CHAPTER ONE

Introduction and literature Review

1.1. Introduction

The therapies that usually used to treat the cancer (as example: chemotherapy), often decreases the numbers of white blood cells in the body. White blood cells fight bacterial, fugal and viral infection. Most patients with cancer who have few white blood cells have very weak immunosystem and can't fight infection well. They get infection easily and as result can die, particularly if the infections are not recognized early and treated.

the spectrum of bacterial and fungal infections Undergo to periodic change and is impacted upon by several factors including the use of antibacterial/antifungal prophylaxis, the use of foreign medical devices and the nature and intensity of the antineoplastic used.

Fever is usually one of the earliest signs of infection. Thus powerful antibiotics used to treat an infection in a patient with cancer whose white blood cells count is low. Combinations of antibiotics that work against many different types of bacteria are successful.

Neutropenic patients, usually their course of treatment interrupted and stopped unless blood cells return to normal. Despite presence of infection, but the burden and causes of this is not known.

The aim of the present study was to analyses the cases of urinary tract infections in patients treated with chemotherapy drug.

1.2. Objectives

1.2.1. Main objective

1. To assess the level of urinary tract infection among cancer patients receiving chemotherapeutic drug at radiation and isotopes center of Khartoum (RICK).

1.2.2. Specific objectives

1. To isolate bacterial pathogen responsible for urinary tract infections (UTI) in patient whom receiving chemotherapeutic therapy.

2. To evaluate antibiotics used to treat such infection.

3. To detect and evaluate the incidence of infection in male and female and among children.

1.3. LITERATURE REVIEW

1.3.1. Urinary tract infection (UTI)

urinary infection is define as bacteriuria, the multiplication of bacteria in urine within the renal tract, with a concentration greater than 10^5 organism/ml is regarded as significant bacteriuria, urinary tract infection remain a major clinical problem over 50 years after introduction of antimicrobial chemotherapy many consultations in general practice are because of urinary infection, Infection of urinary tract may involve anywhere along urinary tract (bladder, kidney, pelvis, parenchyma, or urethra) (Sleigh and Timbury, 1998).

1.3.2. Urinary tract system

Urinary tract makes and store the urine until it's evacuated. The urinary tract is divided into two major divisions: upper, which include (kidney, renal pelvis and ureters) and lower, which include (urinary bladder and urethra) (Koneman *et al*, 1992).

1.3.2.1. Kidney

Two kidneys function to excrete most of waste products of metabolism, which leave the kidneys as urine. They lie behind the peritoneum on the posterior abdominal wall on either side of vertebral column, the right kidney lie slightly lower than the left one and neuron is the functional unite (Snell, 1995).

The kidneys measure about 4 ½ inches long, 2 ½ inches wide, 1 ½ thick (Koneman *et al*, 1992).

1.3.2.2. Ureters

the two ureters are muscular tubes that extend from kidney to the posterior surface of the urinary bladder, the urine is propelled along the ureter by peristaltic contractions of muscle coat, each ureter measure about 10 inch in long, ureters have three constrictions along it's course:

where the renal pelvis (funnel-shaped) joins the ureter, where it is kinked as it crosses the pelvic brim, and where it pierces the bladder (Snell, 2004).

1.3.2.3. Urinary bladder

Urinary bladder is immediately directly behind the pubic bone within the pelvis, it's receptacle for the storage of the urine, in adult maximum capacity of about 500ml, it's strong muscle and it's shape is vary according the amount of urine, empty bladder is pyramidal. Having apex, base, neck and two inferolateral surface (Snell, 2004).

1.3.2.4. Urethra

Urethra is muscular tube for the passage of urine in both sex and semen in male. Extend from the bladder neck to the tip of penis in the male, and the area between the vagaina and pubic bone in female (Qurashi and Taher, 1998).

In female urethra is about 1.5 inch; in male is about 8 inch (Snell, 2004).

1.3.3. Type of infection

Infection is most often due to bacterial from patient's own bowel flora. Transfer to the urinary tract may be via the blood stream, the lymphatics or by direct extension, but the most often via the ascending transurethral rout (Kumar and Clark, 2003).

1.3.3.1. Lower urinary tract infection

Involving of bladder and urethra, involving of urethra is called urethritis, if the bladder is involved called cystitis (Sleigh and Timbury, 1998).

The most typical symptoms are: Frequency of micturition by day and night, Painful voiding (dysuria), Suprapubic tenderness and pain, Haematuria, Smelly urine (Kumar and Clark, 2003).

1.3.3.1.1. Urethritis

Is the infection of urethra, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* are common causes of urethritis and are considered being sexually transmitted (Forbes et al, 1998).

1.3.3.1.2. Cystitis

It's the Infection of the bladder. Episodes of cystitis greatly outnumber those that involve the kidney (Sleigh and Timbury, 1998).

1.3.3.2. Upper urinary tract infection

When the infection extend to the kidney and pelvis (know as pyelitis or pyelonephritis), or to the ureter the most typical symptoms are loin pain and tenderness, with fever and systemic upset, also UTI may present with minimal or no symptoms or may associated with atypical symptoms such as abdominal pain and the typical presentation of lower urinary tract infection (Kumar and Clark, 2003).

1.3.3.2.1. Pyelonephritis

Pyelonephritis refers to inflammation of the kidney parenchyma and pelvis (upper end of the ureter that is located inside the kidney) and is usually caused by bacterial infection.

Of significance, 40% of patients with acute pyelonephritis are bacteremic (Forbes *et al*, 1998)

1.3.4. Pathogenesis

Anything that disrupts normal urine flow or complete emptying of bladder or facilitates access of organisms to the bladder will predispose an individual to infection. The shorter female urethra is less effective deterrent to infection than male urethra. Sexual intercourse facilitates the movement of organisms up the urethra, particularly in females so the incidence of urinary tract infections is higher among sexually active women than among celibate women.

Catheterization is major predisposing factor for UTIs; during insertion of the catheter bacteria may be carried directly into the bladder. Most urinary tract pathogens originate in the fecal flora but only aerobic and facultative species such as *Escherichia coli* possess the attributes required to colonize and infect the urinary tract. Virulence factor of causative organisms such as capsule which inhibiting phagocytosis, pili which enable adherence and bacteria production like heamolysins which cause kidney damage, and urease production which cause pyelonephritis. (Cedric *et al*, 2004)

1.3.5. Causal organisms

The Gram-negative rods *Escherichia coli* is commonest cause of ascending UTIs about 60-90%; this is probably because they are often present in the colon and virulence factors which include: the possession of K antigens and specialized fimbriae (Cheesbrough, 2009).

Staphylococcus saprophyticus is related to sexual active women. *Proteus mirabilis* and klebsiella species are often multiply antibiotic-resistant. *Enterococcus faecalis, Pseudomonas aeruginosa* and *staphylococcus aureus* are cause infection especially after catheterization or instrumentation. Fastidious gram-positive bacteria (e.g. lactobacilli, streptococci, corynebacteria), which require incubation for 24–48 hour in the presence of CO2 for isolation, acute uncomplicated UTI is usually due to one type of organism and Chronic infection is often associated with more than one type of organism (Sleigh and Timbury, 1998).

Obligate anaerobes are very rarely involved, other species may be found e.g.: *Salmonella typhi, Staphylococcus aureus*, and *Mycobacterium tuberculosis* (Cheesbrough, 2009).

1.3.5.1. *Escherichia coli* (*E. coli)*

E. coli is belong to the large group of gram negative rods referred to as enterobacteria, they are cause primary and opportunistic infections in humans belong mainly to lactose fermenting, often referred to as coliforms, they are aerobes and facultative anaerobes, non sporing and motile (Cheesbrough,2009).

E. coli is the cause of 60-90% of urinary tract infection. Certain serotypes of *E .coli* are particularly common in urinary infection (e.g. 02, 04, 06, 07, 018, 075); this is probably because they are often present in the colon, rather than because of inherently high pathogenicity for the urinary tract. Some strains are reputed to more invasive than other. Factor associated with virulence include: the possession of K (capsular) antigen, which inhibit phagocytosis and bactericidal effect of normal human serum, the ability to adhere to uro–epithelium due to specialize fimbriae (Sleigh and Timbury, 1998).

