ANTIBACTERIAL EFFECT OF GREEN TEA EXTRACT AGAINST DRUG RESISTANT *Escherichia coli* ISOLATED FROM URINE SAMPLE OF PATIENTS VISITING TERTIARY CARE HOSPITAL FROM ESTERN NEPAL

A

Dissertation Submitted to the **Department of Microbiology, Central Campus of Technology**, Tribhuvan University, Dharan, Nepal, in Partial Fulfillment of the Requirement for the award of Degree of Masters of Science in Microbiology

(Public Health)

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This is to certify that Mrs. Kabita Giri has completed this dissertation work entitled "**ANTIBACTERIAL EFFECT OF GREEN TEA EXTRACT AGAINST DRUG RESISTANT *Escherichia coli* ISOLATED FROM URINE SAMPLE OF PATIENTS VISITING TERTIARY CARE HOSPITAL FROM ESTERN NEPAL"** as a partial fulfillment of the requirements of M.Sc. degree in Microbiology (Public Health) under our supervision. To our knowledge this work has not been submitted for any other degree.

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CERTIFICATE OF APPROVAL

On the recommendation of the supervisor **Mr. Suman Rai** and **Mr. Shiv Nandan Sah** this dissertation work of **Mrs. Kabita Giri** entitled "**ANTIBACTERIAL EFFECT OF GREEN TEA EXTRACT AGAINST DRUG RESISTANT *Escherichia coli* ISOLATED FROM URINE SAMPLE OF PATIENTS VISITING TERTIARY CARE HOSPITAL FROM ESTERN NEPAL"** has been approved for the examination and is submitted to the Tribbhuvan University in partial fulfillment of the requirements for Master of Science in Microbiology (Public Health).

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ABSTRACT

Urinary tract infections (UTIs) caused by drug resistant (DR) Uropathogenic *Escherichia coli* have become a significant worldwide public health problem. Due to rapid increased in antibiotic resistant, finding alternative antimicrobial agents from plant extracts has received growing interest and employed to control multi drug resistant (MDR). The present study is focused on antibacterial activity of Green tea extract against the virulent Uropathogenic *Escherichia coli*. A laboratory based cross sectional study was conducted to determine the effect of green tea extract against DR Uropathogenic *Escherichia coli* from the urine sample of patient visiting tertiary care hospital. In this review 365 midstream urine samples were collected out of them *Escherichia coli* (45.48%), non-*Escherichia coli* (27.12%) were isolated whereas (27.40%) mid-stream urine samples shown no significant growth from patients with complains of UTIs processed in microbiology laboratory for the isolation and identification by following Bergey's Manuals of identification of bacteria. Female (68.49%) has more *E. coli* isolates then male (31.51%). The identified species was subsequently exposed to selected antibiotics to test for their susceptibility test by using Kirby diffusion method. The minimum inhibitory concentration (MIC) of different antibiotics was determined as recommended by the National Committee of Clinical and Laboratory Standards (2011). Then identification of (19.28%) MDR *Escherichia* coli was done by following prescription of Clinical Laboratory Standard Instructions (CLSI). In this review both non-MDR and MDR Uropathogenic *Escherichia* coli shows high resistance to Amoxicillin, Ciprofloxacine, Cotrimoxazole whereas Cefotaxime; Ceftazidime, Nalidixic acid, Norfloxacin and Tetracycline shown least resistant on the other hand Gentamicine, Amikacin and Nitrofurantonin shown no resistance. Then the effect of green tea extract was performed by preparing the 95% ethanol extract in Soxhlet apparatus follow by phytochemical analysis. In this study phytochemical analysis of Green Tea Extract was done, which shows alkaloids, flavanoids, saponins, terpenes phenolic compound, Cardenolides and Cardiac glycosides. Antibacterial activity of Green Tea Extract against MDR Uropathogenic *Escherichia coli* was performed by making different concentration of green tea extract. Concentration of Green Tea extract of 1.2 gm/ml shown larger clear zone of 12mm. The MIC of Green Tea Extract was found to be 0.6 gm./ml. Based on the present study Green Tea Extracts have great potential as an antimicrobial compounds against DR Uropathogenic *E. coli* due to present of polyphenol compound (catechins). Further work need to be done to identify the biologically active materials of green tea. Studies on in vivo research using human volunteers are lacking.

Key word: *E. coli*, Green tea extract, MDR, Phytochemical analysis, UTI.

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LIST OF ABBREVIATIONS

ATCC	_	American Type Culture Collection
BA	_	Blood agar
CDC	_	Centers for Disease Control and Prevention
CLSI	_	Clinical Laboratory Standard Instructions
CFU	_	Colony Forming Unite
CLED	_	Cystine Lactose Electrode Deficient agar
DR	_	Drug Resistant
EMB	_	Eosin-methylene blue agar
EC	_	Epicatechin
ECG	_	Epicatechin-3-gallate
EGC	_	Epigallocatechin
EGCg	_	Epigallocatechin-3-gallate
ESBL	_	Extended-Spectrum Beta-Lactamase
XDR	_	Extensively drug resistant
ExPEC	_	Extra-intestinal pathogenic *E. coli*
MA	_	MacConkey Agar
MSU	_	Midstream urine
MIC	_	Minimum Inhibitory Concentrations
MDR	_	Multi Drug Resistant
NCCLC Standards	_	National Committee of Clinical and Laboratory
NIH	_	National Institutes of Health
NIHCE	_	National Institute for Health and Clinical Excellence
NA	_	Nutrient agar
UPEC	_	Uropathogenic *Escherichia coli*
UTI	_	Urinary tract infections

CHAPTER I

INTRODUCTION

1.1 Background

Escherichia coli is an important cause of extra-intestinal infections, enteric disease, and systemic infections in humans and animals. Uropathogenic *Escherichia coli* (UPEC), one of the members of the extra-intestinal pathogenic *E. coli* (ExPEC) is a predominant pathogen causing urinary tract infections (UTIs) (Zorc, Kiddoo et al. 2005).These strains harbor a variety of virulence factors that allow them to establish an infection, including adhesins, toxins, host defense avoidance mechanisms and multiple ions acquisition systems (Robinson and Le 2016). Uropathogenic *E. coli* (UPEC) are most commonly associated with human disease. These bacteria are the primary cause of community-acquired urinary tract infections (70–95%) and a large portion of nosocomial UTIs (50%), accounting for substantial medical costs, morbidity and mortality worldwide (Foxman. B. 2014).

Uropathogenic *Escherichia coli* (UPEC) are one of the main bacteria causing urinary tract infections (UTIs).The rates of UPEC with high resistance towards antibiotics and multidrug-resistant bacteria have increased dramatically in recent years and could difficult the treatment (Zorc, Kiddoo et al. 2005; Robinson and Le 2016). UTI is also defined as a combination of bacteriuria with $\geq 10^5$ CFU/ml midstream urine, the presence of white blood cells (leucocytes), with ≥5 WBC per high power field or presence of leukocyte esterase and the presence of clinical signs or symptoms of UTI in the host, including dysuria and frequency or urgency of urination (Ejrnaes, Sandvang et al. 2006).

UTI is the most common bacterial infection; accounting for 25 % of all infections UTI can occur in any age groups however, infection is most common in women (Karki, Tiwari et al. 2004). It is predicted that one half of all women will experience a UTI in their lifetime, and 2003).There are estimated 150 million UTI per year worldwide (Stamm 2001). UTI have been

described since ancient times with the first documented description in the Ebers Papyrus dated 1550 BC (Al-Achi and Antoine 2008). It was described by the Egyptians as *"sending forth heat from the bladder "* (Guinto, De Guia et al. 2010). Effective treatment did not occur until the development and availability of antibiotics in the 1930s before which time herbs, bloodletting and rest were recommended (Al-Achi and Antoine 2008). The Austrian pediatrician Theodor Escherich (1857–1911) discovered the pathogen later to be known as *Escherichia coli* (*E. coli*) in 1885. He isolated it from urine of young girls with symptoms from the lower urinary tract, thus recognizing the significance of urinary tract infections (UTI) in children (Shulman and Friedmann 2007).

Urinary tract infection (UTI) is a serious health problem affecting millions of people each year. It is the most important cause of mortality and morbidity in the world affecting all age groups across the life span (Karki, Tiwari et al. 2004). UTI is the second most common infectious presentation in community practice. Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of six billion US dollars (Foxman 2002). UTI can occur in any populations and age groups however, infection is most common in women in reproductive age (Karki, Tiwari et al. 2004). UTI is a heterogeneous disease, which can be divided into several types of infections such as acute, uncomplicated bacterial pyelonephritis, complicated UTI, recurrent cystitis and asymptomatic bacteriuria (Akortha EE 2008). The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae, Members includes E. *coli*, *Klebsiella, Enterobacter* and *Proteus* (Karki, Tiwari et al. 2004; Iroha, Adikwu et al. 2009).

Gram-negative bacteria are usually tested against specific antimicrobial agents such as Gentamycin, Norfloxacin and Amikacin. The second and third sets of antimicrobial agents are only tested for multiple drug resistant isolates (MDR) (Ullah, Malik et al. 2009). The development of antibiotic resistance in bacteria is a growing problem worldwide. A number of *E. coli* isolates have been collected from urine specimens of patients with UTI that are resistant to antimicrobial agents commonly used to treat UTIs were β-lactams, trimethoprim–sulfamethoxazole, fluroquinolones, nitroforantoin, etc.

(Chomarat 2000; Lee , Han et al. 2005; Ahmad and Aqil 2007). The World Health Organization and the European Commission have recognized the importance of studying the emergence and determinants of acquired anti-microbial resistance and the need to devise appropriate strategies for their control (Naber 2000; Reygaert and Jusufi. 2013; Tariq and Reyaz 2013).

In particular, the Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* are emerging worldwide (Cowan 1999; Lee , Han et al. 2005; Ahmad and Aqil 2007). The ESBL-producing strains are particularly feared as they are resistant to all penicillins, to cepahlosporins (including third and fourth generation agents) and to aztreonam. Furthermore, they are often cross-resistant to trimethoprim/sulfamethoxazole and quinolones. This combination of properties can significantly affect the course and outcomes of infections, both in the community and in the hospital setting (Ahmad and Aqil 2007). Resistance to the antimicrobial agents is recognized as a major global public health problem, infectious diseases are for approximately one–half of all cases of death in different beings. As resistance becomes more common there becomes a greater need for alternative treatments. Therefore, treatment options are replaced with a second or third choice of antibiotics, which are much more expensive. These challenges have been receiving growing interest to find alternative antimicrobial agents from plant extracts that need to be developed and used to control multidrug-resistant bacteria (Cushnie and Lamb 2005; Song and Seong 2007; Reygaert and Jusufi. 2013).

Camellia sinensis is one of the most popular beverages in the world, and has been reported to have antimicrobial effects against various pathogenic bacteria (Taylor, J. et al. 2004; Cushnie and Lamb 2005; Lee , Han et al. 2005). Cultivated in China nearly 5,000 years ago, tea is consumed in greater quantity worldwide than any other beverage except water. The beverage is made from the leaves of the plant *Camellia sinensis*, (family Theaceae). Black, green and oolong teas are all made from this plant but differ in their methods of preparation. It is generally safe, nontoxic, cheap, and available and is a popular drink, traditionally in Asian countries (Reygaert and Jusufi. 2013; Tariq and Reyaz 2013). These properties make it a very good alternative antimicrobial agent. For green tea production, freshly harvested tea leaves of

C. sinensis must be processed with the least amount of oxidation, while oolong and black tea are made from fermented leaves of the same plant. Studies on the antibacterial activity have shown that green tea inhibits the growth of *E. coli* by its polyphenolic components (also known as catechins). The most important catechins in green tea are epicatechin (EC), epigallocatechin-3-gallate (EGCg), epigallocatechin (EGC), epicatechin-3-gallate (ECG). EGC and EGCg have been shown to have the greatest antimicrobial effects (Si, Gong et al. 2006).

Drug resistant Uropathogenic E.coli is becoming worldwide threat to human health. So accurate diagnosis and appropriate use of antimicrobials for treatment and prevention of UTIs is vital to reduce the burden and also to prevent the possible long-term consequences. Several research studies have focused on the effects of green tea on microorganisms. In this present review, antibacterial activity of green tea extracts against drug resistant E. coli (the major pathogen of UTI) isolated from urine samples of patients suffering with UTI. The isolates were also checked for expression of virulence features is also discussed in experimental studies. This helps us to see the antibacterial activity of tea against pathogenic bacteria and to design chemotherapy against the disease caused by them. As present study was designed to reveal the medical importance of green tea extract as an alternative antimicrobial to control Multi Drug Resistant bacteria which is becoming a threat to human health and economic burden worldwide.

1.2 Objectives

1.2.1 General objective

To estimate the antibacterial effect of green tea extract against drug resistant Uropathogenic *E. coli* isolated from urine sample of patients visits in tertiary care hospital from eastern Nepal.

1.2.2 Specific objective

1. To isolate and identify Uropathogenic *E.coli*
2. To estimate MDR Uropathogenic *E. coli*
3. To sort out phytochemical analysis of green tea extract.
4. To determine the antibacterial activity of green tea extract against MDR Uropathogenic *E. coli*.

CHAPTER II

LITERATURE REVIEW

2.1 The Urinary System

The urinary system is a system that maintains the volume and composition of body fluids within normal limits. It consists of the kidneys, which filter the blood, remove the end products of metabolism and excrete the wastes in the urine; ureters carry the urine away from kidneys to the urinary bladder, which is a temporary reservoir and urethra transports the urine from the urinary bladder to the outside (Tortora and Derrickson 2007).Urinary tract infection (UTI) is a term applied to a variety of clinical conditions ranging from asymptomatic presence of bacteria, or fungi in the urine to severe infection of the organs of the system with resultant sepsis (Tanagho and Mcaninch 2004). UTI is defined also as the growth of a known bacterial pathogen more than 10000 cfu/ml tested with a positive dipstick or urinalysis (Zorc, Kiddoo et al. 2005). According to the National Institute for Health and Clinical Excellence (NIHCE) guidelines, urinary tract infection is defined by a combination of clinical features and the presence of bacteria and/ or fungi in urine (National Institute for Health and Clinical Excellence 2007, August).

Urinary tract infection is a mainly causes by bacterial infection that affects any part of urinary tract. In most cases bacteria travel to the urethra and multiply causing kidney infection if not treated (Bethesda 2005; David, Joseph et al. 2008). UTI is the most common bacterial infection, accounting for 25 % of all infections. UTI can occur in any populations and age groups however, infection is most common in women in reproductive age (Karki, Tiwari et al. 2004). Infections are common in women because they have a shorter urethra than men. The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae. Members of this family mostly include *E. coli*, *Klebsiella, Enterobacter* and *Proteus*. *Escherichia coli* is one of the most common bacteria capable of causing infection in humans and animals, particularly urinary tract infections. At the first step of developing

infections, bacteria must bind to the host cells and tissues, in most cases uroepithelial cells (Iroha, Adikwu et al. 2009).

