Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol 7, 2013

Study of Prevalence and Antimicrobial Susceptibilities of Uropathogenic Isolated from Patients in Al-Hillah city

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Abstract

In this study, 200 patients (100 male and 100 female) suffering from Urinary tract infection who presented to the Emergency Department of Babylon Maternity and Children Hospital and Al-Hillah educational Hospital from October\2012 to February\2013 underwent a history and physical examination. In this study, *Escherichia coli* formed the major causative agent (10.8% in male and 13.2% in female) followed by *staphylococcus aureus* (5.4% in male and 4% in female), *Pseudomonas aeruginosa* (4% in male and 4.6% in female).

All isolates were tested for the sensitivity to Amikacin, Chloromphenicol, Nitrofurantoin, Ofloxacin, Norfloxacin, while Pseudomonas aeruginosa shows sensitivity with Norfloxacin, Ofloxacin, Piperacillin, Tobramycin, Proteus species shows sensitivity with Amikacin, Amoxicillin , Cefodizime, Cefoxitin, Cephalexin, Gentamycin, , Norfloxacin, Piperacillin, Tobramycin and Klebsiella pneumoniae isolates shows sensitivity with Amikacin, Norfloxacin, Piperacillin, Tobramycin.

Our investigation showed that from the 200 patients with UTI, 62 male and 84 had pyuria > 5 WBCs/hpf, 84 male and 75 female had pyuria >10 WBCs/mL, 86 male and 64 female had CRP >24 mg/L, 67 male and 70 female had ESR >35 mm/hour, and 69 male and 55 female had WBC >15 000/mL.

Introduction

Bacteria are the most frequent urinary pathogen isolated from 50-90 % of all uncomplicated urinary tract infections [1]. Urinary tract infections are very common infections in humans, with *E. coli* being the dominant pathogen. *E. coli*, the most common member of the family Enterobacteriaceae accounts for 75-90 % of all urinary tract infections in both patients and out patients [**2**]. The identification of nonpathogenic members also needs to detect factors that determine virulence of this organism [3].

The annual incidence of urinary tract infections (UTIs) in the elderly population ranges from 10% in the community to as high as 30% of hospitalized patients [4]. Mortality rates in elderly patients from bacteremia as a result of UTI can be as high as 33% [5]. A study by Plowman et al. (2001) [6] found that over a 12 month period UTIs had the highest incidence (35 %) of all nosocomial infections in a district general hospital, and the majority of patients were over 60 years of age.

UTIs are also the most common infection in long-term care facilities, where they account for 20–60% of all antibiotic prescription use [7]. This large-scale prescription of antibiotics may well contribute to the levels of antibiotic resistance in urinary pathogens [8].

Urinary tract infection (UTI) is a frequent serious bacterial infection among population [9]. UTI is often associated with vesicoureteral reflux or urinary tract obstruction [10], conditions associated with a higher risk of recurrent UTI [11].

Moreover, UTI is believed to be the leading cause of renal scarring [12, 13], one of the most common causes of end-stage renal disease in patients, the presumptive diagnosis of UTI in men is often based on the results of microscopic urinalysis (UA), and most infections remain undiagnosed if tests are not performed routinely to detect them [14, 15].

The sensitivity, specificity, and positive predictive value of the standard UA are so low that only a third to half of patients with positive urine culture results can be identified correctly. Dukes [16. 17] described a more accurate microscopic analysis of centrifuged urine performed with a hemocytometer and reporting cells per cubic

millimeter, herein referred to as hemocytometer white blood cell (WBC) counts.
Stamm [18] defined pyuria as the presence of ≥10 WBCs/μL in centrifuged urine and found it to be very sensitive, identifying 96% of symptomatic adult patients with bacteriuria of ≥1000 colony-forming unit (CFU)/ml.
The present study was undertaken to evaluate a group of febrile infants younger than 2 years of age to assess the usefulness of the WBC count, C-reactive protein (CRP) concentration, erythrocyte sedimentation rate (ESR), and UA for identifying infants at risk for UTI; and antibiotics sensitivity of each pathogen, in addition to compare standard WBC counts and hemocytometer WBC counts in identifying very young infants with positive urine culture results.

