአለሁ፡፡ ይህንን ለማድረግ ስወስን ምንም ዓይነት ቅጣት/ጉዳት እንደማይደርስ ተገቢ የሆኑ ህክምናዎች ሁሉ ለወጣቱ(ቷ) እንደሚደረጉልኝ በማመን ነው፡፡ እነዚህ የስምምነት መሰረቶች ሁሉ በሚገባ በምረዳው ቋንቋ የተገለጸልኝ መሆኑን በፊርማዬ አረጋግጣለሁ፡፡

የወጣቱ(ቷ) ሙሉ ስም ----------
የተመራማሪው ሙሉ ስም----------
የምስክር ሙሉ ስም ---------- ፊርማ----------
ቀን ----------

Annex III: Questionnaire

Addis Ababa University

College of Health Sciences

School of Medicine

Questionnaire to be administered to UTI patients for research entitled **''Phenotypic and molecular characterization of uropathogenic *Escherichia coli* among urinary tract infection patients in selected health facilities of Addis Ababa, Ethiopia''**

01. Name of the Health facility---------- OPD----------Ward----------

02. Questionnaire identification number----------

Introduction

We are interviewing people here in Addis Ababa in different health facilities for a study being conducted by team from Addis Ababa University. The purpose of this study is to assess the phenotypic and molecular characteristics of uropathogenic *Escherichia coli* among urinary tract infection patients in Tikur Anbessa Specialized Hospital, Yekatit 12 Hospital and Zewditu memorial Hospital; which is expected to be helpful for proper treatment and management of urinary tract infections. I am going to ask you some personal questions. Your answers are completely confidential. Your name will not be written on this form, and will never be used in connection with any of the information you tell me. You do not have to answer any question that you do not want to answer, and you may end this interview at any time you want to. However, your honest answers to these questions will help to fulfill the study and to provide information to design appropriate intervention against the disease. We would greatly appreciate your help in responding to this survey. It will take only 5 minutes to fill this questionnaire.

Was the information/ objective clear?

1. Yes 2. No

Would you be willing to participate?

1. Yes 2. No

Thank you.

If the study subject agrees to participate in the study, start to fill the questionnaire.

1. Socio-demographic characteristics (encircle on the answer or fill the blank space)

S. No	Questions	Coding classifications
101	Sex of the patient	1-----Male 2-----Female
102	How old are you?	---------Years
103	What is your completed educational status?	1-----Illiterate 2-----Read and write 3-----Grade 1 to 6 4-----Grade 7 to 8 5-----Grade 9 to 12 6-----Above grade 12 99----No response
104	What is your marital status?	1-----Single 100----Not applicable 2-----Married 3-----Divorced 99----No response
105	What is your monthly personal income?	----------Birr per month 1-----No income 99----No response

2. Clinical data assessment form (Tick on the answer/ Yes or No)

S. No	Clinical data	Answers (response)	
		1. Yes	2. No
106	Dysuria		
107	Urine urgency		
108	Urgency incontinence		
109	Suprapubic pain		
110	Flank pain		
111	Fever ($\geq 38^{o}C$)		
112	Chills		

Amharic version of questionnaire

አዲስ አበባ ዩኒቨርሲቲ

የጤና ሳይንስ ኮሌጅ

የህክምና ትምህርት ቤት

በሽንት ቧንቧ ኢንፌክሽን አምጪ ተህዋሲ (ባክቴሪያ) ወይም ኢኮላይ ተብሎ በሚጠራው ባክቴሪያ ላይ በሚካሄደው ምርምር አዲስ አበባ በሚገኙ በተመረጡ (በተወሰኑ) የጤና ተቋማት በሽንት ቧንቧ ኢንፌክሽን በተያዙ (በታመሙ) ሰዎች የሚሞላ መጠይቅ፡፡