2.2 Infection of urinary system

It is estimated that 150 million people with UTI are diagnosed each year on a global basis, costing in excess of 6 billion dollars in direct health care expenditures (Gupta, Hooton et al. 2011). UTIs are a serious health problem affecting millions of people each year. According to National Institute for Health, it is estimated from surveys of office practices, hospital-based clinics and emergency departments that there are over eight million cases of UTI annually in USA (National Institutes of Health (NIH). 1999). The annual cost to the health care system of the United States attributable to community-acquired UTI alone is estimated to be approximately $ 1.6 billion (Foxman 2002). Asymptomatic bacteriuria in Nigeria was reported as showing prevalence rates of 24 and 6% among rural and urban children, respectively. UTIs are commonly encountered diseases in developing countries with an estimated annual incidence of at least 250 million cases (Nicolle 2009).

It is predicted that one half of all women will experience a UTI in their lifetime, and one in three women will receive antimicrobial therapy for UTI (Foxman 2003). UTI is a heterogeneous disease, which can be divided into several types of infections such as acute, uncomplicated bacterial pyelonephritis, complicated UTI, recurrent cystitis and asymptomatic bacteriuria. Acute UTI is one of the most common bacterial infections among women presenting to primary care ((Akortha EE 2008). Symptomatic UTI are either uncomplicated or complicated. Uncomplicated infections occur in healthy women in the community and are usually caused by Escherichia coli (E. coli). Complicated infections are associated with anatomical, functional or metabolic abnormalities of the urinary tract that disable the natural innate host defense and lead to tissue injury. Major causative organisms for UTI are Escherichia coli, Klebsiella species, Proteus species, Pseudomonas species, Staphylococcus species (Hooton 2012) .

2.3 Uropathogenic E.coli and Urinary Tract Infection

E. coli is a Gram-negative rod that normally colonizes the term infant within hours after birth and form an important part of the normal human gut flora. Certain *E. coli* isolates can cause extra intestinal disease, hence termed ExPEC. Organs targeted are diverse, for example the urinary tract, the central nervous system and the lungs (Kaper and 2004) The mechanisms by which *E. coli* gain access to the urinary tract reflect an exceptional ability to adapt to an environment very different from the gut. They need to alter their metabolism (Smith, Harris et al. 2002), ascend against the flow of urine and adhere to the epithelial layer. The *E. coli* that successfully invade the urinary tract harbor specific factors that enable them to survive. These strains of *E. coli* are commonly named Uropathogenic *E.coli* or UPEC (Foxman 2003).

Uropathogenic *E. coli* (UPEC) are most commonly associated with human disease. These bacteria are the primary cause of community-acquired urinary tract infections (UTI) (70–95%) and a large portion of nosocomial UTIs (50%), accounting for substantial medical costs and morbidity worldwide (Foxman 2003). Recurrent, or relapsing, UTIs are especially problematic in many individuals. UPEC strains act as opportunistic intracellular pathogens, taking advantage of host behavior and susceptibility by employing a diverse repertoire of virulence factors to colonize the urinary tract. It is believed that a primary reservoir of UPEC isolates is within the human intestinal tract, as the isolate responsible for a UTI in a given individual often matches rectal isolates from that same person (Russo 1995). In some cases, dissemination of a single clonal group of UPEC isolates may occur within a community via contaminated food or other consumables (Manges and 2001). Additionally, UPEC strains isolated from sexually active patients often match fecal isolates from their partners, indicating that UTIs can be sexually transmitted (Foxman 2002; Johnson and Delavari 2002).

2.4 Some common virulence factors to UPEC and their major functions

Virulence factor	**Major function**
Flagella	Ascension of *E. coli* in the urinary tract
Type1fimbriae	Adhesion to bladder epithelial cells
P fimbriae	Adhesion to kidney epithelial cells
Dr fimbriae	Cell invasion
F1C fimbriae	Unknown
Cytotoxic Necrotizing Factor 1	Adhesion, invasion, apoptosis of host cells
Hemolysin	Invasion, tissue damage
Secrete auto transporter toxin	Tissue damage
LPS	Immune response activator
Curli fimbria	Adhesion, biofilm formation, invasion
Cellulose	Biofilm formation
Iron and zinc acquisition (several)	Nutrition
Capsule	Resistance to phagocytosis

2.5 Types of Urinary Tract Infection

2.5.1 Lower and Upper UTI on the basis of Anatomic sites of Infections

Lower urinary tract infection is referred to as a bladder infection. The most common symptoms are burning with urination and having to urinate frequently (or an urge to urinate) in the absence of vaginal discharge and significant pain (Nicolle 2009). These symptoms may vary from mild to severe (Lane and Takhar August 2011) and in healthy women last an average of six days (Colgan and Williams 2011-10-01). Some pain above the pubic bone or in the lower back may be present.

People experiencing an upper urinary tract infection, or pyelonephritis, may experience flank pain, fever, or nausea and vomiting in addition to the classic symptoms of a lower urinary tract infection (Lane and Takhar August 2011). Rarely the urine may appear bloody or contain visible pus in the urine (Ronald. S. 2011).

2.5.2 Uncomplicated and Complicated UTI on the basis of Clinical Manifestation

Clinically, UTIs are categorized as uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities (Nielubowicz. GR. and Mobley. HL. 2010; Hooton 2012). These infections are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis) (Hannan. TJ. 2012; Hooton 2012). Several risk factors are associated with cystitis, including female gender, a prior UTI, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility (Hannan. TJ. 2012; Foxman. B. 2014).

Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defence, including urinary obstruction, urinary retention caused by neurological disease, immunosuppression, renal

failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices (Lichtenberger. P. and Hooton 2008; Levison and Kaye 2013).

2.6 Pathogenesis

Community-acquired UTIs usually result from a retrograde ascent of bacteria in the external urethral meatus and/or vaginal introitus to the bladder. However, infection may also occur via the blood or lymph. It is believed that the bacteria are usually transmitted to the urethra from the bowel, with females at greater risk due to their anatomy. The proximity of the urethra to the vagina and rectum allows fecal flora to colonize the periurethral area of women. After gaining entry to the bladder, uropathogenic *E. coli* are able to attach to the bladder wall and form a biofilm that resists the body's immune response. Women with recurrent UTIs have colonization of the vaginal and urethral areas with the uropathogen before the onset of infection (Salvatore, Salvatore et al. june 2011). The relatively short urethra of women in comparison with men and the mechanical effect of sexual intercourse facilitate movement of bacteria into the bladder and explain the 50-times greater UTI rate in women than in men and the connection between UTIs and sexual activity. It thus, appears to be important in the pathogenesis of UTIs in younger women. In addition, use of spermicidal coated condoms dramatically alters the normal bacterial flora and has been associated with marked increases in vaginal colonization with *E. coli* and in the risk of UTI (Braunwald, Fauci et al. 2001).

Following invasion of superficial bladder epithelial cells, UPEC can replicate intracellularly and eventually reemerge from the infected host cells in a manner reminiscent of a lytic virus cycle. Upon exiting the superficial cell, UPEC can interact with and invade surrounding and underlying epithelial cells, leading to the establishment of a quiescent bacterial reservoir within the bladder tissue (Mulvey 2000). The ability of Uropathogenic *E. coli* to flux out of cells and colonize surrounding cells provides them a mechanism to subvert host defense mechanisms and persist in the bladder epithelium for weeks following the acute infection (Schilling, Mulvey et al. 2001).

Several studies have investigated bacterial adherence to uroepithelial cells. Strains of *E. coli* from women with pyelonephritis adhered to epithelial cells in much greater number than did strains of *E. coli* from those with asymptomatic bacteriuria or strains from feces (Svanborg-Eden and Jodal 1979). Larger numbers of bacteria adhere to urothelium or buccal cells of women with recurrent UTIs than in women without UTIs (Svanborg-Eden and Jodal 1979; Schaeffer, Jones et al. 1981). These later data indicate a genetic susceptibility to UTIs determined by adherence of bacteria to uroepithelial cells. A genetic predisposition to UTIs is also suggested by findings of an increased risk of UTI and increased epithelial binding of *E. coli* in women who are non secretors of blood group antigens than in those who do secrete these antigens (Kinane, Blackwell et al. 1982; Lamberg, Adergren B. et al. 1986)

2.7 Clinical Presentation

The clinical symptoms of UTI are dependent on age, stage of infection, host response and type of bacteria causing the infection. Young infants often present with unspecific symptoms like fever, irritability, vomiting, lethargy, or poor feeding. As the children grow older, and in adults, more explicit symptoms like pain upon voiding and increased frequency are present in lower UTI. Upper UTI on the other hand is associated with flank pain and fever. Recently, a meta-analysis evaluated the diagnostic accuracy of UTI signs and symptoms in children. For infants, a history of previous UTI, fever more than 24 hours, suprapubic tenderness and absence of circumcision all increased the likelihood of UTI. For older children, abdominal pain and fever, back pain, new onset of urinary incontinence and dysuria, were most reliable. As UTI is chronic nosocomial and community acquired infection proper identification and timely treatment must be done. Treatment can be proceed by antibiotic susceptibility testing (Shaikh and N. E. Morone. 2007).

2.8 Antibiotic Susceptibility Testing

Antimicrobial susceptibility tests can guide the physician in drug choice and dosage for difficult-to-treat infections. Results are commonly reported as the

minimal inhibitory concentration (MIC), which is the lowest concentration of drug that inhibits the growth of the organism. Reports typically contain a quantitative result in µg/mL and a qualitative interpretation. The interpretation usually categorizes each result as susceptible (S), intermediate (I), resistant (R), sensitive-dose dependent (SD), or no interpretation (NI) (Levinson. W. 2011; Levison and Kaye 2013).

2.9 Choice of Antibiotics for Susceptibility Tests in UTI

A number of considerations are involved in selecting appropriate antimicrobial agents to treat an infection (Ullah, Malik et al. 2009). The purpose of antimicrobial susceptibility testing in urinary infection is to provide in vitro information to assist the clinician in selection the antimicrobials that would be most reliable in inhibiting growth of infection organisms in urine. Susceptibility testing of certain antimicrobials including Nitrofurantoin, Nalidixic acid and Norfloxacin is relevant only for urinary infection as these agents achieve concentrations sufficient to be reliably inhibitory to bacterial growth only in urine (Nicolle 2009). National Committee for Clinical Laboratory Standards (NCCLS) currently recommends the routine testing of urinary isolates include Cinoxacin or Nalidixic acid, Ciprofloxacin, Nitrofurantoin, Norfloxacin, Sulphamithoxazole and Ceftriazone.

All the gram-positive and gram-negative organisms are tested against more than three group antimicrobials: β-lactam antibiotic, Aminoglycosides, Quinolone and other antimicrobial agents such as Cotrimoxazole, Chloramphenicol and Nitrofurantoin. Gram-negative bacteria are also tested against specific antimicrobial agents such as Gentamycin, Norfloxacin and Amikacin. The second and 3rd sets of antimicrobial agents are only tested for multiple drug resistant isolates (MDR) (Ullah, Malik et al. 2009).

2.10 Antimicrobial Drug Resistance

Resistance can be defined as the temporary or permanent ability of an organism and its progeny to remain viable or multiply under environmental conditions that would destroy or inhibit other cells (Smith, Harris et al. 2002).

An antibiotic resistance is defined as the microbe which is sensitive to certain antibiotic starts gaining resistance against it. The MDR strain is defined as the strain that showed resistance to three or more antibiotics among the six commonly prescribed drugs. The World Health Organization and the European Commission have recognized the importance of studying the emergence and determinants of acquired anti-microbial resistance and the need to devise appropriate strategies for their control. This combination of properties can significantly affect the course and outcomes of infections, both in the community and in the hospital setting (Rijal, Tuladhar et al. 2004).

2.10.1 Antibiotic Resistance Mechanisms

An antibiotic is said to be resistant when that antibiotic in prescribed amount and concentration is unable to kill/suppress the growth of the pathogens. The emergence and spread of antimicrobial resistance determinant is a problem of increasing importance worldwide, particularly among nosocomial bacterial pathogens. Bacterial strains are now emerging that is resistance to all currently available antimicrobial drugs. The past two decades have also witnessed a significant increase in clinically important resistance in a variety of bacterial species as well as the emergence of significant pathogens of intrinsically resistant strains previously considered to be of low pathogenicity (Smith, Harris et al. 2002).

The factors playing significant role in the increases and decreases of prevalence of resistant strains include:

- Host and clone specificity
- Plasmid and clone specificity
- Virulence
- Interactions with other commensal flora
- Duration of the selection pressure, and
- Variable gene expression (ASM 2009).

2.11 Urinary Tract Infection in Nepal

UTI is the second most common infectious presentation in community practice. Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of six billion US dollars (Foxman, Barlow et al. 2000). UTI is a common disease among Nepalese population as well as one of the commonest nosocomial infections. According to the annual report published by Department of Health Services (2059/60), morbidity of UTI in Nepal was 1,25,0584. Today, antimicrobial drugs remain the front line therapy for conquering bacterial infection (Forbes, Sahm et al. 2002). UTI is the most common bacterial infection causing illness in females mostly in the developing countries like Nepal due to illiteracy, unhygienic conditions and lack of proper toilet facilities. They are always vulnerable to infections by various organisms. In Nepal, about 20% females experience a single episode of UTI during their lifetime and 3% women have more than one episode of UTI per year (Henry. D. and Isenberg. 2004). Females have a shorter urethra compared to males, explaining the 14 times higher incidence of UTI in women. The most prevalent age group experiencing UTI in females is 21–30 age group. Prevalence of UTI in female is 6% during pregnancy due to anatomical and physiological changes (Department of Health Services 2059/60).

The common pathogens mostly causing UTI are *E. coli, Pseudomonas* spp, *Proteus* spp. *klebsiella* spp, *staphylococcus* spp. According to a study (Basnet *et al.*), the commonest bacterial isolate causing UTI in Nepal were *E. coli* (77.5%), *Klebsiella* spp. (7.1%), *Acinetobacter calcoaceticus* (2.3%), *Pseudomonas aeruginosa* (1.3%) among Gram-negative isolates and *Staphylococcus aureus* (5.7%), *Staphylococcus saprophyticus* (2.3%) and *Enterococcus faecalis* (1.2%) among Gram-positive isolates. While in another study *E. coli* is present between 80 and 90% of UTI and up to 95% of acute pyelonephritis, other isolated Gram-negative rods are *Proteus mirabilis* and *Klebsiella pneumoniae.* Within the Gram-positive organisms *Streptococcus agalacticus* and coagulase negative *Staphylococcus* are found (Henry. D. and Isenberg. 2004; Department of Health Services 2059/60).

