ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *Escherichia coli* ISOLATES OF URINARY TRACT INFECTION FROM PATIENTS VISITING A TERTIARY CARE HOSPITAL OF MORANG, NEPAL

A

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RECOMMENDATION

This is to certify that **Mr Milan Rauniyar** has completed this dissertation work entitled **"ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *Escherichia coli* ISOLATES OF URINARY TRACT INFECTION FROM PATIENTS VISITING A TERTIARY CARE HOSPITAL OF MORANG, NEPAL"** as partial fulfilment the requirements for M. Sc degree in Microbiology (public health) under my supervision. To our knowledge, this work has not been submitted for any other degree.

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On the recommendation of Asst**. Professor Mr Shiv Nandan Sah** this dissertation work of **Milan Rauniyar** entitled **"ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *Escherichia coli* ISOLATES OF URINARY TRACT INFECTION FROM PATIENTS VISITING A TERTIARY CARE HOSPITAL OF MORANG, NEPAL"** has been approved for examination and is submitted for the Tribhuvan University in partial fulfilment of the requirements for M. Sc degree in Microbiology (public health).

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ABSTRACT

Antibiotic resistance among uropathogens is an emerging public health problem. Bacteria may be innately resistant or may acquire resistance to antibiotics. Culture and antibiotics susceptibility testing of urine is an important tool for the diagnosis of infection and monitoring antibiotic resistance patterns of uropathogen. The objective of this study was to isolate and identify *Escherichia coli* and assess their antibiotic resistance pattern. This study was conducted in Surksha Hospital, Biratnagar, among suspected UTI patients from January to June 2021. Altogether, 400 urine samples were analyzed by the semi-quantitative culture method and uropathogens were identified by conventional methods. A total of 109 *E. coli* were tested for antimicrobial susceptibility by the Kirby Bauer disc diffusion method as per CLSI (Clinical and Laboratory Standards Institute) guidelines. Out of 400 samples, 48.75% gave significant growth while 25.5% shows no growth, 24.5 % shows non-significant growth and 1.5 % shows mixed growth. The distribution of UTI is the most common among the age group 16-49 years. *E. coli* was found to be the most predominant isolate (55.5%) followed by Coagulase-negative Staphylococci (CoNS) (12.8%) and *Enterococcus fecalis* (4.1%). Nitrofurantoin was found to be the most effective antibiotic followed by ciprofloxacin and ofloxacin while cephalexin was the least effective. Out of 109 *E. coli* isolates, 90.8% were MDR strains. *E. coli* showed a higher rate of resistance toward commonly used oral antibiotics. However, nitrofurantoin is still active against *E. coli*.

Thus, nitrofurantoin could be the choice for empirical therapy of UTIs.

Keywords: Urinary tract infection, *Escherichia coli*, antimicrobial susceptibility, multi-drug resistant

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LIST OF ABBREVIATIONS

AMPs	Antimicrobial Peptides
CLSI	Clinical and Laboratory Standards Institute
CNF-1	Cytotoxic Necrotizing Factor-1
CoNS	Coagulase Negative Stapylococcci
EPEC	Enteropathogenic *E. coli*
ExPEC	Extraintestinal Pathogenic *E. coli*
HlyA	Alpha Hemolysin
IDAS	Infectious Diseases Society of America
MAR	Multiple Antibiotic Resistance
MDR	Multidrug Resistance
THP	Tam-Horsfall Protein
UPEC	Uropathogenic *E. coli*
UTI	Urinary Tract Infection

CHAPTER -I

INTRODUCTION AND OBJECTIVES

1.1 Background

One of the foremost common infectious maladies is Urinary tract infections (UTIs) which comes after the contamination of the upper respiratory-tract (Ramesh et al2008). Many times, UTIs are related to important mortality and morbidity (Ramesh et al2008). Around the world, individuals analyzed with urinary-tract infections every year are about 150 million people in the world, which expends more than 6 billion dollars to the global economy (Gonzalez and Schaeffer, 1999). Urinary tract infections take place in both sexes' males and females in every age group, Because of the closeness of the short urethra with the anus, it is more common in females, Lack of prostatic secretion. Also, sexual contact between individuals increases the chances of contamination of the female urethra by faecal flora (Adbulkadir et. al. 2009).

Enterobacteriaceae are continuously discovered pathogens causing 84.3% of UTIs (Wada et al., 2009). In the family, *Enterobacteriaceae* (i.e., *Proteus, Klebsiella*, *Enterobacter* and *Citrobacter* spp.), *Pseudomonas* spp., *Enterococcus spp., Streptococci*, *Staphylococci* and *Candida albicans E. coli* is the most common cause of UTI than other organism reported above (Ludwig et al 2000). The nosocomial problem is increased by *Candida*. Yet, the isolation of yeast from urine does not compulsorily every time show infection (Fidel et al 1999).

In both inpatients and outpatients,*Escherichia coli is* the commonest part of the family known as *Enterobacteriaceae,* reporting for 75.0-90.0% of all Urinary Tract-Infections (Dromigny et al 2005). UTI initiation is done by *Escherichia coli* attending inside the digestive tract as a commensal offer to pool and bound serotypes of the *Escherichia coli* answerable for uropathogenicity was historically selected as the Uropathogenic *Escherichia coli* (UPEC) (Raksha et al2003). However, Most of the *Escherichia coli* strains are innocuous to people, but gastroenteritis, (UTI), neonatal-meningitis

and rare conditions, hemolytic uremic- syndrome (HUS), mastitis, septicemia, peritonitis, and gram negative pneumonia are caused by pathogenic strains. In the human colinic microflora E. *coli* is the predominant facultative aerobes. (Todar 2012).

More than 35.0% of nosocomial infections are recorded as hospital-acquired infections from Urinary Tract infections. And among hospitalized patients, they may be the 2nd most commonest source of bacteremia. (Stamm et al 2002). Only for the use of a catheter system, the infection would be even more common. catheterization is usually associated with Nosocomial urinary-tract infection.

Urinary-tract Infections are a worrying issue for female, somewhere in a lifetime up to one-third of all women experience a UTI. If left untreated it may begin pyelonephritis, preterm labour or group B streptococcal infection in the newly born (Morgan 2004). UTIs have a propensity to recur (Foxman 2010). The fact of occurring again within six months in about 25.0% of women who present with an acute UTI, in spite of accepting appropriate antibiotic treatment (Schilling et al 2002). A recurring urinary tract infection can have several causes. inadequate antimicrobial therapy gives rise to Unresolved bacteriuria mostly. Non-compliance, malabsorption, suboptimal drug metabolism, and resistant uropathgens unresponsive to attempted therapy are the outcomes from Sub–therapeutic levels of the antimicrobial agents (Schaeffer et al 1997). Just about 10% of the pregnant mother suffer from UTIs (Bear 1976).

UTIs are a remarkable cause of morbidity even so rarely associated with mortality. vesicoureteral reflux and kidney scarring are the results of Delayed treatment of UTI. In both adults and children, sRenal scarring has been cited as one of the most common causes of end-stage renal disease (De Leon 1997).

sexual intercourse, pregnancy, poor perianal hygiene, urethral reflux, urinary tract obstruction, catheterization instrumentation and neurogenic bladder but in many instances the pathogenesis is equivalents are the causes of urinary tract infection, (Nahar et al 2010).

Most Urinary Tract Infections are not Severe and irreparable damage is not

caused. Though, irreversible tissue destruction with an increased hazard of bacteremia might be caused if the kidneys are involved (Hvidberg et al 2000). can be caused. Urinary-tract infection is the 2nd foremost source of antibiotic consumption in community (Raz et al 2000). About 15.0% of all antibiotic prescriptions are for the management of UTI (Mazzuli 2001). Generally, UTI are treated empirically with antibiotics and laboratory testing was performed only when empirical treatment fails (Alos et al. 2004). Infectious Disease Society of America (IDAS) guiding principle recommend treatment with co-trimoxazole in setting where the resistance bacteria are prevalent for less than 10.0-20.0%, substitute treatment for unfussy UTI in setting with more than 10.0-20.0% resistance to co-trimoxazole might contain a fluoroquinolone, nitrofurantoin and fosfomycin (Warran et al 1999). Co-trimoxazole is widely used as an empirical treatment for urinary tract infections instigated by *E. coli*. However, resistance to co-trimoxazole has increased with a prevalence of resistance which is reported 30 to 50 percent (Gupta et al 2001). Ciprofloxacin belongs to an important class of antibiotic for treatment of UTIs, it has been more active against *E. coli* strains other than usually used agents (for instance) co-trimoxazole and ampicillin (Moniri et al 2003). Resistance to co-trimoxaxole is generally associated with the resistance to ampicillin, cepatholin and tetracycline (Moniri et al. 2003).

The World health organizations (WHO) do have designated antibiotic resistance as an evolving disease. Bacteria can develop resistance naturally or develop resistance to antibiotics. The swift spread of bacterial resistance to antimicrobic agents has led to the hunt for newer and more effective drugs. However, as new drugs emerge, irrational use and abuse lead to bacterial resistance (Omigieet et al2009). The appearance of antimicrobial resistance with inside the remedy of urinary-tract infections is a critical civic fitness concern. High poverty levels, unawareness, poor sanitation practices, and counterfeit and falsified medicines of questionable quality are prevalent, especially in developing countries. Community circulation (Manikandan et al2011). The development of resistance to older drugs such as ampicillin and co-trimoxazole, and the emerging problem of fluoroquinolone resistance can severly limit our antibiotic choice (Karlowsky et al. 2002). Multi-drug

resistance (MDR) bacteria refer to those which are resistance to a vast range of antibiotics with structural independence (at least two or more antibiotics) (CDC 2006). Nowadays, a big concern among the medical and clinical practitioners is the emerging MDR organism and their associated complications in developing world (Guyot et al 1999). These conditions make the treatment more challenging and many even threaten the respective patients lives (Farshad et al 2010).

Urinary Tract Infection is a common condition aliment amongst Nepalese and also common nosocomial infections (Kattel et al 2008). Yearly report of F.Y. (2059/2060) available with the department of health service, illness of UTIs in Nepal was 1,25,058. Culture and susceptibility testing are for proper treatement of UTI ,but this facility is not available in most parts of Nepal. Limited information is available on the occurrence and antibiotic susceptibility patterns of *Escherichia coli* associated urinary-tract infections in Nepal. This consider was conducted to decide the occurrence & antibiotic susceptibility patterns of *Escherichia coli* associated urinary-tract infections in the inpatients and the outpatients at Surksha Hospital, Biratnagar, Nepal. The information will be useful for clinician and health care provider for treatment of *E. coli*-associated urinary tract infections.

1.2 Objectives

1.2.1 General objective

To identify antimicrobial resistance pattern of the *Escherichia coli* inaccessible from suspected urinary-tract infection patients (inpatients & outpatients) attending to tertiary hospital, of Morang, Nepal

1.2.2 Specific objectives

- To isolate and identify *Escherichia coli* from urine-samples collected from UTI patients visiting tertiary hospital, Morang, Nepal
- To determine the antimicrobial susceptibility pattern of *Escherichia coli* isolates.

CHAPTER - II

LITERATURE REVIEW

2.1 Urinary Tract Infection (UTI)

Pathogen colonization of Urinary Tract Infections anywhere along urinary tract, ureter, bladder, kidney and urethra (Shortiffe et al 2006). UTI is a condition in which bacteria colonize and multiply in the urinary tract (Najar et al 2009).

UTIs refers to the event of a microbial pathogen in the urinary-tract, typically classified by site of infections as the bladder (cystitis), kidney (pyelonephritis), or urine (bacteriuria) and may be classified as the asymptomatic or the symptomatic. A UTI which occurs in the usual urogenital tract without previous device use is considered as an “uncomplicated”, but a “complicated disease” with structural or the functional abnormalities, involving devices like indwelling urethral catheters.Acute urogenital tract infections are often diagnosed as asymptomaic. (Gonzalez et al 1999).

