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ANTIMICROBIAL RESISTANCE AND/OR SUSCEPTIBILITY OF BACTERIA IMPLICATED IN URINARY TRACT INFECTION

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ABSTRACT

A total of 272 urine samples were collected from patients examined for urinary tract infections in Mansoura University hospitals or attending Mansoura University outpatients clinics. A positive bacterial growth on CLED, MacConkey and blood agars was detected in 36.77 % of the cases only. *E. coli* was the most predominant pathogen causing urinary tract infection in the examined positive samples (38.09%) followed by *Klebsiella pneumonia* , *Klebsiella oxytoca* , *Pseudomonas areuginosa* , *Staphylococcus aureus, Proteus mirabilis* ,*Enterococcus spp., Proteus vulgaris and Citrobacter freundii* were present in the following frequencies 21.42, 9.52, 9.52, 7.14, 5.95, 4.76, 2.38, and 1.19 respectively. Antibiotic sensitivity tests for Gram negative bacteria and Gram positive bacteria showed that most of the Gram-negative bacteria were highly susceptible to meronem , nitofurantion, norfloxacin and Amikacin. While the Gram-positive bacteria were highly susceptible to meronem and nitofurantion. The effects of volatile oils against highly resistant bacteria isolates showed that marjoram and dianthus were inhibitory to these isolates. Interleukin-8 was taken as a measure of UTI severity. The mean IL-8 levels in urine samples containing *Proteus mirabilis, Proteus vulgaris, Klebsiella oxytoca, Klebsiella pneumoniae, E. coli* and *Pseudomonas areuginosa* were 56.21±5.85 pg/ml, 102.95±47.97 pg/ml, 139.24±40.79 pg/ml, 259.58±61.08 pg/ml, 754.47±279.71 pg/ml and 1200.65±268.36 pg/ml respectively. While, the mean IL-8 levels in urine samples infected with *Staphylococcus aureus* and *Enterococcus spp.* were 100.09±27.95 pg/ml and 110.38±8.83 pg/ml respectively. A statistically significant differences ($P \leq 0.0001$) in IL-8 levels among UTIs caused by Gram positive cocci, Gram negative bacilli, in either males and females were observed.

INTRODUCTION

Urinary tract infection (UTI) is one of the most important causes of morbidity in the general population, and is the second most common cause of hospital visits (Ronald and Pattulo, 1991). They are also among the most common bacterial infections in humans, both in the community and hospital settings (Hooton and Stamm, 1997), and have been reported in all age groups of both sexes. It is characterized by the evidence of uropathogens and pyuria and is accompanied by various clinical manifestations depending on the area of involvement. Although the detection of pyuria in patients with suspected UTI is readily available via laboratory test, but sometimes it is a non-specific indicator of UTI (Johnson and Stamm, 1999) and little is known about the mechanism of neutrophil recruitment into the urine (Ko *et al.*, 2000). Nearly all UTIs are caused by bacteria that enter the urethral opening and

move upwards to the urinary bladders and sometimes to kidneys (Lerner, 1994).

UTIs occur in all populations and ages, however, infection is most common in women, especially sexually active women. About 20% of women experience a single episode of UTI during their lifetime, and 3% of women have more than one episode of UTI per year (Gebre-selassie, 1998). Recurrent infections are common and can lead to irreversible damage of the kidneys, resulting in renal hypertension and renal failure in severe cases (New, 1992).

The choice of antibiotics should depend upon the causative organism and their susceptibility pattern to various antibiotics (Goldstein , 2000). Proper management and prevention of bacteriuria can reduce the incidence of the life-threatening consequences of urinary tract infections. Together , physicians and laboratories must have guidelines and strategies that provide high quality treatment for the patient, while minimizing costs and preventing further emergence of antimicrobial drug resistance (Lo and Smego, 2004 ; Higuchi *et al.*, 2006).

