NAPATA COLLEGE

Medical Laboratory Science

Department of Microbiology

Antimicrobial susceptibility patterns of bacterial isolated from urinary tract infection among patients under chemotherapy and non-chemotherapy in Khartoum state-Sudan "2021"

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BSc Microbiology

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قال تعالي

الَّذِي خَلَقَنِي فَهُوَ يَهْدِينِ (78) وَالَّذِي هُوَ يُطْعِمُنِي وَيَسْقِينِ (79) وَإِذَا مَرِضْتُ فَهُوَ يَشْفِينِ (80)

صدق الله العظيم

سورة الشعراء

DEDICATION

TO THE REAL LOVE THAT PUTS MEANING INTO OUR LIVES

OUR PARENTS

TO WHOM HAVE BEEN ROLEMODELES TO LOOK FORWARD

OUR SIBLINGS

TO THE SMILES DESPITE THE SADNESS AND TO THE STARS IN THE DARKNESS

OUR FREINDS

TO THE GLORIOUS DECEMBER REVOLUTION OF SUDAN

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Firstly, we would like to express our sincere gratitude to our supervisor

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ABSTRACT

Background

Urinary tract infections are the common types of infections in the community and health care settings. Despite the widespread availability of antibiotics, urinary tract infection remains a worldwide therapeutic problem. It is a continuous and significant problem in cancer patients.

Methods

A hospital-based comparative cross-sectional study was conducted on 100 study participants from August to October 2021. Sociodemographic data were collected by a predesigned questionnaire and midstream urine samples collected using simple random sampling technique by using clean, sterile plastic cups and then inoculated onto CLED agar plates and incubated at 37°C for 24 hours. Urine culture was considered significant bacteriuria when colony forming units $\geq 10^5$/mL of voided urine. Identification was done by using standard microbiological methods. Modified Kirby–Bauer disk diffusion technique was applied for antimicrobial susceptibility testing in accordance with CLSI 2018 criteria. Data were entered, cleared, checked and exported to SPSS version 20 for analysis. The results were displayed using tables and figures. Value <0.05 at 95% CI was considered as statistically significant.

Results

The overall prevalence of asymptomatic bacteriuria in cancer patients was 56% and 44% of samples did not grow in media. *Staphylococcus aureus (20%)* was the commonest isolated uropathogenic bacteria followed by *E. coli (18%), Candida albicans (10%), Pseudomonas argionosa (4%), Enterococcus fecalis (2%)* and *Klebsiella species (2%),* in cancer patients.

Conclusions

This study showed a high prevalence of asymptomatic bacteriuria among cancer patients (56%) compared to apparently healthy donors (44%). *E. coli* was isolated predominately.

المستخلص

الخلفية

تعد عدوى المسالك البولية هي الأنواع الشائعة من العدوى في المجتمع المحلي وبيئات الرعاية الصحية. على الرغم من توافر المضادات الحيوية على نطاق واسع، لا تزال عدوى المسالك البولية مشكلة علاجية في جميع أنحاء العالم. وهي مشكلة مستمرة وكبيرة في مرضى السرطان.

الطرق المستخدمة

أجريت دراسة مقارنة مقطعية في المستشفى على 100 مشاركا في الدراسة في الفترة من أغسطس إلى اكتوبر 2021. تم جمع البيانات الاجتماعية الديموغرافية من خلال استبيان مصمم مسبقا وعينات بول من وسط التبول تم جمعها باستخدام تقنية أخذ عينات عشوائية بسيطة باستخدام أكواب بلاستيكية نظيفة ومعقمة ثم زراعتها على أجار(الكليد) واحتضانها عند 37 درجة مئويه لمدة 24 ساعة . تصنف مزرعة البول هامة عندما يكون عدد وحدة تشكيل المستعمرة هو اكبر من او يساوي ≤5^10مل من بول المخرج. تم تحديد البكتيريا باستخدام الطرق الميكروبيولوجية القياسية. مثل تقنية نشر قرص كيربي باور المعدلة ، تم تطبيقها لاختبار قابلية مضادات الميكروبات وفقا لمعايير المختبرات السريرية غير المعززة لعام 2018.تم إدخال البيانات ومسحها وفحصها وتصديرها إلى برنامج الحزمة الإحصائية للنسخة 20 من العلوم الاجتماعية لتحليلها. وعرضت النتائج باستخدام جداول وأرقام. القيمة<0.05 في مستوى الثقة 95٪ اعتبرت ذات دلالة إحصائية.

النتائج

كان الانتشار العام للبكتيريا في البول عديمة الأعراض لدى مرضى السرطان هو 56٪ و 44٪ من العينات لم تنمو في الوسط الزراعي المغذي للبكتيريا. وكانت اكثر البكتيريا المعزولة شيوعا و مسببه لي امراض البول هي المكورات العنقودية الذهبية بنسبة (20)% تليها الاشريكية القولونية بنسبة (18)% و فطريات المبيضات البيض بنسبة (10)%, الزائفة الزنجارية بنسبة (4)%, المكورات المعوية البرازية بنسبة (2)% الكليبسيلا بنسبة (2)% في مرضى السرطان.

الاستنتاجات

أظهرت هذه الدراسة ارتفاع معدل انتشار البكتيريا عديمة الأعراض بين مرضى السرطان هي (56)% مقارنة مع المتبرعين غير مرضى السرطان كانت (44)%. و تمثل البكتيريا الاشريكية القولونية الأكثر شيوعا.

Abbreviation table

NO	Abbreviation	Meaning
1	UTI	Urinary tract infection
2	RICK	Radiation isotope center of Khartoum
3	TAXOTERE	Docetaxel drug
4	Zoladex	Goserelin drug
5	CYCLO	Cyclophosphamide drug
6	SXT	Trimethoprim / Sulfamethoxazole
7	Eloxatin	Oxaliplatin drug
8	CIP	Ciprofloxacin
9	AX-10	Amoxicillin
10	Casodex	Bicalutamide drug
12	CTX	Cefotaxime
13	CAZ	Ceftazidime
14	VA	Vancomycin
15	CN	Cefalexin
16	IPM	Imipenem
17	MEM	Meropenem
18	AK	Amikacin
19	Epirubicin	Anthracycline drug
20	CFM	Cefixime
21	CA PROSTATE	Prostate cancer
22	NOR	Norfloxacine
23	AMX	Amoxicillin
24	AMC	Amoxicillin + Clavulanic acid
25	XELODA	Capecitabine drug
26	MIC	Minimum inhibition concentration

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CHAPTER ONE

CHAPTER ONE

Introduction & Literature review

1. Introduction

1.1 Background

Cancer is a significant cause of death worldwide, and more than half of them occur in developing countries. The most common causes of cancer death are lung, liver, colorectal, stomach, breast cancer, cervical cancer, and leukemia [1].