2.2 Etiological Agents of UTI

Bacteria are the principal causative agents responsible for UTIs, accounts for more than 95.0% cases of UTI, although fungi and viruses are the main source of minor cases of UTIs (Bonadio et al 2001). The causative agent varies by age and associated morbidity (Chang et al 2006). Many unalike organisms can infect the urinary tract, but the most common pathogens are Gram negative bacilli (Wilson et al 2004). The bacteriology of UTIs is very predictable. Many unalike species can source UTIs, but commonly Gram negative facultative anaerobic uropathogenic *Escherichia coli* (UPEC) (Foxman 2010) cause infections in most populations.

Over time, pathogens related to basic urinary tract infections has been remained constant, with *E. coli* recognized as the pathogen in approximately 75.0–90.0% of infections (Omigie et al 2009).

Different enterobacteria, mainly Klebsiella, Proteus mirabilis, and Pseudomonas aeruginosa are the ultimate gram negative urinary pathogens.
The utmost commonly concerned gram-positive bacteria are Enterococci & coagulase negative Staphylococci (CoNS) (eg, *Staphylococcus-saprophyticus*) (Shankel 2007).

Escherichia coli is a major cause of nosocomial UTIs. Gram negative pathogens such as *Pseudomonas* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp. and urease-producing *Klebsiella* spp., *Proteus* spp., *Corynebacterium urealyticum*, and *Providencia* spp. are involved as well (Wagenlehner et al 2008). Because antibiotics cannot penetrate biofilms formed around and within infectious stones, they are often implicated in nosocomial urinary tract infections (Marcus et al 2008). Gram-positive bacteria such as Enterococcus spp. and staphylococcal species. The selection pressure of antibiotics used in hospitalized patients can lead to nosocomial urinary tract infections. Anaerobic bacteria have also been reported in UTIs, but the roles of those bacteria are evidently not defined (Kauffman et al 2000).

Klebsiella pneumoniae is the 2nd foremost cause of gram-negative Urinary Tract Infection. however, it is a much less predominant etiologic agent than UPEC (Rosen et al 2008).

Staphylococcus saprophyticus is a common causative agent of UTI in young women (Minardi et al 2011). A small number of women have been reported to colonize the rectum and colonize the cervix and urethra (Rupp et al. 1992). It commonly causes mild urinary tract infections and is isolated in 3.0% of non-pregnant, sexually-active women of childbearing potential with pyelonephritis (Scholes et al 2005). *Staphylococcus sapropyticus* is a distinguished uropathogen deprived of participation in indwelling catheters, and two other Staphylococci (*S. aureus* and *S.epidermidis*) are often clinically inaccessible from hospitalized patients who have indwelling catheter instead of outpatients (Von Eiff et al2002).

Proteus mirabilis is one of the commonest causes of UTIs in people with indwelling complicated urinary tracts or urinary catheters. Despite its

susceptibility to antibiotics, *Proteus mirabilis* can be hard to eradicate with the antibiotic treatments. As per hypothesis, Bacteria with in the stone matrix is considered to be protected from the antibiotic treatments (Li et al 2002).
Enterococci are usually associated with complex UTIs, in deseased with indwelling urinary catheter or accepting broad-spectrum anti-microbials for another desease (Dimitrov et al 2004).

The UTI causing micro-organism in young men infections are similar to pathogens that cause unfussy infections in females. *Enterococci* and coagulase-negative Staphylococci (CoNS) are more common in older men, most likely due to recent instrumentation or catheterization (Najer et al 2009).

The majority of fungal UTIs, are instigated by the *Candida* spp., and to the less extent by the *Cryptococcus neoformans* and *Aspergillus* spp. (Minardi et al 2011). *Candida* spp.'s positivity was detected in 5.0% of the urine of study from the general hospital and 10.0% of his from tertiary care center (Rivett et al 1986). Most are used by *Candida* spp. , people on a diet are particularly susceptible to fungal UTI, which has been associated with the use of fole catheters, percuataneous nephrostomy and internal stents, drains and diabetics are particulary susceptible to fungal UTI (Minardi et al 2011). Diabetics remain particularly disposed to fungal UTIs (Sobel et al 1999).

Cathter associated UTIs are usually caused by micro-organism from the local patients fecal flora or the hospital environment. *Escherichia coli*, *Enterococcus* spp., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, *Proteus mirabilis* and *Candidaalbicans* are usually associated with cathter associated UTIs (Emoris et al, 1993).

Escherichia coli
In 1885, Thedor Von Escherich who was a paediatrician and scientist first described *E.col*i in an arrangement of pioneering lessons of the intestinal flora of infants revealed a usual microbial occupant of a healthy person which he coined as *Bacterium coli commune*. The name was declared as official only in 1956 and used after, in honour (Kuhert et al 2000).
Gram negative, non-spore-forming, rod-shaped and motile bacteria s are included in the species of *E.coli*. those are approximately 2 µm long and 0.6

µm in breadth, with the cell volume of 0.6-0.7 μm^3 (Darton et al 2007). Those are facultative anaerobes, oxidase negative, ferment glucose and lactoses and sucroses, with an finest growth pH of 6.0-7.0 and at 37^oC. Though, some of the lab strains can be generated up to 49^oC (Fortadar et al 2005).

Escherichia coli strains fall into 4 phylogenetic groups shows the Phylogenetic analysis, can be termed as A, B1, B2 and D (Herzer et al 1990). Extra intestinal infections caused by *Escherichia coli* resulting prominently from group B2 & to less scope, group D. Strains of group A and B1 signify commensal strains and is mainly devoid of virulence determinants (Picard et al 1999). Group A, B1 and D typically assigns the intestinal pathogenic strain (Pupo et al 1997).

The pathogenic strain is generally classified as diarrheagenic *Escherichia coli* or Extra-intestinal pathogenic *Escherichia coli* (ExPEC) (Kaper et al2004). In these broad groups there is a group of strain recognized as pathogenic types that shares very commonest virulence factors and display alike virulence results. (Marrs et al 2005).

Disease of the intestinal tract which is caused by *E. coli* is understood as diarrheagenic *E. coli* (Nataro et al 1998). Diarrhaeogenic *Escherichia coli* strains hardly translocate gastrointestinal epithelium and pathogenic effect of them is typically bounded to pathophysiological changes in the intestinal cells. The main pathotypes of diarrheagenic *Escherichia coli* are enterotoxigenic *Escherichia coli* (ETEC), enteropathogenic *E. coli* (EPEC), Enteroinvasive *E coli* (EIEC), Enteroaggregative *Escherichia coli* (EAggEC), and Wordily adherent *Escherichia coli* (DAEC) (Natro and Kapper et al., 1998). EPEC recently are separated in to the typical EPEC (t-EPEC) as well as atypical EPEC (a-EPEC) (Trabulsi et al. 2002). Some pathogenic forms of diarrhea-causing E.coli cause gastro-enteritis but hardly causes illness out side the intestinal-tract.

ExPEC retains intact the ability to reside in the gut, but has the ability to spread, colonize, and cause disease in another host niches such as blood, central nervous- system, and urinary-tract (Wiles et al. al. 2008). ExPEC

commonly contains strains related with urinary-tract infection (UPEC), neonatal meningitis (MAEC), and bacteremia (Campos et al 2004). Among ExPECs, strains of uro pathogenic *Escherichia coli* (UPEC) are usually related with diseases of humans (Wiles et al 2008). Bacteria of these types cause community-acquired urinary tract infection (UTIs) (75.0-95.0%) and a big proportion of the nosocomial urinary tract infections (UTIs) (50.0%), accounts for substantial medical costs and also death toll worldwide (Foxman et al 2003).

E. coli strains differentiate from UPEC such that UPEC normally inhabits the gastrointestinal tract, they are very good at adapting to live within the urinary tract and the host immune response to be evaded (Foxman 2010).

2.3 Epidemiology of UTI

The occurrence of UTIs differs largely amongst the sexes, with infection occurring 14 times more frequently in females than in males (Presscott et al 2002). Prevalence of bacteriuria do have J-shaped distribution, with more number of incidence in younger age and a steady increase by age in both sexes. By age 60, the prevalence in women is considerably higher than in men (Johnson 1991). The distribution of symptomatic infections takes a slightly different shape. It is most common in women aged 15-29.(approaching 20.0%) (Foxman et al, 2003) Amongst the female aged Eighteen years & elder than 18, the projected percentage of cases was 12.6%; In case of male , this percentage was only 3.0% (Johnson 1991).

The epidemiology of UTIs in child differs by age, sex and other aspects. The number of cases of UTIs is high in the 1st year of life for most of the children (1.0%), but also decreases considerably amongst boys after the infancy (Jakobsson et al 1999).

Urinary Tract Infection's is less prevalent in male population. Males are less affected by UTI due to more distance in between anus (typical source of uropathogens) & urethral meatus, the dry environment of the male urethra, the dimension of male urethra, & the antibacterial activity of prostatic fluid (Lipsky 1989).

The incidence is increased in elderly men and women, particulary among those living institutions where it can be upto 53.0% and 37.0% in women and men, respectively (Neild 2003).

It has been projected that closely 10.0% of the human populace will experience a UTI in their lifetime (Hryniewize et al 2001).

Prevalences of UTI varies according to the season also.The occurrence of *Escherichia coli* UTIs is higher in the summer than in the winter, when temperatures are warmer and sweating is greater than in the winter, which makes urine less and more concentrated and provides more opportunities for bacterial growth. It's for Hang. Conversely, when sweating decreases in winter, urine production is diluted and the bacteria that are growing are washed away. (Nahar et al 2010).

2.4 Host defense

The urinary tract has many specialized defence mechanisms against bacterial colonization that keep the urine sterile.

The urinary-tract (ie, ureter, bladder, urethra and kidney) is a normally sterile, closed space lined with a mucous membrane composed of epithelia known as transitional cells. The vital defence mechanism against urinary tract infections is a constant ante-grade flow of urine from kidneys to bladder that intermittently completely empties the bladder through the urethra. The urinary tract of pathogens is generally cleared by this washout effect of urinary flows. (Cox et al 1961).

Urine itself also has specific antibacterial properties and this is thought to be due to its relatively acidic PH, high osmolarity, high concentration of urea, polymorphonuclear cells, and Tamm-Horsfall protein (THP), which inhibits the adhesion of bacteria to the mucous membrane of the bladder. (McCormick et al 2008).

The epithelial cells lining of urinary tract system produce and secret a number of antimicrobial peptides (AMPs) and proteins, which help to maintain the sterility of the urine through their antimicrobial action. Few AMPs is

constitutively articulated, others are expressed only when the organisms (or the tissues) is injured or unprotected to bacteria. Defensins, cathelicidin, THP, lactoferrin, and lipocalin are antimicrobial protein occupied in the bacterial defence of the urinary tract (Zasloff 2007).

The Abundant protein is THP present in normal human urine, synthesized in the epithelial cells of Henle's loop's ascending limb and in proximal area of distal tubules (Jelakovic et al 1996). THP coats epithelial cells whose major role is to bind type 1 fimbriae to stop bacteria from attaching to the epithelial cells (Pak et al 2001).

The mucosal surface of renal epithelia that is protected by the secretions contains an array of host defence factors which includes immunoglobulins, in which immunoglobulin A (IgA) is a major class (Rice et al 2005).

Infected superficial facet cell layer's exfoliation of the urinary tract starts within hours of interaction and eases the removal of the diseased cells to the urine (Mulvey et al 1998).

Cytokines produced by the bladder mainly through stimulation of the host toll like receptor 4 (TLR4) by bacterial lipopolysaccharides (LPS), that recruit polymorphonuclear neutrophils (PMNs) to help in the removal of bacteria (Hunstad et al 2005).

Lactobacilli in the vagina are protective because they prevent initial colonization with uropathogens (Gupta et al 1998).