Bacterial pathogens stimulate epithelial cells of interstitial tissue and macrophages to secrete proinflammatory cytokines *viz:* interleukin 1 (IL-1 beta), interleukin 6 (IL-6) and interleukin 8 (IL-8) (Hack *et al.*, 1992; Hedges *et al.*, 1992; Agace *et al.*, 1993a&b). It has been demonstrated before that IL-8 levels were elevated in patient with PN and that the level of this chemokines may correlate with the virulence associated traits of the microorganisms (Jacobson *et al.*, 1994). Amongst the many diseases in which IL-8 plays an important role as mediators of inflammation (Baggiolini *et al.*, 2001). UTI were no exception (Agace et al ., 1993 and Ko *et al.*, 2000). The role of chemokines in the development and progression of inflammatory renal diseases, particularly pyelonephritis (PN) has not been thoroughly investigated.

This study investigated the resistance and susceptibility of bacteria implicated in urinary tract infection to some available antimicrobial drugs and evaluated the levels reliability IL-8 as a good marker for the severity of urinary tract infection .

MATERIALS AND METHODS

Samples Collection

During the period between April 2007 to April 2008, a total of 272 urine samples were collected from UTIs patients at Mansoura University hospitals or outpatients clinics. Urine samples were collected in sterile wide mouth containers under complete aseptic condition and transferred immediately to the various laboratory tests or kept at 4° C for further examinations (Warren, 1996 ; NCCLs , 2001).

Isolation and Identification of Bacterial Isolates

Each sample was immediately tested for bacterial density on peptone water. They were inoculated and plated onto CLED agar, blood agar and MacConkey agar plates and incubated for 48 hours at 37 C. Positive culture

was defined if bacterial colony counts was ≥ 10^5 colony forming units/ml. All bacteria were identified to species level according to standared procedures (Bergy's Manual, 1994) ; (Mahon and Manuselis 1995 ; Collee *et al.*, 1996 and Zinsser, 1998).

Antibiotic susceptibility tests

All bacterial strains isolated were tested for antimicrobial sensitivity by standardized disk diffusion technique as adapted and described by Bauer *et al.*, (1986).

Measurements of interleukin-8 (IL-8) level in urine samples:

The urinary IL-8 levels in urine samples from patients with UTI was determined by the Biosource IL-8 EASIA. of a solid phase Enzyme Amplified Sensitivity Immunoassay (EASIA) based on an oligoclonal system in which a blend of monoclonal antibodies (MAbs) directed against distinct epitopes of IL-8 are used.

RESULTS

Of the 272 urine samples cultured, only 100 (36.77%) were positive for UTI. Ages of positive cases ranged from 5 months to 83 years, they including 53 males and 47 females. Gram negative bacteria were the most common cause of UTIs which present in 88.07 % of patients while Gram positive bacteria were present in 11.9 % of patients. The frequency of bacterial pathogens isolated from positive cultures (table.1) indicated that *E. coli* was the most predominant pathogen causing urinary tract infection in human (38.09%) followed by *Klebsiella pneumonia* , *Klebsiella oxytoca* , *Pseudomonas areuginosa* , *Staphylococcus aureus, Proteus mirabilis* ,*Enterococcus spp., Proteus vulgaris and Citrobacter freundii* were present in low frequencies 21.42, 9.52, 9.52, 7.14, 5.95, 4.76, 2.38, and 1.19 respectively. Table (2) shows bacterial species isolated different ages and genders.

Table 1: The frequency of bacterial pathogens isolated from positive cultures.

Bacterial isolates	Number	%
1-E. coli .	32	38.09
2-Klebsiella pneumoniae .	18	21.42
3-Klebsiella oxytoca .	8	9.52
4-Citrobacter freundii .	1	1.19
5- Pseudomonas areuginosa.	8	9.52
6-Proteus vulgaris .	2	2.38
7-Proteus mirabilis .	5	5.95
8-Stphylococcus aureus .	6	7.14
9-Enterococcus spp .	4	4.76
Total	84	100

Most of the Gram-negative bacteria were highly susceptible to meronem , nitofurantion, norfloxacin and Amikacin antibiotics. While the

Gram-positive bacteria were highly susceptible to meronem and nitofurantion (Table 3). *E. coli*, was susceptible to meronem (78.1%) , nitofurantion (81.2%), norfloxacin (59.3%) and Amikacin (78.1%) but Klebsiella pneumoniae was highly susceptible to meronem (83.3%) and norfloxacin (77.7%) and *Staphylococcus aureus* was susceptible to meronem (83.3%) and nitofurantion (100%).

