NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

URINARY TRACT INFECTIONS IN SEXUALLY ACTIVE WOMEN BETWEEN 2018-2021 IN NORTH CYPRUS: A RETROSPECTIVE STUDY

M.Sc. THESIS

SHITTU ANUOLUWA ESTHER

NICOSIA

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APPROVAL

We certify that we have read the thesis submitted by **SHITTU ANUOLUWA ESTHER** titled **"URINARY TRACT INFECTIONS IN SEXUALLY ACTIVE WOMEN BETWEEN 2018-2021 IN NORTH CYPRUS: A RETROSPECTIVE STUDY"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Educational Sciences.

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DECLARATION

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

SHITTU ANUOLUWA ESTHER

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SHITTU ANUOLUWA ESTHER

ÖZET

Kuzey Kıbrıs'ta 2018-2021 Yılları Arasında Cinsel Aktif Kadınlarda İdrar Yolu Enfeksiyonlarının Retrospektif Bir Çalışması.

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İdrar yolu enfeksiyonu, sağlıkla ilgili sorunların %30-40'ını oluşturan, tahmini nokta prevalansı %1,5 ila %1,64 olan dünya çapında bir sorundur ve her ülke ekonomisinde önemli bir etkiye sahip bir hastalığa işaret eder. Araştırma, Yakın Doğu Üniversitesi Hastanesi'nden E. coli üreten Genişletilmiş Spektrumlu Beta-Laktamaz (ESBL) nedeniyle cinsel olarak aktif olan kadınlarda idrar yolu enfeksiyonu prevalansını araştırmak amacıyla yapılmıştır. İşlem, iki fenotipik doğrulama testi, Disk Difüzyon Testi (Kirby-Bauer), E-test ESBL şeritleri (AB Biodisk) ve bir mikrobiyolojik ortam olan Mueller-Hinton agar kullanılarak gerçekleştirildi. Toplam 71 (%40.80) klinik örnek GSBL (Genişletilmiş Spektrum β-laktamaz) için pozitifti ve 103 (%59.91) örnek GSBL için negatifti, ancak araştırma amacıyla sadece GSBL'ye pozitif olanlara odaklandık. Yaş grubu içinde 24-29 yaş için 24 (%33.80) en yüksek, 6 (%8.45) 36-40 yaş en az hasta sayısına sahipti. Bölümler için Üroloji bölümü 26 hasta (%36,62) ile en yüksek sırada yer alırken, bunu dönem için 12 (%16,90) ile Kadın Hastalıkları ve Doğum bölümü izledi. , ebelik ve doğum. Bu çalışmada kullanılan antibiyotiklerin direnç yüzdeleri ise şu şekildeydi; Ampisilin (%87.32), Sefiksim (%92.95), Seftazidim (%100), Amoksisilin/Klavulanat (%38.02), Seftriakson (%92.95), Siprofloksasin (%45.07), Gentamisin (%22.53), Trimetoprim/Sülfametoksazol (%59.15)) ve Nitrofurantoin (%9,85), Piperasilin/Tazobaktam (%19,71).

Anahtar kelimeler: Escherichia coli, Genişletilmiş Spektrumlu β-laktamaz (GSBL), antibiyotikler, idrar yolu enfeksiyonları, çoklu direnç.

ABSTRACT

Urinary Tract Infections in Sexually Active Women between 2018-2021 in North Cyprus: A Retrospective Study.

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Medical Microbiology and Clinical Microbiology Program, Master Thesis, Nicosia, 2022

Urinary tract infection is a worldwide problem which accounts for 30%-40% of health-care related problem with an estimated point prevalence of 1.5% to 1.64%, which signifies a disease of major impact in every country's economy. The investigation was carried out with the sole aim of finding out the prevalence of urinary tract infection among sexually active women due to Extended-Spectrum Beta-Lactamase (ESBL) producing *E. coli* from the Near East University Hospital. The process was carried out with the use of two phenotypic confirmatory test, Disc Diffusion Test (Kirby-Bauer), E-test ESBL strips (AB Biodisk) and a microbiological media, Mueller-Hinton agar. A total of 71 (40.80%) clinical samples were positive to ESBL (Extended Spectrum β-lactamase) and 103 (59.91%) samples negative to ESBL, but for the purpose of the research we focused on only those positive to ESBL. Amongst the age group, 24 (33.80%) for 24-29 years ranked the highest and 6 (8.45%) 36-40 years had the least number of patients. For the departments, Urology department ranked the highest with 26 patients (36.62%) followed by Obstetrics and Gynecology department with 12 (16.90%) for the period, which tells a lot about the two department and their relationship to the issues of female especially pregnancy, midwifery and delivery. The resistance percentage of the antibiotics used in this study were as follows; Ampicillin (87.32%), Cefixime (92.95%), Ceftazidime (100%), Amoxicillin/Clavulanate (38.02%), Ceftriaxone (92.95%), Ciprofloxacin (45.07%), Gentamicin (22.53%), Trimethoprim/Sulfamethoxazole (59.15%), and Nitrofurantoin (9.85%), Piperacillin/Tazobactam (19.71%).

***Key words*: Escherichia coli, Extended Spectrum β-lactamase (ESBL), antibiotics, urinary tract infections, multi-resistance.**

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CHAPTER III

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List of Abbreviations

TRNC: Turkish Republic of North Cyprus

%:Percentage

µg: Micro gram

µl: Micro Liter

HCAIs: Healthcare-Associated Infection

CLSI: Clinical and Laboratory Standards Institute

DNA: Deoxy Ribonucleic Acid

ESBL: Extended Spectrum Beta Lactamase

EAS-NET: European Antimicrobial Surveillance Network

cUTIs: Complicated UTIs

MDR: Multi Drug Resistant

MHA: Muller Hinton Agar

MIC: Minimum Inhibitory Concentration

eCDC: European Center for Disease Prevention and Control

PCR: Polymerase Chain Reaction

CT scan: Computerized Tomography Scan

NAUTIs: Nosocomial Acquired Urinary Tract Infections

UPEC: Uropathogenic *Escherichia coli* strains

XDR: Extremely Drug Resistant

CF: Cystic Fibrosis

CFU/mL: Colony Forming Unit mills per Liter

ICU: Intensive Care Unit

AUC: Acute Uncomplicated Cystitis

AUP: Acute Uncomplicated Pyelonephritis

FQ: Fluoroquinolones

CLED: Cysteine Lactose Electrolyte-Deficient Agar

RND: Resistance-Nodulation-Division

β:Beta

PYR: Pyrrolidone-Arylamidase

EDTA: Ethylenediamine Tetra Acetic Acid

UTI: Urinary Tract Infection

rUTIs:recurrent UTIs

DDD: Diffusion Disk Test

NaOH: Sodium Hydroxide

CLSI: Clinical Laboratory Standard Institute

CHAPTER I

Introduction

1.1 BACKGROUND OF THE STUDY

A Urinary Tract Infection (UTI) is an inflammatory response at the level of the urothelium to fight a bacterial infection. A UTI is almost always associated with bacteriuria, the presence of bacteria in urine, and pyuria, the presence of white blood cells in the urine. Urinary Tract Infection (UTI) is a major worldwide problem in health-care settings usually outpatient clinics, emergency units as well as hospitalized patients [1]. Every year UTI has roughly 150million cases, 70% of women experience this in their lifetime and the other 30% will have recurrent UTIs (rUTIs). UTI also occur amongst 1%-2% of men, older adults and children with 8-10 million visiting the hospital.

In women, they are twice at risk of exposure than men of any age group. Before the age of 24years, approximately one-third of women are diagnosed with a UTI and by the age of 35years of age half of the population develops at least one episode. UTI is an infection from the name that affects the urinary system, when the urethra is affected its called Urethritis, kidneys it's called Pyelonephritis, which involves the renal parenchyma or if the bladder is affected, it's called Cystitis. They are further classified into simple or complicated UTIs. Simple also known as Uncomplicated UTIs are found in young, healthy, non-pregnant women with normal anatomy [3]. While Complicated UTIs, occurs at the upper urinary tract, male anatomy, pregnant women, anatomic abnormalities, urolithiasis, catheterization, malignancy, chemotherapy and immunosuppression.

Due to various prescriptions and diagnostic tests, only bladder infections also known cystitis accounts for more than 10 million office visits, 1 million emergency department visits and more than 2 billion dollars was spent annually on health-care. It's important to note that half of UTI patients come down with clinical depression and about 38.5% suffer from anxiety with an important advance in the quality of life after proper treatment and prophylaxis. These basically affect 30%-50% of women above 50years of age [2].

As mentioned earlier, UTI is a serious public health problem, caused by variety of pathogens, some of which includes; *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus.* The prevalent uropathogens isolated are the uropathogenic *Escherichia coli* (UPEC) strains. Furthermore in European countries, nosocomial acquired urinary tract infections (NAUTIs) sum up to 40% of all hospital-acquired infections and demonstrate the most frequent nosocomial infection. Also, due to antimicrobial resistance uropathogens are changing their features. Some of the underlying host factors that complicates the cause of UTI are, age, diabetes, spinal cord injury or use of catheters.

The relationship between how an organism can attack the urinary tract and the level of infection acquired is play by the virulence factors of the bacteria [1]. As earlier said, females are at higher risk of getting UTIs, because of the shortness of urethra and its closeness to the rectum. Some other risk factors of UTI may include: sexual practices, previous cases of UTI, pregnancy, age and birth control pills. Most common symptoms of this are: frequent urination, pain or burning during urination (dysuria), bloody urine and cramps at the pelvic area or lower abdomen. The presences of UTIs are being determined by a healthcare professional, through physical symptoms, undergoing some examination tests and ordering urine samples if needed. Bacterial infections are known to be treated with antibiotics, but they also have side effects which are; rash, dizziness, nausea and yeast infections.

1.2 STATEMENT OF PROBLEM

Urinary tract infections occurs when bacteria gets into your urine and travels up to the bladder. UTIs cause more than 8.1 million visits to health care providers each year. About 60% of women and 12% of men will have at least one UTI during their lifetime. This infection is more prominent amongst this group of individuals such as; pregnant women, diabetic patients, sexually active females or/and those using catheters. In the past years, there had being a high increase in the number of UTIs among women especially those that are sexually active. These studies therefore aimed at knowing the rate of the infections among the said population in the past two years and the corresponding risk factors.

1.3 PURPOSE OF THE STUDY

The aim of the study is to find out the prevalence of urinary tract infection among sexually active women due to Extended-Spectrum Beta-Lactamase (ESBL) producing *E. coli* in North Cyprus. No data has being reported regarding this group patients with positive ESBL in North Cyprus.

1.4 HYPOTHESES OF THE STUDY

Various studies have shown the prevalence of urinary tract infection in different population especially pregnant women. However little is done on the investigation of this infection in sexually active women in Turkish Republic of Northern Cyprus (TRNC). This study is designed to investigate the prevalence of urinary tract infection among sexually active women in TRNC.

1.5 SIGNIFICANCE OF THE STUDY

Determine the rate of sexually active women between 18-45 years of age with urinary tract infections, which were positive to urine culture.

1.6 LIMITATION OF STUDY

This research work is limited to only sexually active woman that showed symptoms of urinary tract infections.

1.7 SCOPE OF STUDY

The study was carried out based on the data gotten from Near East University Hospital, on the urine cultures of patients that had symptoms of urinary tract infections, basically women between 18-45 years.

1.8 AREA OF STUDY

- This research work was carried out in the microbiological laboratory of Near East University Hospital TRNC.

CHAPTER II

Literature Review

2.1 GENERAL INFORMATION

Urinary tract infection (UTI) is defined as an inflammatory response to pathogenic micro-organisms by the urothelium within the urinary tract (Lusardi *et al.,* 2013). The presence of white blood cells (WBCs) in the urine, demonstrating an inflammatory response is called Pyuria (Lingenfelter E, 2016). The presence of bacteria of at least 105 CFU/mL found in the urine, also a positive result can also be obtained from a dipstick which is generally accepted and found equivalent (Krzysztof *et al.,* 2021). It's also known as a symptomatic inflammation that occurs at the upper and/or lower urinary tract resulting to adherence to the urothelial or internalization of uropathogenic micro-organisms (Han & Lee, 2017). The urinary tract is made of the upper and lower urinary tract. In the upper urinary tract, we have the kidneys and ureters; while in the lower urinary tract, we have the urethra and bladder (Beerepoot *et al.,* 2013).

UTI is an infection from the name that affects the urinary system, when the urethra is affected it's called Urethritis, kidneys it's called Pyelonephritis, which involves the renal parenchyma or if the bladder is affected, it's called Cystitis (Duane *et al.,* 2019). They are further classified into simple or complicated UTIs. Simple or uncomplicated UTIs are found in young, healthy, non-pregnant women with normal anatomy (Anderson *et al.,* 2004). While complicated UTIs occur at the upper urinary tract, male anatomy, pregnant women, anatomic abnormalities, urolithiasis, catheterization, malignancy, chemotherapy and immunosuppression (Gandhi *et al.,* 2009). Bacteria are not found in the urine. It's a byproduct of our filtration system, the kidneys. Without contamination, the urine moves throughout the urinary system. UTI can affect anyone either male or female of any age group, but it's mostly found amongst women (Handley *et al.,* 2002). Due to the shortness of the urethra (the tube is responsible for the transport of urine out of the body) in females and its closeness to the anus (habitation of *Escherichia coli,* major bacteria that causes UTI) (Cai *et al.,* 2012). Risks are known to increase with age (Justice *et al.,* 2004). There are also underlying medical conditions that make one prone to the infection such as, enlarged prostate or bladder (it's a condition that occurs when the bladder falls or shifts from its usual position) (Foxman, 2014).

