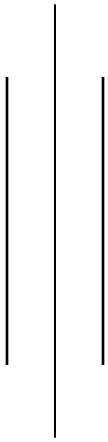
A Research Report
On

PRESCRIBING AND SENSITIVITY PATTERNS OF ANTIMICROBIALS IN UNCOMPLICATED URINARY TRACT INFECTIONS IN FEMALES



Submitted
To

SHAS Research Committee
SCHOOL OF HEALTH AND ALLIED SCIENCES
POKHARA UNIVERSITY, LEKHNATH-12, KASKI
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Submitted
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ACKNOWLEDGEMENT

We take an immense pleasure to acknowledge **Professor Dr.Bishnu Raj Tiwari**, Director Research, School of Health and Allied Sciences, Lekhnath, Kaski, for granting this project.

We would like to express our sincere thanks to **Dr. Suresh Prasad Bastola**, Dean, Faculty of Science and Technology and **Associate Prof. Dr.Nirmala Jammarkatel**, Program Director, School of Health and Allied Sciences, Pokhara University, for providing this opportunity to carry out the project work.

We would like to thanks for Charak Hospital and Research Centre and Western Regional Hospital, Kaski, Pokhara for helping throughout this project despite of their busy schedule and providing space in the hospital to carry out the project work.

Our expression of gratitude also goes to **Mr. Suresh Jaiswal**, Lecturer, School of Health and Allied Sciences, for helping in the microbiology work.

Finally, we would like to take this opportunity to thank all those patients whose participation has made this study possible.

ABSTRACT

Objective: The overuse and misuse of antimicrobials have been related to growing emergence of bacterial resistance worldwide. The aim of this present study was to detect the causative agents of uncomplicated urinary tract infection in females, to assess the pattern of antimicrobial prescription along with the antimicrobial sensitivity pattern.

Methodology: A prospective study was conducted in two hospitals viz. Charak Hospital and Research Centre and Western Regional Hospital, Kaski, Pokhara. Patient information was obtained by interviewing the patients and through their medical record files. Antimicrobials sensitivity testing was performed by disc diffusion.

Result: A total of 175 clean catched midstream urine samples of females who were clinically diagnosed to have UTI were collected, out of which 104 (75.4%) samples grew potential pathogens causing UTI. The study showed that UTI was mostly prevalent in females of age group 20-30. *Escherichia coli* were the predominant (64.4%) bacterial pathogen followed by *Klebsiella* species (13.3%), *Pseudomonas* species (3.8%) and others. Most of the strains of *E. coli* were resistant to cephalexin whereas sensitive to cefpodoxime, amikacin, gentamycin and nitrofurantoin. Most of the urinary isolates showed high degree of resistance to cephalexin, norfloxacin nalidixic acid. Most commonly prescribed antimicrobial was ofloxacin (28.8% in Charak Hospital and Research Centre and 21.8% in Western Regional Hospital) followed by nitrofurantoin (19.2%) and azithromycin(9.6%) in hospital A and cefixime (14.9%), amoxycillin (11.9%) and azithromycin (7.9%) in hospital B. Besides, the combinations of antimicrobials were also found to be prescribed like azithromycin+cefpodoxime, azithromycin+ceftriaxone, gentamycin+azithromycin, gentamycin+ceftriaxone, ofloxacin+cefixime etc. A single antimicrobial was most commonly prescribed in both the hospitals, however, more than three antibiotics were also found to be prescribed in case of hospital B.

Conclusion: This study revealed that *E. coli* was the predominant bacterial pathogen of uncomplicated UTIs in both hospitals. It also demonstrated an increasing resistance to

cephalexin, norfloxacin and nalidixic acid. Thus, formulation of a policy for hospital antimicrobial use is most necessary to ensure safe and efficient treatment of UTIs.

Keywods: *Antimicrobials Sensitivity, Prescribing Pattern, Urinary Tract Infection, Mid Stream Urine, Pathogens.*

1. INTRODUCTION

Urinary Tract Infections (UTI) is a heterogeneous disease, which can be divided into several types of infection, such as acute, uncomplicated bacterial pyelonephritis, complicated UTI, recurrent cystitis and asymptomatic bacteriuria (Akortha and Ibadin, 2010).

Urinary tract infection (UTI) is defined as significant bacteriuria in the presence of symptoms.

The urinary tract is normally sterile. Uncomplicated UTI involves the urinary bladder in a host without underlying renal or neurologic disease. The clinical entity is termed cystitis and represents bladder mucosal invasion, most often by enteric coliform bacteria (eg. Escherichia coli) that inhabit the periurethral vaginal introitus and ascend into the bladder via the urethra.

Sexual intercourse may promote this migration, and cystitis is common in otherwise healthy young women. Urine is generally a good culture medium; factors unfavorable for bacterial growth include a low pH (5.5 or less), a high concentration of urea, and the presence of organic acids derived from a diet that includes fruits and protein. Organic acids enhance acidification of the urine.

Frequent and complete voiding has been associated with a reduction in the incidence of UTI. Normally, a thin film of urine remains in the bladder after emptying, and any bacteria present are removed by the mucosal cell production of organic acids. If the mechanisms of the lower urinary tract fail, upper tract or kidney involvement occurs and is termed pyelonephritis. Host defenses at this level include local leukocyte phagocytosis and renal production of antibodies that kill bacteria in the presence of complement.

Complicated UTI occurs in the setting of underlying structural, medical, or neurologic disease. Patients with a neurogenic bladder or bladder diverticulum and postmenopausal women with bladder or uterine prolapse have an increased frequency of UTI due to incomplete bladder emptying. This eventually allows residual bacteria to overwhelm

local bladder mucosal defenses. The high urine glucose content and the defective host immune factors in patients with diabetes mellitus also predispose to infection.

It is the most common infection experienced by both male and female particularly responsible for discomfort in elderly patients, thus representing a risk of bacteremia, septic shock, respiratory distress syndrome and death (Gradwohl *et al.*, 2005). Infection of adjacent structures such as prostrate and epididymis is also included in this entity. Infection of urinary tract is amongst the most common bacterial infections that prompt patients to seek medical advice second only to infection of respiratory tract (Jha and Bapat, 2005).

It has been estimated that about six million patients visit outpatient departments and about 300,000 are treated in the wards every year for UTI worldwide (Jha and Bapat, 2005). Approximately, 10% of human population gets UTI at some stage during their lives. Nepal, being a developing country, has about 61.4% illiterate people (Jha and Bapat, 2005) who do not have any concept of hygiene and so are always vulnerable to infections by various organisms. According to the annual report of fiscal year (2055/2056) published by Department of health services, 0.46% of total outdoor patients suffered from UTI and this was out of the total population of Nepal (2, 22, 87,417). The geographical distribution of UTI amongst the Nepalese population is 0.57% in the mountains and 0.45% is estimated to be in planes.

Most episodes of UTI are caused by *Escherichia coli* (up to 85%) and *Staphylococcus saprophyticus* (up to 10%), while *Klebsiella pneumoniae* and *Proteus sp*. account for most of the remaining infections (Dimitrov *et al.*, 2004). The major pathogens found in another study were *E. coli* (49%), *S. aureus* (23%), *Proteus sp.* (3.6%), *Klebsiella* (9.71%), *Pseudomonas* (0.8%) and *Citrobacter* (2.8%) (Jha and Bapat, 2005). It has been reported that UTI was more common in females of younger age group as compared to males. The common age group for females was 21-30 years, whereas that for males was 31- 40 years in all the hospitals (Jha and Bapat, 2005).

1.1Prescribing patterns

Prescribing is the act of determining which drug for which patient at an appropriate dosage regimen and with optimal duration of treatment. This is dynamic, highly individualized and involved medical, social and marketing forces. Inappropriate prescribing including supraoptimal or suboptimal choice of medication, higher or lower drug dosage, inappropriate duration, therapeutic duplication, potentially dangerous drug-drug interaction, prescribe an expensive drug when a cheaper and equally effective agent is available, are important health care problem.

Factors influencing prescribing patterns:

System factors: System or exogenous or structural environment factors which affect prescribing pattern are drug policies, hospital formularies, practice organization, influence of pharmaceutical companies, fragmentation of care, over abundance of drug therapy option and information quality.

Prescriber factors: Differences in characteristics of prescriber knowledge ability and experience in the field of specialty would affect the choice of drug to be used. Internal factors that would affect prescribing patterns include an inadequate training or practices, outdated medical knowledge, lack of continuing education, inadequate drug information especially its effectiveness or adverse reaction and drug cost, forgetfulness and temptation to the offer from pharmaceutical industries

Patients or family factors: Patients or families who have a different backgrounds or cultural belief demand for drug or treatment which may be inappropriate; for example, patients demand an injected drug instead of an oral drug because they believed that it is more potent then the oral one and the disease will be cured more rapidly.

UTI are often treated with different broad-spectrum antimicrobials suspecting infection with resistant organisms though a narrow spectrum of activity may be appropriate. In general, β-lactam antibiotics, trimethoprim-sulphamethoxazole (TMP-SMX) and fluoroquinolones are used most often in uncomplicated UTI. In the case of a high-risk patient profile due to complicated factors, quinolones might be added to the armamentarium of treatment. However, the antibiotic of choice is currently highly dependent on the local situation with regard to the antibiotic resistance of the pathogenic bacteria (Matute AJ *et al*., 2004). However, the extensive use of antimicrobial agents has invariably resulted in the development of antimicrobial resistance, which, in recent years,

has become a major problem worldwide (Dimitrov *et al.*, 2004). In UTI, antimicrobial therapy is initiated even before the result of urine culture is available. Hence, there exists a great need for antimicrobial resistance surveillance at local, national and international level from time to time (Khan and Zaman, 2006).

2. OBJECTIVES

This study was carried out in the Microbiology Laboratory of School of Health and Allied Sciences, Pokhara University and Pathology Laboratory of Western Regional Hospital and Charak Hospital and Research Centre.

2.1General

The general objective of study is to determine the prescribing pattern of antimicrobials and their sensitivity pattern in UTI visiting two major hospitals of Pokhara Valley.

