

## Detection of Uropathogens in Urinary Tract Infection and Their Sensitivity to Many Antimicrobial Agents

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### Abstract

Urinary tract infections (UTI) are some of the most common infections experienced by humans, exceeded in frequency among ambulatory patient only by respiratory and gastrointestinal infections. It is also the most common cause of nosocomial infection in adults. A total of three hundred urine sample were collected in age (1-69 years old) in both gender, with (UTI) symptoms referred to AL-Yarmok Teaching Hospital at Baghdad city during the period from January 2010 till August 2010.

The commonest isolates were *Escherichia coli* (*E.Coli*), *Proteus mirabilis* and *Klebsiella pneumoniae* (These represented 49.2%, 22 %, and 16 % of isolates respectively). The percentage of bacteria incidence in females was higher than male in most of examined samples, except in *Klebsiella*; it was higher in males. The most positive case was in elder patients than in children. The infection incidences expressed higher level in summer. It reached to peak from August with percentage of (32 %) and July (24.4 %). The antibiotic sensitivity for streptomycin, lincomycin, Rifampicin, Chloramphenicol, Tetracyclin, Ampicillin, Penicillin, Co-trimoxazole, Amoxicillin, Nalidixic acid and Gentamycin was examined, detected species of bacteria were more sensitive to Chloramphenicol (75.6) and streptomycin (81.4), while the resistances to Tetracycline (100% and Penicillin (100%) were appeared to *Escherichia coli*.

**Key word** : Urinary tract infection, antimicrobial resistance

## Introduction

Urinary Tract Infection (UTI) is a serious bacterial infection causing illness in infants and children. Urinary tract infection is applied to a variety of clinical condition ranging from asymptomatic presence of bacteria in the urine to severe infection of the kidney with resultant sepsis [1]. Urine infection is the most common serious bacterial infection causing illness in infants and children [2]. It is one of the most common bacterial infections encountered by clinicians in developing countries [3]. Various factors make bacteriuria more or less to occur for any individual. These factors are age, gender, race, genetic factors, sexual activity among the teen age girls, and circumcision in boys, nocturnal enuresis and some unhealthy behaviors. UTI is age dependent and bacteriuria is more common at the extremes of life. The Incidence of UTI is bimodal highest during the first year of life and be taking again during adolescence [4]. Most of urinary tract infections are caused by gram-negative bacteria like, *E. Coli*, *Klebsiella species*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter*, and *Serratia*. 90% of UTI cases are caused by gram-negative bacteria while only 10% of the cases are caused by gram positive bacteria. Gram-positive bacteria while only 10% of the cases are caused by gram positive bacteria. Gram-positive bacteria include *Enterococcus*, *Staphylococcus*, and *Streptococcus agalactiae* [5]. *E. Coli* are the most common gram – negative bacteria responsible for UTI [6], 75% to 87% of UTI cases are due to *Escherichia coli* [7, 8]. At least 80% of the uncomplicated cystitis and pyelonephritis are due to *Escherichia coli* [1]. Whereas *Proteus mirabilis* and *Klebsiella pneumoniae* infection accounts 10%, 6% respectively [9,10,11]. Adherence properties of some organisms prevent the normal was hot for of these organisms by bladder emptying and mucosal host defense mechanisms. *Escherichia coli* are virulent due to the presence of p.fimbriae, ganelles on its strains that may attach or adhere on specific receptors of uroepithelial cells and interfere with the washout of bacteria [1]. *Escherichia coli* are predominant in girls, whereas *Proteus mirabilis* and *Klebsiella pneumonia* are likely encountered in boys [9]. Treatment of UTI with the appropriate antibiotic can minimize mortality, morbidity and any renal damage from acute UTI. Choosing the appropriate antimicrobial agents sounds difficult, but advances in the understanding of the pathogenesis of UTI, the development of new diagnostic tests, and the introduction of new antimicrobial agents have allowed physicians to appropriately tailor specific treatment for each patient Sulphamethoxazole, Trimethoprim, Fluroquinolones (e.g. Ciprofloxacin), Nitrofurantoin, amino glycosides (e.g. Gentamicin, Amikacin), cephalosporin and aminopenicillins (e.g. Ampicilline and Amoxicillin). Trimethoprim, Sulphamethoxazole, Cephalosporin and Amoxicillin –clavulanate are considered to be the most acceptable antibiotics for the treatment of UTI in pediatrics in comparison to quinolones, which has an effect on joint development, and first line therapy of amoxicillin which has a high prevalence of resistance to *Escherichia coli* in many communities [4]. Urinary tract infections are second most common type of infection in the body, accounting for a bout 8.1 million visit to health care providers each year [14]. Women are especially prone of UTIs for antibacterial reasons. UTI in men are not common as in women but can be serious when they occur. Many women suffer from frequent UTIs. About 20 percent of young women with a first UTI will have a recurrent infection [15]. Men are less likely than women to have a first UTI. But once a man has a UTI, he is likely to have another because bacteria can hide deep inside prostate tissue. Women are more prone to UTIs than men because in female the urethra is much shorter and closer to the anus [16]. As a woman's estrogen level decreases with menopause, her risk of UTIs increases due to the loss of protective vaginal flora [17]. UTIs are the most frequent bacteria in women [18]. They occur most frequently between the age of 16 and 35 years, with 10 % of woman getting an infection. High incidence of UTI due to *Proteus spp.*, *Klebsiella spp.* And *Enterobacter spp.* Infection is more common among Children with recurrent UTIs and in those treated with antibiotic prophylaxis. Whereas other Uropathogens like *Pseudomonas*, *Serratia* and *Candida* are more common among children with urogenital