2.2 EPIDEMIOLOGY

Urinary tract infection is a worldwide problem which accounts for 30%-40% of health-care related problem with an estimated point prevalence of 1.5% to 1.64%, which signifies a disease of major impact in every country's economy (Suzanne, 2016). Over the years, it's being known to be the leading factor of hospitalization among sexually active women and elderly people (Foxman, 2010). Approximately 150 million cases of UTI every year, of which 70% of women experience this in their lifetime and the other 30%, will have recurrent UTIs (rUTIs) (Johansen *et al.,* 2011). 1%-2% comes down with UTI amongst men and 8-10 million of older adults and children visiting the hospital (Little *et al.,* 2010). Women of any age group are twice at risk of exposure than men (Krieger *et al.,* 2008). Approximately one-third of women are diagnosed with one of the UTIs before 24years of age, while others suffer multiple recurrences and by the age of 35years of age, half of the women population develops minimum one episode of UTI (Foxman, 2010).

Due to various prescriptions and diagnostic tests, only bladder infections accounts for more than 10million office visits, 1 million emergency department visits and more than 2 billion dollars spent annually on health-care (Tambyah & Maki, 2000). It's important to note that half of UTI patients come down with clinical depression and about 38.5% suffer from anxiety with an important advance in the quality of life after proper treatment and prophylaxis. These basically affect 30%-50% of women above 50years of age (Vincent *et al.,* 2010). That's UTI has a great effect on an individual's mental health and sense of well-being (Christiaens *et al.,* 2002). Every year among post-menopausal women, the incidence of UTI varies from 0.07% per person to 0.13% in adults older than 85years (Bleidorn *et al.,* 2010).

2.3 PATHOGENESIS

The interaction between the uropathogens, the host and their pathogenesis involves variety of processes that leads to infection of the urinary tract (Niall & Hugh, 2011). Uropathogens first of all attaches to the epithelial surface, then colonize and spreads all the way through the mucosa causing damage to the tissues (Anderson *et al.,* 2004). Pathogen ascends and gets hooked on to the urinary bladder causing either symptomatic or asymptomatic bacteriuria, these occurs after the first colonization (Coker *et al.,* 2000). In extreme cases, may lead to pyelonephritis and renal

impairment (Handley *et al.,* 2002). The efficiency of the host defense mechanism is due to presence of some virulence factors on the uropathogens membrane which are responsible for bacterial resistance (Zhang *et al.,* 2004).

Among healthy population, most uropathogens that enter into the urinary tract via the urethra into the bladder initiate from the rectal flora (Wu *et al.,* 1996). This process is known as the Ascending route and uropathogens attach primarily to the urothelium of the distal urethra and colonization occurs (Sun, 1996). In patients with urinary catheters and females that uses spermicidal agents; development of this route worsens by soiling around the perineum (Talan *et al.,* 2000). Up to 50% of infections may ascend into the upper urinary tracts in patients with established cystitis and ascending of the bacteria from the bladder through the ureters and into the renal pelvis causes nearly all episodes of pyelonephritis (Schlager *et al.,* 2002). Most bacteria infiltrate the renal parenchyma through the collecting ducts when they get to the renal pelvis and interrupt the renal tubules (Kau *et al.,* 2005).

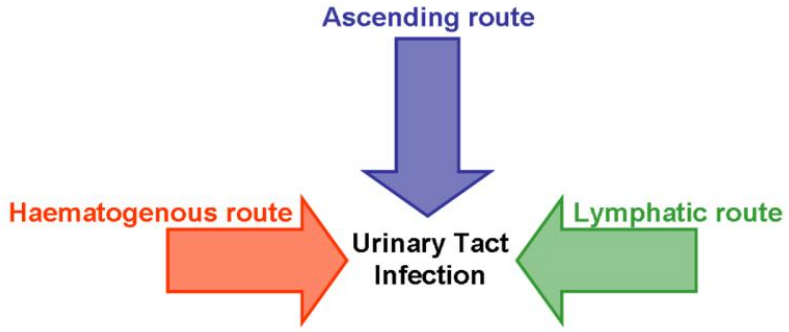


FIG. 1 Urinary tract infections may arise from ascending, haematogenous or lymphatic routes. Ascending routes of infection are most common among patients with an established UTI (Niall & Hugh, 2011).

As mentioned earlier, UTI is a serious public health problem, caused by variety of pathogens, some of which includes; *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus* (Justice *et al.,* 2004). Of all the uropathogens mentioned E. coli is still the most isolated (Khan & Ahmed, 2001). Also, due to antimicrobial resistance uropathogens are changing their features (Stapleton, 2002). Some of the underlying host factors that complicates the cause of UTI are, age, diabetes, spinal cord injury or use of catheters (Ronald, 2003). As a result, complicated UTI has greater consequences

than uncomplicated UTI, and organisms that hardly cause diseases in healthy patients can be the cause of a major disease in hosts with anatomic, metabolic or immunologic underlying diseases (Aydin et al., 2015).

2.3.1 PATHOGENIC BACTERIA

From the name, they are bacteria that cause diseases (Justice *et al.,* 2004). The major pathogenic bacteria are *E. coli* with about 85% acquired from the communities and 50% acquired from hospital related urinary tract infections (Vincent *et al.,* 2010). There are quite a number of subgroups in the *E. coli* species which are isolated from UTI patients regularly such as the O1, O2, O4, O6, O7, O8, O18, O25, O68 and O75 (Wang *et al.,* 2020).

Other pathogenic bacteria are the Gram negative bacteria such as *Klebsiella* and *Proteus*; Gram positive bacteria such as *Enterococcus faecalis* and *Staphylococcus saprophyticus;* the remaining community-acquired infections are caused by this group of bacteria (Aguilar-Duran *et al.,* 2012). For the remaining hospital-acquired infections, they often take place after colonization with *Klebsiella, Enterobacter, Citrobacter, Serratia, Pseudomonas aeruginosa, Providencia, E. faecalis, or S. epidermidis* (Vincent *et al.,* 2010). It's important to note that approximately 10% of Staphylococcus saprophiticus causing UTI in young females is dependent on a patient's age and also determines the kind of infective organisms (Stapleton, 2002).

2.3.2 COMPLICATED UROPATHOGENS

Complicated UTIs (cUTIs) are known not to respond to conventional treatments, also treatment failure is at a higher risk, they require longer time period for antibiotics plus extra work up (Han & Lee, 2017), which is due to major underlying medical conditions or risk factors, such as age and anatomical differences (Malmartel & Ghasarossian, 2016). Uropathogens other than *E. coli* are a major cause of cUTIs and also broad-spectrum antimicrobials (Suzanne, 2016). For the reason that certain organisms after insertion or instrumentation or in combination with medical equipment such as urinary catheters, in patients for renal transplant, those with impaired renal function or radiotherapy, complicated UTIs tends to occur in males/females, pregnant females as a result of obstruction, hydronephrosis or Immunocompromised patients or the elderly (Thanassi *et al.,* 2012). Some of the underlying risk factors that influences complicated UTIs include age, catheterization, diabetes mellitus and spinal cord injury (Rice *et al.,* 2005). The diseases caused

by cUTIs are heterogeneous in nature (Christiaens et al., 2002). When it comes to cUTIs, a small amount of virulent uropathogens (that would normally not cause disease in a normal urinary tract) can cause major damage to an abnormal urinary tract (Boedeker, 2005).

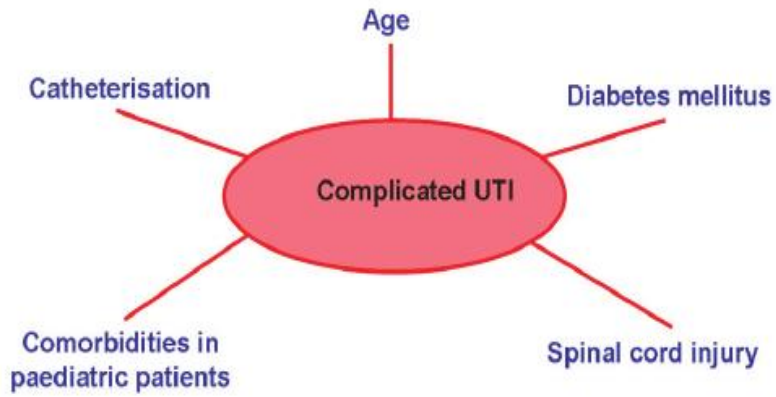


FIG. 2 Predisposing factors of complicated UTIs (Niall & Hugh, 2011).

2.3.3 UNCOMPLICATED UROPATHOGENS

UTIs are categorized into two fractions, complicated UTIs (as explained earlier) or uncomplicated UTIs, which depend on essential host factors and uropathogens (Hooton, 2012). The most commonly encountered infections in the outpatient departments are the uncomplicated urinary tract infections (Aguilar-Duran *et al.,* 2012). They are also the most popular reasons why antibiotics are prescribed after respiratory tract infections (Wang *et al.,* 2020). Uncomplicated UTIs are divided into two groups; acute uncomplicated cystitis (AUC) and acute uncomplicated pyelonephritis (AUP) (Duane *et al.,* 2019). Unlike cUTIs, uncomplicated UTIs are found in patients who have normal, with no history of recent instrumentation, unobstructed genitourinary tract, and have their symptoms restricted to the lower urinary tract (Kranz *et al.,* 2018). In this case, it occurs mostly among young, sexually active women. In recent times, the rate of pathogens-resistance causing uncomplicated UTI has being on the increase greatly (Dason *et al.,* 2011). Over the past 2-3 decades, *E. coli* has being the constant major cause of uncomplicated UTIs (Lin *et al.,* 2012). Some of the symptoms are dysuria, frequent urination, urinary urgency and suprapubic pain. They may also experience fever or tenderness of the costoverteral angle which signifies upper tract involvement (Malmartel & Ghasarossian, 2016). Studies have shown that increase in antimicrobial resistance among uropathogens causes uncomplicated cystitis (Hooton, 2012).

TABLE 1 Uncomplicated Versus Complicated Urinary Tract Infection (UTI) Pathogens.

Uncomplicated UTI Pathogens	Complicated UTI Pathogens
Escherichia coli	*Escherichia coli*
Staphylococcus saprophyticus	*Klebsiella spp.*
Klebsiella spp.	*Enterobacter cloacae*
Enterococcus faecalis	*Pseudomonas aeruginosa*
	Enterococcus faecalis
	Group B streptococci

2.4 BACTERIAL ADHERENCE MECHANISM

Depending on the genetic, biologic and behavioral factors, an individual's vulnerability to UTI is complex (Thanassi *et al.,* 2012). The relationship between host defense factors and bacterial virulence factors can eventually lead to UTI (Humphries *et al.,* 2003). Every species of bacteria has a unique pathogenic mechanism that makes the cause of UTI possible (Weening *et al.,* 2005). Specific bacterial adhesive characteristics are determined by colonization; on the epithelial surface is the receptor selection, and surrounding fluids (Neugent *et al.,* 2020). It's important to note that after vaginal colonization of uropathogens UTI occurs at all times (Thanassi *et al.,* 2012).

2.4.1 VIRULENCE FACTORS

The relationship between how an organism can attack the urinary tract and the level of infection acquired is play by the virulence factors of the bacteria (Costa *et al.,* 2015). Uropathogenic E. coli (UPEC) is found in the bowel flora and the organism's pathogenic strains infect the urinary tract by demonstrating some particular virulence factors that gives adherence permission and colonization of the lower urinary tract (Proft & Baker, 2009). Micro-organism adherence is reliant on three major environmental factors; the adhesive characteristics of the bacteria, the urothelium receptive features and lastly, the fluid present between both surfaces (Berry & Pelicic, 2015). After adhering to the mucosal surfaces, bacteria will move closely and respond suddenly to the host-derived inflammation (Kisiela *et al.,* 2005). On the surface of the bacterial membrane is the adhesins, which are accountable for immediate attachment to the urinary tract tissues (Piatek *et al.* 2005).

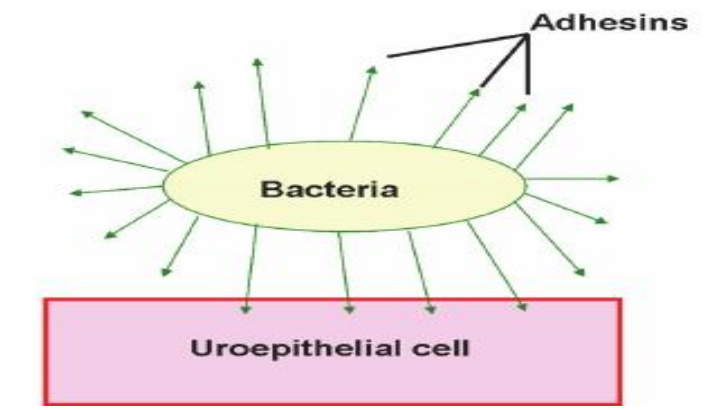


FIG. 3 Adhesins on the uropathogen are responsible for attachment of the bacteria to the uroepithelial cell membrane of the host (Niall & Hugh, 2011).