2.2 Specific

- To study the prescribing pattern in UTI.
- To study the major causative agents of UTI in females.
- To study the antimicrobials sensitivity pattern of causative agents in UTI.

3. LITERATURE REVIEW

3.1 History of Urinary Tract Infection

Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for haemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicemia and Gram-negative pneumonia. Certain strains of *E. coli*, such as O157:H7, O121 and O104:H21, produce potentially lethal toxins. Food poisoning caused by *E. coli* is usually caused by eating unwashed vegetables or undercooked meat. O157:H7 is also notorious for causing serious and even life-threatening complications such as hemolytic-uremic syndrome (HUS). This particular strain is linked to the 2006 United States *E. coli* outbreak due to fresh spinach. Severity of the illness varies considerably; it can be fatal, particularly to young children, the elderly or the immunocompromised, but is more often mild. Earlier, poor hygienic methods of preparing meat in Scotland killed seven people in 1996 due to *E. coli* poisoning, and left hundreds more infected. *E. coli* can harbor both heat-stable and heat-labile enterotoxins. The latter, termed LT contains one A subunit and five B subunits arranged into one holotoxin, and is highly similar in structure and function to cholera toxins. The B subunits assist in adherence and entry of the toxin into host intestinal cells, while the A subunit is cleaved and prevents cells from absorbing water, causing diarrhea. LT is secreted by the Type 2 secretion pathway.

3.2 Introduction of major causative agents of UTI

3.2.1 Escherichia coli

Escherichia coli (commonly abbreviated *E. coli*; named after German pediatrician and bacteriologist Theodor Escherich) is a Gram negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Cells are typically rod-shaped and are about 2 μm long and 0.5 μm in diameter, with a cell volume of 0.6 - 0.7 μm^3. It can live on a wide variety of substrates. *E. coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when *E. coli* lives together with hydrogen-consuming organisms such as methanogens or sulfate-

reducing bacteria. Optimal growth of *E. coli* occurs at 37°C (98.6°F) but some laboratory strains can multiply at temperatures of up to 49°C (120.2°F). Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and the reduction of substrates such as oxygen, nitrate, dimethyl sulfoxide and trimethylamine N-oxide. Strains that possess flagella can swim and are motile. The flagella have a peritrichous arrangement.

Most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and by preventing the establishment of pathogenic bacteria within the intestine. Uropathogenic *E. coli* (UPEC) is responsible for approximately 90% of urinary tract infections (UTI) seen in individuals with ordinary anatomy. In *ascending infections*, fecal bacteria colonize the urethra and spread up the urinary tract to the bladder as well as to the kidneys (causing pyelonephritis), or the prostate in males. Because women have a shorter urethra than men, they are 14-times more likely to suffer from an ascending UTI.

Uropathogenic *E. coli* utilize P fimbriae (pyelonephritis-associated pili) to bind urinary tract endothelial cells and colonize the bladder. These adhesins specifically bind D-galactose-D-galactose moieties on the P blood group antigen of erythrocytes and uroepithelial cells. Approximately 1% of the human population lacks this receptor, and its presence or absence dictates an individual's susceptibility to *E. coli* urinary tract infections. Uropathogenic *E. coli* produce alpha- and beta-hemolysins, which cause lysis of urinary tract cells. UPEC can evade the body's innate immune defenses (e.g. the complement system) by invading superficial umbrella cells to form intracellular bacterial communities (IBCs). They also have the ability to form K antigen, capsular polysaccharides that contribute to biofilm formation. Biofilm-producing *E. coli* are recalcitrant to immune factors and antibiotic therapy and are often responsible for chronic urinary tract infections. K antigen-producing *E. coli* infections are commonly found in the upper urinary tract. *Descending infections*, though relatively rare, occur when *E. coli*

cells enter the upper urinary tract organs (kidneys, bladder or ureters) from the blood stream.

3.2.2 Proteus species

These are Gram-negative, urease-splitting, actively motile, non-capsulated aerobic bacilli, 1-3 × 0.5 μm in size. It is the second most commonly isolated Enterobacteriaceae after *E. coli* in many series. Most common species: *P. mirabilis* (indole negative), causes 90% of infections. Other Proteus spp. is indole positive, e.g., *P. vulgaris* and *P. penneri. P. mirabilis* and *P. vulgaris* Causes ~ 10% of uncomplicated UTIs. It may also cause wound infections, bacteremia and nosocomial pneumonia. This organism splits urea, raising urinary pH (> 8.0) and can cause struvite stone formation.

3.2.3 Klebsiella species

These organisms are named after Edwin Klebs, a 19th century German microbiologist. Klebsiella are nonmotile, rod-shaped, gram-negative bacteria with a prominent polysaccharide capsule measuring 1-2 × 0.5-0.8 μm. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms. Morphologically, Klebsiella species simulate E. coli except that they are nonmotile and possess a polysaccharide capsule. The capsule is responsible for the mucoid appearance of the bacterial colonies and the enhanced virulence of the organism in vivo. They grow on ordinary media; produce pink colonies in MacConkey's agar and mucoid colonies of varying stickiness. They are widely distributed in nature, occurring both as commensals in human and animal intestines as well as saprophytes in soil, water and vegetation.

3.2.4 Pseudomonas species

"*Pseudomonad"* literally means 'false unit', being derived from the Greek *pseudo* (false) and *monas* (a single unit). The term "monad" was used in the early history of microbiology to denote single-celled organisms. They are Gram-negative, rod shaped, aerobic bacilli with one or more polar flagella providing motility. These are non–spore forming bacilli giving positive catalase test. Other characteristics which tend to be associated with *Pseudomonas* species (with some exceptions) include secretion of pyoverdin (fluorescein), a fluorescent yellow-green siderophore under iron-limiting

conditions. Certain *Pseudomonas* species may also produce additional types of siderophore, such as pyocyanin by *Pseudomonas aeruginosa* and thioquinolobactin by *Pseudomonas fluorescens*. *Pseudomonas* species also typically give a positive result to the oxidase test, the absence of gas formation from glucose, glucose is oxidised in oxidation/fermentation test using Hugh and Leifson O/F test, beta hemolytic (on blood agar), indole negative, methyl red negative, Voges–Proskauer test negative, citrate positive.

3.2.5 Staphylococcus species

"Staphylococcus aureus" (literally the "golden cluster seed" or "the seed gold" and also known as *golden staph* and Oro staphira) is a facultatively anaerobic, gram-positive coccus which appears as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. The carotenoid pigment staphyloxanthin is responsible for S. aureus' characteristic golden colour, which may be seen in colonies of the organism. This pigment acts as a virulence factor with an antioxidant action that helps the microbe evade death by reactive oxygen species used by the host immune system. Staph organisms which lack the pigment are more easily killed by host defenses. *S. aureus* was discovered in Aberdeen, Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses.

S. aureus is catalase-positive (meaning that it can produce the enzyme "catalase") and able to convert hydrogen peroxide (H_2O_2) to water and oxygen, which makes the catalase test useful to distinguish staphylococci from enterococci and streptococci. A small percentage of *S. aureus* can be differentiated from most other staphylococci by the coagulase test: *S. aureus* is primarily coagulase-positive (meaning that it can produce the enzyme "coagulase") that causes clot formation, whereas most other *Staphylococcus* species are coagulase-negative. However, while the majority of *S. aureus* are coagulase-positive, some may be atypical in that they do not produce coagulase (the most common organism in patients with nosocomial bacteremia is coagulase-negative staphylococcus). Incorrect identification of an isolate can impact implementation of effective treatment and/or control measures.

Staphylococcus saprophyticus is a coagulase-negative species of *Staphylococcus* bacteria. *S. saprophyticus* is implicated in 10-20% of urinary tract infections in females between the ages of 17-27. It is the second most common cause of UTI. It may also reside in the urinary tract and bladder of sexually active females. *S. saprophyticus* is phosphatase-negative, urease and lipase positive. Until the last decade, coagulase-negative staphylococci occurring in urine specimens were usually regarded as a contaminant. In the early 1970s, more than ten years after the original demonstration of *Staphylococcus saprophyticus* in urine specimens, this species became recognized as a frequent cause of urinary tract infections (UTI). In young women, S. saprophyticus is, after *Escherichia coli*, the second-most-frequent causative agent of acute UTI.

3.3 Clinical Features

3.3.1 General features of a UTI

- Dysuria, frequency, urgency and a sensation of incomplete bladder emptying - a very common presentation.
- Lower abdominal pain - often a presentation in children and young adults.
- Sudden development of incontinence - often a presentation in the elderly.
- Haematuria.
- Enuresis occurring in a previously 'dry' child.
- Non-specifically unwell if previously fit - presentation is seen in infants and elderly.

3.3.2 Sign and symptoms of lower UTI include:

- Dysuria
- Urgency
- Frequency
- Suprapubic tenderness
- Strangury (a condition marked by slow, painful urination, caused by muscular spasms of the urethra and bladder)
- Flank or back pain
- Haematuria
- A change in the smell of urine

Three or more symptoms should be present to make a diagnosis of UTI. When both dysuria and frequency are present the probability of an UTI is >90%.

3.4 Laboratory diagnosis

The commonest method of diagnosing urinary tract infections is the examination of a mid-stream specimen of urine, often referred to simply as MSU. Specimen collection is very important, and clear instructions to patients should be provided. The external genitalia must be washed properly using soap and water. The first portion of urine is voided to wash out any microbes from the distal part of the urinary tract. It is the middle section of the urinary flow that is collected for laboratory analysis

Catheter samples of urine are also frequently examined. Collecting urine samples from babies poses a particular problem. To avoid contamination problems associated with bags, supra-pubic aspirates can be performed. If a patient is suspected of suffering from renal TB, then the number of organisms in the sample will be low. To help in the diagnosis three consecutive early morning mid stream specimens of urine are examined.

