Prospective study of isolates in Urine Samples

FULL TERM REPORT

Submitted in partial fulfilment of the requirements of
Degree of

MASTER OF SCIENCE
(CLINICAL MICROBIOLOGY)

By

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May, 2015

CERTIFICATE

This is to certify that the **Full Term Internship Training** was carried out by **Ms Rupinder Sandhu** under my direct supervision. This is to further certify that this report embodies the original work carried out by the candidate himself/herself and has not been submitted elsewhere in any form or for any other degree/diploma.

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DECLARATION

I hereby declare that work embodied in this Full Term Internship Training report was carried out by me under the direct supervision of **Dr. Anania Arjuna,** Assistant Professor, Dept. of Paramedical Sciences, Lovely Professional University (Phagwara, Punjab). This work has not been submitted in part or in full in any other university for any degree or diploma.

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ACKNOWLEDGEMENT

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Abstract

Urinary tract infection is the most common extraintestinal form of *E.coli* infection. *E.coli*, Gram negative bacillus is the most common cause of UTI infections. UTI is caused by pathogenic invasion of the urinary tract and this will leads to an inflammatory response to urothelium. According to the fact, nearly about 12% of men and 10-20% of women are experiencing an acute symptomatic UTI, and more numbers have developed asymptomatic bacteriuria (Johnson, 1991). Moreover, 100,000 patients of renal infection have become hospitalized in the United States (Johnson, 1991). While all portions of the urinary tract may be affected, but the most common one are infection related with bladder known as cystitis and the renal pelvis called pyelonephritis. This study has been conducted randomly by collecting 75 samples of urine from Escort Hospital, Amritsar. The result has shown that *E.coli* is the common pathogen among others that causing the urinary tract infection. The highest number of *E.coli* is 23 other than *K.pneumoniae* (6), *P.aeruginosa*(4*), E.faecalis*(3), *Proteus mirabilis*(1), and Candida(5).

Keywords*: UTI, Urothelium, bacteriuria, cystitis, pyelonephritis, E.coli, K.pneumoniae, P.aeruginosa, E.faecalis, Proteus mirabilis, Candida spp., bacillus, invension*.

Introduction

Urinary tract infection is the most common extraintestinal form of *E.coli* infection. These UTI infections have become the most common nosocomial infections of hospitals. *E.coli*, Gram negative bacillus is the most common cause of UTI infections. According to the fact, nearly about 12% of men and 10-20% of women are experiencing an acute symptomatic UTI, and more numbers have developed asymptomatic bacteriuria (Johnson, 1991). Moreover, 100,000 patients of renal infection have become hospitalized in the United States (Johnson, 1991). Studies have shown that ESBL (extended-spectrum beta lactamase) are the enzymes that show resistance to most beta-lactam antibiotics such as penicillins, cephalosporins, and the monobactam aztreonam (Soltani et al., 2014). UTI infections are one of the most common bacterial infections in human. There are two predominant pathogens *E.coli* and *K.pneumoniae* that can be easily isolated from the urine sample. This is the fact that ESBLs are clinically significant and detection of this enzyme provides the usage of appropriate antibacterial agents.

UTI is caused by pathogenic invasion of the urinary tract and this will leads to an inflammatory response to urothelium. This infections may be acute or chronic (Griebling, 153). Studies also have shown that the range from 42-100% of mortality rate in misdiagnosing the patients with ESBLs producing UTIs can occur. The purpose of this study was to design and estimate the current prevalence and antimicrobial sensitivities patterns among various drugs producing urinary isolates of mainly E.coli in Escorts Hospital, Amritsar.

1.1 Signs and Symptoms

Lower Urinary Tract Infection

Lower urinary tract infections are usually defined from the patients who are not hospitalized but usually experiencing some symptoms of lower urinary tract infection (Abrams et al., 2002). These include the following-

Increased day time frequency- the complaint by which a patient voids too often during the day.

Nocturia- the complaint about waking up at the night one or more time to void.

Urgency- the complaint of a sudden compelling desire to pass urine which is difficult to consider.

Urinary incontinence- the complaint of any involuntary leakage of urine.

Nocturnal enuresis- the complaint of loss of urine during the sleep on the bed.

Post micturition dribble- in this case an individual feels involuntarily loss of urine after passing out the urine.

Symptoms associated with Sexual intercourse- symptoms such as dyspareunia, vaginal dryness and incontinence that women often face during intercourse.

Other symptoms include vaginal pain, scrotal pain, perineal pain, pelvic pain, bladder pain.

1.2 Pyelonephritis in Women

 Pyelonephritis is the serious bacterial infection occurs in young adult women. It is an infection of the renal pelvis and kidney that caused by pathogen (Colgan and Williams, 2011). This infection often leads to renal scarring. In this case, the bacteria reach the kidney through lower urinary tract and may also through the bloodstream (Fulop, 2014). This may be acute or chronic infection. In acute pyelonephritis , patients will experience fever, chill, flank pain, nausea, vomiting. And in chronic pyelonephritis, it implies recurrent renal infections and development of renal scarring (Grieblling, 153).

Studies have shown that nearly 200,000 hospital admissions and about 250,000 office visits are suffering from this acute infection in USA each year. Moreover, 10,000 Canadian women are suffering from acute pyelonephritis each year (Colgan and Williams, 2011). Nearly 80% chances of an acute pyelonephritis cases is due to *E.Coli* in women. Along with this pathogen, other organisms include Enterobacteriacae, *P.aeroginosa*, Group B Streptococci and Enterococci also occur in this acute infection.

Risk factors for Pyelonephritis

Studies have shown many identified risk factors for acute pyelonephritis. This includes sexual intercourse history in previous 12 months, several sexual partners, oral or rectal sex, recent spermicidal exposure, family history of UTI. Moreover, history of Chlamydia infection, recent antibiotics use, douching, and smoking are also associated with pyelonephritis. In fact, pyelonephritis risk increased nearly 6-fold for recent frequent sexual intercourse and more than 4-fold for UTI history and diabetes (Scholes et al., 2005).

1.3 Cystitis

Cystitis is an inflammatory process of the urinary bladder and it is caused by bacterial infection. The bladder is a storage bag for urine from the kidneys. This infection occurs through the travelling of bacteria up the urethra; infect the urine and results in inflaming of the bladder lining. Even though this causes pain in women, however it is not contagious (Fact sheet). This infection of lower urinary tract system associated with pain, pressure, discomfort with symptoms of more than 6 weeks (Ganerali et al., 2013). Mast cells degranulation involved in the inflammatory process of the bladder. There will be releasing of histamine as well in this inflammatory response.

Moreover, patients with this infection may experience leaky bladder mucous layer. This will leads to the diffusion of potassium into the bladder wall, results in the cascade of depolarization of nerves and muscles. The symptoms that will occur during this process include urgency, frequency, pain, incontinence (Ganerali et al., 2013).
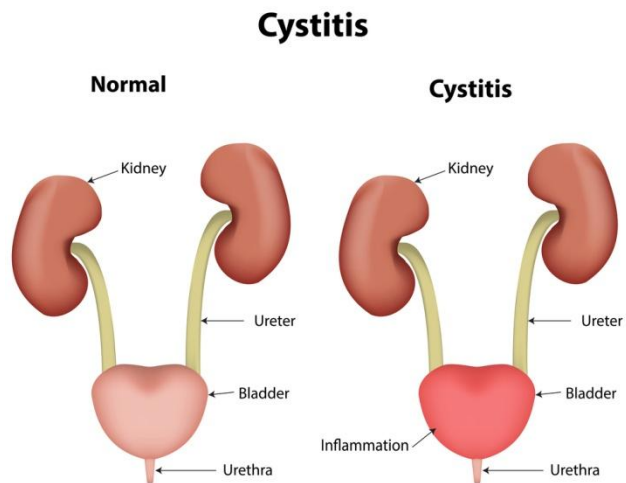
Figure 1.3 showing the normal and inflamed Cystitis (Google Images)

Risk factors

Women are at high risk of getting these UTI infections due to the physical anatomy. This is the fact that women have short urethra than men. This results in easily transfer and travelling of the bacteria to reach the bladder (Mayo Clinic staff). Following are the risk factors-

- Women that are sexually active- this leads to bacterial entry through sexual intercourse.

- Usage of birth control methods- Women who use diaphragms that contain spermicidal agents are at the higher risk of this UTI infections.

- Women who are pregnant also increase the risk of being infected due to the hormonal changes.

- Other risk factor that occurs both in men and women include the changes in immune system. In this the weak immune system of diabetics' patient, cancerous and HIV infected patient will have higher chances of bacterial and viral bladder infections.

Moreover, prolonged use of bladder catheter tubes in case of chronic ill people can result in increased susceptibility to the bacterial infection as well as bladder tissue damage.

