

Laboratory evaluation of urine culture and drug resistance in outpatients clinically suspected of urinary tract infections

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Objectives

To identify common bacterial etiologic agents causing urinary tract infection (UTI) among outpatients with gender and age distribution and susceptibility and resistance patterns of the isolates.

Methods

The study was carried out at Azadi Teaching Hospital, Duhok city, Iraq. Urine samples of 223 outpatients were collected and screened on MacConkey and Blood agar. Antimicrobial susceptibility testing was performed by Kirby Bauer's disc diffusion method. Isolated colonies were processed for biochemical characterization, antibiotic sensitivity test and resistance patterns.

Results

Of the 223 specimens, 126 (56.5%) had a positive urine culture. Of these, 111 (88.1%) were female and 15 (11.9%) male. Infections rate were higher among those aged 20-30 years in both gender. *Escherichia coli* was found to be the most frequent causative agent of UTIs (51.6%) followed by *Enterococcus faecalis* (27.8.3%), *Klebsiella*

pneumonia (9.5%), *Enterobacter aeruginosa* (4.8%), *Staphylococcus aureus* (3.9%) and *Pseudomonas aeruginosa* (2.4%). *Escherichia coli* showed variable susceptibility rate to different antibiotics as 90.5%, 84.7%, 71%, 46.2%, 43.1%, 43.1%, 41.6%, 35.4%, 33.9% and 21.6% of the isolates were found to be susceptible to amikacin, nitrofurantoin, rifampicin, nalidixic acid, trimethoprim, gentamicin, ciprofloxacin, cephalothin, cefotaxime and amoxicillin, respectively. Five different resistotype patterns were found in *Escherichia coli* isolates; common resistotype was 1.

Conclusion

This study showed higher rate of UTI in female than male population in age group of 20-29 years. *Escherichia coli* was the most frequent causative agent and showed multi-drug resistance with different resistotyping patterns. (Rawal Med J 2012;37:268-272).

Keywords

Urinary tract infections, antibiotics, *E. coli*, enterococcus.

INTRODUCTION

Urinary tract infection (UTI) is a heterogeneous disease which can be grouped into: acute uncomplicated UTI (women with signs and symptoms of UTI with no evidence of urological abnormalities), complicated UTI (male sex and/or urological abnormalities), and those with asymptomatic bacteriuria (pregnant women, diabetics).¹ These are caused by a variety of gram negative bacteria that ascend into the urinary tract and establish bacteriuria often at levels more than or equal to 10⁵ colony forming units of bacteria/ml of urine. *Escherichia coli* dominates as the causative agent in all patient populations. Others include *Staphylococcus aureus*, *Enterococcus*, *Enterobacteriaceae* group (*Klebsiella* sp., *Proteus* sp., *Enterobacter* sp., *Serratia* sp., *Citrobacter* sp.,

Salmonella sp and *Pseudomonas* sp.).²

The goals in the treatment of urinary tract infection are to prevent or treat systemic symptoms, to relieve symptoms, to eradicate sequestered infection, to eliminate uropathogenic bacterial strains from fecal or vaginal reservoirs, and to prevent long-term sequelae all at minimal cost, with the lowest rate of side effects, and with the least selection of an antibiotic resistant flora.³ Antimicrobial therapy is initiated even before the results of urine culture are available. Hence, there exists a great need for antimicrobial resistance surveillance at local, national and international level.⁴ In addition, a continuous review of the pattern of microbial isolates causing UTIs and their antimicrobial susceptibility patterns in clinical practice is essential. The aim of the present study was to

determine the incidence and antibiotic susceptibility of etiologic agents recovered and establishment the resistance patterns of the most commonly encountered microbial isolates.

MATERIAL AND METHODS

Two hundred and twenty three patients clinically diagnosed as having UTIs were voluntarily recruited into the purposive study. They were made up of 157 (70.4%) females and 66 (29.6%) males and aged 10-49 years. Patients with concomitant or prophylactic antimicrobial treatment within 7 days before the study, anatomic and functional urinary tract abnormalities, for example, urinary tract obstruction, urinary tract tumors, neurologic bladder disturbances, indwelling urinary catheters or stents; and patients who did not give their informed consent were excluded from the study. Urine specimens were collected from outpatients, at Azadi teaching hospital, Duhok city, Iraq between April and August 2011. The early morning mid-stream urine was collected using sterile, wide mouthed glass bottles with screw cap tops. The time between sample collection and sample analysis did not exceed one hour.

