CHAPTER-I

1. INTRODUCTION

Cancer involves a pathological breakdown in the processes which control cell proliferation, differentiation and death of particular cells due to which group of cells display uncontrolled growth, invasion, and sometimes metastasis (spread to other locations in the body via lymph or blood) (Steward and Kleihues, 2003).

Cancer may affect people at all ages, but the risk for most increases with age. Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells. These abnormalities may be due to the effects of carcinogens such as tobacco smoke, radiation, chemicals, or infectious agents. Other cancer-promoting genetic abnormalities may be randomly acquired through errors in DNA replication, or are inherited (Lewin *et al.,* 2006). Once diagnosed, cancer is usually treated with/or a combination of surgery, chemotherapy and radiotherapy. Chemotherapy, radiation therapy, or a combination of both often cause temporary immune and blood system side effects (American Cancer Society, 2008).

Chemotherapy is a general term for any treatment involving the use of chemical agents to stop cancer cells from growing. Chemotherapy destroys rapidly dividing cells, a characteristic of cancer cells and blood producing bone marrow cells (Essig, 2010). Hence chemotherapy may lead to low blood counts. Generally, white blood cell production is the most sensitive to chemotherapy drugs (Hamilton, 2005).

The type of white blood cell we have in greatest numbers is called the neutrophil.They provide an especially important defense against most types of infections (American Cancer Society, 2008). A reduction in neutrophil count (usually less than $1.5 \times 10^9/l$) is called neutropenia. Chemotherapy-induced neutropenia is not uncommon. The risk of bacterial infection is related to the degree of neutropenia, with counts lower than $0.5 \times 10^9/l$ conferring the highest risk.(Boon *et al.,* 2006).

Infection remains the most common complication of myelosuppressive antineoplastic therapy and is associated with substantial mortality and morbidity despite major advances in supportive care (Rolston, 2009). The most common sites of infection in neutropenic cancer patients include the lung, oropharynx, blood, urinary tract, skin and soft tissues, including the perirectal area. Infections are generally caused by organisms already colonizing the patient, although some of these organisms are acquired after admission to the hospital (Tancheva *et al*., 2009). Severe infections due to Gram-negative bacilli & staphylococci are common in cancer patients (Rosenthal *et al*., 1987).

Urinary tract infection (UTI) is the active infection in any part of the urinary tract beyond distal urethra which is normally bacteriologically sterile (Bhatia and Ichhpujani, 1999). Primarily, the bacteria that colonize in the bowel and are capable of proliferating in urine cause UTI. Urinary tract infection is defined as the condition of multiplication of the organisms in the urinary tract and the presence of more than 10^5 organisms /ml of mid stream urine. Discovering over 10^5 bacteria / ml of voided urine indicates significant bacteriuria, a presumably pathological condition (Forbes *et al.*, 2007).

UTI is the most common bacterial infection in the general population and it is also a common clinical problem in cancer patients. UTI in cancer patients should be considered complicated when it occurs as a result of suppressed immunity (Yeung *et al*., 2009). Many factors increase the susceptibility of immunosuppressed cancer patients to infection. These include erosion of tumor involving the protective integument or mucosa (Zinner,1997), neutropenia during aggressive therapy, altered gut flora because of frequent antibiotic administration, and the various invasive procedures including the long term use of indwelling catheters, and combinations of the aforementioned (Billote,1996).

Antibitotic resistance poses a threat to everyone, but cancer patients are at particular risk. Antibiotic usage for the prevention and treatment of bacterial infections in these

high-risk patients leads to selection pressures resulting in the emergence and spread of resistant organisms (Lewis, 1995). Many organisms acquire several resistance mechanisms, making them multi-drug-resistant (MDR) (defined as resistance to three or more different classes of antibiotics) (Tuladhar, 2001). An increasing trend of resistance to commonly used antibiotics is emerging especially among gram negative organisms like *E. coli, Pseudomonas, and Klebsiella spp* (Khan *et al*., 2004). The current trend of rising trimethoprim-sulphamethoxazole (TMP/SMX) and beta-lactam resistance rates is problematic. Of more concern, however, are the emerging issues of fluoroquinolone resistance (Gupta, 2003). The broad use of fluoroquinolones for antibacterial prophylaxis in neutropenic patients may lead to very high resistance rates among Gram-negative bacilli such as *E. coli* (Maschmeyer et *al.,* 2008).

The present study was conducted with a broad objective to isolate the bacteria causing UTI in cancer patients under chemotherapy and determine the trend of their antimicrobial resistance. Since infections in cancer patients occur mainly due to low blood cell counts, this study was also done to find out the possible association between the occurrence of UTI and the blood cell counts i.e. total WBC count and absolute neutrophil count (ANC) in these group of patients.

CHAPTER – II

OBJECTIVES :

2.1 General Objective :

To determine the incidence of urinary tract infection in cancer patients under chemotherapy visiting OM Hospital and Research Center and to find out the status of MDR strains among the bacterial isolates.

2.2 Specific Objectives :

1. To isolate and identify the causative agents of urinary tract infection in cancer patients under chemotherapy.
2. To analyze the antibiotic susceptibility pattern of the isolated organisms.
3. To find out the prevalence of multi-drug resistant organisms among the urinary isolates.
4. To find out the total WBC count and the absolute neutrophil count in the blood sample of the cancer patients under chemotherapy .
5. To find out the possible association between the total WBC count and UTI in cancer patients under chemotherapy.
6. To find out the possible association between absolute neutrophil count and UTI in these patients.

CHAPTER-III

3. LITERATURE REVIEW

3.1 Cancer

Cancer is a disease of cells that proliferate at inappropriate times and locations in the body. When cells acquire mutations that abolish regulation of the cell division, the cells multiply to form masses that we call tumors. Non-cancerous tumors (benign) are non-invasive and do not affect other tissues, whereas cancerous tumors (malignant) proceed along a destructive pathway. Tumor growth is accompanied by the growth of new blood vessels that nourish the tumor, a process called angiogenesis. Finally, malignant cells can detach from their original location and establish themselves in new locations in the body, in a process called metastasis (Lewin *et al.,* 2006).

About 90% of cancers develop in epithelial cells and are known as carcinomas. Those derived from connective tissue and muscle cells are called sarcomas and those derived from while blood cells are called leukemias and lymphomas (Vander *et al.,* 2001).

3.2 Causes of Cancer:

There are some known *carcinogens*, materials that can cause cancer, but many are still undiscovered. A variety of different and seemingly unrelated environmental factors can cause cancer. They include viruses, a wide variety of chemicals, and both ionizing and ultraviolet radiation. The length and amount of exposure are believed to affect one's chances of developing a disease, such as with cigarette smoking and lung cancer. Genetics can also play an important role in whether an individual develops cancer. Most of these agents however share an important biological property: they can cause damage to or alteration of DNA in cells. This common property suggests that DNA is the essential target of all carcinogenic agents and that cancer arises as a result of changes in the cellular DNA. Other cancer-promoting genetic abnormalities may be randomly acquired through errors in DNA replication, or are inherited, and thus present in all cells

from birth. The heritability of cancers is usually affected by complex interactions between carcinogens and the host's genome (Tannock *et al*., 2007).

Genetic abnormalities found in cancer typically affect two general classes of genes. Cancer-promoting oncogenes are typically activated in cancer cells, giving those cells new properties, such as hyperactive growth and division, protection against programmed cell death, loss of respect for normal tissue boundaries, and the ability to become established in diverse tissue environments. Tumor suppressor genes are then inactivated in cancer cells, resulting in the loss of normal functions in those cells, such as accurate DNA replication, control over the cell cycle, orientation and adhesion within tissues, and interaction with protective cells of the immune system (Kinzler *et al.*, 2002).

Cancer is a disease that disproportionately affects older patients. People aged 65 and older have an 11 fold increase in the incidence of cancer and a 15 fold increase in cancer mortality in comparison to people younger than age 65. With time, there is an increased exposure to possible carcinogens and an accumulation of cellular events, leading to the development of a malignant cell (Kufe *et al*., 2003).

3.3 Cancer treatment:

Treatment of cancer and precancerous lesions has one of three aims: prevention, cure or palliation, depending on the histologic nature of the tumor, stage of disease, and metastasis. Three basic treatment approaches used, either alone or in combination are: surgery, radiation therapy and chemotherapy to prevent both local recurrence and recurrence throughout the body (ACS Professional Education Publication, 1981).

3.3.1 Surgery :

Surgery is the oldest treatment for cancer and has a role in diagnosing pathologic states of cancer patients. Surgery can be simple, safe method to cure patients with solid tumors when the tumor is confined to the anatomic site of origin (Devita *et al.*, 1989). Surgical removal of a tumor must include an adequate margin of normal tissue to allow for local invasive spread (Tannock *et al.*, 2007). Cancer patients are often immunosuppressed by

either their disease or their treatment and are subjected to a variety of opportunistic infections not commonly seen in most general surgical patients (Devita *et al*., 1989).

3.3.2 Radiation treatment :

Radiotherapy means the treatment of cancer with ionising radiation, and for certain localised cancers it may be curative. Ionising radiation can be delivered by radiation emitted from the decay of a radioactive isotope or by high energy radiation beams, usually X-rays and gamma rays (Boon *et al*., 2006)**.** Radiation deposition results in DNA damage manifested by single and double strand breaks in the sugar phosphate backbone of the DNA molecule. Crosslinks between DNA strands and chromosomal proteins also occur (Kufe *et al.*, 2003).

As with every form of treatment, radiation therapy is not without its side effects. It kills mammalian cells mainly by causing damage to DNA, but it is not specific for malignant cells. A major aim of research in experimental radiotherapy thus is to maximize the effect of radiation treatment on the tumor while minimizing the damage to the surrounding normal tissues (Tannock *et al*., 2007).

3.3.3 Chemotherapy :

Chemotherapy is the treatment of cancer with cytotoxic drugs that can destroy cancer cells. Cancer chemotherapy is directed against the disseminated forms of cancer that cannot be controlled by surgery or radiation. As a result, chemotherapy is considered a systemic treatment (Rosenthal *et al.*, 1987).

Most anti-cancer medicines are non-specific and can injure both malignant and normal cells. Hence, chemotherapy has the potential to harm healthy tissue, especially those tissues that have a high replacement rate such as hair, gastro-intestinal lining and blood cells. These cells usually repair themselves after chemotherapy (Rosenthal *et al.,* 1987).

In general, cytotoxic agents have a broader range of intracellular effects than radiotherapy, although they also damage DNA. In order to overcome drug resistance and to limit the side effects of different drugs, chemotherapy is most commonly given as a

combination of agents. Drugs are conventionally given by intravenous injection every 3-4 weeks, allowing enough time for the patient to recover from short term toxic effects before the next dose. Between four and eight such cycles of treatment are usually given in total (Boon *et al*., 2006).

3.3.4 Adverse hematological effects of chemotherapy :

Cancer and its treatment may alter normal hematopoiesis either by direct effects on hematopoietic stem cells or by inhibiting production of and responsiveness to hematopoietic growth factors (Kufe *et al.,* 2003).

3.3.4.1 Myelosuppression :

The most common side effect of cancer chemotherapy is myelosuppression. Myelosuppression is the term used to describe the decrease in numbers of circulating WBC, RBC and platelets (Boon *et al*., 2006).

Chemotherapy destroys cells that divide rapidly, a characteristic of cancer cells. However, bone marrow cells also divide rapidly and are frequently damaged by chemotherapy. As a result, blood counts may fall. With the exception of hormonal therapy, all drugs can cause some injury to the bone marrow (Rosenthal *et al.*, 1987). This not only limits the dose of drug, but also can cause life threatening complications (Boon *et al*., 2006).

The role of treatment in marrow injury and recovery varies both with the drugs employed and with the normal turnover rate of cells of different hematologic lineages. Route and schedule of drug administration, drug metabolism, and pattern of cell sensitivity may influence the pattern of marrow damage (Kufe *et al.,* 2003).

3.3.4.2 Leucopenia (Low white count) :

A reduction in the total numbers of circulating white blood cells is called leucopenia. This may be due to reduction in all types of white blood cells or a reduction in individual

cell types (usually neutrophils or lymphocytes). In turn, leucopenia may occur alone or as part of a reduction in all three hematological lineages (Boon *et al*., 2006).