abnormalities [12]. 87% of Community –acquired UTI is due to *Escherichia coli* and 12% by *Klebsiella*; whereas in hospital acquired UTI 65% are caused by *Escherichia coli* and other pathogens, including *Pseudomonas* [13]. It is very important to recognize and treat UTI rapidly yearly and 60 % having an infection at some point in their lives [19]. The incidence of true UTI in adult male younger than 50 years is low (approximately 5-8 per year per 10,000), with adult woman being 30 times more likely than men to develop a UTI. The incidence of UTI in men approaches that of woman only in men older than 60 years [20]. The aims of this study are detection of bacteria that causes urinary tract infection in some Iraqi patients and determine the sensitivity to many antibiotics

## Materials & Methods

Three hundred urine samples were collected from patients in age ( 1-69 years old ) in both gender , with UTI symptoms referred to AL-Yarmok Teaching Hospital at Baghdad city; were studied during the period from January/ 2010August /2010. Isolation of Uropathogens was performed by a surface streak procedure on both blood and MacConkey agar (Oxoid Ltd. Bashing store Hampaire , uk) using calibrated loops for semi-quantitative method and incubated aerobically at 37c for 24 hours, and those cultures which became negative at the end of 24 hours incubated were further incubated for 48 hours [21].

A specimen was considered positive for UTI if a single organism was cultured at a concentration of  $\geq 10^5$  of U/ml.

Bacteria identification was made using biochemical test, namely indol, citrate, oxidase, H<sub>2</sub>S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, gas production, catalase coagulase and manitol fermentation [22]. All positive cultures were repeated again to confirm and to be accepted as UTI in association with abnormal urinalysis and UTI symptoms. The strains isolated were identified and diagnosed by Grams stain and biochemical reactions which were done by using Api 20 E and API Staph system (bio Meraux, France). In addition to the characteristics of bacterial colonies on culture media. Antibiotic sensitivity was performed for all isolates by discs diffusion method on Nutrient agar (Oxoid). Different antibiotic disc were used [Table 1]. The inhibition zones of antibiotic disc were measured according to the method of baron 1996 [23].

## Results and Discussion

Bacteria species that had been seen in UTIs were of fecal origin . These organisms are a subset of the organisms found in the feces , more than 90% of acute UTIs in patients with normal anatomic structure and function are caused by certain strains of *E.coli* [24] .

