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study of isolation and identification *Escherichia coli* from Urinary tract infection in patients with diabetes mellitus in the Diwaniyah city

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بسم الله الرحمن
الرحيم

((قالوا سبحانك لا
علم لنا إلا ما
علمتنا انك أنت
العليم الحكيم))

صدق الله العلي العظيم

سورة البقرة / الآية (32)

Dedication

After Sixteen years of study

This modest work dedicate

To all who contributed in our arrival to the end of the road

To all who learned us something new

And fueled our thoughts with science & knowledge

To all who stop by our side & help us in every hardship

To our teachers….

And of course to our strength & support

Our family …

Eman , Doaa , Zahraa

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Finally, we extend our sincere thanks and appreciation to the honorable parents and to our brothers and sisters for providing us with great moral support and patience for our suffering. There is very little difference in their right to pray and pray, and there is nothing left except to ask God to extend in their ages and to reconcile them to what pleases them.

Eman , Doaa , Zahraa

Abstract:

This study was completed during a period of six months (8/9/2017 to 13/2/2018) in the bacteriology laboratories of the Diwaniyah Teaching Hospital and the Obstetrics and Gynecology Hospital in Qadisiyah Governorate.

The aim of this study is to evaluate the incidence of urinary tract infection in diabetics and the methods of isolating Escherichia coli for different age groups of both sexes for patients who are visiting and attending the Center for Diabetes and Endocrinology at Diwaniyah Teaching Hospital. A random sample of 100 urine samples was collected for patients with diabetes and had urinary tract symptoms.

The study included age groups between (30-80) years, and the rate of infection among females is higher than males , the total number of cases of the disease was 30 (30%). The females were 16 (53.33%) and 14 (46.66%) of the total population of Escherichia coli.

Try 30 isolates were tested for 10 antibiotics that included almost all known and clinically known antibiotics. Most of these isolates showed similarity in their resistance to most antibiotics such as Amoxicillin / Clavulanic acid (AMC), Rifampicin (RA), Azteronam (ATM), Cefotaxime (CTX), Nalidixic acid (NA) and Trimethoprim (TMP), either Amikacin (AK) has an moderately sensitive .While they were sensitive to antibiotics Chloramphenicol (C), Meropenem (MEM) and Gentamycin (CN) .

Introduction :

- **Urinary tract infection**

Urinary tract infection (UTI) is one of the most common diseases in our community and is caused by many microbes and is widespread among millions of people, both sexes and different age groups. The disease is acquired from nosocomial hospitals and acquired from community (Hryniewicz, *et al.*, 2000). The cause of this disease is bacteria and fungi, Parasites and viruses rarely share . So to the emergence of inflammatory response and acute disease symptoms (Savas, *et al*., 2006).

There are two kinds of urinary tract infections. The upper part of the body and the injury of the lower part, which causes the pathogenic bacteria: *Pseudomonas*, *E.coli*, *Klebsiella*, *aeruginosa* and *Staphylococcus aureus*, have an important role in causing both types of infection. Despite the diversity of the bacteria responsible for the infection, *E.coli* remains the most common cause of the disease. Natural flora in the host system and ease of transmission from the intestinal canal to the urinary tract of humans, especially among children on the other hand (Parvin, *et al*., 2009).

- ***Escherichia coli***

E.coli (Escherichia coli) is one of several types of bacteria that normally inhabit the intestine of humans and animals (commensal organism). Some strains of *E.coli* are capable of causing disease under certain conditions when the immune system is compromised or disease may result from an environmental exposure.
Many patients have problems with urination which need afoly catheter to resolve it The catheter have a risk of contamination with *E.coli* and may lead to UTI (Brooks,et al.,2007)
E.coli , was first described by Theodor *Escherich* in 1885, is a member of family *Enteriobacteriaeace*. It is a gram negative, motile, non-sporing bacillus , produces rose pink colonies on MacConkey Agar, ferments glucose, lactose, trehalose, and xylose , yellow on CLED agar, Positive indole and methyl red tests, Does NOT produce H2S or phenylalanine deaminase, Simmons citrate negative, Voges-Proskauer test negative. Produces yellow colonies on XL-D medium. Further biochemical tests are required for accurate identification.
Modern Taxonomy of *E.coli* :