2.4.2 CHAPERONE-USHER PATHWAY PILI

Through the use pili, most uropathogens initiate UTI that acts an as adhesins to the host and its environmental surfaces, it also speeds up invasion into the tissues of the host and encourages inter-bacterial interactions to form biofilms (Kuehn *et al.,* 1993). In majority of cases, several pathogenic Gram-negative bacteria such as, *E. coli, Klebsiella spp., Proteus spp., Pseudomonas spp., Haemophilus spp., Salmonella spp.,* and *Yersinia spp.,* all demonstrate a large, competent family of adhesive fibers called Chaperone-usher pathway (CUP) pili (Low *et al.,* 2006). Chaperone-usher molecular machinery brings together the CUP pili and they are made up of pilin subunits with unfinished immunoglobulin-like folds that is short of an original carboxyl-terminal, seventh β-strands (Dziva *et al.,* 2004).

Biofilms are known to appear on several kinds of bacteria, nevertheless the pattern of events remains the same for all bacteria throughout the formation stage (Sukumaran *et al.,* 2010). Bacteria demonstrate extracellular polymeric substances that are primarily reversible and afterwards turn out to be irreversible (Roberts *et al.,* 1994). Irreversible attachment of bacteria to a surface serves as a nidus for replication to go on and recruitment of other bacteria (Rice *et al.,* 2005). The bacteria expand into a complicate tower-like structure and become filamentous (up to 70μm in length) and this process is mostly recognized after 24hours. Uropathogen evade the host's immune response due to morphological changes (Cane *et al.,* 2007). Clustered bacteria then separate from the group, become motile and leave the host cell (Boedeker, 2005). When the uropathogen leaves its intracellular environment, the process of adherence and replication

reoccurs and this useful replication process allows bacterial invasion to continue (Qadri *et al.,* 2005).

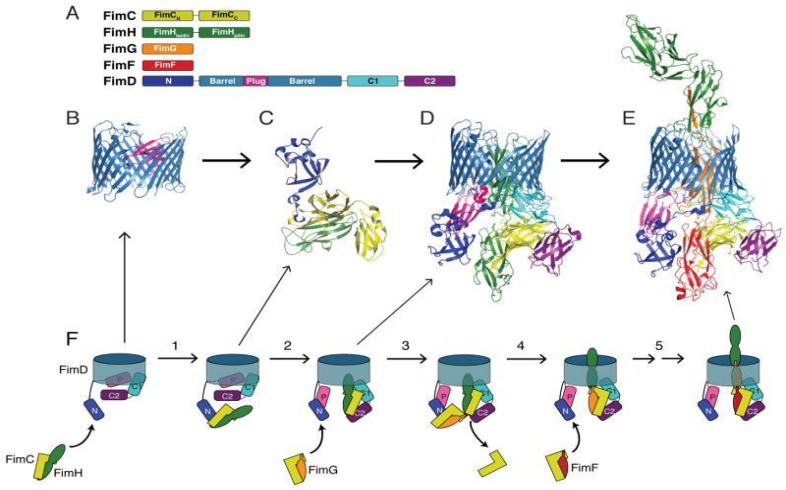


FIG. 4 Pili assembles by the Chaperone-usher pathway of *E. coli* and *Salmonella spp* (Glenn & David, 2018).

2.4.3 VARIATION PHASE

In *E. coli* isolates, which involves irregular periods of piliated and non-piliated adhesins its being observed that environment factors are responsible for speedy changes in pili from in vivo studies (Klumpp *et al.,* 2001). This process of transformation is known as Phase variation.

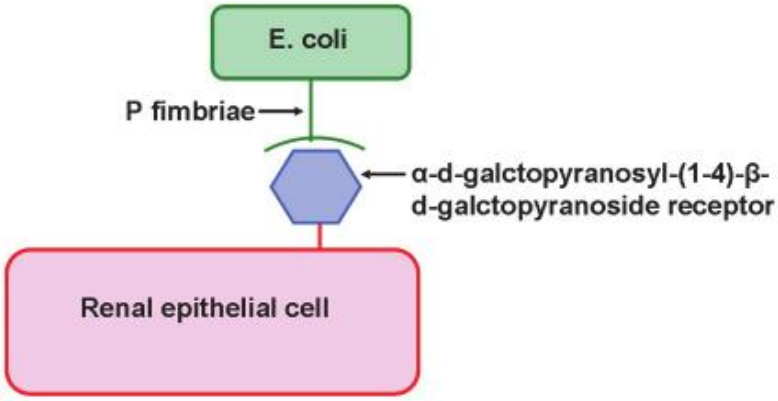


FIG. 5 P fimbriae bind to the α -d-galctopyranosyl-(1-4)- β -d-galctopyranoside receptor on the host's renal epithelial cell via the PapG adhesins (Niall & Hugh, 2011).

A study that was carried out using indirect immune-fluorescence assays of voided urine in patients showed that phase variation of pili from piliated to non-piliated cells showing type 1 pili and P pili (Anantha *et al.,* 2004). The results showed that type 1 and P pili were expressed and subject to phase variation in vivo during acute UTIs (Parsek & Singh, 2003). It's important to note that, the host mucosa in the lower urinary tract speeds up adherence and colonization via the existence of type 1 pili (Mysorekar *et al.,* 2002). Nevertheless, as the infective process progresses and ascends, P pili may be in the majority (Ronald, 2003).

2.5 CLINICAL FEATURES

UTIs are known to cause an irritation (inflammation) of the lining of the urinary tract and eventually become reddish (American Urological Association, 2021). Symptoms are usually common among adults but some of the fundamental symptoms are:

I. Pain in the side (flank), abdomen or pelvic area.
II. Lower pelvis pressure.
III. Frequent urination and incontinence (urine leakage).
IV. Night urination.
V. Painful urination (dysuria) and bloody urine.
VI. Cloudy urine with strong, foul odor.

In tremendous cases, most patients may experience:

I. Pain during sex.
II. Penis pain.
III. Pain at the lower back
IV. Fever (temperature above 100degrees Fahrenheit) with chills.
V. Vomiting.
VI. Mental changes and confusion.
VII. Hypotension (Centers for Disease Control and Prevention, 2021).

2.6 RISK FACTORS

Below are some of the risk factors of associated with women, and most of them experience more than one infection throughout their lifetimes:

I. **Female anatomy:** Urinary tract infections are mostly occurring among women (Oscar et al., 2019). This due to their short urethra (the tube that helps transport urine out of the body) found in females, it's also closer to the anus (the rate of *E. coli* here is on the rise) and the bladder (Ahmed *et al*., 2017).

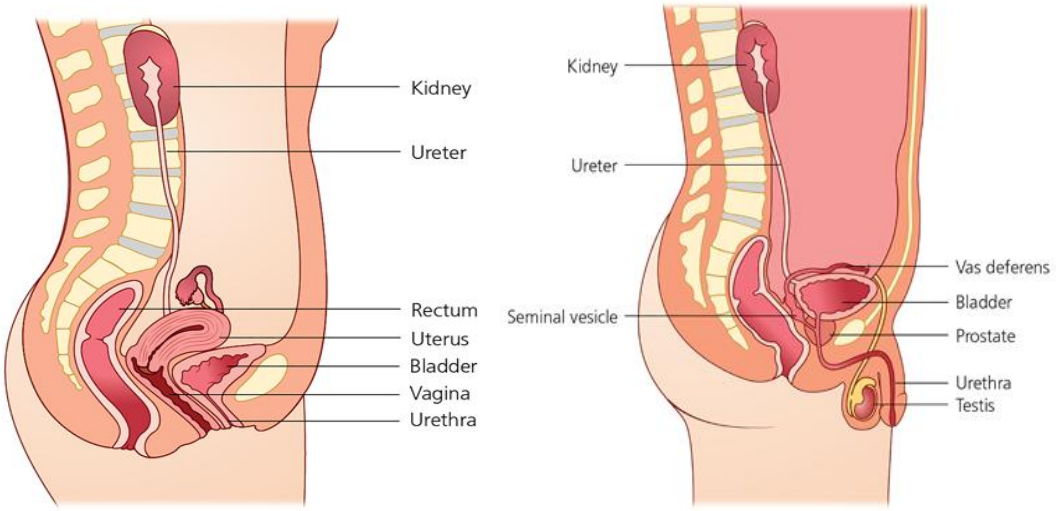


FIG. 6 The female and male urinary tract system respectively

II. **Age:** The older we mature, the higher the risk of developing cystitis, which is basically due to not completely emptying the bladder (Cai *et al.,* 2012). There are some medical conditions that are closely related to this, such as prostate enlargement or prolapsed bladder (a condition where the bladder falls or changes from its usual position) (Gupta *et al*., 2011).

III. **Sexual activity:** The rate of UTIs tends to be higher among sexually active women or those within sexually active age. Also, having more than one sexual partner increases risk of contracting the infection (Gupta *et al*., 2011).

VIII. **Use of birth control pills:** The use of control pills like diaphragms and spermicidal agents increases risk of UTI in women (Centers for Disease Control and Prevention, 2021).

IV. **Menopause:** Post-menopausal women experience a fall in circulating estrogen which causes changes in their urinary tract which makes them susceptible to infections (Merck Manual, 2021).

V. **Immuno-suppression:** Patients with underlying medical conditions such as diabetes, kidney problem, have an impaired immune system which makes it difficult to fight against infections (Ahmed *et al*., 2017).

VI. **Catheterization:** Some people find it difficult passing urine normally, therefore the use of catheter is advised, in turn makes them prone to UTIs. Especially hospitalized patients, those with neurological problems (they find it difficult to control the urge to urinate) and those paralyzed (US Department of Health and Human Services., 2021).

2.7 DIAGNOSIS

Urinary tract infections (UTI) are among the main cause for treatment in adult primary care medicines, leading to a significant amount of prescription of antibiotics (Nicolle *et al*., 2005). A high level of diagnostic accuracy is needed, due to the significant effect of the problem in routine clinical practice (Gould *et al*., 2011, Gandhi *et al*., 2009). The hallmark for the diagnosis of a urinary tract infection is the detection of pathogens in urine samples (using midstream urine) and the presence of clinical symptoms (Hooton *et al*., 2010). Some of the diagnostic tests are:

2.7.1 URINE TEST (URINALYSIS)

It's a test on the urine to check for common signs of a disease or medical conditions. It's the best way to find out a certain disease at an early stage (Andrea *et al*., 2014). Some of which includes; kidney disease, liver disease, diabetes and UTIs (Drekonja *et al*., 2013). Here are three major ways a urine test is being carried out;

I. **Visual exam:** Here, we check for the color and clarity of the urine. If it's bloody, having a red or dark brown color and foamy, could a sign of kidney disease. If it's cloudy, then it's an infection (Gandhi *et al*., 2009).

II. **Microscopic exam:** From the name, it checks for things too small for the eyes to see. Some of these things are: Red blood cells, White blood cells and Bacteria (Gandhi *et al*., 2009).

III. **Dipstick test:** A thin plastic strip is being used and it's treated with chemicals. The strip is dipped into the urine, then the chemicals react with the strip and changes color if the levels are above normal (Gandhi *et al*., 2009). The dipstick test for the:

   a. Acidity or pH, if the acid is abnormal, it's a sign of UTI.
   b. White blood cells, it's a sign of infection or inflammation along the urinary tract.
   c. Nitrites, sign of a bacterial infection.
   d. Bloody urine (Gandhi *et al*., 2009).

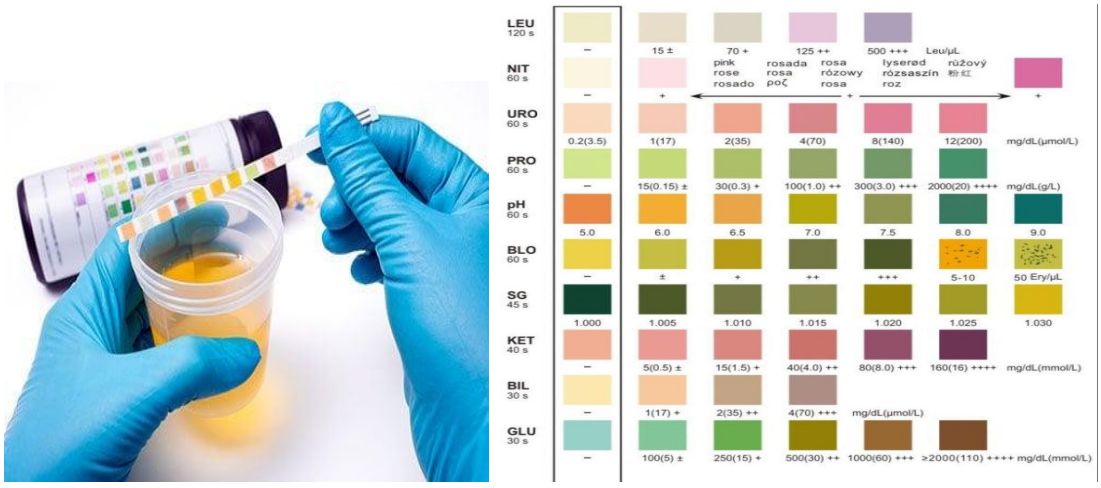


FIG. 7 Urinalysis test strip and result interpretation (Foley & Wasserman, 2021).