10-20 % are caused by coagulase-negative *staphylococcus saprophyticus* 5 % or less are caused by other enterobacteriaceae organisms or enterococci , while in complicated cases of UTI, such as UTIs resulting from anatomic obstructions, or from catheterization the most common causes of UTI are *E.coli* , *Klebsiella pneumoniae* , *Proteus mirabilis* ,

*Enterococcus sp.* , *Pseudomonas aeruginosa* 53 % , 12 % , 6 % , 12 % , 0.4 % [ 25 ] .

Out of 300 urine samples, (250) samples showed a positive culture growth while (50) samples have no growth. Eight different isolates obtained with highest incidence for *E.coli* isolate 123 (49.2%) followed by *Proteus mirabilis* isolate 55 (22%) and *Klebsiella pneumoniae* 40 (16 %), while the lowest incidence was for *Morganella morganie*, *Pseudomonas aeruginosa*, *Citrobacter spp.*, *Staphylococcus aureus* and *Enterobacter spp.* With 10 ( 4% ) , 10 (4%) , 7(2.8% ) , 4 ( 1.6% ) and 1 ( 0.4% ) respectively [ Table 2 ] . In this study, the isolation rate of bacteria from urine was 83.3 % which is comparatively than reports within the country and other part of the world [26].

In our study, results showed that 50 % of the isolates were *E.coli* 7(33.3 %) and *Klebsiella pneumoniae* 4(19 %) while other gram negatives were found in a small number. On the

otherhand, isolated gram positive bacteria were *Staphylococcus aureus* with proportion of 1 (4.8 %) [27].

In other study result showed seven different isolates obtained with highest incidence for *E.coli* 115 (39.7 % ) followed by *Proteus mirabilis* 70 (24.2 % ) and *Klebsiella pneumoniae* 60 ( 20.8 % ) while the lowest incidence was for *Morganella morganie*, *Pseudomonas aeruginosa*, *Citrobacter spp.* and *Staphylococcus aureus* with 19 (6.6% ) ,14(4.9%),10(3.5%) and 1( 0.3%) respectively [28 ] .

*E .coli* is the major a etiological agent in causing UTI which accounts for up to 90% cases [29] In this study, the most frequent Uropathogens were Gram negative which made up (90.8%) of all the isolates.

Regarding sex, the study showed that the percentage of UTI in female was higher than in male four most isolates obtained except in *Klebsiella pneumoniae* was higher in male than female, it was (52.5 %) and its significant [Table 3 ] .

Woman is at great risk for UTI primarily because of the significantly shorter urethra and closer proximity to the rectum. The female genitalia may become colonized with pathogenic bacteria that can more easily enter the urethra. In addition, woman lack the bacteriostatic protection that prostatic secretions offer the male [30]. American women are 30 times more likely to have UTI than men [31] .In our study showed that the percentage of UTI in female was higher than in male four most isolates obtained except in *Klebsiella pneumoniae* was higher in male than female, it was (61.66%) and its significant .

All patients taken were between 1-69 years old, [ Table 4 ] showed the age prevalence of UTI and revealed highest incidence in elder patient than in younger ones (24, 29.2 , 28 ) % were for ages ( 40-49 , 50-59 ,60-69 ) respectively .20-25% of elder women have UTI because of the number of biological factor including estrogen loss, after menopause, the wall of urinary tract thin out weakening the mucous membrane and reducing the ability to resist bacteria , the bladder may lose elasticity and fail to empty completely , besides poor over all health. 5-15% of men older than 50 -65 will have asymptomatic UTI more likely because of prostate problems [32].

In other study showed the age prevalence of UTI in elder patient than in younger (17.99, 28.72, 22.5) % were for age (40-49, 50-59, 60-69) respectively [28]. In other study showed the age range of patients was between 19-39 years is 53.5%, 40-49 is 25.9% [33].

Regarding, month distribution of the UTI obtained in the study. [Table5] the highest incidence was in summer season. It reaches the peak during July and August (56.4%) while (43.6%) distributed on the rest of the months of year .Infection caused by these bacteria has seasonal variation with higher incidence in summer and full in winter and spring may be due to hot weather and bad hygiene management during summer.

our study showed highest incidence was in summer season during July ,August and September about (58.06% ) while( 41.94% ) On the rest of the months [ 34 ] .