Kingdom: *Eubacteria*
Phylum: *Proteobacteria*
Class: Gamma *Proteobacteria*
Order: *Enterobacteriales*
Family :*Enterobacteriace*
Genes: *Escherichia*
Species: *coli*

The species can be differentiated from other members of *Enterobacteria* by biochemical reaction, being a member of *Enterobacteriaeace* it is present as normal flora in the lower intestine of both humans and animals . *E.coli* is a genetically and phenotypically diverse species whose strains are identified on the basis of 'O', 'H' and sometimes 'K' antigens, biochemically and serologically based on toxigenicity , On the basis of intestinal diseases, there are six categories: *Enteroaggregative E.coli* (EAEC), diffusely adherent *E.coli* (DAEC), *Enteroinvasive E.coli* (EIEC), *Enteropathogenic E.coli* (EPEC), *Enterohaemorrhagic E.coli* (EHEC) and *Enterotoxigenic E.coli* (ETEC) (Nataro *et al* , 1998). Over 700 serotypes of *E.coli* are recognized on the basis of O, H, and K antigens.

- **Pathogenesis :**

although a normal gut commensal, many strains of *E.coli* have entero pathogenic mechanisms which can be responsible for diarrhea and other symptoms:

1- Enterotoxins: these are of two types, both plasmid-coded: LT - heat-labile, and ST - heat-stable.
2-Adhesive factors: now known as colonization factor antigens mediated by plasmid-coded pili.
3- Entero-invasiveness: confers the ability to penetrate intestinal epithelial cells.
4- Vero cytotoxin (VT) produces a cytopathic effect on Vero cells. There are two (VT1 and VT2), which are serologically distinct. Strains that produce VT (known as VTEC), notably *E.coli* 0157, cause diarrhea with haemorrhagic symptoms.
5- Attaching - effacing mechanism: some strains adhere to intestinal epithelium to cause erosion (effacement) of the microvilli, resulting in formation of characteristic structures known as pedestals.

- **Virulence factors in *Escherichia coli* urinary tract infection:**

Uropathogenic strains of Escherichia coli are characterized by the expression of distinctive bacterial properties, products, or structures referred to as virulence factors because they help the organism overcome host defenses and colonize or invade the urinary tract. Virulence factors of recognized importance in the pathogenesis of urinary tract infection (UTI) include adhesions (P fimbriae, certain other mannose-resistant adhesins, and type 1 fimbriae), the aerobactin system, hemolysin, K capsule, and resistance to serum killing. Virulence factor expression is more common among certain genetically related groups of E.coli which constitute virulent clones within the larger E.coli population. In general, the more virulence factors a strain expresses, the more severe an infection it is able to cause. Certain virulence factors specifically favor the development of pyelonephritis, others favor cystitis, and others favor asymptomatic bacteriuria. The currently defined virulence factors clearly contribute to the virulence of wild-type strains but are usually insufficient in themselves to transform an a virulent organism into a pathogen, demonstrating that other as-yet-undefined virulence properties await discovery. Virulence factor testing is a useful epidemiological and research tool but as yet has no defined clinical role. Immunological and biochemical anti-virulence factor interventions are effective in animal models of UTI and hold promise for the prevention of UTI in humans. The risk of urinary tract infection (UTI) is higher in diabetics compared to non-diabetics. Diabetes Mellitus (DM) is a metabolic disorder that is described by rising of blood glucose because of incomplete or missing of insulin hormone. The patients with DM have malfunction in bladder which prompt of urine accumulation in its pool which serves a decent situation to the microbes to be develop and cause UTI (Funfstuck, et al., 2012) . Furthermore, a higher glucose level in the urine might make a culture medium for pathogenic microorganisms. In spite of the fact that the connection in the middle of diabetes and bacteriuria has been the subject of a few controlled studies, the relationship between diabetes and UTI hazard has not been analyzed until know (Saber, et al., 2010; Fu, et al., 2014).