1.4 Urethritis

Urethritis is the sexually transmitted disease in men that occurs very frequently. It is characterized by urethral inflammation. This urethral inflammation is caused by *N.gonorrhoeae*. Nearly about 15-40% it is caused by *C.trachomatis* and about 15-25% caused by nongonococcal urethritis in the USA. Patients having the urethritis infection due to genital herpes will have herpetic penile lesions and their sex partners will be having the same urethritis infection due to *T.vaginalis* (handbook).
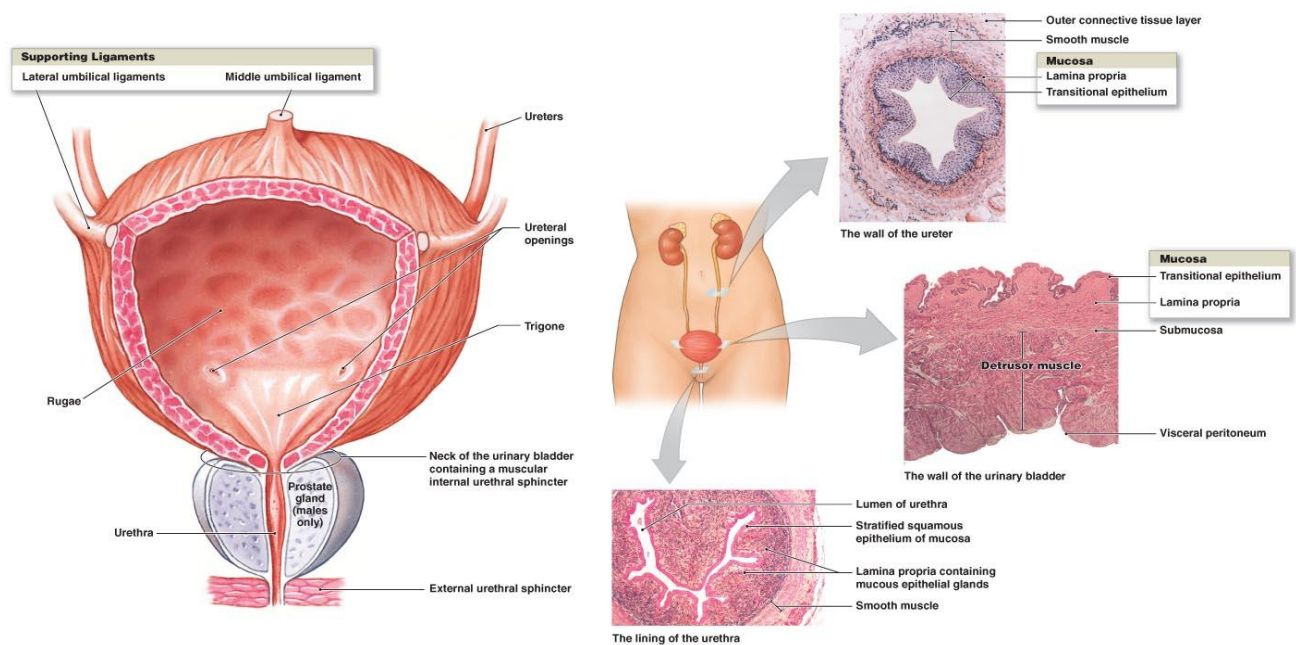

Figure 1.2 showing the structure of Urethra (Google Images)

Risk factors

A prospective cohort study of community-based HIV negative homosexual men in Sydney, New South Wales, Australia has shown some incidence and risk factors for urethral and anal gonorrhea and Chlamydia (Jin et al., 2007).

Risk factors for urethral infections-

In case of univariate analysis, urethral gonorrhea was characterized by risk factors among younger age, sexual contact with a person having gonorrhea, a higher number of casual partners in the past 6 months and a variety of sexual behaviors with these partners that may have led to exposure of the urethra to gonorrhea. In case of multivariate analysis, researchers have found that those who already have incident urethral gonorrhea with younger age, sexual contact with a person who known to have this gonorrhea, and unprotected anal intercourse (UAI) with HIV-positive casual partners (Jin et al., 2007). Moreover, in case of incident urethral Chlamydia, this remains associated with younger age as well and having more insertive oral sex to ejaculation with causal partners.

1.5 Other types of Urinary Tract Infection-

Leukocyturia- In this case, the leukocyte esterase test can be done. The positive test can be made by lysed leukocytes and it can be also negative in case of highly concentrated or collapsed leukocytes. The count of leukocytes per unit volume is affected by the variable amount of urine in each voiding. The range of 5-10// micro liter for a leukocyte count is stated as abnormal in boys above age 3; in girls it ranges from 20-50 micro liters in suspecting the urinary tract infection. Above 50 micro liters, it is clearly considered as abnormal (Utsch et al., 2014).

Bacteriuria- In diagnosis, it requires to have at least 10^5 organisms/ml for urinary tract infection. Bacteriuria means presence of bacteria in urine. Studies have shown that about 30% of urinary tract

infections occur in the age of 1 year in case of boys by *Proteus* species and *Staphylococcus* species in case of girls at the age of 9-15 years. Asymptomatic bacteriuria in case of healthy children of about 0.2-2% shows significant bacteriuria without leukocyturia (Utsch et al., 2014).

Proteinuria- It is the excessive elimination of protein in the urine. This shows the abnormality in tubular and glomerular dysfunction. Proteinuria is defined by the protein concentration of about 30 mg/dL. In case of glomerular proteinuria, more than 3% of the IgG/albumin will occur. And in tubular proteinuria, low-molecular-weight proteins are reabsorbed from the urine in the proximal tubule present in less than normal range (Utsch et al., 2014).

Hematuria- It is defined by the abnormal excretion of blood or erythrocytes in urine. It can be divided as erythrocyturia, red blood cells in the urine and hemoglobinuria is the presence of hemoglobin in the urine

Pyuria- It is defined as the presence of white blood cells or pus cells in the urine.

2.1 Pathogenecity

Mechanism of bladder inflammation in people

Studies have shown that more than 50% of women are experiencing urinary tract infection at least once in their lifetime. The most common cause of the bacterial infection in UTI is uropathogenic *E.coli*. Mostly the cases of infectious cystitis can be easily treated; however hemorrhagic cystitis has become the deadly complicated infection. This infection has been associated with pelvic radiation therapy, chemotherapy, and stem-cell transplant therapy. In this mechanism, type 1 pili and FimH are playing important role in binding to the mannose-coated proteins. These two adhesion factors will express by

uropathogenic *E.coli* which in turn binds to the mannose-coated proteins on the outermost layer of the urothelium, known as umbrella cell on the surface of the cell wall. The replication of UPEC starts taking place within the umbrella cells and results in cell lysing. This will cause more bacteria to release into the urine. In favor to combat the infection, a signaling cascade will be initiated y toll-like receptor 4 which is activated to recruit polymorphonuclear leukocytes (Halder et al., 2014).
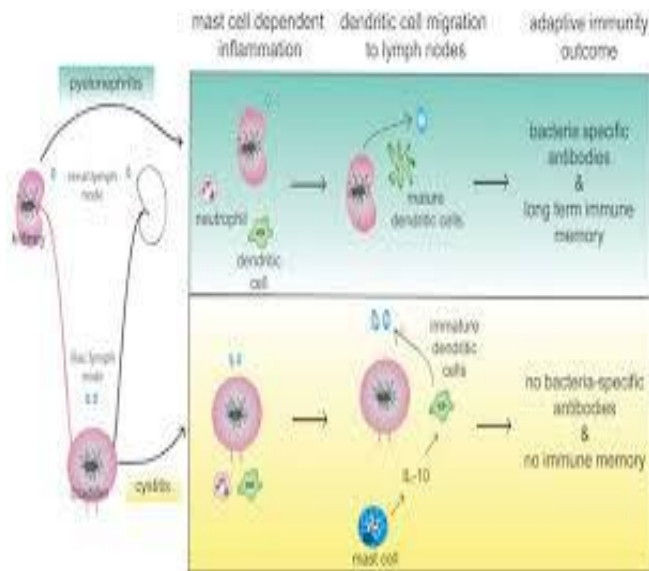
Figure 1.3 showing the mechanism of Pyelonephritis (Google Images)

Moreover, colonization of the urine without having any clinical symptoms is known as asymptomatic bacteriuria (ABU). Even these ABU patients don't need any treatments, but their strains help to prevent further infection in any bacterial virulence. UPEC strains include two groups of virulence factors that enable bacteria to colonize the urinary tract. This includes virulence factors associated with the surface of bacterial cell and virulence factors that secreted and exported to the actionable site (Bien et al., 2012).

1. Surface virulence factors

Adhesive molecules known as adhesins play an important role in determining the pathogenicity. This can occurs directly by triggering the host and bacterial cell signaling pathways, and by delivering the other bacterial products to the host tissues. Moreover, these adhesions can also promote bacterial invasions. Type 1 fimbriae helping bacterial to survive by stimulating mucosal inflammation, promoting invasions and providing growth as biofilm. The type 1 fimbriae binds to the mannosylated glycoproteins uroplankin I a and III a through the adhesin subunit FimH. This will leads to molecular phosphorylation for stimulating invasion and apoptosis. This will also increase the intracellular Ca 2+ level in urothelial cells.

Furthermore, P fimbriae plays as a second most important virulence factor in the Pathogensis of UTI and pyelonephritis. This will adhere to the mucosal and tissue matrix and it is also responsible for the production of cytokines. This fimbriae consists of different protein subunit and it is encoded by the papA-K gene operon. This will result in releasing of ceramide, and will acts as an activation of the immune cell response by toll like receptor 4. This will results in the inflammation of the local area and leads to the development of pain associated with UTI.

In addition, S fimbriae is responsible for causing sepsis, meningitis, and ascending UTIs by facilitating bacterial dissemination within the host tissues. Other virulence factor includes capsule and the lipopolysaccharide. The capsule is going to protect the bacteria from being phagocytosis by the host system. On the other hand, LPS is responsible for inducing nitric oxide and cytokine production to activate the host response.

Studies have shown that flagellated UPEC causes about 70-90% of all urinary tract infections.

Flagella is an organelle responsible for bacterial motility for the interaction of pathogenic *E.coli* strains with the epithelial cells. The flagella is responsible for invading renal collecting duct in case of the pyelonephritis. It also allow bacteria to ascend from the bladder and to initiate kidney infection in humans (Bien et al., 2012).

2. Secreted virulence factors

Toxins are important virulence factors in a variety of *E.coli* related infections. Alpha hemolysin is a lipoprotein secreted virulence factor that associates with pyelonephritis. The alpha hemolysin is a pore forming toxins and this will able to lyse erythrocytes and nucleated host cell to gain enhanced access to host nutrients and iron stores at higher concentrations. At low concentration, this will results in increasing the production of IL-6 and IL-8 and this will also promote the exfoliation of bladder epithelial cells. This also induces endothelial damage, renal vasoconstriction, and permanent renal scarring has become common complication following by alpha hemolysin (Bien et al., 2012).

3.1 Transmission

UTI due to ExPEC strains in food animals- Study have shown that nearly 70-95% of UTI are causing by strains of *E.coli*. There have been find that *E.coli* can passively move through the food chains as well. Virulence factors are another route for the survival of ExPEC (extraintestinal pathogenic *E.coli*).