Having collected the specimen, for routine examination, urine is subjected to a microscopic examination and culture. Urine microscopy reveals the presence of leukocytes, red blood cells, bacteria and "casts". These are proteinaceous deposits formed within the diseased kidney, and shed in the urine. They may be clear (hyaline casts) or may have leukocytes or red cells stuck to their surface. Urine sample containing squamous (skin-type) epithelial cells are considered contaminated. Squamous epithelial cells are not found in the urinary tract.

A small range of bacteria cause urinary tract infections. Nearly all grow on a selective and indicator medium such as CLED agar. A semi-quantitative culture of urine is most often performed. A standard known volume of urine is inoculated, and the number of colonies growing from the sample is used as a guide in diagnosis of urinary tract infections. Typically 1 microlitre of urine is plated, and if more than 100 colonies of a ***single species*** are grown from an MSU sample, then the sample is considered infected, i.e. more than 100,000 cfu/ml. For supra-pubic aspirates, lower microbial counts are considered significant. Growth of more than one species in a sample is taken as an

indication of contamination. Catheter specimens of urine (particularly those from catheters that have been in place for more than a few days) are likely to contain bacteria, sometimes in high numbers. If the patient remains asymptomatic these should ***not*** be treated with antibiotics.

Urine is an excellent bacteriological growth medium. To prevent growth of bacteria in samples, many specimen jars contain measured quantities of boric acid, used to prevent bacterial growth. Alternatively, urine may be refrigerated until it can be examined.

3.5 Complications

When treated promptly and properly, urinary tract infections rarely lead to complications. But left untreated, a urinary tract infection can become something more serious than merely a set of uncomfortable symptoms. Untreated urinary tract infections can lead to acute or chronic kidney infections (pyelonephritis), which could permanently damage your kidneys. Urinary tract infections may be overlooked or mistaken for other conditions in older adults. Young children also have an increased risk of kidney infections. Pregnant women who have urinary tract infections may have an increased risk of delivering low birth weight or premature infants.

In some adults, recurrent UTIs may cause scarring in the kidneys, which over time can lead to renal hypertension and eventual kidney failure. Most of these adults with kidney damage have other predisposing diseases or structural abnormalities. Scarring and future kidney problems are also concerns for children who experience severe or multiple kidney infections. Complications of urinary tract infections would not normally develop if the infection is treated properly. However, sometimes they could develop because treatment was started too late.

Some of the complications of UTI include:

- Kidney failure due to extensive damage to the kidneys.
- Sepsis- This occurs when the infection spreads from the urinary tract to other parts of the body. It is a very bad complication that can lead to death from septic shock if it is not treated urgently and properly.

- In pregnant women, UTI may cause premature delivery of low birth weight or premature infants.
- Stricture - This occurs when there is narrowing of the urethra due to scars formed after the infection heals. Scarring of the urethra will cause difficulty in passing urine.

3.4 Management

- Trimethoprim 300mg orally daily (3 days for women, 14 days for men)
  OR
- Cephalexin 500mg orally 12 hourly (5 days for women, 14 days for men)
  OR
- Amoxycillin/clavulanate 500mg/125mg orally 12 hourly (5 days for women, 14 days for men)
  OR
- Nitrofurantoin 50 mg orally, 6-hourly (5 days for women, 14 days for men)

If there is proven microbial resistance to other medications use:

- Norfloxacin 400 mg orally 12-hourly (3 days for women, 14 days for men)

Optimal duration of therapy for lower cystitis in older patients is still unknown.

For women, regimes vary from 1, 3 and 7-days. Single dose therapy is less effective than longer courses, and is not recommended for management in older adults. Limiting treatment to a minimum reduces adverse effects and cost, therefore where possible a 3-day course should be prescribed.

For men, antibiotic treatment is recommended for 14 days as there is often associated infection of the posterior urethra, prostate or epididymis. Investigations should be done to exclude an underlying urinary tract abnormality.

In immunocompromised patients, a 7-day course may be considered more appropriate.

If relapse occurs, pyelonephritis should be considered, and treatment given for 10 to 15 days.

3.7 Relapse

Most women who have had an uncomplicated UTI have occasional recurrences. About 25 - 50% of these women can expect another infection within a year of the previous one. Between 3 - 5% of women have ongoing, recurrent urinary tract infections, which follow the resolution of a previous treated or untreated episode.

Recurrence is often categorized as either *reinfection* or *relapse*:

Reinfection: About 80% of recurring UTIs are reinfections. A reinfection occurs several weeks after antibiotic treatment has cleared up the initial episode and can be caused by the same bacterial strain that caused the original episode or a different one. The infecting organism is usually introduced through fecal bacteria and moves up through the urinary tract.

Relapse: Relapse is the less common form of recurrent urinary tract infection. It is diagnosed when a UTI reoccurs within 2 weeks of treatment of the first episode and is due to treatment failure. Relapse usually occurs in kidney infection (pyelonephritis) or is associated with obstructions such as kidney stones, structural abnormalities or, in men, chronic prostatitis.

Recurrent UTIs can occur for many reasons, including:

- Problems with the immune system.
- The use of a urinary catheter to empty the bladder.
- Abnormalities in kidneys, ureters, bladder or urethra can cause repeated infections.
- Damage to part of the urinary system.
- Sexual intercourse, which seems to trigger UTI in some women.
- Poor hygiene, such as wiping from back to front after a bowel movement or not changing the underwear often.

3.8 Prevention

To help prevent a urinary tract infection, a woman should:

- Keep the vaginal areas clean, including wiping from the front to back after a bowel movement to prevent contamination of the urinary tract.

- Use tampons and change every three to four hours, instead of sanitary pads. (The pads can act as a culture medium for fecal bacteria, which may then be rubbed against the urinary outlet and invade the bladder).
- Wear cotton undergarments, which allow air circulation and discourage the warm, moist environment needed for bacteria growth. Nylon pantyhose should have a cotton crotch.
- Avoid wearing tight clothes in the genital area, such as control-top pantyhose and skin-tight jeans, as well as extended wearing of a wet bathing suit.
- Urinate before and after intercourse and make sure that the partner's hands and penis are clean.
- Urinate "when you see a bathroom" rather than when the urge to urinate becomes strong.
- Drink plenty of fluids - the equivalent of six to eight 8-ounce glasses - every day to flush bacteria out of your urinary system. This does NOT mean eight glasses of water in addition to everything else you drink.
- Make sure you're getting vitamin C in your diet, either through food or supplements. Vitamin C, or ascorbic acid, makes your urine acidic, which discourages the growth of bacteria. Drinking cranberry juice may also produce the same effect. Cranberry tablets are a more concentrated form of cranberry juice without the sugar content.
- Urinate every two to three hours. Keeping urine in your bladder for long periods gives bacteria a place to grow.
- Of many other means of urinary tract infection prevention, drinking lots of fluid will always be on top of this list for the reason that, hydration aims to flush unwanted bacteria out of your urinary system even before the accumulation of the infection.
- Sugar on the other hand is to be avoided since hyperglycemia may invite the growth of bacteria which is the main cause of urinary tract infection among diabetes inflicted persons.
- Females are then advised to avoid using feminine hygiene sprays and scented douches because they contribute highly to the irritation of the urethra.
- Vaccines are being developed to assist patients' own production of usual infection-fighting prowess. Some researchers are at this time trying injection and oral vaccines

as well as vaccine suppositories that are placed in the vagina all for the same reason of assisting in urinary tract infection prevention.

- If you suffer from urinary tract infections more than three times a year, you can follow one of the therapies given below to prevent another recurrence.
- A low dosage of an antibiotic medication, such as cotrimoxazole or nitrofurantoin, taken daily for six months or longer.
- A single dose of an antibiotic medication taken after sexual intercourse if it is determined that your UTIs are related to sex.
- A short, one- or two-day course of antibiotic medication taken when symptoms appear.

3.9 Antimicrobial Susceptibility Test

In the treatment and control of infectious diseases, especially when caused by pathogens that are often drug resistant, sensitivity (susceptibility) testing is used to select effective antimicrobial drugs. In vitro susceptibility testing usually involves disc diffusion. Selection of antibiotics should be based on antibiotic susceptibility pattern. Periodic evaluation of antimicrobial activity of different antibiotics is essential as the pattern of antibiotic sensitivity may vary over short periods. Increasing antibiotic resistance among urinary pathogens, especially *E coli,* to commonly prescribed drugs like cotrimoxazole has become a global reality. Use of antibiotics by medical practitioners is rampant resulting in increase in resistance to available antibiotics. Isolation of organisms causing UTI and their antibiotic susceptibility is very essential for their appropriate management. The reported positive rate of UTI among Nepalese patients attending general hospitals ranged from 23.1% to 37.4%. Urinary tract disorders in Nepal are estimated to be about seven percent and UTI constitutes majority of these disorders. The antibiotic sensitivity pattern of organisms changes rapidly over a short period. It is especially true for developing countries where antibiotics are prescribed irrationally not only by the medical practitioners but the antibiotics are also purchased directly from the chemists (medicine shop keepers) without prescription. It has been advised that pediatricians should be aware of the rising resistance of urinary pathogens to commonly prescribed antibiotics as well as the profile of antibiotic resistance within their community. Therefore, periodic

evaluation of sensitivity pattern is essential for rational and appropriate use of antibiotics (Rai GK *et al.*, 2008)

4. MATERIALS AND METHODS

4.1. Materials

The materials, equipments, chemicals, reagents, media and antibiotics used in the study are listed below:

4.1.1List of materials:

- Inoculating loop
- Filter paper
- Petri dish
- Detergent
- Test tube
- Glass slides
- Cotton
- Measuring cylinder
- Mask
- Vernier caliper
- Spirit lamp
- Conical flask
- Gloves
- Glass rods
- Labeling stickers
- Beakers
- Forcep

4.1.2List of equipments:

- Microscope
- Autoclave
- Hot air oven
- Water bath
- Electronic balance
- Incubator
- Refrigerator

4.1.3List of chemicals and reagents:

- Crystal violet
- Safranin
- Acetone
- Alcohol
- Iodine
- Barium chloride
- Sulfuric acid

4.1.4Media:

- Muller Hinton Agar
- MacConkey Agar
- Blood Agar
- Triple Sugar Iron Agar
- MR, VP, Indole, Simmons Citrate Agar

4.1.5Antibiotic disc:

- Amoxycillin
- Nalidixic acid
- Cotrimoxazole
- Nitrofurantion
- Ofloxacin
- Ciprofloxacin
- Norfloxacin
- Levofloxacin
- Cefixime
- Cefpodoxime
- Cephalexin
- Amikacin
- Gentamycin
- Ceftriaxone
- Azithromycin

4.2. Methods:

4.2.1 Collection of suspected prescription

The prescription of female patient visiting the hospital with sign and symptoms of UTI were enrolled in our study for the prescribing and sensitivity pattern (Huang and Stafford, 2002).