- **Diabetes mellitus**

Diabetes mellitus (DM) has for quite some time been thought to be an inclining element for UTI and the urinary tract is the fundamental site of the contamination in diabetics with raised risk of complications of UTI. The important

recognized reason for UTI in patients with and without DM is *Escherichia coli* (Saber, *et al*., 2010). Many people with diabetes also have dysfunctional bladders that contract poorly; this allows urine to remain in static pools for long periods of time, providing luxurious ponds for bacteria to grow in (Andriole, 2002). The high prevalence of urinary tract infection among diabetic patients and the evidence of rapid parenchymal involvement emphasize the need for knowledge of the prevalence, clinical awareness of the problem and clarification of its consequences in order to define the magnitude of public health resources required to care for the disease (Akbar, 2001).

The presence of higher glucose concentrations in the urine might present another pathway for UTI development by amplifying bacterial reproduction, which creates a favorable environment for infections (Chen, et al., 2009; Fünfstück, et al., 2012; Geerlings et al., 2000). Patient-related factors such as age, metabolic control and duration of diabetes have also been suggested as increasing the risk of infection among those with diabetes (Brown et al., 2005; Fünfstück et al., 2012; Turan et al., 2008). Moreover, impaired immune response may play a role in the patient's decreased ability to defend against bacterial proliferation (Geerlings, et al., 1999; Valerius et al., 1982). Although previous studies have cited an increased risk of UTIs in subjects with diabetes.

The management of urinary infection in patients with diabetes is essentially the same as patients without diabetes. During the course of a lifetime with diabetes, UTIs would be ranked among the top ten concurrent or complicating illnesses by most experts and patients (Robbins and Tucker, 1994). Antibiotics are usually given empirically before the laboratory results of urine culture are available. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory. Since patterns of antibiotic resistance in a wide variety of pathogenic organisms may vary even over short periods and depend on site of isolation and on different environments, periodic evaluation of antibacterial activity is needed to update this information.

The aim of study :

1- study aimed to assess the occurrence of UTI in diabetic patients in Childbirth and Pediatric Hospital and Diwaniyah Teaching Hospital referred to the type of microbiologically confirmed UTI and Isolation of *Escherichia coli.*

2- The widespread use of antimicrobial agents leads to emergence of drug resistant organisms. Since the pattern of bacterial resistance is constantly changing over years, it is important to monitor the antibiotic susceptibility patterns of isolated organisms to ensure rational use of antibiotics for empirical and definitive treatment of urinary tract infections in the vulnerable group.

Materials and methods :

- **Instruments and Equipment's :**

NO	equipment	company
1.	Triple Beam Balance 2610	TAFESA(Germany)
2.	Incubator	BINDER
3.	Autoclave	HVE-50
4.	Refrigerator	DAIREI
5.	Laminar Air Flow(hood)	Prutscher
6.	Benzene burner	Shndon (England)
7.	Water Distillatory	Gallenkamp (England)
8.	Glass cylinder	AFMA-Jordan
9.	Pyrex® Glass Flask	AFMA-Jordan
10.	Plastic Petri dishes	AFMA-Jordan
11.	Plastic tubes	AFMA-dispo
12.	Loop Full	KD SURGICALS-INDIA
13.	Gloves and mask	Slibrand-China

- **Material and media :**

NO	**Material**	Company
1.	Glycerol	BDH (England)
2.	Blood agar base	HIMEDIA
3.	MacConkey's agar	HIMEDIA
4.	Nutrient broth	CONDA
5.	Mueller-Hinton agar	HIMEDIA
6.	Eosin- Methylene blue	HIMEDIA

