

University of Al-Qadisiyah
College of pharmacy



**Prevalence and Antimicrobial Susceptibility
of Pathogens in Urinary Tract Infections
in Diwaniya city**

BY

Asawer Jawad Abdul-kadim

Huda Rasool Dohan

Supervised by

Prof. Dr. Azhar Noory

To the fountain of patience, optimism and hope ,

To each of the following in the presence of god and his messenger *my mother dear*.

To those who have demonstrated to me what is the most beautiful of *my Brothers life*,

To the big heart *my Father dear*.

To the people who paved our way of science and knowledge All our *teachers Distinguished*

To the taste of the most beautiful moments with *my friends*

I guide this research

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

لَا تَدْرِي لَعَلَّ اللَّهَ يُحْدِثُ بَعْدَ ذَلِكَ أَمْرًا

صدق الله العظيم

الطلاق \ الآية 1

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 60 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 ALQadisiah during the study period (1st July2016- 1st September2016) The antimicrobial sensitivity test was carried out by disc diffusion technique using Muller- Hinton agar. 40 urine samples were cultured positive with a colony count equal or more than 10⁵/ml while 20 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 37.5% followed by Staphylococcus aureus35% whereas Entarobacter showed frequency rate of 7.5%. However, Pseudomonas showed frequency rate 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.

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, nitrofurantoin, 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 w

as aimed to identify the most common etiologic agents responsible for urinary tract infection with determination the antimicrobial sensitivity pattern to the commonly used antibiotics

Experiment work

Instrument / Equipment

Autoclave
Compound light microscope
Electric oven
Digital camera
Hot plate with magnetic stirrer
Incubator
Laminar-flow cabinet
PH meter
Rotary microtome
Sensitive balance
Vortex mixer
Water bath
Water distiller

Culture media used with their remarker

Medium
Blood ager
Brain – heart infusion broth
Eosin – methylene blue (EMB)
MacConkey ager

MacConkey borth
Mannitol salt agar
Muller – Hinton agar
Nutrient agar
Nutrient broth
Simmoms citrate agar
Triple suger Iron agar (TSI)
Urea agar base

Bacterial isolation:

Teaching Laboratories Center between 1st July 2016 and 1st September 2016 . 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 µg), ciprofloxacin(5 µg), gentamicin (15 µg), nalidixic acid (30 µg), nitrofurantion (300 µg), tobramycin (10 µg), imipenem (10 µg), ceftazidime (30 µg)

µg), cefotaxime (30 µg), norfloxacin (10 µg), trimethoprim (5 µg), ampicillin (10 µg) and co-trimoxazole (25 µg).

Collected data were analysed by the Statistical Program for the Social Sciences (SPSS) version [15].

Statistical analysis:

Analysis performed using SPSS [version20]. The data presented as percentages, mean value and standard deviation. Chi square used to calculate significance for frequency, while t test used to determine significance in mean difference. P value of < 0.05 regarded 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 60 patients were attend Teaching Laboratories center in Diwanyia city with presumptive diagnosis

Table (1) Percentage of microbial isolates isolated from urine samples(n = 40).

Bacteria	Number of isolates	percent
Escherichia coli	15	37.5%
Staphylococcus aureus	14	35%
Enterococcus faecalis	3	7.5%
Proteus spp.	2	5%
Pseudomonas Aeruginosa	2	5%
Candida albicans	3	7.5%
Contaminated	1	2.5%

E.coli was significantly the most common isolated organism (37.5%) (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 *Staphylococcus* spp. (35%) and *Pseudomonas aeruginosa* (5%) was seen in this work. This is compatible with the results showed by [21,23]. Other bacteria like *Candida albicans* spp. (7.5%) *Proteus* spp.(5 %), were also isolated in this study. There was no significant [P>0.05] difference in mean age of women with negative urine culture [32.92 ± 14.74 years] and women with positive urine culture [34.54±16.01 years]. In addition, BMI mean value was not significantly [P>0.05] different in women with negative urine culture [25.20 ±3.1] and from those with positive urine culture [25.49±2.87]. However, the mean pus cell scale was significantly [P=0.000] higher in women with culture positive urine [1.78±1.20] as compared to culture negative urine [0.73±0.92], (Table 1).

E. coli demonstrated a resistant rate of > 70% to azitreonam, Termethoprin, Norfloxacin,. However, *E. coli* show a low resistance to Clindamycin, Gentamycin, Chlorothromycin, Amikacin. In addition, a moderate resistant rate [50 to 70%] was demonstrated by *E. coli* to cefaclor, piperillin, cefotaxime, Nalidixic acid, , and Ampicillin, Table3.