01. የጤና ተቋሙ ስም----------ተመላላሽ ታካሚ----------ተኝቶ ታካሚ----------

02. የመጠይቁ መለያ ቁጥር----------

መግቢያ

የጥናቱ ዋና አላማ በአዲሰ አበባ በሚገኙ የጤና ተቋማት ማለትም በጥቁር አንበሳ ሆስፒታል፤ በየካቲት 12 ሆስፒታል እና በዘውዲቱ መታሰቢያ ሆስፒታል የሽንት ቧንቧ ኢንፌክሽን የሚያመጣ ባክቴሪያ(ተህዋስያን) ወይም ኢኮላይ ተብሎ የሚጠራውን ባክቴሪያ ባህሪ ለማጥናት፤ ተህዋስያኑ (ባክቴሪያው) መድሀኒት መለማመዱን እና በሽታው እንዲከሰት ባክቴሪያው የሚጠቀምበት መሳሪያ (virulence factors) አይነት ለመለየት እና ለማጥናት ሲሆን የሽንት ቧንቧ ኢንፌክሽን ለማከም እና ለመከላከል አስፈላጊውን ጤናነክ እርምጃ እንዲወሰድ ለማመቻቸት ነው፡፡ ስለራስዎ አንዳንድ ጥያቄዎችን እጠይቅዎታለሁ፡፡ መልስዎ ሚስጥራዊነቱ ከማንኛውም አካል የተጠበቀ ነው፡፡ ስምዎም በዚህ ፎርም ላይ አይሞላም ወይም አይፃፍም፡፡ ከሌላ ከሚነግሩኝ መረጃ ጋርም አይያያዝም፡፡ መመለስ የማይፈልጉትን ጥያቄ የግድ መመለስ የለብዎትምና መተው (እምቢማለት) ይችላሉ፡፡ በመሆኑም ይህንን ቃለመጠይቅ በፈለጉት ጊዜ ሊያቆሙ ይችላሉ፡፡ ነገር ግን ለጥያቄው እርስዎ የሚሰጡን ቅንና ትክክለኛ መልስ ጥናቱ የተሟላ እንዲሆንና አስፈላጊውን ጤናነክ እርምጃዎች ለመውሰድ በጣም ጠቃሚና አስፈላጊ ነው፡፡ ለጥያቄው ለሚሰጡን መልስ አድናቆታችን (ምስጋናችን) በጣም ከፍ ያለ ነው፡፡ መጠይቁ 5 ደቂቃ ያህል ብቻ ይወስዳል፡፡

ግልጽ ነው ? 1. አዎ 2. አይደለም

ስለዚህ በመጠይቁ ለመሳተፍ ፍቃደኛ ነዎት ? 1. አዎ 2. አይደለሁም

እናመሰግንዎታለን !

በምርምሩ ተሳታፊው ሰው በመጠይቁ ለመሳተፍ ፍቃደኛ ከሆነ መረጃ መሰብሰቡን ይቀጥሉ፡-

1. አጠቃላይ የተጠያቂው መረጃ (መልሱ ላይ ያክብቡ ወይም መልሱን በጽሁፍ ይስጡ)

ተራ ቁጥር	መጠይቅ	የመለያ ኮድ (መልስ)
101	የተጠያቂው ጾታ	1----ወንድ 2----ሴት
102	ዕድሜዎ ስንት ነው	----------ዓመት
103	ያጠናቀቁት ከፍተኛ የትምህርት ደረጃ ስንት ነው	1---አልተማርኩም 2---ማንበብና መጻፍ 3---ከ1-6ኛ ክፍል 4---ከ7-8ኛ ክፍል 5----ከ9-12ኛ ክፍል 6----ከ12ኛ ክፍል በላይ 99---መልስ የለም
104	የትዳር ሁኔታ	1---ያላገባ 100---አይመለከተውም 2----ያገባ 3----አግብቶ የፈታ 99---መልስ የለም
105	የግል የወር ገቢዎ በብር ስንት ይሆናል	----------የኢትዮጵያ ብር 1----የግል ገቢ የለኝም 99----መልስ የለም

2. የክሊኒካል መረጃ መሰብሰቢያ ቅጽ (መልሱ ላይ ምልክት ወይም ራይት ያድርጉ)

ተራ ቁጥር	የክሊኒካል መረጃ	መልስ	
		1. አዎ	2. የለም (አይደለም)
106	ሽንት ሲሸኑ የህመም (የማቃጠል) ስሜት መኖር ወይም ሽንት ለመሽናት መቸገር		
107	ቶሎቶሎ መሽናት		
108	የሽንት መቋጠር መቸገር		
109	የታችኛው የሆድ ክፍል ህመም		
110	የጎን ህመም		
111	ትኩሳት (≥38 ዲግሪ ሰሊሺየስ)		
112	ማንቀጥቀጥ		

Annex IV: Laboratory data form

Result of culture and antimicrobial susceptibility testing of uropathogenic *Escherichia coli*

S. No	Patient code	Isolate	Antimicrobial susceptibility testing; Resistant [R], Intermediate [I] or Sensitive [S]														
			Nitrofurantoin (F)	Norfloxacin (NOR)	Amikacin (AK)	Amoxicillin-clavulanate (AMC)	Nalidixic acid (NA)	Ceftriaxone (CRO)	Tetracycline (TE)	Ciprofloxacin (CIP)	Chloramphenicol (C)	Cefotaxime (CTX)	Gentamicin (CN)	Ceftazidime (CAZ)	Ampicillin (AMP)	Meropenem (MEM)	Imipenem (IM)	Trimethoprim-sulfamethoxazole (SXT)
1	001																	
2	002																	

Annex V: Material Transfer Agreement (MTA)

Material Transfer Agreement

This Material Transfer Agreement (MTA) has been prepared for use by Addis Ababa University, Department of Microbiology, Immunology and Parasitology, Ethiopia and Michigan State University, USA in all transfer of research material (samples, derivatives, and specimens) related to the protocol ''Phenotypic and molecular characterization of Uropathogenic *Escherichia coli* from Urinary tract infection patients in selected Health facilities of Addis Ababa, Ethiopia''

Provider: Belayneh Regasa, Addis Ababa University, Department of Microbiology Immunology and Parasitology, Ethiopia

Recipient: Lixin Zhang, Epidemiology and Biostatistics, Microbiology and Molecular Genetics, Michigan State University, USA

1. Provider agrees to transfer to recipient's designated (provider) the following research materials /specimen. De-identified *Escherichia coli* bacterial isolates

   The research material will only be used for research purposes as described in the protocol by recipient's investigator in designated laboratory for the research project described below, under suitable containment conditions. This research material will not be used for commercial purposes such as screening, production or sale for which a commercialization license may be required. Recipient agrees to comply with all National and International guidelines rules and regulations applicable to the Research Project and the handling of the Research Material.

   a) Are the research materials of human origin?

