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LABORATORY INVESTIGATIONS OF URINARY TRACT INFECTIONS USING BACTERIOLOGICAL AND BIOCHEMICAL METHODS AND THEIR ANTIBIOTICS SENSITIVITY

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Abstract

Urinary tract infections (UTIs) are one of the most common bacterial infections at communities and hospitals settings. In people without anatomical or functional abnormalities, UTIs are generally self-limiting. There are different pathogens that cause urinary tract infections in people of both sexes and ages. Our study aims to identify these pathogens that have antibiotics resistance. The study sample is midstream urine collected from male and female subjects of different age groups and tested by microscopic examinations. Analytical profile index (API) is used for the detection of the Uropathogens as a biochemical test. Different culture media are used in this to identify the type of the bacteria that cause urinary tract infections by using VITEK 2 compact system. The analysis shows that the most common organism cultured from the urine specimen is *Escherichia coli* that is followed by *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus* sequentially. Among the antibiotics tested, patients respond (100%) to chloramphenicol and ciprofloxacin as a treatment of UTIs. Also, they have covered the majority of urinary pathogens. Other treatments are also available for UTIs as follows: tetracycline, gentamycin and kanamycin compose 83% and Ampicillin 67%. Streptomycin, Rifampicin and amoxicillin are less effective 50%.Amikacin is only effective in 21.08% of the patients and has covered the majority of urinary pathogens followed by Imipenem (18.20%). Other less active antibiotics are as follows: Gentamycin (14.69%). Amoxicillin effectiveness is (3.39%), Azithromycin 4.27%and Ampicillin 4.75%. Our study shows that a higher percentage of the patients are 26-35 years. In terms of sex, the results indicated that females are more infected than males.

***Keywords*:** urinary tract infections, bacteriological and biochemical methods, antibiotics sensitivity.

Introduction

Urinary tracts are the most vulnerable part of the body to infection. In addition, people are infected world-wide at any time. Both the upper and lower urinary tract can be infected by different pathogens. People can be infected in hospitals or any other places. *E. coli* is one of the commonest pathogens causing approximately 80 % of UTIs. (Hooton, and Stamm. 1997, 551–581.). *Staphylococcus saprophyticus* is also common uropathogens in uncomplicated UTIs while Gram-negative rods (enterobacteria other than *E. coli* or *Pseudomonas aeruginosa*) and Gram-positive cocci are identified in complicated UTIs (ibid). Fever is fairly common symptom of UTI among children. While among infants, common symptoms of UTIs are poor feeding, vomiting, loose of appetite. Bacteria can cause UTIs when they enter the human body and invade the wall of bladder to form biofilm. Biofilm can resist the body immune reaction (Patterson and Andriole, 1997; Meilandand Hoepelman, 2002). In adults, the common symptoms are burning micturition, upper pubic pain, turbid urine, foul-urine odor, etc. UTIs is complicated in pregnant women (John and MchelL, 2006). UTIs reoccurs frequently in about 33% of the women with acute uncomplicated UTIs (Foxman *et al.,* 2000).

The pathogenesis of UTIs involves complex interaction between an organism, the environment and the potential host. The symptoms of a patient with urinary tract infection depend on age and anatomical location of the infection. Chronic and acute infections of urinary tract lead to high blood pressure, kidney damage and results in death. Chronic manifestations of the UTIs are acute and chronic pyelonephritis (a disease process resulting from the effect of infection of parenchyma and pelvis of the kidney), cystitis, renal carbuncle, arthritis and prostatitis.

If the infection is chronic and acute, it can cause hypertension (high blood pressure), kidney damage and death. The clinical manifestations also vary according to the portion of the infected tract, the etological organism(s), how severe the infection is and the patient's immune reaction to the infection (Foxman and Brown, 2003).

Different methods can be used for the detection of various pathogens in UTI patient Including urinalysis, gram stain procedure, and urine culture. Urine culture is considered the most common and standard method to detect UTIs because it can identify the uropathogen species although it takes a long time to conduct and is expensive. About 70% of urine cultures are negative, with high costs for tests which are not necessary (Deville *et al*., 2004). The urine dipstick test is useful to rule out infections. To achieve more accuracy, an auto-analyzer is used (BMC Urol. 4:4). Thus, the automated analyzers for the detection of UTIs are widely available and negative samples can be sent to clinicians. However, positive samples in this type of tests are cultured for counting colonies and for the identification of the microorganism.