One of the most common infections in cancer patients is urinary tract infections (UTIs)[2, 3].Urinary tract infections are common types of infections in the community and health care settings. Despite the widespread availability of antibiotics, urinary tract infection remains a worldwide therapeutic problem. It is a continuous and significant problem in cancer patients.[1]

Urinary infection is defined as bacteriuria, the multiplication of bacteria in urine within the renal tract, with a concentration greater than 10^5organism/ml is regarded as significant bacteriuria, the urinary tract infection remains a major clinical problem over 50 years. Infection of the urinary tract may involve anywhere along the urinary tract (bladder, kidney, pelvis, parenchyma, or urethra) [4].The infections may be symptomatic or asymptomatic. The infection can result in serious complications if left untreated, like secondary bacteremia and sepsis leading to a rise in the hospital costs and mortality. Although several different microorganisms can cause UTI including fungi and viruses, bacteria are the major causative organisms and are responsible of more than 95% of UTI cases [5] *Echerichia coli* are the most common prevalent organisms of UTI and are solely responsible for more than 80% of these infections [6]

A wide range of bacteria has been reported as a cause of urinary tract infections that among them, Enterobacteriaceae are the most prevalent [3]. The emergence of multiple-drug-resistant (MDR) strains of Gram-negative bacteria causing UTIs has become a serious concern, especially in cancer patients [7] The incidence of infections caused by multi-drug resistant (MDR) bacteria has been increasing throughout the world [9,10] The number of MDR spectrum of microbial infections might increase by the administration of new, stronger immunosuppressive regimens [10].

Infection is a continuous and significant problem in cancer patients due to many factors that increase the susceptibility of immunosuppressed cancer patients to in Cancer patients are at high risk of bacterial infections due to the chemotherapy for cancer patients leads to severe and prolonged immunosuppression [10]. The development of infections caused by multidrug-resistant bacteria has become a major health problem worldwide [12,13].

Adequate treatments of UTI depend on knowledge of the local pattern of causative pathogens, their antibiotic resistance and the associated underling risk factor. The changing pattern of antibiotic susceptibility of bacterial pathogens causing UTI is a growing problem. Sudan is a country in which drug prescription is not under strict control. Consequently many organisms including those causing UTI may ultimately develop high resistance to many antibiotics, that currently in use. Therefore, the knowledge of the local pattern of urinary pathogens and their susceptibility to various antibiotics are essential for selection of appropriate therapy for UTI [7].

1.1.1 Types of urinary tract infections

a. Upper urinary tract infection mainly refers to the kidneys and the tubes that lead from the kidney into the urinary bladder.
b. Lower urinary tract infection refers to the inflammation and infection of the bladder and urethra.

1.2 Literature review

Urinary tract infections (UTIs) are a severe public health problem and are caused by a range of pathogens, but most commonly by Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis and Staphylococcus saprophyticus.clinically, UTIs are categorized as uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities [10].

Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defense, including urinary obstruction, urinary retention caused by neurological disease, immunosuppression, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices[11].

Cancer epidemiology is well-investigated in developed countries; however the cancer burden in Africa is less well documented especially in Sudan because the attention of the health system mainly focuses on communicable diseases such as malaria, tuberculosis and human immunodeficiency virus (HIV)/AIDS [12].

A five years survey of caner prevalence in Sudan was conducted in 2009 to a total of 33,201 patients with cancer were included in a study. The prevalence rate per year was in the range of 5,000-7,000 cancer cases for adults and 300-400 for children. Among adults, slightly more females were diagnosed with cancer than males. By contrast, fewer girls than boys suffered from tumors. During the investigated time frame (2009-2013), the annual number of newly-diagnosed tumors increased among adults, but remained more or less stable in children [12].

1.2.1 Urinary tract infection (UTI)

Urinary infection is defined 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 the introduction of antimicrobial chemotherapy many consultations in general practice are because of urinary infection, Infection of the urinary tract may involve anywhere along urinary tract (bladder, kidney, pelvis, parenchyma, or urethra) [14].

1.2.1.1 Urinary tract system

1.2.1.2 Kidney

Two kidneys function to excrete most of the waste products of metabolism, which leave the kidneys as urine. They lie behind the peritoneum on the posterior abdominal wall on either side of the vertebral column, the right kidney lies slightly lower than the left one and the neuron is the functional unite [15]

1.2.1.3 Ureters

The two ureters are muscular tubes that extend from the kidney to the posterior surface of the urinary bladder, the urine is propelled along the ureter by peristaltic contractions of the muscle coat, each ureter measures about 10 inches in length, ureters have three constrictions along its course where the renal pelvis (funnel-shaped) joins the ureter, where it crosses the pelvic brim, and where it pierces the bladder [16]

1.2.1.4 Urinary bladder

The urinary bladder is immediately directly behind the pubic bone within the pelvis, it's a receptacle for the storage of the urine, in the adult maximum capacity of about 500ml, it's a strong muscle and its shape varies according to the amount of urine, the empty bladder is pyramidal. Having apex, base, neck, and two lateral surfaces [15]

1.2.1.5 Urethra

The urethra is the muscular tube for the passage of urine in both sex and semen in males. Extend from the bladder neck to the tip of the penis in the male, and the area Between the vagina and pubic bone in female [16].In female urethra is about 1.5 inch; in male is about 8 inch [15].

1.2.2 Type of infection

Infection is most often due to bacterial from the patient's bowel flora. Transfer to the urinary tract may be via the bloodstream, the lymphatics, or by direct extension, but most often via the ascending transurethral route [14].

1.2.2.1 Lower urinary tract infection

Involving of bladder and urethra, involving of the urethra is called urethritis the bladder is involved called cystitis [17].

The most typical symptoms are Frequency of micturition by day and night, Painful voiding (dysuria), Suprapubic tenderness and pain, Hematuria, Smelly urine [14].

Urethritis

Is the infection of the urethra, *Chlamydia trachomatis, Neisseria gonorrhea,* and *Trichomonas vaginalis* are common causes of urethritis and are considered being sexually transmitted [18].

Cystitis

It's the Infection of the bladder. Episodes of cystitis greatly outnumber those that involve the kidney [17].

Upper urinary tract infection

When the infection extends to the kidney and pelvis (known as 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 associate with atypical symptoms such as abdominal pain and the typical presentation of lower urinary tract infection [17].

1.2. 3.1 Pyelonephritis

Pyelonephritis refers to inflammation of the kidney parenchyma andpelvis (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 bacteremia [18]

1.2.3.2 Pathogenesis

Anything that disrupts normal urine flow or complete emptying of the bladder or facilitates access of organisms to the bladder will predisposing individual to infection. The shorter female urethra is a less effective deterrent to infection than the 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. [19].

Catheterization is a major predisposing factor for UTIs; during insertion of the catheter, bacteria may be carried directly into the bladder [19, 20].

1.2.3 Causal organisms

The Gram-negative rods *Escherichia coli* is the 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[4].

Staphylococcus saprophyticus is related to sexually active women. *Proteus- mirabilis* and *Klebsiella species* are often multiplied antibiotic-resistant. *Enterococcus faecalis, Pseudomonas aeruginosa,* and *staphylococcus aeruginosa* can cause infection especially after catheterization or instrumentation. Fastidious gram-positive bacteria (e.g. lactobacilli,streptococci, corynebacteria), which require incubation for 24–48 hours 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 [21].Obligate anaerobes are very rarely involved, other species may be found e.g.: *Salmonella typhi, Staphylococcus aureus,* and *Mycobacterium tuberculosis* [17].