2.7.2 URINE CULTURE

It's a test to check for bacteria that cause UTI in a mid-stream collected urine sample. The infection starts in the bladder or urethra, but has a likelihood of affecting other part of the system (Linares *et al*., 2011). The presence of an infection comes with a burning sensation when you pee

or you feel to go, but nothing comes out (Mody and Juthani-Mehta, 2014). After the samples are collected, it's taken to the lab, the sample is dropped on a petri dish containing Mac Conkey agar and stored at room temperature (Drekonja *et al*., 2013). After a few days, if bacteria or yeast is presence, they will multiply. The plate is viewed under a microscope, to check the size, shape and color of the kind of bacteria (Andrea *et al*., 2014). The colonies are also counted, with a minimum 10^5 CFU/ml. The most common cause of UTI is *E. coli*.

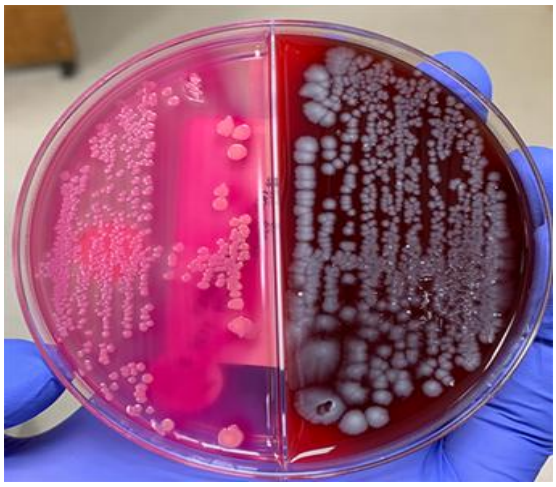


FIG. 8 Diagnosing UTIs with urine culture

2.7.3 CYSTOSCOPY

It's also known as Cystourethroscopy or Bladder scope. It's a procedure to check the health of the urethra and bladder. The test carried via a tube which is inserted into the urethra. In men, it's inserted via an opening at the end of the penis. In women, it's inserted above the vagina. It's different from the usually CT scan because, here the bladder must be empty for the test can be done. The doctor checks for the complete length of the urethra and the bladder for polyps, narrow areas called strictures, for abnormal growth and other related problems. The test is done to:

a. Check for symptoms like bloody urine, overactive bladder, and painful urination.

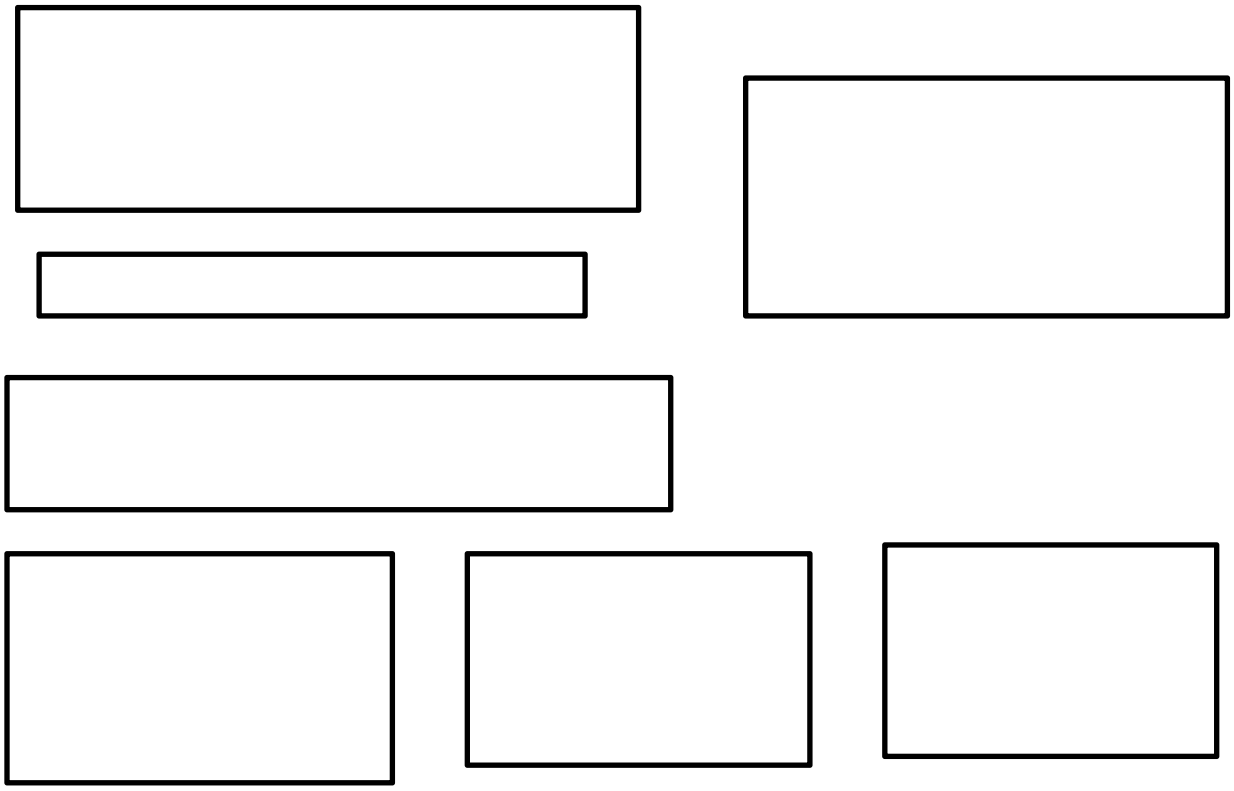
b. Check for recurrent UTIs.

c. Detect bladder diseases like bladder inflammation (cystitis).

d. Treat conditions, the cystoscope helps to remove tumors.

e. Detect prostate enlargement.

Cystoscope is a tube-shaped tool that is inserted into the body. It has light and camera that helps the doctor view the interior of the urinary tract. They are of two types: a standard rigid cystoscope and a flexible cystoscope. The choice of which scope to use is dependent on the reason for examination.



2.8 TREATMENT

Generally, antibiotics are the first line of treatment for all bacterial infections not excluding urinary tract infections (Carol, 2014). The kind of drugs that will be prescribed and the duration of the dose depend on the patient's health condition and the type bacteria present in the urine sample (Wing, 2001).

2.8.1 SIMPLE INFECTIONS: Some drugs that are usually used for simple UTIs are Sulfamethoxazole/Trimethoprim, Nitrofurantoin, Cephalexin and Ceftriaxone (Hooton *et al.,* 2010). There are some groups of antibiotic medicines called Fluoroquinolones; Ciprofloxacin, Levofloxacin and others (Carol, 2014). As the high risks of these antibiotics over shadows the

benefits for treating uncomplicated UTIs, they are not usually used for simple UTIs (Bartoletti *et al.,* 2016).

For some complicated UTIs or kidney infections, fluoroquinolone are prescribed in the absence of no other drug option (Quinlan & Jorgensen, 2017). Like most infections, symptoms of UTIs clear off within few days of using treatment. In the case of uncomplicated UTI, a shorter course of treatment is usually prescribed for at least 1-3 days, when the patient looks healthy (Zalmanovici Trestioreanu, 2010). Note that, this short course depends on the symptoms at the time and medical history. Pain relievers are also prescribed to ease the burning sensation in the bladder and urethra while urinating.

2.8.2 FREQUENT INFECTIONS: In the case of frequent UTIs, treatment recommendations include:

I. For six months or longer, low dose antibiotics are administered.
II. Self-diagnosis and treatment.
III. After sexual intercourse, single dose of antibiotics.
IV. Vaginal estrogen therapy.

2.8.3 SEVERE INFECTIONS: Here patients with extreme cases of UTIs are admitted to the hospital and antibiotics are administered intravenously for up to 2 weeks (Quinlan & Jorgensen, 2017).

If after completing antibiotic dose all symptoms of UTIs clear off, there won't be any need for another urine culture, except in complicated cases (Dalhoff, 2012). A longer course of antibiotics or a different antibiotic would be administered if after the initial dose symptoms don't clear off (Zalmanovici Trestioreanu, 2010).

2.9 PREVENTION

1. **Observing good personal hygiene:** This a major factor in preventing UTIs, especially in women. As earlier mentioned, in the anatomy of women, the urethra is shorter compared to men and makes it easier for bacteria to get into the body (American Urological

Association, 2021). It's also advisable to maintain good hygiene during the menstrual period and wiping from front to back after bowel movement. Also urinating immediately after sex (Duane *et al.,* 2019).

2. **Keeping hydrated:** Drinking enough fluids especially water (approximately 2L per day) helps reduce excess bacteria from the urinary tract by 50% (Mandy A., 2020). Also avoid drinks that can irritate the bladder, such as: alcohol, caffeine drinks and citrus juices.
3. **Birth control:** Changing of birth control pills can reduce risk of developing UTIs, from diaphragm to spermicide or otherwise (Chromek M., 2006).
4. **During sex using a water-based lubricant:** For those that experience vaginal dryness, the use of a water-based lubricant during sex is advised or estrogen-containing vaginal cream in the case of post-menopausal women and avoid spermicide (Jepson R., 2004).
5. **Cranberry:** It juice or supplement has being proven to prevent UTIs (Fu, 2017).

CHAPTER III

Methodology

3.1 RESEARCH DESIGN

All women with urinary tract infection who attended the Near East University Hospital between January 2018 and December 2021 were retrospectively screened for the study.

3.2 STUDY GROUP

The investigation was done at the Near East University; this was centered on the out and in patients of Near East University Hospital. The research was performed in the MICROBIOLOGY LABORATORY of Near East University Hospital. A total of 71 samples for the study was executed from different clinical specimens of hospitalized patients from various departments of the Hospital, these include patients from OPD, ICU, CCU, Emergency and different general wards (Pulmonology, oncology, neurology, gastroenterology, cardiology, general wards of male and female etc.). The study protocol was accepted by the institutional review boards of Near East University. *Escherichia coli* obtained from their clinical specimens and different patients were used for the investigation.

3.3 INCLUSION AND EXCLUSION CRITERIA

Women eligible for the study were between 18-40 years of age, have sexual intercourse on a regular, and must be sexually active, symptoms of urinary tract infections, single or multiple sexual partners, finally with a urine culture of 105 CFUs/ml of uropathogens. Pregnant women, lactating women or those in menopause at the point of test were excluded from the study.

3.4 DATA COLLECTION TOOLS AND EQUIPMENT

The materials used in this research work includes; Antec medical petri-dish, automatic pipette, wire loop, test-tubes, Vitek 2 system, vortex-genie 2, Uri-Trak® 120 Urine Analyzer from EKF Diagnostics, CLINITEK Status®+ Urine Chemistry Analyzer, UF-1000i Automated Urine Particle Analyzer from Sysmex , Cobas u 411 Urine Analyzer from Roche, CLINITEK Status® Connect System, Urisys 1100 Analyzer from Roche, CLINITEK

Advantus® Urine Chemistry Analyzer, Surgical-field autoclave (model M50D), 1000mL conical flask, spatula, masking tape, pH meter, syringe, dispenser, measuring ruler, marker, test tube wrack, sterile swab stick, ERMA INC photoelectric calorimeter, cotton wool, foil paper, biosafety cabinet 2, bunsen burner, lighter/matches, electrical weighing scale (Adam AFP-4100L and Labtech BL 20001), and UNISCOPE SMB930 incubator.

3.5 DATA COLLECTION PROCEDURE

3.5.1 Preparation Of Glucose-Topped MacConkey Agar Plates

1. A bag of 5% glucose intravenous infusion solution (1000mL) was disinfected with a cotton ball impregnated with 70% isopropyl-alcohol and allowed to dry.
2. Using a sterile needle and syringe, 2mL of 5% glucose solution was aspirated.
3. The aspirated solution was placed on the surface of a standard MacConkey agar plate (60x15mm). This was prepared according to the manufacturing company's manual.
4. The solution was regulated by slanting the plate in several directions.
5. Thereafter the plate was left for 1 hour so that the solution could infuse and the surface to dry on the bench at room temperature.

3.5.2 Surface Streak/Calibrated Loop Method

1. The tip was placed over the container to mix the urine sample again after it was brought to the laboratory.
2. The cap of the sample container was removed; the sterile end of a 1- µL inoculating loop (white) was dipped into the urine sample and carefully removed vertically to avoid residual urine on the loop. A single streak was made across the centre, thereafter the inoculum was evenly spread in a zigzag-cross pattern.
3. The end of the same 1- µL loop was re-dipped into the urine sample and carefully removed vertically avoiding any residual urine up the loop.
4. The inoculum was dipped and spread over the surface of a glucose-topped MacConkey agar plate (60x15mm).
5. At 35-37^{0}C for 18-24 hours, the plates were incubated aerobically.