1.3.5.2. *Klebsiella* species

Gram-negative and non motile usually capsulated rods cause UTIs in hospital patients. Antigenic analysis for capsular polysaccharide reveals that more than 80 serotype are recognized (Cheesbrough, 2009).

They grow well on ordinary media, with colonies which are often, but not always, large and mucoid (Sleigh and Timbury, 1998).

1.3.5.3. *Proteus* species

Gram negative pleomorphic motile rods, they grow on selective enteric media (Cheesbrough, 2009).

Proteus mirabilis is main proteus species of medical importance. It causes urinary infection commonly in the elderly and young male often following catheterization or cytoscopy. It is often associated with urinary

stones, probably because this organisms produce ammonia rendering the urine alkaline (Sleigh and Timbury, 1998).

1.3.5.4. *Pseudomonas aeruginosa*

Gram–negative motile aerobic bacilli some strain are capsulated have very simple growth requirement and limited fermentation activity (Sleigh and Timbury, 1998).

Ps. aeruginosa being resistant to infections are often difficult to eradicate due to *Ps. aeruginosa* being resistant to many antimicrobials. Infection with *Ps. aeruginosa* usually following catheterization associated with chronic urinary disease (Cheesbrough, 2009).

1.3.5.5. *Serratia marcescens:*

It has been reported to cause UTIs, and it is gram-negative rods, facultative anaerobe and it is resistant to cephalosporin (Cheesbrough, 2009).

1.3.5.6. *Staphylococcus aureus*

Gram-positive cocci are occurring in group. Non motile, non-capsulated and it is catalase, DNase and coagulase positive, and ferment mannitol, it is rarely cause UTI (Cheesbrough, 2009).

1.3.5.7. *Staphylococcus saprophyticus*

Gram-positive cocci of uniform size occurring in groups but also singly and pairs. They are non-motile and non-capsulated. *S. saprophyticus* cause UTIs in sexually active women. It is coagulase and DNase negative and ferment mannitol. The organism causes are many as one quarter of symptomatic UTIs in women. The surface agglutinins of this pathogen appear to be a key determinant of the virulence promoting it colonizes urinary tract (collee *et al*, 1996).

1.3.5.8. *Enterococcus faecalis*

It is gram-positive cocci, often found accompanying infection with coliforms (Cheesbrough, 2009).

1.3.5.9. Other bacteria

Bacteria species are not primarily in urinary tract but may found in urine e.g. salmonella species, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, *leptospira innterrogans*, Chlamydia and mycoplasma species (Cheesbrough, 2009).

1.3.5.10. Parasites:

Very few parasites can cause UTIs e.g. *Trichomonas vaginalis* which cause urethritis in both male and females, but most often considered as cause of vaginitis. *Onchocerca volvulus*, *Wuchereria bancrofti* and *Schistosoma haematobium* were also uncommon UTI agent (Cheesbrough, 2009)

1.3.5.11. Fungi

Candida albicans usually in diabetic patients and those with immunosuppression, cause bladder infection and source of infection is usually endogenous; however cross-infection may occur (Cheesbrough, 2009).

1.3.5.12. Viruses

Viral cause of UTIs appears to be rare although there are association with hemorrhagic cystitis and renal syndromes (Cheesbrough, 2009).

1.3.6. Background on chemotherapy

Chemotherapy treatment can be used for the following intents: curing, prolonging survival, or palliation, Cancer treatment depends on the type and stage of cancer along with patient characteristics (Kate *et al*, 2010).

1.3.6.1. Principle of chemotherapy

Chemotherapy employs systemically administered drugs that directly damage cellular DNA (and RNA). It kills cells by promoting apoptosis and necrosis. There is narrow therapeutic window between effective treatment of the cancer and normal tissue toxicity, because the drugs are not cancer specific (Kumar and Clark, 2003).

The dose and schedule of the chemotherapy is limited by the normal tissues tolerance, all tissues can be affected however, depending upon the pharmacokinetics of the drug and affinity for particular tissues. The therapeutic effect on the cancer is achieved by a variety of mechanisms which seek to exploit differences between normal and transformed cells. Toxicity to the normal tissue can be limited in some instances by supplying growth factors or by the infusion of stem cell preparations to diminish myelotoxicity (Kumar and Clark, 2003).

1.3.6.2 Classification of cytotoxic drug

Either DNA damaging drug, or Antimetabolites, or DNA repair inhibitors, or Antitublin (Kumar and Clark, 2003).

1.3.6.3. side-effect of chemotherapy

Although chemotherapy kills cancer cells, it can damage normal cells and cause significant side effects, the side effect vary depending on the particular drug, dosage, route of administration and patient characteristics (Kate *et al*, 2010).

Chemotherapy has many potentially serious side-effects and should be used only by trained practitioners, the most common side-effects are:

1.3.6.3.1. Damage and irritation on cells lining the digestive tract which can produce

This may cause Nausea, vomiting or diarrhea. The severity of Vomiting side-effect varies with the cytotoxic and can be eliminated in 75% of

patient by using modern antiemetic (Kumar and Clark, 2003; Kate *et al*, 2010).

1.3.6.3.2. Hair loss:

Many but not all cytotoxic drugs are capable of causing it. Scalp cooling can some time use to reduce it (Kumar and Clark, 2003).

1.3.6.3.3. Bone marrow suppression and immunosuppression

Suppression of the production of red blood cells, white blood cells and platelets occur with the most cytotoxic drug and dose related phenomenon, Anemia and thrombocytopenia are managed by red cell or platelet transfusions but the WBCs have not been successful until the advent of peripheral blood stem cell harvesting (Kumar and Clark, 2003). Neutropenic patients are at high risk of bacterial and fungal infection often from enteric flora; this managed by immediately introduction of broad spectrum antibiotics intravenously (Kumar and Clark, 2003).

1.3.6.3.4. Tiredness.

1.3.6.3.5. Other side-effect is related to certain drug

Cardiotoxicity which related to anthracyclines, and nephrotoxicity which is common with platinum, secondary malignancy (predominantly acute leukemia) with alkylating agent (Kumar and Clark, 2003).

1.3.7. The problem that related with receiving chemotherapy in cancer patient

The treatment of the malignant diseases requires the use of combination chemotherapy in multiple cycles administered to achieve adequate tumor cell kill without life threatening toxicity or the development of tumor cell resistance. The dose of drug needed to achieve adequate tumor cell kill often causes toxicity to normal tissues. Infection is the major cause of morbidity and mortality in patients undergoing antineoplastic chemotherapy (Tancheva *et al*, 2009).

These include defects in humoral and cell-mediated immunity (functional asplenia, hypogammaglobulinemia and phagocyte); mucosal damage resulting from chemotherapy and impairment of central nervous system reflexes, and The most common sites of infection in neutropenic patients include the lung, oropharynx, blood, urinary tract, skin, and soft tissues, including the perirectal area (Tancheva *et al*, 2009).

Several of the cancers chemotherapeutics drugs are used today as immunosuppressant for the treatment of severe systemic autoimmune diseases. This applies to cyclophosphamide5 and methotrexate6, which impair the proliferative and/or effect on functions of peripheral T cells, and this increase susceptibility to viral and bacterial infections. Glucocorticoids are also important components of the chemotherapeutic cocktails; that are used in treating several lymphoproliferative diseases. High doses of Glucocorticoids are prescribed to cancer patient to attenuate chemotherapy-associated nausea and vomiting. Glucocorticoids suppress the production of proinflammatory cytokines such as interferon (IFN), interleukin (IL). (Laurence *et al,* 2008).

1.3.8. Incidence in cancer

Fever in patient with cancer can be due to infection (80%) or the cancer itself (20%). Most infections are due to bacteria, fatal infection usually due to fungal infection (sugar 1990)

The risk of infection is directly related to the depth and length of neutropenia or lowered WBCs count. More than 50% of the patients with neutropenia will become infected. If the absolute neutrophil count is<100/mm^3, approximately 20% of febrile patients will have documented bacteremia (Huges *et al,* 1990).

CHAPTER TWO

2. Materials and Methods

2.1. Study area

Specimens were collected from in Radiation and Isotope Center Khartoum (RICK).