2.5 Risk factors

Due to the short distance between the anus and urethra and a short urethra emptying the bladder, females are at higher risk. Extreme age condition, female gender, bladder catheterization, nephrostomy tubes, previous antibiotic administration, diabetic situation, mechanical obstruction and anatomical abnormalities which promotes urinary statis (e.g., Neurogenic bladder, pregnancy, kidney stones) are the risk factors of UTIs (; Nicolle et al 2009).
The Risk factors for Urinary Tract Infection among females varies by age group. Birth defects and new sexual behaviours are common risk factors in

school-age girls. Risk factors for pre-menopausal females are a history of the UTI, recurrent sexual activity and diaphragm contraceptions use, obesities, sickle cells trait, anatomic congenital abnormality, urinary-tract calculi, neurologic disorder or medical condition requiring indwelling, use of spermicidal agent, increasing parities, diabetes mellitus or repetitive bladder catheterization. In post-menopausal female, common risk aspects includes viginal atrophy, emptying unfinished bladder, underprivileged perineal hygiene, rectocele, cystocele, urethrocele, and uterovaginal prolapse, history of UTIs and type1 diabetic condition mellitus (ACOG practice bulletin, 2008). In women rate of Urinary Tract Infections increases with advancing age, most probably due to the hypoestrogenic state, vaginal epithelium atrophy, decreased voiding, and alteration in the hygiene (Cunningham et al 2005).

Risk factor related with UTIs in healthy male including inter-course with diseased women , homosexuality and absence of circumcision though these factors do not exist in male with UTIs (Mehnert-Kay et al 2005). Healthy young men infected by uro-pahogenic strains have a tendency to be extremely urovirulent (Hooton 1997).

2.6 Virulence factor of *Escherichia coli*

UTIs in healthy hosts cause by the bacteria mostly exhibit distinctive properties recognized as virulence factors-to overcome the usual defences of the urinary system.

Specialized virulence factors in the UPEC strains, enable them to colonize and invade the host, disturb the host defense mechanism, hurt host tissues and/or stimulate a noxious host inflammatory response. UPEC do have the ability to grow extraintestinal which might make them able to cause a variability of diseases, not only UTIs (Zhao et al 2009).

In *E. coli*, virulency is the consequence of cumulative effect of some characteristic or virulency factor (VF), which distinguishes potential pathogens from harmless enteric strains (Johnson 1991b). In a given infection, the individual virulency of the individual strain is calculated by the actual existence of virulency genes present in and also by ecological conditions in the

host (Sharma et al. the 2007).

Adhesion of bacteria to mucosal surfaces of the host is often the critical 1st step in the infectious procedure. This is particularly correct in the condition of urinary tract infection (UTIs) (Svanborg et al 1983). UPEC is able to start infection in the urinary tracts is more likely linked to the expression of adhesive organelles recognized as pili or fimbriae which interacts with the protein on the urinary epithelial cell (Hunstad et al 2005). UPEC elaborates common adhesive organelles like type 1, P, S and F1C pili en-coded by the fim, pap, sfa and foc operons, correspondingly (Wiles et al 2008). Expression of different pili during urinary tract infections appears to likely be coordinated procedure, with E. coli predominantly expressing 1 type of pili at one time (Nowicki et al. 2005). Type 1 and p pili are 2 of the most studied adhesive organelles that are en-coded by more UPEC strain (Lane2008). About 80.0% of UPEC expresses p pili that anchors to the glycolipid of he outer membrane of urothelia cells present in the kidney (Plos et al 1991). P pili are produced by pyelonephritis strains and are oftenly related with condition of acute pyelonephritis (Minardi et al 2011). In the strain which causes cystitis type 1 fimbriae is periodically articulated and the infections are limited to bladder (Connell et al 1996). S fimbriae & F1c fimbriae are exposed to bind to epithelial & endotheila cell from the lower urinary tract and kidney. (Marre et al 1990).

It's been shown that some strain of E. uropathogenic coli exhibit flagella. The flagellates are complex organelles up to 15 µm in length that contributes to bacterial motility (Romos et al., 2004). Flagstone motility and swarm cell differentiations contributes to the virulency of other urinary pathogen, Proteus mirabilis (Allison et al., 1994; Harmon et al., 1989). On the other hand, the role of flagellate in the entry of E into the urinary-tract. E. coli seems to be of secondary importance (Wright et al. 2005).

UPEC was also able to demonstrate the adhesion of Dr. Adhesives binds to the type IV collagen & breakdown accelerating factors (DAF, also CD55) in the kidney. Physician adherence is essential in the development of chronic

pyelonephritis in an experimental model (Goluszko et al. 1997).

An acidic polysaccharide capsule produced by UPEC strains that protects the microbes from phagocytosis by human polymorphonuclear neutrophils (PMNs) and inhibit complement activation (Johnson 2003).

Alpha hemolysin (HlyA) is a pore forming toxin released by *Escherichia coli* (Cavalieri & Snyder 1982). It is principally responsible for the lysis of erythrocytes. Adding to erythrocyte lysis, hemolysin toxin is toxic to a wide range of the host cells, in some ways which may contribute and add up to inflammations, impaired host defenses and tissue damage. Kidneys damage in pyelonephritis can also be caused due to toxic activity as above. About half of the UPEC stains causes upper UTIs, about one-third of which causes lower UTIs, and also about 10% of those fecal isolates produces HlyA (Slavchev et al. 2009)

Cytotoxic necrotizing factor (CNF-1) is encoded by chromosomally *cnf1* (Johnson1991). CNF-1 targets GTP-binding protein of Rho family and induces actin skeleton reorganization, that leads to apoptosis, which also eases bacterial invasion of deeper layer of tissues of the urinary tract (Mills et al. associates 2000). This process allows bacteria to survive in the urinary tract (Rippere-Lampe et al. 2001).

Aerobactin, a bacterial frontier, has recently been implicated in strains of *E. coli* causing pyelonephritis and cystitis (Carbonetti et al. 1986). *Escherichia coli* is allowed to grow in iron-poor environment like as dilute urine and complement-poor serum due to iron transport and absorption system. The aerobactin system has been implicated in strain of *Escherichia coli* due to serious UTIs and other serious infection in animals and human beings, possibly as this promotes the bacterial growth in high concentrations. iron thresholds encountered during infections (Slavchev et al. 2009).

Urease is considered a major virulence factor for UTIs. Urease from Proteus mirbalis and Klebsieall spp. is also considered to be a uropathogenic factor promoting the persistence and formation of kidney stones (Podschun et al.

1993).

Other Enterobacteriaceae including Klebsiella.and the genus Proteus. and Providencia stuartii have been shown to express pili important for both attachment to the urothelium and attachment to urinary catheters (Mobley et al 1987).Adherence to the uroepithelial cell and production of urease is reported to play an significant part in the persistent development and intrusiveness of *Staphylococcus saprophyticus* in the bladder (Hell et al 1998).

2.7 Pathogenesis

UTI is often caused by the normal symbiosis of the adjacent sites and distal urethra. The commonest routes of infection is climbing. Urinary tract pathogens are portion of the common faecal flora. The perianal area is colonized by these bacteria and after that rise to the female vaginal opening, a reservoir for several urinary tract pathogens. In the periurethral region the Colonization spreads, bladder and urethra, strongly depends on the sexual activities (Franz and Horl, 1999). The facilitation of UPEC colonization is possible because UPEC's ability to bind the host tissues in the urinary-tract, allow bacterias to resist the flow of large volumes of urine and allowing UPECs to adhere to urothelial cells. promotes the invasion of Once in the urinary-tract, UPEC preferentially colonize bladder & cause cystitis. However, it can rise through the ureters to the kidney and cause pyelonephritis. Few strategies has been developed by UPEC for the evasion of those innate immune responses, which allows the colonization of pathogen more efficiently and persist in the urinary-tract (Wiles et al. 2008).

2.8 Routes of Infection

Micro-organism could reach the urinary tract by mode of ascending, hematogenous, or lympatic ways.

2.8.1 Ascending route of infection

Generally bacteria infects the urinary-tract by ascending route from urethra. Is The complex process which is related with the bacterial adhesion, virulency,

and also motility possessions and host anatomic, genetic factor and humoral is ascending infection of the urinary tract (Zorc et al 2005). The ascending route accounts for almost 95.0% of cases for UTI (Goldman et al 2000). This is particularly common for *E. coli* and other Enterobacteriaceae (Nadi et al 2006).

2.8.2 Hematogenous route of infection

Endogenous urinary tract infections are limited to a few relatively rare uropathogens, such as yeast (usually Candida albicans), Mycobacterium tuberculosis, Salmonella spp. and Staphylococcus aureus, which cause primary infections elsewhere in the body. Candia albicans readily cause hematologic clinical urinary tract infections but is also an infrequent cause of progressive infection with catheterization or after antibiotic therapy (Grabe et al 2008). The hematogenous spread usually occurs as a result of bacteremia and accounts for only less than 5.0% of UTIs (Forbes et al 2002).

2.8.3 Lymphatic route of infection

In pathogenesis, uropathogen's status of lymphatic spread to urinary-tract is unknown (Hooton 2000).

2.9 Clinical presentation

Urinary tract infections have been traditionally considered acute infections and are usually self-limited. Though, this concept has been challenged by newer evidence that acute bladder infections are the consequence of complex series of host pathogens interactions that leads to the bacterial entry & survival. bacteria, ultimately determining the time course of infection. (Schilling et al., 2002). Generally, UTI also can be classified as asymptomatic bacteriuria, cystitis, or acute pyelonephritis. Cystitis is mainly associated with bladder invasion (Gunther et al 2001).

Fever, burning sensation when urinating, abdominal pain in lower area, itching, ulcers and blisters forming in genital area and pain in the genitals & suprapubic bone, and pyuria usually depends on the oldness of the infected person and where the urinary-tract are located are few symptoms of UTIs

(Amali et al 2009).

In asymptomatic urology, significant bacteriuria is often asymptomatic and requires treatment only in pregnant women, infants, and before urological surgery.(Neild 2003).

Almost 95.0% of all UTIs are cystitis, happening in anatomically typical people and characterized by visit micturition, inadequate voiding, suprapubic torment and burning sensations, (Muhldorfer et al 2001).

Dysuria, frequency, urgency and tenderness and pain over the area of the bladder are generally reported in patients with cystitis. In some individuals, the urine is often cloudy & malodorous, and it is bloody (Forbes et al 2002).
The most severe upper urinary tract diseases acute pyelonephritis includes the kidney's colonization and signifies an infection capable of progressing to bacteremia (Gunther et al 2001). The typical clinical presentation includes fever and flank pain &, commonly, lower tract symptoms (frequency, urgency, and dysuria). Patients may also show signs of systemic infection such as vomiting, diarrhoea, chills, increased heart rate, and pelvic pain. Of significance, 40.0% of patients with acute pyelonephritis are bacterimic (Forbes et al 2002).If pyelonephritis is left untreated might results in renal failures, bacteremia and sepsis (Mehnert-Kay 2005).