6. On the surface of each medium, the numbers of colonies were counted the following day. Each colony growing on the agar plates it's important to note that, they represent 1 colony forming unit (cfu/µL)- according to the loop size, which is equivalent 1000 cfu/mL.
    - We should remember that nutrient agar is the primary medium used for colonies counting.

3.5.3 Microbiological Media

1. Non-selective medium (Blood agar) and selective and differential for Gram-negative rods (MacConkey agar) are most certainly the most recommended and used media for routine urine cultures, according to guidelines. Other options that also stand as a standard media for urine culture may include, cysteine lactose electrolyte-deficient (CLED) agar or chromogenic agar.
2. Blood agar can be used in conjunction with Level-2 advanced bacterial identification for Gram-positives, only if needed. MacConkey agar is a widely used medium for other kinds of bacterial cultures such as cerebrospinal fluid and pus. MacConkey agar with glucose increases fast differentiation amongst glucose-fermenters (especially *Enterobacterales*), which shows a pink colonies in spite of their strengths and weaknesses to ferment lactose, also including the non-fermenters (such as *Pseudomonas* spp. and *Actinobacter* spp.) which the colonies may appear to be colorless.
3. In addition to the standard bacterial media, Sabouraud dextrose agar (SDA) should be added to culture the patient's urine sample in specific care units or if by microscopic examination, yeasts were observed. Nutrient agar, MacConkey agar and glucose-topped MacConkey agar can be used for routine urine culture, but the choice of which to used is based on the resources available and the preferred approach for identification. In majority of cases, blood agar is substituted with nutrient agar so as to reduce costs being that Gram-negative has higher pathogens.

3.6 BIOCHEMICAL IDENTIFICATION OF COMMON BACTERIAL UROPATHOGENS

To decide the kind of species/genus of the concerned bacterium, the positive urine culture is in most cases tagged with different biochemical identification.

3.6.1 Procedure for Basic Identification

1. The capacity to grow on nutrient agar and MacConkey agar plates were examined and registered.
2. The capacity to ferment glucose or lactose on glucose-topped MacConkey agar plates were examined and registered.
3. The capacity to ferment lactose on standard MacConkey agar plates were examined and registered.
4. From an isolated colony, a Gram-stained smear was carried out and examined.
5. A standard oxidase test was carried out and registered for Gram-negative rods.
6. A standard ctalase test was carried out and registered for Gram-positive cocci.
7. A standard pyrrolidone-arylamidase (PYR) and Lancefield test was carried out and registered for catalase-negative Gram-positive cocci. The Lancefield test basically detect streptococci group B or D.

3.6.2 Procedure for Advanced Identification

3.6.2.1 *Enterobacterales*

1. The results of standard tests for indole, citrate, urase, motility, hydrogen sulfide (H_2S) production were carried out and registered.
2. The presence/absence of nitrites from the urine dipstick test was recovered. Its presence points out a positive nitrates reduction test.
3. The results of a viable biochemical identification strip for *Enterobacterales* was carried out and registered in very extreme cases or when needed.

3.6.2.2 Staphylococci (Catalase-Positive)

1. Samples are sub-cultured on blood agar and mannitol salt agar, and then a novobiocin antibiotic disc is placed at the centre of the initial-streaked spot on the blood agar plate (where the heaviest growth is expected to be seen). The blood agar plates were incubated

in a 5-10% CO_2 and the mannitol salt agar plates were air conditioned for 18-24 hours at 35-37^{0}C.

2. The incidence of complete, partial or no hemolysis was carried out and recorded.
3. The capacity to ferment mannitol was also carried out and recorded.
4. The sensitivity of novobiocin was also carried out and recorded.
5. The results of a standard rapid slide agglutination test for *Staphylococcus aureus.* The presence of a clumping factor, protein A and capsular polysaccharides are peculiar to *S. aureus*.
6. The results of a viable biochemical identification strip for staphylococci was carried out and registered in very extreme cases or when needed.

3.6.2.3 Enterococci (Catalase-Negative)

1. Samples are sub-cultured on blood agar and bile esculin agar. The blood agar plates were incubated in a 5-10% CO_2 and the bile esculin agar plates were air conditioned for 18-24 hours at 35-37^{0}C.
2. The incidence of complete, partial or no hemolysis was carried out and recorded.
3. The capacity to grow on a bile medium was also carried out and recorded.
4. The ability to ferment esculin was also carried out and recorded.
5. The results of a standard 6.5% sodium chloride tolerance test was carried out and recorded.
6. The results of a viable biochemical identification strip for streptococci was carried out and registered in very extreme cases or when needed.

3.6.2.4 Glucose Non-Fermenting Gram-Negative Rods

1. For oxidase positive, they were sub-cultured on Cetrimide agar and incubated in air conditions for 18-24 hours at 35-37^{0}C. The productions of pigments were examined.
2. The results of standard tests for indole, citrate, urase, motility, hydrogen sulfide (H_2S) production were carried out and registered.
3. The presence/absence of nitrites from the urine dipstick test was recovered. Its presence points out a positive nitrates reduction test. Nevertheless, it's possible that the absence of nitrites could be as a result of negative nitrate reduction test.

4. The results of a viable biochemical identification strip for non-*Enterobacterales* Gram-negative rods was carried out and registered in very extreme cases or when needed.

3.7 SCREENING METHODS FOR ESBL PRODUCTION

3.7.1 Antibiotic Susceptibility Test

It's a phenotypic confirmatory test which is carried by the use of a Disc Diffusion Test (Kirby-Bauer) for *Klebsiella, E. Coli* and *Proteus Mirabilis*; procedures were followed after the guiding principles of the Clinical Laboratory Standard Institute (CLSI-2010).

1. The antibiotic discs (such as Cefpodoxime (30 µg), Ceftazidime (30 µg), Aztreona, Cefotaxime(30 µg), or Ampicillin (30 µg) can be used) are being placed at definite zone diameter of high level of suspicion for ESBL production.
2. 0.5 McFarland turbidity standard in a 0.85% saline and lawn culture was made from pure culture of proposed bacteria on Muller Hinton Agar Media (Hi-media) and spread using sterile swabs.
3. Agar plates were aerobically incubated for 16-18 hours at 37^0C.
4. *Klebsiella pneumonia* ATCC 700603 (positive control) and *Escherichia coli* strain ATCC 25922 (negative control) was used for quality control.

According to the initial CLSI guidelines, the approved zone diameter is ≤22mm for 10 µg Cefpodoxime disc but it lacks specificity when used to screen *E. Coli* isolates for ESBL production. But was later changed to ≤17mm for isolates with Cefpodoxime, would undergo phenotypic confirmatory tests for ESBL production.

3.7.2 Detection of ESBL Isolates

1. The use of E-test ESBL strips (AB Biodisk) impregananted with Cefotaxime (CT), Cefotaxime/Clavulanate (CTL) And Ceftazidime (TZ), Ceftazidime/Clavulanate (TZL) is used a confirmatory test for ESBL-producing strains, according to the manufacturer's guidelines.
2. The emergence of a phantom zone or deformation of the CT or TZ ellipse or either CT or TZ shows the presence of ESBL. In the presence of Clavulanic acid, the Minimum Inhibitory Concentration (MIC) was condensed by ≥ 3 log 2 dilutions.

3.7.3 Agar supplemented with Clavulanate

1. Here, Mueller-Hinton agar was complemented with 4 µg/ml of clavulanate.
2. On the clavulanate containing agar and on a regular clavlanate free Mueller-Hinton agar plates, antibiotics discs containing ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), and aztreonam (30 µg) were placed on them.
3. For the ESBL production to be considered positive, a divergence in the width of ß-lactam zone of ≥ 10mm on each media.
4. The preparation of fresh plates containing clavulanate is the most important limitations as it effectiveness decreases after 72 hours.

3.8 STATISTICAL ANALYSIS

Qualitative and quantitative data values along with the percentage and mean ± standard deviation (SD) is represented as frequency. The Chi-square test is tested as appropriate on the association between two or more variables. Pictorial explanations of the major results of the study were rendered using an appropriate statistical graph. All statistical analysis was conducted using SPSS version 25.00 statistical packages (SPSS Inc. Chicago, IL, USA). Significance level was accepted to be 0.05.

3.9 ETHICAL APPROVAL

The study protocol was approved by the institutional review boards of Near East University Hospital. The names of the respondents were covered and privacy of data.

CHAPTER IV

Findings and Discussion

The result of this research work shows that 71 (40.80%) clinical samples were positive to ESBL (Extended Spectrum β-lactamase) and 103 (59.91%) samples negative to ESBL were collected from the Near East University Hospital between January 2018 and December 2022. But for the sake of this study, we would be focusing on those positive to ESBL. The study subjects were made up of patients of different age groups and females only attending the various departments of the hospital for *E. coli*.

Table 4.1. Distribution of the gender-based of *E. coli, Klebsiella* and *K. pneumoniae* samples

Gender	Number	%	Valid (%)	Cumulative (%)
Female	71	100	100	100
Total	71	100	100	100

Since the study was basically addressing only females, the total population was 71 patients (100%).

Table 4.2. Distribution of the age-based of *E. coli, Klebsiella* and *K. pneumoniae* samples

Age	Number	%	Valid (%)	Cumulative (%)
18-23	22	30.98	30.98	30.98
24-29	24	33.80	33.80	64.78
30-35	9	12.67	12.67	77.45
36-40	6	8.45	8.45	85.9
41-45	10	14.08	14.08	99.98

The minimum age difference of patients from whom clinical samples were collected ranges from 18-45 years of age. Statistical Analysis of the ages reveals the following frequencies and percentages (%). 22 (30.98%) for 18-23 years, 24 (33.80%) for 24-29 years, 9 (12.67%) for 30-

35 years, 6 (8.45%) 36-40 years and 10 (14.08%) for 41-45 years. From the information above, it shows that age 24-29 years had the highest percentage in response to positive ESBL.

Table 4.3. Distribution of *E. coli, Klebsiella* and *K. pneumoniae* samples from the various hospital departments

Department	Number	%	Valid (%)	Cumulative (%)
Urology	26	36.62	36.62	36.62
Emergency	10	14.08	14.08	50.70
Laboratory	8	11.26	11.26	61.96
Internal Medicine	6	8.45	8.45	70.41
Obstetrics	12	16.90	16.90	87.31
Nephrology	1	1.40	1.40	88.70
Infection	1	1.40	1.40	90.11
Oncology	1	1.40	1.40	91.51
Brain Surgery	2	2.81	2.81	94.32
Anesthesia	1	1.40	1.40	95.72
Rheumatology	1	1.40	1.40	97.12
Orthopedics and Traumatology	1	1.40	1.40	98.52
Cardiology	1	1.40	1.40	99.92

From the above information, it shows that the Urology department ranks the highest with 26 patients (36.62%). Followed by Obstetrics and Gynecology department with 12 (16.90%) for the period. This shows that number is proportional to the requesting department, the two departments that ranked highest are department focused on the female system and their concerns (such as child birth, pregnancy and midwifery).

Table 4.4. Distribution of *E. coli, Klebsiella* and *K. pneumoniae* samples

Micro-Organisms	Number	%	Valid (%)	Cumulative (%)
E. coli	60	84.50	84.50	84.50
Klebsiella spp	1	1.40	1.40	85.90
K. pneumoniae	10	14.08	14.08	99.98

The major pathogens associated with urinary tract infections are *E. coli* and *Klebsiella pneumoniae,* from the table it showed that *E. coli* was present in 60 out of 71 patients (84.50%) and *K. pneumoniae* with 10 (14.08%). Also, other *Klebsiella spp* present in just 1 (1.40%) patients with positive ESBL.

Table 4.5. Distribution of antibiotics among *E. coli, Klebsiella* and *K. pneumoniae* samples

NON-REACTIVE ANTIBIOTICS

Ampicillin

Reactions	Number	%	Valid (%)	Cumulative (%)
Sensitive	0	0	0	0
Resistance	62	87.32	87.32	87.32
Blanks	9	12.67	12.67	99.99

Ampicillin wasn’t sensitive to the micro-organisms amongst patients positive to ESBL, instead a higher percentage were non-reactive 62 (87.32%) and 9 (12.67%) samples yielded no result.

Cefixime

Reactions	Number	%	Valid (%)	Cumulative (%)
Sensitive	0	0	0	0
Resistance	66	92.95	92.95	92.95
Blanks	5	7.04	7.04	99.99

Same as Ampicillin, the micro-organisms had no reaction to Cefixime but 66 (92.95%) samples were resistant to the antibiotics and 5 (7.04%) yielded no result.

Ceftazidime

Reactions	Number	%	Valid (%)	Cumulative (%)
Sensitive	0	0	0	0
Resistance	71	100	100	100
Blanks	0	0	0	0

Of all the samples positive to ESBL, there was no record of reaction and blank to Ceftazidime, they all tested non-reactive to the antibiotics.

REACTIVE ANTIBIOTICS

Ceftriaxone

Reactions	Number	%	Valid (%)	Cumulative (%)
Sensitive	1	1.40	1.40	1.40
Resistance	66	92.95	92.95	94.35
Blanks	4	5.63	5.63	99.98

In the case of Ceftriaxone, the reaction rate is low 1 (1.40%) out of 71 samples and 4 (5.63%) blanked results. The non-reactive was 66 (92.95%).