   Yes [√] No [ ]

   b) If yes, are they collected according to the details in the protocol and in adherence to National Research Ethics Review Committee (NRERC) and Addis Ababa University College of Health Sciences (AAU-CHS) Ethics Review Committee recommendations and their approval.

   Yes [√] No [ ]

2. This research material and its derivatives will be used by recipient's investigator solely in connection with the following research project ("Research Project") described with specificity as follows ''Phenotypic and molecular characterization of Uropathogenic *Escherichia coli* from Urinary tract infection patients in selected Health facilities of Addis Ababa, Ethiopia''

3. In all presentations or written publications concerning the research project, recipient will seek agreement of provider and acknowledge provider's contribution of this research material unless requested otherwise.

4. This research material represents a significant contribution on the part of provider and is considered proprietary to provider. Recipient therefore agrees to retain control over this research material and further agrees not to transfer the research material to other people not under her/his direct supervision without advance written approval of provider. The research material will be disposed of as agreed upon per protocol at the end of completion of the project on 2018.

5. The provider does not take any responsibility for loss, damage, wastage or spoilage of the research material during or after shipment to the address provided by the recipient under conditions agreed to in the protocol on shipment of the samples. This research material is provided as a service to the research community. IT IS BEING SUPPLIED TO RECIPIENT WITH NO WARRANTIES, EXPRESS OR IMPLIED, INCLUDING ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Provider makes no representations that the use of the research material will not infringe any patent or proprietary right of third parties.

6. The recipient shall notify the provider in witting of any intention, improvement, modification discovery or development to the material or the information made by recipient or parties, collaborating with recipient, here in after referred to as "invention". Nothing in this agreement shall, however, be construed as conveying to the provider any rights under any patents or other intellectual property to such invention, other than as explicitly provided herein. At its option the provider shall be entitled to receive sample of any materials derived from the Materials for its own research and evaluation purposes only.

7. The under- signed provider and recipient expressly certify and affirm that the contents of any statements made herein are truthful and accurate.

8. Any additional terms (use an attached page if necessary):

9. The provider maintains, ownership right of the research material and its derivatives unless stated otherwise.

The provider will retain a copy (aliquot) of every sample sent abroad as much as possible for local research needs.

Material Transfer Agreement

Signature page

For Recipient:

Read and Acknowledged by:

Recipient's Investigator

Lixin Zhang, Ph.D

Assistant Professor

Signature

Date 6/1/16

Mailing Address for Material:

East Lansing, MI 48824

U.S.A.
Tel: +1-517-884-2076

Fax: +1-517-432-1130

Duly Authorized

Richard W. Chylla, Ph.D., CLP
Executive Director, MSU Technologies

Signature/ Stamp

Richard W. Chylla
Executive Director
MSU Technologies

Date 5/31/16

Mailing Address for Notices:

B44 Food Safety and Toxicology325 E. Grand River, Suite 350

East Lansing, MI 48823

U.S.A.
Tel: +1-517-355-2186
Fax: +1-517-432-3880

For Provider:

Provider's Investigator

Belayneh Regasa

Signature

Date: 13/05/2016

Mailing Address:

Addis Ababa University, Department of Microbiology, Immunology and Parasitology, College of Health Sciences, School of Medicine, Tikur Anbessa Specialized Hospital, Second floor room number 76,
P.o.Box 9086, Addis Ababa, Ethiopia
Tel: +251911 721624
Fax: +251- 1- 551 30 99

Duly Authorized

Dr. Tamrat Abebe

Signature/ Stamp

DEPARTMENT OF MICROBIOLOGY IMMUNOLOGY, & PARASITOLOGY (DMIP)

Date: 13/05/2016

Mailing Address for Notices:

Addis Ababa University, Department of Microbiology, Immunology and Parasitology, Tikur Anbessa Specialized Hospital, Second floor room number 76,
P.o.Box 9086, Addis Ababa, Ethiopia
Tel: +251911447227

Fax: +251- 1- 551 30 99

Annex VI: Antimicrobial Susceptibility Testing

Method: using Kirby-Bauer disc diffusion method (Cheesbrough, 2006)

Requirements:

Mueller Hinton agar, Antimicrobial discs, Turbidity standard equivalent to McFarland 0.5 *(*Add 0.6 ml of the Barium chloride solution to 99.4 ml of the sulphuric acid solution, and mix), and Control strains *(*are used to test the performance ofthe method. *Escherichia coli* ATCC 25922 was used as control strains)

Procedure:

1. Using a sterile wire loop, touch 3-5 well-isolated colonies of similar appearance to the test organism and emulsify in 3-4 ml of sterile physiological saline or nutrient broth.
2. In a good light match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use). When comparing turbidities, it is easier to view against a printed card or sheet of paper.
3. Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60^{o} to ensure even distribution.
4. With the petri dish lid in place, allow 3-5 minutes (*no longer than 15 minutes*) for the surface of the agar to dry.
5. Using sterile forceps, needle mounted in a holder, or a multidisc dispenser, place the appropriate antimicrobial discs, evenly distributed on the inoculated plate.