Escherichia coli (E. coli)

E. coli belongs 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-spore forming and motile [9]*E. coli* is the cause of 60-90% of urinary tract infections. 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 be more invasive than others. Factor associated with virulence includes the possession of K (capsular) antigen, which inhibits phagocytosis and bactericidal effect of normal human serum, the ability to adhere to uroepithelium due to specializing fimbriae [16].

Klebsiella species

Gram-negative and non-motile usually capsulated rods cause UTIs in hospital patients. Antigenic analysis for capsular polysaccharides revealsthat more than 80 serotypes are recognized [4]. They grow well on ordinary media, with colonies that are often, but not always, large and mucoid [16].

Proteus species

Gram-negative pleomorphic motile rods, grow on selective enteric media [4].Proteus *mirabilis* is main Proteus species of medical importance. It causes urinary infection commonly in the elderly and young males often following catheterization or cystoscopy. It is often associated with urinary stones, probably because these organisms produce ammonia rendering the urine alkaline [17].

Pseudomonas aeruginosa

Gram-negative motile aerobic bacilli some strains are capsulated have very simple growth requirement and limited fermentation activity [17].

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 the following catheterization is associated with chronic urinary disease [4].

Serratiamarcescens:

It has been reported to cause UTIs, and it is gram-negative rods, facultative anaerobe and it is resistant to the cephalosporin [4].

Staphylococcus aureus

Gram-positive cocci are occurring in the group. Non-motile, non-capsulated and it is catalase, DNase, and coagulase-positive, and ferment mannitol, itis rarely causing UTI [4].

Staphylococcus saprophyticus

Gram-positive cocci of uniform size occurring in groups but also singly and in pairs. They are non-motile and non-capsulated. *S. saprophyticus* causes UTIs in sexually active women. It is coagulase and DNase negative and ferments 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 the urinary tract [22].

Enterococcus faecalis

It is gram-positive cocci, often found accompanying infection with coliforms [4].

Other bacteria

Bacteria species are not primarily in the urinary tract but may be found in urine e.g. salmonella species, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, *leptospirainnterrogans*, Chlamydia, and Mycoplasma species [4].

Parasites:

Very few parasites can cause UTIs e.g. *Trichomonas vaginalis* which cause urethritis in both male and females, but most often considered as the cause of vaginitis. *Onchocerca volvulus*, *Wuchereria bancrofti*, and *Schistosoma haematobium* were also uncommon UTI agents [4].

Fungi

Candida albicans in diabetic patients and those with immunosuppression, cause bladder infection, and the source of infection is usually endogenous; however, cross-infection may occur [4].

Viruses

Viral cause of UTIs appears to be rare although there is an association with hemorrhagic cystitis and renal syndromes [4].

1.3 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 [23].

1.3.1 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 a narrow therapeutic window between the effective treatment of cancer and normal tissue toxicity because the drugs are not cancer-specific [14].The dose and schedule of the chemotherapy are limited by the tolerance of the normal tissues, all tissues can be affected, however, depending upon the pharmacokinetics of the drug and affinity for particular tissues. The therapeutic effect on cancer is achieved by a variety of mechanisms that 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 [14].

1.3.2 Classification of cytotoxic drug

Either DNA damaging drugs, or Antimetabolites, or DNA repair inhibitors, or antitublin [14]

1.3.3 Side-effect of chemotherapy

Although chemotherapy kills cancer cells, it can damage normal cells and cause significant side effects, the side effect varies depending on the particular drug, dosage, route of administration and patient characteristics [23].Chemotherapy has many potentially serious side effects and should be used only by trained practitioners, the most common side-effects are:

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 the patient by using modern antiemetic ([14, 23]

Hair loss

Many but not all cytotoxic drugs are capable of causing it. Scalp cooling can sometimes use to reduce it [14].

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[14].Neutropenic patients are at high risk of bacterial and fungal infection often from enteric flora; this is managed by the immediate introduction ofbroad-spectrum antibiotics intravenously [18].

Another side-effect is related to certain drug

Cardiotoxicity which is related to anthracyclines, and nephrotoxicity is common with platinum, secondary malignancy (predominantly acute leukemia) with an alkylating agent [14].

1.3.4 The problem that related to receiving chemotherapy in cancer patient

The treatment of 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 kills often causes toxicity to normal tissues. Infection is the major cause of morbidity and mortality in patients undergoing anti neoplastic chemotherapy [24].

These include defects in humoral and cell-mediated immunity (functional asplenia, hypogammaglobinemia, 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 [24].

Several of the cancers chemotherapeutics drugs are used today as an 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 thus increase susceptibility to viral and bacterial infections [23,24].

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 patients to attenuate chemotherapy-associated nausea and vomiting. Glucocorticoids suppress the production of pro-inflammatory cytokines such as interferon(IFN), interleukin (IL).[25].

1.3.4.1 Incidence in cancer

Fever in a patient with cancer can be due to infection (80%) or cancer itself (20%). Most infections are due to bacteria, fatal infections are usually due to fungal infection [23]. 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/mm³, approximately 20% of febrile patients will have documented bacteremia [26].

1.4 Rationale

One of the most common infections in cancer patients is urinary tract infections (UTI), Clinical diagnosis of UTI in cancer patients under chemotherapy is usually challenging because the underlying pathophysiology is not well known and the presence of common atypical presentations. Due to severe immunosuppression which is an adverse consequence of treatment strategies used to treat cancer, there is a massive increase in the risk of opportunistic infections in cancer patients. Infections are one of the most serious complications and the leading cause of morbidity and mortality in patients with cancer [2]. In Sudan there is a high prevalence of asymptomatic bacteruria and antibiotic susceptibility pattern of bacterial isolates among cancer patients.

Our research aims to identify the most common bacteria that causes UTI among patient under chemotherapy and it would help formulate empirical antibiotic policy in this specific group of patients.

1.5 Objectives

1.5.1 General objective

To determine and update sensitivity profile for the bacterial isolates among chemotherapy patient with Urinary tract infections at the Radiation and Isotope Center of Khartoum (RICK).

1.5.2 Specific objectives of this study are to:

1. Identify bacterial pathogen responsible for urinary tract infections (UTI) on patients under chemotherapy compare to non-cancer
2. Determine the most common bacteria that cause UTI.
3. Evaluate the antibiotics profile between two groups.

CHAPTER TWO

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study Design:

Prospective cross sectional descriptive study conducted during the period from August to October 2021.

2.1.1. Study Area:

Specimens were collected from the Radiation and Isotope Center of Khartoum (RICK) and the Military Hospital Helipad in Omdurman.

RICK was established in 1967 becoming the first cancer institution in Sudan. It is specialized in treatment of cancer patients by using radiotherapy, chemotherapy and hormonal therapy. It also offers social and psychological services.

2.1.2 Study Population:

Inclusion criteria:

Sudanese patients with UTIs signs and Cancer patients under chemotherapy were included.

Exclusion criteria:

Non Sudanese Patients with other diseases.