2.10 Treatment

The treatment of UTI may varies according to patient age, sex, associated disease,infectious agent and whether the problem is in upper or lower urinary tract (Arslan et al 2005). The treatment of UTIs is commonly uses a broad-spectrum antibiotics a different one in which one being a narrow-spectrum of the activity that might be suitable due to concerns about the infections with the resistant organism (Dimitrov et al 2004). The uncomplicated UTIs are treated often using the antimicrobial agent which contains sulfamethxazole drug and trimethoprim drug combinely, trimpehotprim, beta lactamas, fluoroquinolone, nitrofurantoin and fosfomycin trimethamine (Jancel et al 2002). These agents are mainly used due to The tolerability of these agents and also their spectrum

of activities against the suspected uropathogens, and promising phamacoketntic profile (Neu 1992).

a. Beta lactams

Within the past, beta lactam anti-microbial such as to begin with era aminopenicillins (ampicillin, amoxicillin) and the cephalosporins (Cephalexin) were usually utilized to treat UTIs (Jancel 2002). In spite of the fact that the primary era cephalosphorins and aminopenicillins accomplishtall urinary concentrations, they are now notprescribed as to begin with line treatment for UTIs since of resistance and tall repeat rates compared to with other operators. In any case, in certain setting, such as amid pregnancy or when enterococci are suspected, ampicillin or amoxicillin may still be an suitable choice for intense UTI (Warren et al 1999). These specialists ought to be utilized as it were on the off chance that a pee culture archives defenselessness. Cefpodoxime and Cefixime are the cephalosporin of the third generation which offers the significant advantages of longer half-lives, permitting for less doses visits.The Lower *E. coli* rates of resistence were too watched with these actives in comparision to the aminopenicillins and cephalosporins of 1st generation (Jancel et al 2002).

b. Trimethoprim-sulfamethoxazole

The standard treatment in the long time for intense & repetitive urinary tract contaminations is considered to be Trimethoprim-sulfamethoxazole since the action of Trimethoprim-sulfamethoxazole against foremost usual uropathogens and its moo taken a toll and also tolerability. Trimethoprim and sulfamethoxazole's synergistic combination does work at bacterial folate digestion system's two isolated steps, coming about within the hindrance of DNA blend (Jancel et al 2002). Utilize of trimethoprim-sulfamethoxaxole is these days constrained since of far-reaching microbial resistance and ought to as it were be utilized in districts with a recognized moo rate of resistance (<20.0%) affect-ability testing, and after culture (Bean et al., 2008). Be that as it may, in nations where resistances is moo, trimethoprim sulfamethoxazole still can be a substantial first line anti-microbial (Minardi et al 2011).

c. Nitrofurans

Nitrofurans are a gather of compounds categorised by the occurrence of one or more nitro groups on the nitroaromatic or nitro-heterocyclic backbone. Furanzolidone, nitrofurazone and nitrofurantoins are compounds have its place to this class and they all exhibit antibacterial activity and are used clinically to treat various types of infection (Sandegren et al 2008). Nitrofurantoin is the actually first effective and safe antibacterial therapy for UTIs, but activity's spectrum is bounded (Nickel 2005). Another study by Hooton (2003) has shown that the use of nitrofurantoin does not share cross-resistance with more commonly prescribed antibiotics and its wider use is warranted. from a public health point of view as a fluoroquinolone-sparing agent. Nitrofurantoin is administered orally, rapidly absorbed and excreted in the urine to produce therapeutically high concentrations (Conlink 1978). Nitrofurantoin, which includes nitrofurantoin microcrystals and nitrofurantoin monohydrate macrocrystal, is still effective 50 years after its widespread use, for the treatments of UTIs (Warren et al 1999). Nitrofurantoin can be used safely during pregnancy and is not tetragenic (Christensen 2000). However, its use in late pregnancy should be avoided, because of the risk of fetal hemolytic anemia in patients with G6PD deficiency (Sivick et al. 2010). Occasionally, nitrofurantoin can cause acute and dangerous side effects to the lungs. For the treatment of upper UTI is not recommended and is contraindicated in individuals with impaired renal function, as accumulation of metabolites may induce neuropathy (Spring et al. 2001). In comparative studies, nitrofurantoin had a slightly lower cure rate than trimethoprim/sulfomethxazole or fluoroquinolones (Iraveniet et al. 1999). Against non *Escherichia coli* gram negative rods, Proteus and Pseudomonas spp. nitrofurantoin was not that very active (Gupta et al 2001).

d. Fluoroquinolones

The synthetic antimicrobial agent groups form Quinolones which contain nalidixic-acid and fluorinated quinolones (Tavio et al 1999). The treatment of a gram-negative bacilli infection is done by using Fluoroquinolones which are

linked structurally to the acid known as nalidixic amd are strong broad spectrum antibiotics. The fluoroquinolone is used clinically in extensive way due to good oral absorb-ability, overall tolerability and a broad-spectrum of activity. The overview of fluoroquinolone in clinical practices has been related with increased cases of quinolone resistant bacteria, particularly amongst gram-negative bacilli like as *Citrobacter* spp., *Salmonella* spp., *Klebsiella pneumoniae, Serratia marcescens and Pseudomonas aeruginosa, and S. aureus* and(Eom et al 2002). Though, the resistances of *Escherichia coli* to the quinolones remain rare until 1990s; later then the wide spread uses of fluoroquinolone (like as norfloxacin and ciprofloxacin) for the treatment of UTIs is implicated with emergence of resistant strains (Garau et al 1999). The first fluoroquinolone were named norfloxacin, levofloxacin, ciprofloxacin and ofloxacin which were used widely used for the treatment of UTI, (Gupta et al., 2001). Fluoroquinolone are contraindicated in pregnancy because of potential adverse effects of fetal cartilage (Christensen 2000). Although quinolones are highly effective against major micro-organisms of the genitourinary tract, especially in vitro, their clinical features have not demonstrated their advantage in eliminating infections over other agents. In addition, the higher incidence of adverse events compared with other medicines and their low acceptability limit the use of second-line quinolones (Minardi et al. 2011). Furthermore, investigations carried out in many European countries have shown the prevalence of drug resistance of *Escherichia coli* strain to acid known as nalidixic and derivative of them (more than 10.0% and up to 32.6). % in Hungary)(Schito et al 2009).

Higher urinary concentration (>100 times the peak plasma level) enables renal parenchyma to be penetrated efficiently are enabled by those antimicrobial agents, therefore fluoroquinolone are considered operative agent for culture explicit and empiric treatment of both uncomplicated and complicated Urinary Tract Infections. Other benefit of fluoroquinolone over antibiotic agent is their tissue lipophilic possessions. These physochemical properties allow outstanding drug penetration in to the prostate-gland (considered to be 2-4 times the serum level) (Emo et al 2002).

2.11 Prevention of UTI

Patients are to be well-thought-out for preventive measure incorporate ladies, encountering repetitive UTIs, children with auxiliary variations from the norm of the urinary tract or repetitive UTI, patients with spinal rope harm or neurogenic bladder, and patients after renal transplant (Stapletom 2003).

2.12 Antibiotic resistance

Drug resistance is an endless procedure in nature through which organisms can develop tolerance to new environmental conditions. It might be because of pre-existing factors found in the organisms or resultant of acquired factor(s) (Manikandan et al2010).

The human gastrointestinal tract is an critica supply of anti-microbial resistance qualities, which contribute to the support and dissemination of resistance within the environment (Shoemaker et al 2001). The enteric microbes in fecal vegetation are frequently detailed to be exceedingly safe and *E. coli* is detailed to be primary carrier of antimicrobial resistance (Osterblad et al 2000).

The resistance of Antimicrobial organism is presently recognized as an progressively worldwide issue which was watched for the primary time in *E. coli* in 1940 (Ahmed et al 2000).

The predominance of antimicrobial resistance with UTI is expanding and changes agreeing to topographical and territorial area (Khan et al 2006).

There's a slow expanding anti-microbial resistance in nosocomially and communities both obtained UTIs that causes uropathogens. Indeed in ladies with intense the uncomplicated UTIs expanding resistance to co-trimoxazole (15.0-20.0%) , ampicillin (30.0-40.0%) and cephalothin (20.0-30.0%) and is illustrated in causative *Escherichia coli* (Gupta et al 1999).

To most of anti-microbials and chemotherapeutic operators, more of *Escherichia coli* strain was regularly delicate but in later a long time resistance has been experienced in numerous cases. Towards cephaloridine, cephradine,

cephalizolin, tetracycline, fluoroquinolone and gentamycin most of the segregates appeared the resistance (Hameed et al 1995)

MDR bacteria refer to those which are resistance to a vast range of antibiotics with structural independence (at least two or more antibiotics) (CDC 2006). Nowadays, a big concern among the medical and clinical practitioners is the emerging MDR organism and their associated complications in developing world. These conditions make the treatment more challenging and many even threaten the respective patients lives (Farshad et al 2010). *Shigella* was the first organism observed to show resistance to multi drugs (Tenover et al 1996). MDR has been demonstrated in *Escherichia coli, Salmonella enteria serovar Typhimurium, Shigella dysenteriae, Enterococcus faecium, Staphylococcus aureus, Mycobacterium tuberculosis, Stenotrophomonas maltophilia, Xanthomonas, Burkholderia Haemophilus influenza, Pseudomans aeruginosa, Klebsiella pneumoniae and Acinetobacter baumanni,* (Dizidic et al 2008).

Damage file may be a instrument that uncovers spreads of microbes resistance in a provided populace (Krumpermann 1983). A Damage record more noteworthy than 0.2 infers that the strains of such microscopic organisms begin from an environment where a few anti-microbial are utilized (Ehinmidu 2003).

Multiple factors have had led to the occurrence of antibiotic resistance in micro-organisms (Ahmed et al 2000)

a. The wide utilisation of anti-microbial due to tall predominance of irresistible diseases
b. A deficiency of physician
c. Selective endorsing due to taken toll limitations and the weight of pharmaceutical companies' limited time activities
d. Lack of research facility bolsters in rustic areas
e. The troubles in disseminating data with respect to anti-microbial resistance.

CHAPTER-III

MATERIALS & METHODS

3.1 The Materials

The materials, types of equipment, media & reagents that are used here in the study are systematically listed in Appendix I.

3.2 The Methods

3.2.1 Study design

The study conducted was based on the hospital and was a cross-sectional study.

3.2.2 Study site and duration

This study which is cross-sectional was there conducted in Suraksha Hospital Pvt. Ltd for the duration of about 6 months.

3.2.3 Study duration

A cross-sectional and desciptive study was there conducted in January-June 2021' at Surksha Hospital located in Biratnagar, Nepal. The patients referred by doctors as suspected UTIs for culture to the laboratory were the target population in this study.

3.2.4 Laboratory setting

The laboratory setting was done in the Suraksha Hospitals, Department of Microbiology Laboratory and Annapurna Research Center.

3.2.5 Study Variables:

Study variables included types of bacterial pathogens, age, sex, antibiotic-resistance pattern, and in *E. coli* isolates from the clinical samples.

3.2.6 Study population:

The study population for this study was both inpatients (admitted to different wards), and outpatients attending hospital.

3.2.7 Sample size:

The general formula had been used to calculate the sample size assuming the theoretical prevalence rate of 0.5 to avoid the possible bias due to sample size.

Sample size (N) $= 4pq/r^2$

$= 4x0.5x0.5/0.05^2 = 400$

Therefore, estimated 400 clinical samples were included in this study.

3.2.8 Criteria for Sample Selection

All clinical samples from the individuals referred by the physician for culture and Antibiotic susceptibility testing in Microbiology laboratory were included. Inadequately labeled and contaminated samples were rejected.

3.2.9 Data collection

Data on age wise, sex wise and etnic wise of patients were collected from culture and sensitivity request form.

3.2.10 Sample collection and handling

In a sterile, leak-proof, dry, wide-necked and plastic container which was labelled properly, 10-20 ml of clean voided (clean-catch) first morning mid-stream urine was collected which was then processed without delay.

Catheterized specimens or supra-pubic aspirates were collected with the assistance of a clinician from infants and patients who were unable to produce clean-catch mid-stream urine specimens because of urologic or neurologic problems including impaired consciousness. This specimen was processed without delay (i.e. within two hours)

3.2.11 Macroscopic and Microscopic Examination

Specimens obtained were examined macroscopically for its color and turbidity and reported accordingly thereafter observed microscopically by direct Gram stain smear for pus cells, epithelial cells and bacterial cells in relevant specimens.

3.2.12 Culture of specimens

All clinical specimens were inoculated into CLED agar plate. CLED agar plate

was incubated at temperature 37°C for about 24 hours aerobically.

3.2.13 Isolation of the pathogens

Culture plates after incubation were observed for presence of growth. Any significant growth obtained on the primary plates was observed for colony characteristics and was first sub-cultured to obtain pure culture for further processing.