Staphylococcus aureus urinary isolates show a high resistance rate to Aztreonam, Rifampicin, Norfloxacin, Clindamycin. However, *Staphylococcus aureus* was with low resistance rate to ciprofloxacin, Gemifloxacin, Cefixime, Trimethoprin, cefoclar, Table3.

The *Proteus* high rate of resistance demonstrated against Ampicillin[100%], nitrofurantoin [50%] ampicillin [87.5%], Ciprofloxacin [50%] norfloxacin [50%] Aztreonam[50%], Cefixime[50%], amoxicillin [37.5%], and piperillin [50%] High susceptibility was shown to Clindamycin and amikacin,(Table 2).

Table 2. Antibiotic susceptibility of isolates from women with urinary tract infection.

Antibiotic	Resistant rate			
	E. coli (15)	Staph. aureus(14)	Entrobacter (3)	Proteus (2)
	% [No.]	% [No.]	% [No.]	% [No.]
Amikacin	1 (6.6) %	-	1 (33)	-
Nalidixic acid	4 (26)	-	1 (33)	-
Azitreonam	7 (46.6)	5 (35.7)	1 (33)	1 (50)
Amoxicillin	4 (26)	3 (21)	-	-
Tobramycin	3 (20)	-	-	-

Chloromphinc ol	1 (6.6)	2 (14.2)	1 (33)	-
Rifampicin	2 (13.3)	5 (35.7)	-	-
Nitrofurantion	2 (13.3)	4 (28.5)	-	1 (50)
Ampicillin	5 (33.3)	2 (14.2)	-	2 (100)
Ceftriaxone	3 (20)	-	-	-
Ciprofloxacin	3 (20)	5 (35.7)	-	-
Pipercillin	5 (33.30)	-	1 (33)	1 (50)
Norfloxacin	5 (33.3)	8 (57)	-	-
Gemifloxacin	2 (13.3)	2 (14.2)	-	1 (50)
Cefixime	6 (40)	2 (14.2)	2 (66)	1 (50)
Trimethoprim	5 (33.3)	1 (7)	2 (66)	-
Clarithromyci n	1 (6.6)	-	-	-
Clindamycin	1 (6.6)	5 (35.7)	2 (66)	-
Gentamycin	1 (6.6)	4 (28.5)	1 (33)	-
Norfloxacin	5 (33,3)	8 (57)	-	-

Conclusions

Most patients with uncomplicated acute cystitis have cases that are clinically straight forward, and they may not require any laboratory testing beyond urinalysis.

for significant number of patients, however, the clinical history and physical finding alone maybe insufficient to make a definitive diagnosis of UTI , for those patients and for patients with complicated UTI, laboratory tests are necessary to make the diagnosis and to provide specific information's regarding the identify and antimicrobial susceptibility pattern of pathogens .

both the laboratory diagnosis and clinical diagnosis of laboratory test results must be made in light of the method of collection used, clinicians

should specify the method of collection on test requisition forms. of the available laboratory tests, urinalysis is helpful primarily as a means of excluding bacteriuria, but it's not assure gate for culture .

although culture identify pathogens ,the accurate interpretation of culture results requires clinical information that is usually available only to the clinician .

we hope that infectious diseases physicians, in particular will understand both the strength and the limitations of the laboratory based diagnostic studies for UTI that have been reviewed in this article, and we hope that they will incorporate this understanding with current treatment guidelines to optimize patients care.

References

- [1] Foxman, B.; Barlow, R.; D'arcy, H. Urinary tract infection: Self-reported incidence and associated costs; *Ann. Epidemiol.*, 10: 509-515, 2000.
- [2] Mandelle; G. L.; Benntt, J. E.; Dolin, R.; Principles and practice of infectious diseases. 5 th ed, Churchill Livingstone, Philadelphia; pp 881-882; 2005.
- [3] Barret, S.P.; Savage, M.A.; Rebec, M.P.; Guyot, A.; Andrews, N.; Shrimpton, S.B. Antibiotic sensitivity of bacteria associated with community acquired urinary tract infection in Britain; *J. Antimicrob. Chemother.* 44: 359-365, 1999.
- [4] Nicolle, L.E. Uncomplicated urinary tract infection in adult including uncmplicated pyelonephritis; *Urol. Clin. North. Am.* 35(1),1-12, 2008.
- [5] Dromigny, J.A.; Nabeth, P.; Juergens, B.A.; Perrier-Gros-Claude, J.D. Risk factors for antibiotic-resistance *Escherichia coli* urinary tract infection in Dakar; Senegal. *J. Antimicrob. Chemother.* 56, 236-239, 2005.
- [6] Bronsema, D.A.; Adams, J.R.; Pallares, R. Secular trends in rates and etiology of nosocmial urinary tract infections at a university hospital; *J. Urol.* 150,414-416,1993.
- [7] Jacobs, L.G.; Fungal urinary tract infections in the elderly: Treatment guidelines; *Drugs Aging.* 8, 89-96, 1996.