Sampling Unit:

Cancer patients under chemotherapy in RICK from Augusts to October 2021.

2.1.3 .Sample Size:

The sample size was determined according to previous study and literature review 100 samples.

2.1.4 Ethical Consideration:

- Ethical approval from Napata College and RICK.
- Informed consent from patients after explaining the objectives of the study.
- All the information collected remain private and confidential and would only be used for the purpose of study.
- Participation is voluntary and any participant can withdraw at any time during the interview.
- Participants will not be subjected to harm in any way whatsoever.

2.2. Methods

2.2.1. Data collection

Structured questionnaire (Appendix 3) was used to obtain the socioeconomic data and the clinical features from each patient.

2.2.2. Collection of specimens

Precautions in collection of specimens were considered. Mid -stream urine was collected from each patient in sterile screw cap wide neck, leak proof disposable plastic container (10-20 ml).

2.2.3. Sterilization

a. Glassware such as Petri dishes, test tubes and flasks were sterilized in the hot air oven at 160̊ C for one hour.
b. Autoclaving: autoclave for 15 minutes at 121̊ C was used for sterilizations of media solutions. Screw capped bottles, rubber stopper flasks. Autoclaving at 110̊ C for 10 minutes was used for sugar media.
c. Direct flame: platinum loops were sterilized by direct flame.
d. Disinfecting: phenolic disinfectant was used for disinfecting floor, walls of laboratory and alcohol was used for disinfecting the safety cabinet and the benches.

2.2.4. Culture media

The following culture media were obtained from Hi media PVT. Ltd. India, and used throughout the study.

The following media is obtained and prepared in a microbiology laboratory according to manufactures.

Cysteine 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) [4].

Kligler iron agar (KIA)

Used for the differentiation of members of the Enterobacteriaceae based on their ability to ferment dextrose (glucose), lactose, and production of hydrogen sulfides [4].

Simmon's citrate media

Used in the identification of Enterobacteriaceae, based on the utilization of sodium citrate as a sole carbon source [4].

Christensen, urea agar media

Used for testing the urease enzyme activity in the bacterium to differentiate between Enterobacteriaceae members [4].

Deoxyribonuclease agar (DNase)

Used to help in identification of S. aureus which produces Deoxyribonuclease[4].

Mannitol-salt agar

It is a useful differential and selective medium for differentiation Staphylococcus aureus[4].

Muller Hinton agar

For the sensitivity testing, with pH 7.2-7.4 [17]. 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 [4].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 [4,14]

2.2.5 Inoculation of urine specimens

Hundred mid-stream urine (MSU) specimens were collected from patients who undergo chemotherapy treatment, in sterile, dry, wide mouth, leak-proof containers. These specimens were obtained from the Radiation and Isotope Center of Khartoum (RICK).

The specimens were immediately inoculated on Cystine Lactose Electrolyte Deficient agar (CLED) The inoculation was done by a sterile standard calibrated loop. The plates were incubated aerobically at 37̊ C, overnight.

2.2.6 Identification of the isolates

2.2.6.1Colonial morphology:

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

2.2.6.2Gram stain:

A drop of normal saline was placed on a slide. The suspected colonies then emulsified and smeared. The smears should be fixed by dry heat and then cover with crystal violet stain for 30-60 seconds. The stain was rapidly washed with tap water. Stained smear then covers with lugoss iodine for 30-60 seconds. Iodine is immediately washed off and thė smear decolorized with ethanol for few seconds. Suffranin was added to the smear for 2 minutes. The red stain was then washed off with tap water and smear preparation subsequently air-dried and microscopically examine using high-resolution objective power[9].

Catalase test

The differentiation between staphylococci (which produce catalase) from streptococci (non-catalase production) will be made by the catalase test. Catalase acts as a catalyst in the breakdown of hydrogen peroxide into oxygen and water. Using a sterile wooden stick, suspected colonies will be immersed in a tube containing 2ml of 3% hydrogen peroxide [9,14]

- A Positive result should be indicated by the production of air bubbles.
- A negative result will be indicated by no change in the tube.

Oxidase test

Cytochrome oxidase, enzyme in the oxidation reduction pathway, oxidizes the substrate tetramethyl-*p*-phenylene diamine di hydrochloride to indophenol, a purple colored end product. The tested organism was smeared on the filter paper which impregnated with oxidase reagent (1% w /v) (tetramethyl-para-phenylene diamine di hydrochloride). If the organism was oxidase producing the phenylenediamine in the reagent oxidized to a deep purple color within 10 second [9,14]

2.2.7 Antimicrobial susceptibility test

Modified Kirby-Bauer disc diffusion method was performed according to the instructions of NCCLS as follow: plates with Muller- Hinton agar were prepared. The sterilized molten medium was cooled to 45-50̊ C, and poured in sterile, dry Petri

plates on a level surface, to a depth of 4mm. Some representative plates after solidification were incubated at 35̊ C for 24-72 hours to check sterility. The pH of the Muller- Hinton agar was checked (7.4±2 at room temperature) and the presence of any excess surface moister on the medium was removed by keeping the plates inverted in an incubator (35-37̊ C).The inoculums were prepared by transferring 4-5 colonies from only pure cultures, confirmed by Gram's staining with wire loop to 4 ml of sterile normal saline, and the inoculums turbidity was compared with that of McFarland standard (prepared by mixing 0.6 ml of 1% w\v barium chloride and 99.4 ml of 1% v\v sulfuric acid). The sterile nontoxic cotton swab was dipped in to the standardized inoculums and the soaked swab was rotated firmly against the upper inside wall of the tube to get rid of excess fluid. The entire agar surface of the plate was streaked with the swab three times with turning the plate at 60 angles between each streaking; the inoculums were allowed to dry for 3-5 minutes with lid in place. Using sterile forceps the antimicrobial discs were placed and evenly distributed on the inoculated plate. The plate inverted and incubated aerobically at 37̊ C, for 18-24 hours. After overnight incubation the control and the test plates were examined to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate, the diameter of each zone of inhibition was measured in (mm). The end point of inhibition is where the growth starts.[14].

CHAPTER THREE

CHAPTER THREE

3.1Results

During the period from Augusts to October 2021, total of 100 urine specimens investigated of which 50 samples were collected from cancer patients whom received chemotherapy treatment (35) were females70% (15) were males30%. (Table 3.1)(figure3.1). And the other 50 samples were collected from non-cancer patients (33) females 56%and (17) were males 44% (Figure 3.2). The overall distribution of the subjects on our study according to gender showed on (Table 3.1).

From the 50 urine specimens of cancer patients whom received chemotherapy treatment a (28) different isolates were recovered giving 56% and (22) urine specimens were clear 44%. (Figure3.3).

The results of our study showed that the causative organisms of UTI in Gram positive cocci, Staph aureus is the predominant pathogen in patient under chemotherapy (20%) (Figure 3.4) and (Table 3.2) while E.coli is predominant in non-cancer patient (24%) (Figure 3.5) and (Table 3.3). Followed by:

Staph aureus is (20% in patient under chemotherapy and 10% in non-cancer patient). (Figure 3.4 &3.5) and (Table 3.4)

E coli is (18% in patient under chemotherapy and 26% in non-cancer patient). (Figure 3.4 &3.5) and (Table 3.2 & 3.2).