2.2 study population

Fifty urine specimens were collected from patients whom under go with chemotherapeutics treatment.

2.3. Study variables

Frequency of UTI and antimicrobial sensitivity.

2.4. Materials

2.4.1. Instruments

Hot air oven

Incubator

Autoclave

Water bath

Microscope

2.4.2. Media

The following media were obtained and prepared in lab according to manufactures:

1-Cystine lactose electrolyte deficient (CLED) agar medium

Used for culturing of urine sample; because it gives consistent results, can differentiate between lactose fermenting from non lactose fermenting bacteria (the indicator is bromothymol blue) (Cheesbrough, 2009).

2-Kligler iron agar (KIA)

It is used for the differentiation of members of the Enterobacteriaceae on the basis of their ability to ferment dextrose (glucose), lactose and production of hydrogen sulfides (Cheesbrough, 2009).

3- Simmon's citrate media

It's used in the identification of Enterobacteriaceae, based on the utilization of sodium citrate as a sole carbon source (Cheesbrough, 2009).

5-christensen, urea agar media

Testing for urease enzyme activity is important to differentiate Enterobacteriaceae (Cheesbrough, 2009).

6- Deoxyribonuclease agar (DNase)

This is used to help in identification of *S. aureus* which produces Deoxyribonuclease (Cheesbrough, 2009).

7- Mannitol salt agar

It is useful differential and selective medium for differentiation *Staphylococcus aureus* (Cheesbrough, 2009).

8- Muller Hinton agar

Use for sensitivity test with pH 7.2-7.4 (Cheesbrough, 2009).

2.4.3 Reagents and stains

Distilled water

Normal saline

Alcohol 70%

Hydrochloric acid

Kovac´s reagent

Hydrogen peroxide 3%

Oxidase reagent

Crystal violet

Lugol´s iodine

Safranine

2.4.4 Other materials

Sterile containers

Slides

Petri dishes

Benzene burner

Loops (straight + calibrated wire loop)

Racks

Wooden stick

Forceps

Pasteur pipette

Oil immersion

Test tubes

Glass bottles

Antibiotics disks

2.5. Methods

2.5.1. Collection of specimens

Fifty mid stream urine (MSU) specimens were collected from patients whom under go with chemotherapy treatment, in sterile, dry, wide mouth, leak proof containers. These specimens were collected from Radiation and Isotope Center of Khartoum (RICK).

The specimens were immediately inoculated on Cystine Lactose Electrolyte Deficient (CLED).

2.5.2. Culturing of specimens

The specimens were inoculated on plates of Cystine Lactose Electrolyte Deficient media (CLED), by method of streaking. Cultures were incubated at 35-37°C aerobically for overnight.

2.5.3. Identification

2.5.3.1. Colonial morphology

The inoculated media were morphologically examined for size, color, and fermentation of lactose. (CLED) medium contain bromothymol blue though the colonies appeared yellow in acid pH, and green in alkaline pH.

2.5.3.2. Gram stain

A drop of normal saline was placed on slide. The suspected colonies were emulsified and smeared. The smears should be fixed by dry heat and then cover with crystal violet stain for 30-60 seconds. The stain rapidly washed by tap water and tipped the side. Stained smear then cover with lugośs iodine for 30-60 seconds. Iodine immediately washed off and the smear was decolorized with ethanol for few seconds. Suffranin was added to the smear for 2 minutes. The red stain then washed off with tap water and smear preparation subsequently air dried and microscopically examined using high resolution objective power (Cheesbrough, 2009).

2.6.1. Identification of Gram positive cocci

2.6.1.1. Catalase test

The differentiation between staphylococci (which produce catalase) from streptococci (non catalase production) was made by catalase test. Catalase acts as catalyst in the breakdown of hydrogen peroxide to oxygen and water. Using sterile wooden stick, suspected colonies were immersed in tube containing 2ml of 3% hydrogen peroxide (Cheesbrough, 2009; Collee *et al*, 1996)

A Positive result was indicated by production of air pubbling.

A negative result indicated by no change in tube.

2.6.1.2. DNase test

Using sterile loop to inoculate the suspected colonies under a septic condition into DNA media, after overnight, aerobic incubation at 37◦c

hydrochloric acid (1% HCL) was to the spots of an organism. Clear zone around the colonies mean positive result (Cheesbrough, 2009).

2.6.1.3. Mannitol salt agar (MSA)

It is a useful media for identifying staphylococci species, which are able to grow on agar containing 70-100 g/l sodium chloride. Some species of staphylococci are able to ferment mannitol and other cannot ferment mannitol.

The test done by inoculating the organism under test in MSA media which contain phenol red indicator, and then incubated the plate at 37c for 24 hours, and then change in color is observed (Cheesbrough, 2009).

2.6.2. Identification of gram negative rods

2.6.2.1. Indole test

In this test the tested organism produce tryptophanase enzyme which breakdown tryptophan and produce indole, which react with kovac´s reagent and give pink ring. The tested organism was inoculated into peptone water and incubated at 37◦c for overnight, the kovaćs reagent was added. If there is pink ring the result was indicated as positive. If there is no pink ring in the surface the result was indicated as negative (Cheesbrough, 2009; Collee *et al,* 1996).

2.6.2.2. Citrate utilization test

In this test organism has ability to use citrate as only source of carbon. By straight loop apart of tested colonies was emulsified in kosser′s citrate media and incubated at 37◦c for 24 hours.

A blue color with growth indicated as positive, no change in color indicated the negative result (Cheesbrough, 2009).

2.6.2.3. Urease test

In this test organism produce urease enzyme which breakdown urea and produce ammonia, which make the pH of media alkaline, in the presence

of phenol red indicator, the tested organism inoculated in Christensen's urea agar.

Positive: pink color.

Negative: no change in color (Cheesbrough, 2009; Collee *et al*, 1996).

2.6.2.4. Kligler iron agar (KIA)

A tested organism inculcated by sterile straight loop by stepping on the butt then blocked the pore and streaked the slop of the media and incubated at 37◦c for 24 hour. Glucose fermentation indicated by yellow butt, yellow slop indicated the lactose fermentation, gas produce in the end of the tube and H2S produce blacking in the media (Cheesbrough, 2009 and Collee *et al*, 1996).

2.7. Sensitivity testing:

2.7. 1. On Muller Hinton

Use sterile wire loop, touch 3-5 colonies of overnight isolated organism and emulsify in 3ml of normal saline to prepare the suspension, then compared the turbidity of the suspension with the standard. Use sterile swab and socked with the bacterial suspension, excess fluid was removed by pressed the swab against the side of the tube and streaked over Muller-Hinton agar (M.H) on the three directions rotating the plates approximately 60 degree to ensure even distribution. Then allow for 3-5 minute, using a sterile forceps the appropriate antimicrobial discs was placed; the disc should be 15mm from the edge and 25 mm from the next disc (Cheesbrough, 2009; collee *et al*, 1996).

plate was incubated aerobically at 35˚c - 37˚c for 16-18hr, after incubation period the zone of inhibition is measured by using ruler, then using interpretative chart the zone of each disc was measured and reported as sensitive or resistant or intermediate (Cheesbrough, 2009; collee *et al*, 1996)

2.7.2.Antimicrobial drugs

Antimicrobial agents	Symbol	Disc potency	Diameter of zone of inhibition (mm)		
			Susceptible	Intermediate	Resistant
Ampicillin	AMP	10 mcg	≥17	_	≤16
Chloramphenicol	C	30 mcg	≥18	13-17	≤12
Ciprofloxacin	CIP	5 mcg	≥21	16-20	≤15
Erythromycin	E	15 mcg	≥23	14-22	≤13
Gentamicin	GEN	10 mcg	≥15	13-14	≤12
Methicillin	MET	5mcg	≥14	10-13	≤9
Nitrofurantoin	NIT	300mcg	≥17	15-16	≤14
Novobiocin	NV	30mcg	≥17	18-21	≤22
Oxacillin	OX	1 mcg	≥20	11-12	≤10
Penicillin	P	10 units	≥29	-	≤28
Tetracycline	TE	30 mcg	≥15	12-14	≤11

CHAPTER THREE

3. RESULTS

During the period between February and March 2014, total of 50 samples were collected from cancer patients whom receive chemotherapy treatment (29) were female and (21) were male, (78%) of female were infected and (22%) of male were infected, (Table 3.4).