The hematologic complications of most chemotherapeutic agents are leucopenia and thrombocytopenia at 10 days; nadir counts at 14-18 days; recovery of counts at 21-28 days (Kufe *et al*.,2003). Leucopenia generally occurs first because of the short half life of circulating granulocytes (6hrs.). Because most marrows recover within 21 to 28 days, chemotherapy is frequently administered every 3 to 4 weeks (Rosenthal *et al*., 1987).

A low white blood cell count means the immune system isn't as strong as it could be and that the patient is at increased risk for infection (Bowden, 2008).

3.3.4.3 Neutropenia:

Neutrophils constitute the majority (>70%) of blood leukocytes and play a critical role in host defense mechanisms against infection. Thus a decrease in the number of neutrophils places a patient at an increased risk for infection. The number of neutrophils is reported as Absolute Neutrophil Count (ANC). As part of a differential leukocyte count, ANC is calculated as total leukocyte count times the percentage of neutrophils (Schwartzberg, 2006).

An ANC can be calculated by the following formula:

ANC= (% of neutrophils)/100 X WBC count

Neutropenia is defined as an absolute neutrophil count <1500 cells/mm^3 and can be graded as mild (1000–1500 cells/mm^3), moderate (500–1000 cells/mm^3), or severe (<500 cells/mm^3). The risk and severity of neutropenia associated infection is directly proportional to the absolute neutrophil count (ANC) and the duration of neutropenia (Schwartzberg, 2006).

Patients with malignant disease diffusely affecting bone marrow, such as leukemia, mutilple myeloma, lymphoma and metastatic solid tumors often present with neutropenia

and are at greater risk for prolonged neutropenia as a result of cytotoxic chemotherapy. Bacterial and fungal infections are the most common complications of chemotehrapy-induced neutropenia (Schwartzberg, 2006). Bacterial infections are predominant during the early stages of neutropenia, whereas fungal infections are more common in patients with prolonged and severe neutropenia (Rolston, 2009).

However, not all patients with cancer and possible infection present with neutropenia or in critical condition (Rosenthal *et al*., 1987). It is possible for an individual to have a normal total WBC count, but still be neutropenic. In general, however, the WBC is decreased when the neutrophil count is decreased (American Cancer Society, 2008).

3.3.4.4 Common sites of infection in neutropenic patients:

Sites of Infection	Frequency (%)
Bloodstream	20-25
Respiratory tract	25-30
Urinary tract	10-15
Skin infections	10-15
Gastrointestinal tract	5-10
Other sites	1-5

(Rolston, 2009)

3.4 Infection in cancer patients :

Infection continues to be a significant problem in patients with cancer. Recent advances in medical technology, such as hematopoietic stem cell transplantation and the use of intensive chemotherapeutic regimens, have led to the seriously impaired host defense mechanisms that compromise their ability to resist or contain infections (Kufe *et al.*, 2003). Infections in people with cancer are often more serious and more difficult to treat than those in the general population (American Cancer Society,2008).

In the cancer patient, the most likely pathogens include gram positive organisms such as *Staphylococci*, *Pneumococci* and *Streptococci* and gram negative organisms such as Enterobacteriaceae and *Pseudomonas* (Devita *et al*., 1989). The spectrum of bacterial and fungal infection continues to change. Newer opportunistic pathogens are being recognized with increasing frequency. Viral, fungal, and protozoal infections are also becoming increasingly common in immunosuppressed patients (Rolston, 2009).

Patients with cancer are subject to infections as a result of several factors, notably due to obstruction or constriction of airways or ducts, erosion of tumor involving the protective integument or mucosa, alteration of host defenses secondary to infiltration of bone marrow, reduced or altered immunoglobulin or cytokine production, or as a result of chemotherapy (Zinner,1997).

Although the effects of lymphopenia, monocyte dysfunction and humoral deficiency are contributory, the overriding issue in these cancer patients is the absolute granulocyte count. Risk of infection increases significantly when the neutrophil count falls below 1000/mm3 and even more dramatically when below 500/mm3. Prospective studies of patients with granulocytopenia from chemotherapy indicate that only about 30-35% become febrile or infected (Rosenthal *et al.*, 1987).

3.4.1 Some common causative agents of bacterial infections in cancer patients:

Enterobacteriaceae :

Data from several large surveillance studies conducted at major cancer centers both in the United States and Europe indicate that Enterobacteriaceae cause approximately 65% to 80% of documented gram-negative infections in these patients (Saghir *et al.*, 2009), with *Escherichia coli* and *Klebsiella* spp. consistently being among the most common species to be isolated. The bloodstream is the most frequent site of infection, followed by the urinary tract and the lung (Kufe *et al*., 2003).

Pseudomonas aeruginosa :

P. aeruginosa has also been reported to cause a wide variety of infections in immunocompromised cancer chemotherapy patients as it is a common hospital and opportunistic pathogen (Saghir *et al.,* 2009). It is also a cause of catheter-related infections. Pneumonia and bacteraemia are the most common infections, but the urinary tract, skin and gastrointestinal tract are also the sites of infection (Kufe *et al*., 2003). A study showed that mortality rates were higher in cancer patients with nosocomial *Pseudomonas* infections than any other bacterial infections (Ashour *et al*, 2009).

***Acinetobacter* species :**

Acinetobacter species are acknowledged to be opportunistic pathogens and usually cause disease in debilitated patients and in those with severe underlying conditions, including cancer. They are often found in areas of moist skin, including the axilla, groin, and interdigital pockets. Although infections caused by *Acinetobacter* species are relatively infrequent (approximately 4% of all gram-negative infections in cancer patients), infections of nearly all body sites (lungs, meninges, skin, heart valves, biliary tract, urinary tract, bones and joints, peritoneum, skin and soft tissue, and blood) have been reported (Kufe *et al.*, 2003).

Staphylococcus species :

During the past 15 years, a marked increase in the incidence of infections caused by gram-positive organisms has been reported from most major cancer treatment centers. The majority of these are caused by *Staphylococcus* species. Increased use of vascular access devices and other conditions that usurp mechanical barriers to infection (surgery, trauma) are predominantly responsible for the increase in the recovery of these organisms, because they commonly inhabit normal skin (Kufe *et al.,* 2003).

3.5 Urinary Tract Infection :

Urinary tract infection simply means the presence of bacteria undergoing multiplication in urine within the urinary drainage system (Leigh, 1990). From a microbiological perspective, UTI exists when pathogenic microorganisms are detected in the urine, urethra, bladder, kidney, or prostate. In most instances, growth of more than 10^5 organisms per milliliter from a properly collected midstream "clean-catch" urine sample indicates infection (Stamm, 2003). The presence of bacteria in urine is called bacteriuria (Cheesbrough, 2000).

Infection of urinary tract is defined as bacteriuria, the multiplication of organisms in urinary tract and the presence of more than a hundred thousand organisms per ml in the midstream sample of urine (Chakraborty, 2001).

Urinary tract infection is defined as the detection of both bacteriuria 10^5 cfu/ml and pyuria i.e.10 leucocytes/hpf (Goya *et al,* 1997).

3.5.1 Significance of Bacteriuria:

The mere presence of bacteria in urine is called as bacteriuria. But the simple demonstration that bacteria of one or more species are present in the sample of urine is no proof that it has been derived from an infection in the urinary tract. Proof of the urinary tract infection requires the demonstration that potential pathogen is present in freshly voided urine in numbers greater than those likely to result from contamination from the urethral meatus and its invirons. The observations of Kass(1957) suggested that the number taken to indicate significant bacteriuria is about 10^5 cfu/ml . In true infection, in the absence of prior chemotherapy, the number of infecting bacteria is likely to be at least as great as this (Collee *et al,* 1999). Hence, bacteriuria of $10^4 – 10^5$ per ml is considered as doubtful bacteriuria and less than 10^4 is considered as insignificant bacteriuria (Maskell, 1982).

3.5.2 Classification of UTI based on symptoms and levels of infections:

UTIs can also occur without symptoms and with symptoms but with very low bacteria levels.

Asymptomatic bacteriuria

It is defined as the presence of more than 10^5 cfu/ml (i.e. significant numbers of bacteria) of voided urine in patients with no symptoms of urinary tract infection. Many urinary tract infections are asymptomatic but may lead to serious infections and it is not known whether symptomatic UTIs are preceded by asymptomatic bacteriuria (Vejlsgaard, 1966). Severe complications of these UTIs and renal involvement, even without the presence of symptoms, are frequently seen (Forland *et al*, 1977; Ooi, 1974).

Symptomatic urinary tract infection

Symptomatic UTI involving the lower urinary tract is frequently termed acute cystitis and is characterized by urgency, frequency, suprapubic pressure and dysuria, and presence of systemic symptoms such as fever. Symptomatic UTI of upper tract is termed as acute pyelonephritis, which is a serious and common UTI (Leigh, 1990).

3.5.3 Etiological agents of urinary tract infection

Bacteria of only a limited number of species are able to initiate infection in the urinary tract. The causative agents are listed below:

Gram negative *E. coli, P.vulgaris, Proteus mirabilis, Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp, *Serratia* spp., *M. morganii and P. aeruginosa*

Gram positive *S. aureus, S. saprophyticus,* Group B streptococci and *E. faecalis*

Other pathogens *Chlamydia (Chlamydia trachomatis), Mycoplasma (Ureaplasma urealyticum), Candida* spp. *and Mycobacterium tuberculosis.* (Source: Cheesbrough, 2000)

Among the microorganisms causing UTI, *E. coli* is responsible for 74.6%, *Proteus* spp. responsible for 8.0%, *Klebsiella* spp. responsible for 2.0%, *Pseudomonas* spp. is responsible for 2.0%, and other Gram negative organisms are responsible for 13.3% of the total cases of UTI (Herm *et al.,* 2003).

E. coli is the most common infecting organism in patients with uncomplicated UTI (Johnson, 1991). In the complicated UTI that occur in the abnormal or catheterized urinary tract, particularly in hospital patients, *E. coli* is still the commonest causative organism but other members of *Enterobacteriaceae* such as *Klebsiella* spp., *Enterobacter* spp., Indole positive *Proteus* spp. and *Citrobacter* spp. are also frequent (Collins *et al.*, 1986).

Other pathogens include *P. mirabilis*, which is a common cause of urinary tract infections in boys and men, and is associated with renal abnormalities. In hospital patients, *Proteus* spp. may cause chronic UTI in association with the use of instrument (Collins *et al.*, 1986).

Gram positive pathogens such as *E. faecalis, S. saprophyticus* and group B streptococci can also infect the urinary tract. Urinary tract infections due to *E. faecalis* are usually associated with the use of instruments or catheterization (Collins *et al.,* 1986).

3.5.4 Routes of infection

Infectious microorganisms can invade and spread within the urinary tract by following routes:

- **Ascending Route**

For UTIs to occur by the ascending route, enteric gram negative bacteria and other microorganisms that originate in the gastrointestinal tract must be able to colonize the vaginal cavity and/or the periurethral area. Once these organisms gain access to the bladder, they may multiply and then pass up the ureters to the kidneys. Although the ascending route is the most common route of infection in females, ascent in association with instrumentation (eg. Urinary catheterization, cytoscopy) is the most common cause of hospital acquired UTIs in both sexes. UTIs occur more often in women than man, at

least partially because of the short female urethra and its proximity to the anus. (Forbes *et al*., 2007).

- **Haematogenous Route**

UTI may also occur by the haematogenous route (Kunin, 1994). Haematogenous spread usually occurs as a result of bacteremia. Any systemic infection can lead to the seeding of the kidney, but certain organisms, such as *Staphylococcus aureus* or *Salmonella* species are particularly invasive. Although most infections involving the kidneys are acquired by the ascending route, yeast (usually *Candida albicans*), *Mycobacterium tuberculosis*, *Salmonella* species, *Lactospira* species or *Staphylococcus aureus* in the urine often indicate pyelonephritis acquired via haematogenous spread or the descending route. Haematogenous spread accounts for less than 5 % of UTIs (Forbes *et al.,* 2007).

- **Lymphatic Route**

Direct extension of bacteria from the adjacent organ via lymphatics may occur in unusual circumstances such as a severe bowel infection or retroperitoneal abscesses. There is little evidence that lymphatic routes play a significant role in the vast majority of UTI (Schaeffer, 1998).

3.5.5 Pathogenesis

Most UTIs result from the ascending route, only a minority occurs after bacteremia. The first step in the pathogenesis is the colonization of the periurethral tissue with uropathogens. Secondly, these uropathogens may gain access to the urethra. A symptomatic or asymptomatic infection of the bladder may result. A few organisms may finally ascend the ureters to the kidneys. Whether all these steps take place depends on the inoculum size, the virulence properties of the invading microorganism and the defense mechanisms of the host. Micturition is the most important defence mechanism against UTIs and, therefore, obstruction, stasis and reflux of the urinary tract predispose to infection (Sobel and Kaye, 1984).