Researchers have concluded that ExPEC are being transferred to humans by mishandling the food and by undercooked meat products (Singer, 2015).

Food reservoir for *E.coli* causing urinary tract infection- Recent study have shown the strong evidences for the role of foodborne transmission for *E.coli* causing community acquired UTIs. According to the fact, about 6-8 million cases of uncomplicated urinary tract infection occurs in USA annually. Moreover, 130-175 million cases occur globally and more than 80% are correlates with *E.coli*. There have been about 40,000 deaths occurring from sepsis by *E.coli* causing bloodstream infections in USA annually. Studies have found the links between antimicrobial and resistance and specific strains of extraintestinal *E.coli* in animal food products usually chicken meat. Women, who have reported antimicrobial drug-resistant UTIs, consumed a lot of chicken and pork frequently (Vincent et al., 2010).

Transmission of uropathogens through sexual intercourse- Urinary tract infection among females is an acute, and it is mostly caused by bowel flora into the bladder. Having sexual intercourse between the partners may cause the bowel bacteria to move from vaginal to urethra and then ascending into the bladder. Cross sectional survey of this study has estimated that initiating sexual activity of any type will increase the UTI risk of about 3.5-fold.

Study has used pulsed-field gel electrophoresis to compare the outcomes of *E.coli* causing UTIs in sexually active women from their recent male sex partner. Results of the experiment have shown that four men had *E.coli* in their urine and the colony count ranged from 2000 to 10,000cfu/ml. And these four men also complained about having symptoms including urgency, painful urination, chills, and back pain (Foxman et al.,1997).

4.1 Treatment

Treatment for a UTI patient should be based and designed on the basis of patient's medical conditions, the pathogen that is causing the disease and its susceptibility of that particular pathogen towards the infection. Patients having kidney infection are the very ill one and they require special intravenous antibiotics treatment. Patient having cystitis may require only the oral antibiotics. Moreover, UTI infection that caused by pathogens causes STDs may require higher number of oral antibiotics. There must be proper care in case of pregnant and children patients. These two groups should not use antibiotics due to side effects for example using ciprofloxacin and quinolones should be avoided in these two groups. However, penicillins and cephalosporins are considered safe unless the individual is not allergic to these two antibiotics (Rxlist).

In case of Acute pyelonephritis, outpatient treatment is mostly guided for the patients. But in case of serious illness, inpatient therapy is recommended for the patients and will be considered for hospitalization. In case of reappearing the symptoms within two or three days, repeat urine culture is advised. These patients will also advise to go under urinary tract imaging. The main aim of this imaging is to find the structural abnormality from any kind of occult obstruction and abscesses. Study have shown that about 15-30% of patients with acute pyelonephritis are found to be bacteremic and having sepsis (Colgan et al., 2011).

If the prevalence of resistance of community-acquired *E.coli* is 10% or less, then fluoroquinolones can be preferred to the patient. If the resistance rate exceeds 10 percent, then the oral intravenous dose of ceftriaxone or gentamicin should be given to the patient. There are several other antibiotics can be used for the inpatient treatment are fluoroquinolones, aminoglycosides, and cephalosporins (Colgan et al., 2011).

Moreover, study has shown an alternative therapeutic practice for treating and preventing rUTIs. There are many other antibiotics such as trimethoprim-sulfamethoxazole, nitrofurantoin, or cephalexin being used on the bases of low doses for women suffering with UTIs. Furthermore, apigenin, the main component of parsley has shown the diuretic effect in the treatment of UTIs. In addition to this, few studies have shown the effect of garlic in the treatment of interstitial cystitis. Therefore, the combination of garlic oil and parsley in pills can affect bacterial and proliferation synergistically.

In addition to this, cranberry also shows a great effect in treating UTIs. Cranberry contains D-mannose, which will adhere to the bladder epithelium to interfere with the *E.coli* and this will result in washing of the bacterial wash along with the urination. Addition study has also shown that lactobacillus may also help in maintaining the normal flora in women during sexual activity. This will improve the vaginal health and also caused a relief from UTI in women. There is another factor, the formation of nitric oxide after the oxidation of L-arginine with the help of nitric oxide synthase. This nitric oxide is going to exhibits antibacterial, smooth muscle relaxation, hormone releasing, and it will modulate immune system. Furthermore, this can also inhibit the growth of microorganisms, decrease oxidative stress, regenerate the uroepithelial cells of bladder and can prevent the development of new infections after the treatment of UTIs (Mansour et al., 2014).

5.1 Prevention

There is number of categories that can be divided into different ways to prevent urinary tract infection in women. In case of UTIs, infectious bacteria will colonize the vaginal wall and then move to the urethra. Then the infection can travel from the bladder to the kidney. So, we need some preventive

measures to stop or to blocking the traveling of the bacteria (Brusch, 2013). Following are some preventive measures to take-:

1. Hygiene- It is very important to wipe front to back after using the bathroom. Never wipe from back to front and also make sure not to wipe twice with the same tissue. It can transfer the germs back there. While taking shower, it is important to avoid sitting in the water tub for longer time, because the water can be contaminated with the person's own skin florae. Moreover, it is a good idea to use tampons instead of pads. This will keep the bladder opening area drier and will limit the bacterial growth. It is necessary to empty the bladder at least every four hour and never hold your bladder for urination.

2. Clothing- It is necessary to avoid wearing tight-fitting undergarments. This can leads to the accumulation of moisture and for the bacterial growth.

3. Diet- Taking higher amount of fluid is very beneficial. It is important to drink at least one glass of water along your meal. If the person is getting very dark urine other then pale, this implies less consumption of liquid. Cranberry juices are good example and effective in case of younger women.

4. Activities- Having any physical activity, one should empty their bladder frequently and should drink plenty of fluids. During sexual activity, the bladder should be emptied after the intercourse. It is recommended to drink 2 extra glasses of water and patients are advised to take a urinary antiseptic drugs. The woman normal vaginal florae and sperm is important in suppressing the bacterial colonization. So, it is necessary to avoid the use of spermicidal jelly.

6.1 Literature Review

Study related to Extended-Spectrum Beta-Lactamase (ESBL)- producing *E.coli*

There have been many studies reported the increase incidence of urinary tract infections due to ESBL-producing *E.coli.* However, there was no data related to patients having upper ESBL- positive *E.coli* UTI and sepsis. This prospective study has shown and presents the clinical outcomes in patients between January 2008 and September 2011. This study identified and cultured all the strains blood and urine cultures. They have used clinical and laboratory standard Institute double disc method for confirming the presence of ESBL in- vitro (Picozzi, 2014).

Studies have shown that every year, there are about 150 million individuals being affected by urinary tract infections worldwide. A complicated UTI is an infection associated with a condition, such as a structural or functional abnormality of the genitourinary tract. This can also be associated with the presence of an underlying disease that interferes with host defense mechanism. This will leads to increasing the risk of acquiring infection by the presence of an underlying catheter or urinary stent, or by an ileal loop or pouch. Most of the cases, the extended- spectrum beta- lactamase- producing *E.coli* strains are resistant to all penicillins, cephalosporins, and to aztreonam. Furthermore, they are resistant to trimethoprim/sulfamethoxazole and quinolones (Picozzi, 2014).

In this study, 49 patients were studied. Overall, 25 patients culture resulted negative and eleven patients presented positive blood and urine culture for *E.coli*. From these eleven, seven were ESBL positive. These were four women and three men with the median age of 73 years. In this case, patients that were affected by upper UTI due to ESBL- positive *E.coli* presented with pyonephrosis and sepsis (Picozzi, 2014).

Molecular Detection of ESBL for a better therapy plan and infection control program

This study was done to detect the resistant gene types by using polymerase chain reaction and the sequence types by using multilocus sequence typing. The widespread cefotaxime- beta lactamase (CTX) has increased the multi drug resistance of *E.coli*. This has caused the great trouble to the doctors for treating the infection. Study has found that the blaCT X-M-1 group and the blaCTX-M-9 group are the main CTX-M gene types (Shi, Huiqing, 2015).

Few limitations of this study-

First, the clinical information was not acquired and we could not further analyze the risk factors for ESBL *E.coli* infections. Second, the number of strains was not large enough to display epidemiological features. However, this study can provide the reference data to enable the relevant infection control and treatment.

Another prospective comparative study of Cefotaxime and sulbactam versus Cefepimi and Tazobactam in urinary tract infection patients-

According to the study, Cephalosporins are being widely used in UTI, but there is an emerging resistance problem being occurring as well. The purpose of this study was to evaluate efficacy and safety of third generation cephalosporin combined with beta lactamase inhibitors compared with fourth generation cephalosporin. The recent data has shown that about 90% of patients are suffering from cystitis and 10% pyelonephritis. There are 25% chances of being recurrent of this infection (Kaur, Kamalpreet et al., 2014).

Cephalosporins are one of the agents for using as therapy for treating the complicated UTIs. Cefepime and piperacillin tazobactam are being successfully used for the infections that caused by ESBL-

producing *E.coli* or *Kleibsiella* species. Along with this, Cefepime has been useful in treating infections like respiratory tract infection, skin structure and in bacteremia.

Cephalosporin contains a beta- lactam ring, which can be hydrolysed by beta-lactamases. This ensures the resistance of the antibiotic by destroying the beta-lactam ring. The approach to contracting this resistance mechanism has been through the development of beta-lactamase inactivators like clavulanic acid and sulbactam tazobactam molecules with minimal antibiotic activity. However, when combined with safe and efficacious penicillins or Cephalosporins, these inhibitors can serve to protect the familiar beta-lactam antibiotics from hydrolysis by penicillinases (Kaur, Kamalpreet et al., 2014).

The common symptom in the present study noted were increased frequency in 58 patients (96.66%). Most common affected age group was 50-70 year with male predominance. Similar studies have reported same symptoms for cystitis such as dysuria, frequency, urgency, and hematuria and in case of pyelonephritis, it includes fever, chills, and flank pain. The common pathogen was found to be E.coli in this study as well causing 75% of UTIs. This study have shown that the drugs in both generations of cephalosporin combined with beta lactamase inhibitors cefotaxime/sulbactam and cefepime/tazobactam were equally effective and will tolerated in the treatment of UTI (Kaur, Kamalpreet, 2014).