- [8] Zalamanovici, T. A.; Green, H. ;Paul, M. ; Yaphe, J.; Leibovici, L. Antimicrobial agents for treating uncomplicated urinary tract infection in women; *Cochrane Database Syst. Rev.*10(10), 2010.
- [9] Bachelier, C.D.; Bernslein, J.M. Urinary tract infections; *Med. Clin. North Am.* 81, 719-29, 1997.
- [10] Annabelle, T.; Dytan, M. D.; Jennifer, A.; Chua, M. D. Surveillance of pathogens and resistance patterns in urinary tract infections; *Phil. J. Microbiol. Infect. Dis.* 28 (1), 11-14, 1999.
- [11] Acar, J.F.; Goldsein, F.W. Trends in bacterial resistance to fluoroquinolones; *Clin. Infect. Dis.* 24(1), 67-73, 1997.
- [12] Gupta, K.; Sahm, D.; Mayfield, D.; Stamm, W. Antimicrobial resistance among uropathogens that cause community acquired urinary tract infections in women: A nation wide analysis; *Clinic. Infect. Dis.* 33, 89-94, 2001.
- [13] Azra, S.H.; Nair, D.; Kaur, J.; Baweja, G.; Deb, M. Aggarwal, P.; Resistance patterns of urinary isolates in a tertiary Indian hospital; *Ayub Med. Coll. Abbottabad.* 19, 39-41, 2007.
- [14] Sleight, J.D.; Timbury, M.C.; *Notes on Medical Bacteriology.* 2 nd edition, Churchill Livingstone Inc., Broadway, New York; pp. 212-218; 1986.
- [15] Bauer, A.W.; Kirby, W.M.; Sherris, J.C.; Truch, M. Antibiotic susceptibility testing by standarized single disc method; *Am. J. Clin. Path.* 45(4), 493-496, 1996.
- [16] Levitt, P.N. Analysis of pathogens isolated from urinary tract infection in Barbados; *West. Indi. Med. J.* 42, 72-76, 1993.
- [17] Karki, A.; Tiwari, B.R.; Pradhan, S.B. Study on bacteria isolated from urinary tract infection and their sensitivity pattern; *J. Nep. Med. Assoc.* 43, 200-203, 2004.
- [18] Adedeji, B.A.; Abdulkadir, O.A. Etiology and antimicrobial resistance pattern of bacterial agents of urinary tract infections in students of Tertiary Institusions in Yola Metropolis; *Advan. Biol. Res.* 3(3-4), 67-70, 2009.

- [19] Mehr, M.T.; Khan, H.; Khan, T.M.; Iman, N.U.; Iqbal, S.; Adnan, S. E. coli urine super bug and its antibiotic sensitivity: A prospective study; *Med. Sci.* 18(2), 110-113, 2010.
- [20] Kebira, A.N.; Ochola, P.; Khamadi, S.A. Isolation and antimicrobial susceptibility testing of Escherichia coli causing urinary tract infections; *Appl. Biosc.* 22, 1320-1325, 2009.
- [21] Atienza Morales, M.P.; Castellote Varona, F.J.; Romero Portilla, C. Urinary tract infection and antibiotic sensitivity in the south of Albacete; Spain. *An. Med. Interna.* 16(5), 236-238, 1999.
- [22] Ozumba, U.C. Increasing incidence of bacterial resistance to antibiotics by isolates from the urinary tract; *Niger. J. Clin. Pract.* 8(2), 107-109, 2005.
- [23] Muhammed, A.; Muhammed, F.; Muhammed, A-H.; Imam, H.; Imam, A-S.; Muhammed, R. A prospective study of patterns of urinary tract infections and antibiotic sensitivity in rural setting of Mirpurkhas; *Muhammed Medical College.* 1(1), 10-14, 2010.
- [24] Kiffer, C.R.; Kuti, J. L.; Eagye, K. J.; Mendes, C.; Nicolau, D.P. Pharmacodynamic profiling of imipenem, meropenem and ertapenem against clinical isolates of extended-spectrum beta-lactamase producing Escherichia coli and Klebsiella spp. from Brazil; *Int. J. Antimicrob. Agents.* 28(4), 340-344, 2006.