The most prevalent causative microorganism of UTIs is *Escherichia coli* (Johnson,1991). Not all strains of *E. coli* are equally capable of infecting the intact urinary tract. Bacterial virulence factors markedly influence the likelihood that a given strain once introduced into the bladder, will cause UTI (Stamm, 2003). Different virulence factors and O:K:H serotypes of uropathogenic *E. coli* (UPECs) have been described (Johnson, 1991). Adherence of the microorganism to the uroepithelial cell is the most important step in the pathogenesis of UTIs (Svanborg and Godaly, 1997).

Uropathogenic *E. coli* causes 90.0% of the urinary tract infections in anatomically normal, unobstructed urinary tracts. The bacteria colonize from faeces or perineal region and ascend the urinary tract to the bladder. The adhesin that has been most closely associated with uropathogenic *E. coli* is the P fimbriae. The fimbriae bind not only to red cells but to a specific galactose disaccharide that is found on the surfaces uroepithelial cells in approximately 99.0% of the population (Todar, 2008).

Uropathogenic strains of *E. coli* possess other determinants of virulence in addition to P fimbriae. *E. coli* with P fimbriae also possess the gene for Type 1 fimbriae, and there is evidence that P fimbriae are derived from Type 1 fimbriae by insertion of a new fimbrial tip protein to replace the mannose-binding domain of Type 1 fimbriae. In any case, Type 1 fimbriae could provide a supplementary mechanism of adherence or play a role in aggregating the bacteria to a specific manosyl-glycoprotein that occurs in urine. Another factor thought to be involved in the pathogenicity of the uropathogenic strains of *E. coli* is their resistance to the complement dependent bactericidal effect of serum (Todar, 2008).

Uropathogenic strains of *E. coli* usually produce siderophores that probably play an essential role in iron acquisition for the bacteria during or after colonization. They also produce hemolysins, which are cytotoxic due to formation of transmembranous pores in host cells (Todar, 2008).

The K antigens of *E. coli* are capsular antigens. These may be able to promote bacterial virulence by decreasing the ability of antibodies and/or complement to bind to the bacterial surface, and the ability of phagocytes to recognize and engulf the bacterial

cells(Todar, 2008).

The importance of adherence in the pathogensis of UTIs has also been demonstrated with other species of bacteria. Once introduced into the urinary tract, *Proteus* strains appear to be uniquely suited to cause significant disease in the urinary tract. These strains are able to facilitate their adherence to the mucosa of the kidneys. Also, *Proteus* spp. is able to hydrolyze urea via urease production, which results in an increase in urine pH that is directly toxic to kidney cells and also stimulates the formation of kidney stones. Similar findings have been made with *Klebsiella* spp. *S. saprophyticus* also adheres better to uroepthelial cells than does *S. aureus* or *S. epidermidis (*Forbes *et al.*, 2007).

Motility may be important for organisms to ascend to the upper urinary tract against the flow of urine and cause pyelonephritis (Forbes *et al*., 2007)

3.6 Urinary tract infection in cancer patients:

UTI is the most common bacterial infection in the general population and it is also a common clinical problem in cancer patients. While UTI affects all ages, significant difference are seen in age prevalence between the two sexes. While it is common at all ages in females, it occurs mostly at extremes of life in males. Patients with structural or functional abnormalities of the urinary tract, patients who are immunocompromised such as transplant recipients and cancer patients on chemotherapy, are particularly susceptible to UTI (Yeung *et al*., 2009).

UTI in cancer patients should be considered complicated when it occurs as a result of suppressed immunity. The epidemiology and prevalence of UTI in cancer patients are not established; it is believed that the pathogenesis and treatment issues of UTI in cancer patients are no different from those in the general population (Yeung *et al.*, 2009).

3.7Factors that predispose the cancer patients to urinary tract infections

- Local factors such as tumor metastases, that produce obstruction and operative procedures that result in disruption of normal anatomic barriers play an important role in infections occurring in cancer patients. Likewise, urinary tract infections are common in patients with tumors, such as bladder or prostatic carcinoma that obstruct a ureter or the bladder neck causing retention of residual urine. Hydronephrosis, pyonephrosis, chronic pyelonephritis, and cystitis are not uncommon complications in patients with cancer of the genitourinary tract. In these situations, the infection is generally caused by one or more of the microorganisms colonizing the site of obstruction (Kufe *et al*., 2003).

- Chemotherapeutic agents predispose to the development of infections in a variety of ways. Agents that are myelosuppressive produce neutropenia, which is a well-recognized risk factor for infection. Chemotherapeutic agents are also known to interfere with cell-mediated and humoral immunity even when administered in doses that do not generally produce significant myelosuppression (Kufe *et al.*, 2003).
- Foreign bodies, such as urinary catheters, also damage or circumvent normal anatomic barriers, thereby facilitating entry of microorganisms into tissues and the bloodstream (Kufe *et al.*, 2003).

3.8 International Scenario on UTI in cancer patients:

In a retrospective study, patients admitted to Caritas Christi for palliative care with terminal malignant illness (92.2%), and non-malignant illness (7.8%) from May 1997 to October 1998 were included. The most frequent site of infection was the urinary tract (41.0%). *Escherichia coli* was the predominant organism cultured (36.8%) and strains were predominantly ampicillin / amoxycillin and gentamicin sensitive. Urinary tract infections were associated with 5 patients with indwelling urinary catheters (Vitetta *et al*., 2000).

The medical records of all patients with advanced cancer who were enrolled into the palliative care service of a district hospital during the period January 2002 to July 2002 were retrospectively reviewed for infections and the use of antibiotics. The most frequent sites of infection were chest (n=63, 52.5%), urinary tract (n=35, 29.2%), and skin/wound (n=6.5%) (Lam *et al*., 2005).

In a retrospective study done by Homsi *et al.* (2000), three hundred ninety-three patients were admitted to an acute care palliative medicine unit in an 8-month period for evaluation and palliation of cancer-related symptoms and complications. One hundred fifty-two infections and 192 isolates were identified. Sixty-six patients had urinary tract infections. Fifty-three were taking corticosteroids at the time of infection. Only 3 were neutropenic. Urinary tract infections were significantly more common in those taking corticosteroids.

A study was carried out to determine the prevalence of urinary tract infection (UTI) in children on treatment for cancer at the Kenyatta National Teaching and Referral hospital. One hundred and eighty six children between the ages of five and 14 years admitted in Kenyatta hospital with leukaemia or lymphoma were enrolled. The prevalence of UTI was 8.1% (CI=6.1, 10.1). Only five out of 15 patients were symptomatic. *E. coli* and *Klebsiella spp*. were responsible for 93.4% of the infections. Presence of pyuria, defined as five or more pus cells per high power field, had a sensitivity of 80.0%, specificity of 97.1% and a positive predictive value of 70.6% (Munyis *et al*., 1998).

A study was done to analyse the cases of urinary tract infections in patients with hematological malignancies, undergoing combination chemotherapy. 72 patients (48 women and 24 men) with UTI hospitalized in the Clinic of Hematology for the period January 2006-May 2008 were analysed. 36 from 72 patients had history of dysuria. Bacteriuria was detected in 68 from 72 patients. In 49 patients (68%), bacteriuria was significant.19 from 68 patients (26.4% from all the patients) had insignificant bacteriuria. 12 from the patients with significant bacteriuria were asymptomatic. *E.coli* was isolated from urine culture in 44 from 68 patients (64.7%). In the rest 24 patients, *Enterococcus*

faecalis, P. mirabilis, Acineotbacter, P. aeruginosa, K. pneumoniae and mixed infections were detected. Increased number of WBC in urine (pyuria) was found in 70 from 72 patients (Tancheva *et al.,* 2009).

A sample of 100 consecutive febrile neutropenic episodes in cancer patients in Kuwait were studied. Urinary tract infection occurred in 30% of the microbiologically documented cases (Bahar *et al*., 2004).

A study conducted by Ashour *et al.* (2009) examined the microbial spectrum of gram-negative bacteria in various infection sites in patients with leukemia and solid tumors. In both leukemic and solid-tumor patients, gram-negative bacteria causing UTI were mainly *Escherichia coli* (37.8%) and *Klebsiella pneumonia* (31.6%). Out of 98 gram-negative isolates from UTI, 77 isolates were isolated from leukemic patients (78.6%), whereas only 21 isolates were obtained from solid-tumor patients (21.4%).

A retrospective review of febrile neutropenic episodes in patients admitted in Srinagarind Hospital during January 1994-December 1995 was carried out. There were 88 episodes of infection in 60 adult patients with hematological illnesses. UTI (36%) was the most common type of infection (Anunnatsiri *et al.,* 1998).

Carlisle *et al.* (1993) did a cumulative continuous prospective surveillance over a 42-month period in neutropenic patients with hematological and solid malignancies undergoing high-dose chemotherapy. A total of 444 nosocomial infections were identified in 920 neutropenic patients. The rate of bloodstream infection per 100 neutropenic patients was 13.5. Other site-specific rates were: urinary tract, 5.7; respiratory tract, 5.5; thrush, 6.6; skin, 3.4; and gastrointestinal tract, 3.4.

A report described the results of a prospective study of nosocomial infection in 7,714 patients hospitalized during a 24-month period at a cancer treatment center. An overall nosocomial infection rate of 9.3% was observed with site-specific infection rates of 2.6% for urinary tract (Robinson *et al.*, 1984).

In a study conducted by Awidi (1991) in a developing country, 319 episodes of infections in 174 cancer patients over a period of 3 years were reported. 146 episodes appeared in 89 neutropenic patients and 173 in non-neutropenic patients. Most common sites of infection were respiratory tract, kidney and urinary tract and skin. Most commonly isolated organisms in these infections were *Staphylococcus, E. coli, Pseudomonas* and *Streptococcus*.

The frequency of urinary tract infection before and during pelvic radiotherapy was studied prospectively in 172 patients who were not catheterized and had not had instrumentation for at least 4 weeks prior to radiotherapy. The incidence of urinary tract infection prior to radiotherapy was 17% and a further 17% of patients developed a urinary tract infection during radiotherapy (Bialas, 1989).

Records of patients admitted at the National Kidney Institute from Jan 1992 to Aug 1996 with ANC of less than 1000/cumm and who developed fever during the neutropenic period were reviewed. A total of 55 febrile episodes in 41 patients were identified. The underlying disease that was associated with neutropenia was predominantly due to leukemia in 49% of cases and to solid tumors in 12.7%. However infection rate was higher in solid tumors than in leukemia. UTI occurred in 8.3% of the documented cases. The number of documented infections in neutropenic patients is directly proportional to the degree of neutropenia. 45% of total infections were observed at granulocyte counts less than 1000/cumm and only 21.8% occurred at a granulocyte count between 500-1000/cumm (Billote *et al.*, 1996).

3.9 Laboratory Diagnosis of UTI

A sample of urine from a patient with a suspected UTI is the most common type of specimen received by most clinical microbiological laboratories. The schedule for routine examination should be carefully determined with a view to obtaining the necessary diagnostic information with the greatest possible economy of labour and resources (Collee *et.al*, 1999).

3.9.1 Methods of specimen collection and transport

Urine specimen should be adequate and free from urethral and genital tract contamination. Cheesbrough (2000) suggests that whenever possible, the first urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most concentrated and therefore most suitable for analysis.

- **Clean-catch, midstream urine (CC-MSU)**

The least invasive procedure, the clean-catch midstream urine specimen collection must be performed carefully for optimal results. The detail procedure for the collection of sample is mentioned in Appendix IV.

- **Indwelling catheter**

Specimen collection from patients with indwelling catheters requires scrupulous aseptic technique. The catheter tubing should be clamped off above the part to allow collection of freshly voided urine. In catheterized patients, urine should be collected directly from the catheter and not from the collection bag (Forbes *et al*., 2007).

Since urine is itself a good culture medium, all specimens should be processed by the laboratory within 2 hours of collection or be kept refrigerated at 4^0 C until delivery to the laboratory and processed no longer than 18 hours after collection (Vandepitte *et al*., 2003). Transport medium that can be used for urine specimens are 1.8% boric acid, sodium chloride or polyvinylpyrolidine (Pokhrel, 2004).

