Identification of pathogenic bacteria causing

Urinary Tract Infection

Submitted in partial fulfillment of the requirements of the Degree of

MASTER OF SCIENCE

(CLINICAL MICROBIOLOGY)

By

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CERTIFICATE

This is to certify that, the work entitled "Identification of pathogenic bacteria causing urinary tract infection" was carried out by Ms. Parmjeet kaur under my direct supervision. This is to further certify that this report embodies the original work carried out by the candidate herself and has not been submitted elsewhere in any form or for any other degree.

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Abstract:

Urinary tract infections are infections of urinary tract (upper urinary tract or lower urinary tract). It is a second infections acquired in hospitals. This is commonly caused by Gram-negative bacteria. Infection caused by bacteria is more common than fungus. *E.coli* is a prevalent in causing UTI than *Klebsiella* and *Pseudomonas*. Bacteria are gaining resistance to drugs becoming multi drug resistant which makes difficult to treat the patient. Other factors such as age, sex, catheterization, diabetes, renal dysfunction and low immune response are also responsible for UTI. UTIs are more common in women than men and it is seen more in older age. 40% infections are associated with catheterization.

Combination of antibiotic can be given to treat the multidrug resistant UTI.

KEY WORDS: urinary tract infections, multi drug resistant bacteria, *E.coli*, pyelonephritis, cystisitis, uretheritis**.**

Chapter-1

1.1 Introduction:

Urinary tract infections (UTI) are defined as infections of urinary tract. These can occur in any part of urinary system which includes kidney, urinary bladder, and urethra [36]. UTIs are the most common type of health care associated infections or hospital acquired infections [13, 9, 21, 6, 23]. UTI occurs when the immunity of host is weak or compromised then the virulent bacteria adhere, multiply, and colonize in a part of urinary tract [29]. UTI is mostly caused by bacteria but fungi and virus can also cause the urinary tract infection. [25]. UTI is more prevalent in women than men [36, 14, 1]. It is more prevalent in sexually active women [14]. Pregnant women are more likely to get UTI [15,] .the reason behind this is that women have shorter urethra than men. The hormonal changes in women also make her more susceptible for UTI [28]. Some other factors such as age, gender also have important role in urinary tract infections [9, 36, 6, 1, 22]. It is more common in older person than Youngers [36]. Diseases like diabetes, renal problem and liver problem makes person more susceptible for UTIs [30]. Catheterization in hospital also leads to UTIs which are known as catheter associated urinary tract infections (CAUTIs). CAUTIs are the second cause of nosocomial infection [13, 23]. These can be prevented by reducing the catheterization [17].

1.2 Causative organisms:

Urinary tract infections are commonly caused by the gram negative bacteria. These bacteria are normally present in the intestine. But by chance it enters in the urinary tract and cause infection.

The bacteria causing UTI includes *E.coli, Klebsiella, Pseudomonas, Proteus, Enterococcus spp., Staphylococcus* [7]. From these *E.coli* is prevalent in causing UTI[31,5,29]. 80% infections are caused by heterogeneous strains of *E.coli* known as uropathogenic *E.coli* and these are drug resistant strains.[38,2,18,14]This is also caused by fungi and commonly causing fungi is *Candida* [7].

1.3 Urinary tract includes:

Kidney: Kidneys are two in number, small organ lie in abdomen on the sides of the spinal cord. It plays vital role in body important function of it includes filtration of blood and remove the waste materials and excrete as urine. It also regulates the blood pressure, water balance, electrolyte balance, and helps in regulating the temperature of the body.

Ureter: These are thin tube like structures, two in numbers around 10 inches long. These carry urine from kidneys to urinary bladder.

Urinary bladder: Bladder is a small balloon shaped organ lie in pelvic region. In women bladder lies in front of uterus and in men it lies just above the prostate gland. When the bladder is full we experience the sensation that we have to pass urine to empty the bladder.

Urethra: Urethra is thin tube like structure which is connected with bladder and through which the urine pass and excrete through body. Urethra in women is shorter than it is in men.

UTI can involve any part of the tract as mentioned above. When infection is in kidney then it is known as **pyelonephritis.** When it involves bladder then infection is known as **cystitis** and when involves urethra it is known as **urehritis**.

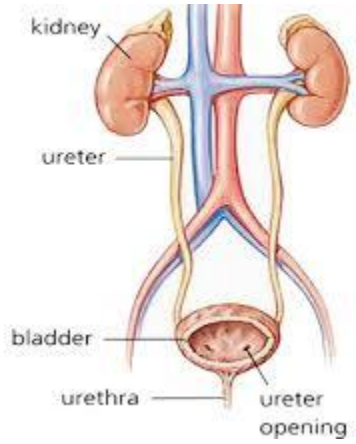

FIG.1.1. Urinary system

1.3 Types of urinary tract infections:

- **Upper urinary tract infections:** Infections which affects upper urinary tract which includes kidney and ureters. Infections of kidney are life threatening if not treated especially when the bacteria moves from kidney to blood. The movement of infectious bacteria from kidneys to blood is known as sepsis. This can leads to serious low blood pressure, shock, and if remain untreated can leads to death.
- **Lower urinary tract infection:** These include infections of lower urinary tract i.e. urethra and bladder.

1.5 Causes of UTI:

Anything that reduces bladder emptying or that causes irritation in the urinary tract those all things can leads to urinary tract infection. Some common causes that can leads to urinary tract infections are:

- **Obstructions:**
  Blockage in the tract due to which there is difficulty in urine passage can leads to urinary tract infections. This obstruction can be due to an enlargement of prostate, stones in kidney, and certain forms of cancer.
- **Sexual activity:**
  During sexual activity pressure on the urinary tract can leads to movement of bacteria from intestine into bladder. After intercourse, in most women bacteria are released along with urine. Usually within 24 hours these pathogenic bacteria is removed from bacteria

- **Unhygienic washrooms:**
  Washrooms should be hygienic especially for women. In hygienic conditions in washroom can lead to UTI because of their close contact of urethra to the surfaces of washrooms.

1.6 Risk factor:

- **Gender :**
  Women are more prone to get UTI because they have shorter urethra as compared to males.
- **Diabetes:**
  Due to diabetes patient becomes more susceptible to UTI.
- **Prolonged use of catheters:**
  Catheters are flexible, thin tubes placed in urinary bladder to drain urine. These are used when someone is unable to urinate normally. Urine drains through the tube into a collecting bag (Maki PG et al, 2001). Long term use of these catheters can increase the risk of urinary tract infections. It make easier for bacteria to enter into the bladder. We can treat this infection by removing the catheters.
- **Use of Spermicide:**
  Use of spermicides can increase the risk of UTI. As they cause skin irritation in some women. This increases the chances of entry of bacteria in urinary bladder.
- **Use of latex Condoms:**
  Use of latex condom can also increase the risk of UTI by increasing the friction during intercourse and irritating the skin.

- **Loss of Estrogen:**
  After menopause, there is a loss of estrogen which leads to changes in the normal flora of the urinary tract or vagina. This change increases the risk of urinary tract infection.

1.7 Symptoms of UTI:

Symptoms of urinary tract infection depend upon that which part is infected.

Symptoms of lower UTI:

Infection of bladder (cystitis): symptoms of this include,

- Inflammation of urethra and bladder,
- Dysuria,
- Urinary urgency,=: sensation of urinating urgently
- Cloudy, bad smelling
- Bloody urine
- lower abdominal pain,
- Mild fever.

Infection of urethra (urethiritis): symptoms include,

- Burning sensation during urination

Symptoms of upper UTI:

Upper UTI includes infection of kidneys. Symptoms of this appear rapidly and may or may also shows symptoms of lower UTI.

- High fever,
- Chills,
- Pain and tenderness in the upper portion of back at waist level and its sides,
- Nausea,
- Vomiting.

Chapter 2

Review of literature

We conform the urinary tract infections when in cultures we get 1000,000 CFU/ml and along with at least one microorganism in urine found during routine microscopy or wet mount [13, 36]. Urinary tract infections are most common infection among the hospital acquired infections, counting more than 40% of nosocomial infections [16, 25]. Urinary tract infections due to catheterization are the second cause of hospital acquired infection in the intensive care units [13]. Indwelling urinary catheters are responsible for approximately 80% of nosocomial infections of urinary tract [16, 5].

Urinary tract infections are more common, severe, and have worse effects in patient with diabetic mellitus type 2 and in this the infections are mostly caused by resistant pathogenic bacteria [26, 12]. Symptoms of urinary tract infections are similar as those in infections in non diabetic patient. There is no indication to treat the patient with diabetic having asymptomatic urinary tract infections [26].

Febrile urinary tract infections a common bacterial infection that may lead to substantial morbidity and mortality especially among the elders [37].