   Note*:* The discs should be about 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. No more than 6 discs should be applied (90 mm dish). Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.
6. Within 30 minutes of applying the discs, invert the plate and incubate aerobically at 35^{o}C for 16-18 hours.
7. After overnight incubation, examine the control and test plates to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts.

Interpretation of zone sizes:

Based on CLSI (CLSI, 2017) criteria using the interpretative chart, interpret the zones sizes of each antimicrobial, reporting the organism as 'Resistant', 'Intermediate/Moderately susceptible', 'Susceptible'.

Annex VII: Collection and transportation of urine specimen

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

Midstream urine (MSU) for microbiological examination is collected as follows (Cheesbrough, 2006):

1. Give the patient a sterile, dry, wide-necked, leakproof container and request a 10-20 ml specimen.

Important: Explain to the patient the need to collect the urine with as little contamination as possible, i.e. a 'clean-catch' specimen.

Female patients: Wash the hands. Cleanse the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect the urine with the labia held apart.

Male patients: Wash the hands before collecting a specimen (middle of the urine flow).

2. Label the container with the date, the name and number of the patient, and the time of collection. As soon as possible, deliver the specimen with a request form to the laboratory.

Annex VIII: DNA genomic lysate procedure

Procedure

- Turn on the UV light in the Biosafety cabinet for at least 10 minutes, then open the barrier, turn on the blower and allow the fan to run for 15 minutes before placing samples in. This ensures proper air flow. Sterilize surface with 70% ethanol.
- Remove isolates of interest from freezer or refrigerator and place in sterilized Biosafety Cabinet.
- Begin by turning on the incubation block and allowing it to warm to 60°C.
- Solution Preparation
    - Prepare 50mL 1X PBS, pH 7.4 solution by adding 5mL 10X PBS, pH 7.4 into 45mL dH_2O
    - Prepare 50mL 1M NaOH by adding 1.998g NaOH in 50mL dH_2O. MAKE FRESH EVERYTIME.
    - Prepare 50mL 0.05M NaOH by adding 2.5mL of 1M NaOH to 48mL dH_2O
    - Prepare 50mL 1M Tris-Cl by adding 7.882g Tris-HCl to 50mL dH_2O
- Label 1.5mL centrifuge tubes respective to the designated sample ID.
- Inoculate, using aseptic technique, a portion of the colonies and place in respective 1.5mL centrifuge tube.
- Add 200µL 1X PBS, pH 7.4 into each tube and mix vigorously by vortex until cells are suspended (*You can add this BEFORE you place your colony in. It may prevent the biofilm from sticking to the tube).*
- Add 800µL 0.05M NaOH and vortex.
- Place samples in 60°C water bath or incubator for 45 minutes (during this time it is suggested you begin labeling the new tubes for the supernatant)
- After 45 minutes add 240µL 1M Tris-Cl to neutralize the NaOH
- Centrifuge at 13,000rpm for 3 minutes at room temperature.
- Transfer 1000µL supernatant into a newly labeled 1.5mL centrifuge tube
    - If some of the sample has not formed a pellet continue to centrifuge for another 3 minutes.
- Place DNA lysate at -20^{o}C until use.

Annex IX: Agarose Gel Electrophoresis procedure

Objective

This will outline the procedure for both preparing the agarose (0.7%-1.5%) for gel electrophoresis as well as preparing the apparatus and UV light camera for imaging. Agarose gel electrophoresis is a process where current is passed through a matrix gel medium (agarose) surrounded by a conductive buffer (TAE) to separate DNA and nucleic acids by size or charge. TAE buffer for electrophoresis is commonly at 1X concentration made from either 10X or 50X concentrations, and is composed of Tris-acetate (pH 8.0) and EDTA which sequesters divalent cations. TBE buffer can be used, but TAE buffer is more efficient when double stranded DNA is involved. The agarose used in this lab is either 1% or 1.5%, but more often the 1.5% is used. Making the agarose is a rather simple procedure, but does require some time and attention if any spills are to be avoided. When casting the gel you will also use GelRed stain as an intercalating nucleic acid stain at 10,000X concentration. This is just as efficient as ethidium bromide without the health risks.