The predominant Uropathogen, are selected for the determination of antimicrobial susceptibility as well as identification of the multidrug resistant (MDR), extensively drug resistant (XDR) and extended spectrum beta lactamase (ESBL) producing isolates. MDR and XDR isolates are identified according to the combined guidelines of the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) (Magiorakos, Srinivasan et al. 2012).UTIs caused by ESBL-producing *E. coli* has been emerged as a serious problem in Nepal. Aminoglycosides and carbapenems can be used as alternative regimens for serious infections caused by MDR *E coli*. Furthermore, it is extremely necessary to formulate a strict antibiotics prescription policy and prudent use antibiotics in our country. For the successful treatment, culture and sensitivity test is essential which is lacking in many parts of Nepal. Early detection and eradication of bacteriuria is very important for prevention of recurrence and complication e.g. chronic pyelonephritis, chronic renal failure etc (Pradhan and Pradhan 2017).

2.12 Green Tea

Tea *(Camellia sinensis)* is the second most widely consumed beverage in the world after water (Macfarlane and 2004) and has been cultivated for thousands of years due to its medicinal benefits and general health promotion purposes. The tea plant is naturally occurring in South China, but is nowadays cultivated in many other regions of the major tea producing countries in the world, like India, Japan, Sri Lanka, Indonesia and Kenia. In general, tea can be divided into three types (percentage of worlds tea production): non-fermented green tea (20%), semi-fermented tea (e.g. oolong tea and white tea) (2%) and fermented black tea (78%) (Sang, Lambert et al. 2011).Additionally, there are more than 300 different kinds of tea that differ regarding the manufacturing process.In particular, consumption of green tea has been associated with a reduction of the risk of cardiovascular disease, some forms of cancer, as well as with the promotion of oral health and other physiological functions such as antibacterial and antiviral activity, neuroprotective properties, anti-hypertensive effects, body weight control and diabetes type 2 prevention (Cabrera, Artacho et al. 2006; Chacko, Thambi et al. 2010).

.Green tea is an infusion of the leaves of the *Camellia sinensis* plant, a member of the Theaceae family (Brown 1999; Higdon and Frei 2003). As a popular drink favoured by Asians, in particular Chinese, green tea has received much global attention for its promotion of human health. A number of studies have shown that green tea possesses a wide range of biological activities including antioxidant activity (Henning SM, Niu YT et al. 2004; Cao H, Kelly MA et al. 2007; Costa ASG, Nunes MA et al. 2012), anti-inflammatory activity (Cao H, Kelly MA et al. 2007), antimutagenic as well as anticarcinogenic activities (Khatiwada J, Verghese M et al. 2006; Jeong H-S, Jang S et al. 2007), and neuroprotective effects (Mandel S, Weinreb O et al. 2004). The major functional constituents of green tea, catechins, account for 8–15% of the dry leaf weight (Yoshida Y, Kiso M et al. 1999). In most cases the beneficial effects have been attributed to the polyphenolic compounds, especially catechins, although a large number of potentially bioactive chemicals are present in tea as well (Ikeda and 2008).

2.12.1 Antimicrobial Properties of Green Tea Extract

Unlike semi-fermented and fermented teas (black and white teas) unfermented green tea contains more catechins (Bhagwat S 2011). A typical green tea infusion of 250 ml hot water with 2.5 g tea leaves approximately contains 620–880 mg of water-extractable solid compounds. About 30–42% of the dry weight of green tea consists of phenolic compounds (Harbowy ME, Balentine DA et al. 1997)(Balentine DA et al 1997) from which EGCG is the most abundant one (up to 50–80% of the total catechins (Harbowy ME et al 1997). Other catechins are present in smaller amounts:- epigallocatechin(EGC)>epicatechin gallate (ECG)>epicatechin (EC)> gallocatechin gallate (GCG)> gallocatechin (GC)> catechin gallate (CG)> catechin (C)> epigallocatechin digallate > epicatechin digallate (Harbowy ME, Balentine DA et al. 1997). Many other components have been identified in tea that might have an effect on human health: theaflavins, thearubigins, theasinesins, gallic acid, quinic acid, theogallin, coumaryl-quinic acid, caffeine, theobromine and theophylline, L-theanine (unique to tea), kaempferol, myricetin and quercetin (Bhagwat S 2011; Sang, Lambert et al. 2011).

The antimicrobial activities of some herbal medicines against different pathogens have been reported from different countries. Green tea (*C. sinensis*), has been reported to have antimicrobial activities against various pathogenic bacteria. Urinary tract infections play a significant role in transmission of drug resistance, as these infections present in asymptomatic form and are caused by opportunistic pathogens of intestinal tract. Studies on the antibacterial activity have shown that green tea inhibits the growth of *E. coli* by its polyphenolic components (also known as catechins). Catechins have been reported to possess strong antioxidant activity and are widely accepted as important antioxidants, which eliminate free radicals (Seeram NP, Henning SM et al. 2006). Epigallocatechin-3-gallate (EGCG) is the major component among the tea catechins and is believed to have a considerable therapeutical potential (Suzuki Y, Miyoshi N et al. 2012).

2.12.2 Mechanism for Antimicrobial Effects of Green Tea

There are different mechanisms for antimicrobial effects of green tea such as:

1.Polyphenols are anti-inflammatory agents that inhibit clinical symptoms of UTIs (Lee Y.S. 2005, Yoon B.I et al 2011) (Lee , Han et al. 2005; Yoon B.I., Ha U.S. et al. 2017).

2.Catechins induce production of cytokines such as IL-12 and IL-1 (Lee , Han et al. 2005).

3.Green tea polyphenols decrease tumor necrosis factor-α gene expression, which is important in pathogenesis of *E. coli* infection (Lee , Han et al. 2005).

4.Catechins, by blocking the connection of conjugated R plasmid in *E. coli*, have bactericidal and antitoxin effects (Lee Y.S. 2005) (Lee , Han et al. 2005).

5.Catechin-copper (II) complexes damage the cytoplasmic membrane of *E. coli* (Hoshino N., Kimura T. et al. 1999; Cho Y., Schiller N.L. et al. 2007).

6.EGC can bind to the ATP site of the DNA gyrase β subunit of bacteria and inhibit the activity of the gyrase enzyme (Hoshino N., Kimura T. et al. 1999; Lee , Han et al. 2005).

7. The bactericidal action of catechin is due to its hydrogen peroxide generation (Arakawa H., Maeda M. et al. 2004).

8. The highest antimicrobial activity of tea is due to presence of catechins and polyphenols which damage the bacterial cell membrane (Cho Y., Schiller N.L. et al. 2007) inhibit the extracellular release of verotoxin from enterohemorrhagic *E. coli* (EHEC) 0157 (Yam T., Hamilton-Miller J. et al. 1998; Sugita-Konishi Y., Hara-Kudo Y. et al. 1999).

2.13 Extraction of Green Tea Extract

Investigating the effects of antimicrobial products on microorganisms is a common procedure carried out in many microbiology laboratory courses, often using antibiotics or disinfectants against common bacterial species. As the issue of antimicrobial resistance continues to grow, there is a renewed interest in deriving antimicrobial products from natural compounds, particularly extracts from plant material (Kaufmann and Christen 2002). Medicinal plants are gaining much interest recently because their use in ethno medicine treating common disease such as cold, fever and other medicinal claims are now supported with sound scientific evidences. The study on medicinal plants started with extraction procedures that play a critical role to the extraction outcomes (e.g. yield and phytochemicals content) and also to the consequent assays performed. A wide range of technologies with different methods of extraction is available nowadays. Most of the phytochemicals from plant sources such as phenolics and flavonoids have been reported to have positive impact on health and cancer prevention (Venugopal and Liu 2012).

High content of phenolic and flavonoids in medicinal plants have been associated with their antioxidant activities that play a role in the prevention of the development of age-related disease, particularly cause by oxidative stress. With regards to the beneficial phytochemicals in medicinal plants and the shift

towards natural products in pharmaceuticals and cosmeticals industry, the research on medicinal plants particularly are as important as the research on conventional drugs (Vongsak, Sithisarn et al. 2013).

2.13.1 Soxhlet Extraction or Hot Continuous Extraction:

In this method, finely ground sample is placed in a porous bag or "thimble" made from a strong filter paper or cellulose, which is place, is in thimble chamber of the Soxhlet apparatus. Extraction solvents is heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reaches the siphon arm the liquid contents emptied into the bottom flask again and the process is continued (Handa, Khanuja et al. 2008).

2.13.2 Strength and Limitation:

This method requires a smaller quantity of solvent compared to maceration. However, the Soxhlet extraction comes with disadvantage such as exposure to hazardous and flammable liquid organic solvents, with potential toxic emissions during extraction. Solvents used in the extraction system need to be of high-purity that might add to cost. This procedure is considered not environmental friendly and may contribute to pollution problem compared to advance extraction method such as supercritical fluid extraction (SFE) (Naudé, De Beer et al. 1998). The ideal sample for Soxhlet extraction is also limited to a dry and finely divided solid and many factors such as temperature, solvent-sample ratio and agitation speed need to be considered for this method (Amid, Salim et al. 2010). Several research studies have focused on the effects of green tea on microorganisms. In the present review, the antimicrobial effect of green tea on *E. coli* (the major pathogen of UTI) is discussed in experimental studies. The present study was designed to check antibacterial activity of tea extracts against bacteria isolated from urine samples of patients suffering with UTI. The isolates were also checked for expression of virulence features. This will helps us to see the antibacterial activity of tea against pathogenic bacteria and to design chemotherapy against the disease caused by them.

CHAPTER III

MATERIALS AND METHOD

3.1 Materials

The materials required used in this study are mentioned in the appendix A and reagents used are mentioned in appendix B.

3.2 Methodology

The prospective cross-sectional study of bacterial Uropathogens was conducted among patients suspected from UTI attending at Birat Medical Hospital and Birat Nursing Home; Biratnagar, Morang, Nepal. The period of this research work was from November 2017 to April 2018.

3.2.1 Sample size

During this study, midstream urine specimens was collected from clinically suspected patients of UTI and processed at bacteriology laboratory of microbiology department of Birat Medical Hospital. The age of patient's ranged from above 16 years. The history of all the patients including age, gender, symptoms was recorded in the data collection form from the requisition form obtained along with the midstream urine for culture.

3.2.2 Specimen collection

The patients attending at Birat Medical Hospital and Birat Nursing Home with clinical features of UTI were given a clean, dry sterile and leak proof container and requested for 5 to 10 ml midstream urine sample and examined immediately. Detailed guidelines for collection of clean catch midstream urine are mentioned in appendix C.

3.2.3 Specimen evaluation

Before proceeding with any testing, the urine specimens were evaluated in terms of their acceptability. Considerations included proper labelling, visible signs of contamination and any transportation delays in getting the specimen to the laboratory. A properly labelled specimen contained patient's full name, date of collection. Single urine specimen was collected from each patient so bacteriological culture was performed first followed by the routine microscopic observation followed by Gram staining procedure.

3.2.4 Macroscopic examination

The observation of color, odor and transparency of the collected urine sample was done.

3.2.5 Microscopic examination

About 10 ml of urine sample was taken in a clean sterile centrifuge tube and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded. The sediment was then examined by wet mount preparation. WBCs in excess of 10^4 cells/ml (>10 cells/ml) of urine was indicate significant pyuria (Cheesbrough 2006). Other examinations in microscopy are RBCs, casts, crystals, epithelial cells and bacteria by making smear of deposit after centrifugation under high power field microscope.

3.2.6 Culture of specimen

Semi quantitative culture technique was used to culture urine specimens and to detect the presence of significant bacteriuria by standard methods (Cheesbrough 2006). An inoculating loop of standard dimension was used to take up approximately fixed (±10% error is accepted) and known volume (0.001ml) of mixed uncentrifuged urine to inoculate on the surface of Cystine lactose electrode deficient (CLED) agar, Nutrient agar (NA), Eosin-methylene blue agar (EMB), 5% Blood agar (BA) and MacConkey Agar (MA). A loopful of sample was touched on the surface of the culture plate, from which the inoculum was spread across the entire plate. Urine specimen was thoroughly

mixed to ensure uniform suspension of bacteria before inoculating the agar plates. The inoculated MA and BA plates was aerobically incubated overnight at 35-37^{o}C (Cheesbrough 2006).

3.2.7 Identification of isolates

Identification of significant isolates was done by using microbiological techniques as described in the Bergy's manual which involves morphological appearance of the colonies, staining reactions, biochemical properties and serotyping if required in specific cases (Cheesbrough 2006). Gram negative i.e Uropathogenic *E.coli* was identified by standard diagnostic procedures like lactose fermentation, motility, indole production and citrate utilization tests after incubation overnight.

3.2.8 Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of the isolates towards the various antimicrobial discs was done by modified Kirby-Bauer discs diffusion technique as recommended by (NCCLS 2011). Antibiotic susceptibility test was performed as follows

1. Inoculum was prepared by taking pure colonies of organisms with the help of sterile inoculating loop to 5 ml Nutrient broth and incubated at 37^{o}C for 4 hours.
2. The prepared inoculum was compared with Mc Farland tube number 0.5. The turbidity was adjusted as necessary by dilution or addition of more colonial growth.
3. A sterile cotton-wool swab was dipped into the suspension and excess liquid was removed by turning the swab against the side of the tube. The inoculum was spread evenly over the entire surface of the already dried (not before 15 minutes) Mueller Hinton Agar plate by swabbing in three directions.
4. The paper\antibiotic discs of selected antibiotics were gently pressed into the organism carpeted Mueller Hinton Agar plate at a

distance of 15 mm away from the edge and 24 mm apart of each other.

5. Plates was inverted and incubated at 37^{o}C for 24 hours (overnight) under aerobic conditions within 15 minutes of disc application.
6. The diameter of zone of inhibition was measured and compared with standardized zone interpretative chart provided by company. The diameters of zones of inhibition was measured to the nearest mm with a ruler, callipers or an automated zone reader.

After performing the antimicrobial susceptibility testing, MDR isolates in pure culture were preserved in 20% glycerol containing Nutrient broth and kept at -70°C until subsequent tests for antimicrobial activity was completed.

Then antibacterial effect of green tea extract against medically different drug Uropathogenic E.coli isolated from urine sample of patients visiting a tertiary care hospital was performed as follows ;

3.2.9 Extract preparation

The samples, Green tea for the present study was collected from Dhamak tea garden located in Jhapa district, Eastern Nepal. The tea samples will be washed and dried in the sun. Sample was made in power form with the help of electric blender. For extract preparation solvent extracts forms are processed separately as described below;

Solvent Extracts (Ethanolic Extracts)/Soxhlet extraction or hot continuous extraction: In this method, finely ground sample will be placed in a porous bag or "thimble" made from a strong filter paper or cellulose, which was placed in thimble chamber of the Soxhlet apparatus. Soxhlet apparatus will be filled with 95% ethanol.. Extraction solvents was heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the process is continued (Handa, Khanuja et al. 2008). The Concentrate

(semisolid paste) was then allowed to dry at room temperature under aseptic conditions. The dried crude extract was stored in sterile air tight labeled bottles at 4^0c until use (Archana and Abraham 2011; Kumar, Thakur et al. 2012).