4.2.2 Inclusion criteria

The female patient visiting the hospital with the following sign and symptoms were enrolled in our study.

- Flank pain
- Urine urgency
- Urine frequency
- Fever
- Rigors
- Burning micturation
- Itching vulva

- History of Discharge
- Painful intercourse
- Foul odor

4.2.3 Exclusion criteria

- Severe anemia (hemoglobin <7 gm/dl.)
- Presence of heart disease with signs of cardiac failure.
- Severe Renal and hepatic diseases.
- Documented tuberculosis with ongoing treatment.
- Associated other severe diseases that require special care or surgical intervention.
- Pregnancy and Diabetes.

4.2.4 Study type

Prospective, Quota and Cohort Female Study

4.2.5 Location

The study was carried out in two hospitals of Pokhara.

1. Western Regional Hospital
   Ramghat-10, Pokhara, Kaski, NEPAL
2. Charak Hospital and Research Centre
   Prithivi Chowk, Pokhara, Kaski, NEPAL

4.2.6 Total number of enrollment

Total no of patients: 175

4.2.7 Noting of patient history and empirical treatment therapy

Performa was filled up with patient name, age, address. Along with them, patient's history of illness with empirical treatment and investigative suggestions were also noted.

4.2.8 Collection of urine sample

Freshly voided mid-stream urine with labia separated of suspected females was collected into a wide mouthed sterile container. Once collected, the sample was transferred to the laboratory without delay within 30 min. If a delay of more than 1-2 hours was to be made

than it was stored in a refrigerator at 4ºC or by collection and transportation in a container with boric acid at bacteriostatic concentration of 1.8% (Collee *et al.*, 2008).

4.2.9 Preparation of culture media

4.2.9.1 Blood Agar Base

40.0 g of agar base was suspended in 1000 ml of distilled water and heated to boiling to dissolve the medium completely. Sterility of the medium was maintained by autoclaving at 15 lbs pressure (121ºC) for 15 min. The medium was then cooled to 50ºC and 5% v/v sterile defibrinated blood was aseptically added. Finally the medium was mixed well and poured into sterile Petri plates.

4.2.9.2Mac-Conkey Agar

51.5 g of agar was suspended in 1000 ml of distilled water and heated to boiling with gentle swirling to dissolve the agar completely. Sterility of the medium was maintained by autoclaving at 15 lbs pressure (121º) for 15 min. Finally the medium was cooled to 45-50ºC and poured into sterile Petri plates.

4.2.9.3Mueller Hinton Agar

38.0 g of agar was suspended in 1000 ml of distilled water and heated to boiling to dissolve the medium completely. Sterility of the medium was maintained by autoclaving at 15 lbs pressure (121ºC) for 15 min. Finally the medium was mixed well before pouring into sterile Petri plates to a depth of 4 mm and was allowed to harden. The pH of the medium was adjusted to 7.2 and stored at 4ºC.

4.2.9.4Triple Sugar Iron Agar

65.0 g of agar was suspended in 1000 ml of distilled water and heated to boiling to dissolve the medium completely. Then, the medium was mixed well and distributed into test tubes. Sterility of the medium was maintained by autoclaving at 10 lbs pressure (115ºC) for 15 min. Finally, the medium was set in sloped form with a butt about 1 inch long.

4.2.9.5Simmons Citrate Agar

24.28 g of agar was suspended in 1000 ml of distilled water and heated to boiling to dissolve the medium completely. Then, the medium was dispensed as desired in test tubes or flasks. Sterility of the medium was maintained by autoclaving at 15 lbs pressure (121ºC) for 15 min.

4.2.9.6Motility Indole Urease (MIU) Medium Base

18.0 g of agar was suspended in 950 ml of distilled water and heated to boiling to dissolve the medium completely. It was then dispensed in 95 ml amounts into flasks and sterilized autoclaving at 15 lbs pressure (121ºC) for 15 min. After that, the medium was cooled to about 50-55ºC and 5 ml of sterile 40% Urea solution (FD048) per 95 ml basal medium was added aseptically. Finally, the medium was mixed well and dispensed into sterile test tubes to get cool in an upright position.

4.2.10 Culture and isolation of pathogens

Clean catch midstream collected urine samples was inoculated on Mac-Conkey and Blood Agar media using calibrated platinum loop following standard bacteriological technique. The primary inoculum was made by a loop and the material was spread into four quadrants of the plate. A platinum or nichrome wire (24 S.W.G. size) loop of 2-4 mm diameter with 2 to 3 inches long wire was first sterilized in the Bunsen flame and cooled by touching an uninoculated part of the medium. Then, a loopful of specimen was gently smeared onto the surface of a well dried plate of medium near the peripheral area. The inoculum was then thinly spread in parallel lines in different segments of the plate. The loop was sterilized between different sets of streaks. Finally, the plate was incubated at 37°C for overnight. Pure bacterial colonies counting 100,000 or more was considered as significant and were subjected to identification based on colony characters and biochemical tests (Uwaezuoke and Ogbulie, 2006).

4.2.11 Bacterial identification

4.2.11.1. Colonial Morphology

The appearance of bacterial colony on the surface of a solid medium was used for the primary identification.

- Colour - Pigment and haemolysin cause change in colour.
- Shape - Circular, irregular or radiate.
- Surfaces - Smooth, wavy, rough, and granular.
- Size - In millimeters.
- Elevation - Flat, elevated, low convex, convex, and umbonate.
- Edges - Entire, undulate, crenated, lobate, ciliate.

- Opacity - Translucent, transparent or opaque.
- Consistency – Mucoid, firm frable, membranous, butyrous.

4.2.11.2 Microscopial morphology

Smears prepared from the bacterial colony were examined by staining methods. Gram's staining divide bacteria into Gram-positive and Gram-negative. It is the most widely used stain in medical bacteriology. The steps in this technique were:

- Primary staining of heat fixed smear of bacterial culture was made with a crystal violet solution for one minute.
- Washing of crystal violet with water and adding dilute solution of iodine and keeping it for one minute.
- Washing with water.
- Decolourisation with an organic solvent (acetone + alcohol) for 10-30 seconds.
- Washing with water.
- Counterstaining with a dye of contrasting colour (dilute safranin or neutral red) for 20-30 seconds.

Gram-positive bacteria resist decolourisation and stain violet while Gram-negative bacteria and other cells (pus cells) are decolourised and stain pink with counterstain.

4.2.12Biochemical test

4.2.12.1 Indole Test

Indole is one of the degradation products of amino acid metabolism. It is useful in *identifying E. coli*. This test was used to determine whether bacteria possess the enzyme tryptophanase which will produce the byproduct indole from the catabolism of tryptophan. Kovac's reagent was used after inoculation and incubation to read the results Kovac's reagent contains HCl and dimethylaminobenzaldehyde (DMABA) dissolved in amyl alcohol, which forms a layer on top of the inoculating medium which causes the red color to be very easily visible and distinguishable. The alcohol in Kovac's reagent floats on the media and another chemical in the reagent react with indole to form a red color in the alcohol layer. A positive test produced pink/purple colour at interface and the organism was E. coli and a negative test remained colourless.

4.2.12.2 Methyl Red/Voges Proskauer Broth

4.2.12.2.1 Voges-Proskauer Test

Some microbes do not produce stable acids from glucose fermentation but instead produce 2,3 butanediol from glucose breakdown and in the process an intermediate chemical acetoin is produced. Two reagents Barritt's A (alpha-naphthol) and Barritt's B (KOH) were added to a 48 hour culture of the MRVP broth. A pipette was taken to aliquot out a small amount of the broth to do the VP test and the broth was returned for further incubation for the MR test if necessary. A wine red color change with the addition of Barritt's reagents A and B is a positive test detecting the presence of acetoin and therefore 2, 3 butanediol; a brown or copper color is negative.

4.2.12.2.2 Methyl Red Test

The methyl red test is used to determine organisms that ferment glucose to a stable acid end product in a great degree, lowering the pH of the system despite the presence of buffer. Media for the methyl red test (MRVP media) was prepared and contained peptone, glucose, and a phosphate buffer. Broth was inoculated and incubated at $30^{\circ c}$ for five days to allow stable acids to be produced. At the end of the fifth day, methyl red indicator was added. Methyl red indicator is red at pH less than 4.4 and yellow at pH above 6.0, so a red result was labeled positive, and a yellow result negative.

4.2.12.3 Citrate Utilization Test

Simmon's Citrate agar is used to determine if an organism can use citrate as its only carbon source using the enzyme citrase (also contains ammonia as the only nitrogen source). Citrate utilization is an aerobic process and a slant tube is used to increase exposure of bacterial growth to the air; inoculate the slant. pH indicator is Bromthymol blue, which is green at neutral pH but turns Prussian blue at pH levels above 7.6

4.2.12.4 Triple Sugar Iron Agar

TSI Agar slants contain 2% polypeptone, 1% lactose, 1% sucrose, 1% glucose, phenol red pH indictor, and ferric ammonium citrate. Utilization of sugars proceeds in much the same way as in sugar broth tests, with acid production changing a pH indicator. Specifically, fermentation of lactose/sucrose and glucose causes the entire tube to be yellow. Fermentation of glucose alone causes the butt of the culture to be yellow, but the shallow slant portion

turns red as glucose is oxidatively exhausted and peptone is metabolized, producing NH_3 resulting in an alkaline pH. Gas production during the utilization of sugar is indicated by fissures or pockets in the slant. Some bacteria also produce hydrogen sulfide (H2S) by reducing thiosulfate in the medium or breaking down cysteine in the peptone. Ferric ammonium citrate reacts with hydrogen sulfide to produce a black precipitate in the butt of the agar. The slants were made from commercially prepared TSI mix and poured to allow the butt of the agar to be four centimeters deep, and the slants were inoculated. The slants were incubated for twenty-four hours at 37°c and tests were read for all three sugars as well as gas and H_2S production.