Materials and methods
Prospectively studied 200 patients (100 male and 100 female) who presented to the Pediatric Clinic or Emergency Department of Babylon Maternity and Children Hospital and Al-Hillah educational hospital from October\2012 to February\2013. All patients were being hospitalized and outpatients.
Every patients underwent a history and physical examination, and a full evaluation for sepsis was performed, including peripheral WBC count and differential; ESR; CRP; blood culture (Blood samples were collected in screw capped tubes containing 20 ml Brain Heart Infusion broth without anticoagulant), Gram stain, and a UA and urine culture. Blood and urine specimens were cultured using standard media (Nutrient agar, MacConkey agar, Blood agar, Kleglar agar, Urea base agar, Peptone media, MR-VP broth and EMB agar) and using Vitic techniques to identification. Blood samples were cultured on aerobic and anaerobic media, also using Elisa technique from DRG (Germany) to determine the other non-cultivable bacteria like *Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Mycoplasma genetalium, and Chlamydia trachomatis*.
All urine specimens were obtained by clean containers with parent's assistance. Eligibility was limited to urine specimens of 3 mL obtained. All urinalyses were performed in a certified clinical laboratory. Specimens were analyzed with both standard UA and hemocytometer WBC counts simultaneously [18].
For the standard UA, specimens were centrifuged at 2000 rpm for 10 minutes and were examined microscopically for pyuria reported as the number of leukocytes per high-power field. For hemocytometer WBC counts, the uncentrifuged urine specimens were examined microscopically on a Neubauer chamber (Neubauer hemocytometer) by the same technician. C-reactive protein test done using latex kit from HUMATEX CRP (Germany) for qualitative and semi-quantitative determination [19]. In this study used 34 different antibiotics to detect the sensitivity of UTI causative agents, using disk diffusion agar using Muller Hinton agar.
Quantitative urine cultures were performed in the Microbiology Laboratory. A loop calibrated to deliver 0.01 mg was used to inoculate plates containing blood agar, nutrient agar, and MacConkey agar. All plates were incubated at 37°C and examined at 24 to 48 hours for colony count and bacterial identification. For standard UA, pyuria was defined as at least 5 WBCs/hfp. For hemocytometer WBC counts, pyuria was defined as at least 10 WBCs/mL, cultures with growth of mixed organisms or nonpathogenic Gram-positive cocci were considered contaminated [20].
The antibacterial susceptibility testing of the isolates was done using the Kirby-Bauer disk diffusion method following the definition of the Clinical and Laboratory Standards Institute (CLSI, 2006) using antibiotics containing discs from Oxoid. Briefly, 20 ml of Mueller- Hinton agar (Himedia medium, India) was prepared and poured into sterile plates. The agar medium was allowed to solidify at room temperature on a flat bench.
Then some few colonies of an 18 h culture of the isolates were streaked on the surfaces of the well-dried agar plates. Then some antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 h to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 35 - 37°C for 24 h. Zones of growth inhibition were then measured to the nearest millimetre and recorded. The mean of triplicate results was taken as the zone diameter.
Results and Discussion
This study investigated the percentage and antibiotic susceptibility patterns of bacterial pathogens isolated from patients with uncomplicated community-acquired UTI. The results showed that most cases of UTIs were caused by bacterial and viral pathogen.
In this study, *Escherichia coli* formed the major causative agent (49%) followed by Pseudomonas aeruginosa (25%), Klebsiella pneumonia (18%) and Proteus species (8%) (Table 1).
This is consistent with the findings of previous studies in which *E. coli, Pseudomonas aeruginosa Klebsiella pneumonia* and others were the predominant pathogen isolated from patients with UTIs [21]. Uropathogenic E. coli is the most common pathogen in cute UTI in previous studies [22].
This study shows the distribution and antibiotic susceptibility pattern of microbial species isolated from patients

with UTIs (Tables 3a, b). These organisms cause a variety of infections including UTIs [23]. Antibiotic resistance is a major clinical problem in treating infections caused by these microorganisms. The resistance to the antimicrobials has increased over the years. Resistance rates vary from country to country [24].

