THE IN VITRO SENSITIVITY PATTERNS PF BACTERIA ISOLATED FROM URINARY TRACT INFECTIONS TO 11 COMMONLY PRESCRIBED ANTIMICROBIAL DRUGS.

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(Received 11 April 2009 , ,Accepted 21 June 2009)

Keywords: Agumentin, *Klebsilla*, trimoxazole.

ABSTRACT

Due to an increase in the number of resistant bacterial strains that cause urinary tract infection (UTI), empirical treatment with the commonly prescribed antimicrobials might be inadequate. This prospective study was conducted on 252 adult patients with uncomplicated urinary tract infection aiming at optimizing the use of empirical antibacterial therapy through identification of the causative bacteria and studying their in vitro sensitivity patterns to 11 commonly prescribed antimicrobial drugs. This study showed that gram negative bacteria were the most frequent cause of UTI accounting for 95.3% of cases, with strains of *Escherichia coli* alone responsible for 50% of all infections. The study also recorded an increase in the rate of infection caused by *Klebsilla* which was reported in 28.6% of cases. Combined drug therapy was not superior to single antimicrobial. Agumentin or Co- trimoxazole were effective against only 78.6% and 43.7% of the tested bacterial strains. Single drug treatment using the aminoglycoside, Amikacin (92.9% bacterial sensitivity rate) or the third generation cephalosporin, Cefotaxime (88.9% bacterial sensitivity rate) might be the first choice for injectable drug treatment and ciprofloxacin (81% bacterial sensitivity) for orai therapy. It is concluded that periodic reevaluation of bacterial etiology and antimicrobial resistance studies and needed for better empirical treatment of uncomplicated UTI through the avoidance of continuous bacterial resistance to such treatment.

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common infectious disease encountered in everyday clinical practice. They account a large proportion of antibacterial drug consumption and have large socio-economic impacts [(1)].

Increasing resistance to commonly prescribed drugs such as ampicillin and trimethoprim-sulfamethaxazolein *Escherichia coli(E. coli)*, the main causative pathogen of urinary tract

infections, has been demonstrated in urinary tract isolates obtained from patients visiting their general practitioners [2-4]. Moreover, in the past few years, fluoroquinolones have been prescribed more frequently for the treatment of such infections. This has been followed by an increase in fluoroquinolone- resistant

E. coli infections, which are difficult to treat [5-6].

Since the majority of the treatments for uncomplicated UTI begins or is done completely empirically, the knowledge of the organisms, their epidemiological characteristics and their antibacterial susceptibility that may vary with time is mandatory. To optimize the use of optimize the etiology andsusceptibility patterns of UTI pathogens in their population. The aim of this study is to assess commonorganisms causing uncomplicated UTI in adults and to examine thepattern of bacterial resistance to 11 commonly used antimicrobials.

MATERIALS AND METHODS

A prospective study carried on at Basrah General Hospital during the period from September to December 2008. A total of 252 adult patients attending the outpatient department with symptoms suggestive of urinary tract infection such as dysuria, frequency and urgency were included in the study. A detailed history was obtained from every patient regarding symptoms, duration of the illness and the presence of any pre-existing disease (e.g. Diabetes mellitus, renal stones, prostatic hypertrophy) and pregnancy in case of female patients. Pregnant females and Diabeticpatients were excluded from the study.

Urine samples were collected by using sterile glass tubes. After cleaning the external genitalia and perineum by soap and water, the midstream urine samples were obtained aseptically and sent immediately to laboratory for microscopical examination and culturing procedures.

Gross examination of every sample for color, turbidity, and presence or absence of blood cells was performed. After centrifugation of 10 ml of each urine sample for 5 minutes, Gram staining and macroscopical examination of the deposit for the presence of red blood cells, pus cells, bacteria, crystals and epithelial cells was carried on.

For culturing of bacteria, standard loop method [7]) was used . A standard sterile metal witha diameter of 5mm was used to take a volume of 0.001 ml of urine from every sample to be streaked separately to each of freshly prepared MacCkonky agar and blood agar plates (for isolation of Gram negative & positive bacteria respectively). The plates were then incubated aerobically at 37˚ C for 24 hours. The growth pattern of the bacteria were then assessed. Bacteria organisms were isolated

and identified according to standard bacteriological procedure [8] Antimicrobial susceptibility was performed on pure colonies of each species to commonly used antimicrobials agents using the disc diffusing method [9] on Mueller-Hinton agar (MH agar). Discs impregnated with theantimicrobials agents of 11 commonly used drugs were placed on the surface of MH agar plate with at least 2.5 cm distance between discs to prevent overlap of the results. After incubation aerobically at 37˚ C for 24 hours, the zone diameter pf inhibition for eachantimicrobials agent was measured and compared with the NCCLS interpretive table [10] to determine sensitivity or resistance.