Table 2: Bacterial species isolated from cases of different ages and different genders.

Patient age	No of cases	Sex		Bacterial Isolates
		M	F	
0 – 10	9	6	3	*E. coli , Klebsiella pneumoniae , Klebsiella oxytoca , pseudomonas areuginosa , proteus mirabilis and Enterococcus spp.*
10-20	5	4	1	*E. coli , Klebsiella pneumoniae , Klebsiella oxytoca and proteus vulgaris.*
20-30	4	3	1	*E. coli.*
30-40	9	3	6	*E. coli , Klebsiella pneumoniae , Klebsiella oxytoca , staphylococcus aureus and Enterococcus spp.*
40-50	20	8	12	*E. coli , Klebsiella pneumoniae , Klebsiella oxytoca , pseudomonas areuginosa , proteus mirabilis and Enterococcus spp.*
50-60	18	6	12	*E. coli , Klebsiella pneumoniae , Klebsiella oxytoca , pseudomonas areuginosa , proteus vulgaris , staphylococcus aureus and Enterococcus spp.*
60-70	9	8	1	*E. coli , Klebsiella pneumonia e, Klebsiella oxytoca , pseudomonas areuginosa , proteus mirabilis and Citrobacter freundii.*
70-80	5	3	2	*E. coli , Klebsiella pneumoniae and staphylococcus aureus.*
80-90	2	1	1	*Klebsiella pneumoniae and proteus mirabilis.*

Table 3: Susceptibility of isolated Gram-negative and Gram-positive bacteria

Bacterial isolates	Total no.	Susceptibility to antimicrobial drugs (%)									
		CN	CTX	SXT	MEM	F	NA	NOR	AK	CEC	AMC
Gram-negative bacteria.											
Escherichia coli	32	62.5	25	25	78.1	81.2	40.6	59.3	78.1	21.8	21.812
Klebsiella oxytoca	8	50	25	—	75	62.5	37.5	50	75	12.5	.5
Klebsiella pneumoniae	18	55.5	22.2	22.2	83.3	55.5	44.4	77.7	61.1	11.1	22.2
Pseudomonas aeuginosa	8	50	—	12.5	62.5	25	12.5	50	75	—	37.5
Proteus vulgaris	2	50	100	—	50	50	50	50	50	50	—
Proteus mirabilis	5	60	40	40	80	40	—	100	60	60	40
Citrobacter freundii	1	100	—	—	—	100	100	100	—	—	—
		CN	SXT	SAM	MEM	F	CL	NOR	CIP	CEC	AMC
Gram-positive bacteria.											
Staphylococcus aueus.	6	66.6	16.6	50	83.3	100	33.3	50	50	16.6	66.6
Enterococcus spp.	4	50	—	50	75	75	25	75	50	—	—

AK = Amikacin (30 μg); AMC = Augmentin(20 μg); CEC = Cefaclor(30 μg); CTX = Cefotaxime(30 μg); CL = Cephalexin(30 μg); CIP = Ciprofloxacin(5 μg); CN = Gentamicin(10 μg); MEM = Meronem(10 μg); NA = Nalidixic acid (30 μg); F = Nitrofurantion(300 μg); NOR = Norfloxacin (10 μg); SXT = Trimethoprim + sulphamethoxazole(1.25 μg); SAM = Unasyn(10 μg).