Procedure

- For a 1% agarose gel weigh 5g of agarose in a medium weigh boat. For a 1.5% agarose gel weigh 7.5g of agarose in a medium weigh boat
    - The final volume made will always be 500mL
- Pour the agarose into the 600mL beaker or 1L Erlenmeyer flask
- Add 500mL TAE buffer to the beaker/flask
- Using the microwave oven:
    - Place the beaker into the microwave and turn the timer to 8 minutes.
    - It is important to watch the beaker constantly because it will begin to boil over when the boiling point is reached. When it begins to boil stop the microwave and allow the liquid to settle. If it looks clear, then remove and pour into marked container. If not close the microwave and continue the cook until clear.
    - It is very important not to let the agarose boil over. It could ruin the microwave as well as losing a large volume of liquid agarose.

- Setting up the electrophoresis apparatus
    - Using the large 20 lane electrophoresis apparatus make sure it has been cleaned before use.

- Take the gel cast and slightly water the rubber seals on the top and bottom before sliding it into place.
- Slowly and carefully push the gel cast so the walls of the cast are perpendicular to the walls of the running apparatus and a water-tight seal is created.
- Place the 20-lane combs in their holds with either the 1mm or 10mm lane side down. It doesn't matter which you use, but the 1mm will fill more easily than the 10mm.
- If you haven't already, place the 1.2% agarose (or whichever percent you will be using) in the microwave and bring to a boil.
    - Ensure that all the agarose has liquefied before pouring
- Once the agarose has liquefied remove it from the microwave using the rubber insulated grips (red) sitting on top of the microwave. The bottle will be extremely hot!
- Pour 100mL of agarose (carefully) into the 125mL Erlenmeyer flask
- Add 10µL 10,000X GelRed stain to the agarose in the flask and mix until it disappears
- IMPORTANT: *let the agarose sit in the flask for 10-15 minutes and cool. If poured into the gel cast while hot it can melt the rubber seals, the combs or damage the apparatus itself. Wait until it has cooled to the point where you can keep your bare hand on it.*
- Once the agarose has cooled pour it into the cast and wait for it to solidify. This can take up to 30 minutes. Prepare your samples in the meantime (next section).
- Once the agarose has solidified very carefully remove the lane combs and avoid cracking or breaking the gel. Lift the combs straight up.
- Remove the cast very carefully by picking it straight up and out of the apparatus. Once out position it so the bottom (part that does not have any lanes) is facing you and the electrodes are on the RIGHT.
    - Since this apparatus is set up so the RED (cathode) will always be on the bottom.
    - ALWAYS RUN TO RED
- Once the cast in facing the correct direction pour the 1X TAE buffer into the apparatus until the gel is covered by at least a few centimeters. DO NOT follow the "Fill Line" mark. It is incorrect.

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

- Running the gel
    - Once the gel has solidified, the 6X loading dye has been added to each sample and the 1X TAE buffer has been added to the apparatus you may load the samples
    - You may load them in whichever order is best for the experiments you run, but loading them follows the same procedure. For easy reference use the gel electrophoresis label sheet.
        - The 100bp ladder will ALWAYS be Lane 1 with 6 µL
    - Each lane will have 4.5µL of sample
    - The best way to go about loading each lane is to stand over the gel and very carefully and slowly dispense the samples into the gel. It is very important that to avoid shooting the sample out of the lane that you DO NOT push the pipette to its second stop. Push the sample into the lane and do not release the plunger until the tip is out of the buffer.
    - Once all the samples have been loaded, carefully move the apparatus over to the power supply.
    - Place the cover on (it can only go one way) and push the electrodes into their receivers
        - The red should be on the bottom, the black on top. ALWAYS RUN TO RED
    - Plug the other end of the cables into their respective ports. Make sure the power supply is OFF when you do this as to avoid a short or electrocution
    - Once all the plugs are in place and the cover is secured turn the power supply on.
        - The running voltage for best results is about 95V
        - At this voltage in a 1.5% gel it will take about 90 minutes, but make sure to check every once in a while to make sure the samples don't run off.

- You should see bubbles coming from the bottom. If you don't there is a bad connection. Turn the power supply off, remove the connectors from the power supply, remove the lid and reconnect everything again. If no bubbles are seen again there is something wrong and the gel should not be run until the problem is discovered.

- Visualizing the gel
  - As the gel is running you will notice the loading dye has separated into three colors; blue, orange and yellow. The yellow indicates 50bp, the blue indicates 100bp and the orange represents 500bp. Once the yellow has reached 2/3 the distance from the bottom of the gel you can turn off the power supply and remove the cables and cover.
  - Have some paper towels ready and remove the gel cast (with the gel) from the apparatus.
    - Make sure to drain the remaining TAE buffer back into the apparatus. No need making a mess.
  - Place the cast on the paper towels and tilt it to collect any remaining buffer.
  - Bring the cast and gel to the UV camera and turn the camera on by pressing the power button at the very top of the machine.
    - The program will not open unless the camera is turned on!
  - Wake the computer up and on the desktop there is an icon "GelCapture." Double click the icon to open the program.
  - Once the program opens there will be several options available for the type of machine to use. On the left hand side there are three tabs. Click on the very top one that says "UV Light Base."
  - After clicking "UV Light Base" the menu will change and two options will come up.
  - Click on "UV Light Base with E-Gel."
    - Although you are not using an actual E-gel, it is the best for capturing with the gel we are using
  - The camera is now on and you can place the gel on the UV light base by removing the top half of the machine (part with the camera is separate from the base).