RESULTS

A total of 252 adult patients with their ages ranged from 19 to 58 years were included in the study.

The majority (172) were females representing 68.3% of patients, the negative 80 were males (31.7%).

Five genera of bacteria were from urine culture. Four were Gram negative bacteria, namely *E. coli, Klebsiella, Pseudomonas auroginosa and Proteus* (collectively affecting 95.3% of patients), and only one Gram positive bacteria, *Staphylococcus* affecting only 4.7% of patients. The isolation rates of specific bacteria in decreasing frequency were:*E. coli*in 50 patients, *Klebsiella*in 28.6%,*Pseudomonas* in 14.3%, *Staphylococcus*4.7%, and finally *Proteus* in 2.4% of the isolated bacteria to the antimicrobial drugs (table 1).

Table1: Antimicrobial sensitivity of the isolated microorganisms

Antimicrobial Drug	E. coli		Klebsiella		Pseudomonas		Staphylococci		Proteus	
	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
Amikacin	91.2	8.8	97.2	2.8	86.1	13.9	100	100	100	0
Cefotaxime	87.3	12.7	91.7	8.3	83.3	16.7	100	0	100	0
Ciprofloxacin	76.2	23.8	84.7	15.3	83.3	16.7	100	0	83.3	16.7
Agumentin	90.5	9.5	76.4	23.6	30.6	69.4	100	0	100	0
Gentamicin	81.7	18.3	76.4	23.6	69.4	30.6	100	0	100	0
Nitrofurantoin	71.4	28.6	19.4	80.6	77.8	22.2	100	0	100	0
Cotrmoxazole	19	81	62.5	37.5	30.6	69.4	58.3	41.7	83.3	16.7
Nalidixic acid	29.3	70.7	12.5	87.5	30.5	69.5	100	0	75	25
Cephalothin	31.7	68.3	15.3	84.7	19.4	80.6	75	25	0	100
Ampicillin	19.8	80.2	22.2	77.8	33.3	66.7	58.3	41.7	0	100
Trimethoprim	4.8	95.2	6.9	93.1	8.3	91.7	33.3	66.7	0	100

Cephalosporins

Cefotaxime, a third generation cephalosporin was found to be more effective than cephalosporin(Keflin), a first generation cephalosporin. Considering all isolatedstrains collectively, only 28 out of total 252 strains were found to be resistant to Cefotaxime (11.1%), compared to 185 resistant strains tocephalothin out of 252 (73.4%).

The resistance pattern of specific bacterial strain to:

a-Cephalothin:

Staphylococci were the most sensitive to cephalothin with only 25% resistant rate, while the most resistant was *Proteus* strains with 100% resistant rate. The resistance rates against cephalothin reported by other bacteria were 84.7% by *Klebsiella*, 80.6% by *Pseudomonas*, and 68.3% by *E. coli* strains.

b- Cefotaxime, the most sensitive bacteria was *Staphylococci* and *Proteus*,each with 100% sensitivity rates, while the most resistant was *Pseudomonas* with 16.7% resistance rate. The resistance rates against Cefotaxime reported by other bacteria were 8.3% by *Klebsiella* and 12.7% by *E.coli* strains.

Aminoglycosides

Both amikacin and gentamicin Showed reasonable effect on all strains with amikacinsuperiority over gentamicin. The overall resistant rate of thetested bacteria to amikacin was only 7.1% (18 out of 252 strains), compared to 20.2% (51 out of 252 strains) to gentamicin

These drugs must be given only for short time periods owing to their serious side effects which include possible damage to ears and the kidneys. The most sensitive organisms to both drugs were *Staphylococci* and Proteus ,both of which showed total sensitivity without any resistant strains. The least sensitive bacteria for both drugs were*Pseudomonas* with 13.9% resistance to amikacin, and 30.6% to gentamicin. *E. coli*showed 8.8% resistant toamikacinand 18.3% to gentamicin *Klebsiella* resistance to amikacin was only 2.8%, and to gentamicin was 23.6%.

Qunilones and Fluoroquinolones

Ciprofloxacin was found in this study to be more effective than nalidixic acid. The overall bacterial resistance to the Qunilone drug, nalidixic acid was 71% which is much higher than the 19% rate reported for the flouroquinolone drug, ciprofloxacin.