3.2.14 Identification of the isolates

Biochemical tests and gram staining was also completed for identification of *Escherichia coli*. In gram staining, these were seen as Gram negative rod shaped.

A different set of biochemical tests was done for identification of the isolated Gram-Negative bacteria. The pure form of the culture was obtained from the primary culture medium and then it was preceded for biochemical tests. SIM, TSI, Methyl red-test, Voges Proskauer (VP) test, Citrate test Indole and Urease test were performed & the pathogens were identified. The identifying biochemical characters of *E. coli* attached in Appendix III.

Culture plates after incubation were observed for the existence of growth. Any significant growth obtained on the primary plates was observed for colony characteristics and were first sub-cultured to obtain pure culture for further processing.

3.2.15 Antimicrobial susceptibility testing

The antimicrobial defencelessness testing of *E. coli* confine was performed by a modified Kirby Bauer disc diffusion method utilizing a commercial disk (HiMedia, India).

A loopful microscopic organisms were taken from a colony when pure culture was obtained and also was exchanged to the tube that contained 5 ml peptone broth and blended gently to get regular suspension. The suspension's turbidity was at that point then balanced to density of McFarland 0.5 in order to homogenize inoculum size.

Then the sterile cotton swab was dipped into the suspension and by gently rolling the swab excess were removed over the tube's surface. Then, bacteria

were distributed evenly over the surface entirely of Muller Hinton's agar (HiMedia, India). Inoculated plate was left for 3-5 min at room temperature and with sterile forceps placed antibiotic plates of the following concentrations on the surface of Muller Hinton Agar (HiMedia, India): Ampicillin (10 mcg), cephalexin (30 mcg), nalidixic acid (30 mcg), ciprofloxacin (5 mcg), ofloxacin (5 mcg), and cotrimoxazole (1.25/23.75 mcg) norfloxacin (10 mcg) and nitrofurantoin (300 mcg) and incubated in 18-24 hours at 37°C. Observation of the zones of inhibition were noted and the measurement of the zones of inhibition were noted and considered to be diameter and noted in millimetres (mm). According to the standard area size interpretation manual of manufacturers the measurement as sensitivity (S) resistance (R) were interpreted.

Percent resistance calculation was done by use of formula **PR = a / b × 100**. Where "**PR**" is the percent of resistance, "**a**" being a number of resistant isolates, and "**b**" being the number of subtypes tested with antibiotics. Percent susceptibility calculation was done by the use of formula **PS = c/d × 100**, where "**PS**" is the percent of susceptibility, "**c**" is the number of susceptible isolates, and "**d**" being the number of isolates tested with antibiotics.

The result was taken according to the newer version of CLSI. The resistance to 2 or more classes of the antimicrobial tested was defined as MDR.

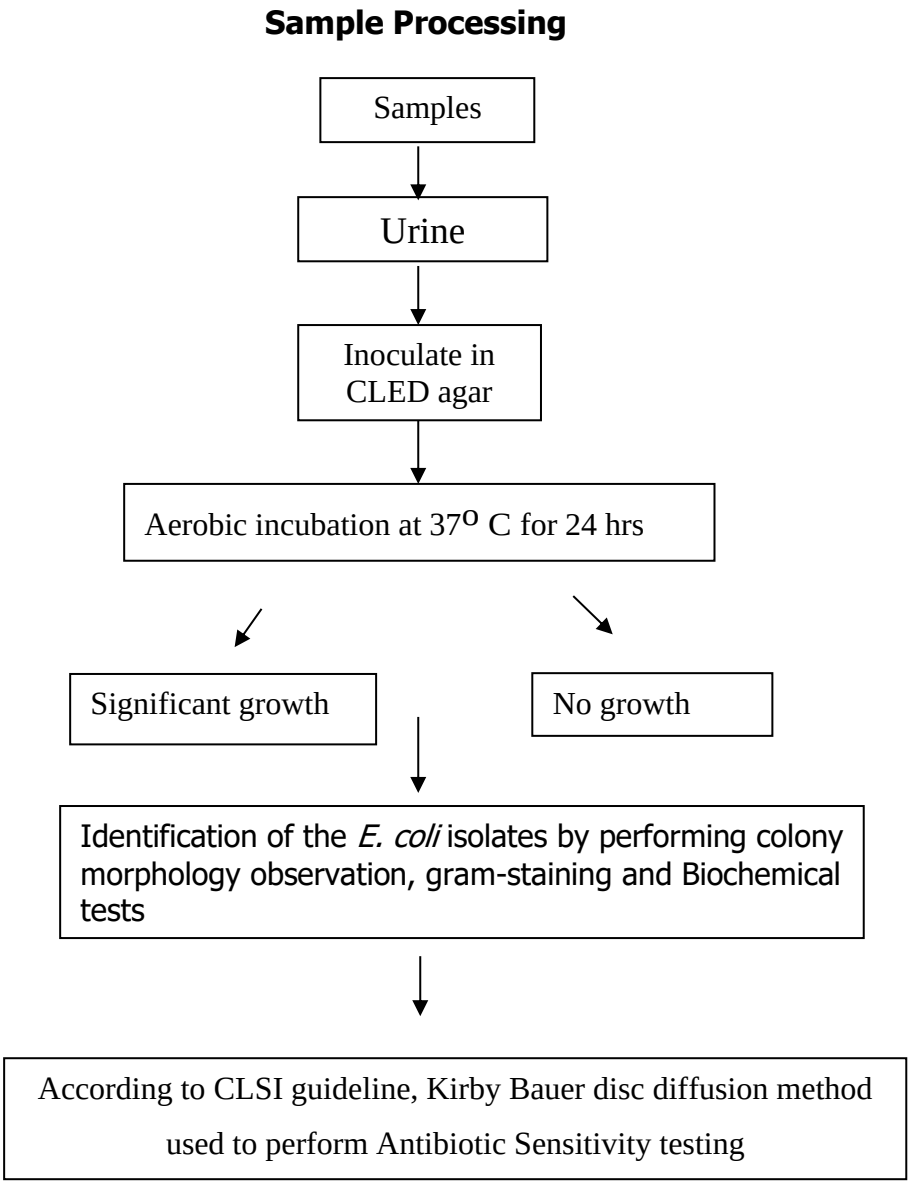


Figure II: Flow diagram of Processing of samples

3.2.16 Data management and analysis

All raw data that are found from the lab investigation was presented in tabulation format in tables defined to discover the major findings. The data was statistically scrutinized by Chi-square (χ^2) test at 5.0 % level of significance by entering the data in the computer-based PASW (The Predictive Analytical Soft Ware), version 18.0, the premier vendor for (Statistical Package for the Social Sciences) program. A p-value of **0.05 or** less is considered statistically important **($p \leq 0.05$).**

CHAPTER –IV

RESULTS

The study was conducted at Suraksha hospital Pvt. Ltd. A total 400 different sample were collected patient visiting as well as admitted patient in the hospital.

4.1 Study Population

The study was done in Suraksha Aspatal Biratnagar Nepal. Study Period was from January 2021 to June 2021 in this study all the sample was cultured for growth of *Escherichia coli*. Higher number of *E. coli* was isolated in female patient. The study was composed of 325(81.25%) female and 75(18.75%) male populations.

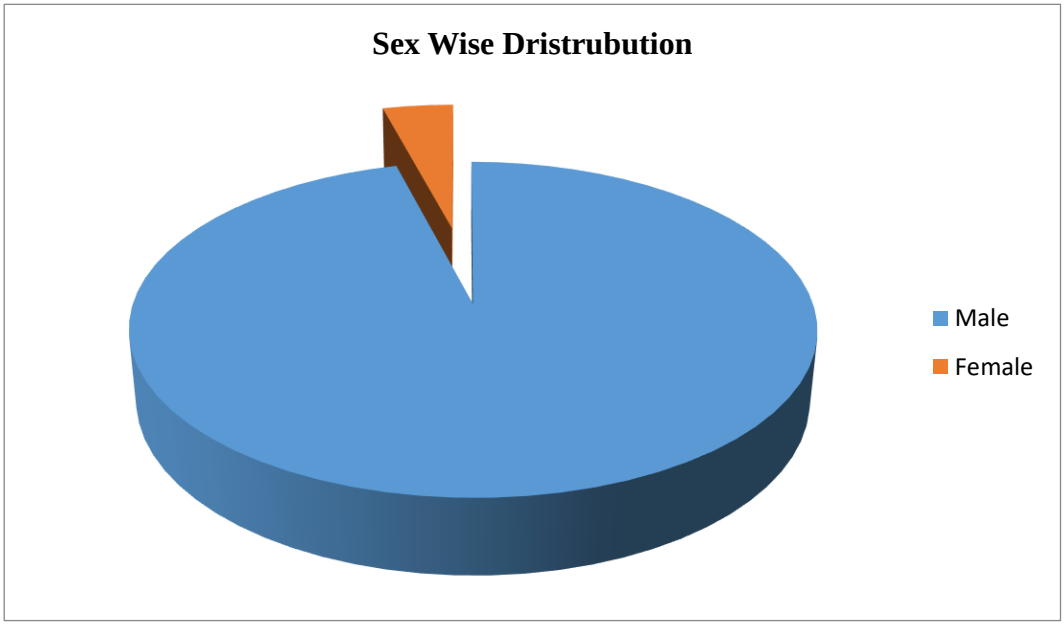


Figure: 4.1 Study Populations

4.2 Culture positive rate in urine samples

Of a total of 400 urine-samples, 195 (48.75%) samples displayed noteworthy growth, while majority of samples' i.e., 102(25.5%) displayed no growth, 97(24.5%) displayed non-important growth, and out of the total, only 6(1.5) samples exhibited growths in mixed ratito.