- Place the gel sideways onto the UV light base so it fits perfectly. It will be sideways when you image it, but that can be rotated later.
- Once the gel in on the base, replace the camera back into position and turn the UV light "ON" by pressing the light bulb on the base itself. This will turn the UV light on and an image will appear on the computer screen.
- The image might be dim, but on the bottom you can alter the sensitivity, brightness and aperture time to get the best picture you can.
    - Simply increasing the sensitivity from "3" to "4" works the best.
- When you have the camera settings where you like click "Capture Image" on the right hand side.
    - This will take you to a new menu where you can edit the image to the extent the program will allow.
    - For our purposes there are only a couple of things to do here:
        - We want to save the image as a ".jpg" file and NOT a bitmap. To do this click on "EXPORT" and an explorer menu will appear and you can either save the image on a flash drive or on the computer itself.
        - You will want to save two images. The first is the image that you originally took. The second will be an inverted image (a negative). To do this click on "INVERT" on the left hand side and save the file as a ".jpg" as previously described.
- After you have imaged the gel and have the pictures you need turn the UV light base OFF (even if you forget to click the button it will turn off automatically when the camera is removed).
- Remove the gel from the base and wrap it in the paper towels it was brought there with and dispose of it in the biohazard waste basket.
- Use distilled water to clean the base with either paper towels or the KimWipes next to the base.
- Close the program
- Clean all the equipment used and place on the drying rack.
- Wipe down any surfaces with 70% ethanol before leaving.

Annex X: Polymerase Chain Reaction (PCR): Quadruplex PCR

Objective

This will outline the procedure for the Quadruplex PCR. Originally developed by Oliver Clermont and his colleges (Clermont *et al*., 2000) and organize *E. coli* samples into appropriate phylogroups. The quadruplex PCR uses published primer sequences for the *arpA*, *chuA*, and *yjaA* genes, as well as, an anonymous DNA fragment *TspE4.C2* found in *E. coli* isolates around the world (Clermont *et al*., 2013). Their presence or absence is indicated with a (+) or (-), respectively. This PCR divides each *E. coli* isolate into one of phylogroups: A, B1, B2 and D (Clermont *et al*., 2000).

Table 2: Primers used for phylogenetics of *E. coli* (Clermont *et al*., 2013; Clermont *et al*., 2000)

Primer Name	Gene Target	Nucleotide Sequence	PCR Product (bp)
chuA.1b chuA.2	*chuA*	5'-ATGGTACCGGACGAACCAAC-3' 5'-TGCCGCCACTACCAAAGACA-3'	288
yjaA.1b yjaA.2b	*yjaA*	5'-CAAACGTGAAGTGTCAGGAG-3' 5'-AATGCGTTCCTCAACCTGTG-3'	211
TspE4C2.1b TspE4C2.2b	*TspE4.C2*	5'-CACTATTCGTAAGGTCATCC-3' 5'-AGTTTATCGCTGCGGGTCGC-3'	152
AceK.f ArpA1.r	*arpA*	5'-AACGCTATTCGCCAGCTTGC-3' 5'-TCTCCCCATACCGTACGCTA-3'	400

Procedure

- On the biosafety cabinet turn the UV light on for 10 minutes, then open, with the blower fan on, and wipe down with 70% ethanol before placing the PCR materials inside. Leave blower fan running for at least 15 minutes to ensure no particulates are moving through the cabinet.
- Use 11 8-strip PCR tubes for this test. This gives you 88 tubes for the following:
    - 1 tube for negative control (primer + water + Supermix)
    - 1 tube for *arpA* positive control (G23)
    - 1 tube for ST131 positive control (Y34511)
    - 1 tube for alternate ST131 positive control (MG6511)
    - 81 samples
- For your convenience use the PCR label sheet provided for easy reference
- Place all materials to be used in the sterilized biosafety cabinet

- **<u>Primer Working Set</u>**
    - If not already made, or haven't been used in a while, make a new working set at the correct concentration (20µM) from the stock 100µM:
        - Get a new 1.5mL centrifuge tube and label with the gene name
        - Add 40µL of the forward primer (Table 1, pg. 1)
        - Add 40µL of the reverse primer (Table 1, pg. 1)
        - Add 120µL EB buffer
    - This PCR can be performed as a multiplex meaning you can include all four gene primers in one reaction. You should have four gene primer working sets at 20µM
        - *ArpA, chuA, yjaA and* TspE4.C2 (you can label this tube as TSP)
    - Add 40uL of each primer working set (20µM) to a 1.5mL centrifuge tube and label "Q-WS"
- Add 20µL Platinum® PCR Supermix (Invitrogen™) to each reaction tube
    - It is suggested that you go through the samples one row at a time to avoid mixing or forgetting samples
- Add 1.3µL Q-WS to each tube.
- Add 1.3µL DNA of isolate to their respective tubes. Close and lock the tube and mix by gently flicking the tube.