Overall, isolates from Latin American countries show the lowest susceptibility rates to all antimicrobial agents followed by Asian-Pacific isolates and European strains. Strains from Canada exhibit the best global susceptibility testing results. In our study, it accounted for approximately all causative agents were belong to Enterobacteriaceae.

This is consistent with the findings of previous studies in which E. coli was the predominant pathogen isolated from patients with community acquired UTIs [25]. However, Klebsiella pneumoniae are rarely encountered in cases of UTI [26]. In the present study 18% of Klebsiella isolates were found to be present among all uropathogens studied.

These isolates shows sensitivity with Amikacin, Norfloxacin, Piperacillin, and Tobramycin which is consistent with the previous data of other community- based studies [27].

Table 1: distribution of bacterial infections related to gender

Type of Bacterial infection	Male		Female	
	no.	%	no.	%
Ureaplasma urealyticum	16	2.4	22	3.3
Ureaplasma parvum	7	1.0	3	0.4
Mycoplasma hominis	8	1.2	19	2.8
Mycoplasma genetalium	19	2.8	4	0.6
Chlamydia trachomatis	17	2.5	17	2.5
Streptococcus pyogenes	28	4.2	16	2.4
Staphylococcus aureus	36	5.4	27	4.0
Staphylococcus epidermidis	24	3.6	40	6.0
Staphylococcus saprophyticus	3	0.4	49	7.3
Escherichia coli	72	10.8	88	13.2
Klebsiella pneumoniae	19	2.8	29	4.3
Pseudomonas aeruginosa	27	4.0	31	4.6
Providenciae	14	2.1	11	1.6
Serratiae marsescens	15	2.2	8	1.2
Total	305	45.6	364	54.4

E. coli isolates showed sensitivity with Amikacin, Chloromphenicol, Nitrofurantoin, Ofloxacin, Norfloxacin and Pseudomonas aeruginosa shows sensitivity with Norfloxacin, Ofloxacin, Piperacillin, Tobramycin, while Proteus species shows sensitivity with Amikacin, Amoxicillin , Cefodizime, Cefoxitin, Cephalexin, Gentamycin, , Norfloxacin, Piperacillin, Tobramycin, it is nearly compatable with Indian, Taiwan, Spain, and Senegal isolates [28, 29].

In this study *E. coli*, Pseudomonas aeruginosa, *Proteus* species and *Klebsiella* isolates are resistant against many antibiotics. Whereas, this drug exhibited low resistance rate in the major part of the world, despite of it's being used for many years [30]. This is probably due to the fact that this antibiotic has been widely used in treating community- acquired UTIs over the past decade in this region [31].

Other study revealed that 92 (65.7%) yielded bacterial growth with *Staphylococcus aureus*, *S. saprophyticus* and *Escherichia coli* having the highest incidence rate of 28.3%, 19.6% and 13.0%, respectively and that partially agreed with our results which was consist 25.69%. it revealed that *E. coli* (20.3%), *Streptococcus agalactiae* (13.4%), *U. urealyiticum* (11.8%), *Staphylococcus epidermidis* (9.7%) [32].

Leila, et al. 2007, publicized the frequency of *Ureaplasma urealyticum* (15%) was higher than that of *Mycoplasma hominis* (10.8%), *Ureaplasma parvum* (4.2%) and *Mycoplasma genitalium* (5%). Other study showed that infection was detected in (44.1%) of oligospermic subject and (74.7%) in azoospermic patients and that agreed with our results (40.3% and 53.3%) respectively. *Staphylococcus aureus* was indicates detected in one hundred and eighteen (68.2%) infected seminal fluids, *Escherichia coli* were detected in thirty one (17.9%)

[33].