Bacterial Uropathogens from patients with UTIs revealed the presence of high levels of single and multiple antimicrobial resistances against commonly prescribed drugs. The increasing rates of resistance to uropathogenic, *E.coli* isolates reported worldwide, antibiotic susceptibility pattern of these isolates revealed that for our patients, [Table 6].

The inhibition zone of the antibiotic discs were measured on nutrient agar for all isolates, the sensitivity of bacterial isolates to the antibiotics was tested, and the results showed that most isolates were resistant to antibiotic in different ranges. *E.coli* which the predominant cause of UTI showed high percentage of resistance to Tetracycline and penicillin ( 100 % ) ,and low resistance to Streptomycin and Chlorompinicol ( 18.6 % , 24.4 % ) respectively . Sensitive to Streptomycin and Chlorompinicol (81.4%, 75.6 % ) respectively.

Other study showed *E.coli* high percentage of resistance to Tetracycline and Amoxicillin (83%, 87%) respectively. [35].

*Proteus mirabilis* which is the second most prevalent pathogen of UTI showed high resistance to Rifampicin (100%) and Lincomycin (94.5%) and low resistance to Chlorompinicol (9%), sensitive to Chlorompinicol (91%).

*Klebsiella pneumoniae* showed high resistance to Rifampicin (100%) and Penicillin (75%) and low resistance to Chlorompinicol (12, 5%), sensitive to Chlorompinicol (87.5%) , Gentamycin (80%) and Nalidixie (80%) .

*Morganella morganii* showed high resistance to Lincomycin, Rifampicin (100% ) respectively and low resistance to Streptomycin (20%), sensitive to Streptomycin (80%) .

*Pseudomonas aeruginosa* showed high resistance to Lincomycin, Rifampicin (100% ) respectively and low resistance to Amoxicillin (40%), sensitive to Amoxicillin(60%) .

*Citrobacter spp.* showed high resistance to Lincomycin, Rifampicin (100% ) respectively and low resistance to Penicillin (42.9%), sensitive to Amoxicillin(71.4%) .

*Staphylococcus aureus* showed high resistance to Streptomycin, Nitrofurantin (100% ) respectively and low resistance to Penicillin, Chloramphenicol, Amoxicillin (25%) respectively, sensitive to Lincomycin(25%) .

*Enterococcus* showed high resistance to Lincomycin, Rifampicin, Penicillin, Amoxicillin and Nitrofurantin, Chloromphenicol, Tetracyclin, Ampicillin and Co-trimoxazole(100%) .

Resistance to antimicrobial agents has been noted since the first use of these agents and is an increasing world-wide problem [ 36 ] .

our study showed higher prevalence rate of resistance to the commonly prescribed antibiotic agent, finding that 100% of *E.coli* and *Klebsiella pneumoniae* isolate were resistance to Amoxicillin and Ampicillin [37].

On the otherhand very levels of resistance were detected to antibiotic such as Ceftriaxone, Nitrofurantoin and Gentamycin and a comparable rate of sensitivity has been reported for these drugs in previous studies done in Ethiopia [ 38 ,39 ] , in Kosovo [40 ], in Iran [41 ] and South Croatia [ 42 ] .Low resistance were observed for these drugs because they are not easily accessible and relatively expensive in price compared to other .Thus ,the drugs could be considered as alternative option in the empirical treatment of UTIs.

our study in India showed that Nitrofurantoin had the best in-vitro susceptibility profile against *E.coli* [43 ] .In previous studies, nitrofurantoin was the most active agent (94%) , followed by Gentamycin and Cefpodoxime ,high rates of resistance to Ampicillin (55%) and Trimethoprim (40%),often in combination were observed in both sets of isolates [44] .

## Conclusion

The isolation of bacterial Uropathogens with a higher resistance rate for commonly use antimicrobial leave the clinicians with very option to choose drug used for empirical oral treatment for UTI was extremely high level of resistance to tetracycline and penicillin.

As drug resistance among pathogens is an evolving process, routine surveillance an monitoring studies should be conducted to proved physicians with knowledge about the most effective empirical treatment of UTIs.

