

Prevalence and Antimicrobial Susceptibility of Pathogens in Urinary Tract Infections

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Abstract

This study was done to assess the bacterial profile and antibiotic susceptibility pattern of urinary tract infections (UTIs) pathogens. For proper identification of causative microbial agents, mid stream urine samples from 459 patients with clinical symptoms suspected to be UTI were collected, cultured and subjected to appropriate biochemical tests. These samples were collected from Teaching Laboratories Center in Baghdad during the study period (1st January 2009 - 1st July 2009). The antimicrobial sensitivity test was carried out by disc diffusion technique using Muller- Hinton agar. 100 urine samples were cultured positive with a colony count equal or more than 10^5 /ml while 359 cases were excluded as they were culture negative or exhibited mixed infections. Overall males to females ratio was 1:3.2. The most prevalence isolates were *Escherichia coli* with frequency rate of 50% followed by *Enterobacter spp.*(12%). Whereas, *Klebsiella spp.*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* showed frequency rate of 9% for each. However, *Proteus spp.*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Acinetobacter spp.* showed frequency rate less than 5%. The majority of isolates were sensitive to imipenem (96%) followed by amikacin(89%) and nitrofurantion(69%) whereas, high level resistance was seen to cotrimoxazole, ampicillin and trimethoprim followed by cefoxitin, nalidixic acid, gentamicin and cefotaxime in decreasing order of frequency.

Keywords: UTI, signs and symptoms, causative agents, antibiotic sensitivity.

Introduction

Urinary tract infection (UTI) is a serious health problem affecting millions of people each year. It is the most important cause of mortality and morbidity in the world affecting all age groups across the life span[1]. UTI may involve only the lower urinary tract or both the upper and the lower tracts[2]. The urethra and urinary bladder are the most frequent sites of infections within the urinary tract[3]. It was found that women were more prone to UTIs than men with the risk of infection related to the frequency of sex[4]. The predominance of *Enterobacteriaceae* and particularly *Escherichia coli* remain the principle pathogen causing UTI, accounting for 75-90% of all UTIs in both inpatients and outpatients[5]. In addition, *Klebsiella spp.*, *Staphylococcus spp.*, *Enterobacter spp.*, *Proteus spp.*, *Pseudomonas spp.* and *Enterococcus spp.* were more often isolated from inpatients [6]. Elsewhere, coagulase negative *Staphylococci* may be a common cause of UTI in some reports [2] whereas anaerobic organisms are rarely pathogens in the urinary tract[7].

Oral antibiotics such as trimethoprim, cephalosporins, nitrofurantion, or a fluoroquinolone substantially shorten the time to recovery. All are equally effective for both short and long term cure rates [8]. Resistance has developed in the community to all of these medications due to their widespread use [9]. Worldwide data showed that there was an increasing resistance noted against amoxicillin, cotrimoxazole and lately, fluoroquinolone[10]. Some authors have found that quinolone resistance was higher in developing countries than in developed nations because of the use of the less active quinolone, such as nalidixic acid and the use of low dosages of more potent compounds such as ciprofloxacin resulting in selection of mutant isolates [11]. It was also found that antibiotic resistance varies according to geographic locations and is directly proportional to the use and misuse of antibiotics [12]. Therefore, it is important to have local hospital based knowledge of the organisms causing UTI and their antibiotic sensitivity patterns. This information would be relevant not only to the local hospital but

would also be a vital regional database [13]. For all of the above reasons, this study was aimed to identify the most common etiologic agents responsible for urinary tract infection with determination the antimicrobial sensitivity pattern to the commonly used antibiotics.

Experimental Work

A total of 459 urine samples were collected from adult patients with various ages, ranged between 18-75 years old (mean \pm SD = 44 \pm 15.244) presented with clinical symptoms of UTI attending Teaching Laboratories Center between 1st January 2009 and 1st July 2009. Midstream urine samples were collected by clean catch method in sterile universal containers and cultured within 30 minutes of collection on MacConkey agar and blood agar media, then incubated aerobically for 18-24 hours at 37°C. Urine culture showing a quantitative count of greater than or equal to 10⁵ colony forming-unit (cfu) per ml of single pathogen was considered as significant bacteriuria [14]. Identification of isolates was done by standard method depending on observation of colony characteristics, Gram-stain as well as using biochemical tests for further identification. Antimicrobial sensitivity test was performed by disc diffusion method (Kirby-Bauer's technique) [15] using Muller-Hinton agar. The following commercially available discs were included: amikacin (30 μ g), ciprofloxacin (5 μ g), gentamicin (15 μ g), nalidixic acid (30 μ g), nitrofurantion (300 μ g), tobramycin (10 μ g), imipenem (10 μ g), ceftazidime (30 μ g), cefotaxime (30 μ g), norfloxacin (10 μ g), trimethoprim (5 μ g), ampicillin (10 μ g) and co-trimoxazole (25 μ g). Collected data were analysed by the Statistical Program for the Social Sciences (SPSS) version 15. Chi-square test or two-tailed Fishers exact test were done wherever applicable with a P-value less than 0.05 was considered as significant.