4.2.13 Preparation of MacFarland turbidity standard

0.5 ml of 0.048 M Barium chloride was added in 66.5 ml of 0.36 N sulfuric acid and 5 ml of it was aliquoted into screw-capped tubes of same size and was stored in dark at room temperature (Shaikh *et al.*, 2005).

4.2.14 Preparation of inoculum

Four or five well isolated colonies of same morphological type were touched with a sterile wire loop, suspended into tubes containing 5 ml of MHB. The medium was then incubated at 35ºC for 2-8 hours until the turbidity reached or exceeded that of 0.5 MacFarland standards (already prepared). If the suspension was exceeded, it was diluted with broth and was visually comparable to the 0.5 MacFarland standards (Shaikh *et al.*, 2005).

4.2.15 Inoculation of MHA plates

A sterile swab was dipped into the broth suspension of the organisms within 15 minutes of standardization. Excess inoculum was removed by rotating the swab several times against the wall of the tube above the fluid level. The entire surface of an MHA plate was then streaked evenly in three/two directions approximately 60 degrees from each other (Shaikh *et al.*, 2005).

4.2.16 Antimicrobial sensitivity test

Antimicrobial sensitivity test was performed by disc diffusion method (Kirby-Bauer's technique) using commercially available disc. The antimicrobial impregnated discs was placed with sterile forceps on the Muller Hinton Agar surface such that each disc was at least 24 mm from the other disc avoiding overlap during incubation. Then, it was incubated at

37°C for 18-24 hours. These test discs will be of the antimicrobials observed in prescription pattern (Khan and Zaman, 2006). At the end of incubation period, the diameter of zones of inhibition around each disc was measured with a Vernier caliper on the back of the plate, with reflected light against a dark non-reflective background. The zone of diameter for each antimicrobial agent was then interpreted as resistant/intermittent/sensitive by comparing with the standard given by the HiMedia Laboratories Pvt. Ltd. (Shaikh *et al.*, 2005).

The test discs of the antimicrobial used were:

Amoxycillin	Cefixime
Nalidixic acid	Cefpodoxime
Cotrimoxazole	Cephalexin
Nitrofurantion	Amikacin
Ofloxacin	Gentamycin
Ciprofloxacin	Ceftriaxone
Norfloxacin	Azithromycin
Levofloxacin	

4.2.17 Noting isolated bacteria and sensitivity pattern

The bacteria responsible for the pathogenesis were noted after isolation of bacteria with culture and biochemical tests. Along with it, the sensitivity pattern of the antimicrobials was also noted down.

4.2.18 Noting final diagnosis and prescribing pattern

The final diagnosis made after seeing the report by the physicians and the drug prescribed after it was noted down.

4.2.19 Data analysis

Statistical Package for Social Sciences for windows (SPSS), version 11.5 was used for statistical analysis. (Akortha and Ibadin, 2010).

5. RESULTS

The research was conducted in the two major hospital of Pokhara valleys which are:

1. Charak Hospital and Research Centre (Hospital A) and
2. Western Regional Hospital (Hospital B).

A total of 175 urine sample were collected, out of these 104 patients' urine were found to have significant bacterial growth. Total six species of bacteria were isolated viz. *E.coli* 67(64.4%), *Proteus species* 3(2.9%), *Klebsiella species* 14(13.3%), *Staphylococcus aureus* 11(10.6%), *Citrobacter species* 5(4.8%) and *Pseudomonas aureginosa* 4(3.8%).

Table 1: Respondence of UTI in relation to age distribution of female patients

		Hospital		Total
		A	B	
Age group	< 10	20.0%	30.9%	26.9%
	10 -20	13.8%	18.2%	16.6%
	20 - 30	36.9%	24.5%	29.1%
	30 - 40	10.8%	15.5%	13.7%
	40 - 50	9.2%	5.5%	6.9%
	50 - 60	3.1%	2.7%	2.9%
	60 - 70	3.1%	2.7%	2.9%
	> 70	3.1%		1.1%
Total		100.0%	100.0%	100.0%

The above given table 1. shows the incidence of UTI in relation to age of the subjects. A higher percentage of females (36.9%) with UTIs were found within the age brackets of 20-30 years in case of hospital A and in hospital B, higher incidence of UTIs were found in the patients below 10 years of age while the subjects above 50 years had the least percentage in both the hospitals (3.1% for hospital A and 2.7% for hospital B). Overall, the highest percentages of incidence of UTIs were found in the patients of age group 20-30 years.

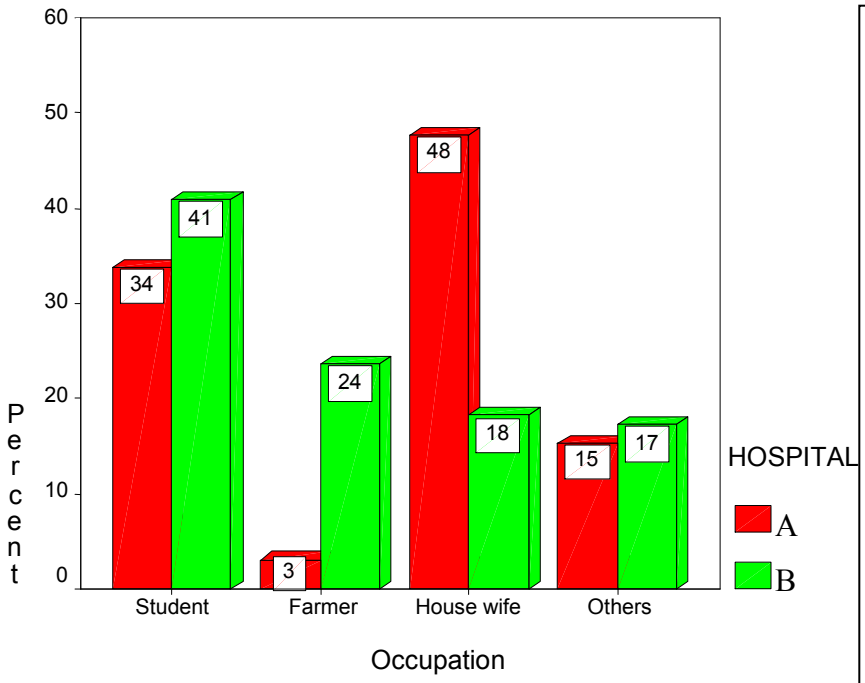

Figure 1: Percentage of patients suffering from UTI based on Occupation

Figure 1. shows the incidence of UTI by occupational groups. UTIs appear to be more prevalent among housewives who constituted 48 % of the UTI patients followed by students (34%), others (15%) and farmers (3%) in the context of hospital A. For hospital B, UTIs appear to be more prevalent among the students (41%) followed by farmers (24%), housewives (18%) and others (17%).