A study of 1540 nursing home residents determined that the risk of hospitalization, length of hospitalization, and length of antibiotic therapy were three times higher in catheterized residents than non catheterized resident[20]. The most notable complication associated with indwelling urinary catheters is the development of nosocomial urinary tract infections, known as catheter associated urinary tract infections (CAUTIs). Most cases of catheter associated bacteriuria or the presence of bacteria in the urine are asymptomatic. However, when an episode of CAUTI becomes symptomatic, the vresulting sequelae can range from mild (fever, uretheritis, and vcystitis) to severe (acute pyelonephritis, renal scarring, calculus vformation, and bacteremia). If these infections are left untreated then these can lead to urosepsis and death [27].

These complicated infections recur and result in long term morbidity due to the presence of encrustation and blockage of catheter by crystalline biofilms that increase resistance to the host immune response and to antibiotic [22].

Second leading causative agent for catheter associated urinary tract infection and asymptomatic colonization is *Candida*, where as it was studied previously that Candida is third leading pathogenic organism causing urinary tract infection [31].

Multi drug resistant *Enterobacteriaceae* (MDRE) and recently carbapenemase producing Enterobacteriaceae (CPE) is spreading which is found as major public health concern in patients with urinary tract infection. A study was conducted on 442 patients out of whom 182

Enterobacteriaceae strains were isolated. In these 160 isolates (87%) were MDRE amnd 5 isolateswere carbapenemase producers (CPE).the most common multi drug resistant isolates obtained were *E.coli* and *Klebsiella pneumonia*[8].

Infection caused by strains of *Klebsiella* producing carbapenemase(KPC) are becoming significant threat in recent years. These infections are associated with high rate of mortality. A patient who are in critical condition they can be treated with two or more drugs shows activity and increases the survival rate [34]

Adenosine-5□□-triphosphate (ATP) is a neurotransmitter and inflammatory cytokine found when there is lower urinary tract infection, in disease condition the normal mechanism of body is disturbed and ATP is produced. ATP also reflects as biomass of the microbes thus this can be used as marker to diagnose the urinary tract infections [11].

E.coli is most commonly causing bacterial infections in humans and animals. It is a prominent cause of urinary tract infections 80% infections are caused by this [3, 25, 28]. The treatment of infections caused by *E.coli* is difficult due to the development of resistance to many antibiotics. These are multi drug resistant *E.coli[*3,25]. In recent years the development of vaccine against uropathic *E.coli* associated urinary tract infections. For the prevention of chronic and recurrent infection caused by multidrug resistant strains vaccine is a viable approach [25].

Pseudomonas aeruginosa is also a Gram-negative pathogen causing infections especially when patient is immune compromised. Then these are commonly leading to nosocomial infections such as UTI, pneumonia and bacteremia. [33].

Urinary tract infections are the most common infection in women caused by bacteria. Use of bacterial based therapeutic vaccines can be the effective strategy to reduce the occurrence of recurrent urinary tract infections [24].

Urinary tract infection acquired in hospitals is the most common cause hospital acquired infections, and 97% it is caused by catheterization mentioned in the report of National Nosocomial Infection Surveillance system [19].

Chapter 3

MATERIAL & METHODS

3.1 Sample collection:

For diagnosis of UTI urine sample is taken. The quality of urine specimen for culture is important to determine the infection. The specimen of choice is the first morning void, since it is generally more concentrated ,due to the length of time the urine was in bladder. The preferred collection method is midstream, clean-catch specimen. Specimen collected from a newly specifically designed sterilized container.

Procedure for collection of midstream urine sample (MSU):

For collection of midstream urine specimen, patient is told to wash his or her hands and genitals.

Precaution to be taken while taking the sample:

Women patient is told to hold, open her labia (entrance of vagina).Men pull back his foreskin. Pass sample urine into toilet. Then without stopping the flow of urine; catch some urine in a sterile bottle. Once she has enough urine in the bottle, finish off passing the rest of her urine into toilet.

Do not open the sterile bottle until you are ready to take sample. do not need to fill the bottle to the top, a small amount will do. (Some specimen bottles contain a preservative, if this is the case, a mark on the bottle will indicate the ideal amount of urine). Avoid touching any part of his or her genitals with the bottle, as this will increase the risk of contamination. Put the cap back on container. The sooner the sample is given in to the laboratory. Within two hours is best. If that is not possible, put the sample in the fridge until you take it to the laboratory.

3.2 Processing of urine sample

After collecting the sample the sample was processed on same day. On first day the culturing was done and on next day identification of organism was done on the basis of growth characters, Gram staining and biochemical tests. Then it was processed further for antibiotic sensitivity testing.

3.3 Culturing of urine sample:

Media used for culturing.

- **Blood Agar**

Blood agar is the enriched medium, is a bright red and opaque medium. Some bacteria are more demanding in the nutrients they require for growth, a quality that results in their being called fastidious. The varieties of complex nutrients found in blood support the growth of most bacteria. Such media are said to be complex and enriched. In addition its role in fostering the growth of difficult to culture organisms, blood agar has a differential function. Bacteria growing on blood

agar can be classified in part on what they do to the red blood cells incorporated into the medium. Bacteria can be differentiated on the basis of hemolytic.

- **CLED Agar**

CLED agar is an abbreviation for cystine lactose- electrolyte deficient agar. CLED agar is a valuable non- inhibitory growth medium used in the isolation and differentiation of urinary organisms. Being electrolyte deficient, it prevents the swarming of Proteus species. Cystine promotes the formation of cystine-dependent dwarf colonies. Lactose fermenters produce yellow colonies on CLED agar, on-lactose fermenters appear blue.

Procedure:

1. Media was prepared and autoclaved at 121^0C for 15mins at 15 lbs pressure.
2. 20-25 ml media was poured in sterile, disposable Petri plates.
3. It was allowed to solidify.
4. All requirements like inoculating loops, sample, plates, slides, burner, normal saline all was kept in laminar flow.
5. Urine sample was mixed properly and inoculating loop was red heated to sterilize it.
6. Label the plates with lab identification number that is given to the sample.
7. With cooled sterilized loop loopful of sample was taken and streaking was done on blood agar, and then again with sterilize loop streak sample similarly on CLED media.
8. Plates were incubated at 37^0C in an incubator for 24 hrs.
9. Wet mount were observed.
10. On next day the growth was observed.

Processing on next day:
Growth was observed. Colonies were identified. Gram staining and biochemical test was done to identify the organism.

3.4 Biochemical testing (IMVIC Tests):

IMVIC reaction is set of four useful reactions that are commonly employed in the identification of members of family Enterobacteriaceae. Enterobacteriaceae are gram negative bacteria that grow in the intestinal tract of humans and other animals. The four reaction are Indole test,Methyl Red test,Voges proskauer test and Citrate utilization test.

- **Indole Test**

Principle of Indole Test:

Certain bacteria decompose aminoacid present in peptone into indole using enzyme tryptophanase.

i. Tryptophanase

Tryptophan ----------⟶ indole + pyruvic acid =NH3

ii. H2O

Production of indole is detected using Ehrlich's reagent or Kovac's reagent. Indole reacts with the aldehyde in the reagent and gives red color. An alcoholic layer concentrates the red color as a ring at the top.

Procedure:

1. Growth was inoculated in the peptone water.
2. It was incubated at 37^0C for 4 hrs.
3. After incubation few drops of kovac's reagent was added.
4. Solution was mixed properly and change in colour was observed.

Interpretation:
Colourless solution- indole test negative
Pink coloured solution- positive test

Example: ***Escherichia coli:*** Positive, ***Klebsiella pneumoniae*****:** Negative.

- **Methyl Red Test**

This test is employed to detect production of sufficient acid during fermentation of glucose by bacteria and maintenance of pH below 4.5.

Principle:
This is to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria produce large amounts of acids from glucose fermentation that they buffering action of the system. Methyl red is a pH indicator, which remains red in color at a pH of 4.4 or less.

Procedure:

1. Sterile tube containing glucose phosphate broth was taken.
2. Sample growth was inoculated into it and mixed properly.
3. Incubation was done for 4-6 hrs at 37C

4. 5 drops of MR reagent was added and mixed.
5. Change in colour was seen and noted.

Interpretation:
Red colour- positive MR test
Yellow colour – negative MR test

Examples: ***Escherichia coli, Proteus vulgaris*****:** Positive, ***Klebsiella*****:** Negative

- **Voges Proskauer Test**

Some bacteria produce acetyl methyl carbinol or its reduction product 2,3 butylene glycol from pyruvic acid in the media. In the presence of alkali and atmospheric oxygen, the small amount of acetyl methyl carbinol present in the medium is oxidized to diacetyl which reacts with peptone of the broth and produces red color.

Principle:
While MR test is useful in detecting mixed acid producers, VP test detects butylenes glycol producers. Acetyl methyl carbinol (acetoin) is an intermediate in the production of butylene glycol. In this test two reagents,40% KOH and alpha-nephthol are added to the test broth after incubation and exposed to atmospheric oxygen. If acetoin is present, it is oxidized in the presence of air and KOH to diacetyl.

Glucose ----------> Acetoin

KOH
Acetoin ----------> Diacetyl +H2O
Atmospheric O2

Alpha -naphthol
Diacetyl + guanidine component of peptone ----------> pink color

Diacetyl then reacts with guanidine component of peptone, in the presence of alpha-naphthol to produce red color. Role of alpha- naphhol is that of a catalyst and a color intensifier.