Results and Discussion

The current study shows the distribution and antimicrobial drugs susceptibility pattern of bacterial species isolated from patients with presumptive diagnosis of UTI. A total of 459 patients were attend Teaching Laboratories center in Baghdad with presumptive diagnosis

of UTI. 359 (78.2%) were not included because they met the exclusion criteria as 344 were gave negative urine culture and 15 showed mixed infection. As a result this study was confined to 100 adult patients (21.8%) with ages ranged between 18-75 years old (mean \pm SD = 44 \pm 15.244). The low rate of growth positivity which is also observed by other researchers [16, 17] may due to the presence of either slow growing organisms or organisms that cannot be grow on the ordinary media. There were 76 females and 24 males. The overall males to females ratio was 1:3.2. This finding was consistent with other reported studies from many parts of the world showing a statistically predominance of females [18,19]. This is usually related to the anatomical and pathogenic factors of females [20]. A total of 100 isolates were obtained from the above patients. Gram-negative bacilli isolated accounted for 85% of the positive cultures, while Gram-positive cocci were 15%.

The frequency of isolated uropathogens was given in table (1). *E.coli* was significantly the most common isolated organism (50%) (P< 0.01). The present finding was in accordance with many other studies [21, 22] who showed predominance of Gram-negative bacteria specially *E.coli* with an isolation rates ranged between 40-69%. This was due to the fact that strains of *E.coli* affecting the urinary tract possess a variety of virulence characteristics that facilitate their intestinal carriage, persistence in vagina and then ascension and invasion of the anatomically normal urinary tract[10]. A high prevalence of *Enterobacter* spp.(12%) and *Pseudomonas aeruginosa* (9%) was seen in this work. This is compatible with the results showed by [21,23]. Other bacteria like *Klebsiella* spp. (9%), *Enterococcus faecalis* (9%), *Staphylococcus aureus*(4%), *Proteus* spp.(4%), *Staphylococcus epidermidis* (2%) and *Acintobacter* spp.(1%) spp. were also isolated in this study.

Table (1)
Percentage of bacterial isolates isolated from urine samples(n =100).

Bacterial isolates	No. of isolates	%
<i>Escherichia coli</i>	50	50
<i>Enterobacter spp.</i>	12	12
<i>Klebsiella spp.</i>	9	9
<i>Pseudomonas aeruginosa</i>	9	9
<i>Enterococcus faecalis</i>	9	9
<i>Proteus spp.</i>	4	4
<i>Staphylococcus aureus</i>	4	4
<i>Staphylococcus epidermidis</i>	2	2
<i>Acinetobacter spp.</i>	1	1
Total	100	100

Table (2) showed that among Gram-negative bacteria, *E.coli* (58.8%) was significantly predominant one ($P < 0.01$) whereas other bacterial isolates showed less frequency rate. Among Gram-positive bacteria, *Enterococcus faecalis* (60%) was significantly predominant one ($P < 0.01$) followed by others. These isolated bacteria have been reported as agents of UTIs and their presence in the sample population was not unusual [18], but the differences in bacterial distribution pattern among different area in the world may be explained by the geographic differences which affect the types of bacterial isolates as well as the changes that occur on bacterial isolates over the years.

Table (2)
Percentage of Gram-negative and Gram-positive bacterial isolates.

Gram-negative isolates	No. of isolates	Percentage
<i>Escherichia coli</i>	50	58.8
<i>Enterobacter spp.</i>	12	14.1
<i>Klebsiella spp.</i>	9	10.6
<i>Pseudomonas aeruginosa</i>	9	10.6
<i>Proteus spp.</i>	4	4.7
<i>Acinetobacter spp.</i>	1	1.2
<i>Enterococcus faecalis</i>	9	60
<i>Staphylococcus aureus</i>	4	26.7
<i>Staphylococcus epidermidis</i>	2	13.3