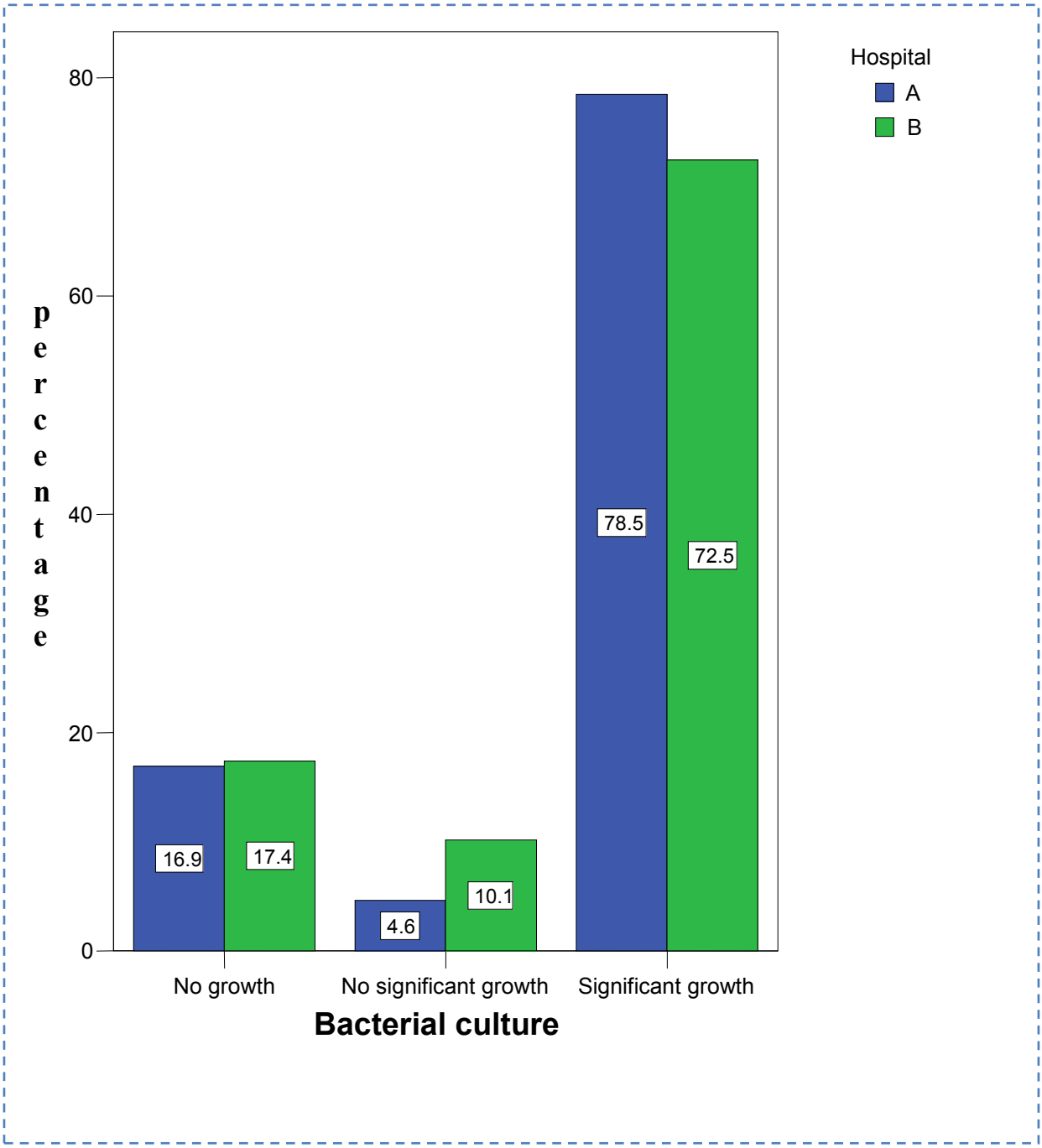

Figure 2: Bacterial growth positive rate in urine samples collected

Figure 2. shows that in hospital A significant growth of bacteria occurred in 51 urine samples (78.5%) out of 65 samples while the bacteria isolates were not found in 14 samples (21.5%). In case of hospital B, the significant growth occurred in 53 samples (72.5%) and 16 cases (27.5%) were found to have no growth of bacteria out of 69 urine samples.

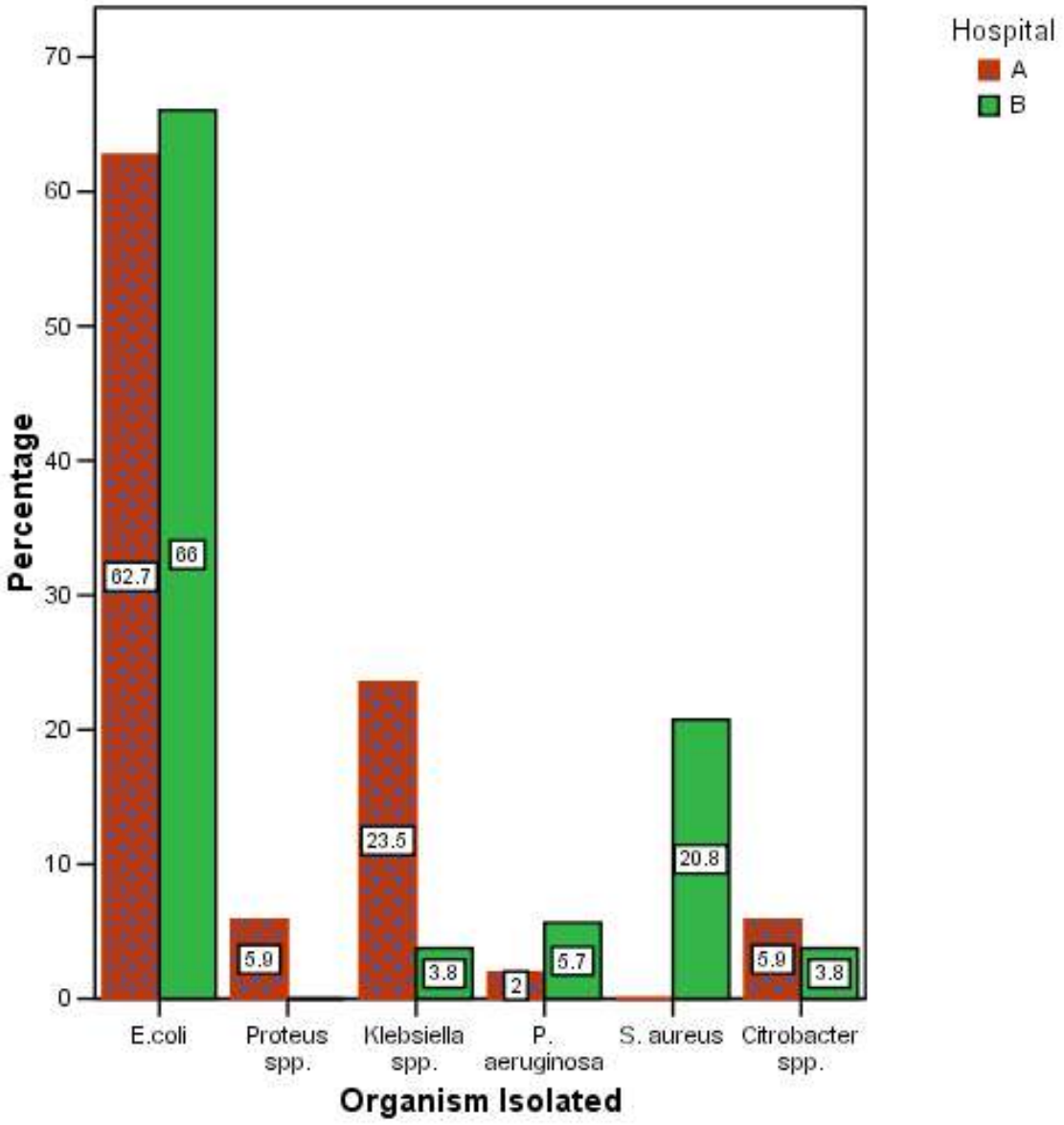

Figure 3: Frequency of different pathogens isolated from urine samples of females with uncomplicated UTI (N = 104) in both hospitals

Total six species of bacteria were isolated viz. *E coli, Proteus, Klebsiella*, *Pseudomonas*, *S. aureus* and *Citrobacter* spp. Among them *E.coli* (62.7% in hospital A and 66% in hospital B) was the most common pathogen causing UTI in both the hospitals followed by *Klebsiella* (23.5%), *Proteus* (5.9%), *Citrobacter* (5.9%) and *Pseudomonas* (2%) in hospital A and *S. aureus* (20.8%), *Pseudomonas* (5.7%), *Klebsiella* (3.8%) and *Citrobacter* (3.8%). However, there was no significant growth of *S. aureus* in hospital A and *Proteus* in hospital B.

The below given tables show the sensitivity pattern of different antimicrobials against the isolated organisms and it was found that most of the antimicrobials were highly sensitive against E. coli. However, ofloxacin, cefixime, norfloxacin, cotrimoxazole were found to be more sensitive against proteus and citrobacter species.

Table 2: Sensitivity patterns of Amoxycillin with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated					
			E.coli	Proteus spp.	Klebsiella spp.	P. aeruginosa	S. aureus	Citrobacter spp.
A	Amoxycillin	Sensitive	45.8%	33.3%	11.1%			
		Intermediate	4.2%	33.3%				50.0%
		Resistant	50.0%	33.3%	88.9%	100.0%		50.0%
	Total		100.0%	100.0%	100.0%	100.0%		100.0%
B	Amoxycillin	Sensitive	52.4%		100.0%	50.0%	42.9%	
		Intermediate	42.9%				42.9%	
		Resistant	4.8%			50.0%	14.3%	
	Total		100.0%		100.0%	100.0%	100.0%	

Table 3: Sensitivity patterns of Nalidixic acid with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated					
			E.coli	Proteus spp.	Klebsiella spp.	P. aeruginosa	S. aureus	Citrobacter spp.
A	Nalidixic acid	Sensitive	45.5%					
		Intermediate	9.1%					
		Resistant	45.5%	100.0%	100.0%			
	Total		100.0%	100.0%	100.0%			
B	Nalidixic acid	Sensitive	44.4%			100.0%	37.5%	
		Intermediate	14.8%		100.0%		25.0%	
		Resistant	40.7%				37.5%	100.0%
	Total		100.0%		100.0%	100.0%	100.0%	100.0%

Table 4: Sensitivity patterns of Nitrofurantoin with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated					
			E.coli	Proteus spp.	Klebsiella spp.	P. aeruginosa	S. aureus	Citrobacter spp.
A	Nitrofurantoin	Sensitive	100.0%	100.0%	90.9%	100.0%		66.7%
		Intermediate						33.3%
		Resistant			9.1%			
	Total		100.0%	100.0%	100.0%	100.0%		100.0%
B	Nitrofurantoin	Sensitive	60.6%		100.0%		37.5%	50.0%
		Intermediate	27.3%			66.7%	37.5%	50.0%
		Resistant	12.1%			33.3%	25.0%	
	Total		100.0%		100.0%	100.0%	100.0%	100.0%

Table 5: Sensitivity patterns of Ofloxacin with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated					
			E.coli	Proteus spp.	Klebsiella spp.	P. aeruginosa	S. aureus	Citrobacter spp.
A	Ofloxacin	Sensitive	51.9%	66.7%	54.5%			66.7%
		Intermediate	14.8%		9.1%	100.0%		
		Resistant	33.3%	33.3%	36.4%			33.3%
	Total		100.0%	100.0%	100.0%	100.0%		100.0%
B	Ofloxacin	Sensitive	46.9%		50.0%	50.0%	37.5%	50.0%
		Intermediate	18.8%			50.0%	50.0%	
		Resistant	34.4%		50.0%		12.5%	50.0%
	Total		100.0%		100.0%	100.0%	100.0%	100.0%

Table 6: Sensitivity patterns of Ciprofloxacin with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated					
			E.coli	Proteus spp.	Klebsiella spp.	P. aeruginosa	S. aureus	Citrobacter spp.
A	Ciprofloxacin	Sensitive	60.7%	100.0%	45.5%			100.0%
		Intermediate	7.1%		27.3%	100.0%		
		Resistant	32.1%		27.3%			
	Total		100.0%	100.0%	100.0%	100.0%		100.0%
B	Ciprofloxacin	Sensitive	51.6%		50.0%	100.0%	66.7%	50.0%
		Intermediate	12.9%		50.0%			50.0%
		Resistant	35.5%				33.3%	
	Total		100.0%		100.0%	100.0%	100.0%	100.0%