Different Diagnostic therapy studies in Urinary Tract infections-

Role of the Quantitative C-Reactive Protein, Erythrocyte Sedimentation Rate and White Blood Cell count in diagnosing Urinary Tract Infections-

This study was done in Qazun, Iran among hospitalized infants and children suffering from urinary tract infections. This study was based on the evaluation of the diagnostic accuracy of the quantitative C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and white blood cell (WBC) count in urinary

tract infection. Mostly, the diagnosis of a UTI in children is often based on the outcomes of a microscopic urine analysis. If the tests are not performed at the time of detection, then the infection can remain undiagnosed. Due to the fact, the general route of infection in the urinary tract is ascending and the pathogens originate from the perineal flora (Ayazi et al., 2013).

Renal parenchymal defects occur in 5-15% of children within one to two years of their first presentation with a UTI, and is associated an increased risks of progressive renal damage. The risk of parenchymal defects most likely diminishes over time. In some studies, renal scarring was present in 8-40% of patients following an event of acute pyelonephritis. As a consequence of the renal scars, patients had increased hypertension and variable degrees of renal failure. The discriminate among upper and lower UTIs has significant medical implications in children younger than two years of age (Ayazi et al., 2013).

Due to all this fact, it is important to do the rapid diagnosis of acute pyelonephritis because early treatment can reduce the risk of renal scarring. Moreover, the study has also used DMSA (dimercaptosuccinic acid renal scintigraphy) for diagnosing an acute pyelonephritis. However, DMSA is an expensive technique and it is not available in all centers to expose the patients to radiation. Also, it is not a perfect differentiator to differentiate old scaring from acute renal involvement. There will be need of another follow up scarring to be performed. In addition, the size of the kidney may become increased due to inflammation.

So, to overcome on these limitations, study has found another attempt to correlate with the DMSA renal scan by locating a blood marker. Therefore, CRP, ESR and peripheral WBC count are simple non invasive tests that can be used for the diagnosis of invasive bacterial infections and for determining the UTI level (Ayazi et al., 2013).

Another study has been done on Urinalysis in Acute care of adults-

Urinalysis is a rapid urine tests for detecting infection on a low costs. However, this test can provide many false positive results including unnecessary antibiotics. These unnecessary antibiotics may cause resistance, diarrhea, side-effects, and allergic reactions. However, this study has helped in planning future work by frame working some important steps. First, it is important to addresses symptoms. Second, it should addresses which patients without UTI symptoms should go for testing for the infection. Thirdly, a urine test must be ordered for the acute care setting. Fourth, it is important to do the rapid test accurately rather than requiring an accurate urine culture. So, in this urinalysis is an indicator of UTI as a poorer method because it provides false positive results (Pallin et al., 2014).

More reliable diagnostic tool, Heparin- Binding protein as a diagnostic biomarker for urinary tract infections in adults-

Diagnosing UTI in particularly critically ill patients with some unspecific and mild symptoms has become very difficult. Previous tests like standard rapid test have some limitations, so there is a need of more reliable diagnostic tools. Therefore, study has been conducted on Heparin- binding protein as a diagnostic and predictive biomarker in all kind of bacterial infections. The results of the rapid dipstick test can also leads to excessive use of antibiotics with higher risk of side effects and many negative outcomes of their adverse reactions (Kjolvmark et al., 2014).

Heparin-binding protein is a protein of 37 kDa and it is being stored in secretory and azurophilic granules of human neutrophils. Releasing of HBP will induce vascular leakage and act as an activator of monocytes. Moreover, HBP also plays an important role in antimicrobial activity and it can also help in clearing the bacteria by direct opsonization. When the level of HBP increases in plasma, cerebrospinal fluid, skin biopsies, it indicates the severe sepsis, bacterial meningitis, and streptococcal skin infection (Kjolvmark et al., 2014).

This study has shown a strong correlation between U-HBP and U-WBC. By proving that HBP is more specific marker than U-WBC, they have shown that U-HBP is the best diagnostic marker for UTI and can also discriminate between cystitis and pyelonephritis (Kjolvmark et al., 2014).

Gram-Stain Plus MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry) for a Rapid Diagnosis of Urinary Tract Infection

Urinary tract infections (UTI) are among the most common infections. Microbiological confirmation of a UTI takes 24–48 h. In the meantime, patients are usually given empirical antimicrobial therapy, sometimes unnecessarily or inadequately. Anticipation of clinically useful information is of the utmost importance, with both diagnostic and therapeutic consequences.

Traditionally, a rapid diagnosis of UTI entailed a Gram stain on urine samples. Several studies conducted mostly in the 1970s and 1980s assessed the usefulness of this stain, which proved to be one of the most rapid, reliable and inexpensive methods for anticipating bacteriuria at $>10^5$ colony forming units/ml. However, the Gram stain has been abandoned as a routine diagnostic test in most microbiology laboratories.

A new technology, Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS), has been recently introduced for the analysis of different biomolecules. This mass spectrometry procedure has been successfully used for the rapid identification of microorganisms already isolated by culture, but has been scarcely employed for diagnostic purposes directly on clinical samples, with the exception of positive blood cultures, and urine samples (Burillo et al., 2014).
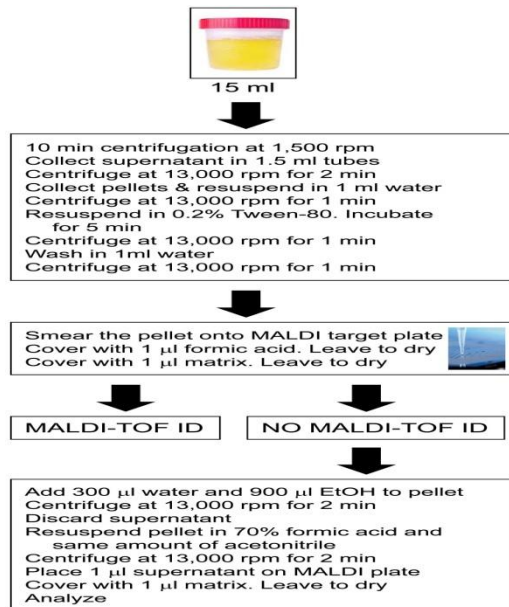
Figure 1.4 is showing the protocol of MALD-TOF (Burillo et al., 2014)

By performing a Gram stain followed by MALDI-TOF MS directly on urine samples we were able to predict the presence or absence of bacteriuria and the causative microorganism in patients with a suspicion of UTI reasonably well and within a working laboratory shift. According to our findings, physicians would receive therapeutically helpful information for 96.1% of samples in less than 1 h. Only in 0.4 out of 10 cases would inappropriate information be provided. However, the only limitation was the sample size in this study. This method requires at least 15 ml of urine sample for performing the test. On the other hand, it is a fast method to do the test of at least 1000 urine sample under an hour. Future studies should address the clinical impacts of our rapid diagnosis algorithm for UTI by examining its capacity to improve the adequacy of treatment in terms of reducing the time of empiric antibiotic treatment or allowing for the earlier withdrawal of unnecessary antibiotics (Burillo et al., 2014).

7.1 Material and Methods

In this part, different approaches to diagnosis of urinary tract infection are going to explain. The laboratory diagnoses are very important to differentiate between infection, colonization, and contamination.

7.2 Collection and Transport of specimen

Collection: First step is to collect the sample. It is very important to collect the sample avoiding any kind of contamination. There are different methods to collect urine on the basis of the diagnostic requirement. However, the most useful method is MSU.

1. Midstream urine (MSU) - A midstream urine sample is appropriate for bacterial culture. The patient should be provided with a wide-mouthed, screwcapped, leak-proof, sterile universal container. It is important to give instructions for proper collection. The patient is advised to clean the area and to void the first part of the urine into the toilet, then to collect the 'midstream' urine in the container, and finally to void the last part into the toilet (Ananthanarayan et al., pg 671). The sample should be collected with a proper labeling as shown in the following figure-

2. Catheter sample urine (CSU)- If the patient has been catheterized, then the sample is collected as follows. First, the area over the catheter should be cleaned by alcohol. Then, the sample is drawn into the universal container with the help of clean needle and syringe.

3. Suprapubic aspirate- This method is used for the infant to collect the sample by using a sterile syringe and needle by puncturing the suprapubic area under sterile conditions.

4. Early Morning Urine- This method is done for investigating the renal tuberculosis, pyelonephritis by collecting the sample on three consecutive days.

7.3 Transport: The sample must be transported at room temperature within half an hour. Or it should be refrigerated at 4 degree Celsius for up to four hours. The sample should not be proceeding after 4 hours for bacterial culture. Because there may be multiplication of contaminating bacteria can occur and give a false positive result. If there is an emergency to process the sample after 4 hours then it has to be provided with 1.8% boric acid and can kept longer (Ananthanarayan et al., pg 671).

After collecting the sample, next step is to apply different laboratory methods to diagnose the sample.

8.1 The following are the different approaches to diagnosis of urinary tract infection in the laboratory:

1. First approach is **by Microscopy**- In this, a microscopy is going to be done to examine any kind of pus or bacteria in urine. An uncentrifuged sample will be examined by direct microscopy for pus cells and bacteria.

2. Second approach is by **Urine Culture**- Urine culture is done by semi-quantitative method on CLED Agar.

Principle- Normally, urine is considered as sterile. However, bacteria may get access to the urinary tract by ascending route through the perineum and then into the bladder. Urinary tract infection more often occurs in females than in males because of shorter and wider urethra.

Procedure-

1. 100 samples of urine were collected randomly from the patients of Escort Hospital.
2. Samples were collected in sterile and screw capped containers.
3. Then each sample was mixed well.
4. Follow that semi-quantitative cultures were on Cystine Lactose Electrolyte Deficient (CLED) agar plates with a 5 mm calibrated loop.
5. Plates are incubated at 37 degree C for 24 hours and colony formation units were counted for the presence of bacteria in urine (Farooqui et al., pg 129-131).
6. Colony count of 10^5/ml is considered as significant.