Procedure for Thermal Cycler

- The program will then run under the following conditions (Clermont *et al*., 2013):
  - (1) denaturation at 94°C for 4 minutes
  - (2) 30 cycles of 94°C for 5 seconds, 59°C for 20 seconds, and 72°C for 5 minutes
  - (3) the program will end
  - Total time: 2 hours 45 minutes (approximately)

Gel Electrophoresis

- After the PCR cycle has completed, run the samples on a 1.2% agarose gel at 95-105V for 2 hours.

Interpretation of Quadruplex PCR for *E. coli* (Clermont *et al*., 2000)

Phylogroup	*chuA*	*yjaA*	*TSPE4.C2*
A	-	-	-
A	-	+	-
B1	-	-	+
B1	-	+	+
B2	+	+	+
B2	+	+	-
D	+	-	-
D	+	-	+

Annex XI: Polymerase chain reaction for virulence genes of *E. coli*

To detect virulence factors of uropathogenic *E. coli,* specific primers were used to amplify sequences of the *fim*, *pap*, *sfa/foc*, *afa*, *hly*, *cnf*, and *aer* operons. Details of primer sequences and predicted sizes of the amplified products are given in Table 1.

Table 1: Primers for uropathogenic *E. coli* virulence genes (Tarchouna *et al*., 2013; Usein *et al*., 2001)

Virulence factor	**Target gene(s)**	**Primer Name**	**Primer Sequence (5'- 3')**	**Size of amplicon (bp)**
Type 1 fimbriae	*fimH*	fimH-f fimH-r	5'-AACAGCGATGATTTCCAGTTTGTGTG-3' 5'-ATTGCGTACCAGCATTAGCAATGTCC-3'	465
P fimbriae	*papC*	pap1 pap2	5'-GACGGCTGTACTGCAGGGTGTGGCG-3' 5'-ATATCCTTTCTGCAGGGATGCAATA-3'	328
S and FIC fimbriae	*sfa/focDE*[h] *region*	sfa1 sfa2	5'-CTCCGGAGAACTGGGTGCATCTTAC-3' 5'-CGGAGGAGTAATTACAAACCTGGCA-3'	410
Afa adhesins	*afaC*[c]	afa-f afa-r	5'-CGGCTTTTCTGCTGAACTGGCAGGC-3' 5'-CCGTCAGCCCCCACGGCAGACC-3'	672
Hemolysin	*hlyCA region*	hly s hly as	5'-AGATTCTTGGGCATGTATCCT-3' 5'-TTGCTTTGCAGACTGTAGTGT-3'	556
Cytotoxic necrotizing factor	*cnf*	cnf s cnf as	5'-TTATATAGTCGTCAAGATGGA-3' 5'-CACTAAGCTTTACAATATTGA-3'	693
Aerobactin	*iucC*	aer s aer as	5'-AAACCTGGCTTACGCAACTGT-3' 5'-ACCCGTCTGCAAATCATGGAT-3'	269

Sample: DNA lysates

Panel	PCR	Primer/s	Annealing Temprature	Positive Control	Negative control
1	Multiplex PCR	*fim H, afa* and *pap*	60^{o}C	J96, K10	Distilled water
2	Multiplex PCR	*sfa* and *aer*	55^{o}C	J96, Cl_{1212}	Distilled water

Procedure

1. Add 20µl Platinum® PCR Supermix (Invitrogen™) to 96 well plates or 8 rack PCR tubes
2. Add 2µl of primers
3. Add 1.5µl of DNA lysates
4. Mix by using microplate centrifuge
5. Perform Multiplex PCR using T100™ Thermal cycler (BIO RAD) & PTC-200 Peltier Thermal cycler (MJ Research).
6. Run 1.2% agarose gel electrophoresis at 95 volt for 2 hours
7. Take the photograph of gel using E-Gel Imager (life technologies, USA)

Sample: DNA lysates

Panel	PCR	Primer/s	Annealing Temprature	Positive Control	Negative control
1	Single-plex PCR	*cnf*	45°C	J96	Distilled water
2	Single-plex PCR	*hly*	50°C	J96	Distilled water

Procedure

1. Add 20µl Platinum® PCR Supermix (Invitrogen™) to 96 well plates or 8 rack PCR tubes
2. Add 1.5µl of primers
3. Add 1.5µl of DNA lysates
4. Mix by using microplate centrifuge
5. Perform Multiplex PCR using T100™ Thermal cycler (BIO RAD) & PTC-200 Peltier Thermal cycler (MJ Research).
6. Run 1.2% agarose gel electrophoresis at 95 volt for 2 hours
7. Take the photograph of gel using E-Gel Imager (life technologies, USA)

Note: The Platinum® PCR Supermix (Invitrogen™) contains 22U/mL complexed recombinant Taq DNA polymerase with Platinum® Taq Antibody, 22mM Tris-HCl (pH 8.4), 55mM KCl, 1.65mM $MgCl_2$, 220µM dGTP, 220µM dATP, 220µM dTTP, 220µM dCTP, and stabilizers.