3.9.2 Routine Examination of Urine:

Routine examination of urine includes:

- Macroscopic appearance of urine
- Microscopic examination of urine
- Chemical examination of urine
- Culture of urine
- Interpretation of culture reports

- Antibiotic Susceptibility of testing of causative organism

3.9.3 Macroscopic examination of urine

Color and turbidity of urine is noted in the very initial step (Cheesbrough, 2000).

3.9.4 Microscopic examination of urine

Microscopic examination of the urine is an indispensable tool in the diagnosis of genitourinary disorder. Urine is examined microscopically as a wet preparation to detect:

- Significant pyuria
- Erythrocytes
- Epithelial cells
- Casts
- Yeast cells
- Bacteria (provided the urine is freshly collected)

Pus cells

These are round 10 to 15 µm in diameter cells that contain granules in them. In urinary infections, they are often found in clumps (Cheesbrough, 2000). The observation of pus cells in urine is indicative of bacteriuria but may result from any inflammatory disorder of the urinary tract. Increased number of leukocytes in the urine, principally neutrophils, are seen in almost all renal diseases and diseases of the urinary tract. Normal urine contains 2-3 pus cells/hpf. Pyuria is considered significant if more than or equal to 5 pus cells are seen per HPF in urine sediments. The other criteria for significant pyuria defined by various workers is the leukocyte count of >=10 leukocytes per HPF (Robins *et al.*, 1975; Hallstorm *et.al*; 1975; Kuinn 1987).

Erythrocytes

Erythrocytes are found in small numbers in normal urine. In normal male and female, occasional red cells (0-2/hpf or 3-12/µL) may be seen on microscopic examination of the sediment. Under high power, unstained RBC appears as pale discs, usually 7 µm in

diameter. Increased number of erythrocytes (haematuria) in the urine may be present in renal diseases like glomerulonephritis, extra renal disease like acute appendicitis, urinary schistosomiasis, leptospirosis, infective endocarditis, malignancy of urinary tract and hemorrhagic conditions. The finding of RBC count greater than 3/hpf is considered as abnormal (Froom *et al,* 1986; Steward *et al,* 1985; Wargotz *et al,* 1987). The study done by Froom *et al* (1987) concluded that the examination of the urine sediment by HPF method is not sufficiently sensitive to be used as a screening test for the detection of UTI in asymptomatic subjects. Microscopic haematuria may be present in 40.0% to 60.0% of patients with UTI (Faro and Fenner, 1998).

Epithelial Cells

It is normal to find few epithelial cells in urine. These cells are cuboidal in shape having small nuclei and granular cytoplasm. When seen in large number, however, they usually indicate inflammation of the urinary tract or vaginal contamination of the specimen (Cheesbrough, 2000). Normally few cells (3-5/hpf) from genitourinary tract can be found in urine due to sloughing off of old cells (Godkar, 2001). Increased number of tubular epithelial cells suggests tubular damage. It can occur in pyelonephritis, acute tubular necrosis, salicylate intoxication and kidney transplants rejection (Godkar, 2001). Wargotz *et al* (1987) reported that greater than or equal to five squamous epithelial cells per high power field is considered as abnormal.

3.9.5 Bacteriological examination of urine

Bacteriological culture of the urine is the only accurate way of diagnosing bacteriuria. Quantitative or semi-quantitative techniques are to be preferred but the particular one chosen will depend upon the resources of the laboratory. The accurate methods of counting bacteria, e.g. the pour-plate technique or the surface viable count are time-consuming and expensive in use of materials. Most of the laboratories use a semi-quantitative technique. The standard loop, filter-paper strip, dip-spoon and dip-slide are all useful means of examining large numbers of urine specimens but they differ considerably in the amount of medium used and in performance time (Leigh, 1990).

- **Standard Loop Method**

An inoculating loop of standard dimensions is used to take up a small, approximately fixed and known volume of mixed uncentrifuged urine and inoculate on to an agar culture medium. The plate is incubated, the number of colonies is counted and this number is used to calculate the number of viable bacteria per ml of urine. Thus, if a 0.002ml loopful of urine yields 25 colonies, then the approximate number of cfu per ml of urine will be 25 x 500 = 12,500. Such a count should be reported as 10^4-10^5 colonies/ml (Collee *et al*, 1999).

3.10 Antimicrobial Susceptibility Testing

The primary goal of antimicrobial susceptibility testing is to determine whether the bacterial etiology of concern is capable of expressing resistance to the antimicrobial agents that are potential choices as therapeutic agents for managing the infection. According to Greenwood (2000), since therapy of infection begins before laboratory results are available, antibiotic susceptibility testing primarily plays a supplementary role in confirming that the organism is susceptible to the agent that is being used.

WHO recommended modified Kirby-Bauer disc diffusion technique is used by most laboratories to test routinely for antimicrobial susceptibility. Using this test, antimicrobial resistance is detected by allowing the antibiotics to diffuse from a point source, commonly in the form of an impregnated filter paper disc, into an agar medium that has been seeded with the test organism. Visible growth of bacteria occurs on the surface of the agar where the concentration of antibiotic has fallen below its inhibitory level for the test strain (Collee *et al*, 1999). Following incubation, the diameter of the zone of inhibition around each disc is measured in millimeters.

3.11 Antibiotics used in UTI

Antibiotics are the mainstay of treatment for UTI. A variety of antibiotics are available and choices depend on many factors like type of UTI including whether the infection is complicated or uncomplicated or primary or recurrent. Treatment decisions are also based on the type of patient (eg. Man or woman, a pregnant or non-pregnant women, child, hospitalized or non-hospitalized patients, immunocompromised patients etc.).

- **Nitrofurantoin**: Nitrofurantoin is active against most members of *Enterobacteriaceae*, but not against *Pseudomonas* spp. It is most active at acid pH. It is primarily a bacteriostatic compound, but may be cidal at higher concentrations and in acidic urine. Its activity is enhanced at lower pH. It antagonizes the action of nalidixic acid. Susceptible bacteria appear to enzymatically reduce nitrofurantoin to generate reactive intermediates which damage DNA.

- **Sulphonamides:** The sulphonamides are extremely useful for the treatment of uncomplicated urinary tract infection caused by *E. coli* in domiciliary practice. *S. pneumoniae*, ß-hemolytic streptococci and *P. mirabilis* are almost always sensitive to the sulphonamide; those almost always resistant include *E. faecalis*, *P. aeruginosa*, indole-positive *Proteus* spp. and *Klebsiella* spp. Trimethoprim-sulfomethaxole (TMP-SMX) has been the standard therapy for UTI, however, *E. coli* is becoming increasingly resistant to medication (Simon *et al*, 2000).

- **4-quinolone antibacterials:** Fluoroquinolones have become popular treatments for patients with uncomplicated UTI because of *E. coli*'s emerging resistance to other common medications. Nalidixic acid is active against several different types of Gram negative bacteria, whereas Gram-positive organisms are resistant. However, the new Fluoroquinolone derivatives show superior activity against *Enterobacteriaceae* and *P. aeruginosa*, and their spectrum includes S*taphylococci* but not S*treptococci*. Extensive studies with Norfloxacin have demonstrated as a drug with a promising future in the treatment of urinary infections. The Infectious Diseases Society of America (IDSA) guidelines recommend the use of Fluoroquinolones (e.g. Ciprofloxacin, Norfloxacin and Ofloxacin) as first-line agents in communities with greater than 10.0% to 20.0% resistance rates to TMP-SMX (Naber, 2000).

- **Cephalosporins:** Cephalosporins including Cephalexin (Keflex), Cefuroxime (Ceftin), and Cefixime (Suprax), can also manage UTIs. Increasing resistance, however, has limited their effectiveness. First generation Cephalosporins (Cephalexin) are very active against Gram positive cocci (except enterococci and Nafcillin-resistant staphylococci) and moderately active against some Gram negative rods (primarily *E. coli*, *Proteus* spp. and *Klebsiella* spp.) The second generations Cephalosporins (Cefuroxime) are active against organisms covered by first generation drugs including *Klebsiella* spp. and *Proteus* spp., but not *P. aeruginosa.* Third generation Cephalosporins have their enhanced activity against Gram negative rods, especially that of Ceftazidime and Cefoperazone against *P. aeruginosa.* Fourth generation Cephalosporins include Cefepime and Cefpirome. Cefepime has enhanced activity against *Enterobacte*r spp. and *Citrobacter* spp. (Nicolle, 2003)

- **Aminopenicillin:** Among the most important Penicillins are Ampicillin and Amoxicillin, which are active against some enterobacteria. These are systemically absorbed oral drugs that are excreted in high concentrations in urine (Brooks *et al*, 2004). They have been used frequently in the past for the treatment of UTI, but emergence of resistance in up to 30.0% of common urinary isolates has lessened the utility of these drugs (Hooton and Stamm, 1997).

- **Aminoglycosides:** The aminoglycosides inhibit protein synthesis by interfering with the genetic transcription and finally causes cell membrane disruption. They are bactericidal in action. Gentamicin is active against many strains of Gram positive and Gram negative bacteria, including some strains of *P. aeruginosa*. Amikacin inhibit many Gram negative enteric bacteria (Hugo and Russell, 1993).

3.12 Antibiotic Resistance :

Antibiotics today are the front-line therapeutic means for the medical intervention in an infection, which plays a central role in the control and management of infectious diseases. However, due to misuse and overuse of antibiotics, most clinically relevant bacterial pathogens have acquired a selection process to adapt to the pressures of antimicrobial attack, so that certain strains are now no longer susceptible to one or more of these antimicrobial agents (Hugo and Russell, 1993).

Multiple drug resistance (MDR) bacterial isolates have been frequently reported from different parts of the world as an emergence of treatment problem. The MDR strain is defined as the strain that showed resistance to three or more antibiotics belonging to different structural classes (Tuladhar, 2001).

All bacterial resistance strategies are encoded by one or more genes and these resistance genes are readily shared between strains of the same species, between species of different genera, and even between more distantly related bacteria. Therefore, resistance may spread to a wide variety of bacteria, and any single organism can acquire multiple genes and become resistant to the full spectrum of available antimicrobial agents (Hugo and Russell, 1993).

In recent years resistance to first-line antibiotics such as Ampicillin, Tetracyclines, Chloramphenicol and Sulphonamides has been increasing. The abundant use of antimicrobial drugs favors the persistence and growth of drug-resistant bacteria, including *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp. *and Serratia* spp. (Brooks *et al.*, 2004).

3.13 Antibiotic resistance in cancer patients :

Resistance is an especially vexing problem for people with impaired immune systems, such as AIDS, and cancer patients, and recipients of organ transplants. In recent years, there has been marked increase in the incidence of antibiotic resistance against Gram-negative bacilli. Multidrug-resistance among *P. aeruginosa, K. pneumoniae* and

Enterobacteriaceae species, with emerging resistance, is an important cause of morbidity and mortality in hospitalized critically ill patients and patients with underlying medical condition such as neutropenia and immunosuppressants (Saghir *et al.,* 2009).

A study conducted by Alain Cometta, M.D., and his colleagues at the Centre Hospitalier Universitaire Vaudois in Lausanne, Switzerland, and reported in the April 28, 1994, New England Journal of Medicine, showed that increase in antibiotic resistance parallels increase in antibiotic use in humans. They had examined a large group of cancer patients given antibiotics called fluoroquinolones to prevent infection. The patients' white blood cell counts had been very low as a result of their cancer treatment, leaving them open to infection. Between 1983 and 1993, the percentage of such patients receiving antibiotics rose from 1.4 to 45. During those years, the researchers isolated *Escherichia coli* annually from the patients, and tested the microbes for resistance to five types of fluoroquinolones. Between 1983 and 1990, all 92 *E. coli* strains tested were easily killed by the antibiotics. But from 1991 to 1993, 11 of 40 tested strains (28 percent) were resistant to all five drugs (Lewis, 1995*).*

Study of Ashour *et al*. examined the microbial spectrum of gram-negative bacteria in various infection sites (i.e. respiratory, gastro-intestinal, urinary and blood stream infections) in patients with leukemia and solid tumors. The most frequently isolated gram-negative bacteria were *Klebsiella pneumonia* (31.2%) followed by *Escherichia coli* (22.2%). The antimicrobial resistance patterns of different gram-negative isolates from cancer patients were examined. Isolates of *Escherichia coli, Klebsiella, Enterobacter*, *Pseudomonas, and Acinetobacter* species were resistant to most antibiotics tested including non-β-lactam antibiotics such as aminoglycosides (gentamicin) and quinolones (ciprofloxacin, levofloxacin). All gram-negative species examined were highly resistant to third-generation cephalosporins. In addition, isolates exhibited simultaneous resistance to more than one non β-lactam drug. *Escherichia coli* isolates were also highly resistant to ampicillin, ampicillin-sulbactam, aminoglycosides, and other antibiotics. El Kholy *et al.* (2003) had also reported that *Escherichia coli* isolates from cancer patients in Egypt exhibited a low susceptibility pattern**.**

CHAPTER-IV

4. MATERIALS AND METHODS

4.1 MATERIALS

The materials required for this study are listed in Appendix VI.