Volatile oils activity against highly resistant isolated bacteria shown in (Table 4) indicated that the marjoram inhibitory effect with *Enterococcus spp. (Es.1), Klebsiella oxytoca (Ko.3) and Pseudomonas areuginosa (Pa.2)* with a recorded inhibition zone (15mm, 12mm and 10mm respectively) and the other isolates gave lower inhibition zone. Dianthus showed inhibitory action against *Klebsiella pneumoniae (Kp.3), Pseudomonas areuginosa (Pa.2), Enterococcus spp. (Es.1), Klebsiella oxytoca (Ko.3)* and *Klebsiella pneumoniae (Kp.8)* and the highest inhibition zones were 13 mm which recorded with *Klebsiella pneumoniae (Kp.3)*. Mean while, thyme, carawaya and eucalyptus show no inhibitory effect aganist all tested bacterial isolates.

Table 4. Diameter of inhibition zone(mm) of Bacterial isolates to various volatile oils.

Diameter of inhibition zone(mm) / Bacterial isolates	Eucalyptus	Cummin	Marjoram	Carawaya	Dianthus	Anise	Thyme
E. coli (Ec.9)	0	10	8	0	9	0	0
Klebsiella pneumoniae (Kp.3)	0	11	9	0	13	0	0
Klebsiella pneumoniae(Kp.8)	0	8	9	0	10	0	0
Klebsiella oxytoca (Ko.3)	0	11	12	0	10	0	0
Pseudomonas areuginosa (Pa.2)	0	9	10	0	12	0	0
Pseudomonas areuginosa (Pa.3)	0	0	0	0	0	0	0
Pseudomonas areuginosa (Pa.5)	0	0	0	0	8	0	0
Enterococcus spp. (Es.1)	0	11	15	0	12	10	0

IL-8 levels in urine of patients with UTI due to Gram negative bacteria.

The results presented in figure (1) showed that the mean IL-8 levels in urine samples infected with *Proteus mirabilis, Proteus vulgaris, Klebsiella oxytoca, Klebsiella pneumoniae, E. coli* and *Pseudomonas areuginosa* were 56.21, 102.95, 139.24, 259.58, 754.47 and 1200.65 pg/ml respectively.

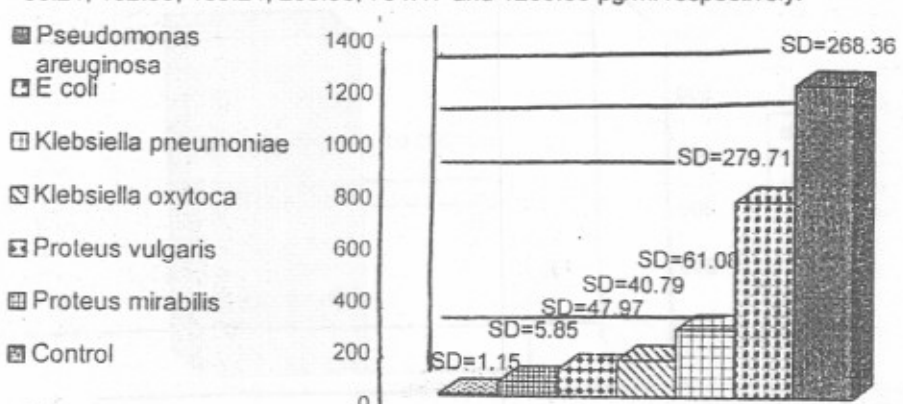

Figure 1. IL-8 levels in urine of patients with UTI due to Gram negative bacteria (pg/ml).

While the mean IL-8 levels were 5.81 pg/ml in control urine samples. A significant differences ($P<0.05$) between the mean values of IL-8 in urine samples infected with Gram negative bacteria and control urine samples is evident.

IL-8 levels in urine of patients with UTI due to Gram positive bacteria.

The results depicted in figure (2) revealed that there were significant differences between the mean values of IL-8 in urine samples infected with Gram positive bacteria ($P<0.05$) as compared to control urine samples, where the mean IL-8 levels in urine samples infected with *Staphylococcus aureus* and *Enterococcus spp.* were 100.09 pg/ml and 110.38 pg/ml respectively but in control was 5.81 pg/ml.