The VITEC system (biomerioux –VITEC 2 compact) is prototype and introduced to clinical laboratories in 1979 and

since then has been evaluated extensively (Doern *et al*., 1997).

The UTI treatment relies on the resistant and the sensitivity of antibiotics such as ciprofloxacin, Amoxycillin, Trimethoprime. However, a prolonged use of antibiotics has side effects on the patient and the pathogen may develop resistance through mutational changes. In contrast, in patents with frequent infections, low-dose antibiotics may be recommended for preventative measures. Intravenous daily antibiotics or prolonged antibiotics course may be effective for frequent and complicated infections. Unfortunately, Ambekar *et al.* (2008) state that many uropathogens are resistant to a large number of antibiotics because of the abuse, over or uncompleted dosage, non-prescribed use and easy access to antimicrobial medications.

The study aims mainly to determine the type of bacteria which cause UTI according to age and sex. The study also attempts to investigate the patients' antibiotic susceptibility through cultural and biochemical methods.

Materials and Methods

This study is carried out in the microbiology department in Par Hospital in Erbil city in Iraq. The urine samples are collected from 860 outpatients suspected of having a UTI, who did not receive antimicrobials within the previous month. There are 591 (66.62%) samples from female patients and 296 (33.38%) from male patients. The patients age range is 2-55 years.

Collection and processing the urine samples

The samples are clean catch mid-stream urine collected in a sterile container. Then, these samples are sent to the microbiology laboratory to be processed within two hours (hr) of collection. Two types of agar media are used to culture the samples in a semi-quantitative standard program. The first is blood agar and the second is MacConkey II Agar. Incubators are used to incubate the culture plates at 35–37°C in ambient air for 24 hrs. After two hours of culturing, the culture plates are monitored for the appearance of bacterial colonies. The growth considered to be significant or non-significant according to the plate count method. Urine samples are also centrifuged to obtain urine sediment which is directly analyzed under light microscope for red blood cells (RBCs), leukocytes, epithelial cell, casts, crystals, and parasites. A few count of RBCs, pus cells (0–5/high power field), and epithelial cells can be found in sediment of normal urine. A more delicate count is reported for Epithelial cell in a low-power field: "few," "moderate," or "many".

Measurement and Calculation

To count Colony of growing and/or dividing bacterium cells numerically per milliliter of urine, the colony count method is used. It is a quantitative method to measure and can differentiate between true bacteria from contaminations of bacterial (Hamdan *et al*., 2011). Hamdan *et al.* (2015) state that the latter often occurs when the mid-stream or "clean-catch" urine samples are not collected probably. People are affected by UTIif colony-forming units (CFUs)/ml of mid-stream urine is $>10^5$. This amount is diagnostic and UTI is described as urine culture plates which grow bacterial colonies of $\geq 10^5$ CFU/mL in every sample of mid-stream urine.

Bacterial identification and susceptibility testing

VITEK 2 Compact System is used to identify and measure the antimicrobial susceptibility of bacteria which are cultured from the urine samples. First, CLED agar is used for urine culture and then incubated at temperature 35–37°C in 5% CO_2 following a period of 18 hr incubation; the growth of bacteriais first identified by their colony morphology and then applying the procedure of gram staining. For the formulation of a standardized saline inoculum suggested to identify the VITEK, the culture plates with a growth of significant bacterial colonies are utilized. For this purpose, a special ID: Gram-negative ID card (GN Reference 21 341) and Gram-positive ID card (GP Reference 21 342) are employed.

Biochemical Examination

To confirm the pathogens species, two sets of tests are conducted on the selected culturally based colonies. The first include analysis by the microscopic and by microbiology, and biochemical hydrolysis of starch, lipid, caseins. The second tests are triple sugar iron agar, oxidase, catalase, nitrate reduction, indole production, methyl red, voges-proskauer, citrate utilization, ureaset).

Because of their accuracy, Analytical Profile Index (API) strips are utilized for the identifications which are extensive databases based, standardized, quick, safe and easy-to-use. The kits are strips with about 20 tests of miniature biochemical. These tests contain a variety of substances which are quick and safe to use.