4.2 METHODS

The study was conducted in Om Hospital and Research Center, Chabahil, Kathmandu from February 2009 to July 2009 in joint collaboration with Central Department of Microbiology, Tribhuvan University, Kirtipur.

One hundred and twenty seven mid-stream urine samples were collected from cancer patients undergoing chemotherapy. The samples collected were subjected to routine examination, culture and antibiotic susceptibility tests. 127 blood samples were also collected from the same patients and their total WBC count and differential count was determined by using the hematology analyzer. Absolute neutrophil count (ANC) was then calculated by using the following formula :

ANC = % of neutrophil count X total WBC count

Data analysis: P-value was calculated by using Winpepi software (Version 7.9, 2008)

4.2.1 Selection of patients

All hospitalized cancer patients under chemotherapy were studied. However, patients already undergoing anti-microbial therapy and those who had recently received blood transfusion were excluded from the study. No discrimination was made on the basis of age or gender.

4.2.2 Blood sample

4.2.2.1 Specimen collection

Each cancer patient was asked to fill a questionnaire for their clinical history during sample collection.

- Using a tourniquet, a suitable vein was located in the arm.
- Wearing gloves, the vein puncture site was thoroughly disinfected using 70% ethanol; the area about 5 cm diameters was cleansed and allowed to dry.
- Using a sterile syringe and needle, about 1-2 ml of blood was withdrawn from a patient. The blood was kept in test tube labelled with the name and number of the patient and the date and time of collection.

4.2.2.2 Calculation of blood cell count

Anticoagulated blood was processed through automated hematology analyser to measure different hematological parameters i.e. total WBC count and differential count (Boon *et al*., 2006). The detailed procedure for the calculation of the blood cell counts using a hematology analyzer is mentioned in Appendix IX.

4.2.3 Urine sample

4.2.3.1 Specimen Collection

Each patient was given a sterile, dry, wide-necked leak-proof container for collection of 10-20 ml of first morning clean-catch mid-stream urine. The patient was given instructions for the collection of CC-MSU. The detailed procedure is mentioned in Appendix IV. The container was labelled with date, name and number of the patient and the time of collection and was delivered to the laboratory along with the request form as soon as possible.

4.2.3.2 Routine Examination

Routine examination of urine included the physical examination of urine, detection of sugar and albumin in urine and microscopic examination of the urine deposit following standard methodology (Godkar, 2001).

4.2.3.3 Macroscopic examination

The specimen obtained in laboratory was observed for its color and turbidity.

4.2.3.4 Detection of sugar and albumin in urine

The test for the detection of sugar and albumin in the urine was done by Uristrix strip method.

Fresh urine sample was collected in a clean dry container. Then it was mixed well immediately before testing, then reagent strip was completely dipped in the urine specimen for few seconds and the change in color in the test area was noted after 3 seconds. It was then visually compared to the corresponding color charts on the bottle label at the times specified. The strip was held close to the color blocks and was matched carefully.

4.2.3.5 Microscopic examination

The urine specimen was examined microscopically as a wet preparation primarily for detection of the pus cells. WBCs in excess of 10^4 cells/ml (>10 cells/ ml) of urine will indicate significant pyuria. 1 WBC / LPF correspond to 3 cells/µl (Cheesbrough, 2000).

For WBC count, 3ml of urine was taken in a clean sterile test tube with the help of dropper and centrifuged at 3000 rpm for 10 minutes and then the supernatant was discarded. The deposit was then well mixed and was placed on a clean slide and covered with a cover slip. It was then examined under the microscope using 40X objectives for viewing the pus cells in urine (Cheesbrough, 2000).

4.2.3.6 Culture of specimen

The urine sample was cultured onto the MacConkey agar and Blood agar medium by the semi-quantitative culture technique using a standard loop. The method used for loop standardization is given in the Appendix III.

- A loopful of sample was touched to the centre of the plate, from which the inoculum was spread in a line across the diameter of the plate.

- Without flaming or re-entering urine, the loop was drawn across the entire plate, crossing the first inoculum streak numerous times to produce isolated colonies.
- The MacConkey and Blood agar plates were incubated aerobically at 35-37^{0}C overnight.
- The approximate numbers of colonies were counted and the number of bacteria, i.e. cfu/ml of urine was estimated in accordance to the volume of urine inoculated previously. For example, 100 colonies on inoculating 0.001 ml of urine would correspond to 10^5 cfu/ml.

The bacterial count was reported as

- Less than 10^4/ml organisms, not significant.
- 10^4-10^5/ml organisms, doubtful (suggest repeat specimen).
- More than 10^5/ml organisms, significant bacteriuria.

However if the culture indicated the appearance of $\geq$3 organism types with no predominating organism, this was interpreted as due to possible contamination of the specimen and asked for another specimen. In addition to the previously described guidelines, a pure culture of *S. aureus* was considered significant regardless of the number of cfu (Forbes *et al,* 2007).

4.2.3.7 Identification of the Isolates

Identification of significant isolates was done by using microbiological techniques as described in the Bergey's manual which involves morphological appearance of the colonies, staining reactions and biochemical properties (Baron and Finegold, 1990).

Each of the organism was isolated in pure form before performing biochemical and other tests. Gram staining of an isolated colony was done from primary culture. The Gram-staining procedure is mentioned in the Appendix III.

For gram negative organism a speck of single isolated colony from MacConkey agar and for gram positive the same from blood agar was transferred into the nutrient broth

and incubated at 37ºC for 4 hours. It was then subcultured on dried nutrient agar plate and incubated at 37ºC for 24 hours. Thus obtained overnight incubated culture of organism on nutrient agar was used to perform catalase, oxidase, other biochemical tests and antibiotic susceptibility test.

Gram-positive organisms were identified primarily on the basis of their response to gram's staining, catalase, oxidase and coagulase tests.

The biochemical tests used for the identification of gram-negative bacterial isolates include Catalase test, Oxidase test, Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Oxidative-Fermentative test, Triple Sugar Iron (TSI) test, Motility test and Gas production tests.

The composition and preparation of biochemical media and reagents used in the biochemical tests are mentioned in the Appendix II. The procedure for performing biochemical tests are mentioned in Appendix V.

4.2.4 Antibiotic susceptibility testing

The antibiotic sensitivity testing was performed according to the recommended Kirby-Bauer sensitivity testing method (NCCLS, 1999).

- Mueller Hinton agar was prepared and sterilized as instructed by the manufacturer.
- The pH of the medium 7.2-7.4 and the depth of the medium at 4 mm (about 25 ml per plate) were maintained in petridish.
- Using a sterile wire loop, a single isolated colony of which the sensitivity pattern is to be determined was touched and inoculated into a nutrient broth tube and was incubated for 2-4 hrs.
- After incubation in a good light source, the turbidity of the suspension was matched with the turbidity standard of MacFarland 0.5.
- Using a sterile swab, a plate of MHA was inoculated with the bacterial suspension using carpet culture technique.

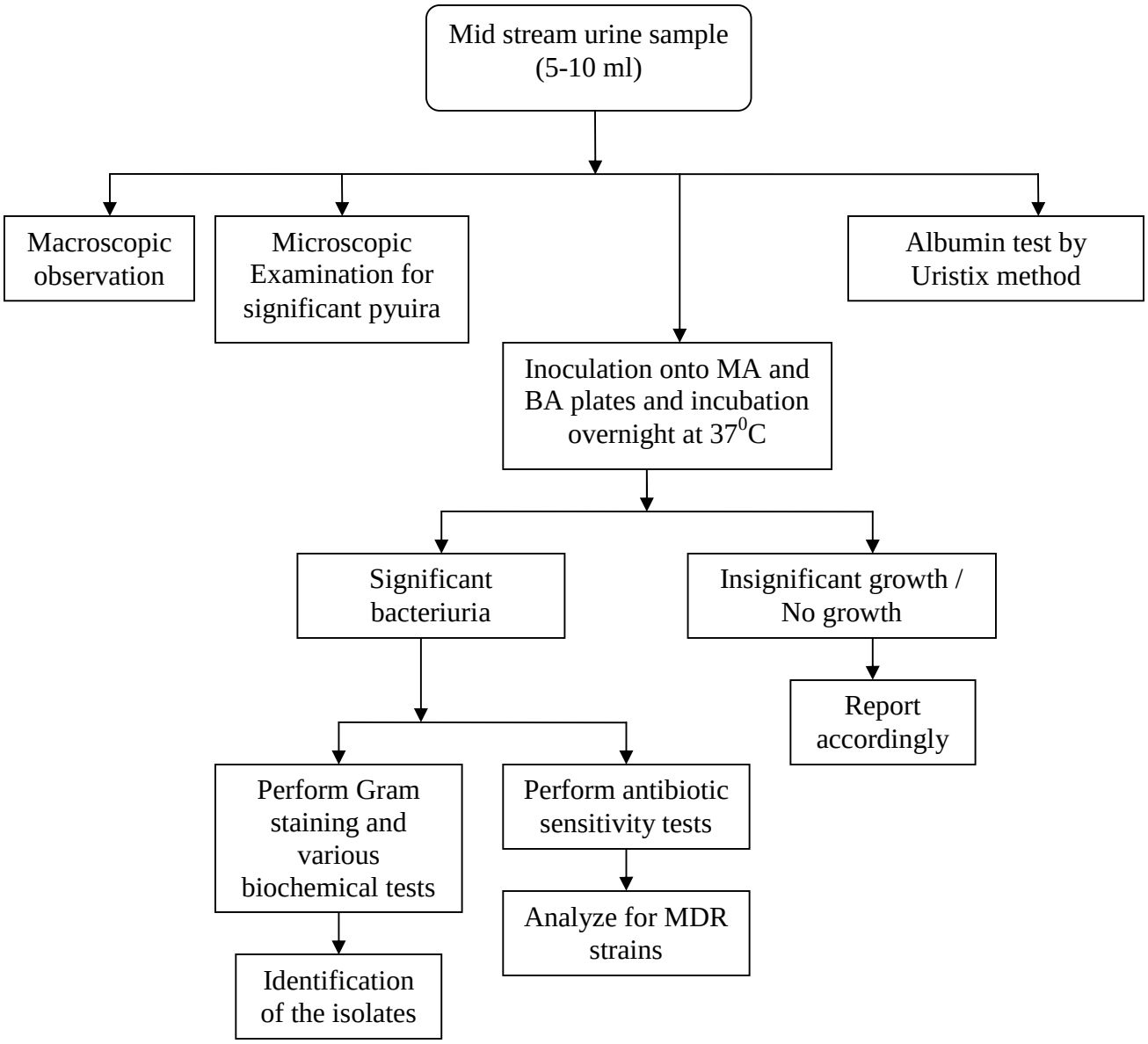
- Using sterile forceps, appropriate antimicrobial discs (6 mm diameter) was placed, evenly distributed on the inoculated plates, not more than 6 discs were placed on a 90 mm diameter petriplate.
- Within 30 minutes of applying the discs, the plates were taken for incubation at 35^0C for 16-18 hrs.
- After overnight incubation, the plates were examined to ensure confluent growth and the diameter of each zone of inhibition in mm was measured and results interpreted.

4.2.5 Purity plate

The purity plate was used to ensure that the inoculation used for the biochemical tests was pure culture and also to see whether the biochemical tests are performed in an aseptic condition or not. Thus, while performing biochemical tests, the same inoculum was subcultured in nutrient agar plate and incubated. The media was then checked for the appearance of pure growth of organisms.

4.2.6 Quality control for test

Quality of each test was maintained by using standard procedures. The quality of each agar plates prepared was tested by incubating one plate of each lot on the incubator. Quality of sensitivity tests was maintained by maintaining the thickness of Mueller-Hinton agar at 4mm and the pH at 7.2-7.4. Similarly antibiotic discs containing the correct amount as indicated were used. Strict aseptic conditions were maintained while carrying out all the procedures.