Figure 1a is showing the lactose fermenter of E.coli on CLED Agar

4. **Gram Stain**- Once, the culture is done. Next step is to do the Gram-stain. This step is important to identify the bacteria on the basis of Gram positive and Gram negative and to study their morphology.

Gram Stain is based on various theories which are discussing as follows-

1. Gram-positive bacteria have a thick peptidoglycan layer showing below in figure 1b and these cells have more acidic protoplasm. So they will retain the primary dye and appear blue in color.

2. On the other hand, Gram negative bacteria contain lipid layers showing below in figure 1b and these lipid layers will make the primary dye to permeable and will take the counterstain. These will appear pink in color (Ananthanarayan et al., pg 13).
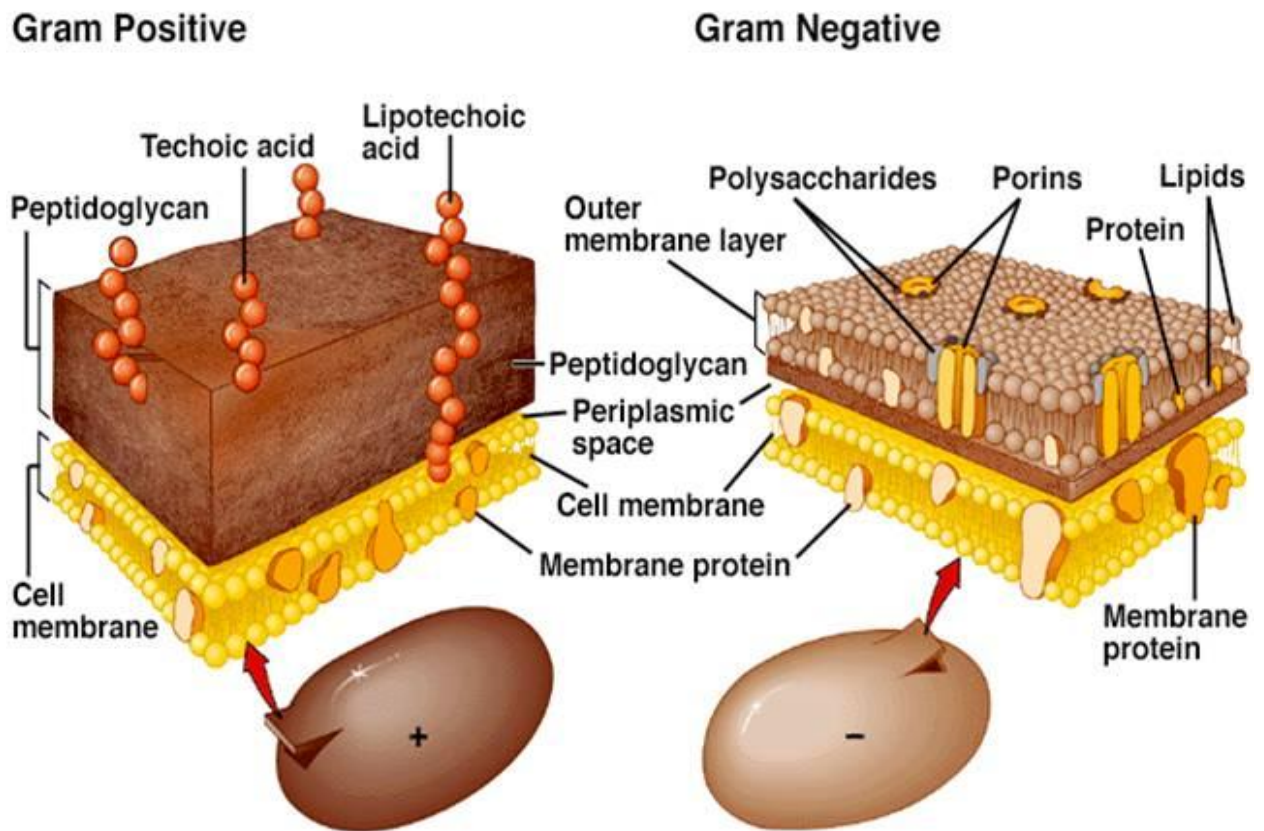

Figure 1b showing difference between Gram Positive and Gram Negative Bacteria's cell

Membrane (Google Images)

5. **Biochemical Test**- Once we done with the Gram-stain, next important step is to do the biochemical test to confirm the organism. There are different biochemical test being tested for identifying the organism on the basis of their motility, color change, gas production being explained in the following-

   ➢ Indole- This test is based on p-dimethylaminobenzaldehyde also known as Kovac's Reagent. There will be a formation of red ring when indole reacts with this aldehyde group. This test is done to identify the presence of enzyme tryptophanase in bacteria which hydrolyses and deaminates tryptophan with the production of indole, pyruvic acid, and ammonia (Dr.Renuka, SRL).

      ✓ Procedure:

      • A pure growth of an organism is inoculated aseptically in peptone water and incubated over night at 35-37 degree C.

      • Add 0.5 ml of Kovac's reagent and shake gently.

      • Observe the changes

   ➢ Urease Test- This test is done to detect the presence of an enzyme urease which is possessed by many organisms. These organisms will hydrolyse urea with the release of ammonia and CO_2 (Dr. Renuka, SRL).

      ✓ Procedure:

         • Inoculate a suspension of the organism to be tested on the urease slant.

         • Incubate for 24 hrs at 37 degree C.

         • Observe the change of light orange color to pink color in positive tests.

➢ Citrate- Citrate is utilized by certain organism as an external source of carbon. Citrate is an intermediate of Kreb's cycle. The medium also contains ammonium salt and these organisms used ammonium salts as a nitrogen source. This will leads to the formation of an alkaline environment which turns bromothymol blue to intense blue with a pH of nearly about 7.6 (Dr. Renuka, SRL).

   ✓ Procedure:

   • Inoculate the single colony by streaking at the surface of the citrate slant.

   • Incubate at 35 degree C for 24 hours.

   • Observe the color change from green to blue in positive tests.

➢ TSI (Triple Sugar Iron Test)- This test is done to differentiate Gram negative bacilli on the basis of their abilities to utilize carbohydrates fermentatively and to release hydrogen sulphide. This medium contains glucose, sucrose, and lactose. Phenol red detects pH and ferrous sulphate detects the formation of hydrogen sulphide. If there will be a fermentation of lacose and glucose by an organism then the slant will be acid and yellow in color. If there is no fermentation of glucose or lactose then the slant will remains alkaline in nature and remains red in color. The production of H_2S will results in blackening of the medium (Dr. Renuka, SRL). Positive test is as shown below.

   ✓ Procedure:

   • Inoculate the well-isolated colony and stab through the center of the medium deep into the tube.

   • Incubate at 35 degree C for 24 hours.

Positive TSI

➢ Methyl Red Test (MR)- This test is used to determine the hydrogen ion concentration when an organism ferments glucose.

✓ Procedure:

- Inoculate 0.5 ml of glucose phosphate broth with a single colony.

- Incubate at 38 degree C for 24 hours.

- Add one drop of methyl red indicator and observe the formation of red ring.

➢ Mannitol Motility Test- This test is done to differentiate bacteria on the basis of their motility and their ability to ferment mannitol. In this case, motile bacteria will produce diffused growth throughout the medium and non-motile will grow only on the line of inoculation. The color will change from red to yellow (Dr. Renuka, SRL).

✓ Procedure:

- Inoculate the well-isolated colony and stab through the center of the tube.

- Incubate at 38 degree C for 24 hours.

MTM Positive Test

> Oxidase Test- This test is based on the enzyme known as cytochrome oxidase enzyme. This enzyme will transfer the electron from the transport chain to Oxygen and it will reduce it to water. In case of positive test, it gives purple color. This is done to detect the presence of *P.aeruginosa* as it is a positive test for this organism.

✓ Procedure:

- This is a simple strip test which can be done very easily.

- Take an oxidase strip with the help of a sterile forceps and simple touch with the isolated colony to see the color change.

➢ Catalase Test- This test is done to identify the organism that produces catalase enzyme.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$
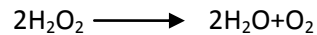
In this test, a bubble will form and resulting in the production of oxygen gas as a positive test. This test is positive for *E.coli*, *Staphylococcus aureus*. This test can be done by two methods.

a. Slant Method

b. Slide Method

✓ Procedure:

a. Slant method

- Inoculate an isolated colony in the test tube.

- Incubate for 24-48 hours at 37 degree C.

- Add 3-4 drops of 3% hydrogen peroxide.

- Examine the presence or absence of bubbling.

b. Slide Method

- Take a clean slide and divide into two parts.

- Place a small drop of normal saline on each side and inoculate a small amount of culture.

- Emulsify the colonies and make a smooth smear of pea size.

- Then add few drops of hydrogen peroxide over the test smear, leave the negative control as it is and observe for the bubble formation.

➢ Coagulase Test- This test is based on the enzyme, coagulase that clots blood plasma and prevents phagocytosis. Coagulase enzyme will reacts with prothrombin in the blood and convert fibrinogen to fibrin. This test is positive for *Staphylococcus aureus*. This test is done by two methods as well.

  ✓ Procedure

  a. The slide coagulase test

    - Take a clean slide and divide into two parts.

    - Place a small drop of distilled water on each side.

    - Inoculate few colonies of tested organism and emulsify them.

    - Add a drop of citrated plasma on the test side and leave negative side as it is.

    - This will result in clumping within 5-10 minutes.

  b. The Tube Coagulase Test

    - Three test tubes are taken. One as the test and other two as positive and negative test tube.

    - Each tube is filled with 1 ml of 1 in 10 diluted rabbit plasma.

    - Add the bacteria in the test tube.

    - Add 0.2 ml of *Staphylococcus aureus* to the positive test.

    - And sterile broth to the negative tube.