Amoxicillin/Clavulanate

Reactions	Number	%	Valid (%)	Cumulative (%)
Sensitive	5	7.04	7.04	7.04
Resistance	27	38.02	38.02	45.06
Blanks	39	54.92	54.92	99.98

From the table above, it shows that only 5 samples (7.04%) where sensitive to Amoxicillin/Clavulanate, 27 samples (38.02%) where resistant to the antibiotics and 39 samples (54.92%) when tested with Amoxicillin/Clavulanate showed no result.

Ciprofloxacin

Reactions	Number	%	Valid (%)	Cumulative (%)
Sensitive	32	45.07	45.07	45.07
Resistance	32	45.07	45.07	90.14
Blanks	1	1.40	1.40	91.54
Less Sensitive	6	8.45	8.45	99.99

The table shows that the rate of reaction and non-reactive is directly proportional to each other with 32 samples (45.07%), only 1 (1.40%) blank and 6 (8.45%) samples were less sensitive and not completely non-reactive.

Gentamicin

Reactions	Number	%	Valid (%)	Cumulative (%)
Sensitive	54	76.05	76.05	76.05
Resistance	16	22.53	22.53	98.58
Blanks	1	1.40	1.40	99.98

In the case of Gentamicin, a higher percentage of the sample were sensitive to the antibiotics with 54 (76.05%) with just 1 (1.40%) blank and 16 (22.53%) were resistant to Gentamicin.

Trimethoprim/Sulfamethoxazole

Reactions	Number	%	Valid (%)	Cumulative (%)
Sensitive	29	40.84	40.84	40.84
Resistance	42	59.15	59.15	99.99
Blanks	0	0	0	0

Here, 29 (40.84%) samples were sensitive to Trimethoprim/Sulfamethoxazole and 42 (59.15%) were resistant to the antibiotics, didn't show any sign of reaction. They also all yielded results either positive or negative, no blank results.

Nitrofurantoin

Reactions	Number	%	Valid (%)	Cumulative (%)
Sensitive	53	74.67	74.67	74.67
Resistance	7	9.85	9.85	84.52
Blanks	10	14.08	14.08	98.60
Less Sensitive	1	1.40	1.40	100

The table shows that the rate of reaction of the uropathogens is 53 (74.67%) and non-reactive with 7 samples (9.85%), only 10 (14.08%) blank and 1 (1.40%) sample was less sensitive and not completely non-reactive.

Piperacillin/Tazobactam

Reactions	Number	%	Valid (%)	Cumulative (%)
Sensitive	55	77.46	77.46	77.46
Resistance	14	19.71	19.71	97.17
Blanks	1	1.40	1.40	98.57
Less Sensitive	2	2.81	2.81	100

The table shows that the rate of positive reaction of the uropathogens to the antibiotics appeared on 55 (77.46%) samples and non-reactive with 14 samples (19.71%), only 1 (1.40%) blank and 2 (2.81%) samples were less sensitive and not completely non-reactive.

CHAPTER V

Discussion

A pathogen that produces extended spectrum lactamases (ESBLS) is a member of the Enterobacteriaceae family, especially *Escherichia coli* and *Klebsiella pneumoniae.* They are mostly found in healthcare settings and communities. They always find new defense mechanisms known as the resistance mechanisms, to be able to withstand the antibiotics. The enzymes produced by the ESBL- Enterobacterales can breakdown and destroy the effectiveness of some of the commonly used antibiotics used in treating infections such as Penicillins and Cephalosporins. In 2017, it was predicted at about 197,400 cases were positive to ESBL from the hospitalized patients and 9,100 deaths cases estimated. These infections affects people exposed to healthcare, those in the hospitals and nursing homes. Amongst the healthy population, it affects those with urinary tract infections. Their resistance to the antibiotics poses them at the edge of fewer options. Most cases of widespread infections caused by ESBL-producing pathogens like urinary tract infections, needs more profound treatments regime. Alternatively to the usual oral antibiotics at home, patients are now expected to be hospitalized and administered intravenously carbapenem antibiotics.

As a result, they are often associated with serious infections and high mortality rates. A very essential cause of UTI has being recorded over the years to be the multidrug-resistant ESBL *E. coli* (CTC-M enzyme type). The strains of ESBL are known for their ability to hydrolyze as much beta-lactam antibiotics as possible effectively not excluding the 3rd generation cephalosporins and monobactams. As ESBL producing strains acquire a transmissible form of antibiotic resistance it is being reported that antibiotics (clavulanic acid, sulbactam, and tazobactam) generally inhibit them. In opposition to ESBL positive, it shows that penicillin and cephalosporins that were used over the years no longer works. The correlation of β-lactamase and esbl genes (CTX-M, TEM, SHV, OXA and AMP-C types) pass through a process of change at the nucleotide and amino acid sequence, along the line four classes A, C, D (serine based mechanism) and B (metallo β-lactamase) were noticed. Pathogenic enterobacteria causes a severe antibiotic management problem especially those produced from ESBL, as their genes are known to be moved easily from one organism to another via plasmids. Nosocomial organisms

containing ESBL are becoming more rampant than community acquired infections, specifically found in the urinary tract. Among nosocomial isolates, a study carried out in South Indian showed the high prevalence of multidrug resistant CTX-M type ESBL. It was deduced from North Indian tertiary referral hospital, the presence of blaNDM-1 gene from Clinical isolates of *Actinobacter baumannii.* Most urinary tract infections, respiratory tract infections, endocarditis are known to be caused by ESBL producers leading to high cost of treatment, morbidity and mortality. In recent times, the spread of ESBL-producing organisms has being a contributing factor to fecal carriage, intestinal colonization, international travel and household member transmission in public health. A proportional study was carried and it was reported that 68.78% of gram negative bacteria were discovered to be ESBL producers. New antibiotics and combinations would be introduced to conquer the ongoing danger posed by ESBL pathogens, as they are now resistant to the usual antibiotics used for treatment.

The worldwide prevalence of Extended-Spectrum Beta-Lactamase ESBL is rising on a regular, bringing to urgency the need for optimized detection techniques. The European Antimicrobial Resistance Surveillance System (EARSS) in an annual report in 2008, they came to a conclusion that in almost all European countries, the resistance of *E.Coli* to extended spectrum cephalosprins has increased drastically since 2001.

In a report, the clinical presentation, management, and clinical outcome in seven patients affected by upper UTI due to ESBL positive *E. coli* were looked into. It was advised that an early recognition and treatment of acute infections of the kidney, patients with suspected urinary tract obstruction were of supreme importance. There was a noticeable rise in ESBL positive due to the use of regular and commonly used antibiotics and in most cases resistance to the antibiotics.

The samples collected from the Near East University Hospital included females that where positive to ESBL from January 2018-December 2021, which came to 71 (40.80%) patients and 103 (59.91%) negative to ESBL. We decided to focus on the female patients because they are more at prone to infections such as the urinary tract infections and other relating infections than the male gender. As explained earlier, due to their shorter urethra and its closeness to the bladder and rectum.

The results were group based on their ages from 18-45 years of age because females between these groups are either sexually active or premenopausal stage. It was deduced that patients between ages 24-29 had the highest number in relation to positive ESBL with 24 (33.80%) samples. The difference between the earlier mentioned age group and 18-23 years of age is by approximately 3% lesser with 30.98%. The least age group was 36-40 years of age with 6 (8.45%) samples. The third highest was 41-45 years of age of 10 (14.08%) samples, most women in this age group are usually close to their menopausal age or very few already at that stage, it could also be because of their past sexual relationship and lastly at these stage, they also have dry vaginal, which makes them prone to urinary tract infections.

Based on their department, Urology came out to be the highest requesting department with 26 (36.62%) samples out of 71. The department from its name focus on the concerns of urinary tract infections in the male and female but for the purpose of this study, we would be focusing on the female. Followed by the obstetrics and gynecology department with 12 (16.90%) samples. As earlier mentioned, department focusing on the concerns of women (such as pregnancy, midwifery, and childbirth) ranked the highest. Other departments such as Infection, Oncology, Nephrology, Rheumatology, Cardiology and Anesthesia, all had 1 (1.40%) sample each. The other departments like Emergency, Laboratory and Internal Medicine had 10 (14.08%), 8 (11.26%) and 6 (8.45%) respectively.

In the case of the kind of micro-organisms detected, *E. coli* was present in 60 (84.50%) samples and *K. pneumoniae* was present in 10 (14.08%), this proof that *E. coli* is a truly predominant uropathogen.

Antibiotics were also tested with these micro-organisms to know which is sensitive and resistance. For the non-reactive antibiotics, Ampicillin, was resistant in 62 (87.32%) samples and 9 (12.67%) came out with no result. For Cefixime, 66 (92.95%) samples were resistant and 5 (7.04%) samples came out with no result. Of all the samples positive to ESBL, there was no record of reaction and blank to Ceftazidime, they all tested non-reactive to the antibiotics. For the reactive antibiotics, it shows that only 5 samples (7.04%) where sensitive to Amoxicillin/Clavulanate, 27 samples (38.02%) where resistant to the antibiotics and 39 samples (54.92%) when tested with Amoxicillin/Clavulanate showed no result. In the case of Ceftriaxone, the reaction rate is low 1 (1.40%) out of 71 samples and 4 (5.63%) blanked results. The non-

reactive was 66 (92.95%). The rate of reaction and non-reactive of Ciprofloxacin is directly proportional to each other with 32 samples each (45.07%), only 1 (1.40%) blank and 6 (8.45%) samples were less sensitive and not completely non-reactive. In the case of Gentamicin, a higher percentage of the sample were sensitive to the antibiotics with 54 (76.05%) with just 1 (1.40%) blank and 16 (22.53%) were resistant to Gentamicin. Here, 29 (40.84%) samples were sensitive to Trimethoprim/Sulfamethoxazole and 42 (59.15%) were resistant to the antibiotics, didn't show any sign of reaction. They also all yielded results either positive or negative, no blank results. The rate of reaction of the uropathogens to Nitrofurantoin is 53 (74.67%) and non-reactive with 7 samples (9.85%), only 10 (14.08%) blank and 1 (1.40%) sample was less sensitive and not completely non-reactive. The rate of positive reaction of the uropathogens to the antibiotics, Piperacillin/Tazobactam appeared on 55 (77.46%) samples and non-reactive with 14 samples (19.71%), only 1 (1.40%) blank and 2 (2.81%) samples were less sensitive and not completely non-reactive.

CHAPTER VI

Conclusion and Recommendation

CONCLUSION

Finally, our findings support the prevalence of *E. coli* primarily amongst urinary tract infections patients' especially female patients. Results from the study verified the prevalence of ESBL-producing pathogens amongst UTI patients. It also showed that the most commonly used antibiotics for treatment are becoming less effective. In the case of Amoxicillin, a higher percentage of patients that were tested yielded no results to the antibiotics either positive or negative.

RECOMMENDATION

As earlier mentioned, the rise of multiple bacteria antibiotic resistance is becoming bothersome by the period especially in UTI. In hospital settings, urologists should be advised to act timely as the cases of Multi-Drug resistant *E. coli* and UTI as a result of ESBL positive *E. coli* are interestingly being encountered. The management of this kind of infections is really important for future referencing, in other to curb the rise of new antibiotic resistance patterns.

REFERENCE

Abdelrahman A., A. T. (2019). Methods of ESBLs Detection in Clinical Microbiology Lab. Virology & Immunology Journal.

Aguilar-Duran S., Horcajada J.P., Sorli L., Montero M., Salvadó M., GrauS., Gómez J., Knobel H. (2012). Community-onset healthcare-related urinary tract infections: comparison with community and hospital-acquired urinary tract infections. . *J Infect* , 64:478–483.

Ahmed H, Davies F, Francis N, et al. (2017). Long-term antibiotics for prevention of recurrent urinary tract infection in older adults: systematic review and meta-analysis of randomised trials. *BMJ Open* , 7: e015233.

Al Naiemi, N., J. L. Murk, P. H. M. Savelkoul, C. M. J. VanderbrouckeGrauls, and Y. J. Debets-Ossenkopp. 2009. Extended-spectrum beta-lactamases screening agar with AmpC inhibition. Eur. J. Clin. Microbiol. Infect. Dis. 28:989–990.

American Urological Association, U. C. (2021.). *What is a Urinary Tract Infection (UTI) in Adults?*

Anantha R.P, McVeigh A.L, Lee L.H, Agnew M.K, Cassels F.J, Scott D.A, Whittam T.S, Savarino S.J. (2004). Evolutionary and functional relationships of colonization factor antigen i and other class 5 adhesive fimbriae of enterotoxigenic Escherichia coli. . *Infect Immun.* , 72:7190–7201. .

Anderson, G. G., Dodson, K. W., Hooton, T. M. and Hultgren, S. J. (2004). 'Intracellular bacterial communities of uropathogenic Escherichia coli in urinary tract pathogenesis'. *Trends Microbiology* , 12(9), 424-430. .