Flowchart

Figure 1 : Flow diagram for processing of urine sample

(Source: Cheesbrough, 2000)

CHAPTER -V

5. RESULTS

5.1 STUDY POPULATION

A total of 127 cancer patients undergoing chemotherapy were considered for this study. Incidence of lung cancer was found highest among the cancer patients (20.5%) which was followed by leukemia (16%) and stomach cancer (14%). Cancer of cervix were found in 11% of the study population while cancer of prostate and ovary accounted for 9% each of the study population.

Table 1 : Distribution of patients according to the type of cancer:

Type of Cancer	No. of cases	%
Lungs	26	20.5
Leukemia	20	16
Stomach	18	14
Cervix	14	11
Prostate	11	9
Ovary	11	9
Breast	5	4
Gall bladder	5	4
NHL	4	3
Colon	3	2
Bladder	3	2
Oesophagus	2	1.5
Vulva	2	1.5
Hepatocellular carcinoma	2	1.5
Pancreas	1	1
Total	**127**	**100**

5.2 AGE AND GENDER WISE DISTRIBUTION OF THE CANCER PATIENTS

Out of 127 cancer patients, 62 were males and 65 were female patients. Highest number of patients were found in the age group 60-70 (32.3%) followed by the age group (50-60) and (70-80) which covered19.7% and 16.5% of the study population respectively.

Table 2 : Age and sex-wise distribution of the cancer patients

Age group	No. of cancer patients			
	Male	**Female**	**Total**	**%**
0-10	2	0	2	1.6
10-20	0	0	0	0
20-30	3	2	5	3.9
30-40	5	7	12	9.5
40-50	5	14	19	14.9
50-60	10	15	25	19.7
60-70	23	18	41	32.3
70-80	12	9	21	16.5
>80	2	0	2	1.6
Total	**62**	**65**	**127**	**100**

5.3 CLINICAL PATTERN OF RESULTS

5.3.1 Growth pattern of bacteria in urine samples

Out of 127 mid-stream urine samples, 26 (20.5%) showed significant bacterial growth whereas the majority of samples (79.5%) didn’t show any significant growth. The results are shown in table 3.

Table 3 : Pattern of significant bacteriuria in cancer patients

Sample	Bacteriuria				Total
	Significant	%	Insignificant	%	
Mid stream urine	26	20.5	101	79.5	127

5.3.2 Genderwise distribution of significant bacterial growth from urine samples

Among the total 26 isolates, higher prevalence of significant bacterial growth were seen in females (65.4%) than in males (34.6%). However there was no statistical significance found between the gender of the patient and the incidence of urinary tract infection (shown in Table 4).

Table 4: Pattern of genderwise significant bacterial growth from urine samples

UTI	Gender-wise occurrence				Total	p-value (Chi-square test)
	Male	%	Female	%		
Significant	9	34.6	17	65.4	26	P = 0.104
Insignificant	53	52.5	48	47.5	101	
Total	**62**		**65**		**127**	

Pearson Chi-square test (P>0.05)

5.3.3 Pattern of age and sex-wise distribution of significant bacteriuric cases:

Highest number of significant bacterial growth was found in the age group 70-80 (38.5%) which was followed by the age group (50-60) and (40-50) that accounted for 23.1% and 19.2% of the significant cases respectively. The results are shown in table 5.

Table 5: Age and sex-wise distribution of the significant bacteriuric cases:

Age Group (yrs)	Bacteriuria		Total	%
	Male	Female		
0-10	-	-	-	-
10-20	-	-	-	-
20-30	-	1	1	3.8
30-40	1	-	1	3.8
40-50	2	3	5	19.2
50-60	-	6	6	23.1
60-70	2	3	3	11.5
70-80	4	4	10	38.5
>80	-	-	-	-
Total	**9**	**17**	**26**	**100**

5.3.4 Prevalence of UTI according to the type of cancer:

This study showed that the patients with ovarian cancer had higher prevalence of UTI (19.2%) followed by those with lung cancer (15.4%). Leukemia, cancer of prostate, gall bladder and cervix each accounted for 11.5% of the significant bacteriuric cases.

Table 6: Pattern of significant bacterial growth according to the type of cancer

Type of cancer	No. of significant bacteriuric cases	%
Lungs	4	15.4
Ovary	5	19.2
Leukemia	3	11.5
Prostate	3	11.5
Kidney	1	3.8
Vulva	1	3.8
NHL	1	3.8
Gall bladder	3	11.5
Rectum	1	3.8
Cervix	3	11.5
Breast	1	3.8
Total	**26**	**100**

5.3.5 Distribution of significant bacteriuric cases with the number of chemotherapy cycles

Among the 26 culture positive cases, maximum occurrence of UTI (50%) were found in those patients who had received 6-8 chemotherapy cycles followed by those who had received 4-6 cycles (38.5%).

Table 7 : Distribution of significant bacteriuric cases with the number of chemotherapy cycles

No. of cycles	No. of significant bacteriuric cases	%
0-2	-	-
2-4	2	7.7
4-6	10	38.5
6-8	13	50
>=8	1	3.8
Total	**26**	**100**

5.4 MICROSCOPIC OBSERVATION OF URINE

5.4.1 Microscopic observation of pus cells against the culture result

Among the total 127 urine samples, 28 (22%) showed significant pyuria (>5pus cells/hpf) while the remaining 99 samples (78%) showed insignificant or no pyuria. Out of the 28 significant pyuric cases, only 18 samples (64.3%) gave culture positive results. Among the 99 cases of insignificant pyuria, 8 (8.1%) gave culture positive results. The results are shown in Table 8.

Table 8: Microscopic observation of pus cells against the culture result.

Culture	Significant pyuria (>5/hpf)	Insignificant pyuria (<5/hpf)	Total (%)
Positive	18 (64.3%)	8 (8.1%)	26
Negative	10 (35.7%)	91 (91.9%)	101
Total	**28 (22%)**	**99 (78%)**	**127 (100.0)**

5.5 BIOCHEMICAL PATTERN OF THE RESULTS

5.5.1 Comparison of albuminuria with culture result

Out of the total 127 samples, 16 (12.6%) were positive for albumin test and among them 9 samples (56.2%) showed significant growth on culture. The remaining 111 samples (87.4%) were negative for albumin test and among these 17 (15.3%) samples gave culture positive results. The results are shown in table 9.

Table 9: Correlation of albuminuria with the culture result in cancer patients

Culture	Albumin Positive (≥1+)	Albumin Negative (<1+)	Total (%)
Positive	9 (56.2%)	17 (15.3%)	26
Negative	7 (43.8%)	94 (84.7%)	101
Total (%)	**16 (12.6%)**	**111 (87.4%)**	**127 (100.0)**

5.5.2 Correlation of total WBC count with the culture result :

The normal range for total count is between 4,000–10,000 cells/mm^3. Out of 127 cancer patients, 105 (82.7%) had the normal total count and among these 19 (18.1%) urine samples gave positive growth results. Similarly, the remaining 22 samples (17.3%) had total count less than the normal range and among these 7 (31.9%) urine samples gave positive growth results. The result was found to be statistically insignificant (shown in table 10).

Table 10: Correlation of UTI with the Total WBC Count:

UTI	Total count (<4000)	Total count (> 4000)	Total	P- value
Positive	7 (31.9%)	19 (18.1%)	26	P =0.147
Negative	15 (68.1%)	86 (81.9%)	101	
Total	**22 (17.3%)**	**105 (82.7%)**	**127**	

Pearson Chi-square (P> 0.05)

5.5.3 Correlation of Absolute Neutrophil Count with the culture result :

The normal value for the absolute neutrophil count is ANC>1500 cells/mm^3. Out of the 127 cancer patients, 113 (89%) had the normal value for ANC while the remaining 14 (11%) were neutropenic. Out of the 113 patients with normal ANC, 21 samples (18.6%) gave culture positive results while out of the 14 neutropenic patients, only 5 (35.7%) gave culture positive results. The result was found statistically insignificant suggesting that there is a no correlation between the Absolute Neutrophil Count of the patient and the presence of significant bacteriuria in the urine sample. The results are shown in table 11.

Table 11: Correlation of UTI with Absolute Neutrophil Count:

UTI	ANC <1500	ANC >1500	Total	P-value
Positive	5 (35.7%)	21 (18.6%)	26	P =0.134
Negative	9 (64.3%)	92 (81.4%)	101	
Total	**14 (11%)**	**113 (89%)**	**127**	

Pearson Chi-Square (P> 0.05)

5.6 MICROBIOLOGICAL PATTERN OF RESULTS

5.6.1 Pattern of bacteria isolated from culture positive urine samples according to Gram's stain

Out of the total 26 isolates, 25 (96.2%) were found to be Gram negative bacilli and the remaining 1 (3.8%) was found to be a Gram positive cocci. The results are shown in table 12.

Table 12: Pattern of bacteria isolated from culture positive urine samples according to Gram's staining

Isolated organism	No. of isolates	%
Gram positive bacteria	1	3.8
Gram negative bacteria	25	96.2
Total	**26**	**100.0**

5.6.2 Pattern of microbial isolates from urine sample

A total of 26 organisms were isolated from culture positive urine samples of cancer patients under chemotherapy. Among the isolated organisms, gram negative organisms were predominant. *S. aureus* was the only Gram positive species isolated. Among the gram negative isolates, *E. coli* was found to be the most predominant with 19 (73%) isolates. The results are shown in tables 13.

Table 13: Pattern of different species of bacteria isolated from urine of cancer patients

Type of organisms	No. of cancer patients	%
Staphylococcus aureus	1	3.9
Escherichia coli	19	73
Klebsiella oxytoca	2	7.7
Citrobacter freundii	2	7.7
Acinetobacter spp.	1	3.9
Pseudomonas aeruginosa	1	3.9
Total	**26**	**100.0**

5.7 ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE ISOLATES

5.7.1 Antibiotic susceptibility pattern of the Gram positive isolate

Among the 26 bacterial isolates, only one gram positive bacteria was isolated and it was identified as *Staphylococcus aureus*. The isolate was resistant to most of the antibiotics used but it was found susceptible to Amikacin and Novobiocin. The results are shown in table 14.

Table 14: Antibiotic susceptibility pattern of the gram positive isolate:

Antibiotics used	**Sensitive**		**Intermediate**		**Resistant**		**Total**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Amoxycillin	-	-	-	-	1	100.0	1
Cephalexin	-	-	-	-	1	100.0	1
Nalidixic acid	-	-	-	-	1	100.0	1
Norfloxacin	-	-	-	-	1	100.0	1
Nitrofurantoin	-	-	-	-	1	100.0	1
Ofloxacin	-	-	-	-	1	100.0	1
Ciprofloxacin	-	-	-	-	1	100.0	1
Cotrimoxazole	-	-	-	-	1	100.0	1
Cloxacillin	-	-	-	-	1	100.0	1
Ceftazidime	-	-	-	-	1	100.0	1
Amikacin	**1**	**100.0**	-	-	-		1
Novobiocin	**1**	**100.0**	-	-	-		1

5.7.2 Antibiotic susceptibility pattern of Gram negative isolates

Among the common antibiotics used against all Gram negative isolates, Amikacin was found to be the most effective drug with a susceptibility of 84% (21/25) followed by Ceftazidime and Nitrofurantion; each of which had the susceptibility of 76% (19/25). Most of the gram negative isolates were found to be resistant to the commonly used antibiotics with amoxycillin and nalidixic acid showing maximum resistance of 96% each. The results are shown in table 15.

Table 15: Antibiotic sensitivity pattern of the gram negative isolates:

Antibiotics used	**Sensitive**		**Intermediate**		**Resistant**		**Total**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Amoxycillin	1	4.0	0	0	24	96.0	25
Cephalexin	3	12.0	0	0	22	88.0	25
Nalidixic acid	1	4.0	0	0	24	96.0	25
Norfloxacin	5	20.0	1	4.0	19	76.0	25
Nitrofurantoin	**19**	**76.0**	1	4.0	5	20.0	25
Ofloxacin	5	20.0	2	8.0	18	72.0	25
Ciprofloxacin	6	24.0	1	4.0	18	72.0	25
Cotrimoxazole	4	16.0	0	0	21	84.0	25
Ceftazidime	**19**	**76.0**	3	12.0	3	12.0	25
Amikacin	**21**	**84.0**	1	4.0	3	12.0	25
Cefixime	7	28.0	1	4.0	17	68.0	25

5.7.3 Antibiotic susceptibility pattern of individual gram negative isolates

5.7.3.1 Antibiotic Susceptibility pattern of *E. coli* isolates:

The antibiotic susceptibility pattern of *E.coli* showed that 84.2% of isolates were susceptible to both Nitrofurantoin and Amikacin followed by Ceftazidime which had the susceptibility of 79%. The results are shown in table 16.