    - Incubate the tubes at 37 degree C.

    - Positive test tube will be seen as gelling of the plasma.

6. **Antibiotic Susceptibility Testing**- This is the following step after the biochemical test to check the susceptibility of an antibiotics against a particular organism explained as follows-

   Antimicrobial sensitivity testing is done to determine the susceptibility of isolates of pathogenic bacteria to antibiotics. This is a fast and simple disc method to check the susceptibility to antibiotics and chemotherapeutic agents. This test is done to treat and control the infection which is caused by any pathogen. This helps to select an effective antimicrobial drug (Ananthanarayan et al., pg 635).
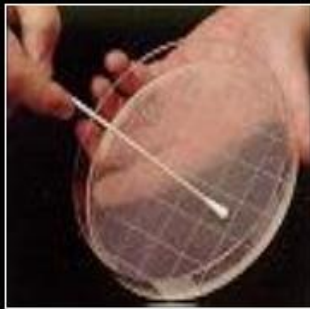
   The method that is used to check the antibiotic sensitivity test is Kirby- Bauer disk diffusion method.

   Kirby- Bauer method: This method is a common method and the procedure is discussing in the following ways-
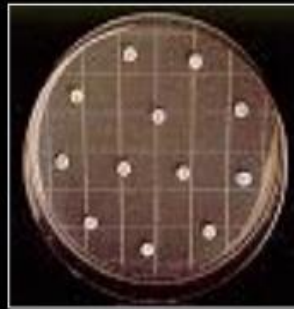
   1. This test is done on the Mueller-Hinton agar.

   2. A suitable dilution of pure culture will be prepared by taking 4-5 colonies in the normal saline.

   3. Then the suspension of the test bacterium will be inoculated on the Mueller-Hinton agar plates as a lawn by using sterile cotton swabs.

   4. Let the plates dry for 30 minutes at 37 degree C.

   5. Following that an antibiotics discs will be applied on the surface with the help of sterile forceps.

   6. Keep the plates for overnight for incubation at 37 degree C.

   7. The degree of sensitivity is determined by measuring the zones of inhibition of growth around the discs as showing in the following figure.

8. The growth can be only inhibited where the bacterium has shown the susceptibility to the antibiotics and it will show resistant around no inhibition (Ananthanarayan et al., pg 635).

Figure 2a showing the antibiotic susceptibility testing methods.

Figure 2b showing the sensitivity and resistant of an antibiotic

There are number of factors that influence on the diameter of the zone of inhibition. These are as follows-

- Diffusibility of the drug

- Disc concentration

- Nature and composition of the medium

- Medium's thickness

- Presence of inhibitory or stimulatory substance

- pH and time of incubation

Mechanism of action of antimicrobial agents- There are different categories of antimicrobial agents based on their principle mechanism of action. This includes as the following-

- interference with cell wall synthesis eg. Beta lactum

- inhibition of protein synthesis eg. Tetracyclines

- interference with nucleic acid synthesis eg. Rifampin

- inhibition of a metabolic pathway eg. Sulphamethoxazole

Moreover, bacteria may get the resistant to antimicrobial agents through mutation or genes through other organism. A gene can provide resistant to a bacterium through the following points-

- enzyme that can destroy the antibacterial drug

- it can interrupt the target site by expressing the efflux system

- by producing an alternative metabolic pathway that evades the action of the drug.

7. **VITEK 2 Compact System**- This is very last step to check the susceptibility manually by identifying the organism on the basis of turbidity and colorimetric.

Principle- The VITEK 2 is an automated microbiology system utilizing growth- based technology. The system is based on turbidity and colorimetric reagent cards that are incubated and interpreted results (Sukhjinder, Scientific Officer). There are substrates on the tray which utilizes the enzymes and result in changing the color on reaction. Turbidity is based on the identification on the basis of susceptibility and the colorimetric is based on biochemical to detect the spore-forming Gram- positive bacilli.

Reagent Cards

There are 64 wells in reagent card and each well contains an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis and growth in the presence of inhibitory substances. The card has an optically clear film on both sides that maintains the level of oxygen transmission and prevents the contact with the organism-substrate admixtures. Each card has different bar codes that contain information on product type, lot number, expiration date, and a unique identifier (Pincus, bioMerieux, Inc).

Figure 3a and 3b shows the example of Colorimetric card and turbidity card.

Figure 3a. VITEK 2 GP COLORIMETRIC Identification Card.

Figure 3b. VITEK 2 N280 Turbidity Identification Card.

There are different panels available for the identification of different organism as follows:

1. GN- Gram- negative fermenting and non-fermenting bacilli

2. GP- Gram-positive cocci and non-spore-forming bacilli

3. YST- Yeasts and Yeast- like organisms

4. BCL- Gram-positive spore- forming bacilli

5. N280- Lactose fermenter

6. N281- Non Lactose fermenter

7. P628- *Staphylococcus* and *Enterococcus*

8. ST01- *Streptococcus*

   Procedure

   1. A flexiloop is used to pick 4-5 isolated colonies and then emulsified in 3.0 ml of normal
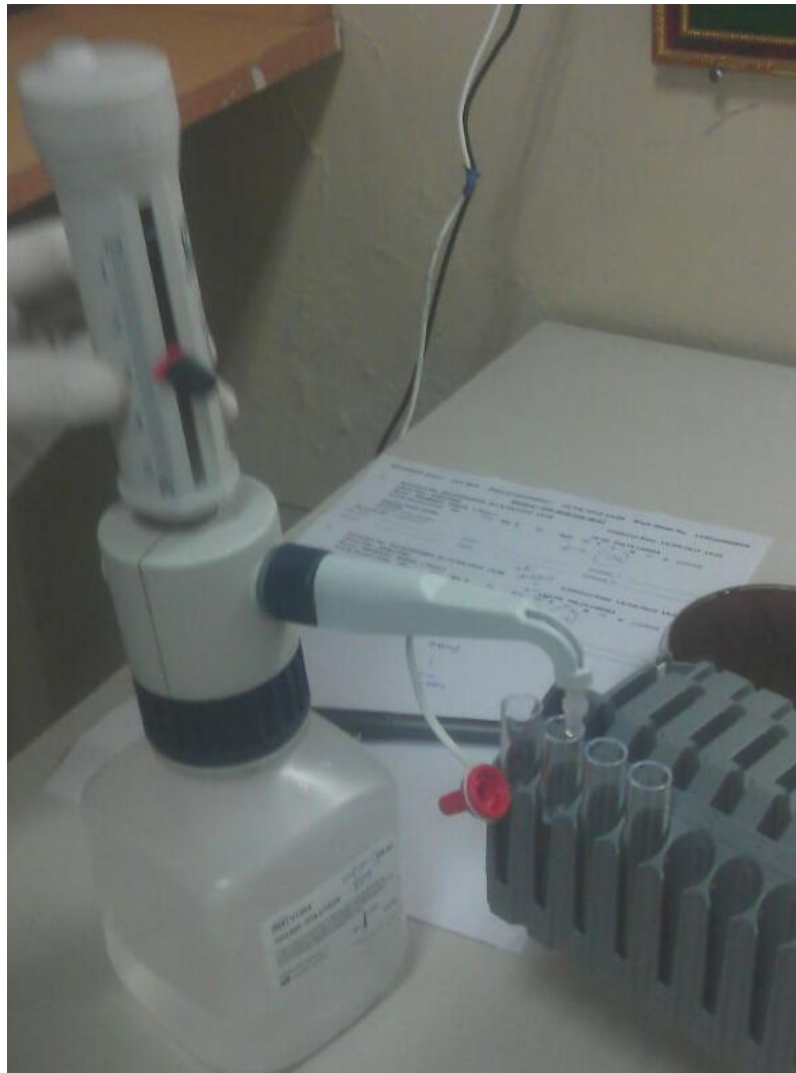   saline (pH 5.0-7.2) showing in the following figure-

   Figure4 shows the Dispensette that contains normal saline.

2. All the test tubes (polystyrene) should be arranged in Cassette, a special rack to hold the test tubes.

3. The turbidity is adjusted accordingly (Table 1) and can be measured with the help of a turbidity meter known as the DensiChek as shown in the figure-

Figure 5 shows the DensiChek used for measuring the turbidities.

Table 1: Suspension Turbidities Used for Card Inoculation.

Product	Turbidity Range
GN	0.53-0.63
GP	0.53-0.63
YST	2.0-2.20

4. Followed this, identification cards are going to be inoculated with microorganism suspensions as shown below-

Figure 6 shows cards inoculation for identifying the microorganism.

5. Then load the cards and suspension tubes into the Automated Transport system.

6. Take the reading following the day. A VITEK 2 Compact takes about 8-12 hours to indentify the organism and the susceptibility test (Sukhjinder, SRL)

9.1 Results and Observations

Data Collection

Table 2 shows the Number of Cases of Urinary Tract Infection in January, February, and March collectively.

Months	January,2015	February,2015	March,2015
Total number of cases	30	30	15
Positive	14	15	08
Negative	16	15	07

Table 3 shows different sex with UTI.

Months	January	February	March
Female	10	9	6
Male	4	6	2

Table 4 shows the Total number of Organisms in three months.