The sensitivity and resistance patterns of the uropathogens isolates to different antibiotics were illustrated in Tables (3, 4, 5). The isolated bacteria exhibited significantly wide differences ($P < 0.01$) in their susceptibility to the tested antimicrobial antibiotics. Table (3) reveals that majority of Gram-negative bacteria showed susceptibility towards imipenem (95.3 %) followed by amikacin (91.8 %), nitrofurantion (70.6 %) and tobramycin (52.9 %). Ampicillin was found least effective drug (9.4%) followed by cefoxitin (18.8 %). Other tested antibiotics were effective only for less than half of Gram-negative bacterial isolates. On the other hand, Gram-positive cocci as demonstrated in Table (4) exhibited complete sensitivity to imipenem (100 %) followed by cefoxitin (86.7 %), amikacin (73.3 %), ampicillin (73.3 %) and nitrofurantion (60 %). On contrary, complete resistance was noted against cotrimoxazole and trimethoprim followed by cefotaxime (6.7 %) and nalidixic acid (13.3 %). The rest antibiotics were found effective only for less than half of the Gram-positive bacterial isolates. The percentage susceptibility and resistance of all the isolates (when considered together) to the different antimicrobial agents was shown in Table (5). Imipenem was found to be very effective against most of the isolates (96 %) ($P \leq 0.01$) followed by the amikacin which showed 89% of sensitivity for those organisms while nitrofurantion and tobramycin were effective against 69% and 52% of uropathogen isolates, respectively. In contrast, highest resistance was recorded against cotrimoxazole (83 %), ampicillin(81%)and trimethoprim (80 %), followed by cefoxitin (71 %), nalidixic acid (69 %), gentamicin (68 %), cefotaxime (67 %), ciprofloxacin (57 %) and norfloxacin (54 %) in decreasing order.

The results of the antibiotic susceptibility tests showed that imipenem (carbapenem) were the most effective as well as drug of choice against both Gram-negative and Gram-positive isolates used in this study as more than 95% of isolates were sensitive to imipenem. Similar result was obtained by other worker [24] who found that sensitivity of uropathogens to imipenem was 99.96 %. Nevertheless, it is advocated that imipenem should be used as a

last line antibiotic to prevent the occurrence of carbapenem resistance. On the other hand, amikacin has tremendous effect against most uropathogens (89 %) with the resistance rate no more than 11 %. This was in consistent with the study done by [20] who observed resistance rate of 7%. Accordingly, it can be recommends amikacin to be prescribed as the empirical treatment for UTI.

Table (3)
Antimicrobial drugs susceptibility profile of Gram-negative bacteria (n=85).

Antibiotic	Sensitive no.	Sensitive %	Resistant no.	Resistant %
AK	78	91.8	7	8.2
CIP	37	43.5	48	56.5
GM	26	30.6	59	69.4
NA	29	34.1	56	65.9
F	60	70.6	25	29.4
TOB	45	52.9	40	47.1
IPM	81	95.3	4	4.7
FOX	16	18.8	69	81.2
CTX	32	37.7	53	62.3
NOR	39	45.9	46	54.1
TMP	20	23.5	65	76.5
AM	8	9.4	77	90.6
COT	17	20	68	80

Amikacin(AK), ciprofloxacin(CIP), gentamicin(GM), nalidixic acid(NA), nitrofurantion(F), tobramycin(TOB), imipenem(IPM), cefoxitin(FOX), cefotaxime(CTX), norfloxacin(NOR), trimethoprim(TMP), ampicillin(AM) and cotrimoxazole(COT).

Table (4)
Antimicrobial drugs susceptibility profile of Gram-positive bacteria (n=15).

Antibiotic	Sensitive no.	Sensitive %	Resistant no.	Resistant %
AK	11	73.3	4	26.7
CIP	6	40	9	60
GM	6	40	9	60
NA	2	13.3	13	86.7
F	9	60	6	40
TOB	7	46.7	8	53.3
IPM	15	100	0	0
FOX	13	86.7	2	13.3
CTX	1	6.7	14	93.3
NOR	7	46.7	8	53.3
TMP	0	0	15	100
AM	11	73.3	4	26.7
COT	0	0	15	100

Amikacin(AK), ciprofloxacin(CIP), gentamicin(GM), nalidixic acid(NA), nitrofurantion(F), tobramycin(TOB), imipenem(IPM), cefoxitin(FOX), cefotaxime(CTX), norfloxacin(NOR), trimethoprim(TMP), ampicillin(AM) and cotrimoxazole(COT).