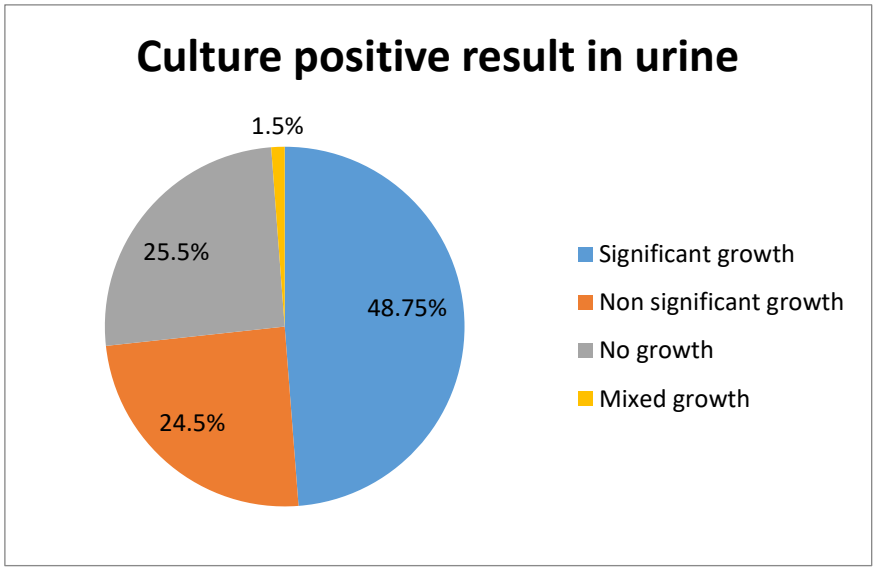


Figure 2: Culture positive rate in urine samples

Table 4.1:Spectrum of uropathogens isolate from urine sample

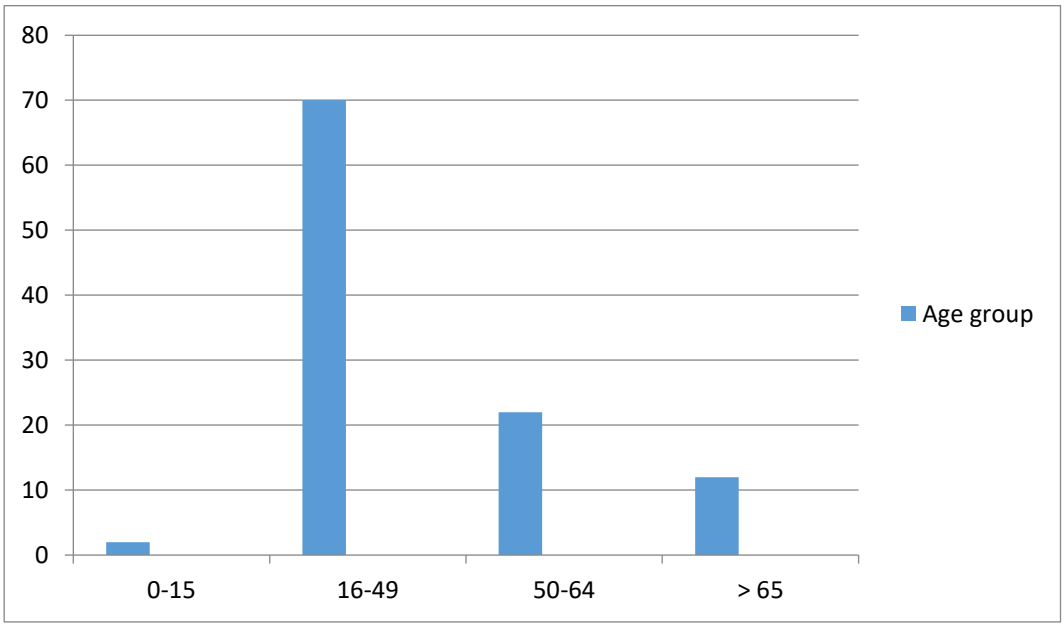
Uropathgens	**Total isolates**	**Percent**
Escherichia coli	109	55.8
Coagulase negative Staphylococci(CoNS)	25	12.8
Enterococcus fecalis	8	4.1
Pseudomonas aeruginosa	12	6.1
Serratiaspp.	4	2.0
Candida spp.	5	2.56
Klebsiella pneumonia	24	12.3
Staphylococcus aureus	4	2.0
Citrobacter spp.	2	1.0
Enterobacter spp.	1	0.5
Salmonella Typhi	1	0.5
Total	**195**	**100.0**

4.3 Age Wise Distribution of Uropathogens

The distributions of *Escherichia coli* was found to be the commonest in age cluster of 16 years to 49 years.

Escherichia coli is the commonest uropthogen that are isolated from female population compared to male population and it is statistically noteworthy ($p<0.05$). Isolation of CoNS is commonest amongst the male population; whereas, it is statistically insignificant ($p>005$).

Table 4.2: Age wise distribution of *Escherichia coli* isolates from sample of urines

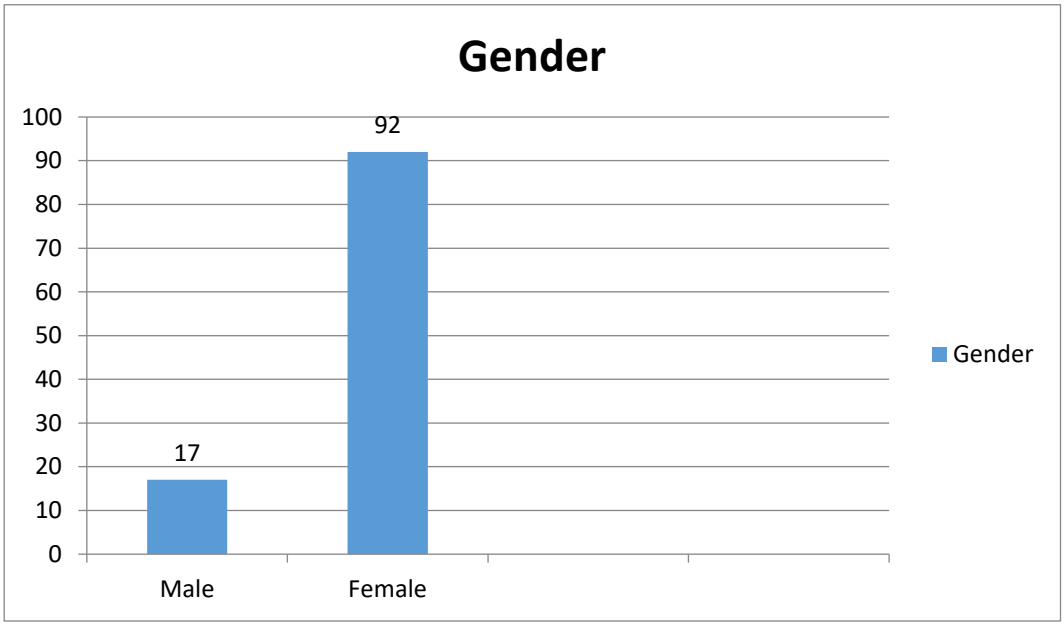


4.4 Sex wise Distribution of Uropathogens

The distribution of *Escherichia coli* was detected to be most common in females.

E. coli is the commonest uropathogen isolated from the female population compared to male population and it is statistically vital ($p<0.05$). Isolation of CoNS is commonest amongst the male population; whereas, it is statistically insignificant ($p>005$).

Table 4.3: Sex wise distribution of *E. coli* isolates from urine samples



4.5 Antibiotic susceptibility pattern of *E. coli*

The majorities of *Escherichia coli* displayed susceptibility to nitrofurantoin (94.5%) trailed by ciprofloxacin and followed by ofloxacin with a susceptibility of about 50.5% for each one. Cephalexin (7.3%) was the drug to be least effective trailed by ampicillin (18.3%). Co-trimoxazole, Norfloxacin and nalidixic acid was also found to be only effective in lesser than halfer of *E. coli* isolate.

Table 4.4 : Antibiotic susceptibility patterns of *Escherichia coli*

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin	20 (18.3)	89 (81.7)
Cephalexin	8 (7.3)	101 (92.7)
Nalidixic acid	23 (21.1)	86 (78.9)
Ciprofloxacin	55 (50.5)	54 (49.5)
Ofloxacin	55 (50.5)	54 (49.5)
Norfloxacin	51 (46.8)	58 (53.2)
Co-trimoxazole	50 (45.9)	59 (54.1)
Nitrofurantoin	103 (94.5)	6 (5.5)

CHAPTER-V

DISCUSSION

This particular study was conducted amongst patients suspected of UTIs, visiting Surksha Hospital, Biratnagar, Nepal. Four hundred midstream urine samples subjected to culture and 109 were *E. coli. E. coli* were further subjected to antimicrobial susceptibitiy testing.

In this study, 48.75% of urine samples from patients with suspected UTI showed noteworthy growth. The outcomes were consistent with reports by another researchers from country Nepal (Chhetri et al. 2008) and the rest of the world (Bashar et al. 2009). The larger part of urine samples showed no development (25.5%). This might be because the patient has been treated with antibiotics that inhibit or kill the growth of bacteria(Okonofua et al 1989), or slow growing organism and those organisms that was unable to grow on the routine-culture media used (Kattel et al 2008). Dimitrov et al., 2003 found that only 24.5% urine specimens yielded significant growth of uropathogens. Similarily (Tsegaye et al2011). Reported low rate of significant growth of uropathogens from urine samples. However, Saeed and Mohameed et al 2010 and Arjunan et al 2010 found 55.8%, 64.2% and 47.5% significant growth of uropathogens, respectively.

In this study, the most predominant organism isolated among UTIs patients attending Surksha Hospital were *E. coli* (55.5%). The study shows that the most common isolates was *E. coli* (55.5%), CoNS (12.1%), *Enterococcus fecalis* (4.1%), *Pseudomonas aeruginosa* (4.0%) and *Klebsiella* spp. (12.6%) in a study from Biratnagar.

In our study, *Escherichia coli* were isolated as the major isolates and reported for 55.5% of the total uropathogens. This finding agreed with other study done by Kattel et al 2008 in Nepal. This result do agrees with other studies conducted at international level that specified the conclusion that the gram negative bacteria mostly *Escherichia coli* was the most common bacteria isolated in people with UTIs (Ahmed a et al 2011). However, this varies from

the report of Ehinmidu, 2003 and Aboderin et al, 2009 which did report *Pseudomonas aeruginosa* and *Klebsiella* spp., correspondingly as the major bacterias. Whereas,; Bobos et al 2010 and Hryniewicz et al 2001 did still report more high incidence of *E. coli* 71.3%, 73.0% and 76.8% respectively in urine sample.

In the current study 55.5% of the micro-organisms were *E. coli*, but there was a significant difference between males and females: among males only 24.5% of the pathogens were *E. coli* compared to 81.5% among females ($p<0.05$). The incidence of CoNS in our study is high 12.1 However, the incidence of *Klebsiella* spp. in our study is low 12.8%, with predominance in men at 4.4% vs 2.3% in women ($p>0.05$).

In contrary to other studies finding where 2nd most reported isolates were *Klebsiella* spp. (Bahandin et al 2011), in this study was CoNS which is in agreement with the finding of Enayat et al 2008 and Zia and Hassan Shahi, 2010. Our conclusions demonstrated that these pathogens plays vital role in UTI and 21.3% of UTI in our study were caused by coagulase negative S*taphylococcus* (CoNS) and there was no important differences between male and female ($p>0.005$). The frequency of this type of UTI in our study was not sex dependent.

Coagulase negative Staphylcocci (CoNS) was the second most commonest pathogen in our study, whereas *Enterococcus* spp. was the 3rd most cause of UTI. Al Benwen et al 2010 did report that *Streptococcus agalactiae* as second commonest isolates after *Escherichia coli* in the study done by them on 56,506 sample of urines. Khan et al 2001 did report *Candida* spp., the 2nd commonest isolates after *Escherichia coli.* Factors like the changing patient population, more extensive use and misue of antimicrobial agent would contribute to the change in the bacterial profile of UTIs (Brosnema et al 1993). Adedeji and Adbulkadir, 2009 did report *Staphyloccus saprophyticus* as the 2nd commonest causes of UTIs with the rate of isolation of 23.8%. *Staphylococcus saprophyticus* is typically found in infection among sexually active young women (Chessbrough, 2006). However, in our study in present study the incidence of CoNS was higher in male as compared to female, though,

isolations of CoNS with sex of patients can be statistically unimportant ($p>0.05$).In males, the reason is not yet clear for the higher prevalences of CoNS, though the considered risk factors in males are lack of circumcision, HIV infection and receptive anal intercourse (Orrett et al 2006).

In our study *Enterococcus* spp. is the third most commonest pathogen. Alike reporting done by (Manjunath et al 2011) that shows the main uropathogens of UTIs now being the gram positive bacteria. Often *Enterococcus* is problematic in complicated UTIs, in population with indwelling urethral Cathers, or in people broad-spectrum antibiotics being received for another infection (Dimitrov et al., 2003). The higher prevalence of *Enterococcus* (7.3%) in this study is consistent with the statistic that the patient in this study was with indwelling cather or treated with broad-spectrum antibiotic for another infection.

In our study, out of total 202 uropathogens, 66.3% were gram negative bacilli, 31.2% were gram positive cocci and 2.5% were yeast cells. This study is alike to study done by (Khattak et al., 2006), 66.7% isolates were gram negative bacilli, 27.8% were gram positive cocci, and 5.6% were yeast cells. Van Norstrand et al., 2000, also found gram negative bacilli in 67.0%, gram positive cocci in 25.0% and yeast cells in 8.0%. Astal, 2005, found 88.8% gram negative bacilli, 8.7% gram positive cocci and 2.4% yeast cells.

The study exposed that women (81.25%) were more assailable to UTIs than the men population (18.75%), which is also alike to another studies (Manjunath et al 2011). The increased cases of UTIs in females is due to the effects of anatomical factors, hormonal changes, and urodynamic disturbances that occur with increasing age (Bobos et al 2010).