Principle of the test

The API range is a standard test which is a miniaturized version of availed identification techniques. Up till now. These techniques are complicated to conduct and hard to read. The API 20Eplastic strip have twenty mini-test chambers which contain dehydrated media. These media are compositions which chemically defined in every test. They are often used for the detection of enzymatic activity which in most cases related to ferment carbohydrate or catabolism of proteins or amino acids through the inoculated organisms.

To rehydrate every wells, a suspension of bacteria is employed in addition to the incubation of the strips. While incubated, metabolic activity changes color and the changes are either spontaneous or emerge by adding reagents. All the test results, positive and negative, are assembled to produce a profile number; this number is then compared to commercial codebook (or online) profile numbers to determine the type of the bacteria.

Antimicrobial sensitivity testing (Kirby-bauer method)

Antibiotics are the usual treatment for uropathogenes and tested by Kirby-Bauer method to measure the response of the entire isolated organisms to this treatment.

Sterile Mueller Hinton agar plates are prepared and different discs of antibiotic are chosen. This is followed by the incubation of identified pathogens at 37C for one hr. Sterile cotton swabs is inoculated on the Mueller Hinton agar plate surfaces for each test organism and each incubated test organisms and rotated the plates 60^ three times following each streaking.

Finally, the swab is rotated around the agar edge. The cultures are then dried on the plates for 5-10 minutes at air temperature. Different antibiotics discs are put on the agar medium surface through agent pressure by the use of a sterile forceps over the discs to make a better contact and to effectively spread the antibiotics into the medium. It is important to ensure that the antibiotic disc touches the medium. Then for about 16-18 hours at 37c, the plates are incubated the opposite position.

Statistical analysis

When the patients consulted the hospital OPD, their demographic and clinical characteristics such as gender, age, medical history and vital signs are recorded. Descriptive statistics is the method used to analyze the data by Chi square test at ($P<0.01$). The prevalence of dominance of the antimicrobial resistance equals the number of the positive results in the entire study samples.

Result

A total of 860 urine sample are collected and analyzed in the microbiology department of Par Hospital in Erbil city from the period of (June2018 to November 2018). Out of the 860 samples, 402 (46.74%) show noteworthy growth for bacteria causing UTI. The females are 231 (57.46%) while male are 171(42.53). There is higher prevalence of UTI in both genders in age group (26-35) while the lowest rate of infection is seen in the age group of (2-5)111 as clarified in table 1:

Table 1 : Distribution of UTI as per gender and age

Age in years	Male		Female	
	No.	%	No.	%
2-5	13	7.60	8	3.46
6-15	22	12.87	16	6.93
16-25	13	7.60	38	16.45
26-35	33	19.30	42	18.18
36-45	18	10.53	49	21.21
46-55	28	16.37	39	16.88
Above 55	44	25.73	39	16.88
	171	42.53%	231	57.46%