Andrea G.H., M. E. (2014). Urinary Tract Infection and Asymptomatic Bacteriuria Guidance. *Antimicrobial Stewardship Subcommittee of Pharmacy and Therapeutics Committee of the Nebraska Medical Center.*

Aydin A., Ahmed K., Zaman I., Khan M.S., Dasgupta P. . (2015). Recurrent urinary tract infections in women. . *Int Urogynecol J* , 26:795–804.

Bartoletti R, Cai T, Wagenlehner F.M, Naber K, Bjerklund Johansen T.E. . (2016). Treatment of Urinary Tract Infections and Antibiotic Stewardship. *Eur Urol Suppl* , 15:81-87.

Beerepoot M.A., Geerlings S.E., Van Haarst E.P., Van Charante N.M., Ter Riet G. (2013). Nonantibiotic prophylaxis for recurrent urinary tract infections: a systematic review and meta-analysis of randomized controlled trials. . *J Urol* , 190:1981–1989.

Berry J.L, Pelicic V. . (2015). Exceptionally widespread nanomachines composed of type IV pilins: the prokaryotic Swiss Army knives. . *FEMS Microbiol Rev.* , 39:134–154.

Birgul K (2010) Investigation of extended spectrum beta lactamase production of bacteria by direct urine inoculation. African J Microbiol Res 4: 1087-1090.

Bleidorn, J., Gágyor, I., Kochen, M. M., Wegscheider, K. & Hummers-Pradier, E. (2010). Symptomatic treatment (ibuprofen) or antibiotics (ciprofloxacin) for uncomplicated urinary tract infection? results of a randomized controlled pilot trial. *BMC Med.* , 8, 30.

Boedeker E.C. (2005). Enteric infections. *Curr Opin Gastroenterol.* , 21:1–3.

Briongos- Figuero LS, Gómez- Traveso T, Bachiller- Luque P, Domínguez- Gil González M, Gómez- Nieto A, Palacios- Martín T, et al.(2012) Epidemiology, risk factors and comorbidity for urinary tract infections caused by extended- spectrum beta- lactamase (ESBL)- producing enterobacteria. Int J Clin Pract; 66: 891- 896.

Cai T, Mazzoli S, Mondaini N, et al. . (2012). The role of asymptomatic bacteriuria in young women with recurrent urinary tract infections: to treat or not to treat? *Clin Infect Dis* , 55: 771–777.

Cane G, Moal V.L, Pages G, Servin A.L, Hofman P, Vouret-Craviari V. (2007). Up-regulation of intestinal vascular endothelial growth factor by Afa/Dr diffusely adhering Escherichia coli. *PloS one.* , 2:e1359.

Carol A. Kauffman. (2014). Diagnosis and Management of Fungal Urinary Tract Infection. *Infect Dis Clin* , 28: 61–74.

Centers for Disease Control and Prevention. (10/21/2021.). Urinary Tract Infection. *(https://www.cdc.gov/antibiotic-use/community/for-patients/common-illnesses/uti.html)* .

Christiaens, T. C. et al. (2002). Randomised controlled trial of nitrofurantoin versus placebo in the treatment of uncomplicated urinary tract infection in adult women. . *Br. J. Gen. Pract.* , 52, 729–734 .

Coker, C., Poore, C. A., Li, X. and Mobley, H. L. . (2000). 'Pathogenesis of Proteus mirabilis urinary tract infection', . *Microbes Infection* , 2(12), 1497-1505. .

Costa T.R, Felisberto-Rodrigues C, Meir A, Prevost M.S, Redzej A, Trokter M, Waksman G. . (2015). Secretion systems in Gram-negative bacteria: structural and mechanistic insights. *Nat Rev Microbiol.* , 13:343–359. .

Dalhoff A. . (2012). Global fluoroquinolone resistance epidemiology and implictions for clinical use. *Interdiscip Perspect Infect Dis* , 2012:976273.

Dason S, Dason J.T, Kapoor A. . (2011). Guidelines for the diagnosis and management of recurrent urinary tract infection in women. . *Can Urol Assoc J* , 5:316-322.

Drekonja D.M., Rector T.S., Cutting A., Johnson J.R. (2013). Urinary tract infection in male veterans: treatment patterns and outcomes. *JAMA Intern Med.* , 173:62-68.

Duane S, Vellinga A, Murphy A.W, Cormican M, Smyth A, Healy P, et al. . (2019). COSUTI: a protocol for the development of a core outcome set (COS) for interventions for the treatment of uncomplicated set (COS) for interventions for the treatment of uncomplicated urinary tract infection (UTI) in adults. *Trials* , 20.

Dziva F, van Diemen P.M, Stevens M.P, Smith A.J, Wallis T.S. . (2004). Identification of Escherichia coli O157: H7 genes influencing colonization of the bovine gastrointestinal tract using signature-tagged mutagenesis. . *Microbiology.* , 150:3631–3645.

Foley, K.F., and J. Wasserman. . (2021). Are unexpected positive dipstick urine bilirubin results clinically significant? A retrospective review. *Lab Med 45.1 Winter* , 59-61.

Foxman B. . (2014). Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. . *Infect Dis Clin North Am* , 28: 1.

Foxman, B. (2010). The epidemiology of urinary tract infection . *Nat. Rev. Urol.* , 7, 653–660.

Fu, Z. L. (2017). Cranberry reduces the risk of urinary tract infection recurrence in otherwise healthy women: A systematic review and meta-analysis. *The Journal of Nutrition* , 147(12), 2282-2288.

Gandhi T., Flanders S.A., Markovitz E., et al. . (2009). Importance of urinary tract infection to antibiotic use among hospitalized patients. . *Infect Control Hosp Epidemiol.* , 30:193-195.

Glenn T. W and David G. Thanassi. (2018 Mar). Pili Assembled by the Chaperone/Usher Pathway in Escherichia coli and Salmonella. *EcoSal Plus* , 8(1): 10.1128.

Gould C., Umscheid C., Agarwal R., Kuntz G., Pegues D.,. (2011). Guideline for prevention of catheter-associated urinary tract infections. *Healthcare Infection Control Practices Advisory Committee (HICPAC).* .

Gupta K., Hooton T.M., Naber K.G., et al. (2011). International Clinical Practice Guidelinesfor the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin Infect Dis* , 52: 103-120.

Han L.Y., Lee Y.S. . (2017). Risk factor affecting recurrence of cystitis after Urovaxom treatment for female patients with recurrent cystitis. . *Neurourol Urodynamics* , 36: S104.

Handley, M. A., Reingold, A. L., Shiboski, S. and Padian, N. S. . (2002). 'Incidence of acute urinary tract infection in young women and use of male condoms with and without nonoxynol-9 spermicides'. *Epidemiology* , 13(4), 431-436.

Harwalkar A, Sataraddi J, Gupta S, Yoganand R, Rao A, et al. (2013) The detection of ESBL-producing Escherichia coli in patients with symptomatic urinary tract infections using different diffusion methods in a rural setting. J Infect Public Health 6: 108-114.

Hooton T., Bradley S., Cardenas D., Colgan R., Geerlings S., Rice J., Saint S., Schaeffer A., Tambayh P.,Tenke P., Nicolle L. (2010). Infectious Diseases Society of America Guidelines for the diagnosis, prevention, and treatment of catheter-associated urinary tract infections in adults. *Clin InfectDis.* , 50: 625-663.

Hooton T.M. . (2012). Clinical practice. Uncomplicated urinary tract infection. *N Eng J Med* , 366:1028–1037.

Hooton T.M., Bradley S.F., Cardenas D.D., Colgan R., Geerlings S.E., RiceJ.C., Saint S., Schaeffer A.J., Tambayh P.A., Tenke P., Nicolle L.E. (2010). Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. . *Clin Infect Dis* , 50:625–663.

Humphries A.D, Raffatellu M, Winter S, Weening E.H, Kingsley R.A, Droleskey R, Zhang S, Figueiredo J, Khare S, Nunes J, Adams L.G, Tsolis R.M, Baumler A.J. (2003). The use of flow cytometry to detect expression of subunits encoded by 11 Salmonella enterica serotype Typhimurium fimbrial operons. *Mol Microbiol.* , 48:1357–1376. .

Jepson, R. (2004.). Urinary tract infections (UTIs). *Cochrane Database Syst Rev.*

Johansen T.E., Botto H., Cek M., Grabe M., Tenke P., Wagenlehner F.M., Naber K.G. (2011). Critical review of current definitions of urinary tract infections and proposal of an EAU/ESIU classification system. . *Int J Antimicrob Agents* , 38(Suppl):64–70.

Justice, S. S., Hung, C., Theriot, J. A., Fletcher, D. A., Anderson, G. G., Footer, M. J. and Hultgren, S. J. . (2004). 'Differentiation and developmental pathways of uropathogenic Escherichia coli in urinary tract pathogenesis. *Proc Natl Acad Sci U S A,* , 101(5), 1333-1338.

Kau, A. L., Hunstad, D. A. and Hultgren, S. J. . (2005). 'Interaction of uropathogenic Escherichia coli with host uroepithelium', . *Curr Opin Microbiology* , 8(1), 54-59.

Kenneth S, Thomson (2010) Extended-spectrum-β-lactamase, AmpC, and Carbapenemase J.Clin Microbiol 48: 1019-102.

Khan, S. W. and Ahmed, A. . (2001). Uropathogens and their susceptibility pattern: a retrospective analysis. *J Pak Med Assoc* , 51(2), 98-100.

Kisiela D, Sapeta A, Kuczkowski M, Stefaniak T, Wieliczko A, Ugorski M. . (2005). Characterization of FimH adhesins expressed by Salmonella enterica serovar Gallinarum biovars Gallinarum and Pullorum: reconstitution of mannose-binding properties by single amino acid substitution. *Infect Immun.* , 73:6187–6190. .

Klumpp, D. J., Weiser, A. C., Sengupta, S., Forrestal, S. G., Batler, R. A. and Schaeffer, A. J. . (2001). Uropathogenic Escherichia coli potentiates type 1 pilus-induced apoptosis by suppressing NF-kappaB'. *Infect Immun* , 69(11), 6689-6695.

Kranz J, Schmidt S, Lebert C, Schneidewind L, Mandraka F, Kunze M, et al. . (2018). The 2017 update of the German clinical guideline on epidemiology, diagnostics, therapy, prevention, and management of uncomplicated urinary tract infections in adult patients. Part II:therapy and prevention. *Urol Int* , 100:271–278.

Krieger J.N., Lee S.W., Jeon J., Cheah P.Y., Liong M.L., Riley D.E. (2008). Epidemiology of prostatitis. . *Int J Antimicrob Agents* , 31(Suppl 1):S85–90.

Krzysztof C., Magdalena Broś-Konopielko, Justyna Teliga-Czajkowska. (2021). Urinary tract infection in women . *Menopause Rev* , 20(1): 40-47.

Kuehn, M. J., Ogg, D. J., Kihlberg, J., Slonim, L. N., Flemmer, K., Bergfors, T. and Hultgren, S. J. (1993). Structural basis of pilus subunit recognition by the PapD chaperone . *Science,* , 262(5137), 1234-1241. .

Lin E, Bhusal Y, Horwitz D, et al. . (2012). Overtreatment of enterococcal bacteriuria. . *Arch Intern Med* , 172:33–38.

Linares L.A., Thornton D.J., Strymish J., et al. . (2011). Electronic memorandum decreases unnecessary antimicrobial use for asymptomatic bacteriuria and culture-negative pyuria. *Infect Control Hosp Epidemiol.* , 32:644-648. .

Lingenfelter E, D. Z. (2016). ED pharmacist monitoring of provider antibiotic selection aids appropriate treatment for outpatient UTI. *Am J Emerg Med* , 34, 1600-1603.

Little P., Merriman R., Turner S., Rumsby K., Warner G., Lowes J.A., Smith H., Hawke C., Leydon G., Mullee M., Moore M.V. (2010). Presentation, pattern, and natural course of severe symptoms, and role of antibiotics and antibiotic resistance among patients presenting with suspected uncomplicated urinary tract infection in primary care: observational study. *BMJ* , 340:b5633.

Low AS, Holden N, Rosser T, Roe AJ, Constantinidou C, Hobman JL, Smith DG, Low JC, Gally DL. (2006). Analysis of fimbrial gene clusters and their expression in enterohaemorrhagic Escherichia coli O157:H7. *Environ Microbiol.* , 8:1033–.

Lusardi G., Lipp A., Shaw C. . (2013). Antibiotic prophylaxis for short-term catheter bladder drainage in adults. *Cochrane Database Syst Rev* .

Malmartel A., Ghasarossian C.H. . (2016). Bacterial resistance in urinary tract infections in patients with diabetes matched with patients without diabetes. . *J Diabetes Complications* , 30: 705-709.

Manoharan A, Premalatha K, Chatterjee S, Mathai D; SARI Study Group (2011) Correlation of TEM, SHV and CTX-M extended-spectrum beta lactamases among Enterobacteriaceae with their in vitro antimicrobial susceptibility. Indian J Med Microbiol 29: 161-164.

Mandy A., S. V. (2020, December 15). Urinary Tract Infections (UTIs) in Women. *GoodRx health* .

Merck Manual. (10/21/2021.). Overview of Urinary Tract Infections (UTIs). . *(https://www.merckmanuals.com/home/kidney-and-urinary-tract-disorders/urinary-tract-infections-utis/overview-of-urinary-tract-infections-utis)* .