Several media and tests were used for the isolation, identification and testing the susceptibility of the isolates for common used antibiotics. These were Blood agar (with 5-7% defibrinized blood), MacConkey agar, Nutrient agar and Mannitol salt agar. Simmons citrate agar, kligler Iron Agar (KIA), Mueller-Hinton agar, Sulfide formation indole production, Motility Test (SIM), Nutrient agar, Methyl Red-Voges Proskauer broth, Coagulase, Catalase, Urease, Oxidase tests (Difco, USA) were used for the identification. All samples were cultured quantitatively on blood and MacConkey agar by calibrated loop technique, using a standard 1 microliter of urine inoculums. The plates were incubated at 37°C for 18-24 hours in an incubator. The plates were read the following day but extended to 48 hrs if there was no bacterial growth within 24 hrs. Significant bacteriuria was defined as the growth of $\geq 10^5$ CFU/ml of a single species cultured from urine. Isolated colonies were subjected to Gram staining technique and biochemical tests for identification.⁵

Antibiotic susceptibility tests were carried out on isolated and identified colonies of bacterial isolates using commercially prepared antibiotic sensitivity disc (Oxoid, England) using modified Kirby-Bauer method according to CLSI guidelines, using Mueller-Hinton agar standard media. The inhibition zone standards for antimicrobial susceptibility were considered from tables for interpretative zone diameters of Clinical and Laboratory Standards Institute (CLSI).⁶ Resistance typing patterns were done only for *Escherichia coli* isolates.

RESULTS

Of the 223 urine specimens cultured, 126 (56.5%) were positive, of these 111(88.1%) were from females and 15 (11.9%) from males. There was higher prevalence of UTI in both genders in age group 20-29 years (Table 1).

Table 1. Age and gender distribution.

Age groups (Years)	Male Number (%)	Female Number (%)	Total Number (%)
(10-19)	4(3.2)	33(26.2)	37(29.4)
(20-29)	5(4.0)	43(34.2)	48(38.2)
(30-39)	5(4.0)	25(19.8)	30(23.8)
(40-49)	1(0.7)	10(7.9)	11(8.6)
	15(11.9)	111(88.1)	126(100)

Escherichia coli was commonest isolate following by *Enterococcus faecalis* (Table 2).

Table 2. Bacteriological profile of UTI.

Pathogens	Male (%)	Female (%)	Number of isolates
<i>Escherichia coli</i>	6(40)	59(53)	65(51.6)
<i>Klebsiella pneumonia</i>	1(6.7)	11(9.9)	12(9.5)
<i>Enterococcus faecalis</i>	3(20)	32(28.8)	35(27.8)
<i>Enterobacter aeruginosa</i>	2(13.3)	4(3.6)	6(4.8)
<i>Staphylococcus aureus</i>	2(13.3)	3(2.7)	5(3.9)
<i>Pseudomonas aeruginosa</i>	1(6.7)	2(1.8)	3(2.4)
Total	15 (100)	111(100)	126(100)

There was high susceptibility to amikacin and increased resistance to amoxicillin were noted in all isolates (Table 3). On other hand, the susceptibility

rate of varies bacterial isolates to other antibiotics was variable.

Table 3. Antimicrobial susceptibility of Escherichia coli isolates.

Antibiotics	Symbol	Disc Potency (ug)	(* %)
Amikacin	AK	10	90.5
Nitrofurantoin	F	10	84.7
Rifampicin	RF	5	71
Nalidixic acid	NA	30	46.2
Gentamicin	CN	10	43.1
Trimethoprim	TMP	10	43.1
Ciprofloxacin	CIP	100	41.6
Ciphalothin	KF	30	35.4
Cefotaxime	CTX	30	33.9
Amoxicillin	AX	10	21.6

* %: Susceptibility rate

Table 4 shows the resistotyping patterns of Escherichia coli isolates to commonly used antibiotics. It was found that all of the isolates were resistant to more than one antibiotic.

Table 4. Resistance patterns of Escherichia coli isolates.