- **Antibiotics :**

NO	**Antibiotics**	**Company**
1.	Gentamycin (CN) 10mcg	CONDA pronadisa
2.	Meropenem (MEM) 10mcg	CONDA pronadisa
3.	Azteronam (ATM) 30mcg	CONDA pronadisa
4.	Cefotaxime (CTX) 30mcg	CONDA pronadisa
5.	Chloramphenicol (C) 30mcg	CONDA pronadisa
6.	Trimethoprim (TMP) 5mcg	CONDA pronadisa
7.	Rifampicin (RA) 5mcg	CONDA pronadisa
8.	Nalidixic acid (NA) 30mcg	CONDA pronadisa
9.	Amikacin (AK) 30mcg	CONDA pronadisa
10.	Amoxicillin /Clavulanic acid (AMC)30mcg	CONDA pronadisa

Methods :

Samples collection

A total of 100 urine samples were collected from 100 donors with symptoms of urinary tract infection and diabetic patients from the Diabetes and Endocrinology Center at Diwaniyah Teaching Hospital in Qadisiyah City from the period 8/9/2017 to 13/2/2018.

Midstream urine samples (to avoid contamination of natural flora present in this area) were collected by sterile container and recording information (sex and age).

To investigate the *E.coli* bacteria, take a drop of urine and plant it by Loop full and plan on the surface of the dishes of the blood agar base and MacConkey agar and incubate the dishes for incubator (24-48h) at 37c°. And transferred to an EMB dish, then incubated dishes at 37c° for 24h . After this, a swab of the cultured bacteria in an EMB dish is taken with a sterile loop full and placed in a nutrient broth tube for the purpose of sample conservation.

To study the sensitivity and resistance of bacteria causing urinary tract infections to some antibiotics used to treat urinary tract infections.

Preparation of culture media :

Culture media were prepared according to the company's instructions for each medium, and the pH was determined and sterilized according to the type of agricultural medium by autoclave at 121c° for 2h. And then saved the agricultural media after pouring in dishes or tubes and according to the requirements of experiment in the refrigerator at a temperature of 4c ° until use.

Preparation of the culture media

1- Preparation of blood agar base

Is a medium used for the growth and differentiation of the blood-analyzing bacteria (Cruickshank *et al*.,1975) prepared according to the manufacturer's instructions.

2- Preparation of MacConkey agar

It is used for the growth of intestinal bacteria and for the identification of fermented bacteria of non-fermented lactose sugar (Atlas *et al*., 1995). It is prepared according to manufacturer's instructions.

3- Preparation of EMB agar

This medium was used to differentiate *E.coli* isolates from other *enterobacteria*. On this medium, *E.coli* colonies appear with green metallic shine coloration.

4- Preparation of nutrient broth

Used to save the sample after planting on an EMB dish. It is prepared according to manufacturer's instructions.

5- Preparation of Mueller-Hinton agar

Mueller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method (Satcher *et al.* , 1993).

Isolation and Activation of bacteria:

Upon arrival to the laboratory the samples were cultured on the nutrient agar. The pure colony that resulted from the first inoculation was cultured into two plates of MacConkey agar and blood agar to selective and differentiate the resulted colony.

These entire two agars were incubated aerobically at 37c° and checked after 24h.

The samples were activated by planting them on nutrient broth and then incubation at 37c° for 24h.

Group Test IMViC :

Indole test :

The indole test is a qualitative procedure for determining the ability of bacteria to produce indole by deamination of Tryptophan .A rad ringof indophenol is a sign of the positive outcome , and if green ring is evidence (Koneman *et al* . , 1988).

Methyl red test:

The methyl red test is a test to detect the susceptibility of bacteria to ferment a sugar glucose within 24 h when developed in the food medium containing a sugar producing a large amount of acids .Turns the color of the medium to the sampler on positive test(Brown ,2007).

Voges _Proskaur test:

The voges _proskaur test is used to detect the susceptibility of bctebaria to the analysis of glucose in part and the composition of the central compound **Acetyl methyl carbinol** when developing on the sugar_containing medium.Turn the center color to red color on the positive test(Brown ,2007).