Table 16: Antibiotic Susceptibility pattern of *E. coli*

Antibiotics used	**Sensitive**		**Intermediate**		**Resistant**		**Total**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Amoxycillin	1	5.3	-	-	18	94.7	19
Cephalexin	3	15.8	-	-	16	84.2	19
Nalidixic acid	1	5.3	-	-	18	94.7	19
Norfloxacin	4	21.0	1	5.3	14	73.7	19
Nitrofurantoin	**16**	**84.2**	-	-	3	15.8	19
Ofloxacin	4	21.0	2	10.5	13	68.5	19
Ciprofloxacin	5	26.3	1	5.3	13	68.5	19
Cotrimoxazole	3	15.8	1	5.3	15	78.9	19
Ceftazidime	**15**	**79.0**	2	10.5	2	10.5	19
Amikacin	**16**	**84.2**	-	-	3	15.8	19
Cefixime	6	31.6	1	5.3	12	63.1	19

5.7.3.2 Antibiotic Susceptibility pattern of *Citrobacter freundii:*

Both isolates of *Citrobacter freundii* were highly susceptible to Amikacin (100%) while Nitrofurantoin and Ceftazidime showed the susceptiblity of 50% each. All the other drugs used were found to be resistant. (Table 17)

Table 17: Antibiotic Susceptibility pattern of *Citrobacter freundii*

Antibiotics used	**Sensitive**		**Intermediate**		**Resistant**		**Total**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Amoxycillin	-	-	-	-	2	100.0	2
Cephalexin	-	-	-	-	2	100.0	2
Nalidixic acid	-	-	-	-	2	100.0	2
Norfloxacin	-	-	-	-	2	100.0	2
Nitrofurantoin	1	50.0	-	-	1	50.0	2
Ofloxacin	-	-	-	-	2	100.0	2
Ciprofloxacin	-	-	-	-	2	100.0	2
Cotrimoxazole	-	-	-	-	2	100.0	2
Ceftazidime	1	50.0	-	-	1	50.0	2
Amikacin	**2**	**100.0**	-	-	-	-	2
Cefixime	-	-	-	-	2	100.0	2

5.7.3.3 Antibiotic Susceptibility pattern of *Klebsiella oxytoca :*

Both isolates of *K. oxytoca* were susceptible to Nitrofurantoin, Ceftazidime and Amikacin while only one isolate was found to be susceptible to Cefixime. All the other drug used were resistant to *K. oxytoca.*

Table 18: Antibiotic Susceptibility pattern of *K. oxytoca:*

Antibiotics used	**Sensitive**		**Intermediate**		**Resistant**		**Total**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Amoxycillin	-	-	-	-	2	100.0	2
Cephalexin	-	-	-	-	2	100.0	2
Nalidixic acid	-	-	-	-	2	100.0	2
Norfloxacin	-	-	-	-	2	100.0	2
Nitrofurantoin	**2**	**100.0**	-	-	-	-	2
Ofloxacin	-	-	-	-	2	100.0	2
Ciprofloxacin	-	-	-	-	2	100.0	2
Cotrimoxazole	-	-	-	-	2	100.0	2
Ceftazidime	**2**	**100.0**	-	-	-	-	2
Amikacin	**2**	**100.0**	-	-	-	-	2
Cefixime	1	50.0	-	-	1	50.0	2

5.7.3.4 Antibiotic Susceptibility pattern of *Acinetobacter spp.:*

Most of the drugs used against *Acinetobacter spp*. were resistant other than Cotrimoxazole to which it was susceptible. Amikacin had showed intermediate activity towards the isolate. The results are shown in table 19.

Table 19: Antibiotic Susceptibility pattern of *Acinetobacter spp.:*

Antibiotics used	**Sensitive**	**Intermediate**	**Resistant**	**Total**
	No.	**No.**	**No.**	
Amoxycillin	-	-	1	1
Cephalexin	-	-	1	1
Nalidixic acid	-	-	1	1
Norfloxacin	-	-	1	1
Nitrofurantoin	-	-	1	1
Ofloxacin	-	-	1	1
Ciprofloxacin	-	-	1	1
Cotrimoxazole	**1**	-	-	1
Ceftazidime	-	-	1	1
Amikacin	-	1	-	1
Cefixime	-	-	1	1

5.7.3.5 Antibiotic Susceptibility pattern of *Pseudomonas aeruginosa:*

The single isolate of *P. aeruginosa* was found susceptible to Norfloxacin, Ofloxacin, Ciprofloxacin, Ceftazidime and Amikacin. Amoxycillin, Cephalexin, Nalidixic acid, Nitrofurantoin, Cefixime and Cotrimoxazole were resistant towards the isolate. The results are shown in table 20.

Table 20: Antibiotic Susceptibility pattern of *Pseudomonas aeruginosa:*

Antibiotics used	**Sensitive**	**Intermediate**	**Resistant**	**Total**
	No.	**No.**	**No.**	
Amoxycillin	-	-	1	1
Cephalexin	-	-	1	1
Nalidixic acid	-	-	1	1
Norfloxacin	**1**	-	-	1
Nitrofurantoin	-	-	1	1
Ofloxacin	**1**	-	-	1
Ciprofloxacin	**1**	-	-	1
Cotrimoxazole	-	-	1	1
Ceftazidime	**1**	-	-	1
Amikacin	**1**	-	-	1
Cefixime	-	-	1	1

5.7.4 Antibiotic resistance pattern of the bacterial isolates :

Out of the 26 isolates from cancer patients under chemotherapy, 23 (88.5%) were found to be MDR strains with resistance to 3 or more classes of drugs. Out of 19 isolates of *E. coli*, 16 (84.2%) were multidrug resistant. Both the isolates of C*itrobacter freundii* and *K. oxytoca* and a single isolate of *P. aeruginosa*, *Acinetobacter spp.* and *S. aureus* were also multidrug resistant. The results are shown in table 21.

Table 21: Antibiotic resistance pattern of the bacterial isolates from urine samples

Organisms isolated	**Total isolates**	**Resistance to**					
					MDR Strains		
		0 Drug	**1 Drug**	**2 Drug**	**3 Drugs**	**>3 Drugs**	**Total (%)**
E. coli	19	0	1	2	13	3	**16 (84.2)**
Citrobacter freundii	2	0	0	0	2	0	2 (100.0)
K. oxytoca	2	0	0	0	2	0	2 (100.0)
Acinetobacter spp.	1	0	0	0	1	0	1 (100.0)
P. aeruginosa	1	0	0	0	1	0	1 (100.0)
S. aureus	1	0	0	0	1	0	1 (100.0)
Total	26	0	1	2	**20**	**3**	**23 (88.5)**

Photograph 1 Significant growth of *E. coli* on MacConkey agar

Photograph 2 Antibiotic susceptibility test of an MDR strain of *Escherichia coli*

Photograph 3 Biochemical tests for *E. coli*

[From left to right : Fermentative (A); Oxidative(B) ;VP–ve (C); MR+ve (D); Urease –ve (E), Citrate -ve (F); Indole +ve and motile (G); TSI: Acid/Acid + gas(H)]

Photograph 4 Automated Hematology Analyzer (Sysmex KX-21)

CHAPTER – VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

This study was conducted among cancer patients under chemotherapy in OM Hospital and Research Centre, Kathmandu, Nepal. One hundred twenty seven mid stream urine samples were collected and subjected to routine examination and then processed for culture and sensitivity. Meanwhile, the blood cell count of the patients were also monitored before they received the new cycle of chemotherapy. The results obtained were tabulated in the previous chapter. In this chapter, the results are discussed and compared with the findings of other investigators.

The results of this study revealed that the incidence of UTI was found to be 20.5% in cancer patients under chemotherapy . Out of the 26 culture positive cases, 19.2% (5/26) of them were isolated from those with ovarian cancer. Similarly patients with lung cancer showed 15.4% (4/26) probability of UTI while leukemia and cancer of prostate, cervix and gall bladder; each accounted for 11.5% (3/26) of the significant cases. Hence incidence of UTI was found more in solid tumor patients than in patients with leukemia. This finding is similar to a study done in National Kidney Institute which concluded that infection rate was higher in solid tumors than in leukemia (Billote *et al.*, 1996). However, Ashour *et al*.(2009) had reported that out of 98 gram-negative isolates from UTI, 77 were isolated from leukemic patients (78.6%) whereas only 21 isolates were obtained from solid-tumor patients (21.4%).

Microbial growth pattern:

Altogether 127 urine samples from cancer patients under chemotherapy were obtained for this study. Out of the 127 test samples, 26 samples (20.5%) showed significant bacterial growth whereas majority of samples i.e. 101 (79.5%) didn't show any significant growth. Study done by Tancheva *et al*.(2009) on urinary tract infections in patients with

hematological malignancies undergoing combination chemotherapy revealed that 68% had significant bacteriuria. But similar study carried out in children on cancer treatment at the Kenyatta National Teaching hospital showed the prevalence of UTI to be 8.1% (Munyis *et al.*,1998). Study of Vitetta *et al*. (2000) have concluded that the most frequent site of infection among the patients admitted to Caritas Christi with terminal malignant illness was the urinary tract (41.0%). Similar study by Lam *et al*.(2005) had found that urinary tract infections constitute for 29.2% of all the infections occurring in the cancer patients.

This study have thus demonstrated that there is a greater susceptibility of cancer patients under chemotherapy to UTI. Many factors increase the susceptibility of immunosuppressed cancer patients to UTI. These include erosion of tumor involving the protective integument or mucosa (Zinner,1997), neutropenia during aggressive therapy, altered gut flora because of frequent antibiotic administration, and the various invasive procedures including the long term use of indwelling catheters, and combinations of the aforementioned (Billote,1996).

Table 4 has revealed that the percentage of urine samples showing significant bacterial growth is higher in females (65.4%) than in males (34.6%). Women suffer more from this complication probably due to short urethra , proximity to anal or colon dysfunction (Tancheva *et al.,* 2009). However, the higher number of growth positivity seen in females was found to be statistically insignificant ($P>0.05$) suggesting that there is no direct correlation between the gender and incidence of UTI in cancer patients under chemotherapy.

The majority of the patients included in the study belonged to age group (60-70) and (50-60) which represented 32.3% and 19.7% of the study population respectively (Table 2). The majority of UTI occurred in the age group (70-80) followed by age group (50-60) and they accounted for 38.5% and 23.1% of the total significant bacteriuric cases respectively (Table5). Among males, highest growth positivity was found among the age group of (70-80) while among females it was (50-60) years. This indicated that UTI in cancer patients under chemotherapy are quite common in later stages of life.

Among the 26 culture positive cases, maximum occurrence of UTI (50%) were found in those patients who had received 6-8 chemotherapy cycles followed by those who had received 4-6 cycles (38.5%) thus suggesting that the incidence of UTI increases with each cycle of chemotherapy. Study done by Mendonca *et al*.(2009) had reported 49 infection episodes (63.2% urinary, 18.4% neutropenic fever and 18.4% diverses), mainly between course 1–2 (39%) and course 3–4 (38%) of chemotherapy. Their study have also concluded that progressive chemotherapy, but not the neutrophil count, was an independent factor for infection.

Pattern of Aetiological agents:

Among the total 26 bacterial isolates, 25 (96.2%) were Gram negative rods and the remaining 1 (3.8%) was found to be Gram positive cocci. Altogether 6 species of bacteria were isolated in this study. *E. coli* was the most predominant isolate accounting for 73% (19/26) of the total bacterial isolates. It was followed by *Klebsiella oxytoca* and *Citrobacter freundii;* each accounting for 7.7% (2/26) of the total isolates. Such a high prevalence of *E.coli* isolated in this study was similar to many other studies done in UTI in cancer patients. Similar study done by Tancheva *et al.,* (2009) also found that the most frequent causative agents of UTI in cancer patients undergoing combination chemotherapy was *E. coli* which accounted for 64.7% of all the bacterial isolates. Study done in Kenyatta National Teaching hospital reported that *E. coli* and *Klebsiella spp.* were responsible for 93.4% of the urinary tract infections (Munyis *et al.,* 1998). Ashour *et al*.(2009) have also reported that in both leukemic and solid-tumor patients, the gram-negative bacteria causing UTI were mainly *Escherichia coli* (37.8%) *and Klebsiella pneumonia* (31.6%). This is reminiscent of the study by Espersen *et al*.(1984) who demonstrated that UTI due to *Escherichia coli* were the most frequent infections in patients with myelomatosis.