Resistance patterns	Resistance spectrum phenotypic	Number (%)
Resistotype 1	AX, CN, KF, NA, CTX, TMP	3 (27.2)
Resistotype 2	AX, CIP, KF, NA, CTX, TMP	2 (18.2)
Resistotype 3	AX, CN, CIP, KF, NA, CTX	2 (18.2)
Resistotype 4	AX, CN, CIP, KF, NA, CTX, TMP	2 (18.2)
Resistotype 5	AX, CN, CIP, KF, NA, CTX, TMP, F	2 (18.2)
Total		11 (100)

Out of 65 isolates, 11 isolates with multiple-drug resistant belonged to 5 distinct resistotype patterns; common resistotype was 1.

DISCUSSION

Urinary tract infection is one of the common causes for seeking medical attention in the community and

effective management commonly relies on the identification of the type of organisms and the selection of an effective antibiotic agent to the organism in question. The most frequent causative agent in this study was Escherichia coli (51.6%) followed by Enterococcus faecalis (27.8%) and Klebsiella pneumonia (9.5%). Another study conducted in Duhok City, Iraq, reported uropathogens prevalence among outpatients of 46.5% for E coli; followed by Proteus spp. (30.7%), Enterococcus faecalis (7.8%), Staphylococcus saprophyticus (7.4%), Klebsiella spp, (4.4%) and Pseudomonas aeruginosa (3.2%).⁷ Another study from Pakistan reported the most frequent causative agent of UTIs was E.coli (66.97%) followed by Enterococci (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).⁸

Prevalence of different pathogens is dependent on several population attributes, sample size and hygienic conditions of the patients. Therefore, a stable pattern in this regard cannot be predicted. This is why data vary when different studies are compared. Hsueh et al, during the period 1993-1998 reported Candida spp. (23.6%), E coli (18.6%) and Pseudomonas aeruginosa (11.0%), however, in 1999, E.coli (18.4%) replaced Candida spp. (14.3%) as the top ranking pathogen causing UTIs.⁹

The present study showed the prevalence of UTIs was 88.1% and 11.9% in females and male gender, respectively. It was previously found that UTIs is always a very common phenomenon among the women.¹⁰ Half of all women develop a UTI during their lifetimes.¹¹ In the present study, age group of 20-29 year in both genders were found to be very much prone to UTI. In other study, a much greater prevalence of this infection was observed in 56.0% female and 52.1% male cases belonged to the age group >51 years of age.¹² Pseudomonas aeruginosa was isolated from about 2.4% in the present study, 3% in Duhok; Iraq,⁷ 7.3% in Pakistan,⁸ 3.5% in Canada,¹³ 1.5% in Texas,¹⁴ 2% in Nigeria,² and 2.9% in Croatia.¹⁵

Based on the antibiogram results of our study, E coli was the most recovered bacteria Its greatest susceptibility rate was found in 90.5% to amikacin, followed sequentially by nitrofurantoin (84.7%)

and to rifampicin (71%). Thus, these drugs could be considered as satisfactory options in the empirical treatment of UTI. Also, all the E coli isolates were less susceptible to amoxicillin, cefotaxime and cephalothin. Furthermore, the later finding were 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 area. Similar findings were clarified by AL-Derzi et al except they found low resistant rate of E coli isolates against cephalixin (11.8%) and ciprofloxacin (23.3%).⁷ Our findings are also consistent with other investigators.^{16,17} The susceptibility rate of E coli isolates to Sulpha drugs was low to moderate as 43.1% in our study. Similar data were reported by others.^{2,9,15} This is important as these drugs are frequently used for empirical treatment in suspected cases of UTI.

We determined the susceptibility test of E coli to selected antibiotics and found most of the isolates were multiple resistant. Out of 65 E. coli isolates, 11 isolates of multi-drug resistant occurred twice or more were belonged to 5 distinct resistotype patterns. Resistotype 1 was predominant has much higher frequency rate comprising 27.2% of the isolates. Resistant strains have been reported from Iran,¹⁸ Scotland,¹⁹ India,²⁰ and Nigeria.²¹

CONCLUSION

E. coli was the commonest causative agent of UTI in women aged between 20-29 years. Most of these isolates showed multiple antibiotic resistances with different resistotype patterns. The isolation of bacterial uropathogens with a higher resistance rates for commonly used antimicrobials leaves the clinicians with very few options to choose drug used for empirical treatment of UTIs. Our urge physicians on the need of re-evaluation of empiric treatment of UTI, monitoring studies and continued surveillance at local level. Ultimately, introduction of molecular-based methods is essential primarily because of difficulties in detecting strains with low-level resistance by conventional methods.

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