Table 7: Sensitivity patterns of Norfloxacin with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated					
			E.coli	Proteus spp.	Klebsiella spp.	P. aeruginosa	S. aureus	Citrobacter spp.
A	Norfloxacin	Sensitive	41.4%	66.7%	45.5%			66.7%
		Intermediate	13.8%					
		Resistant	44.8%	33.3%	54.5%	100.0%		33.3%
	Total		100.0%	100.0%	100.0%	100.0%		100.0%
B	Norfloxacin	Sensitive	27.3%		100.0%		25.0%	
		Intermediate	27.3%				50.0%	
		Resistant	45.5%				25.0%	100.0%
	Total		100.0%		100.0%		100.0%	100.0%

Table 8: Sensitivity patterns of Levofloxacin with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated		
			E.coli	Klebsiella spp.	S. aureus
B	Levofloxacin	Sensitive	62.5%		60.0%
		Intermediate	25.0%	100.0%	20.0%
		Resistant	12.5%		20.0%
	Total		100.0%	100.0%	100.0%

Table 9: Sensitivity patterns of Cefixime with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated					
			E.coli	Proteus spp.	Klebsiella spp.	P. aeruginosa	S. aureus	Citrobacter spp.
A	Cefixime	Sensitive	43.5%	66.7%	37.5%			66.7%
		Intermediate	4.3%					
		Resistant	52.2%	33.3%	62.5%	100.0%		33.3%
	Total		100.0%	100.0%	100.0%	100.0%		100.0%
B	Cefixime	Sensitive	34.6%		100.0%	50.0%	85.7%	50.0%
		Intermediate	19.2%					
		Resistant	46.2%			50.0%	14.3%	50.0%
	Total		100.0%		100.0%	100.0%	100.0%	100.0%

Table 10: Sensitivity patterns of Gentamycin with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated					
			E.coli	Proteus spp.	Klebsiella spp.	P. aeruginosa	S. aureus	Citrobacter spp.
A	Gentamycin	Sensitive	100.0%	66.7%	91.7%	100.0%		66.7%
		Intermediate			8.3%			
		Resistant		33.3%				33.3%
	Total		100.0%	100.0%	100.0%	100.0%		100.0%
B	Gentamycin	Sensitive	75.0%		100.0%	100.0%	50.0%	50.0%
		Intermediate	12.5%				25.0%	
		Resistant	12.5%				25.0%	50.0%
	Total		100.0%		100.0%	100.0%	100.0%	100.0%

Table 11: Sensitivity patterns of Azithromycin with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated					
			E.coli	Proteus spp.	Klebsiella spp.	P. aeruginosa	S. aureus	Citrobacter spp.
A	Azithromycin	Sensitive	84.6%	100.0%	100.0%	100.0%		66.7%
		Intermediate	3.8%					33.3%
		Resistant	11.5%					
	Total		100.0%	100.0%	100.0%	100.0%		100.0%
B	Azithromycin	Sensitive	100.0%				100.0%	
	Total		100.0%				100.0%	

Table 12: Sensitivity patterns of Cotrimoxazole with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated					
			E.coli	Proteus spp.	Klebsiella spp.	P. aeruginosa	S. aureus	Citrobacter spp.
A	Cotrimoxazole	Sensitive	64.3%	100.0%	33.3%			100.0%
		Resistant	35.7%		66.7%			
	Total		100.0%	100.0%	100.0%			100.0%
B	Cotrimoxazole	Sensitive	56.0%				50.0%	
		Intermediate	12.0%			100.0%	25.0%	
		Resistant	32.0%		100.0%		25.0%	100.0%
	Total		100.0%		100.0%	100.0%	100.0%	100.0%

Table 13: Sensitivity patterns of Cefpodoxime with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated			
			E.coli	Klebsiella spp.	Pseudomonas aeruginosa	S. aureus
A	Cefpodoxime	Sensitive	100.0%			
	Total		100.0%			
B	Cepodoxime	Sensitive	40.0%		100.0%	60.0%
		Intermediate	20.0%			40.0%
		Resistant	40.0%	100.0%		
	Total		100.0%	100.0%	100.0%	100.0%

Table 14: Sensitivity patterns of Cephalexin with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated			
			E.coli	Klebsiella spp.	S. aureus	Citrobacter spp.
A	Cephalexin	Sensitive	33.3%			
		Resistant	66.7%	100.0%		
	Total		100.0%	100.0%		
B	Cephalexin	Sensitive	18.8%	50.0%	20.0%	
		Intermediate	25.0%	50.0%	40.0%	
		Resistant	56.3%		40.0%	100.0%
	Total		100.0%	100.0%	100.0%	100.0%

Table 15: Sensitivity patterns of Amikacin with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated					
			E.coli	Proteus spp.	Klebsiella spp.	P. aeruginosa	S. aureus	Citrobacter spp.
A	Amikacin	Sensitive	100.0%	100.0%	100.0%	100.0%		66.7%
		Resistant						33.3%
	Total		100.0%	100.0%	100.0%	100.0%		100.0%
B	Amikacin	Sensitive	81.3%		100.0%	100.0%	75.0%	50.0%
		Intermediate	15.6%				25.0%	
		Resistant	3.1%					50.0%
	Total		100.0%		100.0%	100.0%	100.0%	100.0%

Table 16: Sensitivity patterns of other antibiotics with respective isolated organism in hospital A and B

HOSPITAL			Organism Isolated	
			E.coli	Citrobacter spp.
A	Other antibiotic	Resistant		100.0%
	Total			100.0%
B	Other antibiotic	Sensitive	12.5%	
		Intermediste	12.5%	
		Resistant	75.0%	100.0%
	Total		100.0%	100.0%

Table 17: Overall sensitivity Pattern of antimicrobials in hospital A and hospital B

Hospital A	Cefpodoxime (100%) > Amikacin (98.0%) > Nitrofurantoin (95.9%) > Gentamycin (94.1%) Azithromycin (88.1%) > Cotrimoxazle (60.9%) > Ciprofloxacin (58.1%) > Ofloxacin (53.3%) > Cefixime (44.7%) = Norfloxacin (44.7%) > Nalidixic Acid (35.7%) > Amoxycillin (33.3%) > Cephalexine (25.5%).
Hospital B	Azithromycin (100%) > Amikacin (80.3%) > Gentamycin (72.3%) > Ciprofloxacin (56.8%) > Nitrofurantoin (54.2%) > Levofloxacin (53.3% > Amoxycillin (53.1%) > Cotrimoxazole (48.5%) > Cefixime (47.1%) > Ofloxacin (45.7%) > Cefpodoxime (45.5%) > Nalidixic Acid (40.4% > Norfloxacin (31.0%) > Cephalexine (20.8%) > Other antibiotics (11.1%).

According to table 17, the most sensitive antimicrobial against the isolated organisms was cefpodoxime in hospital A and azithromycin in hospital B whereas highly resistant antimicrobial was cephaleximе in both the hospitals**.** Regardless the etiology Nitrofurantoin, Gentamycin and Cepodoxime are more sensitive than the frequently used antimicrobial agents like Quinolones (Ciprofloxacin, Ofloxacin and Norfloxacin).

Table 18: Frequency of antibiotics prescribed in respective hospitals

		HOSPITAL		Total
		A	B	
Treatment	Amikacin	3.8%		1.3%
	Amoxycillin		11.9%	7.8%
	Azithromycin	9.6%	7.9%	8.5%
	Cefpodoxime	1.9%	2.0%	2.0%
	Cefixime	3.8%	14.9%	11.1%
	Ofloxacin	28.8%	21.8%	24.2%
	Ceftriaxone	1.9%	5.9%	4.6%
	Gentamycin	5.8%	1.0%	2.6%
	Ciprofloxacin		6.9%	4.6%
	Clotrimazole		2.0%	1.3%
	Metronidazole		1.0%	.7%
	Cloxacillin		1.0%	.7%
	Levofloxacin		1.0%	.7%
	Nitrofurantoin	19.2%		6.5%
	Azithromycin Cefpodoxime		2.0%	1.3%
	Azithromycin Amoxycillin		2.0%	1.3%
	Azithromycin Ceftriaxone		1.0%	.7%
	Cefixime Azithromycin	1.9%	10.9%	7.8%
	Gentamycin Azithromycin	1.9%		.7%
	Gentamycin Cefixime	1.9%		.7%
	Gentamycin Ceftriaxone	5.8%		2.0%
	Nitrofurantoin Doxycycline	1.9%		.7%
	Ofloxacin Azithromycin	1.9%		.7%
	Ofloxacin Cefixime	3.8%		1.3%
	Ofloxacin Metronidazole		1.0%	.7%
	Ceftriaxone Cefixime	1.9%		.7%
	Ceftriaxone Azithromycin	3.8%	1.0%	2.0%
	Gentamycin Ampicillin Azithromycin		1.0%	.7%
	Cefixime Azithromycin Ofloxacin		1.0%	.7%
	Clotrimazole Cefixime Azithromycin		1.0%	.7%
	Clotrimazole Metronidazole Cefixime Azithromycin		1.0%	.7%
	Ciprofloxacin Azithromycin		1.0%	.7%
Total		100.0%	100.0%	100.0%

The table 18 shows that the most commonly prescribed antimicrobial was ofloxacin (28.8% in hospital A and 21.8% in hospital B) followed by nitrofurantoin (19.2%) and Azithromycin(9.6%) in hospital A and Cefixime (14.9%), Amoxycillin (11.9%) and Azithromycin (7.9%) in hospital B. Overall, the most commonly prescribed drug in both the hospitals was found to be ofloxacin (24.2%). Also, during the study we found that the combination of antibiotics was also prescribed.