Procedure:
1. Sterile tube of glucose phosphate broth was taken.
2. The sample was added to this and incubated it for 4-6 hrs.
3. To the test tube 0.6ml of alpha-naphthol was added.
4. To this 0.2 ml of 40% KOH was added and mixed properly.
5. Tube was allowed to stand for 15 min.
6. Then change in color was seen.

Interpretation:
Red colour- positive test
Colourless – negative test

Examples: ***Escherichia coli:*** Negative, ***Klebsiella pneumoniae :***Positive

- **Citrate Utilization Test**

This test detects the ability of an organism to utilize citrate as the sole source of carbon and energy. Bacteria are inoculated on a medium containing sodium citrate and a pH indicator bromothymol blue. The medium also contains inorganic ammonium salts, which is utilized as a sole source of nitrogen.

Principle:

Citrate ----------> Oxaloacetate + Acetate

Oxaloacetate ----------> Pyruvate + CO_2

$CO_2 + Na + H_2O$ ----------> Na_2CO_3

Utilization of citrate involves the enzyme citritase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and CO_2. Production of NA_2CO_3 as well as NH_3 From utilization of sodium citrate and ammonium salt respectively results in alkaline pH. This results in change of medium color from green to blue.

Procedure:

1. Sterile tube containing citrate media (Simmon's citrate agar) was taken.
2. Sample was inoculated on the media.
3. Then incubated at 37C for overnight.
4. Change in colour was observed.

Interpretation:

Green colour- negative citrate test

Blue colour – positive citrate test

Examples: ***Escherichia coli:*** Negative, ***Klebsiella pneumoniae****:* Positive

- **Urease Test**

Urease broth is a differential medium that tests the ability if organisms to produce an exoenzyme called urease that hydrolyzes urea to ammonia and carbon dioxide. The broth contains two pH buffers, urea, a very small amount of nutrients for the bacteria, and the pH indicator is phenol red. Phenol red turns yellow in an acidic environment, fuchsin in an alkaline environment. If urea in the broth is degraded and ammonia is produced, an alkaline environment is created and the media turns pink. Many enteric can hydrolyze urea; however only a few can degrade urea rapidly these are known as rapid urease positive organisms.

Principle:

Many organisms especially those that infect the urinary tract, have a urease enzyme which is able to Split urea in the presence of water to release ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water to form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink.

$(NH_2)CO + H_2O \text{ ----------> } CO_2 + 2NH_3$

Procedure:

1. Loop was sterlized and then allowed to cool.
2. Loopful organism with the loop was taken aseptically.
3. Sterile urea slant tube was taken.
4. The slant of the medium is inoculated by streaking the surface of the agar in zigzag manner.
5. Again the neck of the urea tube was sterilized on flame and placed in the test tube rack.
6. Tightened the cap and incubated at 37ºC for 24-48 hours.
7. Obtain the tubes from the incubator and observe the color change.

Examples: P***roteus, Cryptococcus:*** Positive***, Escherichia coli:*** negative

3.5 Antibiotic sensitivity Testing

Antibiotic sensitivity testing is done to determine which antibiotic will treat the patient. By this we check the sensitivity of bacteria towards antibiotics. This will help to treat the patient with infection.

Kirby-Bauer method

This method was used to do the antibiotic sensitivity. It is a type of disc diffusion method. Mueller-Hinton agar was used for this. The organism is spread on media and antibiotic disc are placed on it. During incubation the antibiotic is diffused into the medium and will not allow the organism to grow. We can see the clear zone around the antibiotic disc. This zone is known as clearance zone or zone of inhibition. This zone is measured. In this method antibiotic disc are placed on spreaded growth, no control organism is used in this.

Procedure:

1. The growth was spread on sterilized MH media.
2. Then the antibiotic disc was put on it with the help of sterilized forceps.
3. Plates were labeled and then it was incubated for overnight at 37C.

Chapter 4

Observation:

4.1 Growth characters of E.coli:

- On CLED media it showed yellow growth (small colonies). Colour of medium is also changed.
- On blood agar it showed small grayish green colonies.
- On XLD media conformation was done it showed green metallic growth.

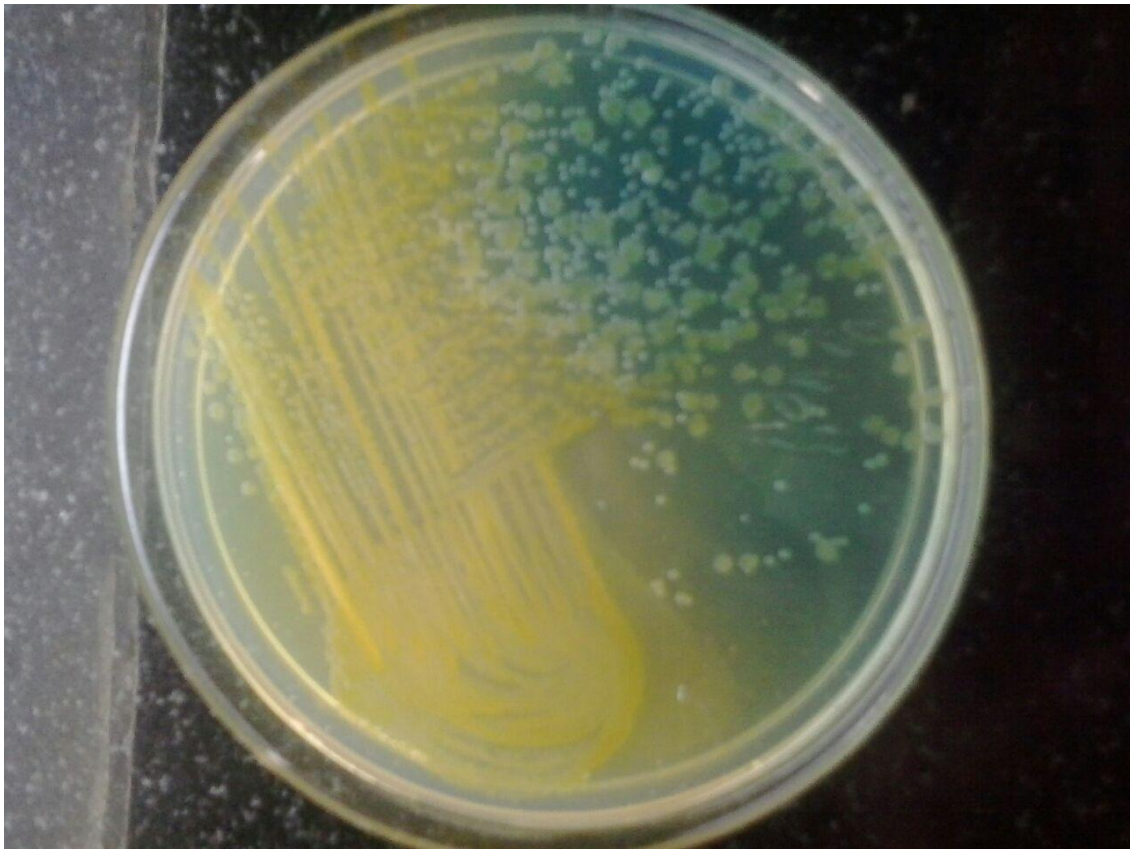

Fig .4.1 Growth of *E.coli* on CLED medium(yellow colonies)