Urease test:

The urease test is used to detect the susceptibility of bctebaria to the production of the urease enzyme, which is dependent on the analysis of in the middle, resulting in **ammonia(NH3),carbon dioxide (CO2)** and **water (H2O).** Changing the color of the medium from yellow to pink indicates a positive test (MacFaddin,2000).

Oxidase test:

The ability of bacteria to produce cytochrome oxidase can be determined by the addition of the oxidase test reagent or test strip to colonies that have grown on a plate medium .The appearance of violet indicates a positive test (Barron and Sydney , 1990) .

Catalase test:

Catalase is the enzyme that breaks hydrogen peroxide (H2O2) into H2O and O2. It is easy to test for this enzyme in bacteria. A test culture is exposed to 3% H2O2. If catalase is present, H2O2 is broken down to H2O and O2 .The oxygen is detected as a steady evolution of gas bubbles from the culture .The appearance of gas bubbles on slide surface is evidence of a positive test . (Barron and Sydney , 1990) .

Lactose fermentation:

It was used to differentiate lactose fermenters from non-lactose fermenters. Bacteria were streaked on MacConkey agar plates prepared and incubated at 37c° for 24 h. Pink color colonies indicates lactose fermentation.

Citrat utilization test:

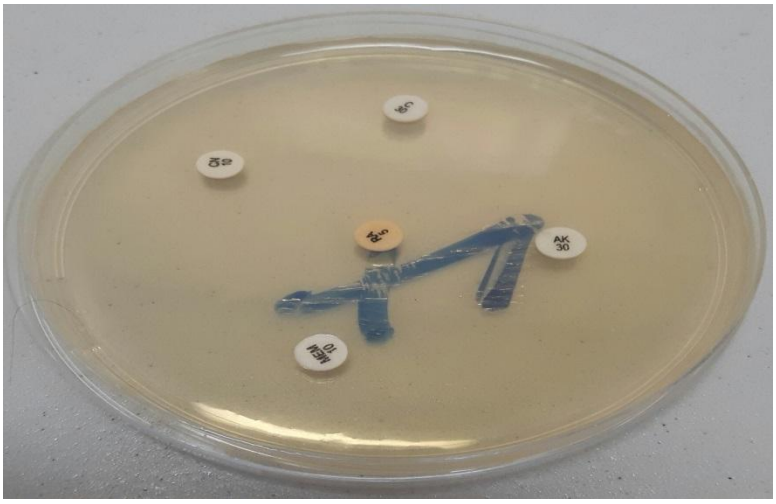
This test is used to detect the susc**e**ptibility of bacteria to the consumption of jackets as a single source of carbon. Changing the color of the medium from green to blue indicates that the test is positive, when the medium retains the color the green detector ,the result is negative (Brown ,2007).

Identification of isolated bacteria:

All samples of urine culture were verified within an hour of sampling. They were inoculated on blood agar as well as MacConkey agar and an EMB dish and incubated at 37c° for 24 h . A positive specimen was considered for UTI Bacterial identification was based on standard culture, morphological and biochemical characteristics of isolate (Nitzan, *et al*., 2015) .

Antibiotic Sensitivity test:

Prepared the Muller-Hinton agar according to the manufacturer's instructions, then leave the media to cool at 45-50c ° and pour in dishes to a depth of 4ml and kept at 4c° and were used within one week of preparation. (4-5) isolated and pure colonies were transferred to a tube containing 5ml of Nutrient broth using a sterile Loop and incubated for 2-4h and 37c°. Then a sterile cotton swap was plunged into the nutrient broth tube Then leave the dishes to dry and the lid is sealed for 15 minutes at room temperature. After the plate surface dried, the antibiotic tablets were placed using sterile forceps on the surface of the Muller-Hinton dish. five tablets can be placed on the surface of the dish. The dishes were incubated 15 minutes after placing the antibiotic tablets in the incubator for 24-18 h and at 35-37c° figer (1) . The diameter of each inhibition zone was measured to the nearest millimeter. The inhibition measures were then compared with specific measures recommended by the National Committee for Clinical Laboratory Standers. Accordingly, measurements of the inhibition zones were interpreted as sensitive (NCCLS , 1988 ; Satcher *et al* ., 1993) .