Table 2: distribution of viral infections related to gender

Type of Viral infection	Male	Female	Male	Female
	%	no.	%	no.
	No.	%	No.	%
Rubella	5	3.3	11	7.3
Herpes simplex I	11	7.3	18	12.0
Herpes simplex II	22	14.7	39	26.0
Cytomegalovirus	6	4.0	1	0.7
Human papilloma virus	14	9.3	2	1.3
Adeno-associated virus	1	0.7	0	0.0
Epstein-Barr virus	5	3.3	15	10.0
Total	64	42.7	86	57.3

Table 2 revealed the percentage of viral infection among male and female suffering from urinary tract infection. It is showing that Herpes simplex II was the most common cause (22 male and 39 female) followed by Herpes simplex I as shown in table 2 above.

In Greece Viral infection was detected in 143/172 (83.1%) of the total samples for at least one herpes virus: HSV-1, 2.5%; VZV, 1.2%; EBV, 45%; CMV, 62.5%; HHV-6, 70%; HHV-7, 0% in the normal semen samples and HSV-1, 2.1%; VZV, 3.2%; EBV, 39.1%; CMV, 56.5%; HHV-6, 66.3%; HHV-7, 0% in the abnormal semen samples [34].

The frequency of HSV, which was observed only in the 2.3% of the normozoospermic males, is at variance with the findings of Kapranos et al. but close to those demonstrated by Bezold, with the difference that they were detected in infertile males [35].

The detection rate of HSV-1/2 DNA in ejaculates from infertile patients in the current study was relatively high (3.7%). However, HSV was associated with the strongest effect of any of the pathogens on semen parameters. HSV infections had significantly reduced sperm concentration, sperm motility, motile sperm concentration, total motile sperm count, neutral α-glucosidase and citrate concentrations. Thus, HSV-infection of the male genital tract could explain some cases of male infertility, due to its association with decreased semen quality and that agreed with our results [36].

CMV is a member of the herpesvirus family and can cause a variety of teratogenic effects in newborns as well as a clinical illness in adults resembling infectious mononucleosis (Drew, et.al 1999). Its presence and persistence in semen has been reported previously. In the current study, CMV was detected in semen of infertility patients (20.42%). Detection of a low level of CMV in semen could reflect a recent infection in this individual. We did not observe a significant association of CMV DNA with semen parameters in this study, although other study revealed that there was a trend for lower motile sperm and **α**-glucosidase concentrations in the CMV-infected group, indicating that CMV infection could have a modest effect on semen quality, perhaps by affecting epididymal function. Previous studies have not shown an association of CMV infection with a reduction in semen parameters and that can explain the low ratio of CMV infection [37].

Human papillomaviruses represent a group of small DNA viruses that induce epithelial cell proliferation. More than 35 types of HPV infect the genital tract. DNA from HPV has been associated with invasive squamous cell cancers of the genital tract and anus [38]. HPV is primarily transmitted through direct epithelial contact, but high risk HPV types have been detected in both semen and spermatozoa, as well as in the vas deferens [39]. HPV was associated with a significant decrease in total sperm count, and a statistically non-significant trend for lower total motile sperm count and neutral α-glucosidase concentrations. A previous study reported an association between HPV and reduced sperm, EBV, a member of the herpesvirus family, causes infectious mononucleosis and has been associated with Burkitt's lymphoma. EBV is found in semen and is thought to be sexually transmitted [40].

Table 3a: Antimicrobial potency and spectrum of 13 selected antimicrobial agents tested against most frequently occurring UTI pathogens.

Microorganism	Antimicrobial agent / % sensitive strains												
	AK	AX	AMC	AZM	B	PY	CDZ	FOX	ZOX	CL	C	CLR	DA
Escherichia coli	80	33	25	26	-	-	-	-	10	20	90	-	-
Pseudomonas aeruginosa	65	17	36	-	19	-	10	15	15	55	15	10	-
Klebsiella pneumoniae	87	8	14	34	-	10	-	-	10	5	-	10	-
Proteus species	93	71	98	16	20	55	80	95	35	75	25	55	25
Streptococcus pyogenes	80	80	-	5	-	10	95	-	10	15	80	75	5
Staphylococcus aureus	90	-	10	1	5	10	50	-	-	-	-	10	-
Staphylococcus epidermidis	-	10	45	30	-	-	50	25	80	50	45	70	-
Staphylococcus saprophyticus	70	64	18	-	34	28	64	18	15	29	67	90	-
Providencia stuartii	76	67	76	48	15	-	19	-	42	19	65	10	38
Serratia marcescens	80	80	84	27	15	45	62	86	20	76	20	46	16