The incidence of infection among the different age was (26%) in children and (74%) in adult, (Table3.5).

From the 50 urine specimens investigated, 23 different isolates were recovered giving a percentage of (46%), and 27 urine specimens (54%) were clear, (figure 3.1). The predominant isolate were *S. aureus* (26.1%) followed by *E.coli* (21.7%), *S. epidermidis* (17.4%), *K. pneumoniae* (13%), *p. mirabilis* (13%) and *Ps. aeruginosa* (8.7%), (Figure 3.2).

In-vitro sensitivity test for four antimicrobial agents by Kirby-Bauer method were carried out on (10) Gram positive organisms and (13) of gram negative organisms.

S. aureus susceptibility, using four antibiotics, showed that Chloramphenicol highly active (100%), followed by fusidic acid (83%), penicillin (67%), and methicillin (50%), (Figure 3.3).

S. epidermidis susceptibility, using four antibiotics, showed that gentamicin highly active (100%), followed by Chloramphenicol (75%), penicillin (25%) and then methicillin (0%), (Figure 3.4).

E.coli susceptibility, using four antibiotics, showed that Chloramphenicol is highly active (80%), followed by ampicillin and nitrofurantoin (60%), and ciprofloxacin (40%), (Figure 3.5).

Proteus mirabilis susceptibility, using four antibiotics, showed that Chloramphenicol, ciprofloxacin and tetracycline were highly active (100%), followed by ampicillin (67%), (Figure 3.6).

Klebsiella pneumoniae susceptibility, using four antibiotics, showed that ciprofloxacin were highly active (100%) followed by Chloramphenicol, and tetracycline (67%), and nitrofurantoin (0%), (Figure 3.7).

Pseudomonas aeruginosa susceptibility, using four antibiotics, showed that Chloramphenicol and ciprofloxacin were highly active (100%), followed by ampicillin (50%), nitrofurantoin (0%), (Figure 3.8).

Table 3.1: Morphological characteristic on CLED agar and gram reaction

Organisms	CLED	Gram stain
E. coli	Moderate, moist, yellow L.F colonies	Gram- negative bacilli
S. aureus	Small, moist, yellow L.F colonies	Gram- positive cocci
Ps. aeruginosa	Moderate, moist, blue-green NLF colonies	Gram- negative bacilli
K. pneumonia	Large, mucoid, yellow L.F colonies	Gram- negative bacilli
S. epidermidis	Small, moist, yellow LF colonies	Gram- positive cocci
P. mirabilis	Moderate, moist, blue-green NLF colonies	Gram- negative bacilli

Table 3.2: Biochemical tests of isolated Gram- negative bacteria

species	Biochemical tests						
	Indole test	Urease test	Citrate test	KIA			
				slope	butt	Gas	H2S
E. coli	positive	Negative	Negative	Y	Y	+	–
K. pneumoniae	Negative	Positive	Positive	Y	Y	+	–
Ps. aeruginosa	Negative	Negative	Positive	R	R	–	–
P. mirabilis	Negative	positive	Positive	R	Y	+	+

Table 3.3: Biochemical test of isolated Gram- positive bacteria

Species	Biochemical tests		
	Mannitol fermentation	Catalase test	DNase test
S. aureus	positive	Positive	positive
S. epidermidis	negative	positive	negative

Table 3.4: Incidence of urinary tract infection among both male and female whom receive chemotherapeutic drug:

Gender	Urinary tract infection	
	frequency	percentage
Female	18/23	78%
Male	5/23	22%

Table 3.5: Incidence of urinary tract infection among adult and children whom receive chemotherapeutic drug:

Age	Urinary tract infection	
	frequency	percentage
adult	17/23	74%
children	6/23	26%

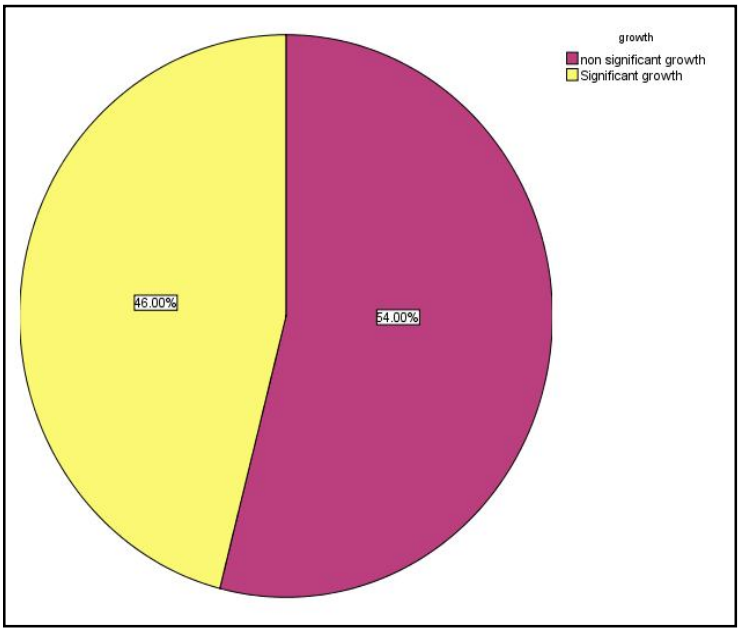

Figure 3.1: Bacterial growth on CLED media for primary isolation of causative agents.

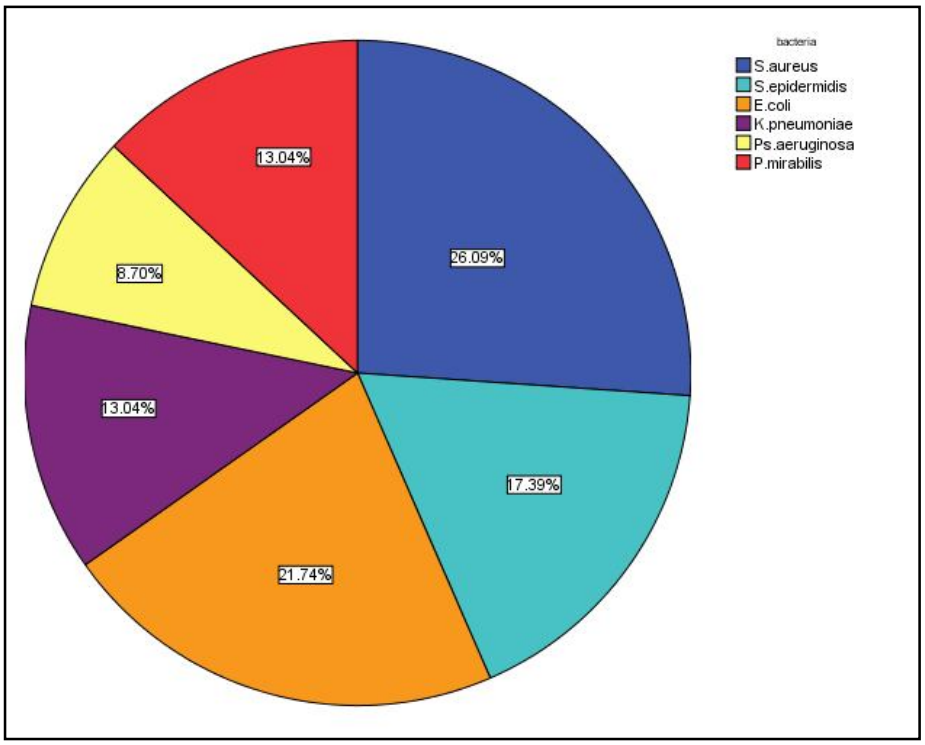

Figure 3:2: Percentage of the isolated bacteria that cause urinary tract infection.