3.2.10 Phytochemical Screening of Green Tea Extract

The aqueous extracts of tea were subjected to phytochemical analysis to screen for the presence of secondary metabolites such as alkaloids, saponin, phenolics, tannins, anthraquinones, cardenolides, terpenes, flavonoids and cardiac glycosides. The phytochemical screening was carried out using standard procedures (Herborne 1973; Trease and Evans 1989; Ajaiyeoba, Onocha et al. 2003; Jigna and Sumitra 2007). Brief description is shown in Appendix G.

3.2.11 Antibacterial activity of extracts

1. Agar well diffusion assay was the key process used to evaluate the antibacterial potential of plant extracts.
2. Petri dishes (100mm) containing 18ml of Mueller Hinton Agar was seeded with 100 µl inoculum of bacterial strain (inoculum size was adjusted so as to deliver a final inoculum of approximately 10 CFU/ml with 0.5 Mac Farlands standards).
3. Wells of 8 mm diameter was cut into solidified agar media using a sterilized cup-borer.
4. Different concentrations of Green tea extracts (0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, and 1.6 mg/ml) was prepare.
5. 1 ml of each extract was poured in the respective well and the plates was incubated at 37°C overnight.
6. The zone of clearance around each well after the incubation period confirms the antimicrobial activity of extract.
7. The experiment was performed in triplicate and antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition in mm, produced by each extract at the end of incubation period.

3.2.12 Minimum inhibitory concentration

1. Tea liquors that presented inhibitory properties *in vitro* in the screening activity was evaluated for their MIC using the Agar well diffusion method. Different concentrations of Green extracts (0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6 and 1.8gm./ml) was loaded in individual wells.
2. Plates was observed after 24-48 hrs incubation for appearance of zones of inhibition around the discs.
3. Antibacterial activity will evaluated by measuring diameter of zones of inhibition (in millimeters) of bacterial growth.
4. The MIC was determined as the lowest drug concentration that inhibited growth, as recommended by the National Committee of Clinical and Laboratory Standards (NCCLS 2011).

3.2.13 Quality control

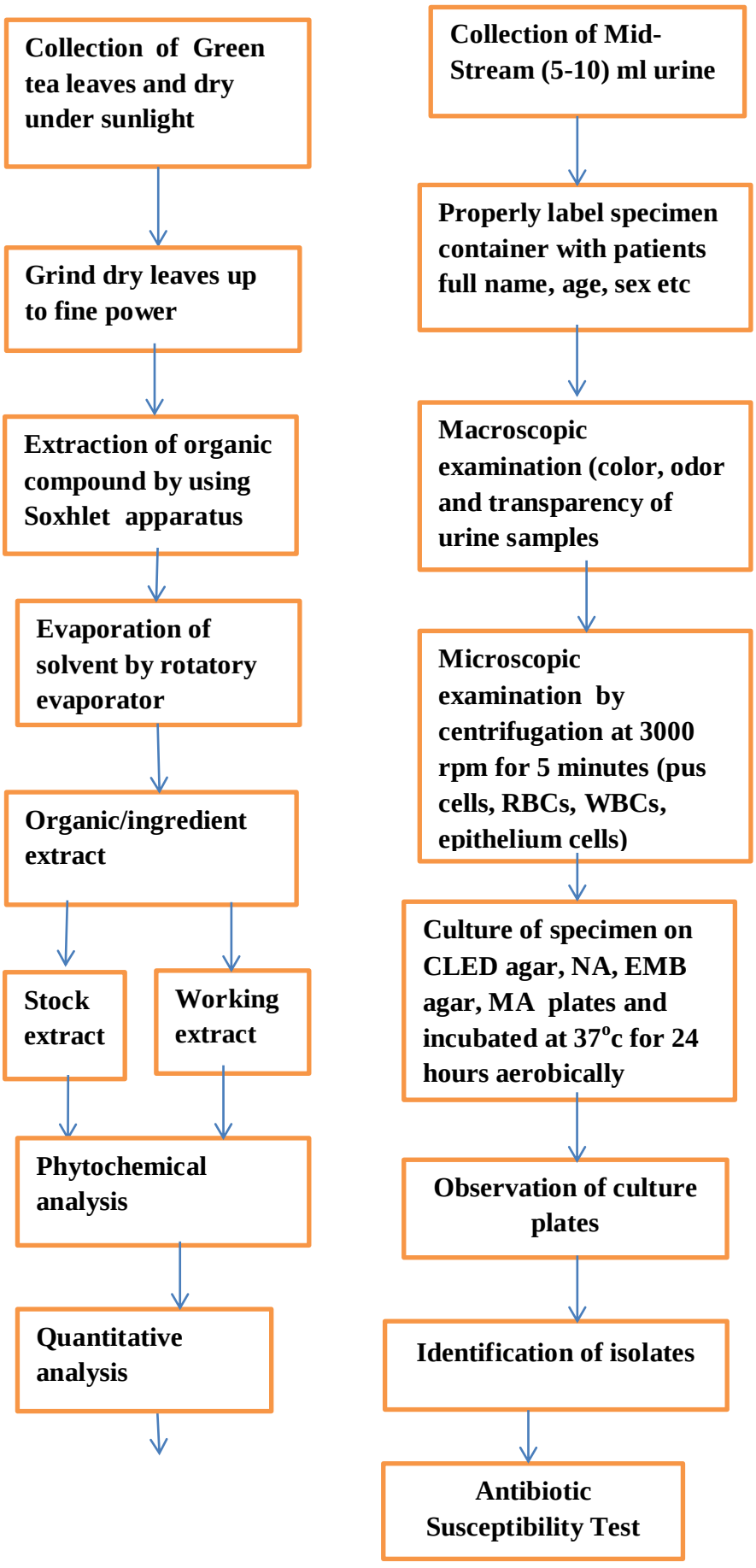
1. To obtain reliable microbiological results it is necessary to maintain quality control. During this study, quality control was applied in various areas.
2. During sample collection, aseptic technique was followed for collecting midstream urine in sterile bottles in order to avoid contamination.
3. During sample processing all the tests were carried out appropriately in aseptic conditions.
4. Laboratory equipment like incubator, refrigerator, autoclave and hot air oven were regularly monitored for their efficiency. The temperature of the incubator and refrigerator was monitored every day.
5. While using readymade dehydrated media, the manufacturer's instructions for preparation, sterilization and storage were followed to prevent the alteration of the nutritional, selective, inhibitory and biochemical properties of media.

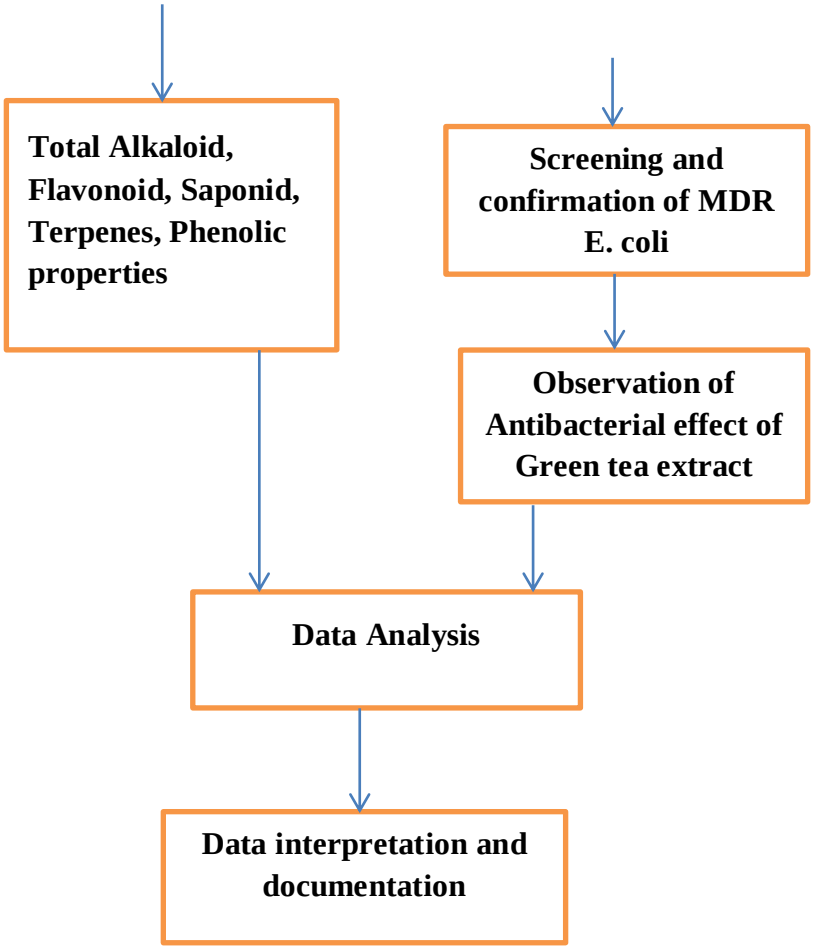
6. Reagents and culture media were regularly monitored for their manufacture and expiry date and proper storage.
7. The performances of newly prepared media were properly labelled with preparation date, expiry date, name of media, and were tested using control species of bacteria (i.e. known organisms giving positive and negative reactions) and stored as mentioned by manufacturer. For stains and reagents, whenever new batches of them were prepared or a control smear was stained to ensure correct staining reaction.
8. stains and reagents, whenever new batches of them were prepared or a control smear will be stained to ensure correct staining reaction.
9. The Soxhlet extraction process heats the solvent (ethanol) to boiling temperature (>78°C). The evaporated ethanol is contained within the apparatus by the condenser unit; however the apparatus should be placed under a fume hood in case of escape. Due to the continuous running of water and a heat source, it is not advisable to leave the apparatus unattended overnight. Personal protective equipment, including gloves, should be used when handling the plant extract, as it may be an irritant to the skin.
10. During green tea extract preparation and phytochemical screening all the tests will be carried out appropriately in aseptic conditions.
11. Control strains of *E. coli* (ATCC 25922) was used for the standardization of the Kirby-Bauer test and also for correct interpretation of zone of diameter (NCCLS 2011).

3.2.13 Data Analysis

Data entry, checking and validation were done. All data are entered in MS Excel and finally analysed by SPSS Software version 20.

3.2.14 Research Design





Figure; Flow chart of Research Design

CHAPTER IV

RESULS

4.1 Study Population

Out of 365 samples taken, 115 samples were from males (32%) and 250 samples were from females (68%).

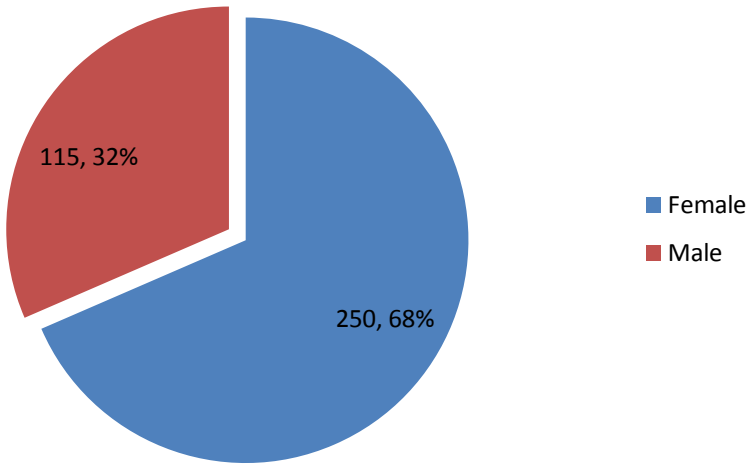


Figure 1: Study population of sample population

4.1.1 Bacterial growth pattern in UTI patients

Out 365 of mid-stream urine samples was collected from patients attending hospital. Among them 166 *Escherichia coli*, 99 non *Escherichia coli* were isolated whereas 100 mid-stream urine samples showed no growth in Cysteine Lysine Electrode Deficient Agar (CLED).The results are shown in Table 1.

Table 1. Bacterial growth pattern in UTI patients

Bacteria	**Number (%)**
Escherichia coli	166 (45.48%)
Non *Escherichia coli*	99 (27.12%)
No growth	100 (27.40%)
Total	**365 (100%)**

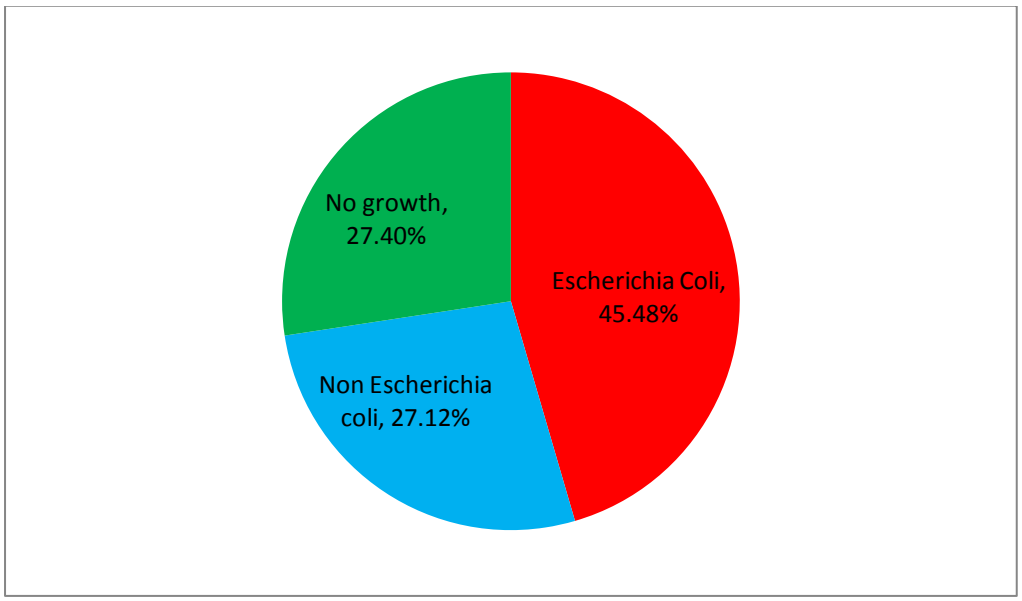


Figure 2: Bacterial growth pattern in UTI patients

4.2 Calculation of Sensitivity, Specificity, Positive and Negative Predictive Value and Efficiency

Table 2. Calculation of sensitivity, specificity, positive and negative predictive value and efficiency

Test	True positive (a)	False positive (b)	False Negative (c)	True Negative (d)
RBC counts	104	64	68	129
Pus cell counts	151	17	37	160

Calculation of Sensitivity

Sensitivity can be calculated as:

Sensitivity = a/(a+c) × 100%.

Sensitivity of RBC count = 104/(104+68) × 100% = 60.47%.

Sensitivity of pus cell count = 151/ (151+37) × 100% =80.32%

Calculation of Specificity

Specificity can be calculated as

Specificity = d/(b+d) × 100%.

Specificity of RBC count = 129/ (64+129) × 100% = 66.84%.