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**Table (1): The antibiotics discs used to study the sensitivity of the isolate**

Antibiotics	Concentration $\mu\text{g}\backslash\text{disc}$	Company
Streptomycin	10	Oxoid(England)
Lincomycin	15	Oxoid(England)
Nitrofurantin	300	Oxoid(England)
Rifampicin	5	Oxoid(England)
Chloramphenicol	30	Oxoid(England)
Tetracycline	30	Oxoid(England)
Ampicilline	25	Oxoid(England)
Penicillin	10 IU	Oxoid(England)
Co-trimoxazole	25	Oxoid(England)
Amoxicillin	25	Oxoid(England)
Nalidixic acid	30	Oxoid(England)
Gentamycin	10	Oxoid(England)

**Table (2): Types of isolates isolated from patients with (UTI) and their percentage**

isolates	NO. of isolates	percentage
<i>E.coli</i>	123	49.2
<i>Proetus mirabilis</i>	55	22
<i>Klebsiella pneumoniae</i>	40	16
<i>Morganella morganie</i>	10	4
<i>Pseudomonas aeruginosa</i>	10	4
<i>Citrobacter spp.</i>	7	2.8
<i>Staphylococcus aureus</i>	4	1.6
<i>Enterobacter spp.</i>	1	0.4
<b>Total</b>	<b>250</b>	<b>100</b>

**Table (3): Sex prevalence in positive case s with (UTI)**

Isolates	No. of isolates	Male		Female	
		NO	%	No	%
<i>E.coli</i>	123	53	43.1	70	56.9
<i>proetus mirabilis</i>	55	22	40	33	60
<i>Klebsiella pneumoniae</i>	40	21	52.5	19	47.5
<i>Morgarella morganie</i>	10	4	40	6	60
<i>Pseudomonas aeruginosa</i>	10	2	20	8	80
<i>Citrobacter spp.</i>	7	3	42.9	4	57.1
<i>Staphylococcus aureus</i>	4	0	0	4	100
<i>Enterobacter spp.</i>	1	0	0	1	100
<b>Total</b>	<b>250</b>	<b>105</b>		<b>145</b>	

**Table (4): Age prevalence in patients with (UTI)**

Age (years)	No. of positive cases	%
1-9.	20	8
10-19.	15	6
20-29.	8	3.2
30-39.	4	1.6
40-49.	60	24
50-59.	73	29.2
60-69.	70	28
<b>Total</b>	<b>250</b>	<b>100</b>



**Table ( 5 ): Monthly prevalence of ( UTI ) \ 2010**

Month	Positive case	Negative case	Total	% of positive
January	2	8	10	0.8
February	10	14	24	4
March	17	9	26	6.8
April	16	6	22	6.4
May	32	5	37	12.8
June	32	3	35	12.8
July	61	2	63	24.4
August	80	3	83	32
Total	250	50	300	100