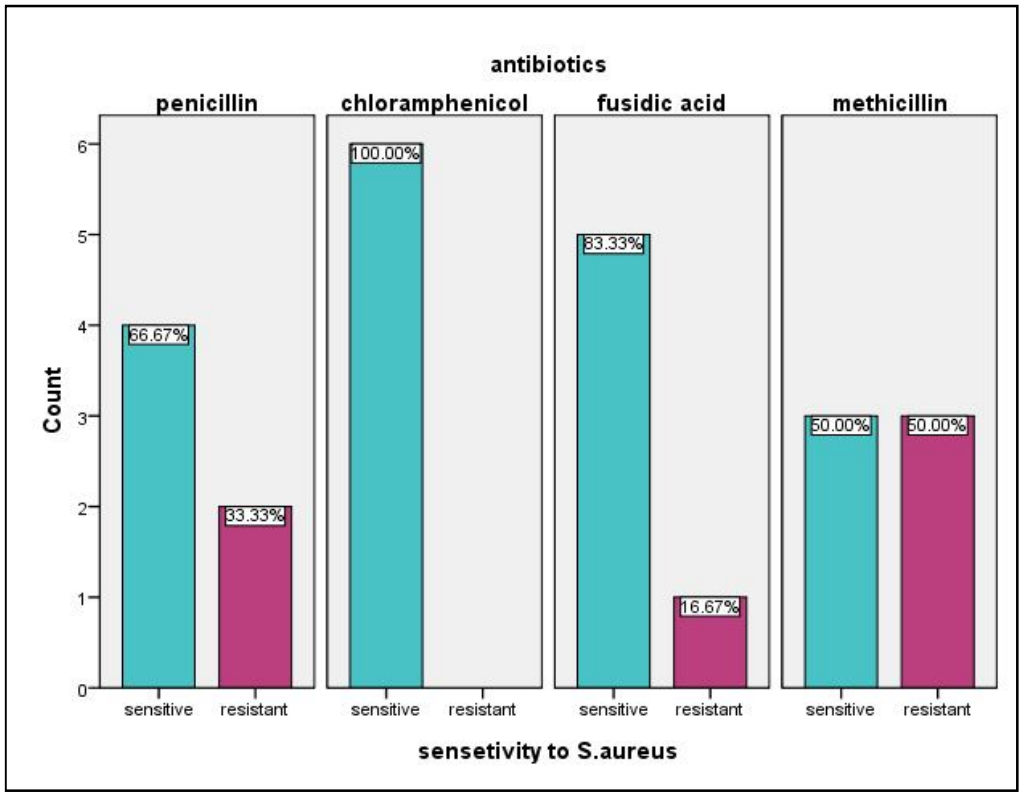

Figure 3.3: Antimicrobial susceptibility test for *S. aureus*

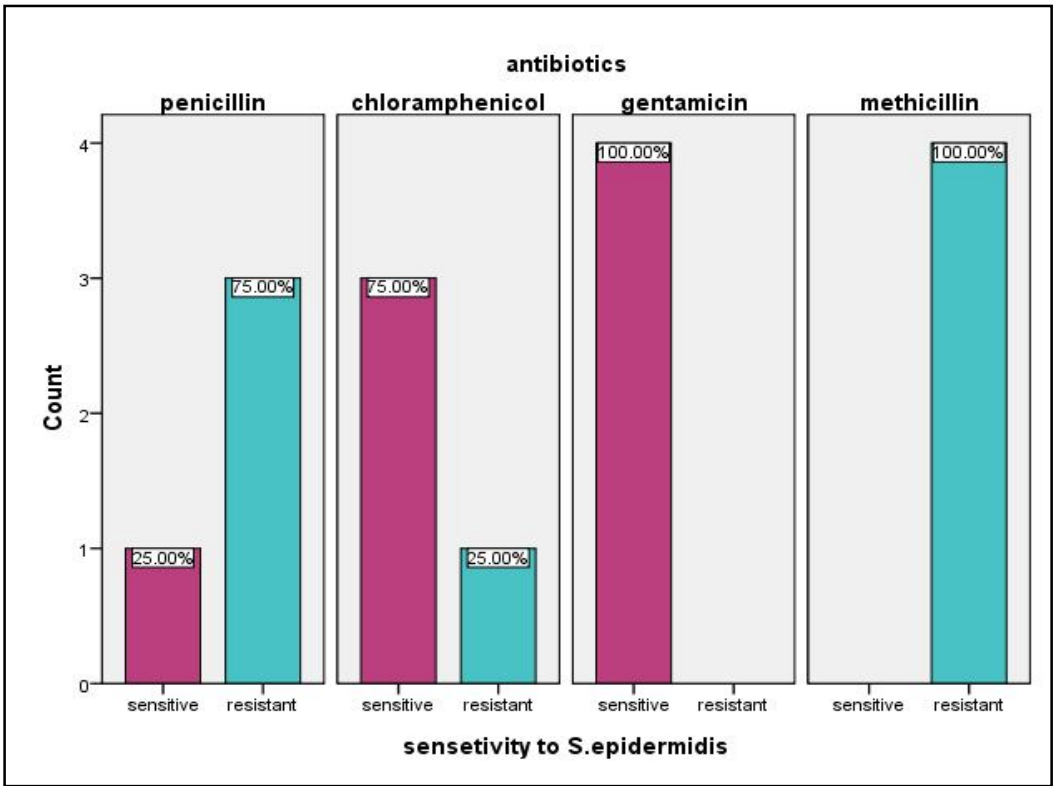

Figure 3.4: Antimicrobial susceptibility test for *S. epidermidis*

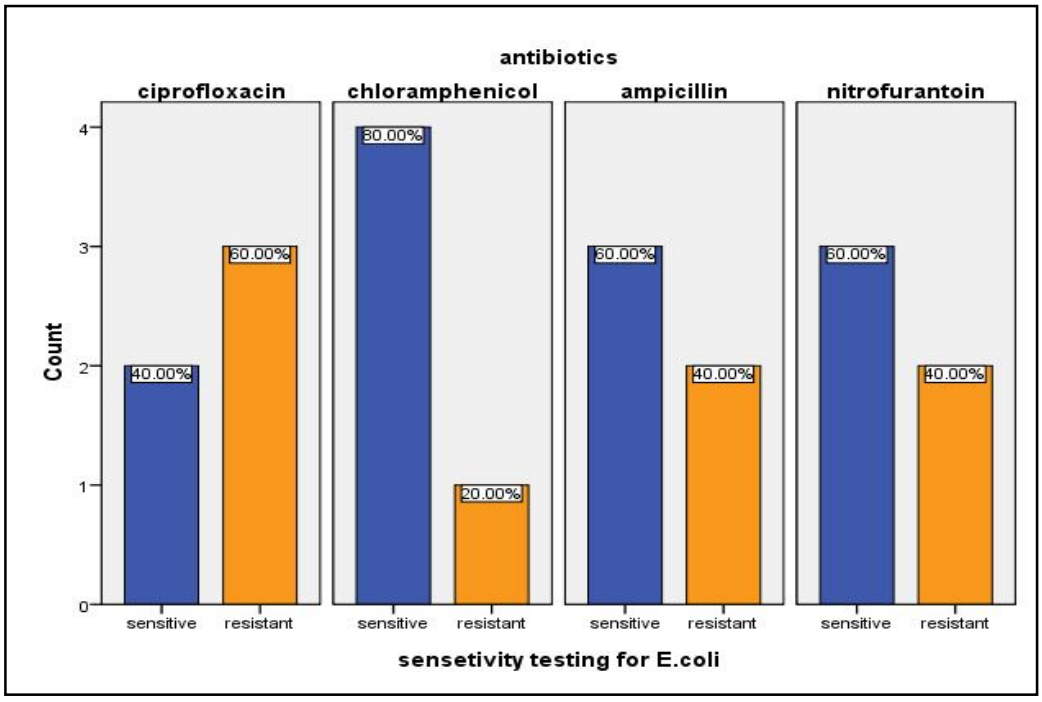

Figure 3.5: Antimicrobial susceptibility test for *E.coli*

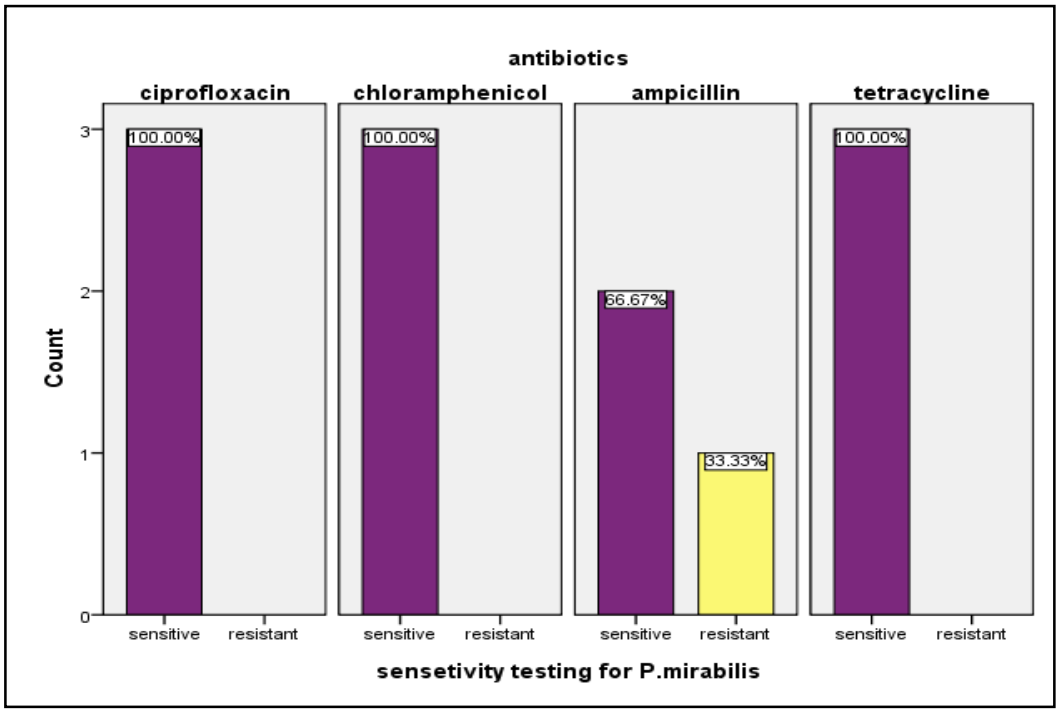

Figure3.6: Antimicrobial susceptibility test for *P. mirabilis*

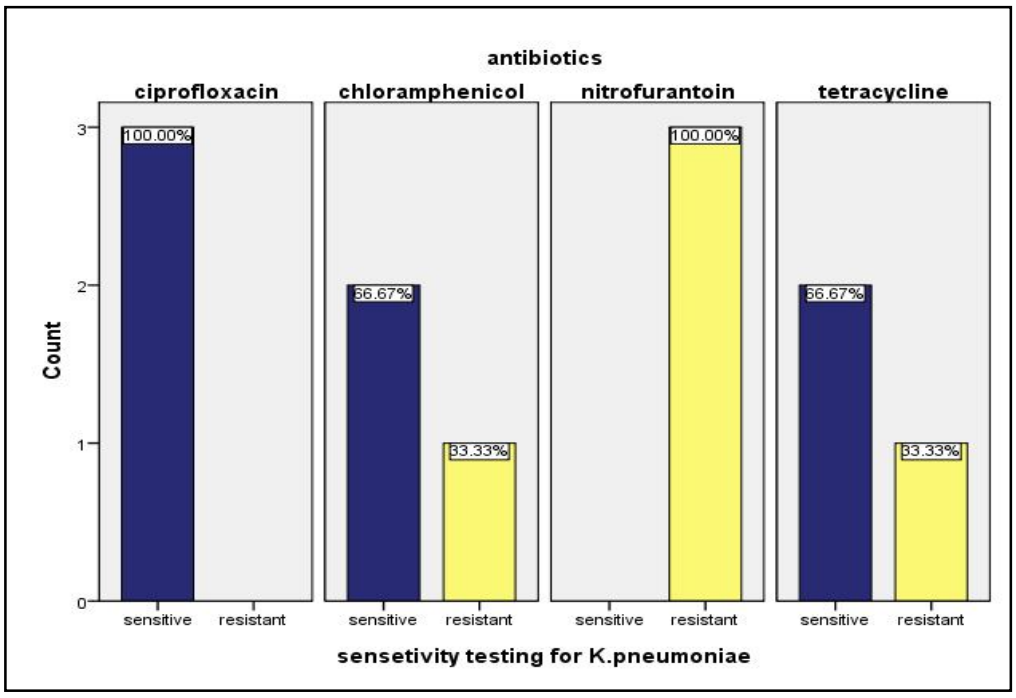

Figure 3.7: Antimicrobial susceptibility test for *K. pneumoniae*

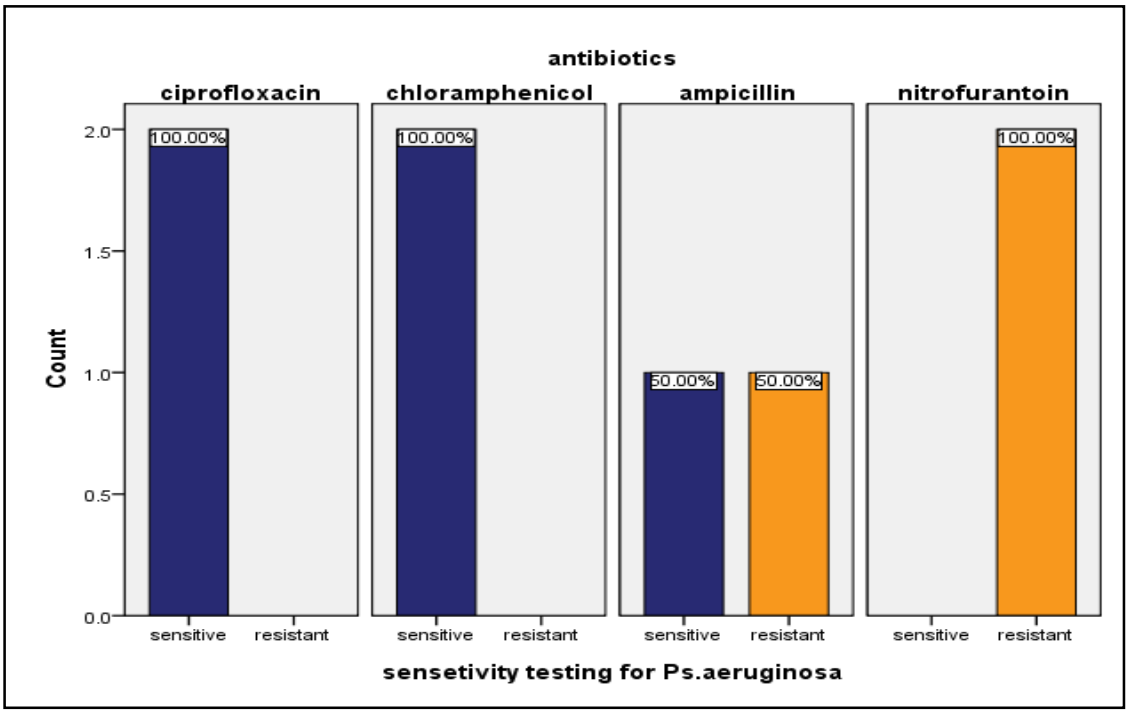

Figure 3.8: Antimicrobial susceptibility test for *Ps. aeruginosa*

Chapter four

Discussion

The main objective of the present study was to isolate the main common pathogens that cause urinary tract infections (UTI) among patients receiving a chemotherapeutic drug.

Fifty specimens were collected for patients, prepared and adopted different standardized tools and methods for the realization of the problem through isolation and identification of bacterial strains which cause UTI.

The study relived that gram positive pathogens are commonly isolated from the patients and *Staphylococcus aureus* was predominant microorganism recovered (26.6%) disagree with (Purewal *et al*, 2011).

Followed by *E.coli (21.7%)* agree with (Purewal *et al*, 2011) but disagree with (Tancheva1 *et al*, 2009) whom found that *E.coli* is the common pathogen isolated, then *S.epidermis* (17.4%), *K.pneumonia* (13%), *pr.mirabilis* (13%) and *Ps.aeruginosa* (8.7%) in agree with (Tancheva1 *et al*, 2009) and disagree with (Purewal *et al*, 2011) .