Mody L., Juthani-Mehta M. (2014). Urinary tract infections in older women. *JAMA.*, 311:844-854.

Mysorekar, I. U., Mulvey, M. A., Hultgren, S. J. and Gordon, J. I. (2002). 'Molecular regulation of urothelial renewal and host defenses during infection with uropathogenic Escherichia coli',. *J Biol Chem,* , 277(9), 7412-7419.

Neugent M.L, Hulyalkar N.V, Nguyen V.H, Zimmern P.E, De Nisco N.J. . (2020). Advances in understanding the human urinary microbiome and its potential role in urinary tract infection. . *mBio* , 11:e00218-20.

Niall F.D., Hugh D. F. (2011). The Pathogenesis of Urinary Tract Infections. In D. A. (Ed.), *Clinical Management of Complicated Urinary Tract Infection* (pp. 7: 101-113). Ireland : InTech.

Nicolle L., Bradley S., Colgan R., Rice J., Schaeffer A., Hooton T. (2005). Infectious Diseases Society of America Guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. . *Clin Infect Dis.* , 40: 643-654.

Nicolle L. E. (2005) AMMI Canada Guidelines Committee. Complicated urinary tract infection in adults. Can J Infect Dis Med Microbiol. 16:349- 60.

Oscar Storme, José Tirán Saucedo, Arturo Garcia-Mora, Manuel Dehesa-Dávila and Kurt G. Naber . (2019). Risk factors and predisposing conditions for urinary tract infection. *Ther Adv Urol* , Vol. 11: 19–28.

Overdevest I. T., W. I. (2010). *Laboratory Detection of Extended-Spectrum-Beta-Lactamase-Producing Enterobacteriaceae: Evaluation of Two Screening Agar Plates and Two Confirmation Techniques.* JOURNAL OF CLINICAL MICROBIOLOGY.

Parsek, M. R. and Singh, P. K. (2003). 'Bacterial biofilms: an emerging link to disease pathogenesis', . *Annu Rev Microbiol,* , 57, 677-701.

Piatek R, Zalewska B, Bury K, Kur J. (2005). The chaperone-usher pathway of bacterial adhesin biogenesis-from molecular mechanism to strategies of anti-bacterial prevention and modern vaccine design. *Acta Biochem Pol.* , 52:639–646.

Proft T, Baker E. N. (2009). Pili in Gram-negative and Gram-positive bacteria - structure, assembly and their role in disease. . *Cell Mol Life Sci.* , 66:613–635.

Picozzi S, Ricci C, Gaeta M, Macchi A, Dinang E, Paola G, et al. (2013) Do we really know the prevalence of multi- drug resistant Escherichia coli in the territorial and nosocomial population? Urol Annals;5:25-29.

Qadri F, Svennerholm A.M, Faruque A.S, Sack R.B. . (2005). Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. . *Clin Microbiol Rev.* , 18:465–483.

Quinlan J.D, Jorgensen S.K. . (2017). Recurrent UTIs in women: How you can refine your care. . *J Fam Pract* , 66:94- 99.

Rice JC, Peng T, Spence J.S, Wang H.Q, Goldblum R.M, Corthesy B, Nowicki B.J. . (2005). Pyelonephritic Escherichia coli expressing P fimbriae decrease immune response of the mouse kidney. *J Am Soc Nephrol.* , 16:3583–3591.

Roberts J.A, Marklund B.I, Ilver D, Haslam D, Kaack M.B, Baskin G, Louis M, Mollby R. (1994). The Gal(alpha1-4)Gal-specific tip adhesin of Escherichia coli P-fimbriae is needed for pyelonephritis to occur in the normal urinary tract. *Proc Natl Acad Sci U S A.* , 91:11889–11893.

Rodríguez-Baño J, López-Cerero L, Navarro MD, Díaz de Alba P, Pascual A (2008) Faecal carriage of extended-spectrum beta-lactamase-producing Escherichia coli: prevalence, risk factors and molecular epidemiology. J Antimicrob Chemother 62: 1142-1149.

Ronald, A. (2003). The etiology of urinary tract infection: traditional and emerging pathogens. *Dis Mon,* , 49(2), 71-82. .

Schlager, T. A., Hendley, J. O., Bell, A. L. and Whittam, T. S. . (2002). Clonal diversity of Escherichia coli colonizing stools and urinary tracts of young girls. *Infect Immun,* , 70(3), 1225-1229.

Stapleton, A. . (2002). 'Urinary tract infections in patients with diabetes'. *Am J Med* , 113 Suppl.

Sukumaran S.K, Fu N.Y, Tin C.B, Wan K.F, Lee S.S, Yu V.C. (2010). A soluble form of the pilus protein FimA targets the VDAC-hexokinase complex at mitochondria to suppress host cell apoptosis. *Mol Cell.* , 37:768–783.

Subashini K., K. K. (2013). *Screening and Identification of Extended Spectrum β-lactamase (ESBL) Pathogens in Urine Sample of UTI Patient's.* Trop Med Surg. 1:3.

Sun, T. T. (1996). Epithelial growth and differentiation: an overview. *Mol Biol Rep* , 23(1), 1-2.

SUZANNE E. GEERLINGS. (2016). Clinical Presentations and Epidemiology of Urinary Tract Infections. *Microbiology Spectrum* , 4(5):UTI-0002-2012.

Talan, D. A., Stamm, W. E., Hooton, T. M., Moran, G. J., Burke, T., Iravani, A., Reuning Scherer, J. and Church, D. A. (2000). Comparison of ciprofloxacin (7 days) and trimethoprim-sulfamethoxazole (14 days) for acute uncomplicated pyelonephritis pyelonephritis in women: a randomized trial. *JAMA* , 283(12), 1583-1590. .

Tambyah, P. A. & Maki, D. G. . (2000). The relationship between pyuria and infection in patients with indwelling urinary catheters: a prospective studyof 761 patients. . *Arch. Intern. Med.* , 160,673–677 .

Thanassi D.G, Bliska J.B, Christie P.J. (2012). Surface organelles assembled by secretion systems of Gram-negative bacteria: diversity in structure and function. . *FEMS microbiology reviews.* , 36:1046–1082.

US Department of Health and Human Services, Office of Population Affairs. (10/21/2021.). Urinary Tract Infection (UTI). . *(https://www.hhs.gov/opa/reproductive-health/fact-sheets/urinary-tract-infections/index.html)* .

Vincent, C. et al. . (2010). Food reservoir for Escherichia coli causing urinary tract infections. . *Emerg. infect. Dis.* , 16, 88–95.

Wang T., Wu G., Wang J. (2020). Comparison of single-dose fosfomycin tromethamine and other antibiotics for lower uncomplicated urinary tract infection in women and asymptomatic bacteriuria in pregnant women: a systematic review and meta-analysis. . *Inter J Antimicrob Agents* , 56: 106018. .

Weening E.H, Barker J.D, Laarakker M.C, Humphries A.D, Tsolis R.M, Baumler A.J. . (2005). The Salmonella enterica serotype Typhimurium lpf, bcf, stb, stc, std, and sth fimbrial operons are required for intestinal persistence in mice. *Infect Immun.* , 73:3358–3366.

Wing D.A. . (2001). Pyelonephritis in pregnancy: treatment options for optimal outcomes. . *Drugs* , 61:2087–2096.

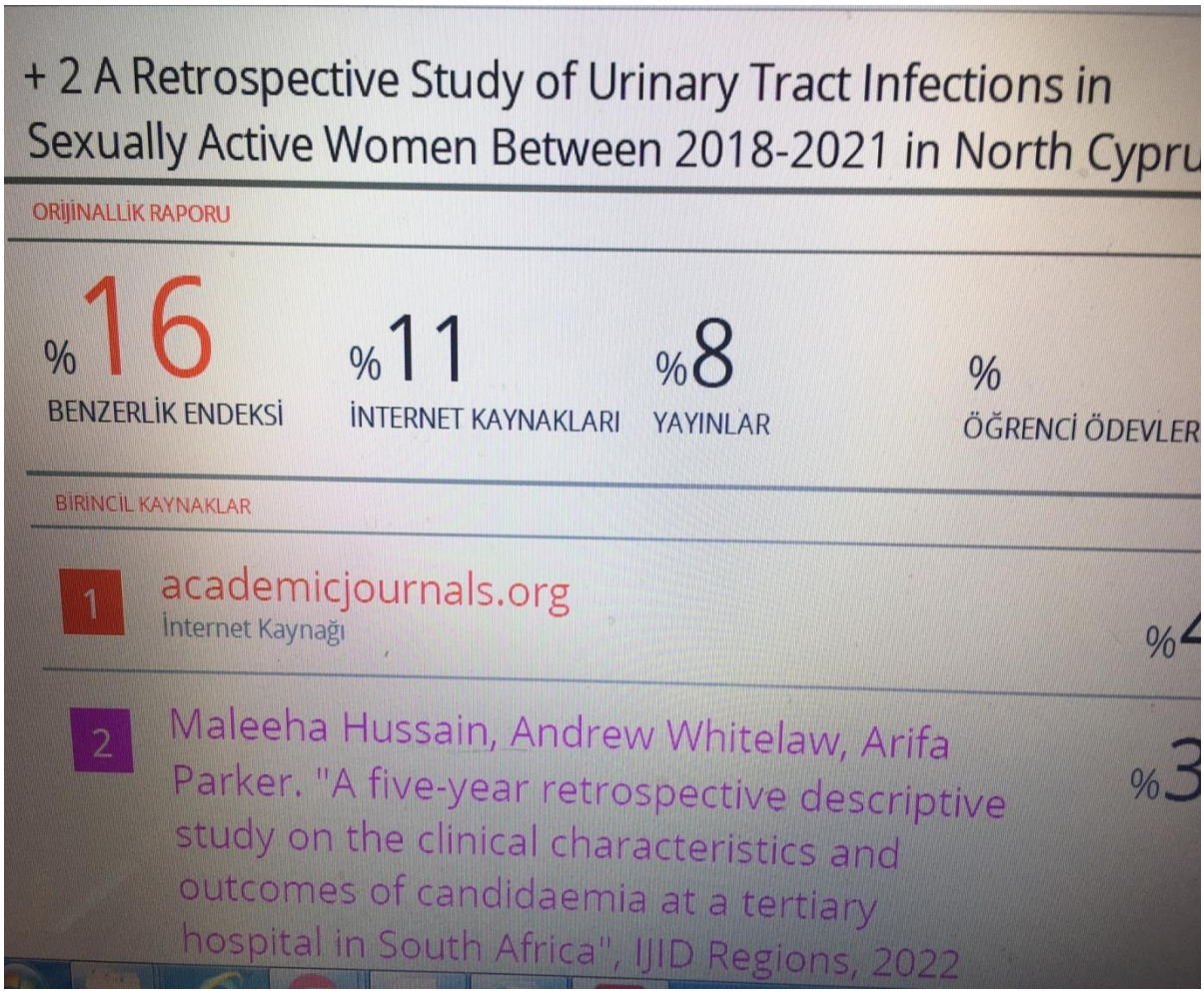
Wu, X. R., Sun, T. T., Medina, J. J. . (1996). In vitro binding of type 1-fimbriated Escherichia coli to uroplakins Ia and Ib: relation to urinary tract infections. *Proc Natl Acad Sci U S A,* , 93(18), 9630-9635. .

Zalmanovici Trestioreanu, A. G. (2010). Antimicrobial agents for treating uncomplicated urinary tract infection in women. *The Cochrane Database of Systematic Reviews* , (10), CD007182.

Zhang, D., Zhang, G., Hayden, M. S., Greenblatt, M. B., Bussey, C., Flavell, R. A. and Ghosh, S. (2004). A toll-like receptor that prevents infection by uropathogenic bacteria',. *Science,* , 303(5663), 1522-1526.

http://www.rivm.nl/earss/result/Monitoring_reports/

Appendices I



Appendices II

CURRICULUM VITAE

Full Name	Anuoluwa Esther	Surname	Shittu
Place of Birth	Lagos, Nigeria	Date of Birth	27-03-1998
Nationality	Nigerian	Telephone Number	+905338454632
Email	shittuanuoluwa@gmail.com		

Education level

	Name of the Institution	Year
Post Graduate/Specialization	Medical and Clinical Microbiology Near East University, North Cyprus	2021
Under Graduate	Microbiology Landmark University, Nigeria	2014-2018
High School	The Lord's College, Nigeria	2008-2014

Job experience

Duty	Institution	Duration
Food Microbiologist	De Royal Mills and Foods Limited, Karu, Abuja	January 2017 to June 2017
Research Assistant	International Research Centre of Excellence, Institute of Human Virology, Nigeria	November 2018 to September 2019.
Research Volunteer	International Research Centre of Excellence, Institute of Human Virology, Nigeria	October 2019 to January 2021
Laboratory Assistant	Zanklin Research Centre, Bingham University, Nasarawa State, Nigeria.	October 2020 to January 2021

Foreign language

Language	Reading	Speaking	writing
English	Very good	Fluent	very good
Turkish	Basic	Basic	Basic

Computer knowledge

Program	User proficiency
All seven modules in Microsoft office	Very good
Specialized in web design	Good
Photoshop	Very good
Flash software	Good