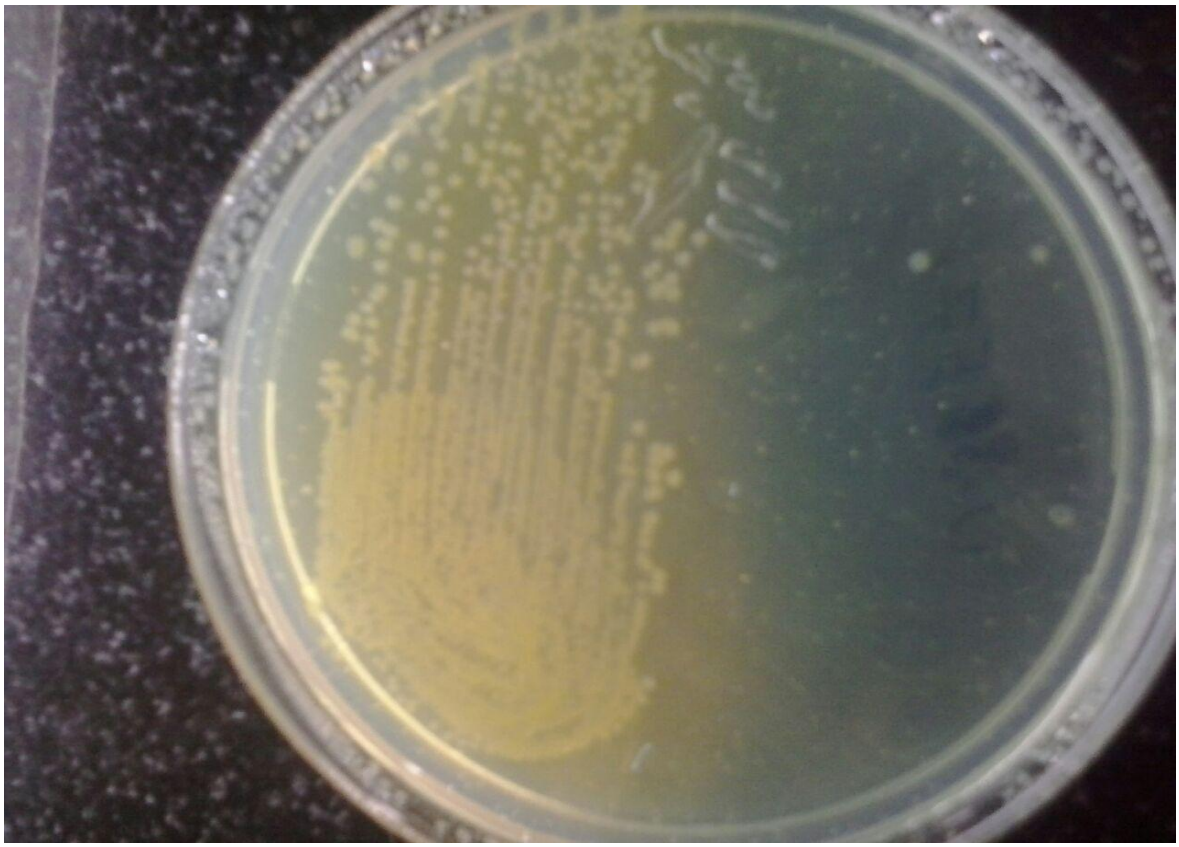

Fig.4.2 Growth of *E.coli* on CLED medium (yellow colonies)

Fig.4.3 Growth of *E.coli* on blood agar

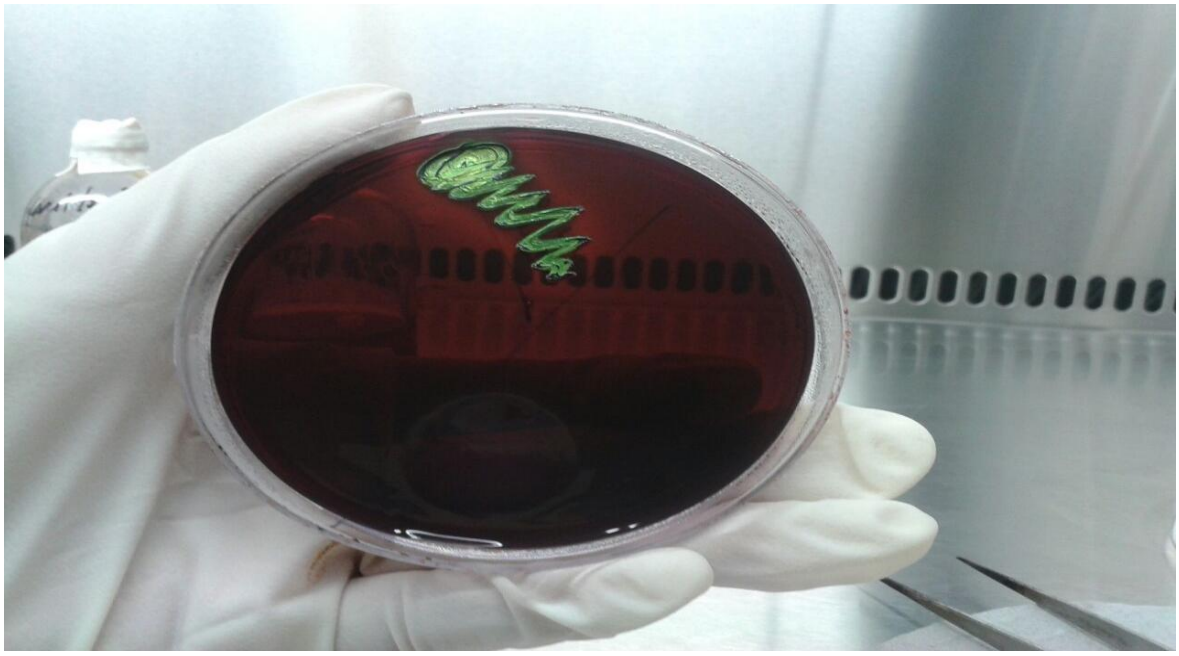

Fig.4.4 Growth of *E.coli* on XLD media (conformation of *E.coli*)

4.2 Growth of klebsiella:

- On CLED agar it showed large mucoid yellow colonies.
- On blood agar it showed large mucoid greenish grey colonies.

Fig 4.5 Growth of *Klebsiella* on CLED media.

4.4 Growth of Pseudomonas:

- On CLED media it showed the white and bluish mucoid colonies with fruity smell.

Fig.4.6 Growth of *Pseudomonas* on CLED media

Fig. 4.7 Growth of *Pseudomonas* on CLED media

4.5 Biochemical testing of isolated strains:

Fig 4.8 Tubes showing Biochemical tests

Fig. 4.9 Tubes showing biochemical testing

Fig4.10 Indole negative

***Klebsiella pneumoniae*:** Negative

Fig.4.11 Indole positive

Escherichia coli: Positive,

Fig.4.12 Citrate negative

***Escherichia coli**:* Negative,

Fig.4.13 Citrate positive

Klebsiella pneumoniae: Positive

Fig.4.14 Urease negative

Fig.4.15 Urease positive

***Escherichia coli*: negative**

Chapter-5

5.1 Results

For the analysis of urinary tract infections 200 samples were diagnosed out of which 80 samples were positive shows the growth of bacteria and 110 samples shows no growth as these were sterile. In these the male patients were 113 out of which 41 male patients were having urinary tract infection and 72 patients were sterile. We have total 87 samples of female out of which 39 was positive, showing the growth of bacteria and 48 was sterile having no infection. In this data of patient the age factor also shows a role. In my study 43 patient with age of >60year was positive, 22 patient with age 40 -60years and 11 patient of was of age group of 30-40years and 5 patients was of age of 20-30years.

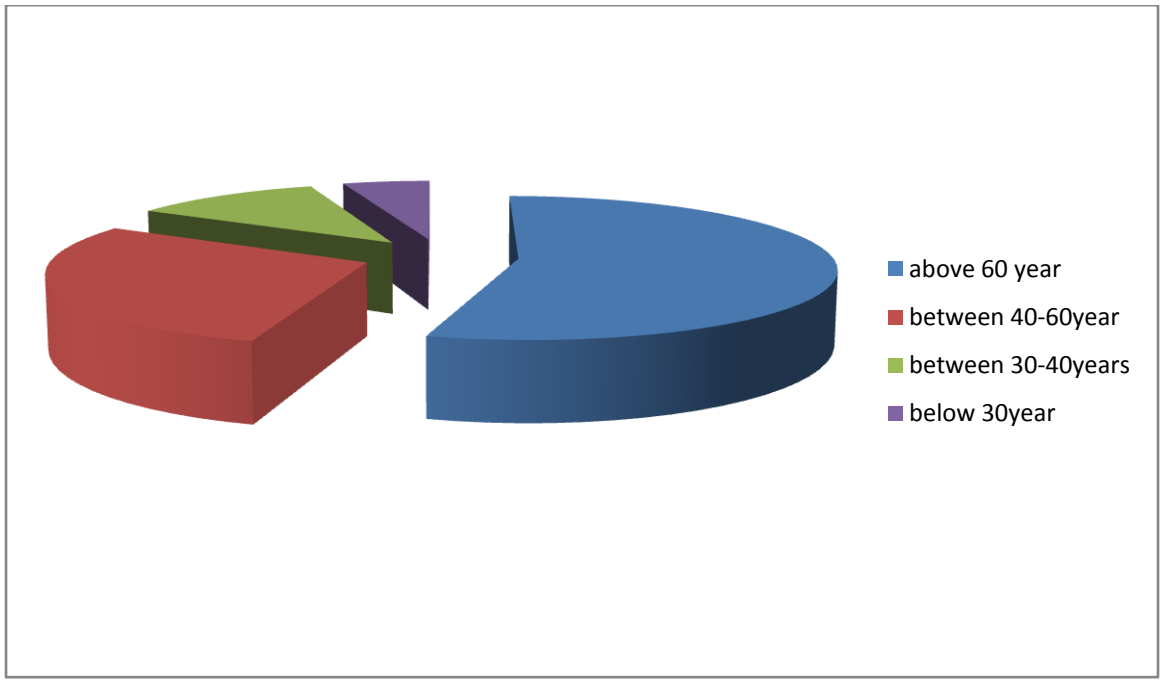

Fig. 5.1 Chart shows the percentage of age groups showing the positivity.

Other problems related to patient were also studied like liver problems, diabetes, renal problems, surgery and other heart related problems. After analysis of data of patient got the result t5hat 13 patient were of liver disfunction or fatty liver, 7 patient were diabetic, 20 patient were having renal problemand 2 patient have history of heart disease ande 12 patient was having history of surgery. Identification of grown bacteria was done which shows the bacteria was *E.coli, Klebsiella, Pseudomonas*.