Annex XII: Plasmids extraction using Wizard® Plus Minipreps DNA Purification System

1. Transfer the culture cells to a 1.5ml microcentrifuge tube and resuspend the cell pellet in 250µl of Cell Resuspension Solution.
2. Add 250µl of Cell Lysis Solution and mix by inverting the tube 4 times. The cell suspension should clear immediately.
3. Add 10µl of Alkaline Protease Solution and mix by inverting the tube 4 times. Incubate for 5 minutesat room temperature.
4. Add 350µl of Neutralization Solution and mix by inverting the tube 4 times.
5. Centrifuge the bacterial lysate at maximum speed (around 14,000×*g*) in a microcentrifuge for 10 minutes at room temperature.
6. Transfer the cleared lysate (approximately 850µl) to the prepared Spin Column by decanting. Avoid disturbing or transferring any of the white precipitate with the supernatant. *Note:* If the white precipitate is accidentally transferred to the Spin Column, pour the Spin Column contents back into a sterile 1.5ml microcentrifuge tube and centrifuge for another 5-10 minutes at maximum speed. Transfer the resulting supernatant into the same Spin Column that was used initially for this sample. The Spin Column can be reused but onlyfor this sample.
7. Centrifuge the supernatant at maximum speed in a microcentrifuge for 1 minute at room temperature. Remove the Spin Column from the tube and discard the flow through from the Collection Tube. Reinsert the Spin Column into the Collection Tube.
8. Add 750µl of Column Wash Solution, previously diluted with 95% ethanol, to the Spin Column.
9. Centrifuge at maximum speed in a microcentrifuge for 1 minute at room temperature. Remove the Spin Column from the tube and discard the flow through. Reinsert the Spin Column into the Collection Tube.
10. Repeat the wash procedure using 250µl of Column Wash Solution.
11. Centrifuge at maximum speed in a microcentrifuge for 2 minutes at room temperature.
12. Transfer the Spin Column to a new, sterile 1.5ml microcentrifuge tube, being careful not to transfer any of the Column Wash Solution with the Spin Column. If the Spin Column has Column Wash Solution associated with it, centrifuge again for 1 minute at maximum speed.
13. Transfer the Spin Column to a new, sterile 1.5ml microcentrifuge tube.

14. Elute the plasmid DNA by adding 100µl of Nuclease-Free Water to the Spin Column. Centrifuge at maximum speed for 1 minute at room temperature in a microcentrifuge.
15. After eluting the DNA, remove the assembly from the 1.5ml microcentrifuge tube and discard the Spin Column.
16. Cap the microcentrifuge tube and store the purified plasmid DNA at –20°C or below.

Gel electrophoresis

Agarose gel electrophoresis is performed in 0.7% agarose. Mix 20µl purified plasmids with 5µl of 6x blue loading dye on parafilm and load 25µl sample slot in agarose gel. Electrophoresis is carried out at 12V/cm and usually requires about 4 hours for the 6x blue loading tracking dye in the sample to migrate 12cm. Take the photograph of gel using E-gel imager (life technologies, USA).

Annex XIII: Institutional Review Board (IRB) ethical clearance

ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCES (IRB)
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Institutional Review Board

ANNEX 3
Form AAUMF 03-008

IRB's Decision

Meeting No: 007/2016 Date: 10 August , 2016

Protocol number: 042/16/DMIP Assigned No.

Protocol Title: Phenotypic and molecular characterization of uropathogenic E. Coli from urinary tract infection patients in selected health facilities of Addis Ababa , Ethiopia		
Principal Investigator:	Belayneh Regassa	
Institute:	College of Health Sciences, AAU	
Elements Reviewed (AAUMF 01-008) :	☑ Attached ☐ Not attached	
Review of Revised Application ☐ Yes ☐ No	Date of Previous review:	
Decision of the meeting:	☑ Approved ☐ Approved with Recommendation ☐ Resubmission ☐ Disapproved	

I. Elements approved-
1. Protocol Version No:
2. Protocol Version Date:
3. Informed consent Version No. :
4. Informed Consent Version Date

II. Obligations of the PI-
1. Should comply with the standard international & national scientific and ethical guidelines
2. All amendments and changes made in protocol and consent form needs IRB approval
3. The PI should report SAE within 10 days of the event
4. End of the study, including manuscripts and thesis works should be reported to the IRB

III. TO NERC ☐

Institution Review Board (IRB) Approval: Period from 11 August 2016 to 10 August 2017
Follow up report expected in

3 Months ___ 6 months ____ 9 months__✓_____one year____

Chairperson, IRB
Dr. Yimtubezenash Wtanfanuel
Signature
Date: 11/8/16

Institutional Review Board Office (IRB) Faculty of Medicine ADDIS AB

DECLARATION

I undersigned declare that this PhD thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for the thesis has been duly acknowledged.

Principal investigator: Belayneh Regasa Dadi

Signature: _____

Date of submission: _____

	Signature	Date
Advisors:		
Dr. Tamrat Abebe (PhD)	_____	_____
Dr. Lixin Zhang (PhD)	_____	_____