This study in accordance with the study of other researcher [25] who showed strong activity of nitrofurantion against more than 60% of organisms responsible for UTI. With this evidence, nitrofurantion can be suggested as the drug of choice for empirical treatment. The percentage sensitivity of the most isolated organisms to the commonly used antibiotics for UTI, namely ampicillin and cotrimoxazole was low. It is obvious that cotrimoxazole is no more useful against uropathogens as only 17% of the studied isolates were susceptible to this drug. High incidence of resistance to these drugs has also been reported by other worker in developing countries [26, 27]. This observation may due to the irrational use of first line antibiotics at primary health care level which is the leading cause of increasing resistance to these commonly used drugs. The previous in vitro sensitivity tests showed no resistant urinary pathogens to norfloxacin [28]. On contrary, the uropathogen isolates in this work showed increasing resistance to norfloxacin (54%) which indicates that they can no more be opted for treating UTI. In this study, uropathogens showed resistant also to

antibiotics like new quinolones, third generation cephalosporins. This is in harmony with the results of [29] who showed significantly high resistance to ciprofloxacin in the same study setting. High level of resistance to trimethoprim may be due in part to misuse of this drug as it was recommended to be taken at night to ensure maximal urinary concentrations and increase its effectiveness.

Table (5)

Antibiotic sensitivity and resistance of 100 uropathogens (irrespective of isolates).

Antibiotic	Sensitive %	Resistant %
AK	89	11
CIP	43	57
GM	32	68
NA	31	69
F	69	31
TOB	52	48
IPM	96	4
FOX	29	71
CTX	33	67
NOR	46	54
TMP	20	80
AM	19	81
COT	17	83

Amikacin(AK), ciprofloxacin(CIP), gentamicin(GM), nalidixic acid(NA), nitrofurantion(F), tobramycin(TOB), imipenem(IPM), ceftoxitin(FOX), cefotaxime(CTX), norfloxacin(NOR), trimethoprim(TMP), ampicillin(AM) and cotrimoxazole(COT).

Conclusions

Based on the findings of this study, it is concluded that UTI is affected females more than males. The main organism causing UTI is *E.coli* followed by *Enterobacter spp.* Almost all isolates are resistant to commonly prescribed antibiotics. Therefore, antibiotics should only be commenced after performing culture sensitivity test because most of the UTI patients are treated blindly with different antibiotics. A high percentage of resistance was found to cotrimoxazole, ampicillin and trimethoprim. Therefore in blind therapy of suspected UTIs, imipenem, amikacin and

nitrofurantion were the drugs of choice. Hence, new antimicrobial should be used with more caution and wide spread use of antibiotic therapy should be stopped.

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الخلاصة

أجريت هذه الدراسة لتحديد أنواع البكتيريا المسببة لخمج المجاري البولية ودراسة حساسيتها للمضادات الحيوية في المرضى الذين لديهم أعراض مرض خمج المجاري البولية . ولغرض تشخيص العوامل الميكروبية المسببة للمرض، جمعت عينات وسط الادرار من 459 مريض ثم زرعت واجريت عليها الفحوصات الكيمياحيوية المناسبة، حيث تم الحصول على هذه العينات من المختبرات التعليمية المركزية في بغداد خلال مدة الدراسة والتي كانت بين الأول من كانون الثاني 2009 والأول من حزيران 2009. كما تم إجراء فحص الحساسية للمضادات الحيوية باعتماد تقنية الانتشار بواسطة الاقراص باستخدام وسط مولر هنتون . اظهرت 100 عينه مأخوذة من مجموع عينات المرضى، زرع مايكروبي موجب ويتعداد بلغ 10^5 اواكثر (مستعمرة/ مل ادرار) وقد تم اهمال باقي المرضى البالغ عددهم 359 وذلك لعدم ظهور زرع مايكروبي او لظهور مزارع مايكروبية مختلطة لديهم . كانت نسبة الذكور الى الاناث 1:3.2. كانت بكتريا الاشيريشيا كولاي هي الحي المجهري الاكثرسيادة وبنسبة تعداد (50%) تلتها بكتريا الانتيروبيكتري وبنسبة تعداد (12%). بينما أظهرت كل من *Klebsiella spp.*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* نسبة تعداد بلغت 9% لكل منهم. بينما تواجدت البكتريا المعزولة الاخرى (*Proteus spp*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Acintobacter spp.*) بنسبة اقل من 5% لكل منهم. اظهرت اغلبية العزلات حساسية ضد الامينينيم (96%) يليه في التأثير كل من الاميكاسين (89%) والنايتروفورانشن (69%) بينما لوحظت اعلى نسبة للمقاومة ضد كل من كوتراي موكساسول والامبيسلين والترايمثريم تلتها تأثيرات كل من السيفوكستين وحامض النالدكسك والجنتاميسين والسيفوتاكسيم على التوالي.