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5.2 Result of cultures:

Table 5.1. Result of culture:

S.NO.	Name of bacteria	NO. of positive samples
1.	*E.coli*	59
2.	*Klebsiella*	13
3.	*Pseudomonas*	6
4.	*Enterococcus*	2
	total	80

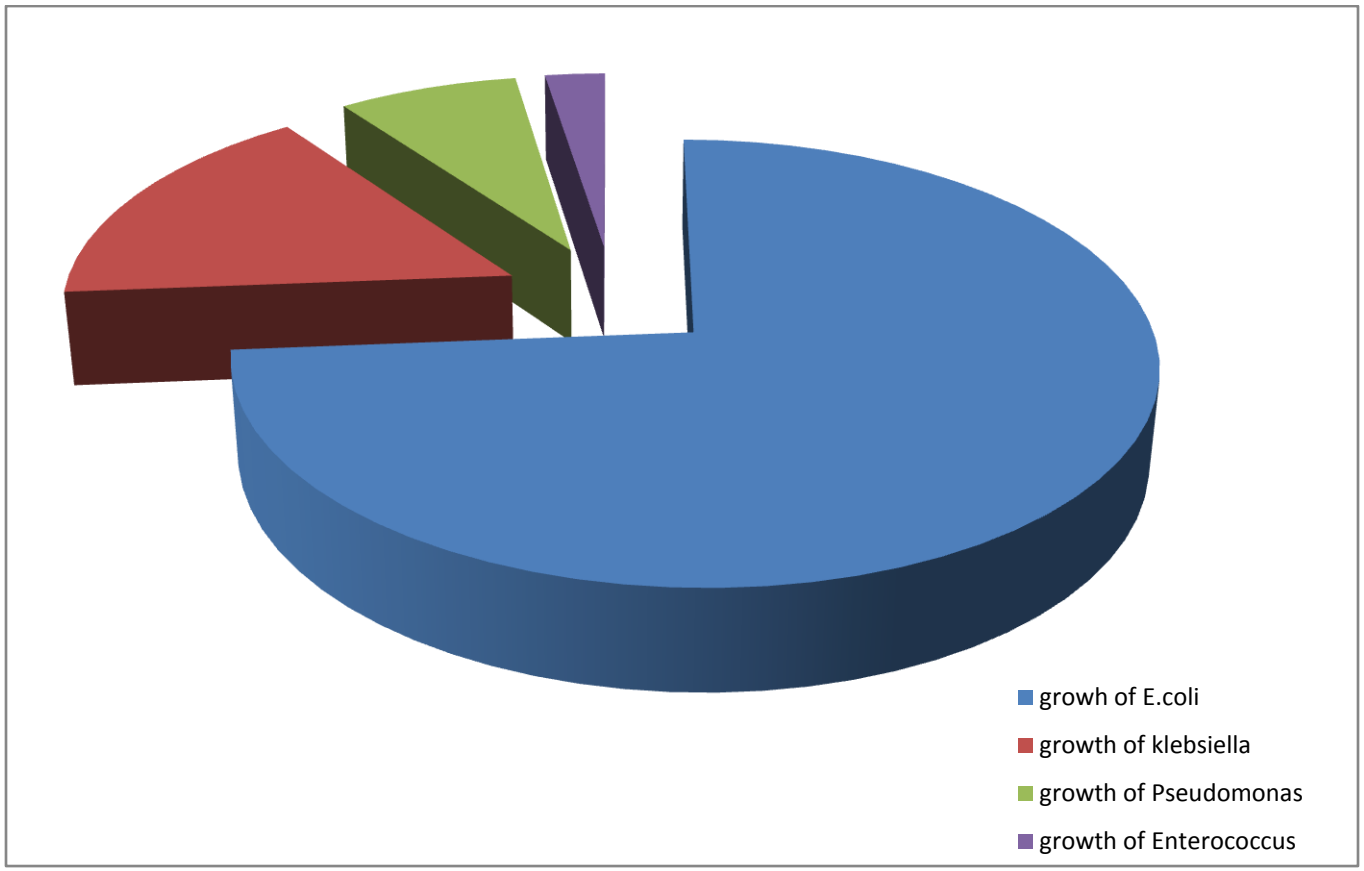

Fig.5.2 Chart showing the percentage value of prevalence of particular bacteria

5.3 RESULT OF ANTIBIOTIC SENSITIVITY TESTING

Antibiotic sensitivity was also done for all samples. Which shows some antibiotic were resistant some were intermediate sensitive and some were sensitive.

Table 5.2. Reference range for sensitivity:

S.No.	Name of antibiotic	Diameter for resistance	Diameter for intermediate sensitive	Diameter for sensitive
1.	Ciprofloxacin (5mcg)	15mm	16-20mm	21mm
2.	Norfloxacin (10mcg)	12mm	13-16mm	17mm
3.	Ofloxacin (1mcg)	10mm	11-12mm	13mm
4.	Ampicillin+ clavulanate (10mcg)	13mm	14-16mm	17mm
5.	Colistin (10mcg)	12mm	13-15mm	16mm
6.	Fosfomycin (200mcg)	10mm	--------	11mm
7.	Gentamycin (10mcg)	12mm	13-14mm	15mm
8.	Piperacillin+ tazobactum(100/10mcg)	17mm	18-20mm	21mm
9.	Amikacin(30mcg)	14mm	15-16mm	17mm
10.	Levofloxacin(5mcg)	15mm	16-18mm	19mm