Candida albicans (10% in patient under chemotherapy and 0% in non-cancer patient) (Figure 3.4 &3.5) and (Table 3.2).

Pseudomonas is (4% in patient under chemotherapy and 4% in non-cancer patient) (Figure 3.4 &3.5) and (Table 3.2 & 3.3).

Enterococcus fecalis (2% in patient under chemotherapy and 2% in non-cancer patient) (Figure 3.4 &3.5)

Klepsiella spp (2% in patient under chemotherapy and 10% in non-cancer patient). (Figure 3.4 &3.5)

In-vitro sensitivity test for antimicrobial agents by Kirby-Bauer method were carried out show the following result:

S. aureus susceptibility showed that Meropenem highly active (100%) followed by Gentamycin (80%), and Nalidixicacid (40%). (Table 3.4).

Enterococcus fecalis showed that Gentamycin (100%) and Ciprofloxacin. (Table 3.4).

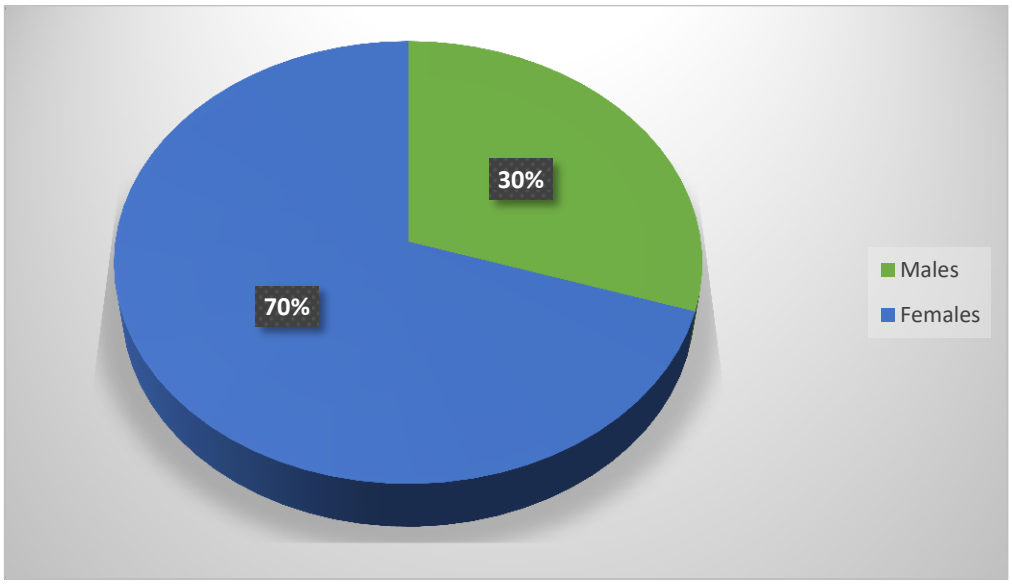
E.coli susceptibility showed that Meropenem highly active (100%) followed by Gentamycin (70%), and Ciprofloxacin (42%). (Table 3.4).

Pseudomonas susceptibility showed that Ciprofloxacin (100%), Gentamycin (100%) were highly active followed by Meropenem (50%). (Table 3.4).

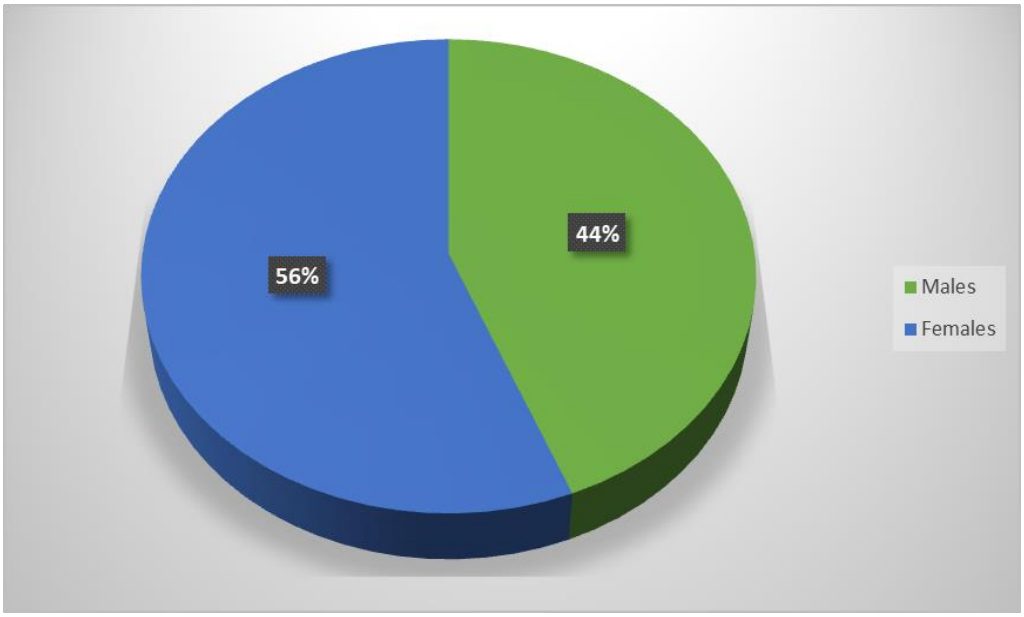
Klebsiella susceptibility showed that Meropenem (100%), Nalidixic acid (100%) and Ciprofloxacin (100%) were highly active. (Table 3.4).

Status	Male	Female
Chemotherapy	15	35
Non –cancer	17	33
Total	100	100

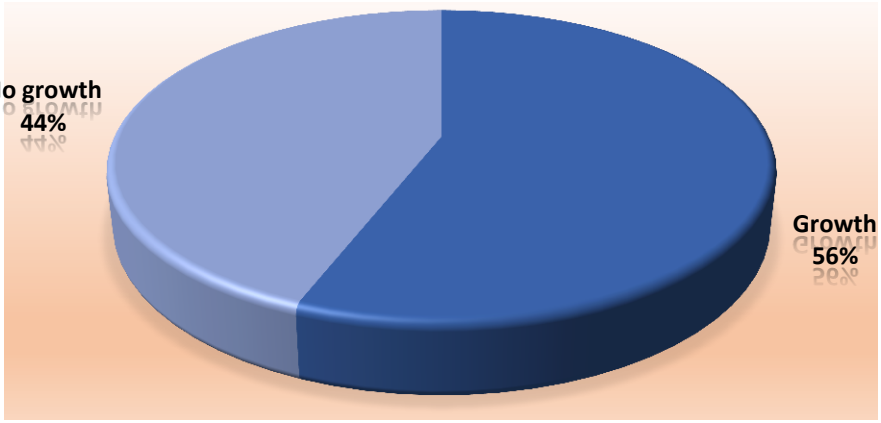
Table (3.1) show distribution of the subjects according to gender.