Figer (1) Mueller-Hinton agar dish and placing antimicrobial agents

Results and discussion :

A total of 100 mid-stream urine samples from diabetic patients were collected between the age range of 30-80 years which consists of 40 male (40%) and 60 female (60%) the total number of cases of the disease was 33 (30%). The females were 16 (53.33%) and male 14 (46.66%) of the patients were having asymptomatic bacteriuria of the total population of *Escherichia coli*.

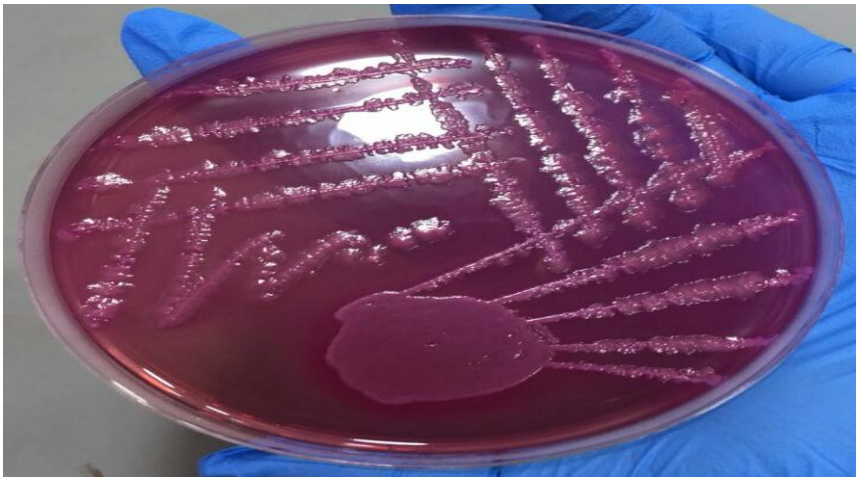
When the data were classified into different age and gender groups, it appeared that the cases of Urinary Tract Infections (UTIs) were more in women than men. The morphological characteristics of the isolated bacterial colonies were studied after their development in MacConkey agar blood agar base and the study included (shape, size and color). The results showed that the number of isolates belonging to *E.coli* bacteria was 30 isolates and 30% *E.coli* bacteria, which constitute the largest proportion of infections of urinary tract infections. The ecological diagnosis of the growing *E.coli* colonies on the MacConkey agar plate was characterized by its small pink size due to its ability to ferment the lactose sugar contained in the medium components figer (2) . As the growth of *E.coli* bacteria on the blood agar base figer (3), the colonies were characterized as non-blood- Bacteria on the secretion of hemolysis . After implantation of *E.coli* on the EMB dish, which is considered Selective Media of *E.coli*, will grow green colonies on the dish figer (4) . After that sample is taken from EMB dish by a sterile Loop Full and the sample was placed in the Nutrient broth .

The antibiotic sensitivity, which included almost all of the known antibiotics, was studied by taking a sample of Nutrient broth without Glycerol tube and planting them on a Mueller-Hinton agar dish and placing antimicrobial agents in the dish for the sensitivity and resistance of each antimicrobial used . The antibiotic sensitivity pattern has been determined by the zone of inhibition and classified as resistant, moderately sensitive and sensitive plate (5) . Try 10 isolates were tested for 30 isolates that included almost all known and clinically known antibiotics. Most of these isolates showed similarity in their resistance to most antibiotics such as Amoxicillin / Clavulanic acid (AMC), Rifampicin (RA), Azteronam (ATM), Cefotaxime (CTX), Nalidixic acid (NA) and Trimethoprim (TMP), either Amikacin (AK) has an moderately sensitive .While they were sensitive to antibiotics

