Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued four times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Khaled M. Khleifat
Department of Biology,
College of Science,
Mutah University,
Mutah, Jordan

Tel: + 00962 3237 2380/3222

J. Med. Sci., 6 (1): 5-11
January-March, 2006

Urinary Tract Infection in South Jordanian Population

[1]Khaled M. Khleifat, [1]Muayad M. Abboud,
[2]Sharaf S. Omar and [1]Jafar H. Al-Kurishy

Urinary tract infections were investigated in order to screen for the linked causative bacterial agent. One hundred seventy patients with clinical symptoms of urinary tract infections were examined. It was found that 119 of the patients had actual laboratory evidence for urinary tract infections. One hundred and nine of them were discovered by the routine work and the remaining 10 patients were detected by the filtration of 50 mL of urine through a filter paper and culturing the filter paper due to the low counts of bacteria in their samples. The result of different examination test suggest that the *Escherichia coli* is responsible for the large proportion of infection (53.24%), followed by other strains like *Enterococcus faecalis* (24.05%), *Proteus* sp. (19.537%), *Staphylococcus aureus* (19.206%), *Staphylococcus epidermidis* (7.8%), *Staphylococcus saprophyticus* (13.2%), *Klebsiella* sp. (11.96%), *Enterobacter* sp. (5.128%), *Pseudomonas aeruginosa* (3.4%), *Citrobacter* sp. 1.92% and *Serratia marcescens* (0.8%). The most spreaded bacteria in female was *Escherichia coli*, while the most spreaded bacteria in male was *Proteus* sp. The proportion of urinary tract infections in female was 81%, while the proportion in male was 19%. The proportion of infection in married male was 10%, while in single male was 9%, compared with a proportion of 55% in married female and 26% in single female. For the treatment of urinary tract infection the antibiotic Ciprofloxacin (5 μg disk^{-1}) was found to be most effective antimicrobial agents against all isolated bacteria strains, while Oxacillin (1 μg disk^{-1}) was found to be the least effective.

Key words: Urinary tract infection, serotype, Jordan

[1]Department of Biology, College of Science,
Mutah University, Mutah, Jordan
[2]Department of Nutrition and Food Sciences,
College of Agriculture, Mutah University, Mutah, Jordan

ANSInet
Asian Network for Scientific Information

INTRODUCTION

The urinary system consists of the kidneys, ureters, bladder and urethra. The function of the kidney is to remove liquid waste from the blood in the form of urine, as well as keeping a stable balance of salts and other substances in the blood. Additionally it produces hormone that aids the formation of blood cells. Ureters are narrow tubes that carry urine from the kidney to the bladder. The later is a triangle-shaped chamber in lower abdomen. Urine is stored in the bladder and emptied through the urethra[1].

The amount of urine varies, depending on the fluids and the foods, being consumed by a person. It's volume at night being produced is about half that formed in the daytime. All area of the upper urinary tract system are sterile through the urethra hosts except resident microflora that colonizes its transitional epithelium. Normal urine is sterile (microorganisms-free). It contains fluids, salts and waste products but no microorganisms[2].

Urinary Tract Infection (UTI) can be caused by different microorganisms. The urethra and urinary bladder are most frequently the sites of infection in urinary tract, and the resulting infection referred to as urethritis and cystitis. The most common etiological agents of this infection are Gram-negative bacteria, particularly those which are normally occurring in the gastrointestinal tract. This infection is attributed to an accidental contamination of the urinary tract with the fecal matter which appears to be the most important means of this infection transmission[1].

The most common bacteria which are responsible for UTI is *Escherichia coli*. Many other UTI-causing genera are also isolated from patients with variable degree of infection such as *Klebsiella, Enterobacter, Proteus, Serratia* and *Pseudomonas aeruginosa*[1]. The latter one mostly occurred after the catheterization procedures because the catheter may carry microorganisms from the extremities of the genitourinary tract causing a contamination of the inner tissues and resulting in urethritis and cystitis[1].

Urinary tract infection is the condition where bacteria or infection occurred any where in the urinary system[3]. Infections occurred when there is blockage to the normal flow of urine or if bacteria are introduced from outside the urinary tract. An infection occurs when microorganisms cling to the opening of urethra and begin to multiply[3]. From there, some microorganisms often move on to the bladder, and if the infection is not treated properly, microorganisms may then travel to the kidneys. Bacteria may get into the urinary tract or the urine from the skin around the rectum and genitals[4]. The most common organism causing the urinary tract infection UTIs is *E. coli* which posses special appendages (fimbriae) that enable the bacterium to adhere to the urinary tract epithelium cells. Recent molecular assay have identified 29 virulence factor genes of *E. coli*[5]. On the other hand, other pathohgen adhere to the uroepithelial cells through different mechanisms including production of urease enzyme which catalyzes the hydrolysis of urea in the urine causing the formation of bladder or kidney stones.