The activities of antibiotics inspected in the particular study against *E. coli* in female population are as follows, and is in decreasing order: nitrofurantoin > ciprofloxacin = ofloxacin > norfloxacin > co-trimoxazole > nalidixic acid > ampicillin > cephalexin. For isolates from male populations , as in order is: nitrofurantoin > ciprofloxacin = ofloxacin>norfloxacin=co-trimoxazole > ampicillin > nalidixic acid > cephalexin. The higher rate of antibiotic

resistance to all agents observed in male in comparisioin to female. This is may be due to the complicated nature of UTI in male (Lipsky et al, 1989). Also, UTI in male patient may be associated with more antimicrobial resistant pathogens (Sahm et al 2001). The similar higer rates of antibiotic resistance to male patients were detected by Bean et al., 2008 and Sahm et al 2001.

UTI affects all age groups, many study proved that sexually active person have more chance having UTI (Foxman et al 1997). The present study also supports this likelihood as more than 50.0% UTI positive individual were between 16 and 49 years. It is also found that women aged greater than 49 years were more affected than age group 1-15 years but less than 16-49 years. This is might because during post menopause their significance change occurs in urethra when *E. coli* can outnumber the Lactobacilli (normal flora) and may easily cause UTI (Schaeff 2001).

Isolation of *E. coli* from females and outpatients were higher than that of males and inpatients. However, isolation of *E. coli* according to sex of patient and type of patients were statistically insignificant ($p>0.05$). As for the patients' status, this study discovered that there was a higher rate of community-based than nosocomial *E. coli* associated UTIs cases.

This discrepancy may be due to improper hygienic conditions within the community, our household, lack of proper health and sanitation education and improper personal hygienic practice. Similarly, 24 (22.0%) were male, whereas 85 (78.0%) were female. This is comparable to the study done by Khan and Zaman, 2006, where 18 (17.6%) were males and 84 (82.4%) were females. As far for the inpatients cases, it is well documented that many factors, including poor patients care in the hospitals, catherization and other surgical procedures linked to lower abdomen, bowel region are highly associate with UTI in inpatients (Stamm and Norby, 2001; Raz, 2001; Sottoo et al., 2001).

The tool that indicates the prevalence of the bacterial resistance in a provided population is known as MAR index (Krumpermann 1983). A MAR index when larger than 0.2 implies that these bacterial strains came from the

environment using certain antibiotics (Ehinmidu 2003). The *E. coli* MAR indices found in the study are an indication that a huge part of the isolates were exposed to multiple antibiotics.

In our study most *Escherichia coli* isolates were resistant to ampicillin (81.7%) also be a lot like other study (Farshad et al 2011).
The general quinolone resistance of *Escherichia coli* was 57.7%. The increased resistance of quilon against *Escherichia coli* might reflect the overuse of quilon to treat urinary tract infections (Saleh et al. 2009). Other factors can be the widespread use of fluoroquinolones in animal feed (specially in poultry), and the subsequent transmission of resistant strain from animals to humans (Miller et al 2004).

In 1997, Acar and Goldsein found that quinolone resistance is lower in developed countries than in developing countries due to the use of less active quinolones, like as nalidixic acid, and the use of lower doses of other quinolones. More effective compounds like ciprofloxacin lead to the selections of mutant strains.

Another study from Poland showed that the drug resistance pattern of *E. coli* bacteria in the hospital was similar to the pattern of resistance in the isolated community, except for those of extended-spectrum B-lactam production (ESBL). Other Enterobacteriaceae species are more resistant when isolated in a hospital setting (Hryniewicz et al. 2001). According to this study, MDR is generally associated with ESBL production, both in community and hospital isolation cases.

Nitrofurantoin is an effective urinary tract antiseptic that should not be used for other types of infections. It did not affect antibiotic use in any other infections (Bosch et al., 2011). In this particular study, about 5.5% of isolates was resistant to nitrofurantoin. These data are consistent with (Bahadin et al. 2011). Nitrofurantoin was considered to be one of the effective drugs in our study. Nitrofuarantion was considered to be the most operative antibacterial agent in UTIs caused by *Escherichia coli* from different studies done in Nepal (Sharma et al. 2011), found to be 80.0%, 76.0% and 61.1. % resistance to *E.*

coli to nitrofurantoin, respectively. However, in this study, nitrofurantoin was observed to be the most efficient and effective antibacterial agent.

Nitrofurantoin is an effective urinary antiseptic that should not be used for other types of infections. This did not affect antibiotic use in any other infections (Bosh et al. 2011). Nitrofurantoin had less than 20.0% resistance rate in this study. Therefore, it should be used as the first choice treatment in our facility.

Nitrofurantoin as an option for empirical therapy has been considered by many authors (Karlowsky et al 2002). Nitrofurantoin resistance among *E. coli* strains due to urinary tract infections remains lower even after more than 50 years of widespread use (Mazzulli et al2001). The reasons for emerging lack of resistance are not fully understood but may comprise limited use for UTIs, limited systemic absorption, and the need for multiple gene mutations. for bacteria to develop resistance (Nicolle et al 2006). However, nitrofurantoin demonstrated poor in vitro activity against Enterobacterieacea other than *E. coli* (Farrell et al 2003). Moreover, this antibiotic doesn't penetrate into tissue and couldn't be used to treat infections with suspected tissue involvement (such as pyelonephritis).

Resistance is a natural biological phenomenon that responds to micro-organisms to the selective pressure of antimicrobial drugs. Resistance may be inherent (Ahmed et al 2008). In this particular study, antibacterial agents displayed that *Escherichia coli* has high resistance to usually used antibiotics such as cephalexin, amipicillin, nalidixic acid, ciprofloxacin, ofloxacin, norfloxacin and co-trimoxazole. The resistance ratio of *Escherichia coli* for usually used antibiotics were: ampicillin (81.7%), cephalexin (92.7%), nalidixic acid (78.9%), ciprofloxacin (49.5%), ofloxacin (49.5%)), norfloxacin (53.2%), cotrimoxazole (54.1%).) and nitrofurantoin (5.5%).

Similary, in a study conducted in India in 200 *Escherichia coli* isolates from symptomatic incidents of UTIs attending outpatient department of Karnatake institute of medical sciences (KIMS) hospital, the resistance rate reported were: ampicillin (91.5%), cephalexin(76.0%), nalidixic acid (93.0%),

ciprofloxacin (83.0%), ofloxacin (83), norfloxacin (85.0%), co-trimoxazole (72.0%) and nitrofurantoin (76.0%) (Kausar et al 2009). The resistance rate was higher than present study except for cephalexin. Similarly, study done in England, the rates of resistance reported were: ampicillin 55.2%, ciprofloxacin 11.9%, cephalexin 10.0% and nitrofurantoin (5.9%) (Bean et al 2008). These resistance rates were lower for first three antibiotics than those obtained in present study. Whereas for nitrofurantoin the resistance rate is almost same. The high values observed in this study can be explained by the extensive, frequent and unrestrained use of antimicrobials.

A study by the North American UTIs Collaborative Alliance determined the susceptibility of usually used antibiotics to treat UTIs caused by *Escherichia coli*. Urinary isolates found from outpatients in different geographic regions in the United States and Canada, total resistance to ampicillin was 37.7% trailed by co-trimoxazole 21.3%, ciprofloxacin 5.5% and nitrofurantoin 1.1% (Zhanel et al 2006). The resistance for all these antibiotics observed in our particular study was higher in comparision to those in the North American study.

Since the resistance rate of UPEC strain to antimicrobials is gradually increasing, in order to minimize resistance development, prior to antimicrobial therapy it is important to investigate antimicrobial susceptibilities of pathogens (Eryimaz et al 2010).

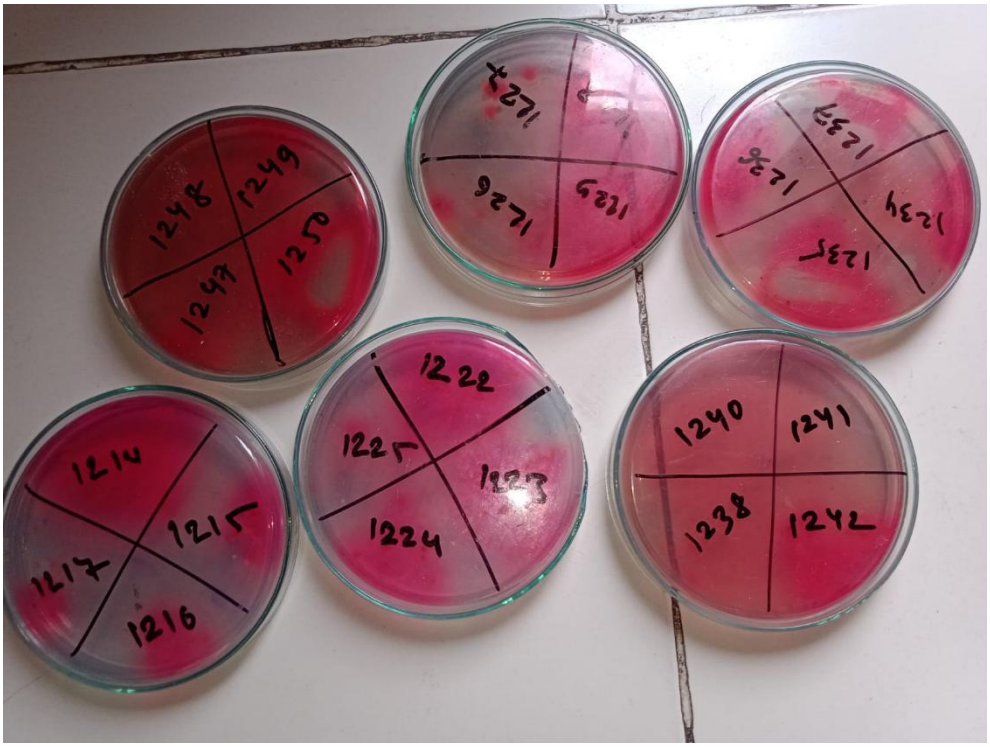
Increased resistance of co-trimoxazole to *Escherichia coli* is reported in other studies conducted in Nepal & other countries (Jadhave et al. 2011). Their rates are higher than those reported in our study. Ampicillin resistance in *Escherichia coli* was 81.7%, comparable to other studies (Khadri et al. 2011). Resistance to ampicillin in urinary tract pathogens is due to its continued use for many years (Hasan et al. 2011). Previously, ampicillin was reported to be ineffective against urinary tract pathogens (Sahm et al. 2001). Based on our findings, antibiotics such as ampicillin and co-trimoxazole are not recommended for initial empiric treatment of UTIs.

The higher rate of resistance to all antibiotics used in this study, with the exception of nitrofurantoin, could be explained by the high and uncontrolled

use of these antibiotics in our institute. control. Moreover, all antibiotics are available in medical stores without a doctor's prescription in our country. More factors have contributed to this higher rate of resistance, including the misuse of antibiotics by health professionals, unqualified practitioners and the public, and the misuse of antibiotics by the public (antibiotics can also be bought even without a prescription), poor quality drugs, lack of sanitation explaining the spread of resistant bacteria, and inadequate surveillance (lack of information from antibiotic testing) Routine mapping of bacterial strains and monitoring of isolates and surveillance of antibiotic resistance, all of which are critical to good clinical practice and sound policies against resistance. birth (Okeke et al. 1999).

The level of resistance to common antibiotics of *Escherichia coli* strains was high in inpatients compared to outpatients. This may be because hospital uropathogens are exposed to more broad-spectrum antibiotics, are more resistant and therefore more difficult and time-consuming to treat (Magalit et al. 2004). The high prevalence of antibiotic resistance in isolated pathogens in hospitals can be explained by the selective effect of treating a patient with multiple antibiotics, which can lead to amplification of resistance in some organisms (Archibald et al. 1997). Pathogens in hospitals have developed more resistance to drugs and are therefore more difficult and time consuming to treat (Magalit et al. 2004).