Specificity of pus cell count = 160/ (17+160) × 100% =90.39%.

Calculation of Positive Predictive Value (PPV)

PPV can be calculated as

PPV = a/(a+b) × 100%.

PPV of RBC count = 104/ (104+64) × 100% = 61.90%

PPV of pus cell count = 151/ (151+17) × 100% = 89.88%

Calculation of Negative Predictive Value (NPV)

NPV can be calculated as

NPV = d/ (c+d) × 100%.

NPV of RBC count = 129/ (68+129) × 100%= 65.48%

NPV of pus cell count = 160/ (37+160) × 100%=81.21%

4.3 Age and sex wise pattern of *Escherichia coli* isolates in UTI patients:

Among 166 isolated Uropathogenic *Escherichia* coli, age between (20-30) showed highest number of bacteria isolated i.e. (64.86%) in the study followed by (30-40) age group (55.37%). Age group between 0-10 (20%) has least Uropathogenic *Escherichia* coli. Female has more (68.49%) *E. coli* isolate then male (31.51%). Results are shown in Table 3.

Table 3. Age and sex wise pattern of *Escherichia coli* isolates in UTI patients

Age	**sex**				**Total**
	Infected Male	**Total Male**	**Infected Female**	**Total Female**	
0-10	0	2	1	3	1 (20%)
10-20	2	4	11	22	13 (50%)
20-30	6	11	18	26	24 (64.86%)
30-40	29	39	38	82	67 (55.37%)
40-50	9	16	9	25	18 (43.90%)
50-60	12	30	21	69	33 (33.33%)
60-70	2	7	3	12	5 (26.32%)
70-80	1	3	3	10	4 (30.77%)
80-90	1	3	0	1	1(25%)
Total	**62**	**115**	**104**	**250**	**166**
Percentage		**(31.51%)**		**(68.49%)**	

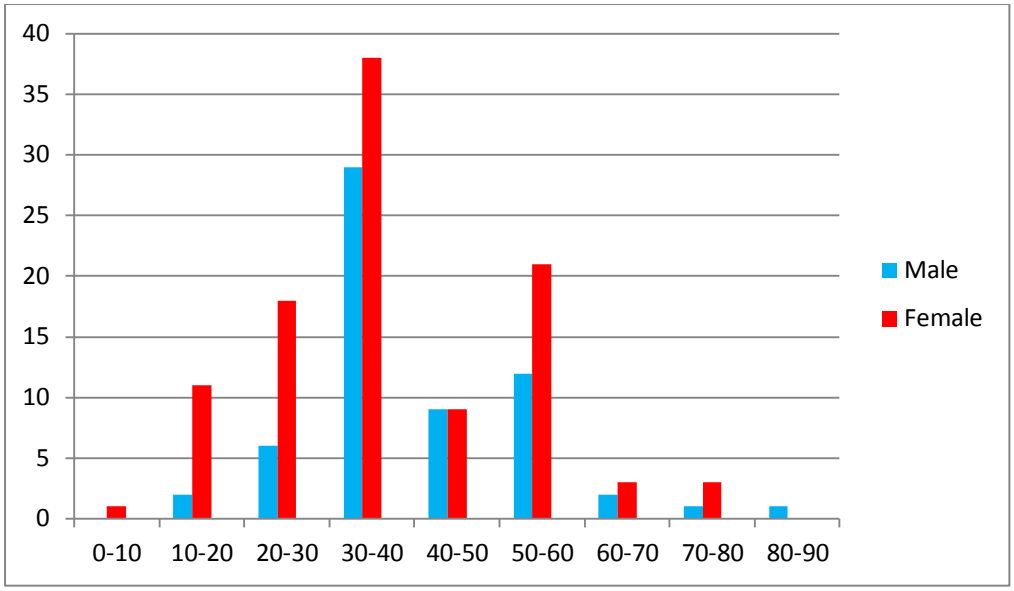


Figure 3: Age and sex wise pattern of *Escherichia coli* isolates in UTI patients

4.4 Pattern of Multi Drug Resistant Uropathogenic *Escherichia* coli isolated in UTI patients

Among 166 Uropathogenic *Escherichia* coli isolates from the patients attending hospital 32(19.28%) were MDR *Escherichia* coli. The MDR strain is the strain that showed resistance to three or more antibiotics among the six commonly prescribed drugs. The result is shown in Table 4.

Table 4. Multi Drug Resistant Uropathogenic *Escherichia* coli isolated in UTI patients

Isolates	**Number of isolates (%)**
Uropathogenic *Escherichia* coli	134 (80.72%)
MDR *Escherichia* coli	32 (19.28%)

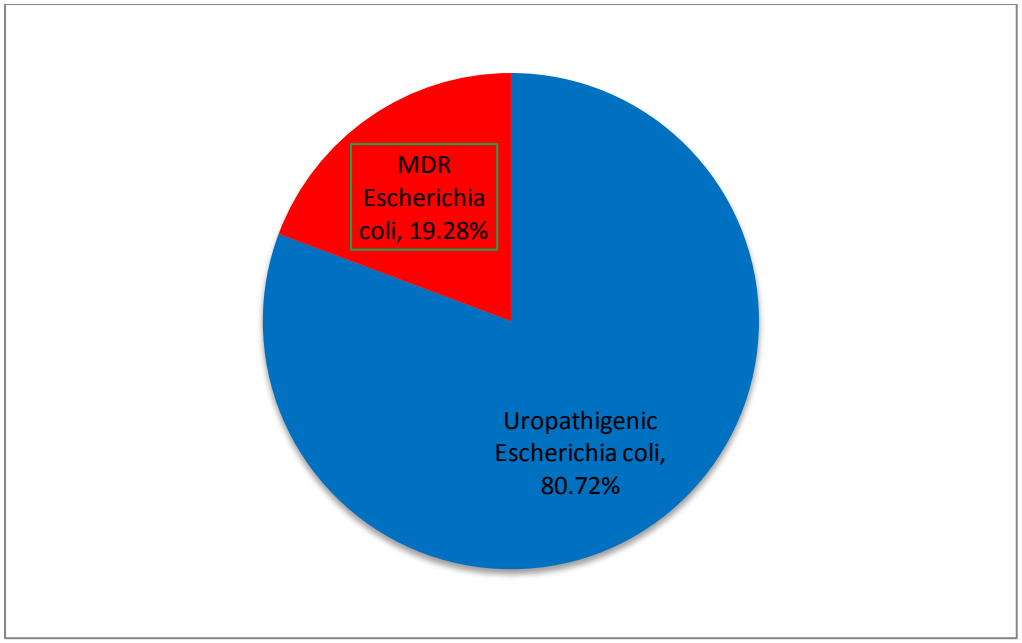


Figure 4: Pattern of Multi Drug Resistant Uropathogenic *Escherichia* coli isolated in UTI patients

4.5 Age and sex wise pattern of Multi Drug Resistant *Escherichia coli* isolates in UTI patients

Among 32 isolates of Multi Drug Resistant Uropathogenic *Escherichia* coli, age group from (30-40) showed highest number of bacteria isolated i.e (26.87%) followed by (40-50) age group (22.22%). Age group between (0-10) and (70-80) no MDR Uropathogenic *Escherichia* coli. Female has more (65.63%) *E. coli* isolate then male (34.37%). Results are shown in Table 5.

Table 5. Age and sex wise pattern of Multi Drug Resistant *Escherichia coli* isolates in UTI patients

Age	Sex				Total
	Infected Male	Total Male	Infected Female	Total Female	
0-10	0	0	0	1	0
10-20	0	2	2	11	2(15.38%)
20-30	1	6	3	18	4(16.67%)
30-40	7	29	11	38	17 (26.87%)
40-50	2	9	2	9	4 (22.22%)
50-60	1	12	2	21	3 (9.09%)
60-70	0	2	1	3	1 (20%)
70-80	0	1	0	3	0
80-90	0	1	0	0	0
Total	**11**	**62**	**21**	**104**	**32**
Percentage (34.37%)			**(65.63%**		

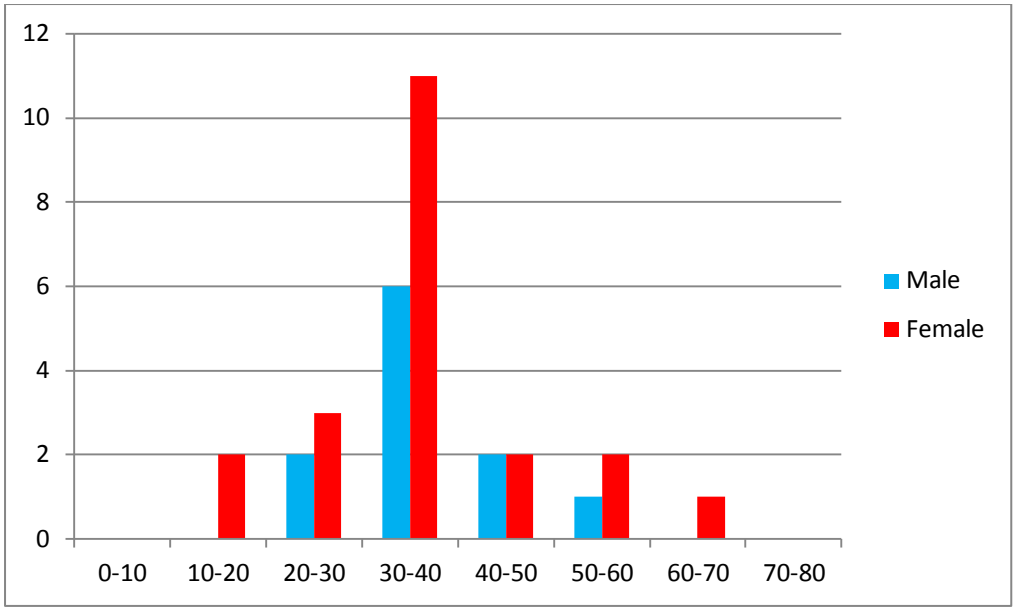


Figure 5: Age and sex wise pattern of Multi Drug Resistant *Escherichia coli* isolates in UTI patients

4.6 Antimicrobial susceptibility pattern of Uropathogenic *Escherichia coli.*

Among 166 isolates of Uropathogenic *Escherichia* coli, Amoxicillin (AMX 10 mcg), Ciprofloxacin (CIP 30 mcg) and Cotrimoxazole (COT 25mcg) showed (97.59%) and (38.55%) highest resistant respectively. Gentamicine (GEN 10 mcg) (7.22%) and Amikacin (Ak 10 mcg) (7.83%) shown least resistant whereas Nitrofurantonin (NIT 100mcg) shown no resistant. Results are shown in Table 6.

Table 6. Antimicrobial susceptibility pattern of Uropathogenic *Escherichia coli*

Antibiotic	Resistance (%)
Amikacin (Ak 10mcg)	13 (7.83%)
Amoxicillin (AMX 10mcg)	162 (97.59%)
Cefixime (CFM 30 mcg)	47 (28.31%)
Cefotaxime (CTX 30 mcg)	13 (7.83%)
Ceftazidime (CAZ 30 mcg)	35 (21.08%)
Ceftriaxone (CTR 30mcg)	14 (8.43%)
Cefpodoxime (CPD 30mcg)	15 (9.03%)
Ciprofloxacin (CIP 30 mcg)	64 (38.55%)
Cotrimoxazole (COT 25mcg)	64 (38.55%)
Gentamicin (GEN 10mcg)	12 (7.22%)
Nalidixic acid (NA 30mcg)	49 (29.52%)
Nitrofurantonin (NIT 100mcg)	0
Norfloxacin (NX 10mcg)	49 (29.52%)
Oflaxacin (OF 5mcg)	14 (8.43%)
Tetracycline (TET 10mcg)	17 (10.24%)

4.7 Antimicrobial susceptibility pattern of Multi Drug Resistant Uropathogenic *Escherichia* coli:

Among 32 isolates of Multi Drug Resistant Uropathogenic *Escherichia* coli, Amoxicillin (AMX 10mcg), Ciprofloxacin (CIP 30mcg) and Cotrimoxazole (COT 25mcg) showed (96.87%), (90.62%) and (87.50%) highest resistant respectively. Cefotaxime (CTX 30mcg), Cefpodoxime (CPD 30mcg) and Ceftazidime (CAZ 30 mcg) showed least resistant (37.5%), (21.87%) and (12.50%) respectively whereas Gentamicine (GEN 10 mcg), Amikacin (Ak 10mcg) and Nitrofurantonin (NIT 100mcg) shown no resistant. Results are shown in Table 7. **Table 7. Antimicrobial Table 7 susceptibility pattern of least resistant Multi Drug Resistant Uropathogenic *Escherichia* coli**

Antibiotic	Resistance (%)
Amikacin (Ak 10mcg)	0
Amoxicillin (AMX 10mcg	31 (96.87%)
Cefixime (CFM 30 mcg)	21 (65.62%)
Cefotaxime (CTX 30 mcg)	12 (37.5%)
Ceftazidime (CAZ 30 mcg)	4 (12.50%)
Ceftriaxone (CTR 30mcg)	13 (40.62%)
Cefpodoxime (CPD 30mcg)	7 (21.87%)
Ciprofloxacin (CIP 30 mgc)	29 (90.62%)
Cotrimoxazole (COT 25mcg)	28 (87.50%)
Gentamicin (GEN 10mcg)	0
Nalidixic acid (NA 30mcg)	22 (68.75%)
Nitrofurantonin (NIT 100mcg)	0
Norfloxacin (NX 10mcg)	22 (68.75 %)
Oflaxacin (OF 5mcg)	14 (43.75%)
Tetracycline (TET 10mcg)	17 (53.12%)

4.8 Phytochemical Analysis of Green Tea Extract

Qualitative Analysis of Green Tea Extract was performed in this study. Result is shown in Table 4.8.

Table 8 Qualitative Analysis of Green Tea Extract

Quantitative Analysis	**Result**
Alkaloids	Brownish precipitate was observed
Flavonoids	Formation of pink color solution
Phenolic compounds	Formation of green color solution
Saponins	Appearance of foam
Terpenes	Formation of reddish brown color at the interface
Anthraquinones	Not observation of a delicate pink rose
Cardenolides	Presence of brown ring at the interface
Cardiac glycosides	Presence of brown ring at the interface

4.9 Antibacterial Activity of Green Tea Extract Against MDR Uropathogenic *Escherichia coli*

Antibacterial Activity of Green Tea Extract Against MDR Uropathogenic *Escherichia coli* was performed in this study by making different concentration of green tea extract as shown in Table 9. Concentration of Green Tea extract of 1.2 gm./ml shown 12mm larger clear zone followed by 1.0, 1.2 and 1.4 gm./ml of 10mm clear zone. Whereas concentration of 0.2, 0.4, 1.8 gm./ml and control shows no clear zone. The MIC of Green Tea Extract was found to be 0.6 gm./ml. Results are shown in Table 9.