**(Table 6): The sensitivity of Uropathogens to many antibiotic**

Antibiotic		<i>E.coli</i> (%)123	<i>Proetus mirabilis</i> (%)55	<i>Klebsiella species</i> (%)40	<i>Mogarella morgnii</i> (%)11	<i>Pseudomonas aeruginosa</i> 10(%)	<i>Citrobacter spp.</i> 7(%)	<i>Staphylococcus aureus</i> 4(%)	<i>Enterococcus</i> 1(%)
Streptomycin	Sensitive	100(81.4)	10(18.2)	30(75)	8(80)	3(30)	3(42.9)	0	1(100)
	Resistant	23(18.6)	45(81.8)	10(25)	2(20)	7(70)	4(57.1)	4(100)	0
Lincomycin	S	23(18.6)	3(5.5)	20(50)	0	0	0	1(25)	0
	R	100(81.4)	52(94.5)	20(50)	10(100)	10(100)	7(100)	3(75)	1(100)
Nitrofurantin	S	73(59.4)	35(63.6)	20(50)	5(50)	2(20)	2(28.6)	0	1(100)
	R	50(40.6)	20(36.4)	20(50)	5(50)	8(80)	5(71.4)	4(100)	0
Rifampicin	S	50(40.70)	0	0	0	0	0	3(75)	0
	R	73(59.4)	55(100)	40(100)	10(100)	10(100)	7(100)	1(25)	1(100)
Chloromphenicol	S	93(75.6)	50(91)	35(87.5)	5(50)	3(30)	2(28.6)	3(75)	1(100)
	R	30(24.4)	5(9)	5(12.5)	5(50)	7(70)	5(71.4)	1(25)	0
Tetracycline	S	0	35(63.6)	20(50)	3(30)	3(30)	3(42.9)	2(50)	1(100)
	R	123(100)	20(26.4)	20(50)	7(70)	7(70)	4(57.1)	2(50)	0
Ampicilline	S	75(61)	30(54.5)	20(50)	2(20)	2(20)	2(28.6)	2(50)	1(100)
	R	48(39)	25(45.6)	20(50)	8(80)	8(80)	5(71.4)	2(50)	0
Penicillin	S	0	15(27.3)	10(25)	4(40)	3(30)	4(57.1)	3(75)	0
	R	123(100)	40(72.7)	30(75)	6(60)	7(70)	3(42.9)	1(25)	1(100)
Co-trimozoles	S	15(12.2)	22(40)	18(45)	5(50)	3(30)	2(28.6)	2(50)	1(100)
	R	108(87.8)	33(60)	22(55)	5(50)	7(70)	5(71.4)	2(50)	0
Amoxicillin	S	15(12.2)	32(58.2)	18(45)	5(50)	6(60)	3(42.9)	3(75)	0
	R	108(87.8)	23(41.8)	22(55)	5(50)	4(40)	4(57.1)	1(25)	1(100)
Nalidixie acid	S	73(59.4)	18(32.7)	32(80)	4(40)	6(60)	3(42.9)	2(50)	1(100)
	R	50(40.6)	37(67.3)	8(20)	6(60)	0(0)	4(57.1)	2(50)	0
Gentamicin	S	75(61)	15(27.3)	32(80)	5(50)	5(50)	3(42.9)	2(50)	0
	R	8(39)	40(72.7)	8(20)	5(50)	5(50)	4(57.1)	2(50)	1(100)

## التحري عن الممرضات المسببة لالتهاب المجاري البولية وحساسيتها للمضادات الحياتية

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### الخلاصة

التهاب المجاري البولية هي من اغلب الإصابات الشائعة التي تصيب الإنسان ، وتكرارها أكثر من إصابات الجهاز التنفسي والهضمي . وكذلك هي المسبب الغالب للإصابات من داخل المستشفيات في البالغين. من مجموع 300 عينة إدرار جمعت من مرضى تتراوح أعمارهم بين ( 1-69 ) سنة ولكلا الجنسين الذين يعانون من التهابات المجاري البولية الوافدين إلى مستشفى اليرموك التعليمي / بغداد خلال المدة من كانون الثاني/2010 الى غاية آب /2010. اغلب العزلات كانت *E.coli* ، *Proteus mirabilis* ، *Klebsiella pneumoniae* ( 49.2%-22%-16.22% على التوالي ) . وكانت النسب المئوية للعزلات البكتيرية في الإناث أكثر من الذكور في العينات المفحوصة ماعدا *Klebsiella pneumoniae* كانت أعلى في الذكور . وكانت اغلب العينات ايجابية في المرضى البالغين أكثر من الأطفال . وجد إن الإصابة البكتيرية كانت عالية في فصل الصيف ووصلت أعلى مستوياتها ما بين ( 32% ) وتموز ( 24% ) . وكانت منها البكتريا الحساسة للمضادات الحيوية ( ستربتومايسين ، لنكومابسين ، نتروفورانتين ، ريفامبسين ، كلورامفينيكول ، تتراسايكلين ، امبيسيلين ، نالدكسك اسد ، جنتاميسين ) قد اختبرت وكانت بكتريا القولون الأكثر حساسية للستربتومايسين ( 81.4% ) والكلورامفينيكول ( 75.6% ) بينما الأكثر مقاومة للمضادات الحيوية تتراسايكلين ( 100% ) وبنسلين ( 100% ) .

الكلمات المفتاحية: التهاب المجاري البولية وحساسيتها للمضادات الحياتية