AK \Amikacin, AX \Amoxicillin, AMC \Amoxicillin + Clavulanic acid, AZM \Azithromycin, , B \Bacitracin, PY \Carbenicillin, CDZ \Cefodizime, FOX \Cefoxitin, ZOX \Ceftizoxime, CL \Cephalexin, C \Chloromphenicol, CLR \Clarithromycin, DA \Clindamycin

Table 3b: Antimicrobial potency and spectrum of 13 selected antimicrobial agents tested against most frequently occurring UTI pathogens.

Microorganism	Antimicrobial agent / % sensitive strains												
	E	CN	K	L	ME	F	NOR	OFX	OX	T	P	PRL	RA
Escherichia coli	88	20	20	-	5	-	-	-	10	20	90	-	-
Pseudomonas aeruginosa	55	10	20	10	10	-	10	15	15	55	15	10	-
Klebsiella pneumoniae	80	-	-	25	-	10	-	-	10	5	-	10	-
Proteus species	100	60	90	10	20	55	80	95	35	75	25	55	25
Streptococcus pyogenes	30	80	80	95	20	90	90	95	85	95	25	5	75
Staphylococcus aureus	20	5	10	10	-	70	95	-	15	50	5	-	-
Staphylococcus epidermidis	-	10	-	25	5	-	-	70	-	10	-	5	45
Staphylococcus saprophyticus	65	88	-	5	15	10	95	-	16	15	80	81	12
Providencia stuartii	75	14	18	10	16	35	19	10	5	-	-	28	24
Serratia marcescens	90	57	80	17	35	40	64	67	54	68	30	19	5

E \Erythromycin, CN\Gentamycin, K\Kanamycin, L\Lincomycin, ME\Methicillin, F\Nitrofurantoin, NOR\Norfloxacin, OFX \Ofloxacin, OX\Oxacillin, T\Oxytetracyclin, P\Penicillin G, PRL \Piperacillin, RA\Rifampim.

These results were done according to performance standards for antimicrobial disk susceptibility tests, CLSI (formerly NCCLS). The antibiotic which not including in CLSI chart, FDA approved performance standards for antimicrobial discs obtained from drug manufactures [41].

Table 4: The Results of Diagnostic Test in Presence of a Positive Urine Culture.

Diagnostic Test	Male		Female	
	No.	%	No.	%
Standard UA > 5 WBCs/hpf	62	62	84	84
Hemocytometer WBC Counts >10 WBCs/mL	84	84	75	75
CRP > 24 mg/L	86	86	64	64
ESR > 35 mm/h	67	67	70	70
Peripheral WBC > 15 000/mL	69	69	55	55

CRP >20 mg/L, ESR >30 mm/hour, and WBC >15 000/mL are key findings in various studies on febrile infants [25]. The diagnostic value of these parameters for predicting serious bacterial infection in febrile infants, CRP is an acute phase reactant which opsonizes invading pathogens. Levels of CRP increase within 6 hours of an inflammatory stimulus, and may rise up to 1000-fold, measurement of CRP provide direct index of acute inflammation, in contrast to CRP, the ESR is an indirect measure of the acute phase response [42].

Our investigation showed that from the 200 patients with UTI, 62 male and 84 had pyuria > 5 WBCs/hpf, 84 male and 75 female had pyuria >10 WBCs/mL, 86 male and 64 female had CRP >24 mg/L, 67 male and 70 female had ESR >35 mm/hour, and 69 male and 55 female had WBC >15 000/ml. Our results corroborate the findings of Crain and Dar-Shong [43, 44] whose sample was similar to ours.

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