PHOTOGRAPHS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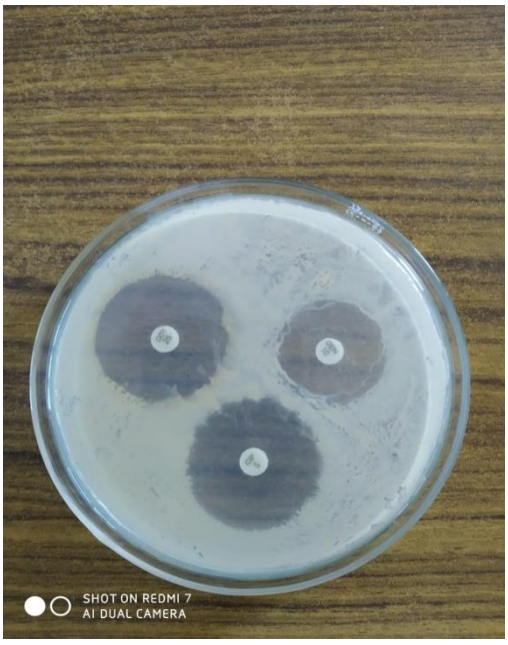
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CHAPTER- VI

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This particular study showed that *Escherichia coli* being the commonest cause of UTIs in both outpatients & inpatients in Surksha Hospital, Nepal. Urinary tract infection is more commonest in outpatients in comparison to inpatients. UTIs is more common in the women population than the male population. The current study highlight the resistance of *E. coli* isolates recovered from samples of urines obtained from suspected UTI patients to widely prescribed oral antibiotics. Most amongst the *Escherichia coli* isolates are highly resistant to generally prescribed antibiotics (ampicillin, cephalexin, quinolones and co-trimoxazole). This may be due to the injudicious use of antibiotics. It is therefore the prescription of these agents as empiric therapy for suspected UTIs should be avoided. *E. coli* isolates retained susceptibility to nitrofurantoin, but once the organism is identified, nitrofurantoin should be considered the preferred therapeutic agent.

6.2 Recommendations

1. As resistance rate of all antibiotics (expect for nitorfurantion) is over 40.0%, polices on prescribing antibiotics must be considered.

2. More than ninety percent MDR strains of *Escherichia coli* was isolated from suspected UTIs patients. Therefore, suspected patients are only treated with antibiotics after obtaining result of antimicrobial susceptibility testing.

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APPENDIX–I

Materials and Equipment's

List of equipments, materials and reagents

Autoclave

Incubator

Hot air oven

Microscope

Refrigerator

Weighing machine

Gas burners

Glassware's

Inoculating loops and wires

Sterile cotton swabs

Sterile Petri-plates

Forceps and rulers

Biosafety cabinet

APPENDIX–II

Media and reagents

Microbiological media

Blood agar

MacConkey agar

Mueller Hinton agar

CLED Agar

Nutrient Agar

Sulphide Indole Motility medium

Simmons's Citrate agar

Urea agar

Triple sugar Iron agar

MR/VP medium

Reagents/stains

Catalase reagent (3% H_2O_2)

Oxidase reagent (1% tetramethyl p-phenylenediaminedihydrochloride)

Kovac's reagent

Gram's reagent

Normal saline

Antibiotic discs:

Ampicillin

Cephalexin

Nalididic Acid

Ciprofloxacin

Ofloxacin

Noorfloxacin

Co-trimoxazole

Nitrofurantoin

Mueller Hinton agar

Beef Extract	2.00gm
Acid Hydrolyaste of casein	15.50gm
Starch	1.50gm
Agar	17.00gm
Distilled water	1000ml
Final PH	7.3+-0.1 at 25^{o}C

MacConkey agar

Peptones (Meat and Casien)	3.00gm
Pancreatic digest of gelatin	17.00gm
Lactose Monohydrate	10.00gm
Bile Salts	1.500gm
Sodium Chloride	5.00gm
Crystal Violet	0.001gm
Neutral Red	0.030gm
Agar	13.500gm
Distilled Water	1000ml
PH	7.1+-0.2 at 25^{oc}

CLED Agar

Peptone	4 g/l
'Lab Lemco' powder	3 g/l
Tryptone	4 g/l
Lactose	10 g/l
L-Cystine	128 mg/l
Bromothymol blue	20 mg/l
Agar No. 1	15 g/l

APPENDIX-III: Procedure of isolation of bacteria

1.Isolation of E. coli

The plates were incubated at 37°c for 24 hours. E. coli colonies were identified on
the basis of colony characteristics on Nutrient Agar, Gram's reaction and biochemical tests.

2. Subculture on NA

Green metallic sheen colonies from EMB were sub cultured on NA and incubated
for 24 hours at 37°c. Large, round, greyish white colonies having raised, entire,
opaque surface were indicative of E. coli.

3.Gram's staining

Isolated colony selected for staining:

1. Smear was made from pure culture by emulsifying a colony in normal saline
and heat fixed.
2. Smear flooded with crystal violet for 1 mint.
3. Wash with water
4. Add Gram's Iodine for 1minute.
5. Wash with water.
6. Decolorize with absolute alcohol for 10-15secs.
7. Wash with water
8. Flood with safranin for 1minute.

Wash with water, blot dry and examine under oil immersion objective of the microscope.

4.Indole test:

The bacterial colony was inoculated on tryptone broth and then incubated at 37^0C
for 24 hours. After 24 hours of incubation, 1ml of Kovac's reagent was added. Appearance of red color (red ring) on the top of media indicates positive indole

test.

Principle:

This test is used to determine the ability of bacteria to oxidize the tryptophan by producing tryptophanase enzyme.

5.MR-VP test:

The bacterial colonies were inoculated into MR and VP broth and incubated at 37 0 C for 24 hours. After incubation, 5 drops of methyl red indicator were added to MR broth and mixed well for MR test. The positive test was indicated by the development of red color, and negative with yellow color. For VP test, 5 drops of Barritt's reagent was added to VP broth and shaken well. Positive test is indicated by the development of pink red color.

Principle of MR test:

The principle of this test is to detect the ability of bacteria to produce and maintain sufficient stable acid from glucose fermentation which is indicated by

MR indicator.

Principle of VP test:

This test detects the ability of bacteria to produce a neutral end product, acetyl methyl carbinol (acetoin) from glucose fermentation.

6. Citrate utilization test:

A bacterial colony was stabbed on the butt of the Simmons citrate agar and then streaked on slant by a sterile inoculating needle. Then the inoculated media were incubated at 37 0 C for 24 hours. A positive test was indicated by the growth of organism and change of color of media from green to blue. Bromothymol blue is green acidic (pH 6.8 and below) and blue when alkaline (pH 7.6 and higher).

7. Catalase test:

3% H 2 O 2 was taken in a clean and dry test tube (3ml). A small amount of culture from nutrient agar plate was added and mixed with the help of glass rod. Positive test is indicated by the formation of bubbles of oxygen gas.

Principle:

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. Bubbles of oxygen are released if the organism is catalase

producer.

8. Carbohydrate fermentation test

Procedure:

I. Preparation of Carbohydrate Fermentation Broth

Weigh and dissolve trypticase, Sodium chloride, and Phenol red in 100 ml distilled water and transfer into conical flasks. Add 0.5% to 1% of desired carbohydrate into all flasks. Insert inverted Durham tubes into all tubes, the Durham tubes should be fully filled with broth. Sterilize at 115 0 C for 15 minutes.Do not overheat the Phenol red Carbohydrate fermentation broth. The overheating will result in breaking down of the molecules and form compounds with a characteristic colour and flavour. The process is known as caramelization of sugar(the browning of sugar). Transfer the sugar into screw capped tubes or fermentation tubes and label properly.

II. Inoculation of Bacterial Culture into the Phenol Red Carbohydrate Broth

Aseptically inoculate each labeled carbohydrate broth with bacterial culture (keep-on inoculated tubes as control tubes). Incubate the tubes at 18-24 hours at37 o C.Observe the reaction.

Principle:

When micro-organisms ferment carbohydrate an acid or acid with gas are produced. Depending up on the organisms involved and the substrate being fermented, the end products may vary. Common end-products of bacterial fermentation include lactic acid, formic acid, acetic acid, butyric acid, butylalcohol, acetone, ethyl alcohol, carbon dioxide and hydrogen.The production of the acid lower the pH of the test medium, which is detected bythe colour change of the pH indicator. Colour change only occurs when sufficient amount of acid is produced, as bacteria may utilize the peptone producing alkaline

by products.

9.Antibiotic susceptibility test

In vitro susceptibility of the pure bacterial species to fifteen different antibiotics was determined using Kirby- Bauer disk diffusion technique using Muller-Hinton agar and antibiotic discs as described by the National

Committee for Clinical Laboratory Standards (CLSI, 2006). One ml of each bacterial isolates prepared directly from an overnight agar plates adjusted to0.5 McFarland Standard was inoculated using sterile swab into each of the Petri-dishes containing Mueller Hinton agar and were allowed to stand for 30 minutes for pre-diffusion of the inoculated organisms.Antibiotic discs were seeded into the Petri-dishes containing Mueller-Hintonagar (MHA) for each bacterial isolate. The AST of the isolates towards various antimicrobial discs was done by modified Kirby-Bauer M2-A9 disc-diffusion method as recommended by Clinical Laboratory Standard Institute
(CLSI) using MHA as follows:

1. MHA was prepared and sterilized as instructed by the manufacturer.
2. The pH of the medium was adjusted to 7.2-7.4 and the depth of the medium at 4mm (about 25 ml per plate) was maintained in Petri-dish.
3. Using a sterile wire loop, a single isolated colony whose susceptibility pattern is to be determined was touched and inoculated into MHB tube and was incubated at 37oC for 2-4 hrs.
4. After incubation, the turbidity of the suspension was matched with the McFarland standard tube number 0.5 (which is equivalent to 10 to power 4 organisms).
5. Using a sterile swab, an MHA plate was inoculated with the matched suspension using a carpet culture technique.
6. The plate was then allowed to stand for 20-30 minutes for the pre-diffusion of the inoculated organisms.
7. Using clean and sterile forceps, the above-mentioned antibiotic discs (6 mm)
were placed on the MHA. The discs were placed at the considerable distance apart from each other on a 90 mm Petri-dish. Then the plate was incubated at 37 ºC for 24 hrs.
8. After incubation, the plates were observed for zone of inhibition and the diameters of inhibition zones were measured in millimetres (mm). The measurement was interpreted as sensitive and resistant according to the manufactures standard zone size interpretative manual of CLSI (2006).

The percentage resistance was calculated using the formula PR=a/b×100,where ‘PR’ was percentage resistance, ‘a’ was the number of resistant isolates
and ‘b’ was the number of isolates tested with the antibiotic. The percentage sensitivity was calculated using the formula PS=c/d×100, where ‘PS’ was percentage sensitivity, ‘c’ was the number of sensitive isolates and ‘d’ was the number of isolates tested with the antibiotic.

APPENDIX IV

Antibiotic susceptibility test

The following steps are involved in AST by Kirby Bauer disc diffusion method.

- At least 3-5 well isolated colonies of the same morphological type were selected from a growth positive agar plate culture.
- The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4 to 5 ml of nutrient broth medium.
- The broth culture was incubated at 37^{o}c until it achieved the turbidity of 0.5 McFarland standards (usually 2 to 6 hours).
- After standardization of turbidity, using a sterile cotton swab, a lawn culture was made by streaking the swab over the entire sterile MHA surface.
- Antibiotic disc were applied by pressing gently using sterile forceps to ensure complete contact with agar surface and placed at least 20 mm apart from each other.
- The plates were inverted and placed in an incubator at 37^{o}c within 15 minutes after the discs were applied.
- After 16-18 hours of incubation, each plate was examined for the complete zone of inhibition (as judged by the unaided eye). Zones were measured to the nearest whole millimetre, using a ruler, which was held on the back of inverted Petri-plate.