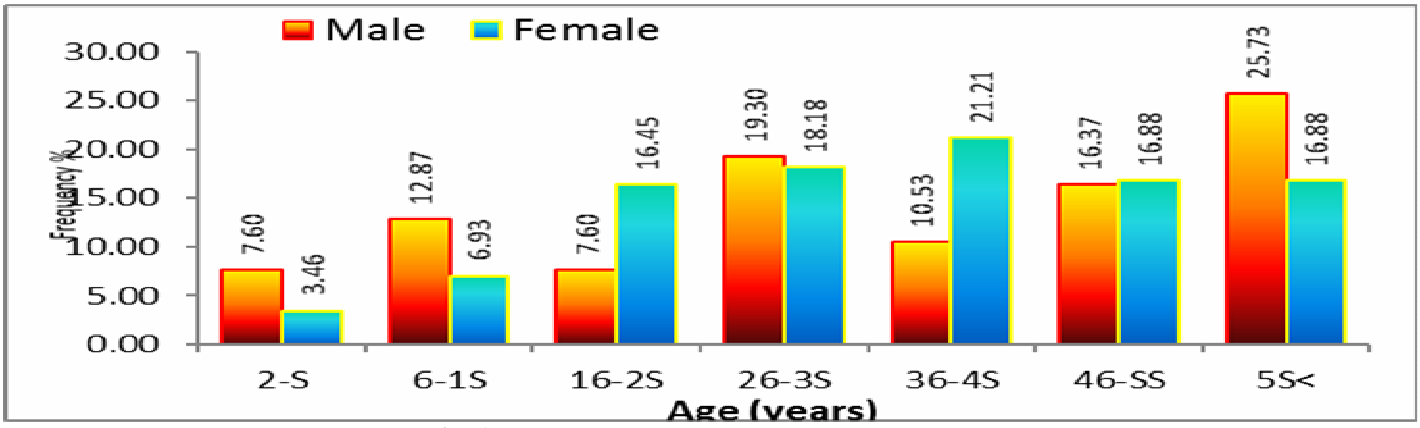


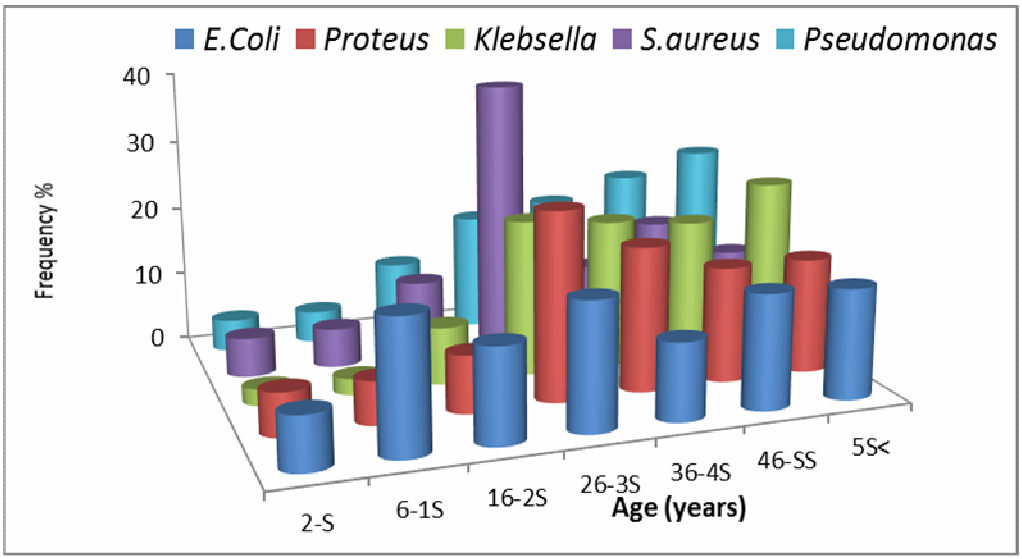
Fig. 1 : Distribution of UTI as per gender and age .

Among the bacteria, *Escherichia coli* is the most common isolate which causes urinary tract infections (45.52%) and the development of multidrug resistant among pathogens that causes complicated UTI followed by *Klebsiella pneumonia* (21.39%) *Pseudomonas aeruginosa* (16.41%) *Proteus mirabilis* (12.19) and *Staphylococcus aureus* (4.59%) as can be seen in Table 2.

Table 2 : UTI prevalence among different age group with infecting agent.

Age	*Escherichia coli*		*Proteus mirabilis*		*Klebsiella pneumonia*		*Staphylococcus aureus*		*Pseudomonas aeruginosa*	
	No.	%	No.	%	No.	%	No.	%	No.	%
2-5	13	7.10	3	6.12	2	2.33	1	5.56	3	4.55
6-15	32	17.48	3	6.12	2	2.33	1	5.56	3	4.55
16-25	23	12.56	4	8.16	7	8.14	2	11.11	7	10.61
26-35	31(10)	22.40	13	26.53	19	22.09	7	38.89	11	16.67
36-45	19	10.38	10	20.41	18	20.93	2	11.11	12	18.18
46-55	28	15.30	8	16.33	17	19.77	3	16.67	14	21.21
55<	27	14.75	8	16.33	21	24.42	2	11.11	16	24.24
	183	45.52%	49	12.19%	86	21.39%	18	4.59%	66	16.41%

Table 3 : Bacteria distribution Escherichia



The isolated uropathogens are sensitive to various antibiotics. There is high susceptibility to amikacin (21.08%) for all isolates followed by imipenem (18.20%) and increased resistant to amoxicillin are noted in all isolates (3.9%) and ampicillin (4.75%).On the other hand, the susceptibility rate of various bacterial isolates to other antibiotics is variable as in Table 3:

Table 4 : Antibiotic sensitivity of the bacteria-

Pathogen		AK	AMp	AMOX	AZ	CIP	GEN	IMP	NA	PI	TET	X^2
E.coli	No	80.9	17.2	55	21.6	28.8	55.5	94.2	11.9	22.50	36.40	167**
	%	19.08	4.06	12.97	5.09	6.79	13.09	22.22	2.81	5.31	8.58	
Proteus	No	89.8	33.2	12.8	32.4	93.5	78.9	91.3	19.2	77.80	0.00	153**
	%	16.98	6.28	2.42	6.13	17.68	14.92	17.26	3.63	14.71	0.00	
Pseudomonas	No	93.9	22.5	0.00	0.00	65.7	88.9	94.5	25.3	22.50	0.00	122**
	%	22.72	5.44	0.00	0.00	15.9	21.51	22.86	6.12	5.44	0.00	
Klebsiella	No	89.20	22.50	14.00	22.30	68.80	76.80	99.60	47.30	23.50	27.80	185**
	%	18.14	4.58	2.85	4.53	13.99	15.62	20.25	9.62	4.78	5.65	
S. aureus	No	90.9	9.2	2	10.4	30.3	10.6	1.1	2.1	42.9	27.8	311**
	%	39.99	4.05	0.88	4.58	13.33	4.66	0.48	0.92	18.87	12.23	
		21.08%	4.75	3.39	4.27	13.74	14.69	18.20	6.82	7.61	5.45	16**

X^2=Chi Square value. **= high significant at (P ≤ 0.01).

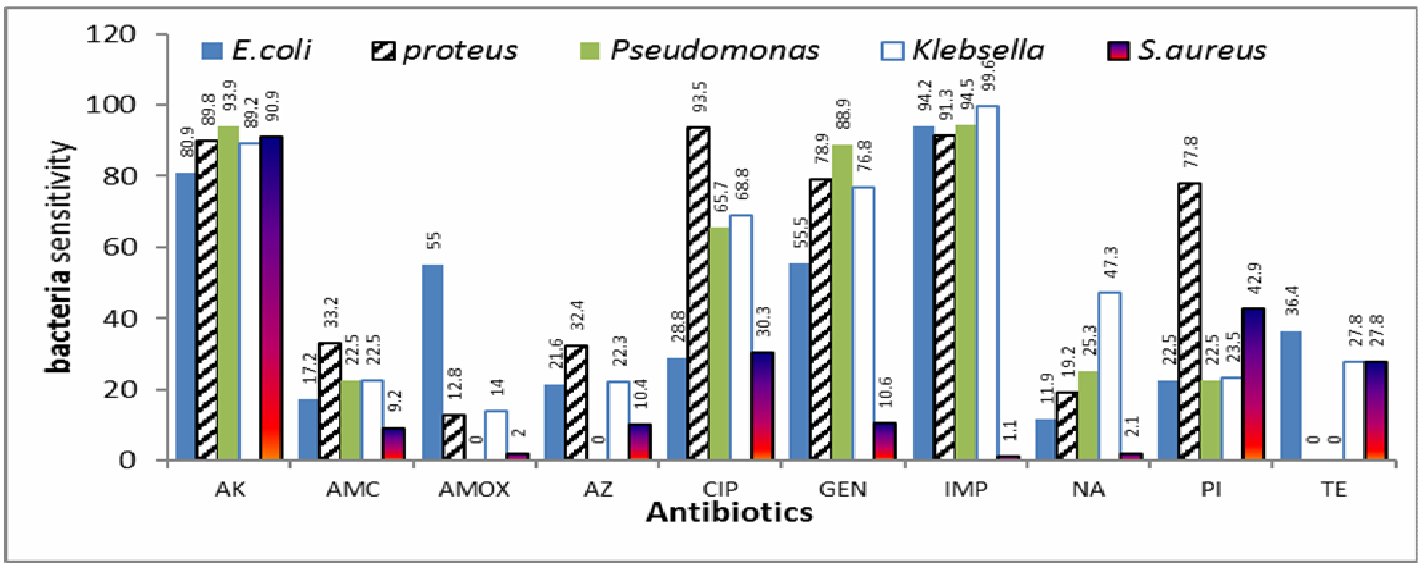


Table 4 : sensitivity to the various antibiotics

Discussion

Urinary tract infection is one of the most common causes affecting millions of people every year. It can be classified into two categories, uncomplicated UTI such as acute cystitis in women who non-pregnant, or premenopausal). The second is complicated UTI which happens when there are predisposing factors. However, the

second types should be considered in pregnant or post-menopausal females and men (Dytan, 1999).