Table 9. Antibacterial Activity of Green Tea Extract against MDR Uropathogenic *Escherichia coli*

Concentration of Green Tea extract (gm./ml)	Formation of Clear Zone diameter (mm)
0.2	-
0.4	-
0.6	6
0.8	8
1.0	10
1.2	10
1.4	10
1.6	12
Control	-

CHAPTER: V

DISCUSSION

Urinary tract infections are most common problem throughout the world and in the study area as well. In addition, bacterial resistance to commonly used antibiotic agents is widespread phenomenon all over the world. UTI is the most common bacterial infection; accounting for 25% of all infections. UTI is a common disease among Nepalese population as well as one of the commonest nosocomial infections. According to the annual report published by Department of Health Services (2059/60), morbidity of UTI in Nepal was 1,25,0584. Today, antimicrobial drugs remain the front line therapy for conquering bacterial infection (Forbes, Sahm et al. 2002). UTI can occur in any age groups however, infection is most common in women. The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae, Members includes E. *coli*, *Klebsiella*, *Enterobacter* and *Proteus* (Karki, Tiwari et al. 2004; Iroha Adikwu et al 2009).

Among them Uropathogenic *E. coli* are most commonly associated with UTI. These bacteria are the primary cause of community-acquired urinary tract infections (UTI) (70–95%) and a large portion of nosocomial UTIs (50%), accounting for substantial medical costs and morbidity worldwide (Foxman 2003). As Drug resistant Uropathogenic *E.coli* is becoming worldwide threat to human health. So accurate diagnosis and appropriate use of antimicrobials for treatment and prevention of UTIs is vital to reduce the burden and also to prevent the possible long-term consequences. Several research studies have focused on the effects of green tea on microorganisms.

In this present review, drug resistant *E. coli* (the major pathogen of UTI) was isolated from urine samples of patients suffering with UTI. The study was performed in Birat Medical Hospital and Birat Nursing Home; Biratnagar, Morang, which are the one of most referred hospitals in eastern Nepal. The antibacterial activity of green tea extracts against drug resistant *E. coli* was

Our study was carried out at microbiology laboratory of Central Campus of Technology, Hattisar, Dharan, Sub-metropolitan city.

In this study, out of 365 midstream urine samples, collected from patients attending hospital, 115 samples were from males (32%) and 250 were female (68%). Among them *Escherichia coli* (45.48%), non-*Escherichia coli* (27.12%) were isolated whereas (27.40%) mid-stream urine samples shown no significant growth. In similar study in 20 11, out of 429 isolates accounts more than 95% bacterial species among them (59.2%) were *E.coli*. In another study by (Dash. M, Padhi. S et al. 2013) of the total 300 complaints of UTI, the overall prevalence of UTI was 346 (90.1%), the most frequently isolated species was *E. coli* (61.3%).

In this study, Microscopic examination of urine samples for WBCs, RBCs, Epithelial cells, pus cell and presence of casts and crystals help in determination of the type of urinary tract infection in the patients and by gram's staining we can distinguish Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell wall), which stains purple while Gram-negative bacteria have a thinner layer (10% of cell wall), which stains pink. Similarly different biochemical reactions for distinguishing pathogenic *Escherichia coli* were performed in which the isolates was found to be indole, methyl red, lactose fermentation, Gas formation from glucose, Motility test, and Lysin decarboxylase positive and other rest tests such as VP-Voges-proskauer, Citrate utilization, Urease, H2S formation on TSI agar and Inositol negative.

Likewise on calculating Sensitivity of RBC count and pus cell count we found (60.47%) and (80.32%) respectively and Specificity of RBC count and pus cell count were (66.84%) and (90.39%). Similarly PPV of RBC count and pus cell count were (61.90%) and (89.88%) and NPV of RBC count and pus cell count were calculated (65.48%) and (81.21%) respectively.

In this study among 166 isolated Uropathogenic *Escherichia* coli, age between (20-30) showed highest number of bacteria isolated i.e. (64.86%) followed by (30-40) age group (55.37%).and age group between (0-10) has least Uropathogenic *Escherichia* coli (20%). Female has more (68.49%) *E. coli* isolate then male (31.51%). In similar study of the age group analysis shows

that the female patients in the range of 20-30 years had highest prevalence rate (27.8%) and then the least was found in age group more than 80, this might be due to reason that female in the reproductive age groups has a high prevalence rate of UTI and similarly the incidence of symptomatic UTI is high in sexually active young women (Dash. M, Padhi. S et al. 2013). The uropathogens found in this study are similar to uropathogens identified in other studies conducted in different parts of the World (Farajnia .S, Alikhani. MY et al. 2009). The similarities and differences in the type and distribution of uropathogens may result from different environmental conditions and host factors, and practices such as healthcare and education programmed, socioeconomic standards and hygiene practices in each country (Amin. M, Mehdinejad. M et al. 2009).

Among 166 isolates of Uropathogenic *Escherichia* coli, Amoxicillin (AMX 10 mcg), Ciprofloxacin (CIP 30 mcg) and Cotrimoxazole (COT 25mcg) showed (97.59%) and (38.55%) highest resistant respectively. Gentamicine (GEN 10 mcg) (7.22%) and Amikacin (Ak 10mcg) (7.83%) shown least resistant whereas Nitrofurantonin (NIT 100mcg) shown no resistant i.e. these are highly susceptible to Uropathogenic *Escherichia* coli. In similar study order for resistance pattern is Ampicillin > Cephalexin > Cotrimoxazole > Norfloxain > Cefixime > Ceftriaxone > Ciprofloxaxin > Azithromicin > Ofloxacin > Gentamicin > Nitrofurantoin > Amikacin. which show that isolates of most of the species exhibited a high rate of resistance to Ampicillin, Cephalexin, Cotrimoxazole, and so on. Resistance to antibiotics develops due to its frequent misuse (Nerurkar. A, Solanky. P et al. 2012).

The regional variation of resistance to antibiotics may be explained in part by different antibiotic practices. The influence of excessive and inappropriate use on the development of antibiotic resistant strains particularly broad spectrum antibiotics prescribed empirically. Antibiotic susceptibility test reveals that higher percentage of susceptibility for Amikacin (94%), followed by Nitrofurantoin (92.8%), Gentamicn (88.55%) and least for Ampicillin (25%). Second to Amikacin is the Nitrofurantoin which is considered as an appropriate agent for first-line treatment of community acquired UTIs, it can be administered orally and is highly concentrated in urine; it may therefore be

the most appropriate agent for empirical use in uncomplicated UTI. Aminoglycosides (amikacin, gentamicin, and netilmicin) have also shown a decreasing resistance trend against E. coli from the year 2007 to 2009. Aminoglycosides being injectables are used restrictively in the community-care setting and hence have shown better sensitivity rates (Sood. S and Gupta. R 2012).

Among 166 Uropathogenic *Escherichia* coli isolates from the patients attending hospital 32 i.e. (19.28%) were MDR *Escherichia* coli. The MDR strain is the strain that showed resistance to three or more antibiotics among the six commonly prescribed drugs. Among 32 isolates of Multi Drug Resistant Uropathogenic *Escherichia* coli, age group from (30-40) showed highest number of bacteria isolated i.e. (26.87%) followed by (40-50) age group (22.22%). Age group between (0-10) and (70-80) has no MDR Uropathogenic *Escherichia* coli. Female has more (65.63%) *E. coli* isolates then male (34.37%).In simillar study among total 739 *E. coli* isolates subjected for antimicrobial susceptibility testing, 480 (64.9%) isolates were found multidrug resistant (MDR) and 37 (5.0%) isolates were extensive drug resistant (XDR). Increasing pattern of resistance of urinary tract pathogens against common antibiotics in Nepal have also been reported by other researchers (Sharma. A, Shrestha. S et al. 2011; Singh. SD, Madhup. SK et al. 2013)but MDR rates and drug resistance pattern among pediatric isolates from Nepal was not available (Sharma. A, Shrestha. S et al. 2011).

Among 32 isolates of Multi Drug Resistant Uropathogenic *Escherichia* coli, Amoxicillin (AMX 10mcg), Ciprofloxacin (CIP 30 mcg) and Cotrimoxazole (COT 25mcg) showed (96.87%), (90.62%) and (87.50%) highest resistant respectively. Cefotaxime (CTX 30 mcg), Cefpodoxime (CPD 30mcg) and Ceftazidime (CAZ 30 mcg) showed least resistant (37.5%), (21.87%) and (12.50%) respectively whereas Gentamicine (GEN 10 mcg), Amikacin (Ak 10 mcg) and Nitrofurantonin (NIT 100mcg) shown no resistant. In similar study MDR isolates were resistant to ampicillin (100%), amoxicillin clavulanate (84.7%), cephalexin (81.6%) and ciprofloxacin (80.6%) respectively. However, MDR isolates were susceptible towards amikacin (87%), imipenem (92%) and piperacillin tazobactam (81%). It is observed that ampicillin,

cephalexin, ciprofloxacin and cefixime were poorly effective against Uropathogenic *E coli*. Only 13% of the isolates were found susceptible to all the antibiotics tested. Cephalosporin, the commonly prescribed antibiotic as empirical therapy in pediatric and adults, resistance to this group of antibiotics was found high. Almost 45% of *E coli* isolates were resistant to at least one cephalosporin and monobactam. Similar rates of antimicrobial resistance was documented in the study from Bangladesh (Masud. MR, Afroz. H et al. 2014), Iran (Mirsoleymani. SR, Salimi. M et al. 2009-2012) and India (Sood. S, Gupata. R et al. 2012). However, compared to previous reports from Nepal, we observed a considerable increase in resistance against penicillins, aminoglycosides, quinolones and ceftriaxone (Sharma. A, Shrestha. S et al. 2011)

In this study phytochemical analysis of Green Tea Extract was done. In quanlitative analysis of Green Tea Extract the substances like Alkaloids, Flavonoids, Phenolic compounds, Saponins, Terpenes Cardenolides and Cardiac glycosides were found and Anthraquinones was not found as shown in Table 4.8. In similar study the secondary metabolites tested were alkaloids, saponin, phenolics anthraquinones, cardenolides, terpenes, flavonoids and cardiac glycosides. Cardenolides were present in green and black tea but absent in orthodox tea. The presence of the secondary metabolites (alkaloids, terpenes, saponins, flavonoids, cardiac glycosides, cardenolides anthroquinones and phenols) in tea partly enhances the antimicrobial and antiparasitic activity of the green tea. The antimicrobial and antiparastic activities shown by tea metabolites are in line with the previous work of antifungal, antioxidant and larvicidal activities of compounds isolated from the heartwood of *Mansonia gagei*. Owing to the presence of these secondary metabolites worth to note that their presence depends on many factors; season, rain, collection time, part collected and other agronomic factors (Tiew. P, Ioset. J.R et al. 2003)

In this study antibacterial activity of Green Tea Extract against MDR Uropathogenic *Escherichia coli* was performed by making different concentration of green tea extract as shown in Table 9. Concentration of Green Tea extract of 1.2 gm./ml shown 12mm larger clear zone followed by 1.0, 1.2

and 1.4 gm./ml of 10mm clear zone. Whereas concentration of 0.2, 0.4, 1.8 gm./ml and control shows no clear zone. The MIC of Green Tea Extract was found to be 0.6 gm./ml. In similar study by (J. O, Obwoge. J et al. 2014). The tea extracts were found to be more effective in the tested bacteria than they were on fungi. Green and orthodox tea extracts showed important inhibition of *Salmonella typhimurium* and *Escherichia coli* gram positive bacteria, at the concentrations of 200mg/ml and 400mg/ml. The Green tea extract inhibited *Escherichia coli* at a concentration of 200mg/ml and had an inhibition diameter of 14mm.

CHAPTER-VI

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

This study revealed the presence of Uropathogenic *E. coli*, which is capable of causing various urinary tract infections in both gender of any age group in their lifetime. Early detection and eradication of infection is very important for prevention of complication and recurrence. So, knowledge of the most common causative agents of infection and their antimicrobial susceptibility pattern is very essential for the administration of empirical therapy. Drug resistant and multi drug resistant Uropathogenic *E. coli* is worldwide burning issues. Due to rapid increased in antibiotic resistant, finding alternative antimicrobial agents from plant extracts has received growing interest and employed to control multi drug resistant pathogens. The present study was focused on antibacterial activity of Green tea extract against the virulent Uropathogenic *E.coli*. In this study phytochemical analysis of Green Tea Extract shows alkaloids, flavanoids, saponins, terpenes phenolic compound, Cardenolides and Cardiac glycosides. Antibacterial activity of Green Tea Extract against MDR Uropathogenic *Escherichia coli* was performed by making different concentration of green tea extract. Concentration of Green Tea extract of 1.2 gm./ml shows larger clear zone of 12mm. The MIC of Green Tea Extract was found to be 0.6 gm./ml. Based on the present study Green Tea Extracts have great potential as an antimicrobial compounds against DR Uropathogenic *E. coli* due to present of polyphenol compound (catechins).As present study reveals the medical importance of green tea extract as an alternative antimicrobial to control drug resistant bacteria which are becoming a threat to human health and economic burden worldwide. Further work need to be done to identify the biologically active materials of green tea.

6.2 Recommendations

- This study was confined on Birat Medical Hospital, Birtanagar and may not reveal the total picture of the whole country therefore systematic prospective surveillances of this type of study should be carried out throughout the year covering wide geographical region in order to obtain information regarding seasonable, geographical, and ethnic variation of pathogens.
- In this study the infection rate is found to be higher in female than in male. Howevere , both sex groups should be equally aware and take care about the UTI.
- As drug resistance among pathogens is an evolving process. Routine surveillance and monitoring studies should be conducted to help physicians to provide most effective empirical treatments.
- Resistance to antibiotics develops mainly due to its frequent misuse so to overcome drug resistance in bacteria includes reduce use of antibiotics, use of synergistic combinations, lunching health camping about antibiotics and improving the hygienic measures in an individual is necessary.
- The results obtained in this study provide preliminary evidence of the significance of secondary metabolites of tea and and their pharmacological effects. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.
- This study can be useful in the comparative studies of the presence of bioactive principles present in tea with its other clones and population, belonging to different climatic conditions. This data can also help us to choose the superior race of this valuable shrub with greater quantity and quality of medically and therapeutic important phytochemicals.