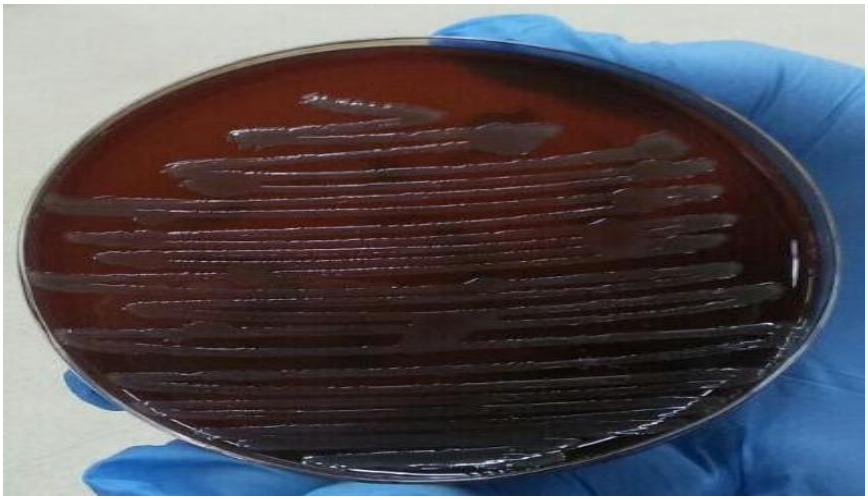
Chloramphenicol (C), Meropenem (MEM) and Gentamycin (CN) (such as table.

Antibiotic	resistant	sensitive	result
TMP	21	9	R
NA	23	6	R
CTX	23	7	R
ATM	24	5	R
AMC	30	-	R
CN	13	16	S
RA	30	-	R
MEM	-	30	S
C	-	30	S
AK	6	7	N

antibiotic	Resistance [R]	Sensitivity [S]
TMP	70%	30%
NA	76.66%	20%
CTX	76.66%	23%
ATM	80%	16%
AMC	100%	-
CN	43.33%	53.33%
RA	100%	-
MEM	-	100%
C	-	100%
AK	20%	23.33%



Figer(2) growing *E.coli* colonies on the MacConkey agar



Figer(3)growing *E.coli* colonies on the blood agar

Figer(4) growing *E.coli* colonies on the EMB agar

Figer (5) antibiotic sensitivity pattern has been determined by the zone

Sample	Age	Sex
1.	51	Male
2.	48	Female
3.	53	Female
4.	55	Female
5.	44	Female
6.	43	Female
7.	46	Female
8.	81	Male
9.	59	Female
10.	75	Female
11.	64	Female
12.	42	Female
13.	43	Female
14.	39	Female
15.	55	Female
16.	67	Female
17.	80	Female
18.	74	Female
19.	33	Female
20.	72	Male
21.	44	Male
22.	53	Male
23.	51	Male
24.	57	Male
24.	40	Male
25.	43	Male
26.	52	Male
27.	49	Male
28.	57	Male
29.	44	Male
30.	57	Male

Sex	Sample	percentage
Male	14	46.66%
Female	16	53.33%

- **Biochemical tests :**

Biochemical tests of *E.coli*	MR	KIA Medium				Ox	Mot	Cit	Ind	Urea	Vp
		slope	Butt	H2S	Gas						
Result	+	y	y	-	+	-	-	-	+	-	-

- Ind = indole test
- Cit = Citrate Utilization test
- Mot = motility test
- Ox = Oxidase test
- H2S = Hydrogen Sulphide
- MR = Methyle– Red test
- VP = Voges – Proskauer test
- Y = Yellow

Conclusion :

UTI are common among patients with diabetes mellitus. In these patients, UTI are more severe, caused by more resistant pathogens, and is associated with worse outcomes than in patients without diabetes. Treatment should be offered only to symptomatic cases, as Asymptomatic Bacteriuria (ASB) is a common finding, and antibiotic treatment in such cases serves mostly to increase bacterial resistance. Treatment should be tailored according to severity of infection and culture results. Further studies are needed to improve the treatment of patients with diabetes and UTI.

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