Table 19: Frequency of antibiotics prescribed in hospital A and hospital B

No. of antimicrobials		HOSPITAL	
		A	B
	1 antibiotic	75.0%	77.2%
	2 antibiotics	25.0%	18.8%
	3 antibiotics	00.0%	3.0%
	> 3 antibiotics	00.0%	1.0%
Total		100.0%	100.0%

The above table describes that mostly single antibiotic was found to be prescribed for the treatment of UTIs in both hospitals (75% in hospital A and 77.2% in hospital B). In most cases, due to resistance against the isolated organisms, antibiotic previously used was changed and more sensitive one was prescribed. More than 3 antibiotics were least prescribed and found only in hospital B (1%). Overall, only one antibiotic was found to be commonly prescribed in both hospitals (76.5%) to prevent problems like antibiotic resistance and drug-drug interaction**.**

According to figure 4, we found that 37 % of the patients visiting hospital B were empirically prescribed on the basis of urine R/E report. No culture and sensitivity test was done for such patients. Azithromycin, Cefixime, and Ofloxacin were most frequently empirically prescribed antibiotics. More than two antibiotics were also prescribed to some patients. The order of empirically prescribed antibiotics are Azithromycin (14 %) = Cefixime + Azithromycin (14 %) > Amoxycillin (11 %) = Cefixime (11 %) = Ofloxacin (11 %) > Ceftriaxone (5 %) = Cepodoxime = Ciprofloxacin = Clotrimazole = Cloxacillin = Azithromycin and Cepodxime = Cefixime and Metronidazole = Cefixime Azithromycin and Ofloxacin = Clotrimazole, Cefixime and Azithromycin = Clotrimazole, Metronidazole, Cefixime and Azitromycin = Ciprofloxacin and Azitromycin (2 %). In hospital A, we did not find prescribing antibiotics merely on the basis of urine R/E. Though, some patients were prescribed on the basis of urine R/E report in extreme condition, it was changed according to culture sensitivity reports.

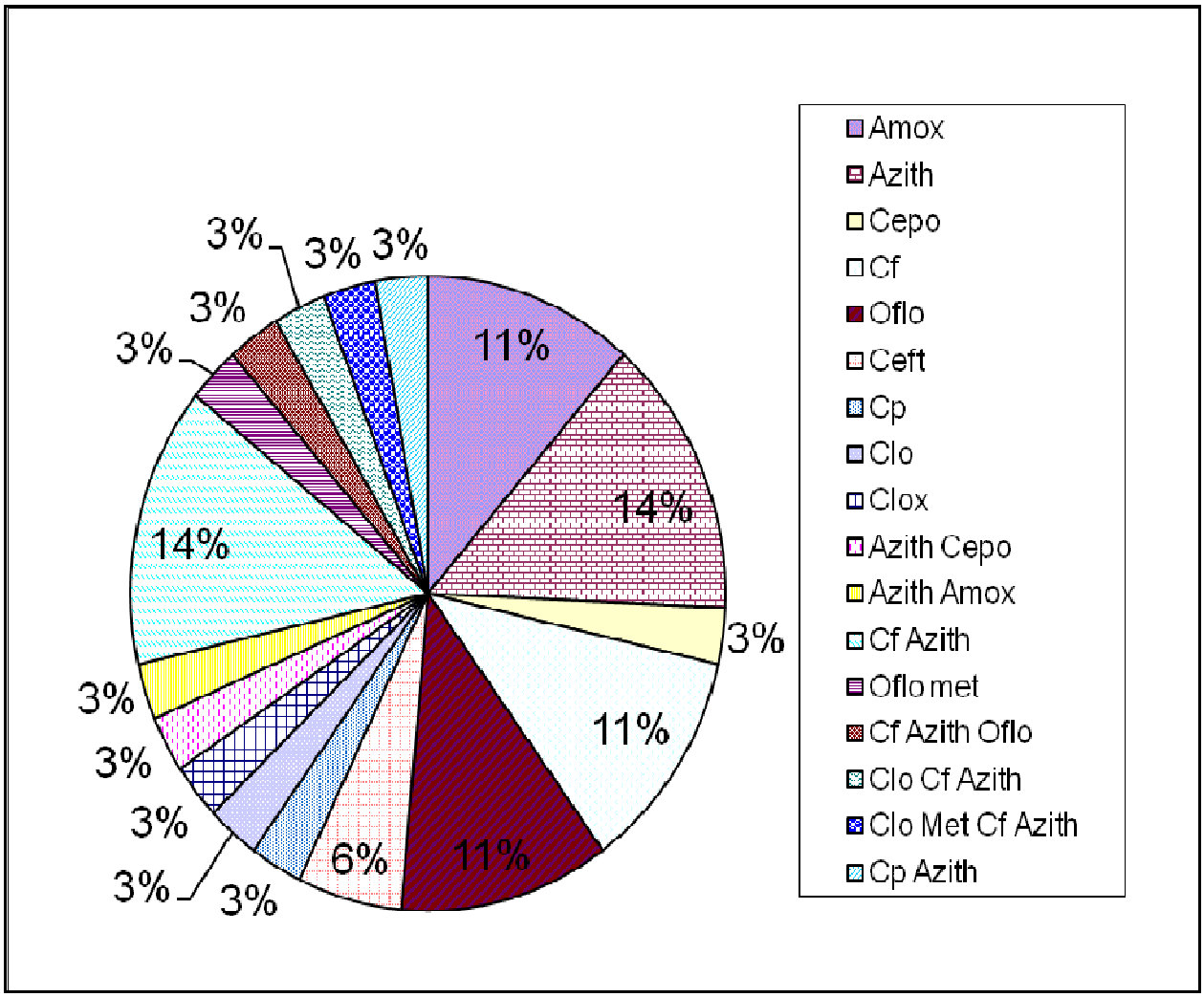

Figure 4: Emperical prescription of antibiotics in hospital B on the basis of urine routine examination only

6. DISCUSSION

The antibiotic sensitivity pattern of organisms changes rapidly over a short period. It is especially true for developing countries where antibiotics are prescribed irrationally not only by the medical practitioners but the antibiotics are also purchased directly from the chemists (medicine shop keepers) without prescription. It has been advised that medical practitioners should be aware of the rising resistance of urinary pathogens to commonly prescribed antibiotics as well as the profile of antibiotic resistance within their community. Therefore, evaluation of sensitivity pattern is essential for rational and appropriate use of antibiotics.

An analysis of the data for different aspects reveals the pattern of UTI prevalent according to age, organism affecting and the antimicrobials used and are effective. The observations are mostly concurrent with the reports available in the literature though some significant differences were observed which are reported in this study. A total of 175 urine sample were collected, out of these 104 patients' urine were found to have significant bacterial growth. Total six species of bacteria were isolated viz. *E.coli*, *Proteus species*, *Klebsiella species*, *Staphylococcus aureus, Citrobacter species* and *Pseudomonas aureginosa*. Among them, most prevalent organism found was *E. coli* (64.4%), which is confirmatory to the study done by Das RN *et al.* 2006 and Basnet BB *et al.* 2009. Higher prevalence of *E. coli* followed by *Klebsiella* (13.5%) and *S. aureus* (10.6%) in this study resembles to the various studies done by different scientists in different parts of the world. 89.4% of gram negative bacilli are responsible for UTI and the only gram positive bacteria responsible for UTI was *S. aureus* which constitute 10.6% in our study. *E. coli* is dominant for outpatients as well as indoor patients. In our study, *S. aureus* was not a causative agent for UTI in hospital A, similarly *Proteus species* was not a causative agent for UTI in hospital B. Amongst the antimicrobials used, most of the organisms were sensitive to amikacin, cefpodoxime, gentamycin and azithromycin whereas cephalexin, nalidixic acid and norfloxacin were found least sensitive.

The combinations of antibiotics were also found to be prescribed like (azithromycin + cefpodoxime), (azithromycin + ceftriaxone), (gentamycin + azithromycin), (gentamycin + ceftriaxone), (ofloxacin + cefixime), etc. A single antibiotic was most commonly prescribed in both the hospitals, however, more than three antibiotics were also found to be prescribed in case of hospital B.

The patients of age group less than 10 have high incidence of UTI in WRH. That may be due to the poor hygienic condition of the patients visiting WRH because most of the patients visiting WRH are of poor economic condition and of rural area who don't care about the personal hygiene.

The prevalence of UTI was found to be high in housewife in Charak than WRH because most of the patients visiting WRH were of rural area and due to the social burden they feel shy to share the sign and symptoms of UTI and so they don't visit hospitals even though they have UTI.

Nitrofurantoin, Aminogylcosides and Cepfpodoxime were found to be more sensitive than Quinolones because of the misuse and abuse of antibiotics among the general population as a empirical treatment therapy which has favored the emergence of resistance strains.

7. SUMMARY AND RECOMMENDATIONS

In two hospitals, the incidence of UTI was maximum in females of age group 20-30. Altogether, six species of bacteria were isolated, *E .coli*, *S. aureus*, *Klebsiella*, *Proteus*, *Pseudomonas* and *Citrobacter*. The most common organism was *E. coli* wheras least prevalent organism was *Pseudomonas* and *Proteus* species. Among the antimicrobials tested for sensitivity in the urine culture in both the hospitals. Aminoglycosides, Nitrofurantoin are most sensitive while frequently used qunolones have increasing incidences of resistances.

For uncompleted UTI single safe and effective antibiotics prescription is recommended as per standard guidelines.

8. CONCLUSION

1.E.coli is the most commonest bacteria causing UTI followed by Gram negative like k.pneumoniae, Acinitobacter spp., Citrobacter spp., and gram positive bacteria like S.aureus, CONS and Enterococcus

2.Bacteriuria itself is not a disease but the normal flora of the human body is extremely important as a key part of host defences against infection.

3.Bacteriuria alone is rarely an indication for antibiotic treatment therefore doctors are encouraged to ask their patients to do urine culture when they suspect UTI, in order to give the best treatment.

4.In patients presenting with symptoms or signs of UTI who have a history of fever or back pain the possibility of UTI should be considered.

5.Empirical treatment with an antibiotic should be started and urine culture including all positive confirmed cases of UTI either for Escherichia coli, Staphylococcus aureus, or other uropathogens performed to guide the choice of antibiotic.

6.A widespread screening program for UTI should be implemented to know the exact prevalence of UTI.

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