The precentage of urinary tract infections among patients whom receive chemotherapeutic drug is (46%) this result agree with (Kenneth, 2009) whom found that bout (45%) where positive and in disagree with (Tancheval *et al*, 2009) their result show (68%) where positive, also we found that the distribution of disease among female (62%) higher than male (28%) disagree with (Hayami *et al*, 2013) where their study show (88%) of infected where female.

Chloramphenicol was highly active against gram negative bacteria (84.6%), followed by ciprofloxacin (77%) agree with (Theodore, 2007).

Chloramphenicol (100%) were highly active against gram positive agrees with (Theodore, 2007).

Susceptibility test for *E. coli* show that Chloramphenicol is active (80%) disagree with (Boris *etal*, 2010) reported that activity of Chloramphenicol (54%) followed by ampicillin and nitrofurantoin (60%) agree with

(Hayami *et al*, 2013) report the activity (65%), then ciprofloxacin (40%) disagee with (Hayami *et al*, 2013) whom report activity (85%).

Susceptibility test for *Proteus mirabilis* show that Chloramphenicol, tetracycline and ciprofloxacin are highly active (100%) disagree with (Iwon and Stefania, 2007), followed by ampicillin (67%) agree with (Iwon and Stefania, 2007) that report ampicillin activity (61%).

Susceptibility test for *klebsiella pneumoniae* show that ciprofloxacin is highly active (100%) agree with (Archana and Harsh, 2011) that report activity of ciprofloxacin (90%) followed with Chloramphenicol and tetracycline (67%) disagree with (Archana and Harsh, 2011) that report activity (40%).

Susceptibility test for *staphylococcus aureus* show that Chloramphenicol is highly active (100%) disagree with (Adebola and Josiah, 2008) followed with fusidic acid and penicillin and then methicillin agree with (Adebola and Josiah, 2008).

Susceptibility test for *Pseudomonas aeruginosa* show that Chloramphenicol and ciprofloxacin were highly active (100%) agree with (Jonathan and Jiyoung, 2011), and show (50%) resistance to ampicillin disagree with (Jonathan and Jiyoung, 2011) and (100%) resistance to nitrofurantoin agree with (Jonathan and Jiyoung, 2011) that reported (96%) resistance.

Susceptibility test for *S. epidermidis* susceptibility show that gentamicin is highly active (100%) disagree with (Hellmark *et al*, 2009) that report activity of gentamicin is (21%), followed by Chloramphenicol, penicillin, and (100%) resistance to methicillin agree with (Hellmark *et al*, 2009).

Conclusion

- Of the 23 positive samples *S. aureus* and *E.coli* are the most frequent.
- Females are more infected than males.
- Adults are more infected than children.
- Chloramphenicol, gentamicin and ciprofloxacin have good effect against causative agents and recommended as the first line of treatment.

Recommendations

- For more accurate description of the etiology of urinary tract infection on patients whom receive chemotherapeutic drug further well designated studies are needed with increased number of samples.
- Patients should also be followed for infection of urinary tract at regular time.
- To control drug resistance, the use of antibiotics should be restricted and be given only after doing culture and sensitivity test.
- Further scanning and sensitivity testing is recommended.
- Radiation and Isotope Center of Khartoum, was about to establish microbiology laboratory but they should speed to establish of appropriate microbiological laboratory to allow the fast and early and good diagnose of such infection.

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APPENDIXES

Appendix 1

Cystine lactose Electrolyte deficient (CLED) agar

Ingredients

Peptic digest of animal tissue	4g/l
Casein enzyme hydrosylate	4g/l
Beef extract	3g/l
L-cystine	0.128g/l
Bromothymol blue	0.02g/l
Agar	15g/l

Preparation

Suspend 36.15 grams in 1000 ml D.W.

Heat the media until boiling to dissolve it completely. Sterilize by autoclaving at 15 Ibs pressure at 121c for 15 minutes.

Appendix 2

Pepton water

Ingredient

Peptic digest of animal tissue	10g
Sodium chloride	5g

Preparation

15g of powder dissolve in 1 liter of D.W then sterilize by autoclaving at 15 Ibs pressure at 121◦c for 15 minute

Appendix 3

Urea agar base (Christensen)

Ingredient

Peptides digest of animal tissue	1g
Dextrose	1g

Sodium chloride	5g
Mono-potassium phosphate	0.80g
Phenol red	0.012g
Agar	15g

Preparation

24g of powder dissolve in 1L of D.W then sterilize by autoclaving at 15 Ibs pressure at 121◦c for 15 minutes then cool and add aseptically 50 ml of 40% urea, mix and pour in tube in vertical position.

Appendix 4

Simmons citrate agar

Ingredient

Magnesium sulphate	0.2 g
Ammonium dihydrogen phosphate	0.2 g
Sodium ammonium phosphate	0.8 g
Sodium citrate tribasic	2 g
Sodium chloride	5 g
Bromothymol blue	0.08 g

Preparation

Suspend 23 gram in 1 litre of D.W. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121ºC for 15 minutes.

Appendix 5

Kligler iron agar (KIA)

Ingredient

Peptic digest of animal tissue	15g
Beef extract	3g
Yeast extracts	3g
Protease peptone	10g
Lactose	10g

Dextrose	1g
Ferrous sulfate	.20g
Sodium chloride	5g
Sodium thiosulfate	.3g
Phenol red	.024g
Agar	15g

Preparation

57.5 of powder dissolve in 1L of D.W and sterilize by autoclave at 15 Ibs pressure at 121◦c for 15 minutes then cool and pour in tube in slop slant position

Appendix 6

Mannitol salt agar

Ingredient

Meats extract	1g
Casein peptone	5g
Sodium chloride	75g
D.mannitol	10g
Phenol red	.025g
Agar	15g

Preparation

111g of powder dissolve in 1L of D.W and sterilize by autoclave at 15 Ibs pressure at 121◦c for 15 minutes then cool and pour in petridishes.

Appendix 7

DNase agar

Ingredient

Casein enzyme hydrolysate	15g
Papic digest of soya bean meal	5g
Deoxyribonucleic acid	2g

Sodium chloride	5g
Agar	15g

Preparation

24g powder dissolve in 1L of D.W and sterilize by autoclave at 15 Ibs pressure at 121◦c for 15 minutes then cool and pour in petridishes.

Appendix 8

Mueller Hinton agar

Ingredient

Beef infusion	300 g
Casein hydrolysate	17.5 g
Starch	1.5 g
Agar	10 g
pH approximately	7.4

Preparation

Suspend 35 grams in I litre of distilled water. Bring to the boil to dissolve the medium completely. Sterilize by autoclaving at 121º C for 15 minutes.

Appendix 9

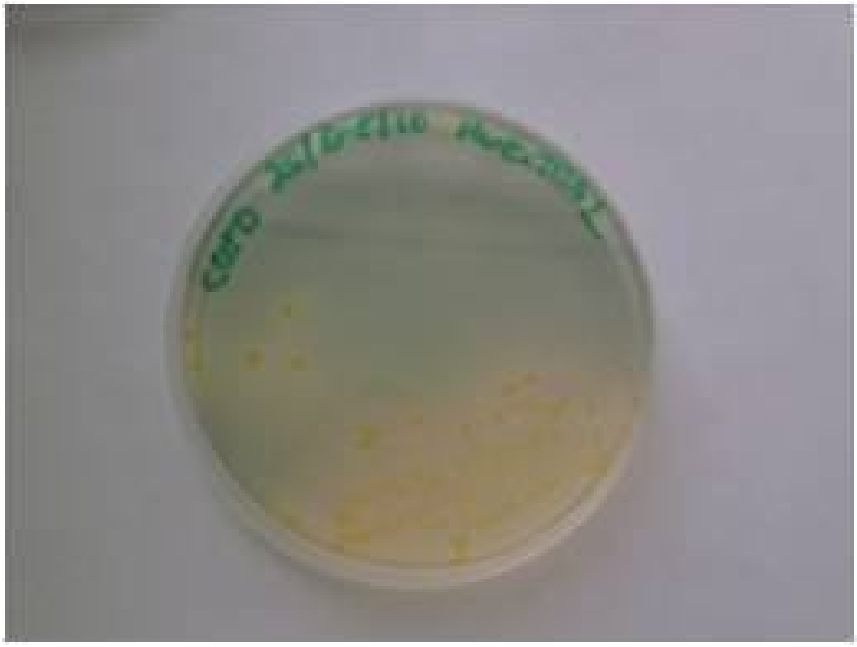
McFarland turbidity standard

Prepare 1% v/v solution of sulphuric acid by adding one ml of concentrated sulphuric acid to 99 ml of D.W. and mix well.