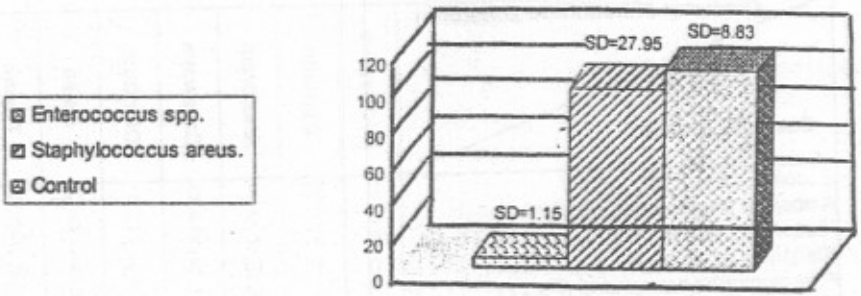

Figure 2. IL-8 levels in urine of patients with UTI due to Gram positive bacteria (pg/ml).

IL-8 levels in urine of patients with UTI with different gender.

The results depicted in figure (3) revealed that the mean IL-8 levels in males and females were 285.59 pg/ml and 580.32 pg/ml. Meanwhile, there was a significant difference ($P<0.05$) between mean IL-8 level in males and females.

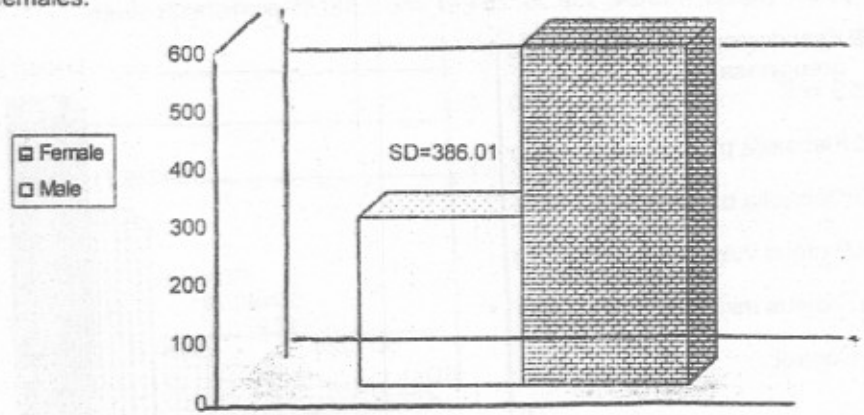

Figure 3. IL-8 levels in urine of patients with UTI with different gender (pg/ml).

IL-8 levels in urine of patients with UTI with different age.

The results presented in figure (4) showed that the mean IL-8 levels in the different age group as 1(0-20y), (>80y), (20-40y), (60-80y) and (40-60y) were 137.55, 142.77, 368.85, 580.62 and 589.11 respectively. So there were significant differences (P<0.05) between the mean values of urinary IL-8 in the different age groups.

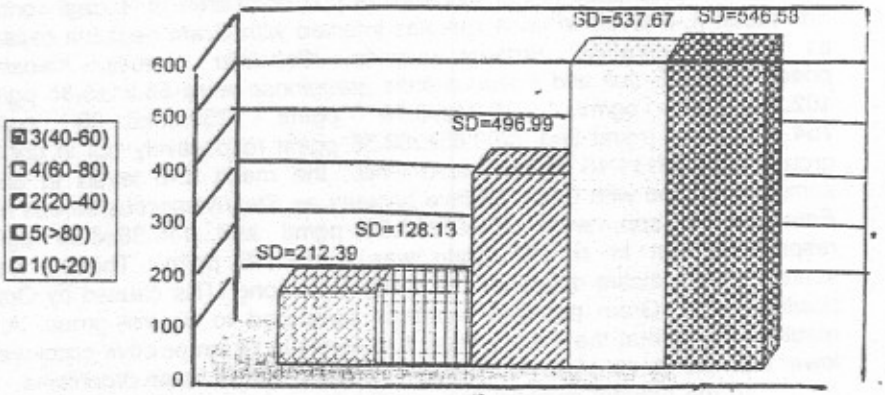

Figure 4. IL-8 levels in urine of patients with UTI with different age (pg/ml).