CHAPTER VII

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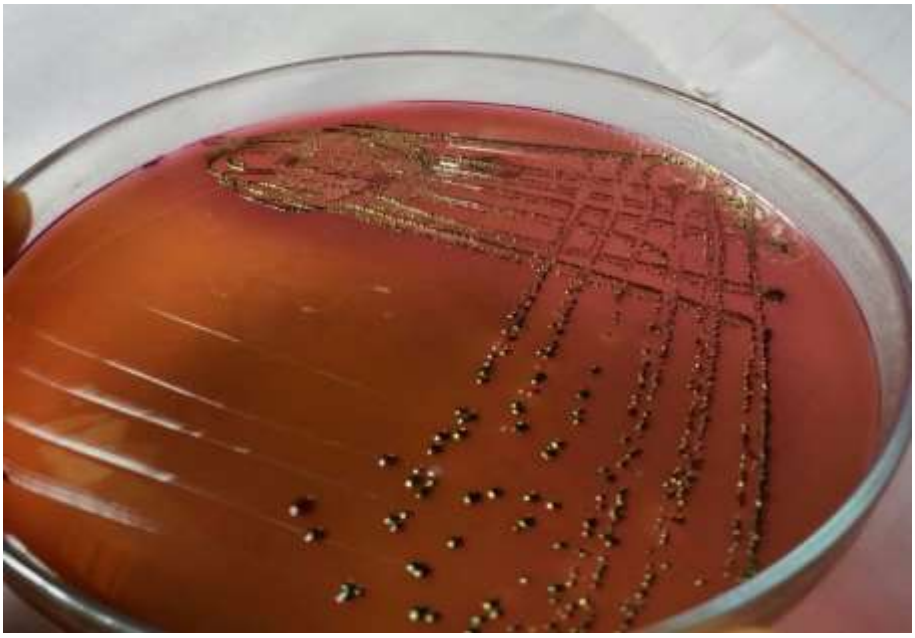
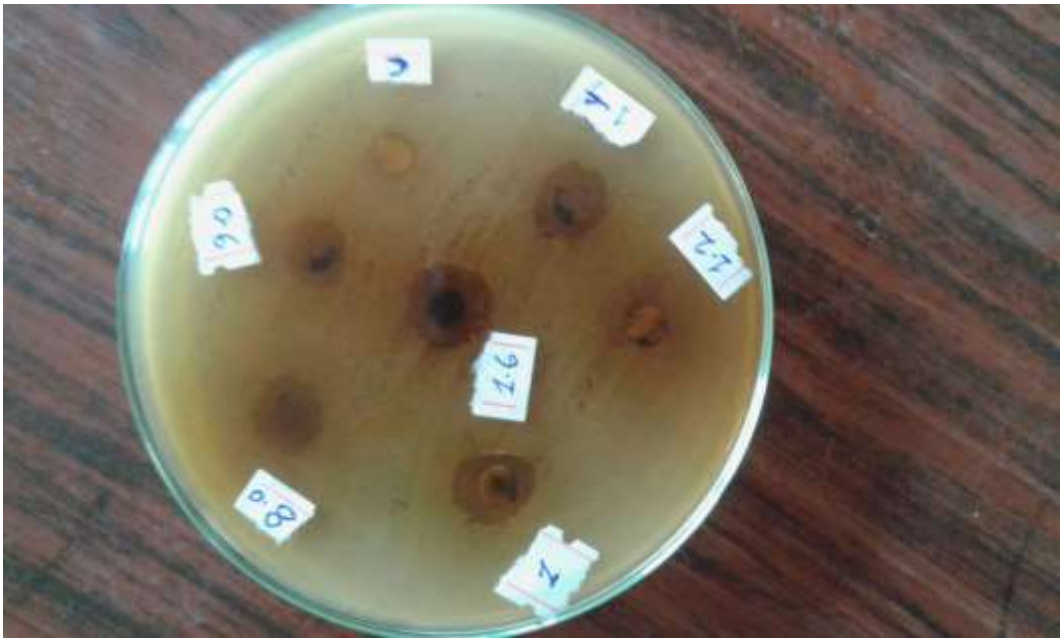
PHOTOGRAPHS

Photograph 1 : Growth of *E. coli* isolated from urine sample in CLED agar plate



Photograph 2 : Growth of *E. coli* isolated from urine sample in EMB agar plate

Photograph 3 : Antibiotic Susceptibility Test (AST) MDR *Escherichia coli* on MHA plate



Photograph 4 : Antibacterial activity of Green Tea Extract against MDR *Escherichia coli* on MHA plate

APPENDIXES

APPENDIX-I

1 List of materials

A. Equipments

1. Instruments

Autoclave	Hot air oven Centrifuge Incubator
Digital electronic balance	Refrigerator
Digital thermometer	Microscope
Evaporatory vacuum pump	Soxhlet apparatus

2. Glass wares

Conical flasks	Petridishes
Funnel	Pipette
Glass rod	leak proof bottles
Measuring cylinder	Slides

3. Others

Burner

containers

Cotton

Gas cylinder

Match box

Inoculating loop

Price tag

Wire guage

B. Microbiological media

Blood Agar

Cysteine Lysine Electrode Deficient Agar

Eosin Methylene Blue Agar

MacConkey Agar

MR-VP medium

Muller Hinton Agar

Nutrient Agar

Nutrient Broth

Simmons Citrate Agar

Sulphur Indole Motility Agar

Triple Sugar Iron Agar

Urea Broth

C. Chemical and reagents

Absolute (95%) alcohol

Gelatin agar	3% Hydrogen peroxide
starch	Burritt's reagent
Iodine solution	Barium chloride
Distilled water	Crystal violet
Ethanol	Gram's iodine
Methanol	kovac's reagent
Ferric chloride	Safranine
Hydrochloric Acid	Ammonia Sulphate
Chloroform	Lead Acetate
Sodium Hydroxide	Acetic Acid
Sulphuric Acid	Olive Oil

D. Antibiotics discs

Amikacin (Ak 10mcg)

Amoxicillin (AMX 10mcg)

Cefixime (CFM 30 mcg)

Cefotaxime (CTX 30 mcg)

Ceftazidime (CAZ 30mcg)

Ceftriaxone (CTR 30mcg)

Cefpodoxime (CPD 30mcg)

Ciprofloxacin (CIP 30 mcg)

Cotrimoxazole (COT 25mcg)

Gentamicin (GEN 10mcg)

Nalidixic acid (NA 30mcg)

Nitrofurantonin (NIT 100mcg)

Norfloxacin (NX 10mcg)

Oflaxacin (OF 5mcg)

Tetracycline (TET 10 mcg)

E. Miscellaneous

Aluminium foil, Cotton, Immersion oil ,Distilled water

Inoculating loop, Dropper, Lysol, Forceps, Sprite, Test tube holder,

APPENDIX-II

Composition and preparation of different culture media

The culture media used were from Hi-Media Laboratories Pvt. Limited, Bombay, India.

(All composition are given in gram per liter and at 25^{o}c temperature)

1. Blood agar (BA)

Blood agar base (infusion agar +5-10) % sheep blood)

Ingredients	gm/liter
Protease peptone	15.0 g
Liver extract	2.5 g
Yeast extract	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Distilled water (D/W)	1000 ml
Final PH (at 25^{o}c) 7.4±0.2)	

2. Cysteine Lactose Electrolyte Deficient Agar

Ingredients	gm/liter
Lactose	10.0 g
Pancreatic digest of gelatin	4.0 g
Pancreatic digest of casein	4.0 g
Beef extract	3.0 g
L- Cystine	0.128 g
Bromothymol blue	0.02 g
Agar	15 g
Distilled water (D/W)	1000 ml
Final PH (at 25^{o}c) 7.4±0.2)	

3. Eosin Methylene Blue (EMB) Agar

Ingredients	gm/liter
Peptone	10.0 g
Lactose	5.0 g
Dipotassium hydrogen phosphate	2.0 g
Eosin Y	0.4 g
Metheylene blue	0.065 g
Agar	15.0 g
Distilled water (D/W)	1000 ml
Final PH (at 25^{o}c) 7.4±0.2)	

4. MacConkey agar (MA)

(Without Sodium taurocholate, salt and crystal violet)

Ingredients	gm/liter
Peptone	20.0 g
Lactose	10.0 g
Sodium taurocholate	5.0 g
Sodium chloride	5.0 g
Neutral Red	0.04 g
Agar	20.0 g
Distilled water (D/W)	1000 ml
Final PH (at 25^{o}c) 7.4±0.2)	

5. Muller Hinton Agar (MHA)

Ingredients	gm/liter
Beef. Infusion form	300.0 g
Casein Acid Hydrolysate	17.5 g
Starch	1.5 g
Agar	17.0 g
Distilled water (D/W)	1000 ml
Final PH (at 25^{o}c) 7.4±0.2)	

6. Nutrient Broth (NB)

Ingredients	gm/liter
Peptone	10.0 g
Sodium chloride	5.0 g
Beef extract	10.0 g
Yeast extract	1.5 g
Agar	12.0 g
Distilled water (D/W)	1000 ml

Final PH (at 25^{o}c) 7.4±0.2)

7. Nutrient Agar (NA)

Ingredients	gm/liter
Peptone	10.0 g
Sodium chloride	5.0 g
Beef extract	10.0 g
Yeast extract	1.5 g
Distilled water (D/W)	1000 ml

Final PH (at 25^{o}c) 7.4±0.2)

B. Composition and preparation of different biochemical test media

1. MR-VP Medium

Ingredients	gm/liter
Buffer peptone	7.0 g
Dextrose	5.0 g
Dipotassium phosphate	5.0 g
Distilled water (D/W)	1000 ml

Final PH (at 25^{o}c) 7.4±0.2)

2. Sulphide Indole Motility (SIM) Medium

Ingredients	gm/liter
Beef extract	3.0 g
Peptone	30.0 g
Peptonized Iron	0.2 g

Sodium Thiosulphate	0.025 g
Agar	3.0 g
Distilled water (D/W)	1000 ml

Final PH (at 25^{o}c) 7.4±0.2)

3. Simmon Citrate Agar

Ingredients	gm/liter
Magnesium Sulfate	0.2 g
Mono-ammonium phosphate	1.0 g
Dipotassium phosphate	1.0 g
Sodium Citrate	2.0 g
Sodium chloride	5.0 g
Bromothymol Blue	0.08 g
Agar	15.0 g
Distilled water (D/W)	1000 ml

Final PH (at 25^{o}c) 7.4±0.2)

4. Triple Sugar Iron (TSI) Agar

Ingredients	gm/liter
Peptone	10.0 g
Tryptone	10.0 g
Sodium chloride	5.0 g
Beef extract	10.0 g
Yeast extract	1.5 g
Lactose	10.0 g
Sucrose	10.0 g
Dextrose	1.0 g
Ferrous Sulphate	0.2 g
Sodium Thiosulphate	0.3 g
Phenol Red	0.024 g
Agar	12.0 g
Distilled water (D/W)	1000 ml

Final PH (at 25^{o}c) 7.4±0.2)

5. Urea Agar Base

Ingredients	gm/liter
Peptone	1.o g
Sodium chloride	5.0 g
Dextrose	1.0 g
Dipotassium Phosphate	1.2 g
Mono-potassium Phosphate	0.8 g
Phenol Red	0.012 g
Agar	15.0 g
Distilled water (D/W)	1000 ml

Final PH (at 25^{o}c) 7.4±0.2)

6. Staining and Test reagents

1. For Gram's Stain

a) Crystal Violet solution

Ingredients	Amount
Crystal Violet	20.0 g
Ammonium Oxalate	9.0 g
Ethanol or Methonal	95 ml
Distilled water (D/W)	1000ml

b) Lugol's Iodide

Ingredients	Amount
Potassium Iodide	20.0 g
Iodide	10.0 g
Distilled water (D/W)	1000 ml

c) Acetone-Alcohol Decoloriser

Ingredients	Amount
Acetone	500 ml
Ethanol (absolute)	475 ml
Distilled water (D/W)	25 ml

d) Safranin (Counter Stain)

Ingredients	Amount
Safranin	10.0 g
Distilled water (D/W)	1000 ml

2. Normal saline

Ingredients	Amount
Sodium chlorine	0.85 g
Distilled water (D/W)	1000 ml

3. Biochemical Test Reagents

a) Catalase Reagent (For Catalase Test)

Ingredients	Amount
Hydrogen peroxide	3 ml
Distilled water (D/W)	97 ml

b) Oxidase Reagent (impregnated in a Whatman's No. 1 filter paper) (For Oxidase Test)

Ingredients	Amount
Tetramethyl p-Phenylenediaminedihydrochloride (TPD)	1 g
Distilled water (D/W)	1000 ml

c) Kovac's Indole Reagent (For Indole Test)

Ingredients	Amount
Isoamyl alcohol	30 ml
p-dimethylaminobenzaldehyde	2.0 g
Conc. Hydrochloric acid	10 ml

d) Methyl Red Solution (For Methyl Red Test)

Ingredients	Amount
Methyl Red	0.05 g
Ethyl alcohol (absolute)	28 ml Distilled
water (D/W)	22 ml

e) Barrit's Reagent (For Voges-Proskauer Test)

Solution A

Ingredients	Amount
α- naphthol	5.0 g
Ethyl alcohol (absolute)	100 ml

Solution B

Ingredients	Amount
Potassium hydroxide	40.0gm
Distilled water	1000ml

4. McFarland solution(No.0.5)

0.5 ml of 0.048 M $BaCl_2$ (1.17% W/V $BaCl_2.H_2O$) as added to 99.5 ml of 0.18 M H_2SO_4 (1%W/V) with constant stirring. The McFaraland standard was thoroughly mixed to ensure that it is evenly suspended. Using matched cuvettes with a 1cm light path and water as a blank standard, the aborbance was measured in a spectrophotometer at a wavelength of 625nm. The acceptable range for the turbidity standard is 0.08-0.13. The standard was distributed into secrew-cap tubes of the same size and volume as those used to prepare the test inoculum. The tubes were sealed tightly to prevent loss by evaporation and protected from light at room temperature. The turbidity standards may be stored for up to 6 months, after which time they should be discarded.

APPENDIX-III

Method of collection of mid-stream urine

Mid-stream urine (MSU) for microbiological examination is collected as follows;

Women

Women who are ambulatory should;

1. Wash her hands thoroughly with soap and dry them.
2. Undress in a suitable room, spread the labia and cleanse the vulva and labia thoroughly using sterile cotton gauze pads and warm soapy water wiping from front to rear.
3. Rinse thoroughly with warm water and dry with a sterile cotton gauze pad.
4. Pass urine, discarding the first part of the stream. Collect the remaining urine in the sterile container, the lid as soon as the urine has been collected.
5. Collect the clean-catch midstream urine, in the close container, to the health personnel for prompt delivery to the laboratory.

For bedridden patients, the same procedure is followed, except that a nurse must assits the patient.

Men

A man who is ambulatory should;

1. Wash his hands.
2. Pull back the foreskin (if not circumcised) and pass a small amount of urine out of a sterile container.
3. Still holding back the foreskin, pass most of the remaining urine into a sterile container. This is a mid-stream urine specimen.
4. Place the cover on the container and hand over to the nursing staff for prompt delivery to the laboratory.

For bedridden patients, the same procedure is followed, except that a nurse must assits the patient.

Infant and children

Child should be given water or other liquid to drink. The external genitalia should be cleaned. The child can be seated on the lap of the mother, nurse or ward attendant, who should then encourage the child to urinate and collect as much urine as possible in sterile container. The container should then be covered and delivered to the laboratory for immediate processing.