(Figure3.1) show Gender in patient under chemotherapy



(Figure3.2) show Gender in non-cancer patie



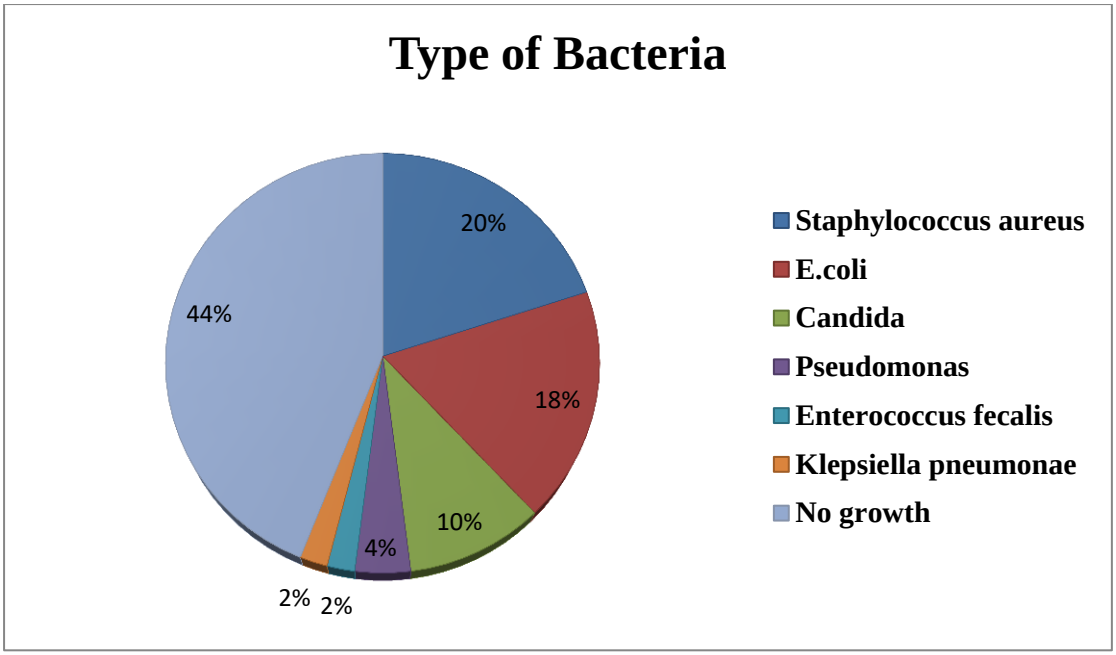
(Figure 3.3) show percentage of growth and non-growth

Isolated bacteria		Percentage
Staphylococcus aureus	10	20.0
E.Coli	9	18.0
Candida	5	10.0
Pseudomonas	2	4.0
Enterococcus fecalis	1	2.0
Klepsiella pneumonae	1	2.0
NO growth	22	44.0
Total	50	100.0

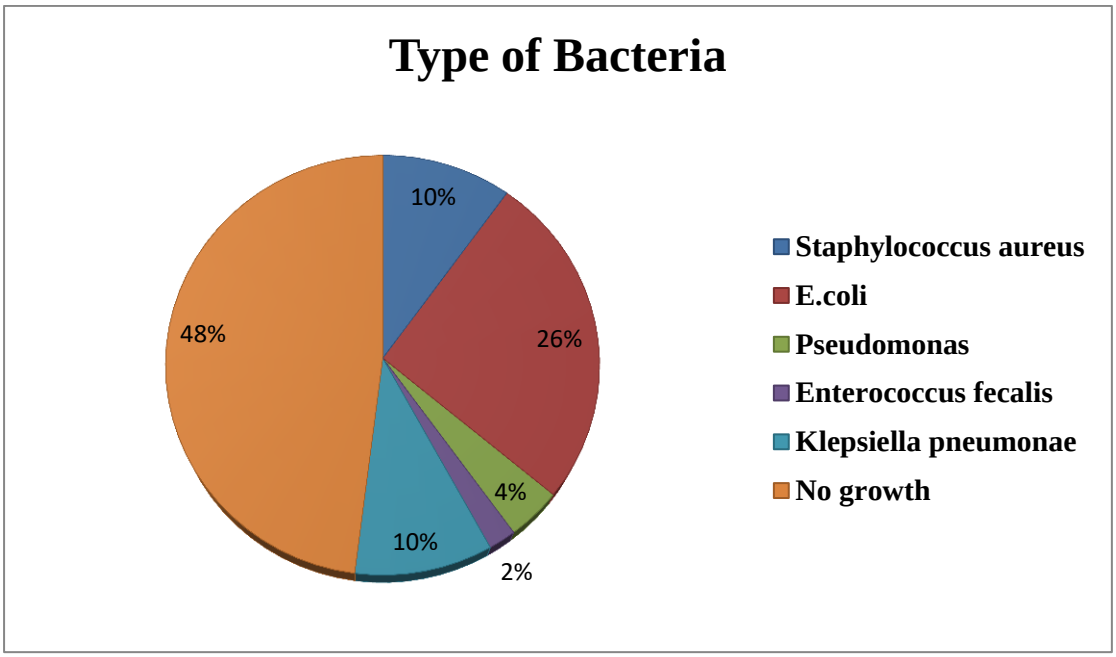
Table(3.2) show percentage of isolated bacteria among patient Under chemotherapy

Isolated bacteria		Percentage
Staphylococcus aureus	5	10
E.Coli	13	26
Pseudomonas	2	4
Enterococcus fecalis	1	2
Klepsiella	5	10
no growth	24	48
Total	50	100.0

Table (3.3) show percentage of isolated bacteria among non cancer patient



(figure3.4) Show type of bacteria in patient under chemotherapy



(figure3.5) Show type of bacteria in non-cancer patients

	Sensitive		Resistance	
Isolated	Chemotherapy	Non-chemotherapy	chemotherapy	Non-chemotherapy
E.coli	MEM:100% CN:70% CIP:42%	IPM:91% CIP:25% MEM:91%	AX:44% CIP:22%	NA:50% CN:50% CIP:8%
pseudomonas	CIP:100% CN:100% MEM:50|%	MEM:100% IPM:100% AMC:50% AK:100%	AMC:50%	AMC:50% CIP:50%
Klepsiella	MEM:100% NA:100% CIP:100%	IPM:100% MEM:100% CIP|:100% NA:40%	AMC:100%	AMC:60% NA:60%
Staph	MEM:100% CN:80% NA:40%	M,EM:60% CN:80% NA:40%	TPZ:70% NA:50%	NA:80% TPZ:40%
Enterococcus	CN:100% CIP:100%	AMC:100% CIP:100% CN:100%	CTX:100% AMC:100%	VA:100%

(Table 3.4) show the result of antimicrobial susceptibility testing in both patient under chemotherapy and non-chemotherapy patient

CHAPTER FOUR

CHAPTER FOUR

4.1 Discussion

This study was carried out to assess the burden of asymptomatic bacteriuria and associated risk factors which could be the possible causes for UTI in cancer patients attending at radiation isotope center of Khartoum (RICK) .In this study, the overall prevalence of asymptomatic bacteriuria in cancer patients was 28 out of 50 patients (56 %), and non-cancer patient was 25 out of 50 (50%).