DISCUSSION

This study revealed that Gram negative bacteria were the most common cause of UTIs which present in 88.07 % of patients while Gram positive bacteria were present in 11.9 % of patients. *E. coli* was the most predominant pathogen causing urinary tract infection in human (38.09%). The other pathogens namely: *Klebsiella pneumonia* , *Klebsiella oxytoca* , *Pseudomonas areuginosa* , *Staphylococcus aureus, Proteus mirabilis* ,*Enterococcus spp., Proteus vulgaris and Citrobacter freundii* were present in low frequencies 21.42, 9.52, 9.52, 7.14, 5.95, 4.76, 2.38, and 1.19 respectively. This study was consistent with other studies which showed that *E. coli* was the most frequent cause of UTIs at all ages (Garcia and Nager, 2002; Maherzi *et al.*, 1978; Abbott, 1972; Tamim *et al.*, 2003; Bergstrom *et al.*, 1972; Ginsberg and McCracken, 1982). Yuksel *et al.*, (2006) reported that the most causative agents was *E. coli* (87%of cases) followed by *Klebsiella pneumonia* (10%) and this agree with this study.

The results of the present study showed that the susceptibility rate of urinary isolates was highest for meronem (76.19 %), followed by amikacin (70.27 %), nitrofurantion (66.60 %), norfloxacin (64.28) and gentamicin (58.33 %). Meanwhile, the resistant rate of urinary isolates was highest for cefaclor (71.42 %) and trimethoprim-sulphamethoxazole (70.23 %). Adeyemo

and colleagues (1994) reported that all urinary isolates were poorly susceptible to trimethoprim-sulphamethoxazole and ampicillin, but exhibited good susceptibility to nalidixic acid, nitrofurantion, and ofloxacin. Yuksel *et al.*, (2006) showed that nitrofurantion was the most active agent against *E. coli* (2.2% resistant isolates), followed by amikacin (4.9 %), ceftriaxone (7.5 %) and ciprofloxacin (12%) and this agree with this study.

The results of the present study revealed that the mean urinary IL-8 levels was remarkably higher in cases of UTI compared to normal control. The mean IL-8 levels in urine samples infected with Gram negative bacteria as *Proteus mirabilis, Proteus vulgaris, Klebsiella oxytoca, Klebsiella pneumoniae, E. coli* and *Pseudomonas areuginosa* were 56.21±5.85 pg/ml, 102.95±47.97 pg/ml, 139.24±40.79 pg/ml, 259.58±61.08 pg/ml, 754.47±279.71 pg/ml and 1200.65±268.36 pg/ml respectively but in control group was 5.81±1.15 pg/ml. Mean while, the mean IL-8 levels in urine samples infected with Gram positive bacteria as *Staphylococcus aureus* and *Enterococcus spp.* were 100.09±27.95 pg/ml and 110.38±8.83 pg/ml respectively but in control group was 5.81±1.15 pg/ml. There were statistically significant differences ($P \leq 0.0001$) among UTIs caused by Gram positive cocci, Gram negative bacilli as compared to control group. Also results showed that the mean urinary IL-8 levels in Gram positive cocci were lower than the mean urinary IL-8 levels in Gram negative bacilli organisms.

In the present investigation results showed that the mean IL-8 levels of males and females were 285.59±386.01 and 580.32±570.34 respectively there was a significant difference ($P \leq 0.0001$) between mean IL-8 levels in males and females. The mean IL-8 levels in the different age group as 1 (0-20y), 5 (>80y), 2 (20-40y), 4 (60-80y) and 3 (40-60y) were 137.55±212.39, 142.77±128.13, 368.85±496.99, 580.62±537.67 and 589.11±546.58 respectively. Mean while there were significant differences ($P \leq 0.0001$) between the mean values of urinary IL-8 in the different age groups.