APPENDIX-IV

Gram-staining procedure

First devised by Hans Christian Gram during the late 19^{the} century, the Gram stain can be used effectively to divide all bacterial species into two large group: those that take up the basic dye, crystal violet (Gram- positive) and those that allow the crystal dye to was out easily with the decolourizer alcohol or acetone (Gram- negative). The following steps are involved in Gram-stain.

1. A thin film of the material to be examined was prepared and dried.
2. The material on the slide was heat fixed and allowed to cool.
3. The slide was flooded with crystal violet stain and allowed to remain without drying for 1 minute.
4. The slide was rinsed with tap water, shaking off excess.
5. The slide was flooded with iodine solution and allowed to remain on the surface without drying for twice as long as the crystal violet was in contact with the slide surface.
6. The slide was rinsed with tap water, shaking off excess.
7. The slide was flooded with alcohol acetone decolorizer for 10 seconds and rinsed immediately with tap water until no further color flows from the slide with the decolorizer.
8. The slide was flooded with counter-stain (safranine) for 1 minute and washed off with tap water.
9. The slide was blotted between two clean sheets of bibulous paper and examined microscopically under oil immersion at 100X.

APPENDIX-V

Methodology of biochemical tests used for identification of bacteria

A. Catalase test

This test is performed to demonstrate the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide. During aerobic respiration, in the presence of oxygen, microorganism produce hydrogen peroxide, which is lethal to the cell itself.

Procedure：A small amount of a culter from Nutrient Agar plate was taken in a clean glass slide and about 2-3 drops of 3% H_2O_2 was put on the surface of the slide. The positive test is indicated by the formation of the active bubbling of the oxygen gas. A false positive reaction may be obtained if the culture medium contains catalase (e.g. Blood Agar) or if an iron wire loop is used.

B. Oxidase test

This test is performed for the detection of cytochrome oxidase in bacteria which catalyzes the transport of electrons between electron donors. In the presence of redox dye Tetramethyl-p-phenylenediaminedihydrochloride, the cytrochrome oxidase oxidizes it into a deep purple colored end product Indophenol which is detected in the test. The test is used for screening species of Enterobacteriaceae, in which all the species give negative reaction.

Procedure：A piece of filter paper was soaked with few drops of oxidase reagent (Whatman`s No.1 filter paper impregnated with 1% tetramethyl- p-phenylenediaminedihydrochloride). Then the colony of the test organism was smeared on the filter paper. The positive test is indicated by the appeareance of blue- purple color within 10 seconds.

C. Indole production test

This test detects the ability of an organism to produce an enzyme: ‘tryptophanase’ which oxidizes tryptophan to form indolic metabolites indole, skatole(methyl indole) an indole acetic acid. The enzyme tryptophanese catalyses the deamination reaction attacking the

tryptophan molecule in its side chain and leaving the aromatic ring intact in the form of indole.

Procedure : A smooth bacterial colony was sabbed on SIM cthe inoculated media was incubated at 37^0 c for 24 hours. After 24 hours incubation, 2-3 drops of Kovac's reagent was added. Apperence of red colour on the top of media indicates indole positive. Indole if present combines with the aldehyde present in the reagent to give a red color in the alcohol layer. The color reaction is based on the presence of the pyrrole structure present in indole.

D. Methyl Red Test

This test is performed to test the ability of an organism to produce and maintain stable acid in product from the fermentation of glucose to give a capacity of the system. Medium used in study was Clark and Lubs medium (MR/VP broth, PH 6.9). Methyl red is an indicator which is already acid and well denote charges in degree of acidity by color reactions over a PH range of 4.4 -6.0.

Procedure : A pure colony of the test organism was inoculated into 2ml of MR/VP medium and was incubated at 37^0c for 24 hours. After incubation, about 5 drops of methyl red reagent was added and mixed well. The positive test was indicated by the development of bright red color, indicating acidity and negative with yellow color.

E. Voges- Proskauer (VP) test

The principle of this test is to determine the ability of some organisms to producce a acettnol, a neubiyl methyl carbinol, a neutral end product (acetoin) or its reduction product 2, 3-butanediol during fermentation of carbohydrates. An oraganism of the Enterobacteriaceae group is usually either methyl red positive and Voges-Proskauer test negative or methyl red negative and Voges-Proskauer test positive. Voges-Proskauer test for acetone is used primarily to separate *E. coli* from *Klebsiella* and Enterobacter species.

Procedure : A pure colony of the test organism was inoculated into 2 ml of MR\VP medium and was incubated at 37^0c for 24 hours. After incubation, about 5 drops of Barrit's reagent was added and shaken well for maximum aeration and kept for 15 minutes, positive test is indicated

F. Citrate utilization test

This test is performed to detect whether an organism utilizes citrate as a sole source of carbon for metabolism with resulting alkalinity. The medium used for citrated fermentation (Simmon's Citrate medium) also contains inorganic ammonium salts. Organism capable of utilizing citrate as its sole carbon source also utilizes the ammonium salts present in the medium as its sole nitrogen source, the ammonium salts are broken down to ammonia with resulting alkalinity.

Procedure : A loopful of test organism was streaked on the slant area of Simmon's Citrate Agar medium and incubated at 37^0c for 24 hours. A positive test was indicated by the growth of organism and change of media by green to blue, due to alkaline reaction. The PH indicator bromothymol blue has a PH range of 6.0-7.6, i.e. above PH 7.6; a blue color develops due to alkalinity of the medium.

G. Motility test

This test is done to determine if an organism was motile or non-motile. Bacteria are motile by means of flagella. Flagella occur primarily among the bacilli; however a few cocci forms are motile. Motile bacteria may contain single flagella. The motility media used for motility test are semisolid, making motility interpretations macroscopic.

Procedure : Motility of organism was tested by hanging drop and cultural method. In cultural method, the test organism was stabbed in the SIM medium and incunated at 37^0c for 48 hours. Motile organisms migrate from the stabline and diffuse into the medium causing turbidity

whereas non- motile bacteria show the growth along the stabline, and the surrounding media remains colorless and clear.

H. Triple Sugar Iron (TSI) Agar Test

The TSI agar is usedto determine the ability of an organism to utilize specific carbohydrate incorporated in the medium (glucose, sucrose and lactose in concentrations of 0.1% and 1.0% respectively), with or without the production of gas (indicated by cracks in the media as well as an air gap at the bottom of the tube) along with determination of possible hydrogen sulghide production (detected by production of black color in the medium). A PH indicator (phenol red) included in the medium can detect acid production from fermentation of these carbohydrates and it gives yellow reaction at acidic PH, and red reaction to indicate an alkaline surrounding.

Procedure : The test organism was streaked and stabbed on the surface of TSI and incubated at 37^0c for 24 hours. Acid production limited only to the butt region of the tube is indicative of glucose utilization, while acid production in slant and butt indicates sucrose or lactose fermentation. The result are interpreted as follows;

a. Yellow/Yellow (Acid/Acid), Gas, H_2S→ Lactose/sucrose fermentation H_2S producer.
b. Red/Yellow (Alkaline/Acid), No gas, No H_2S→ Only Glucose, not Lactose/Sucrose fermenter, not aerogenic, No H_2S production.
c. Red (Alkaline)/No change→ Glucose, Lactose and Sucrose non-fermenter.
d. Yellow (Acid)/No change→ Glucose- Oxidiser.
e. No change/ No chande → Non-fermenter.

I. Urea Hydrolysis test

This test demonstrates the urease activity present in certain bacteria which decomposes urea, releasing ammonia and carbondioxide. Ammonia thus produce charges the color of indicator (phenol red) incorporated in the medium.

Procedure : The test organism was inoculated in a medium containing urea and the indicator phenol red. The inoculated medium was incubated at 37^0c overnight. Positive organism shows pink red color due to breakdown of urea to ammonia. With the releaseof ammonia the medium become alkaline as shown by a change in color of the indicator to pink.

APPENDIX-VI

Different Biochemical Reactions for Distinguishing Pathogenic *Escherichia coli*

Test/substrate	Result
Indole production	Positive
Methyl red	Positive
VP-Voges-proskauer	Negative
Citrate utilization	Negative
Urease	Negative
Lactose fermentation	Positive
Gas formation from glucose	Positive
H2S formation on TSI agar	Negative
Motility	Positive
Lysine decarboxylase	Positive
Inositol	Negative

APPENDIX-VII

Phytochemical screening of Green Tea Extract

Anthraquinones: 1g of each tea sample was shaken with 10ml of ferric chloride solution with 5ml of hydrochloric acid (HCI). Each mixture was heated in a water bath for 10-15min, filtered and allowed to cool. The filtrate was extracted with chloroform and shaken gently. The clear layer at the base was pipetted into test tubes and 2ml of ammonia sulphate added. An observation of a delicate pink rose indicated the presence of anthraquinones.

Cardenolides: 4g of each tea sample was extracted in the test tube with 80% ethanol, and appropriately labeled. They were divided into two portions for Kedde's test and Keller-Killian's test. For Kedde's test, five drops of 10% lead acetate was added to each of the tubes, followed by five drops of distilled water and chloroform. The contents was evaporated to dryness in a water bath. 5% sodium hydroxide was added to each residue and then 2% of 3,5 dinitrobenzoic acid. For Keller-Killian's test, six drops of 10% lead acetate, water and chloroform was added to each test sample. The mixture was evaporated to dryness in the water bath and subsequently six drops of concentrated sulphuric acid were added. For Keller-Keillan's test, a brown ring indicated the presence of cardenolides, while for Kedde's test a brown to purple colour was indicates of cardenolides.

Phenolics: To 2ml of aqueous tea extract, 1ml of 1% ferric chloride solution was added. Blue or green color formation was an indication of phenols.

Flavonoids: 2g tea was extracted in 10ml water. To 2ml filtrate four drops of concentrated hydrochloric acid (HCI) followed by 0.5g magnesium turnings was added. After 3min magenta or pink color formation indicated the presence of flavonoids. The test was repeated by using 2g of tea extracted in 10ml ethanol.

Terpenes: To 2ml of aqueous extract, 5mg chloroform, 2ml acetic anhydride, concentrated HCI was added carefully to form a layer. Redish brown colour at the interface was an indication of terpenes.

Cardiac glycosides: To 2ml of ethanoic filtrate, 1ml glacial acetic acid and 1-2 drops of ferric chloride will be added followed by 1ml of concentrated sulphuric acid. Presence of brown ring at the interface indicated the presence of cardiac glycosides.

Saponins: 5ml of each aqueous tea extract was be placed into a test tube and diluted with 5ml of distilled water. The mixture was shaken vigorously for 2min, persistence appearance of foam lasting for 5min or the forming of emulsion when olive oil was added confirmed the presence of sponins.

Alkaloids: 2g of tea extract was hydrolyzed with 2ml hydrochloric acid (HCI) solution by heating in water bath for 10 min, allowed to cool and 5ml of filtrate was reacted with five drops of Dragendoff's Mayer's Wagner's reagents (18mM I_2,18mM KI). Alkaloids were recorded as present in the sample if turbidity or brownish precipitate was observed.

APPENDIX-VIII

Zone size interpretative chart based on results obtained using Muller Hinton Agar

Antimicrobal Agents used	Symbol	Disc Content (mcg)	Resistant (mm or less)	Intermediate (mm)	Susceptible
Amikacin	AK	10	15	16-18	19
Amoxicillin	AMX	10	19	-	20
Cefixime	CFM	30	19	-	20
Cefotaxime	CTX	30	14	15-22	23
Ceftazidime	CAZ	30	19	20-22	23
Ceftriaxone	CTR	30	19	20-22	22
Cefpodoxime	CPD	30	19	-	20
Ciprofloxacin	CIP	5	15	16-20	21
Cotrimoxazole	COT	25	10	11-15	16
Gentamicin	GEN	10	12	13-14	15
Nalidixic acid	NA	30	12	13-16	17
Nitrofurantonin	NIT	100	14	15-16	17
Norfloxacin	NX	10	12	13-16	17
Oflaxacin	0F	5	12	13-15	16
Tetracycline	TET	30	14	15-18	19

Source; Product Information Guide, Hi- Media Laboratories Pvt, Limited, Bombay, India

APPENDIX-IX

SPSS output

1. Urine analysis according to bacterial isolates

A. Macroscopic Observation

		Bacteria Isolated			Total
		E. coli	No Growth	Non-E. coli	
Color of urine	Deep yellow	36	15	21	72
	Light yellow	70	42	35	147
	Pale yellow	60	43	43	146
Total		166	100	99	365

		Bacteria Isolated			Total
		E. coli	No Growth	Non-E. coli	
Appearance	Clear	85	59	51	195
	Slightly turbid	36	24	28	88
	Turbid	45	17	20	82
Total		166	100	99	365

B. Microscopic Observation

Epithelial cell					
Count					
		Bacteria Isolated			Total
		E. coli	No Growth	*Non-E. coli*	
Epithelial cell	0	4	0	0	4
	2	12	4	6	22
	3	46	36	25	107
	4	50	21	27	98
	5	28	24	20	72
	6	13	5	7	25
	7	9	6	7	22
	8	4	2	7	13
	11	0	2	0	2
Total		166	100	99	365

Pus cell					
Count					
		Bacteria Isolated			Total
		E. coli	No Growth	*Non-E. coli*	
Pus cell	0	20	7	6	33
	1	4	4	6	14
	2	43	25	32	100
	3	36	27	15	78
	4	30	19	20	69
	5	21	11	9	41
	6	6	5	2	13
	7	4	0	6	10
	8	1	1	0	2
	9	1	0	1	2
	10	0	1	0	1
	11	0	0	2	2
Total		166	100	99	365

RBC					
Count					
		Bacteria Isolated			Total
		E. coli	No Growth	*Non-E. coli*	
RBC	0	64	27	32	123
	1	14	18	10	42
	2	45	26	21	92
	3	23	17	19	59
	4	10	4	6	20
	5	7	5	8	20
	6	2	2	1	5
	7	1	1	0	2
	8	0	0	2	2
Total		166	100	99	365

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2. Antibiotic Susceptibility Pattern of Uropathogenic *Escherichia coli*

Name of Antibiotic	Antibiotic Susceptibility Testing	
	R	S
AK	13	153
AMX	162	4
CAZ	35	131
CFM	47	119
CIP	64	101
C0T	64	102
CPD	7	159
CTR	14	152
CTX	13	153
GEN	12	154
NA	49	117
NX	49	117
NIT	-	166
OF	14	152
TET	17	149

3. Antibiotic Susceptibility Pattern of Multi Drug Resistant Uropathogenic *Escherichia coli*

Name of Antibiotic	Antibiotic Susceptibility Testing	Antibiotic Susceptibility Testing
	R	S
AK	0	32
AMX	31	1
CAZ	4	28
CFM	21	11
CIP	29	3
C0T	28	4
CPD	7	25
CTR	13	19
CTX	12	20
GEN	0	32
NA	22	10
NX	22	10
NIT	0	32
OF	14	18
TET	17	15