This finding of cancer patient showed a higher prevalence of bacteriuria than those reported in Saudi Arabia (35.8%), India (34.7%, 32%), and Sudan (31.6%) (A. F. Kantor , E. Akortha and O. Ibadin).This variation of bacteriuria from other studies might be due to the difference in the characteristics of the study population, quality of sampling, culturing techniques, geographical distribution, and diagnostic techniques.

The frequency of Gram-negative bacteria isolated from cancer patients in Egypt was 17.2% (Purewal *et al*,) from a urine sample, which is higher than our study. However, in our study (15.83%), Gram-negative bacteria were found from the urine of cancer patients.

The study relived that gram positive pathogens are commonly isolated from the patients and *Staphylococcus aureus* was predominant microorganism recovered (20%) disagree with(Mansoor Sirkhazi) and (H. Fukushima ,*et al*) followed by *E. coli (18%)* agree with (M. Sirkhazi, A. Sarriff, *et al*) 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(Qurashi M. A. and Taher O. A.) and disagree with (Worku Ferede)that found the most predominant bacteria was *Staphylococcus aureus* (22%).

The percentage of urinary tract infections among patients whom receive chemotherapeutic drug is (56%) this result is higher than (K. H. Kim) whom found that about (45%) where positive and lower than (Tancheva1 *et al*) their result show (68%) where positive, also we found that the distribution of disease among female (70%) higher than male (30%)

The higher prevalence of asymptomatic bacteriuria in females might be due to women's urethra is short and located near the anus that allows relatively easy passage of bacteria into the bladder and urethral opening.

Many factors were assessed as risk factors for bacteriuria in cancer patients. However, no evidence was found to support the association between asymptomatic bacteriuria and sex, age, and cancer types. Correspondingly, a previous study by Fukushima et al. and (Li-Min Sun). showed that neither of the above factors mentioned which similar to our study had no effect on the occurrence of bacteriuria On the contrary, bladder cancer had shown statistically significant association with urinary tract infection in a study done by (Richards *et al*.). A study by Fan et al. in 2017 had also identified an association between prostate cancer and bacteriuria(Worku Ferede). Another study by Sun et al. in 2013 had also confirmed the association among urinary tract cancers and asymptomatic bacteriuria(Li-Min Sun). This difference might be due to the variation in study design and characteristics of the study population. In our study, catheterization and previous surgery and other independent variables were not significantly associated with bacteriuria. Consistently, surgery had been found to be less important in a study by Kim et al. in patients with bladder cancer(Dong Hyeon Lee).

4.2 Conclusion

The prevalence of asymptomatic bacteriuria among cancer patients (56%) was higher than that of compare group (50%). This higher prevalence of asymptomatic bacteriuria in cancer patients might be due to the immunocompromised state of cancer patients by cancer chemotherapy agents. Out of 28 positive samples Staphylococcus aureus and Escherishia coli are the most common causative agent of Urinary Tract Infection in patient under chemotherapy. Females are more infected than males. Sulfamethoxazole, Flucloxacillin and ciprofloxacin have good effect against causative agents and recommended as the first line of treatment .Bacterial infection with MDR organisms which may be persist due to immune suppression which alter metabolism and toxicity contribute to nosocomial infection.

4.3 Recommendations

- For more accurate description of the etiological agent of urinary tract infection on patients under chemotherapy there should be further studies and researches
- Patients should also go for routine screening for urinary tract infection 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 recommended.
- Application of infection control to avoid cross transmission of Urinary tract infection pathogen.

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Appendices:

Appendix 1

Questionnaire

Program: Medical laboratory science

Department: Microbiology

Semester: 8

Batches: 2

Academic Year: 2021-2022

Age: _____

Gender: Male □ Female□

Diabetic: Yes □ No □

Hypertensive: Yes□ No□

Antibiotic Administration: Yes □No□

Type of cancer: _____

Type of chemotherapy: _____

Appendix 2

Informed Consent

Program: Medical laboratory science

Department: Microbiology

Semester: 8 Batch: 2

Academic Year: 2021-202

اقرار موافقة للمشاركة في البحث

نجري بحث لدراسه بعنوان الكشف عن البكتريا المسببه لأمراض الجهاز البولي لدى المرضى تحت العلاج الكيماوي في ولايه الخرطوم و قد تم اختياركم في البحث لأخذ المعلومات و العينات اللازمه لغرض البحث.

أنت\ي مدعو\ه للمشاركه في هذا البحث:

-يتم الكشف داخل المعمل باستخدام الطرق المعمليه المتخصصه.

-لا توجد اثار جانبيه من الاشتراك في هذا البحث.

- هذا البحث يساعد في التعرف على البكتريا المسببه لأمراض الجهاز البولي و انسب مضاد حيوي يمكن استخدامه للعلاج منها.

في حال الموافقه على المشاركه في الدراسه,نتعهد بسريه هذه المعلومات و ألا يتم استخدامها الا في العلم و سيبقى اسمك سري جداً.

لقد قرأت استماره القبول هذه و فهمت مضمونها.و بناءاً عليه أوافق على الاشتراك فيه.كما اعرف تمام المعرفه بأنني حر في الانسحاب من هذا البحث متى شئت حتى بعد التوقيع على الموافقه دون أن يؤثر ذلك على العنايه الطبيه المقدمه لي.

اسم المشترك توقيع المشترك

Appendix 3

- **Cysteine lactose Electrolyte deficient (CLED) agar**

Ingredients

Lactose 10gm

Pancreatic digest of gelatin 4gm

Pancreatic digest of casein 4gm

Beef extract 3gm

L-Cysteine 0.128gm

Bromothymol blue 0.02gm

Agar 15gm

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.

- **Urea agar base (Christensen)**

Ingredient

Urea 20gm

Sodium chloride 5gm

Mono-potassium phosphate 2gm

Peptone 1gm

Dextrose 1gm

Phenol red 0.012gm

Agar 15gm

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.

- **Simmons citrate agar**

Ingredient

Sodium chloride 5 gm

Sodium citrate (dehydrate) 2gm

Magnesium sulphate 0.2 gm

Ammonium dihydrogen phosphate 1 gm

Dipotassium phosphate 1 gm

Bromothymol blue 0.08 gm

Agar 15 gm

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.

- **Kligler iron agar (KIA)**

Ingredient

Peptone 15gm

Beef extract 3gm

Yeast extracts 3gm

Protease peptone 5gm

Lactose 10gm

Glucose 1gm

Ferrous sulfate 0.20gm

Sodium chloride 5gm

Sodium thiosulfate 0 .3g

Phenol red 0.024g

Agar 12g

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.

- **Mannitol salt agar**

Ingredient

Beef extract 1gm

Casein peptone 5gm

Sodium chloride 75gm

Mannitol 10gm

Phenol red 0.025gm

Agar 15gm

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 petri dishes.

- **DNase agar**

Ingredient

Casein enzyme hydrolysate 15g

Soya peptone 5gm

Deoxyribonucleic acid 2gm

Sodium chloride 5gm

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 petri dishes.