Other bacteria isolated in this study were those of *S. aureus, P. aeruginosa and Acinetobacter* spp.; each accounting for 3.9% (1/26) of the total isolates. Tanchvea *et al.*(2009) had also reported detection of *E. faecalis, Pseudomonas aeruginosa, Klebsiella*

pneumoniae, P. mirabilis, and Acinetobacter as etiologic pathogens of UTI in these patients.

Microscopic observation of urine:

The microscopic examination of urine was done by wet mount preparation. The intention of microscopy was to determine the number of pus cells. Finding of equal to or more than five leukocytes per HPF is of great importance for urinary tract infection diagnosis (Merila *et al.,* 1987). In this study, significant pyuria was observed in 22 % (28/127) of the samples. Out of 28 cases of significant pyuria, 18 (64.3%) cases were culture positive and 10 (35.7%) were culture negative. Similarly, out of the remaining 99 cases of insignificant pyuria, 8 (8.1%) were culture positive.

Jenson (2004) has reported that pyuria may be present in less than 20% of patients with urinary tract infections. It is because patients with neutropenia often fail to develop sign and symptoms of infection because of a blunted inflammatory response. ; and hence those with urinary tract infections may not have localized symptoms such as dysuria. Similar study done at M.D Anderson Hospital have also found that the signs and symptoms of infection were frequently absent in neutropenic patients and hence their report concluded that only 11% of neutropenic patients with UTI had pyuria (Rosenthal, 1987). However, Tancheva *et al.*(2009) has reported that an increased number of WBC in urine (pyuria) was found in 70 from 72 patients with UTI.

In this study, it was seen that 8.1% of the culture positive urine samples would have been interpreted falsely as being negative, if only microscopy for pyuria was used. A urinalysis and culture should therefore be done even if there is no pyuria (Jenson, 2004). This result of insignificant pyuria with positive culture could have appeared due to early urinary tract infection or presence of asymptomatic UTI or due to neutropenia in some patients. As mentioned earlier, neutropenia may also lead to lack of classic signs and symptoms such as pyuria in urinary tract infections. Out of 28 significant pyuric cases, there were 10 false positive results (significant pyuria with negative culture result) and this could be

due to prior use of antibiotics by the patients or presence of bacteria which were unable to grow on the routine culture media.

Biochemical pattern of results:

In this study, out of the total 127 samples, only 16 samples were positive for albumin test while the remaining 111 were negative. Among the 16 positive cases, 9 (56.2%) of them gave culture positive results while among the 111 negative cases, 17 (15.3%) of them gave culture positive results. There are various conditions in which protein (albumin) appear in urine, UTI is one of them. According to the North Thames Regional Guidelines for Diagnosis and Management of Urinary Tract Infection, if dipstick proteinuria is consistently more than 1+, then this may indicate UTI and a MSU specimen for culture should be taken. So, it could be concluded that detection of protein (albumin) in urine is also important for diagnosis of UTI and mid-stream urine sample should be cultured in cases when there is absence of significant pyuria.

The normal range for total WBC count is between 4,000 – 10,000 cells/mm^3. Out of 127 cancer patients, 105 (82.7%) had the total WBC count within the normal range and among these, 19 (18.1%) urine samples gave culture positive results. Similarly, the remaining 22 (17.3%) samples had total WBC count less than the normal range and among these 7 (31.9%) urine samples gave culture positive results. The result was found to be statistically insignificant ($P>0.05$) thus indicating that there is no association between the total WBC count and the occurrence of UTI in cancer patients under chemotherapy.

The overriding issue in these cancer patients is the absolute granulocyte (neutrophil) count. Risk of infection increases significantly when the neutrophil count falls below 1000/mm^3 and even more dramatically when below 500/mm^3 and the condition is known as neutropenia. Since very few severely neutropenic patients were involved in this study, it was not possible to correlate the incidence of UTI with severe neutropenia when ANC<500. Hence instead, this study was carried out to find the association between the incidence of UTI and mild neutropenia; the condition when ANC is <1500.

Out of 127 cancer patients included in this study, only 14 (11%) were found to be neutropenic with ANC<1500 while the remaining 113 (89%) had a normal range for ANC. Among the 14 neutropenic patients only 5 (35.7%) gave culture positive results while among the 113 non-neutroepnic patients, 21 (18.6%) of them gave culture positive results. The result was found to be statistically insignificant (P>0.05) suggesting that there is no direct correlation between the Absolute Neutrophil Count of the patient and the occurrence of UTI. The reason behind such a result may have been due to the lesser number of neutropenic patients included in the study. Had the number of neutropenic patients been increased, then may be a significant association could have been established between the two factors. Schwartzberg (2006) has reported that infection is common and most likely when ANC count falls below 500 cells/mm^3 and since very few patients with ANC less than 500 were present in this study, it could also have been a contributing factor for having an insignificant result. Rolston (2009) has also reported that cancer patients undergoing chemotherapy are more prone to having bactraemic and respiratory infections at the time of severe neutropenia. This could also have been another likely factor which have led to an insignificant result in this study.

Antibiotic sensitivity profile:

Antibiotics have revolutinized cancer treatment by enabling the use of more aggressive therapies. This had led to dramatically higher survival rates for this medically vulnerable group as a while but the loss of effective antibiotics would have immense ramifications.

In this study, the only isolate of *S. aureus* was found resistant to most of the common antibiotics used including Cloxacillin, Nitrofurantoin, 3rd generation Cephalosporins (Cefixime and Ceftazidime) and the Fluoroquinolones. However, Amikacin and Novobiocin were found to be effective towards the isolate.

Among gram negative organisms isolated, an emerging pattern of resistance was seen with most of the commonly used antibiotics. In this study, Amikacin was found to be the most effective antibiotic against gram negative bacteria with sensitivity of 84%. Second most effective antibiotics against gram negative bacteria were found to be Ceftazidime

and Nitrofurantoin each of which showed sensitivity of upto 76%. On the other hand, Amoxycillin and Nalidixic acid were found to be the least effective drugs; each of which had resistivity as high as 96 %. Similarly, the 1st generation cephalosporin Cephalexin showed 88% resistance while resistance to a 3rd generation cephalosporin Cefixime was found to be 68%. Cotimoxazole was also less effective with resistance of 84%.

Surprisingly, Fluoroquinolones were also found to be highly resistant towards the isolated gram negative organisms. 72% of the isolates were resistant against both Ofloxacin and Ciprofloxacin while Norfloxacin showed 76% resistance. Possible causes of higher resistance to flouroquinolones may be due to the widespread use of fluoroquinolones by general practitioners. The broad use of fluoroquinolones for antibacterial prophylaxis in neutropenic patients may also have lead to very high resistance rates among Gram-negative bacilli such as *E. coli* (Maschmeyer, 2008). Other studies have also reported increasing fluoroquinolone resistance among *E.coli* /gram negative isolates. A study conducted in Pakistan to find out the prevalence of UTI among prostate cancer patients receiving pelvic radiotherapy has reported 66.6% resistance to ciprofloxacin (Tunio *et al.*, 2009).

Multidrug resistance profile:

Multiple drug resistant (MDR) bacterial isolates have been frequently reported from different parts of the world as an emergence of treatment problem. The MDR strain is defined as the strain that showed resistance to three or more antibiotics belonging to different structural classes (Tuladhar, 2001). Antimicrobial resistance is an evolving and growing problem in cancer patients and has been associated with an increased rate of clinical failure. The emergence of MDR is clearly related to the quantity of antibiotics and how they are being used.

In this study, 23 out of 26 (88.5%) bacterial isolates were found to be multidrug resistant (table 21). Among the 19 *E. coli* isolates, 16 (84.2%) were MDR strains. Both isolates of *Citrobacter freundii* and *Klebsiella oxytoca*, the single isolate of *Pseudomonas aeruginosa, Acinetobacter spp.* and *S. aureus* were also multi-drug resistant. This alarmingly high rate of emergence of MDR strains in cancer patients undergoing

chemotherapy may be due to frequent antibiotic administration by physicians to avoid/ cure infections in this vulnerable patient group.

6.2 CONCLUSION

The incidence of urinary tract infection in cancer patients under chemotherapy attending OM Hospital and Research Centre was found to be 20.5% and the prevalence of MDR strains among the isolated bacterial pathogens was 88.5%. The most predominant organism isolated was *E.coli*. The most effective drugs for gram negative isolates were Amikacin, Ceftazidime and Nitrofurantoin. This study has also revelaed that there was no significant relationship between the total WBC count, absolute neutrophil count and the occurrence of UTI in these group of patients.

CHAPTER – VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY :

This study was conducted with the purpose of determining the incidence of urinary tract infection in cancer patients under chemotherapy at Om Hospital and Research Centre, Chabahil, Kathmandu. The study was done on 127 cancer patients. Following are the major findings from the study :

- The results of the present study showed the incidence of UTI in cancer patients under chemotherapy to be 20.5%.
- Growth positivity with regards to gender wise distribution of the urine samples showed higher growth positivity in females than in males. However, the result was found statistically insignificant.
- The predominant bacteria causing UTI among cancer patients were found to be gram negative (N=25/26) which constituted 96.1% of the total bacterial isolates. Only a single isolate of gram positive bacteria was isolated which accounted for 3.9% of the total isolates.
- Altogether 6 different species of bacteria were isolated from the culture positive samples. *E. coli* (19/26) was found to be the most predominant isolate (73%). Among Gram positive, *S. aureus* was the only isolate.
- Among the total 127 urine samples, 28 (22%) showed significant pyuria and among these 18 (64.3%) gave culture positive results.
- Out of the total 127 urine samples, 16 (12.6%) samples were positive for albumin test and among them 9 (56.2%) showed significant growth on culture.
- Amikacin was found to be the most effective drug against Gram negative bacteria with a susceptibility of 84% followed by Ceftazidime and Nitrofurantoin each of which had effectivity upto 76%. The only isolate of gram positive bacteria was found sensitive to both Amikacin and Novobiocin.

- Of the total 26 bacterial isolates from cancer patients under chemotherapy, 23 (88.5%) were found to be MDR strains.
- Out of 19 isolates of *E. coli*, 16 (84.2%) were multidrug resistant. Both the isolates of C*itrobacter freundii* and *K. oxytoca* and the single isolates of *P. aeruginosa* and *Acinetobacter* spp. were also multidrug resistant.
- The only isolate of *S. aureus* was also found to be multidrug resistant.
- 22 (17.3%) out of the 127 cancer patients had their total WBC count less than the normal range and among them 7 (31.9%) had significant bacteriuria. Similarly, the remaining 105 (82.7%) patients had the total WBC count in the normal range and among these 19 (18.1%) urine samples gave culture positive results. The result was found to be statistically insignificant.
- Of the 127 cancer patients, 14 were found to be neutropenic. Among the 14 neutropenic patients, 5 (35.7%) gave culture positive results while among the remaining 113 non-neutroepnic patients, 21 (18.6%) of them gave culture positive results. The result was found to be statistically insignificant suggesting that there is no statistical relationship between the Absolute Neutrophil Count of the patient and the occurrence of UTI in these group of patients.
- Incidence of UTI was more prevalent with the increasing number of chemotherapy cycles as maximum number of culture positive results were seen in cycles (6-8) followed by (4-6).

7.2 RECOMMENDATIONS :

1. As this study was confined to OM Hospital and Research Centre, Kathmandu, it doesn't reveal the picture of whole country, therefore this type of study should be conducted in other parts of the country.
2. Infection is common among cancer patients due to aggressive cancer treatment. Hence, study of infections at other sites such as those of respiratory tract, skin, blood-stream, gastrointestinal tract etc should also be done.

3. The sample size must be increased as far as possible to get the significant result between the blood cell count and the incidence of UTI in cancer patients under chemotherapy.
4. High resistance observed in this study warrants the needs of surveillance of resistant pattern of antimicrobial agents. Testing is required not only for therapy but also to monitor the spread of resistant organisms or resistance genes throughout the hospital and community.
5. Neutropenia is a significant intrinsic risk factor that should be addressed in surveillance programs. Infection control and infectious diseases practitioners may need to modify techniques for surveillance, control, and management of infection in this population.

CHAPTER – VIII

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