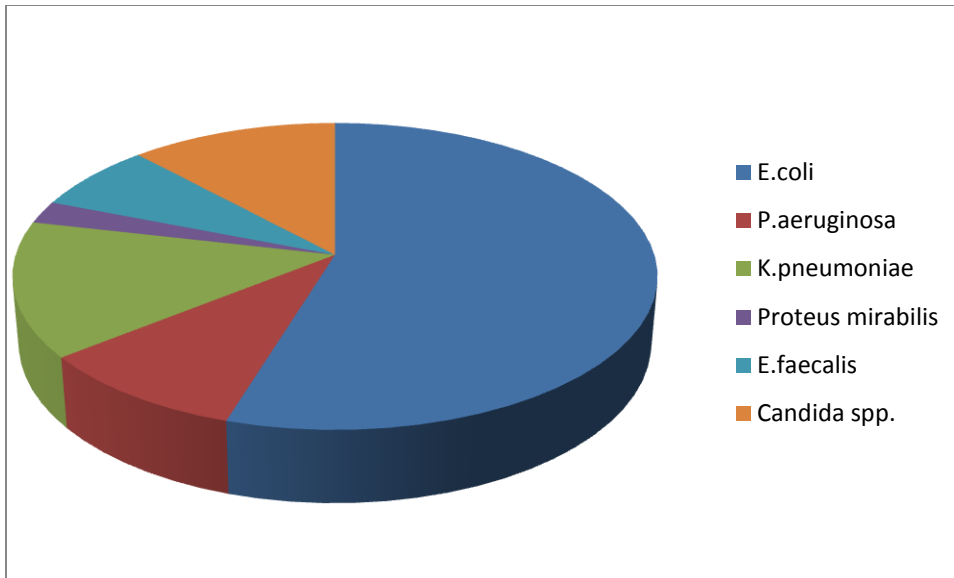| Organisms	Total number of Organism
E.coli	23
P.aeruginosa	4
K.pneumoniae	6
Proteus mirabilis	1
E.faecalis	3
Candida spp.	5

Figure 7 shows the high number of *E.coli* vs other organisms.

Table 5 shows the Antimicrobial sensitivity in different organisms.

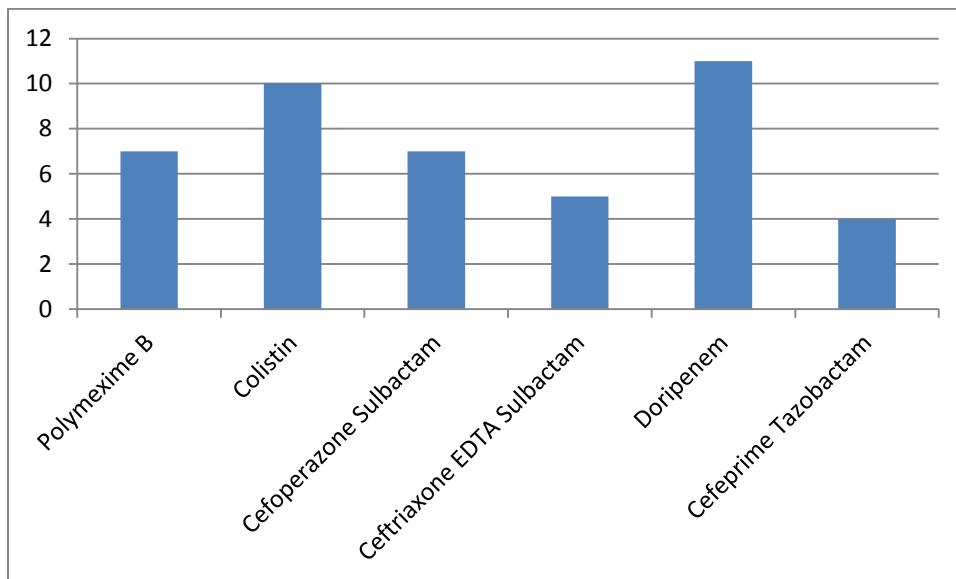| Antimicrobial	No. of Sensitivities
Polymexime B	7
Colistin	10
Cefoperazone Sulbactam	7
Ceftriaxone EDTA Sulbactam	5
Doripenem	11
Cefeprime Tazobactam	4

Figure 8 showing the bar chart of Antimicrobial Sensitivity.

Biochemical Results

Table 6 shows the Biochemical Results of *E.coli, K.pneumoniae. P.aeruginosa, Proteus mirabilis*.

Biochemical	*E.coli*	*K.pneumoniae*	*P.aeruginosa*	*Proteus mirabilis*
Indole	+ve	-ve	-ve	-ve
Urease	-ve	+ve	-ve	+ve
Citrate	-ve	+ve	+ve	+ve
TSI	Gas/Acid	Acid/gas/+ve	Alkaline/gas/-ve	Acid/Alkaline/H$_2$S
MR	+ve	-ve	-ve	+ve
MTM	Fermenter/motile	F/NM	NF/NM	NF/M
PPA	-ve	-ve	-ve	+ve
Oxidase	none	none	+ve	-ve

Table 7 shows the colony characteristics-

observations	organism	Colony characters
	E.coli	Large, elevated yellow, opaque, with a center slightly darker, yellow agar, Lactose fermenting colonies.

| | *Kleibsiella* | Large, yellow or yellowish white,highly mucoid, Elevated, lactose fermenting colonies. |

|		
	Candida Spp.	White to cream Colored Colonies, Smooth, Glabrous, Yeast like appearance.
	*Proteus mirabili*s	Small opaque, bluish-green colonies, non-lactose fermenting colonies, produce fishy smell.

10.1 Result Interpretation

This study has been conducted randomly by collecting 75 samples of urine from Escort Hospital, Amritsar. The result has shown that *E.coli* is the common pathogen among others that causing the urinary tract infection. This can be seen from the results. Table 2 showing the number of cases of January in which out of 30 cases, 14 are positive and 16 are negative. Total numbers of cases of February are 30 in which 15 are positive and 15 cases are negative. Lastly, in March it has shown that out of 15 cases, 8 are positive and 7 are negative. Table 4 shows that the highest number of *E.coli* is 23 other than *K.pneumoniae* (6), *P.aeruginosa*(4), *E.faecalis*(3), *Proteus mirabilis*(1), and Candida(5). Figure 7 is showing the pie chart of these organisms in which *E.coli* is the one covering most of the area. Table 5 is showing the antimicrobial sensitivity of these different organisms in which Doripenem is the one showing highest sensitivity towards these microbes as seen in figure 8. Following that table 6 is explaining the biochemical test of particular organism in which *E.coli* is indole positive, urease and citrate are negative. There is a gas and acid production in TSI, it is fermenter and motile in MTM, and negative in case of PPA as shown in the figure below.
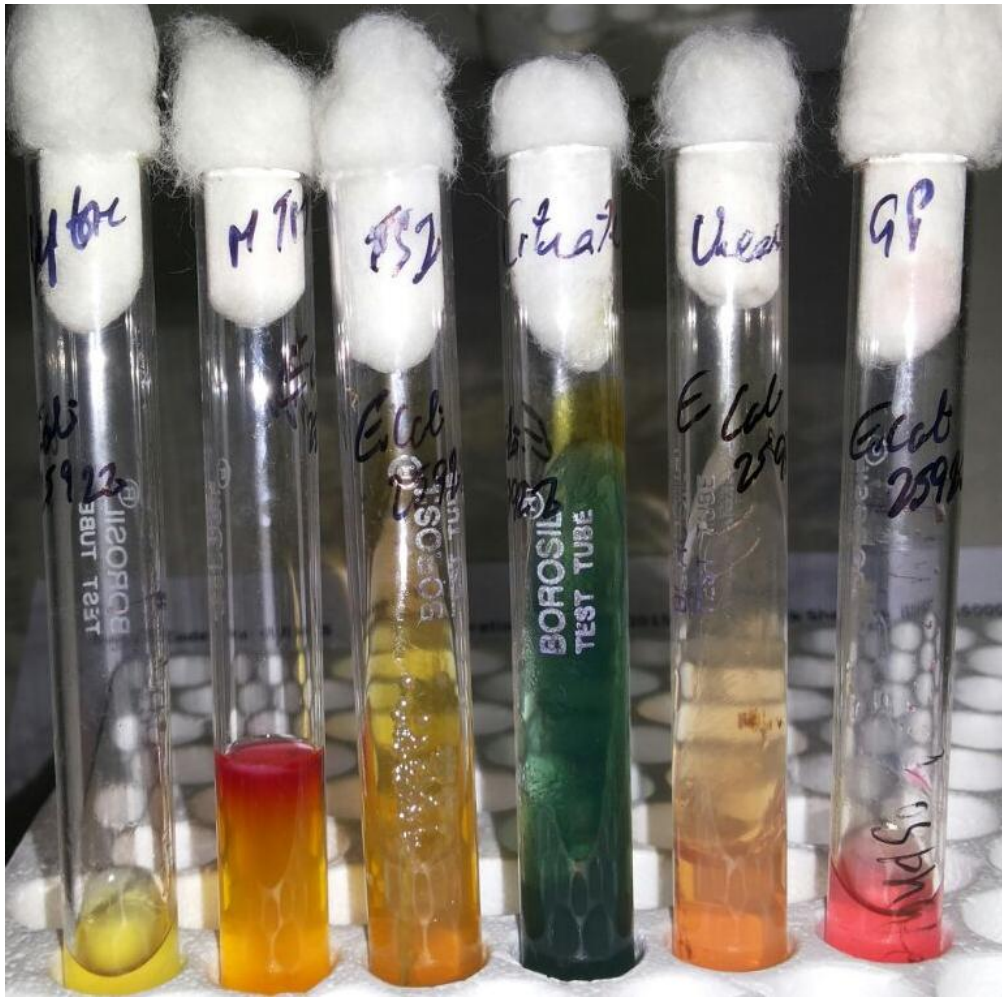
11.1 Discussion-

Urinary tract infection has become one of the greatest challenges in today's health care system. Due to the fact, nearly half of all people will experience a UTI in their lives at some point. In results, I have found that *E.coli* is the most common cause of urinary tract infection which accounts for up to 80% of infection in UTI patients. The data that I collected randomly for three months from different wards of Escort Hospital has shown 23 cases of *E.coli*, 6 cases of *K.pneumoniae*, and 4 cases of *P.aeruginosa*. The other organisms such as *Proteus mirabilis*, *E.faecalis*, and Candida spp accounts for 1 case, 3 cases, and 5 cases. This prospective study has proved that result of *E.coli* from Escort Hospital has shown the similar results of other studies. According to the recent study, it has been found that *E.coli* is the most common cause of UTI which accounts for up to 70% of UTI associated infections (Mach et al., 2012). Another study stated that about 130-175 million cases of UTI are mostly 80 % correlates with E.coli (Vincent et al., 2010). Moreover, study has also shown the highest cases of women having urinary tract infection instead of men. This is due the fact, as the results shows in table 3 where it says higher number of females is 25. On the other hand, the total number of male is 12. The reason behind women is more prone to UTI due to the shorter distance between the urethra and the anus.