The pathogens of urinary tract infection involve complex interaction of organisms, the environment and the potential host. Gram negative aerobic organisms are the organisms involved in urinary tract infections. These organisms usually originate from the normal microbial flora and 80-90% of the pathogens of urinary tract infection involve a complex interaction of organisms, the environment and the potential host. Gram negative aerobic organisms are the organisms involved in these types of infections. Gram negative bacteria considered the most microorganisms effecting individuals and lead to cause UTI.

This study report that the main causes *Escherichia coli* isolate are as follows: (45.52%), *Klebsiella pneumonia* (21.39) *Pseudomonas aeruginosa* (16.41%) *Proteus mirabilis* (12.19) and *Staphylococcus aureus* (4.59%) such bacteria colonize the uroepithelial mucosa with pillifimbriae and adhesion. Complicated catheter-associated urinary tract infections are due to Escherichia coli and Proteus mirabilis.

Al-Derzi and Salih's study in Duhok city in Iraq reveals that uropathogenes prevalence among outpatients of (46.5%) for *E. coli*, by *Proteus* spp. (30.7%), *Enterococcus faecalies* (7.8%) *Klebsiella* spp. (4.4%) and *Pseudomonas aeruginosa* (3.2%) (2009).

In Pakistan, a study finds that the most frequent reason for UTIS is *E. coli* (66.97%) followed by *Enterococcus* (8.26%) *Candida* spp. and *Pseudomonas* spp. (7.34% each), *Klebsiella* spp. (5.50%) *Enterobacter* spp. (2.75%), *Proteus* spp. and *Morganella* spp. (0.91 % each) (Bashir, 2008)

The present study finds that the prevalence of UTIS is (46.74%) in female 57.46% and 42.53% in males. It is previously found that UTIS is always a very common phenomenon among the women (Ho, 2008) Dromigny *et al.* (2005) indicate that half of the women develop a UTI during their life time.

In relation to gender of patients the prevalence of UTI is significantly higher in females than in males. Females are more likely to develop UTI than in males. More than two-third of the incidence is seen in females (70.53%) and quiet lower than one-third is in male (29.47%).These findings are in accordance with earlier studies on UTI (Sharma *et al.*, 2013).

In the present study, the age group of 26-35 years in both genders are the highest incidence to UTI and the lowest incidence of UTI found in age group between (2-5) years.

In another study, a much greater prevalence of this infection is observed in 56.0% females and 52.1% male causes belong to the age group >51 years of age (Joseph *et al.*, 2008).

Based on the antibiotic sensitivity results of our study, *E. coli* is the most recovered bacteria. Its greatest susceptibility rate is found in amikacin (21.08%). It is followed by imipenem (18.20%) and gentamicin (14.69%). Thus these drugs could be considered as a satisfactory options and can be prescribed to treat the patients with UTI. Also all the *E. coli* isolates are less susceptible to amoxicillin (3.39%) ampicillin (7.75%) and azithromycin (4.27%) Furthermore, the later finding are also observed among other bacterial isolates than *E. coli* evolving great importance and implies that these antibiotics cannot be used as empirical therapy for UTI particularly in the study. Similar findings are classified by Al-Derzietal except they find a low resistant rate of *E. coli* isolates against cephalexin (11.8%) and ciprofloxacin (23.3). The increased resistant to ampicillin and amoxicillin suggests that a change in prescription practice may be necessary.

Conclusion

It is concluded that gram-negative organisms are the statistically significant uropathogens. *E. coli* is the most common uropathogen isolated from all age groups and both sexes. Our findings are at par hospital with other studies conducted in Pakistan. *Staphylococcus aureus* is a common gram-positive organism responsible for urinary tract infections. The study shows that one of the common health problems in females is UTI mainly in reproductive age women. The age group of 26-35 years are found to be the high prevalence for developing UTI. It is found that a variety of bacteria could be cause urinary tract infection in the samples of patients of Par Hospital in Erbil city Iraq. *Escherichia coli* is the most predominant bacterial isolates. Amikacin is found to be the most effective drug that can be used in the treatment of UTI patients. Most of the isolates shows antimicrobial resistant. This study shows a large number of isolates resistance to Ampicillin and amoxicillin.

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