- **Mueller Hinton agar**

Ingredient

Beef extract 2gm

Casein hydrolysate 17.5 gm

Starch 1.5 gm

Agar 17 gm

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.

- **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 ($BaCl_2 . H_2O$) 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.

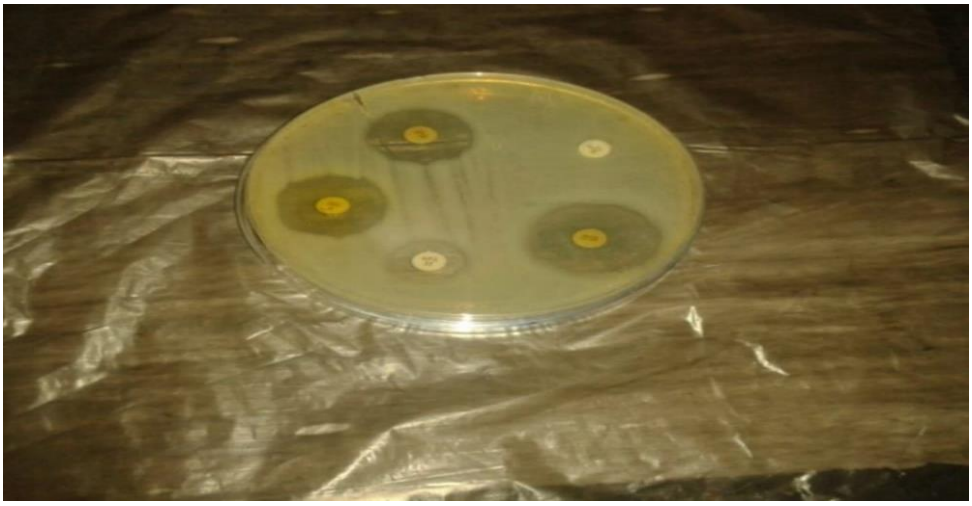
Transfer a small volume of the turbid solution to a screw-cap bottle of the same type as used for preparing the test and control inoculum.

Appendix 4

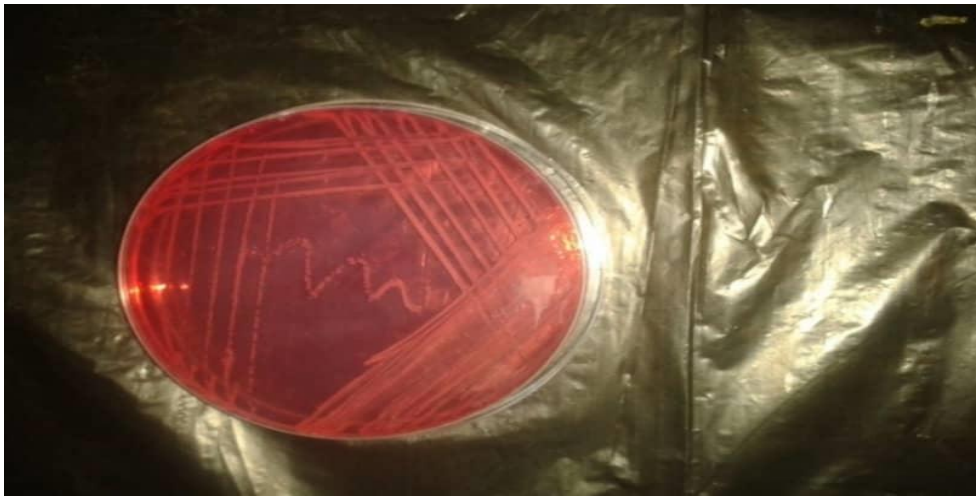
Characteristic reaction of *E.coli* on the biochemical tests

Characteristic reaction of *Ps.aeruginosa* on the biochemical test

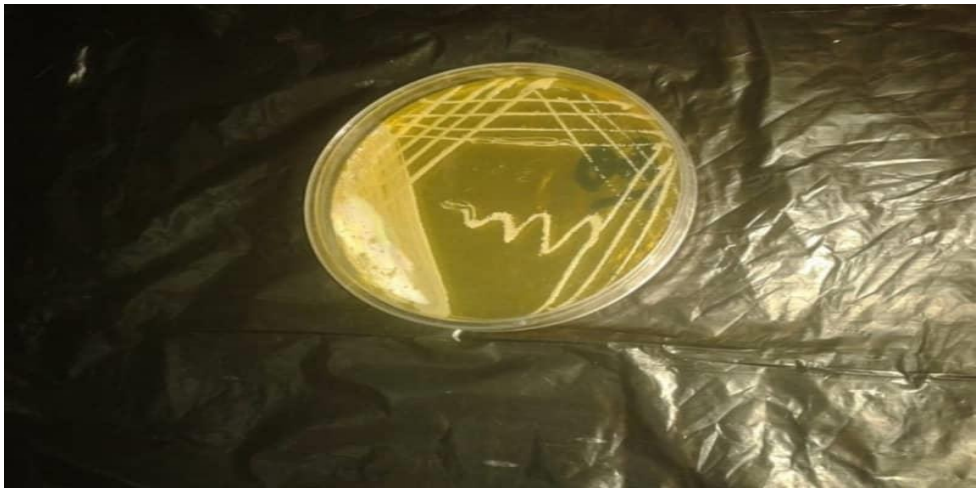
Characteristic reaction of *K.pneumoniae* on the biochemical test



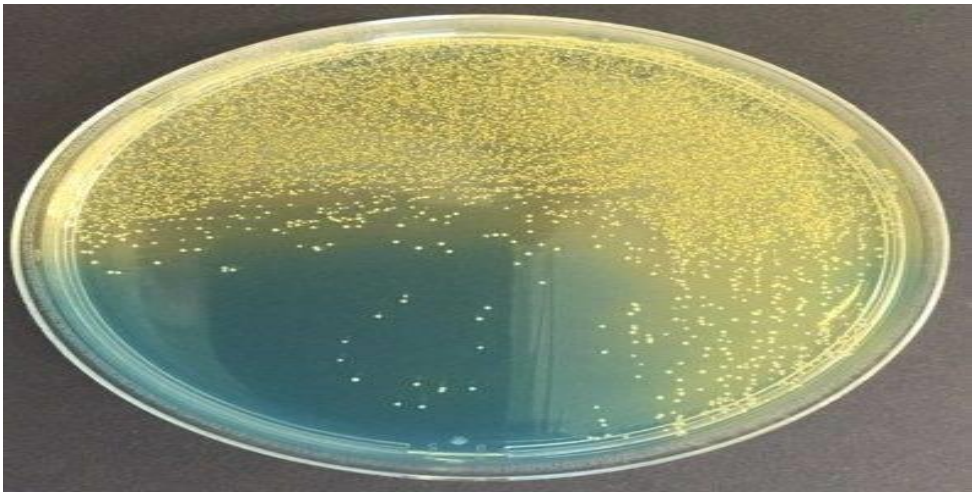
Disc diffusion method on muller hinton agar and the zone of inhibition was measured by millimeter ruler



Red,non mannitol fermenting colonies of *S.fecalis* on mannitol salt agar



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



Lactose fermentation on CLED