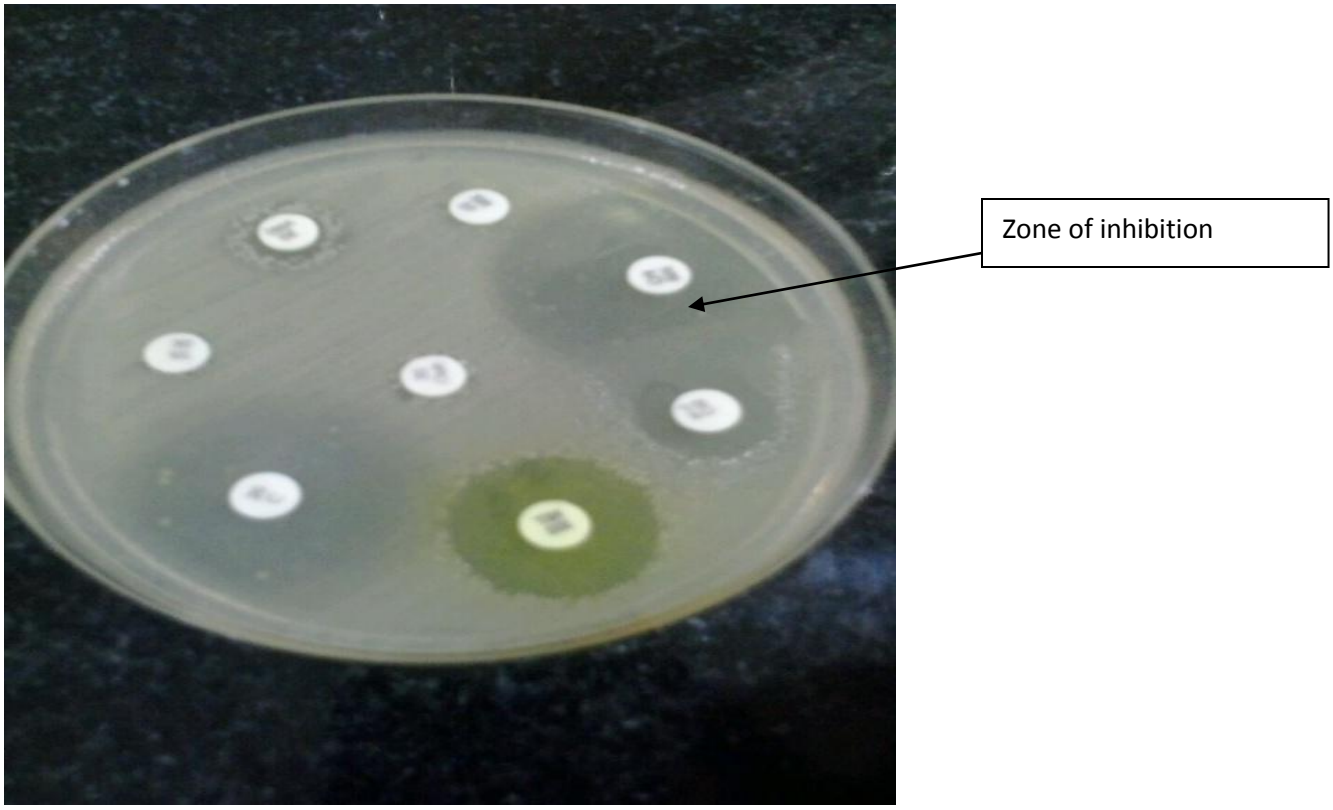

Fig.5.3 Antibiotic sensitivity against *E.coli*

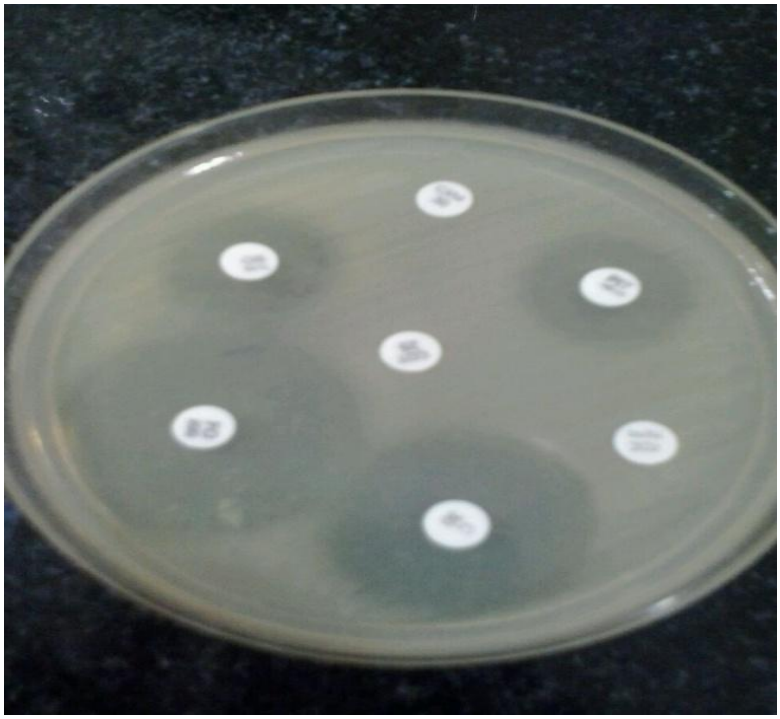

Fig 5.4 antibiotic sensitivity against *Klebsiella*

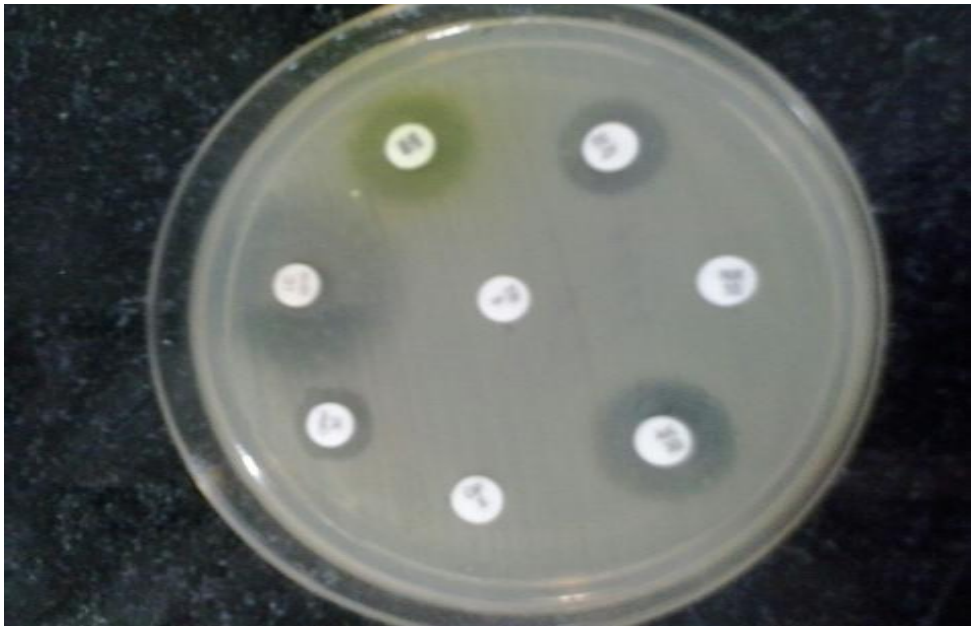

Fig 5.5 Antibiotic sensitivity against *Pseudomonas*

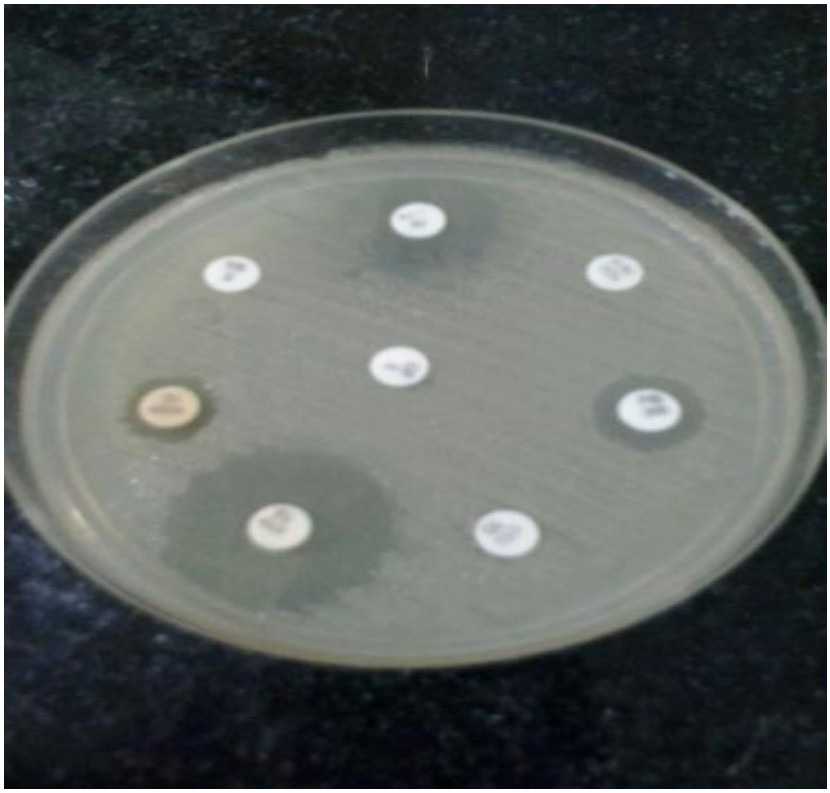

Fig 5.6 Antibiotic sensitivity *E.coli*

Table5. 3. Result of antibiotic sensitivity for *E.coli*

S.o.	Name of antibiotic	Antibiotic sensitivity
1.	Ciprofloxacin	Resistant in 99% samples
2.	Fosfomycin	Sensitive
3.	Amoxycillin+ Clavulanate	resistant
4.	Norfloxacin	Resistant
5.	Ofloxacin	Resistant
6.	Cefoperazone+ Sulbactam	Sensitive in 40% Intermediate sensitive in 60%
7.	Gentamycin	Sensitive in 60%

Table 5.4. Antibiotic sensitivity result for *Klebsiella*

S.no.	Name of antibiotic	Antibiotic sensitivity
1.	Ciprofloxacin	Resistant
2.	Fosfomycin	Sensitive
3.	Amoxycillin+ Clavulanate	Resistant
4.	Norfloxacin	Sensitive in 50%
5.	Ofloxacin	Resistant in 90%
6.	Cefoperazone+ Sulbactam	Resistant in 80%
7.	Gentamycin	Sensitive
8.	Colistin	Sensitive

Table 5.5. Antibiotic sensitivity result for *Pseudomonas*

S.no.	Name of antibiotic	Antibiotic sensitivity
1.	Ciprofloxacin	Sensitive
2.	Fosfomycin	Sensitive
3.	Amoxycillin+ Clavulanate	Resistant
4.	Norfloxacin	Sensitive
5.	Amikacin	Intermediate sensitive
6.	Piperacillin+ tezobactum	Sensitive
7.	Levofloxacin	Sensitive
8.	Ofloxacin	Resistant

Above result were obtained for antibiotic sensitivity testing.

Chapter-6

Discussion and conclusion:

After analysis of all data of patients we come to know that UTI are more common in women than men. Age factor also affect the occurrence of urinary tract infection. In my study 43 patient with age of >60year was positive, 22 patient with age 40 -60years and 11 patient of was of age group of 30-40years and 5 patients was of age of 20-30years.

Study shows that urinary tract infection was seen more in age >60. Infection in age group of <30 was rare. Other problems related to patient were also studied like liver problems, diabetes, renal problems, surgery and other heart related problems. After analysis of data of patient got the result t5hat 13 patients were of liver dysfunction or fatty liver, 7 patients were diabetic, 20 patients were having renal problem and 2 patients have history of heart disease and 12 patients was having history of surgery. We can say the other problems also affect the occurrence of urinary tract infection. Renal problems in which there will be inflammation and urine will not be passed properly which can ultimately result to urinary tract infection. As we know that kidney filters blood and urine formation occurs and through ureters the urine reached into bladder where it stores urine and when bladder is full then we have sensation of passing urine. Through urethra we pass the urine to outside the body.

Urinary tract infections are more common in women than men as they have shorter urethra and change in hormone. These are more prone in elders than younger. UTI are more seen in older age that is above 60 year in which the immunity level of patient is decreased which make person more susceptible for UTI. Thus this is less commonly seen in younger persons. These can be seen in children due to low i8mmunity and their unhygienic conditions.