In case of antimicrobial sensitivities among different organisms, Doripenem has shown the highest antimicrobial sensitivity. Antibiotics are the substances that produced by any microorganism and it has the ability to kill or inhibit the growth of other microorganism. Antimicrobial susceptibility test are used to measure the ability of an antibiotic that will inhibit the microbial growth in vitro. Moreover, study has shown that Doripenem with a susceptibility of 97.4% is at least two-fold more potent than other drugs such as imipenem against *E.coli* (Gales, Ana Cristina et al., 2011). According to the study, doripenem is a 1 beta- methylcarbapenem and it has been approved as a broad-spectrum antibiotic for treating complicated urinary tract and intra-abdominal infections (Pillar, Chris M et al., 2015). Doripenem is an agent that used to treat infections caused by Gram- negative bacteria. This has proved

the correlation between the sensitivity of Doripenem against Gram negative bacteria from Escort study in relates to other studies in USA.

Diagnosis by culturing has been done on CLED agar in Escort's Microbiology Lab. This is due to the reason that CLED is the cystein lactose electrolyte deficient medium. It is a valuable non-inhibitor growth medium and is being used for isolating and differentiating urinary tract infection causing organism. It has the capacity to prevent the swarming of *Proteus* species. *Proteus* swarm due to the presence of multiflagella. There are lactose fermenter and non- lactose fermenter organisms in case of UTI. *Kleibsiella, E.coli*, Enterobactor are the lactose fermenting bacteria and produces pink colonies. On the other hand, *Proteus* and *Pseudomonas* are non- lactose fermenter. *Pseudomonas* is further characterizing by four different pigments. These are Pyocyanin (bluish green), Pyoverdin (greenish yellow), Pyorubin (reddish brown), and Pyomelanin (brown to black).

12.1 Conclusion

In conclusion, it is possible to say that urinary tract infections are an important cause of illness in humans. *E.coli* has become the most common pathogen causing this infection. While all portions of the urinary tract may be affected, but the most common one are infection related with bladder known as cystitis and the renal pelvis called pyelonephritis. It is very important to know the symptoms and follow the treatment as per prescribed. Even though there are many routine and manual tests to detect the infection caused by particular organism, but there is always need for another molecular techniques which can be done easily and get the results more quickly for diagnosis.

References

1. Abrams, Paul et al. "The Standardisation of Terminology of Lower Urinary Tract Function- Report From the Standardisation Sub. Committee of the International Continence Society." *Neurology and Urodynamics*. 2002. 21:167-178. <ncbi.nlm.nih.gov>

2. Ananthanarayan and Paniker.2013. "Textbook of Microbiology." Hyderabad, India. Pg 3-688.

3. Ayazi, Parviz et al. "Diagnostic Accuracy of the Quantitative C. Reactive Protein, Erythrocyte Sedimentation Rate and White Blood Cell Count in Urinary Tract Infection Among Infants and Children." *Malays J Med Sci*. 2013 Oct: 20(5): 40-46. <ncbi.nlm.nih.gov>

4. Burillo, Almudena et al. "Gram-Stain Plus MALDI-TOF MS (Matrix- Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry) for a Rapid Diagnosis of Urinary Tract Infection." *PloS one*. 2014; 9(1): e86915. <ncbi.nlm.nih.gov>

5. Bien et al. "Role of Uropathogenic E.coli Virulence factors in Development of Urinary Tract Infection and Kidney Damage*." International Journal of Nephrology*. 2012. Article ID 681473: 1-15. <ncbi.nlm.nih.gov>

6. Brusch, John C. "Prevention of Urinary Tract Infections in Women." 2013 June. <emedicine medscape.com>

7. Colgan and Williams. "Diagnosis and Treatment of Acute Pyelonephritis in Women." Physician. 2011 September; 84(5): 519-526. <www.aafp.org>

8. "Cystitis." Fact Sheet. 2014 April. <betterhealth.vic.gov.au>

9. "Cystitis." Mayo Clinic Staff. 2012 April. <Mayoclinic.org>

10. Farooqui, Radar Jehan et al. "Urinary Tract Infection." *Journal of the college of Physicians and Surgeons Pakistan*. 2011, Vol.21 (12): 741-744. www.JCPSP.pK.

11. F Jin et al. "Incidence and Risks factors for urethral and anal Gonorrhoea and Chlamydia in a cohort of HIV-negative homosexual men: the health in men study." *Sex Transm Infect*. 2007 April; 53(2): 113-119. <ncbi.nlm.nih.gov>.

12. Foxman. "Epidemiology of Urinary Tract Infections: Incidence, morbidity, and Economic Costs." *The American Journal of Medicine*. 2002. Vol 113(1A). <Pubmed>.

13. Foxman et al. "Transmission of Uropathogens between Sex Partners." *The Journal of Infectious Diseases*. 1997; Vol 175(4): 989-92. <ncbi.nlm.nih.gov>.

14. Fulop. "Acute Pyelonephritis." 2014. <medicine.medscape.com>.

15. Gales, Ana Cristina et al. "Antimicrobial activity of doripenem against Gram-negative pathogens result from INVIT A-A-DORI Brazilian Study." *Braz J Infect Dis*. Vol 15 no. 6 Salvador. Nov/Dec. 2011. www.scielo.br.

16. Generali et al. "Intraversal Heparin: Interstitial Cystitis (Painful Bladder Syndrome)." *Hosp Pharm*. 2013 Nov. Vol. 48(10): 822-824. <ncbi.nlm.nih.gov>.

17. Griebling. "Urinary Tract Infection in Women." *Urologic Diseases in America Interin Compendrum*. 2004. 153-180. <urology.ucla.edu>.

18. Halder et al. "Mechanisms of hemorrhagic cystitis*." Am J Clin Exp Urol*. 2014:2(3): 199-208. <ncbi.nlm.nih.gov>.

19. Johnson, James R. "Virulence Factors in E.coli Urinary Tract Infection." *Clinical Microbiology Reviews*. Jan. 1991. Pg 80-128. <Pubmed>.

20. Karlowsky et al. "Trends in Antimicrobial Resistance among Urinary Tract Infection Isolates of E.coli from Female Outpatients in the United States." *Antimicrobial Agents and Chemotherapy*. Aug 2002. Vol. 48(8). 2540-2545. <ncbi.nlm.ncbi.gov>.

21. Kaur, Kamalpreet et al. "Evaluation of Efficacy and Tolerability of Cefotaxime and Sulbactam Versus Cefepime and Tazobactam in Patients of Urinary Tract Infection- A prospective Comparative Study." *J Clin Diagnose Res*. Nov 2014. Vol. 8(11): HCO5-HCO8. <ncbi.nlm.nih.gov>.

22. Kjolvmark, Charlott et al. "Heparin Binding Protein: A Diagnostic Biomarkers of Urinary Tract Infection in Adults." *Open Forum Infect Dis*. March 2014. Vol. 1(1). <ncbi.nlm.nih.gov>.

23. Mansour et al. "Efficient and Cost- Effective Alternative Treatment for Recurrent Urinary Tract Infection and Interstitial Cystitis in Women: A two –case Report." 2014. Pg 1-4. www.hindawi.com.

24. Mach, Kathleen E et al. "Biosenser diagnosis of urinary tract infections: A path to better treatment." *Trends Pharmacol Sci*. 2011 June. Vol.32(6): 330-336. <Pubmed>.

25. Pallin, Daniel J et al. "Urinalysis in Acute Care of Adults: Pitfalls in Testing and Interpreting Results." *Open Forum Infect Dis*. March 2014. Vol. 1(1). <ncbi.nlm.nih.gov>.

26. Picozzi, Stefeno C. et al. "Extended Spectrum beta-lactamase- positive E.coli causing complicated upper urinary infection: urologist should act in time." *Urol Ann*. June 2014. Vol. 6(2): 107-112. <ncbi.nlm.nih.gov>.

27. Pillar, Chris M et al. "In Vitro Activity of Challenging Infections Caused by Gram-Negative Bacteria, against Recent Clinical Isolates from the United States." *American Society for Microbiology*. 2015. Vol. 59. <aac.asm.org>.

28. Pincus, David H. "microbial Identification using the biomerieux Vitek 2 System." Biomerieux, Inc.Mo. USA. www.store.pda.org.

29. Scholes et al. "Risks Factors Associated with Acute Pyelonephritis in Healthy Women." *Ann Intern Med*. Jan 2004. Vol. 142(1): 20-27. <ncbi.nlm.nih.gov>.

30. Singer. "Urinary Tract Infections attributed to diverse ExPEC Strains in food animals: evidence and data gaps*." Front Microbiol*. 2015. Pg 6-28. <Pubmed>.

31. Shi, Huiqing et al. "Epidemiology of CTX-M type extended Spectrum beta-lactamase ESBL-producing nosocomial- E.coli infection in China." *Ann Clin Microbiol Antimicrob*. 2015.Vol. 14(4). <ncbi.nlm.nih.gov>.

32. Soltani, Rasool et al. "Antimicrobial susceptibility pattern of extended- spectrum beta-lactamase- producing bacteria causing nosocomial urinary tract infections in an Iranian referral teaching hospital." *J Res Pharm Pract*. 2014. Vol. 3(1): 6-11. <ncbi.nlm.nih.gov>.

33. "Urinary Tract Infection." www.Rx List.com.

34. Vincent et al. "Food Reservoir for E.coli Causing Urinary Tract Infections*." Emerg Infec Dis.* Jan 2010. Vol. 16(1): 88-95. <ncbi.nlm.nih.gov>.

35. Warren, John W et al. "Guidelines for Antimicrobial Treatment of Uncomplicated Acute Bacterial Cystitis and Acute Pyelonephritis in Women." *Clin Infec Dis*. 1999. Vol. 29: 745-58. <Pubmed>