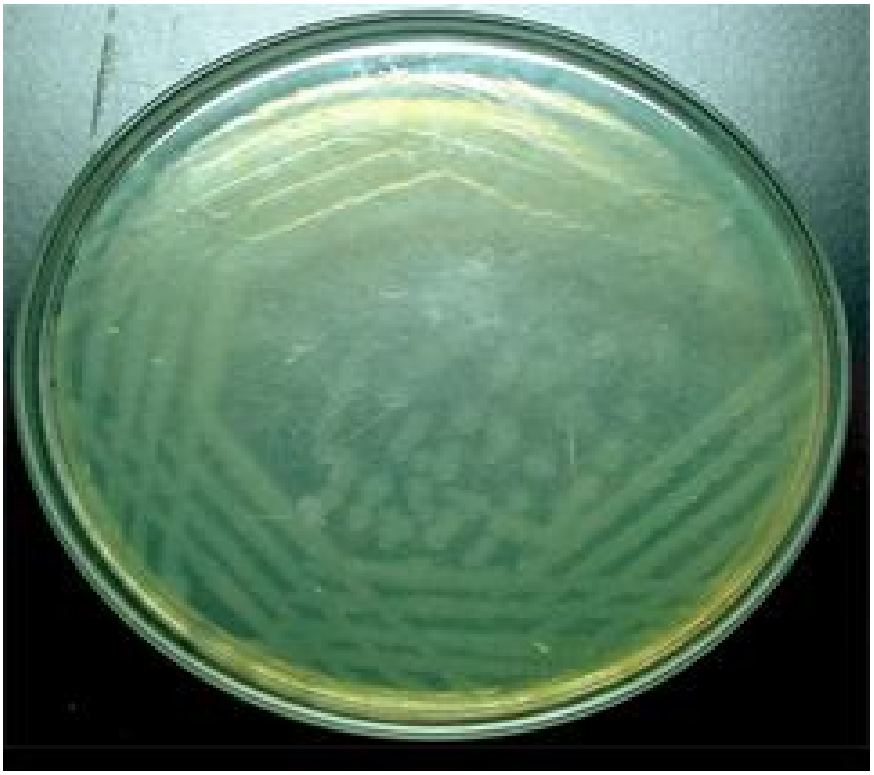
Prepare 1% w/v solution of barium chloride by dissolving 0.5 g of dehydrate barium chloride (BaCl2. H2O) in 50 ml of D.W.

Add 0.6 ml of barium chloride solution to 99.4 ml of the sulphuric acid solution, and mix.

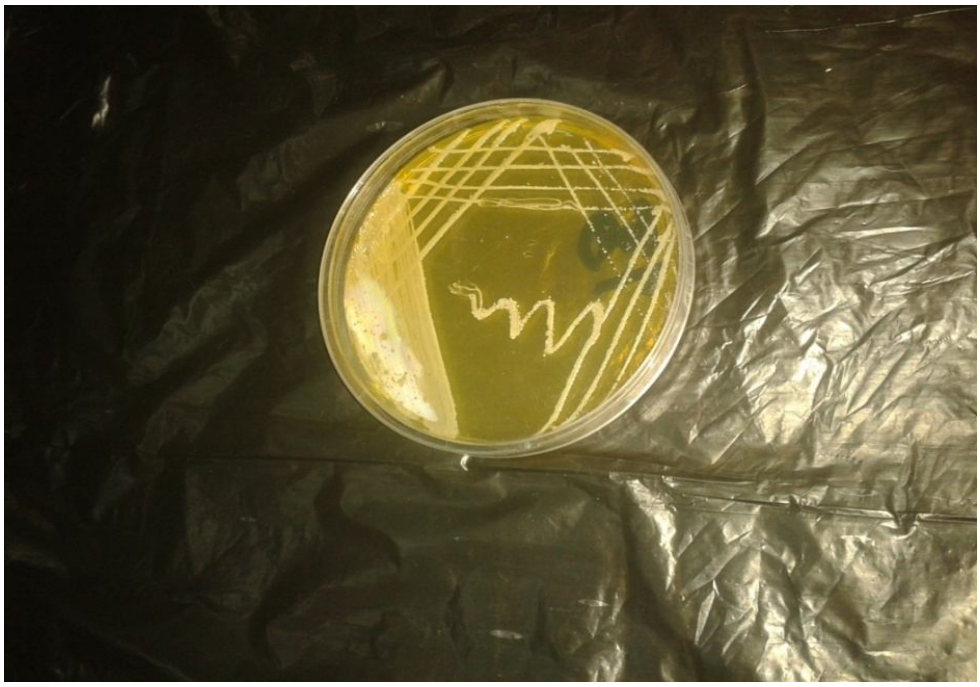
Transfere asmall volume of the turbid solution to a screw-cap bottle of the same type as used for preparing the test and control inocula.



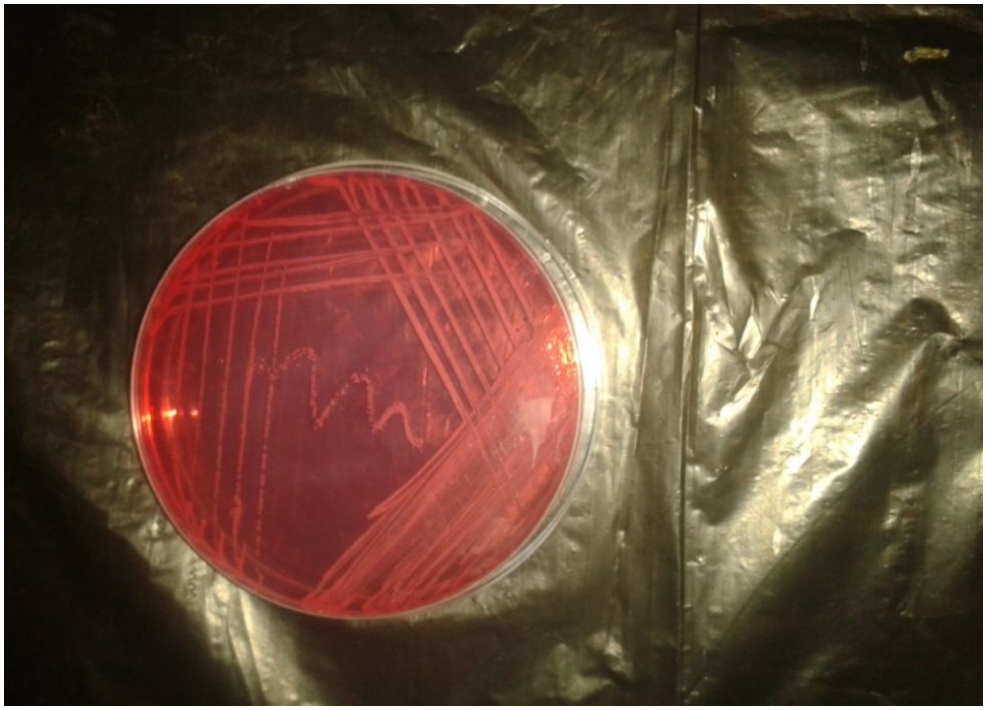
Yellow, lactose fermenting colonies of *S. aureus*



Blue-green, non-lactose fermenting colonies



Yellow, mannitol fermenting colonies of *S. aureus* on mannitol salt agar (MSA)



Red, non-mannitol fermenting colonies of s. epidermidis on mannitol salt agar (MSA)

Characteristic reaction of *E. coli* on the Biochemical tests

Characteristic reaction of *K. pneumoniae* on the Biochemical tests

Characteristic reaction of *Ps. aeruginosa*
On the Biochemical test

Characteristic reaction of *P. mirabilis*
On the Biochemical tests

Disc diffusion method on Muller Hinton agar and the zone of inhibition was measured by millimeter ruler