We have the *Enterococcus* bacteria in our gastrointestinal tract by chance it can transfer to urinary tract and can cause urinary tract infection. Bacteria can also enter through contact of urethra to con5tam8nated surface like unhygienic washrooms or toilet sheets. Bacteria enter in urinary tract adhere to the epithelial lining of cells and there it colonize. In result it cause inflammation and damage to the organs like urinary bladder which is known as cystitis and if it reached to kidneys and damage the kidney which is known as pyleonephritis. Urinary tract infections occur when the immunity level of person is low and when patient is unable to empty the bladder completely.

Urinary tract infection is commonly caused by Gram negative bacteria. Bacteria which can cause urinary tract infection include *E.coli, Klebsiella, Pseudomonas, Proteus, other Enterococcus spp.* Sometimes it can be caused by other pathogenic bacteria which are transferred from other infected organ to urinary tract. This can be *Mycobactrium tuberculosis* and other. There are many symptoms by which it can be diagnosed that person is having UTI. 90% of urinary tract infections are caused by *E.coli*.

Patient may have the inflammation, pain in lower abdomen; burning sensation during passage of urine etc. this can be diagnosed by symptoms of patient, urine analysis, ultrasound, and cytoscopy of urinary tract

In urine analysis we can do wet mount and urine culture, and antibiotic sensitivity can be done. By doing the antibiotic sensitivity we can come to know that which antibiotic are resistant and which are sensitive. This will help doctor to prescribe the drugs to patient to treat him. Now days the more bacteria are becoming resistant against many antibiotics due to which it is difficult to treat the patient.

Conclusion:

Urinary tract infections are infections of urinary tract. These are mostly caused by pathogenic bacteria. It is a second infection in infections which are acquired in hospital. 90% infections are caused by *E.coli*. Many factors are responsible for urinary tract infections. Women are more prone for urinary tract infections than men as they are having shorter urethra and hormonal changes. Age factors also have a effect, old aged persons are more prone than younger due to weaker immunity level. Long term use of catheters is also one of the causes of UTI. So to prevent UTI we should maintain personal hygiene, avoid long term use of catheters, etc.

Long term use of catheters should be avoided. Treatment should be done by giving the combination of antibiotics. Hospital wards, Operation Theater and washrooms should be sterilized properly.

References:

1. Allocati N[1], Masulli M, Alexeyev MF, Di Ilio C. , Escherichia coli in Europe: an overview Int J Environ Res Public Health, 2013 Nov 25; 10(12):6235-54. doi: 10.3390/ijerph10126235

2. Ariel R Brumbaugh and Harry LT MobleyPreventing urinary tract infection: progress toward an effective *Escherichia coli* vaccine

Expert Rev Vaccines. 2012 Jun; 11(6): 663–676.

3. Ahmed Al-Badr[1,*] and Ghadeer Al-Shaikh[2] , Recurrent Urinary Tract Infections Management in WomenSultan Qaboos, Univ Med J. 2013 Aug; 13(3): 359–367.

Published online 2013 Jun 25.

4. C. A. Czaja,[*] B. N. Rutledge, P. A. Cleary, K. Chan, A. E. Stapleton,[†] W. E. Stamm , Urinary Tract Infections in Women With Type 1 Diabetes Mellitus: Survey of Female Participants in the Epidemiology of Diabetes Interventions and Complications Study Cohort, J Urol. 2009 Mar; 181(3): 1129–1135.

5. Donelli G[1], Vuotto C, Biofilm-based infections in long-term care facilities, Future Microbiol. 2014; 9(2):175-88. doi: 10.2217/fmb.13.149

6. David Medina-Bombardó[#1, 3] and Antoni Jover-Palmer[#2] , Does clinical examination aid in the diagnosis of urinary tract infections in women? A systematic review and meta-analysis, BMC Fam Pract. 2011; 12: 111

7. Dilista Piljic,[1] Humera Porobic-Jahic,[1] Dragan Piljic,[2] Sead Ahmetagic,[1] and Rahima Jahic[1] Chateter-associated Urinary Tract Infections in AdultsMater Sociomed, 2013; 25(3): 182–186.

8. Eshetie S[1], Unakal C[2], Gelaw A[2], Ayelign B[3], Endris M[2], Moges F[2], Multidrug resistant and carbapenemase producing Enterobacteriaceae among patients with urinary tract infectionat referral Hospital, Northwest Ethiopia, Antimicrob Resist Infect Control. 2015 Apr 17; 4:12. doi: 10.1186/s13756-015-0054-7. eCollection 2015

9. Enrico Magliano, [1] Vittorio Grazioli, [1] Loredana Deflorio, [1] Antonia Isabella Leuci, [1] Roberto Mattina, [2] Paolo Romano, [2]and Clementina Elvezia Cocuzza [3], Gender and Age-Dependent Etiology of Community-Acquired Urinary Tract Infections, ScientificWorldJournal. 2012; 2012: 349597.

10. Eshetie S[1], Unakal C[2], Gelaw A[2], Ayelign B[3], Endris M[2], Moges F[2], Multidrug resistant and carbapenemase producing Enterobacteriaceae among patients with urinary tract infection at referral Hospital, Northwest Ethiopia, Antimicrob Resist Infect Control. 2015 Apr 17; 4:12. doi: 10.1186/s13756-015-0054-7. eCollection 2015

11. . Gill K[1,2], Horsley H[3,4], Kupelian AS[5,6], Baio G[7], De Iorio M[8], Sathiananamoorthy S[9,10], Khasriya R[11,12], Rohn JL[13,14], Wildman SS[15], Malone-Lee J[16,17], Urinary ATP as an indicator of infection and inflammation of the urinary tract in patients with lower urinary tract symptoms,

BMC Urol. 2015 Feb 21; 15(1):7. doi: 10.1186/s12894-015-0001

12. Hamdan HZ[1], Kubbara E[2], Adam AM[3], Hassan OS[4], Suliman SO[5], Adam I[6], Urinary tract infections and antimicrobial sensitivity among diabetic patients at Khartoum, Sudan, Ann Clin Microbiol Antimicrob. 2015 Apr 21; 14(1):26

13. Hidalgo Fabrellas I[1], Rebollo Pavón M[2], Planas Canals M[3], Barbero Cabezas M[4]; colaboradores , Incidence of urinary tract infections after cardiac surgery: comparative study accordind to catheterization device, Enferm Intensiva. 2015 Mar 21. pii: S1130-2399(14)00095-9. doi: 10.1016/j.enfi.2014.10.004.

14. Hamdan Z Hamdan,[1] Abdel Haliem M Ziad,[2] Salah K Ali,[3] and Ishag Adam[4], Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital, Ann Clin Microbiol Antimicrob. 2011; 10: 2.

Published online 2011 Jan 18. doi: 10.1186/1476-0711-10-2

15. Joanna Matuszkiewicz-Rowińska,[1] Jolanta Małyszko,[2] and Monika Wieliczko[1] ,Urinary tract infections in pregnancy: old and new unresolved diagnostic and therapeutic problems, Arch Med Sci. 2015 Mar 16; 11(1): 67–77.

Published online 2015 Mar 14. doi: 10.5114/aoms.2013.39202

16. Jain P[1], Parada JP, David A, Smith LG , Overuse of the indwelling urinary tract catheter in hospitalized medical patients, Arch Intern Med. 1995 Jul 10;155(13):1425-9

17. Jennifer Meddings,[1] Mary A M Rogers,[1] Sarah L Krein,[1,2] Mohamad G Fakih,[3] Russell N Olmsted,[4] and Sanjay Saint[2,1] , Reducing unnecessary urinary catheter use and other strategies to prevent catheter-associated urinary tract infection: an integrative review, BMJ Qual Saf. 2014 Apr; 23(4): 277–289.

Published online 2013 Sep 27. doi: 10.1136/bmjqs-2012-001774

18. Justyna Bien, [1] Olga Sokolova, [2] and Przemyslaw Bozko [3], Role of Uropathogenic *Escherichia coli* Virulence Factors in Development of Urinary Tract Infection and Kidney Damage, Int J Nephrol. 2012; 2012: 681473.

Published online 2012 Mar 8. doi: 10.1155/2012/681473

19. Josef Peter Guggenbichler, [1] Ojan Assadian,[*, 2] Michael Boeswald,[3] and Axel Kramer[4] , GMS Krankenhhyg Interdiszip. 2011; 6(1): Doc18.

Published online 2011 Dec 15. doi: 10.3205/dgkh000175

20. Kunin CM[1], Douthitt S, Dancing J, Anderson J, Moeschberger M, The association between the use of urinary catheters and morbidity and mortality among elderly patients innursing homes, Am J Epidemiol. 1992 Feb 1; 135(3):291-301

21. Kevin B Laupland,[1] Sean M Bagshaw,[2] Daniel B Gregson,[3] Andrew W Kirkpatrick,[4] Terry Ross,[5] and Deirdre L Church[6] , Intensive care unit-acquired urinary tract infections in a regional critical care system, Crit Care. 2005; 9(2): R60–R65.