Both drugs showed excellent activity against *Staphylococci* without any resistantstrains. Other bacterial strains studied showed the following resistance rates: *E.coli* 23.8% to ciprofloxacin and 70.7% to nalidaxic acid, *Klebsiella*15.3% to ciprofloxacin and 87.5% to nalidaxic acid, *Pseudomonas* 16.7% to ciprofloxacin and 69.5%, to nalidaxicacid ,and finally *Proteus* 16.7% to ciprofloxacin and 25%to nalidaxic acid.

Ampicillin

This study showed that ampicillin, a semisynthetic penicillin had low activity the tested bacteria. The rate of resistant strains of all bacteria to ampicillinwas found to be 76.2% (192 from 252 strains). Only *Proteus* strains responded fully to ampicillin, while the gram-positive *Staphylococci* showed 41.7% resistance. For gram-negative bacteria, *E.coli*wasfound to be the least sensitive bacteria to ampicillin with resistance rate of 80.2%, followed by *Klebsiella* with 77.8% resistance, and finally*Pseudomonas* with 66.7% resistance rate.

Nitrofurantoin

This drug is absorbed well and excreted in urine. Adverse effects limit doses so therapeutic levels are achieved only in the urine. This study reported that 40.5% (102 from 252) of the isolated bacteria were resistant to nitrofurantoin All *Staphylococci* and *Proteus* strains responded fully to nitrofurantoin. The greatest resistance was found by *Klebsiella*80.6% followed by *E. coil*28.6% and finally *Pseudomonas*22.2%.

Combined therapy

In the present, study theantibacterial effects of two commonly used combination therapy tested. The first combination drug was Agumentin (a combination of the semisynthetic antibiotic amoxicillin plus the β-lactamase inhibitor, calvulaic acid) and the second one was Co-trimoxazole (a combination of trimethoprim plus sulfamethaxazole) .The overall bacterial sensitivity to Agumentin was found to be greater than that to Co-trimoxazole since the bacterial resistant rates to Agumentin was only 21.4% compared to 56.3% due to co-trimoxazole. When trimethoprim (Methprim) alone was tested the overall resistance rate had reached 92.6%.

The resistance pattern of specific bacterial strain to:

a- Agumentin:

All *Staphylococci* and *Proteus* strains were sensitive to Agumentin, while the least sensitive bacteria was *Pseudomonas* with resistance rate reported in 69.4% of strains. *E. coli* and *Klebsiella* strains showed 9.5% and 23.6% resistance rates respectively.

b-Co-trimoxazole:

the most sensitive bacteria were *Proteus*(16.7 resistance), and the most resistant was *E.coli* (81% of the strains resistants). The resistance of other bacteria to co- trimoxazole were 69.4% due to *Pseudomonas*, 41.7% due to *Staphylococci*, and 37.5% due to*Klebsiella*, The overall sensitivities of the tested bacterial strains to 11 antimicrobial agents uscd in this study are represented in table 2.

Table2: Percentages of sensitivity and resistance of the isolated bacteria to specific

Antimicrobial drug	Sensitive bacterial strains		Resistant bacterial strains	
	Number	Percent	Number	Percent
Amikacin	234	92.9	18	7.1
Cefotaxine	224	88.9	28	11.1
Ciprofloxacin	204	81	48	19
Gentamicin	201	79.8	51	20.2
Agumentin	198	78.6	54	21.4
Nitrofurantion	150	59.5	102	40.4
Co-trimoxazole	110	43.7	142	56.3
Nalidixic acid	73	29	176	71
Cephalothin	67	26.6	185	73.4
Ampicillin	60	23.8	192	76.2
Trimethoprim	18	7.1	234	92.9

DISCUSSION

Urinary tract infection (UTI), the presence of bacteria in urine along with symptoms of infection, is a very common medical problem. Females are more prone to urinary tract infection than males due to short female urethra and the proximity of anal region with its contaminants[11]. This was also true in the present study in which 68.3% of patients were females and the remaining 31.7% were males. Female to male ratio was 2.2:1.