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دراسات على حساسية ومقاومة البكتريا المتورطة فى عدوى القناة البولية للمضادات الميكروبية

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أجريت هذه الدراسة على٢٧٢ عينة بول تم جمعها من المرضى المقيمين بمستشفيات جامعة المنصورة والمترددين على العيادات الخارجية بجامعة المنصورة.ووجد أن ٣٦,٧٧ ٪ فقط من عينات البول التى تم جمعها أعطوا نمو بكتيرى ايجابي على وسط مغذى من الكليدأجار. كما اثبتت نتائج هذه الدراسة أن إشيريشياكولاى "*Escherichia coli*" هى أكثر أنواع البكتيريا التى تصيب الجهاز البولى حيث أنها تمثل ٣٨,٠٩% من اجمالى العينات التى اعطت نمو بكتيري ايجابي . اما بقية البكتريا وهى كليبسيلانومونيا *Klebsiella pneumonia*، كليبسيلاوكسى توكا *Klebsiella oxytoca* ، سودوموناس إيروجينوزا *Pseudomonas areuginosa* ، ستافيلوكوكاس أوريوس*Staphylococcus aureus* ، بروتيوس ميرابيلس *Proteus mirabilis*، إنتيروكوكاس سبيشز *Enterococcus spp*، بروتيوس فالجارز *Proteus vulgaris*، ستروباكتر فروندای *Citrobacter freundii* تمثل المعدلات الآتية على التوالى (٢١,٤٢، ٩,٥٢، ٩,٥٢، ٧,١٤ ، ٥,٩٥ ، ٤,٧٦، ٢,٣٨، ١,١٤%). وتم عمل اختبار حساسية لجميع المعزولات البكتيرية فأوضحت هذه الدراسة أن البكتريا العصوية السالبة تبدى حساسية عالية تجاه الميرونام ،النيتروفيورانتوين ،النورفلوجساسين وأميكاسين ولكن البكتريا الكروية الموجبة تبدى حساسية عالية تجاه الميرونام والنيتروفيورانتوين. وبعمل اختبار حساسية لبعض البكتريا التى اعطت مقاومة عالية تجاة المضادات الحيوية المستخدمة باستخدام بعض الزيوت العطرية فأثبتت نتائج الدراسة ان هذه البكتريا اعطت حساسية تجاه القرنفل والبردقوش. وبدراسة تأثير العامل المناعي انترليوكين- ٨ (IL-8) فى عينات البول التى أعطت نمو بكتيرى إيجابى ، أظهرت الدراسة أن المعزولات البكتيرية العصوية السالبة وهى بروتيوس فالجارز ، بروتيوس ميرابيلس ، كليبسيلاوكسى توكاو ، كليبسيلانومونيا ، إشيريشياكولاى ، سودوموناس إيروجينوزا كان متوسط تركيز الإنترليوكين هو ٥٦,٢١ ، ١٠٢,٩٥ ، ١٣٩,٢٤ ، ٢٥٩,٥٨ ، ٧٥٤,٤٧ ، ١٢٠٠,٦٥ بيكوجرام لكل ملى على التوالى ، ولكن متوسط تركيز الإنترليوكين فى حالة المعزولات البكتيرية الكروية الموجبة وهى ستافيلوكوكاس أوريوس ، إنتيروكوكاس سبيشز هو ١٠٠,٠٩ ، ١١٠,٣٨ بيكوجرام لكل ملى على التوالى ومن خلال هذه الدراسة نجد ان هناك فروق معنوية ($P \leq 0.00001$) بين عدوى الجهاز البولى" Urinary tract infection" الناتجة عن المعزولات البكتيرية العصوية السالبة و المعزولات البكتيرية الكروية الموجبة عند مقارنتها بعينات الكونترول ، ولذلك يمكن اعتبار تركيز الإنترليوكين دليل جيد لتحديد مدى الإصابة بعدوى الجهاز البولى للإنسان.