Published online 2005 Jan 6. doi: 10.1186/cc3023

22. Lona Mody, MD, MSc and Manisha Juthani-Mehta, MD, Urinary Tract Infections in Older Women, JAMA. 2014 Feb 26; 311(8): 844–854.

doi: 10.1001/jama.2014.303

23. Meltem Isikgoz Tasbakan,[1] Raika Durusoy,[2] Husnu Pullukcu,[1] Oguz Resat Sipahi,[1] and Sercan Ulusoy[1] , Hospital-acquired urinary tract infection point prevalence in Turkey: Differences in risk factors among patient groups,

Ann Clin Microbiol Antimicrob. 2013; 12: 31.

Published online 2013 Nov 4. doi: 10.1186/1476-0711-12-31

24. M. F. Lorenzo-Gómez, B. Padilla-Fernández, F. J. García-Criado, J. A. Mirón-Canelo, A. Gil-Vicente, A. Nieto-Huertos, and J. M. Silva-Abuin, Evaluation of a therapeutic vaccine for the prevention of recurrent urinary tract infections versus prophylactic treatment with antibiotics, Int Urogynecol J. 2013 Jan; 24(1): 127–134.

25. Moriel DG, Schembri MA[1], Vaccination approaches for the prevention of urinary tract infection, Curr Pharm Biotechnol. 2013; 14(11):967-74.

26. Nitzan O[1], Elias M[2], Chazan B[3], Saliba W[2] , Urinary tract infections in patients with type 2 diabetes mellitus: review of prevalence, diagnosis, and management, Diabetes Metab Syndr Obes. 2015 Feb 26; 8:129-36. doi: 10.2147/DMSO.S51792. eCollection 2015.

27. Niël-Weise BS[1], van den Broek PJ, da Silva EM, Silva LA , Urinary catheter policies for long-term bladder drainage, Cochrane Database Syst Rev. 2012 Aug 15;8:CD004201. doi: 10.1002/14651858.CD004201.pub3

28. . O Akingbade,[1] S Balogun,[1] D Ojo,[1] P Akinduti,[2] P O Okerentugba,[3] J C Nwanze,[4] and I O Okonko[3] , Resistant plasmid profile analysis of multidrug resistant Escherichia coli isolated from urinary tract infections in Abeokuta, Nigeria, Afr Health Sci. 2014 Dec; 14(4): 821–828. doi: 10.4314/ahs.v14i4.8

29.Olin SJ[1], Bartges JW[2], Urinary Tract Infections: Treatment/Comparative Therapeutics, Vet Clin North Am Small Anim Pract. 2015 Mar 27. pii: S0195-5616(15)00027-3. doi: 10.1016/j.cvsm.2015.02.005.

30. Orna Nitzan, [1, 2, 3] Mazen Elias, [2, 4] Bibiana Chazan, [1, 2] and Walid Saliba[2,4] ,Urinary tract infections in patients with type 2 diabetes mellitus: review of prevalence, diagnosis, and management, Diabetes Metab Syndr Obes. 2015; 8: 129–136.

Published online 2015 Feb 26. doi: 10.2147/DMSO.S51792

31. Padawer D[1], Pastukh N[2], Nitzan O[3], Labay K[3], Aharon I[3], Brodsky D[2], Glyatman T[2], Peretz A[4] ,Catheter-associated candiduria: Risk factors, medical interventions, and antifungal susceptibility, Am J Infect Control. 2015 Apr 24. pii: S0196-6553(15)00183-2. doi: 10.1016/j.ajic.2015.03.013

32. Stickler DJ[1], Zimakoff J, Complications of urinary tract infections associated with devices used for long-term bladder management,J Hosp Infect. 1994 Nov; 28(3):177-94

33. Sharma G[1], Rao S[1], Bansal A[1], Dang S[1], Gupta S[1], Gabrani R[2], Pseudomonas aeruginosa biofilm: potential therapeutic targets,

Biologicals. 2014 Jan; 42(1):1-7. doi: 10.1016/j.biologicals.2013.11.001. Epub 2013 Dec 3.

34. Tumbarello M[1], Trecarichi EM[2], De Rosa FG[3], Giannella M[4], Giacobbe DR[5], Bassetti M[6], Losito AR[2], Bartoletti M[4], Del Bono V[5], Corcione S[3], Maiuro G[2],Tedeschi S[4], Celani L[2], Cardellino CS[3], Spanu T[7], Marchese A[8], Ambretti S[9], Cauda R[2], Viscoli C[5], Viale P[4]; ISGRI-SITA (Italian Study Group on ResistantInfections of the Società Italiana Terapia Antinfettiva), Infections caused by KPC-producing Klebsiella pneumoniae: differences in therapy and mortality in a multicentre study, J Antimicrob Chemother. 2015 Apr 21.

35. Tazebew Emiru,[1] Getenet Beyene,[2] Wondewosen Tsegaye,[2] and Silabat Melaku[3] , Associated risk factors of urinary tract infection among pregnant women at Felege Hiwot Referral Hospital, Bahir Dar, North West Ethiopia, BMC Res Notes. 2013; 6: 292.

Published online 2013 Jul 25. doi: 10.1186/1756-0500-6-292(sexualactive,pregatwome)

36. Theresa A Rowe[1] and Manisha Juthani-Mehta[*, 1], Urinary tract infection in older adults, Aging health. 2013 Oct; 9(5): 10.2217/ahe.13.38.doi: 10.2217/ahe.13.38

37. van der Starre WE[1], van Nieuwkoop C[2], Thomson U[1], Zijderveld-Voshart MS[1], Koopman JP[1], van der Reijden TJ[1], van Dissel JT[1], van de Vosse E[1], Urinary proteins, vitamin d and genetic polymorphisms as risk factors for febrile urinary tract infection and relation with bacteremia: a case control study, PLoS One. 2015 Mar 25; 10(3):e0121302. doi: 10.1371/journal.pone.0121302. eCollection 2015.

38. Zhang L, Foxman B, Molecular epidemiology of *Escherichia coli* mediated urinary tract infections, Front Biosci. 2003; 8:e235–e244

Published online 2011 Oct 10. doi: 10.1186/1471-2296-12-111

Wesite links:

1. http://www.medicinenet.com/urinary_tract_infection/article.htm
2. http://www.cdc.gov/hai/ca_uti/uti.html
3. http://www.healthline.com/health/urinary-tract-infection-adult
4. www.cdc.gov/ecoli/general/index.html
5. https://www.cdc.gov/ncidod/dbmd/diseaseinfo/urinarytractinfections-t.html
6. https://www.cdc.gov/hai/orgaism/gram-negative-bacteria.html

Appendix

Composition of blood agar:

Peptic digest of animal tissue	-	5g
Sodium chloride	-	5g
Beef extract	-	1.5g
Yeast extract	-	1.5g
Agar	-	15g
Distilled water	-	1000ml
pH	-	7.4 ± 0.2

After autoclaving the media add 20ml in 500ml blood.

Composition of CLED media:

Peptic digest of animal tissue	-	4g
Casein enzymic hydrolysate	-	4g
Beef extract	-	3g
Lactose	-	10g
L- Cystine	-	0.1 – 8g
Bromothymol blue	-	0.02g
Agar	-	15g
Distilled water	-	1000ml
pH	-	7.3 ± 0.2

Composition of Simmon's citrate agar:

Magnesium sulphate	-	0.20g
Ammonium dihydrogen phosphate	-	1g
Dipotassium phosphate	-	1g
Sodium citrate	-	2g
Sodium chloride	-	5g
Bromothymol blue	-	0.08g
Agar	-	15g
Distilled water	-	1000ml
pH	-	6.8 ± 0.2

Composition of Muller-Hinton agar:

Casein acid hydrolysate	-	17.50g
Beef heart infusion	-	2g
Starch soluble	-	1.5g
Agar	-	17g
Distilled water	-	1000ml
pH	-	7.3 ± 0.1

Composition of Urease medium:

Peptic digest of animal tissue	-	1.5g
Dextrose	-	1.0g
Sodium chloride	-	5g
Phosphate	-	2g
Phenol red	-	0.012g
Agar	-	15g
Distilled water	-	1000ml
pH	-	6.8 ± 0.2

Composition of Gram stain:

A. 1% Crystal violet solution:
   - Crystal violet - 1g
   - Distilled water - 100ml

B. Gram's iodine solution:
   - Iodine - 1g
   - Potassium iodide - 2g
   - Distilled water - 300ml

C. Acetone

D. 0.5% Safranin solution

Composition of XLD (Xylose Lysine Deoxycholate Agar) media:

Yeast extract	-	3g
Lysine hydrochloride	-	5g
Xylose	-	3.7g
Lactose	-	7.5g
Sodium chloride	-	5g
Sodium deoxycholate	-	2.5g
Sodium thiosulphate	-	6g
Ferric ammonium citrate	-	0.8g
Phenol red	-	0.08g
Agar	-	15g
Distilled water	-	1000ml