Most bacterial urinary tract infections are caused by commensal colonic gram- negative aerobic bacteria with strains of*E. coli* beingmost frequent etiologic agents[1,12-13]. The remaining gram-negative urinary bacteria include *Klebsiella, Pseudomonas auroginosa,* and *Proteus mirabilis*, all are enterobacteria .From gram-positive organisms, the most frequently encountered bacteria are coagulase-negative*Staphylococci* and enterococci (group D Streptococci). In the study, the majority of microorganisms isolate were gram negative bacteria 95.3%, compared to only 4.7% due to gram-positive bacteria, namely *Staphylococci*. The most commoncause of UTIs reported in the current study was *E.coli* which was isolated in 50% of the cases. This result was in accordance with other studies [14,14].*Klebsiella*, with a rate of 28.6% came in the second rank as the causative agent of UTI following *E.coli* in this study. This rate of klebsiella isolation seems to be much higher than the average rates of 8-10% reported in the literatures[1,11,16].

All forms of bacterial UTI require antimicrobial therapy. A variety of antibiotics and chemotherapeutic drugs are available nowadays both as single drugs or in combination. There is on single regimen to be addressed as the best one since the choice depends on many factors, like whether the infection is primary or recurrent or simple infection or complicated infection from underlying renal disease. Other or factors in the patient also will dictate the type of antimicrobial therapy, like is childhood age ,presence of diabetes, pregnancy, and whether the patient is hospitalized or not[15]. The rates bacterial resistant to antimicrobials are continually changing worldwide, especially by strains of *E. coli*. The most common not The of continually changing worldwide, especially by strains of common cause of UTIs [13,16]. This increased bacterial resistance to the commonly used antimicrobial drugs are mainly due to indiscriminate widespread use of antibiotics in humans and animal feed. The results of this study had pointed that taking the sensitivity pattern of all the five bacterial species tested, single drug treatment using Amikacin or Cefotaxime (with bacterial sensitivity rates of 92.9% and 88.9% respectively) is superior to the combined therapy using Agumentin or Co- trimoxazole with 78.6% and 43.7% sensitivity rates respectively. Because the bacterial resistance to antimicrobial drugs is continuously changing, the

trend to prescribe the initial empirical treatment for UTI such as trimethoprim sulfamethaxazole or ampicillin would be inadequate and should be avoided.

The study recommends the need for periodic re-evaluation of bacterial etiology and the pattern to avoid resistance to empirical.

دراسة الحساسية الدوائية للبكتيريا المعزولة من التهابات المجاري البولية لـ 11 نوع من المضادات المكروبية.

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الخلاصة

نظراً لحدوث زيادة في عزل عترات بكتيرية لأدوية المضادات الحياتية في مرضى التهاب المجاري البولية فأن المعالجة التجريبية بالعقاقير المضادة الشائعة الاستعمال يمكن ان يكون غير كافياً لعلاج تلك الألتهابات.

تم اجراء الدراسة على 252 مريضاً بالغاً يعانون من التهابات المجاري البولية البسيطة بهدف نتائج المعالجة التجريبية من خلال التعرف على البكتريا المسببة للالتهابات ودراسة انماط الحساسية الدوائية لـ 11 مضاد جرثومي شائع الوصف.

اظهرت الدراسة ان البكتيريا السالبة لصبغة كرام هي السبب الأكثر ترددا لألتهابات المجاري البولية وبنسبة 95.3% من الحالات مع سلالات الـ *Escherichia coli* بمفردها وهي مسؤولة عن 50 % من جميع الألتهابات.

سجلت الدراسة ايضاً زيادة في نسبة الأصابة المسببة بواسطة جرثومة *Klebsiella* والتي تم تسجيلها في 28.8% من الحالات.

المعالجة الدوائية المزدوجة لم تكن أكثر فاعلية من استخدام المضادات الحياتية المنفردة.

أن عقار Co-trimoxazole أوAgumenentin كانا فعالين ضد فقط 78.6% و 43.7% من عترات البكتريا التي تم دراستها على التوالي.

المعالجة الدوائية المفردة بعقار Amikacin من مجموعة Aminoglycoside والذي أظهر نسبة فعالية 92.9% ضد البكتريا أو عقار Cefotaxine من مجموعة الجيل الثالث لـ Cephalosporin والذي أظهر 88.9% فعالية ضد البكتريا قد يكون هو الاختيار الأول لمعالجة التهابات المجاري البولية عن طريق الحقن. بينما عقار ciprofloxacin مع 81% فعالية ضد البكتريا للمعالجة الفموية.

استنتجت الدراسة بأن أعادة التقييم الدوري للمسببات البكتيرية وبنمط مقاومة البكتريا للأدوية المضادة للميكروبات يحتاج إليها لتحسين العلاج التجريبي لالتهابات المجاري البولية.

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