Urinary Tract Infection (UTIs) are common clinical problem. Various methods were applied in an attempt to isolate causative microorganisms. The pathogens traditionally associated with UTIs are changing many of their features, particularly antimicrobial resistancy. The etiology of UTIs is also affected by the host factors such as age, diabetes, spinal cord injury, catheterization and sex[6,7]. Consequently, complicated UTIs has a more diverse etiology than uncomplicated UTIs and organisms that rarely cause disease in healthy patients can cause significant disease in hosts with anatomic, metabolic and immunologic underlying disturbance such as age, diabetes, spinal cord injury, catheterization and sex[6,7]. Consequently, complicated UTIs has a more diverse etiology than uncomplicated UTIs and organisms that rarely cause disease in healthy patients can cause significant disease in hosts with anatomic, metabolic and immunologic underlying disturbance.

The majority of acute community acquired uncomplicated infections are caused by some members of the family *Enteriobactericeae*, *Enterococci, Staphylococci*[8] as well as fungal pathogens[9]. Colonization of the pathogenic microorganisms is determined by specific bacterial adhesive characteristic, the receptor repertoire on the epithelial surface and the surrounding fluids. Genotypic traits for epithelial cells receptivity have been identified as an important susceptibility factor in UTIs[3].

Many studies have shown that the high incidence of bacterial UTIs occurred in children, with 71% of these infections caused by *Escherichia coli* in both sex. *Proteus* sp. is identified as the causative agent in 24.8% of the total isolation in boys against 2.8% in girls. Generally UTI infections were diagnosed more in girls (35.3%) than in boys (18.3%)[10].

On the other hand other studies reported that the using of catheter resulted in the increase of UTI infections. In this case *E. coli* showed 79% of the total UTI infections. Also, increase in the ratio of infection with opportunistic bacteria such as coagulase negative Staphylococci (*epidermidis* and *saprophyticus)*, as well as enterococcal and *Klebsiella* sp. were detected[11].

MATERIALS AND METHODS

Isolation and identification of UTI isolates: One hundred seventy urine samples from UTI patients were collected from medical center of Mutah University at Jordan. For the isolation of UTI-causing strains, loop full of urine sample was streaked on to nutrient agar plate and incubated at 37°C for 24 h. Next day individual colonies were selected and identified on the bases of morphological, cultural and biochemical characteristics.

Identification of gram negative bacteria: Morphological characteristics, gram-staining, capsule staining (Anthony's method) and motility test were conducted. To study the growth pattern, different growth media including MacConky's agar, Eosine methylene blue agar (BioM laboratories, USA) were used. For biochemical characteristics identification, sugar fermentation including lactose, glucose, mannitole, maltose, sucrose and xylose, TSI, IMVIC (indole, MR, VP, citrate) and nitrate tests were conducted[12].

Identification of gram positive bacteria: For morphological and motility studies the same procedure made with gram negative was conducted. To study the growth pattern, different growth media including MacConky's agar No. 3, Nutrient agar, Brain heart infusion agar, Mannitole salt agar and blood agar base (Oxoid) supplemented with 5% sheep blood were used. For biochemical characteristics, sugar fermentation, oxidase, catalase, coagulase, novobiocin, optochin, bactracin and bile esculin and DNAs test, sensitivity test were performed.

Serological test: Serological tests[1,13,14] were used to classify the *E. coli* into two important serotypes O157 and H7 in which more important serotypes associated with the urinary tract infections.

Filter paper test: This test[13] was conducted to screen urine cultures by using a filter paper dilution system particularly when other isolation methods gave negative results to confirm if there is real UTI-causing bacteria or not.

Maintenance of clinical isolate: Stock cultures were maintained in vails by growing the UTI isolates in 3 mL nutrient broth and next day overlaying with 3 mL 40% glycerol. Vials were then freezed at -70°C.

Determination of antibiotic resistance profile: UTI isolates were subjected to antibiotic resistance screening by disc diffusion method. For this purpose, lawn of UTI isolates was made on nutrient agar plates with the help of wire-loop. Then commercially available antibiotic discs were placed on lawn of culture and plates were incubated at 37°C for 24 h. Next day presence or absence of zone of inhibition around the antibiotic discs was observed. Antibiotics used were ciprofloxacin and nilidixic acid (DNA synthesis inhibitors), ampicillin, vancomycin, cephalothin, amoxicillin and oxacillin (cell wall synthesis inhibitors with different pathways), chloroamphenicol (inhibiting of protein-synthesis), cefoperazone (inhibition of mucopeptide synthesis in the cell wall and β-lactamase inhibitor).

RESULTS

Morphological studies: Microscopical examination of the bacteria isolated from the UTIs infected patients revealed the presence of both gram-positive and negative bacteria. The number of UTIs-infected samples were 119. Based on morphological characteristics, these UTI-causing bacteria were composed of 40.206% *Micrococaceae*, 24.05% *Streptococcus*, (Gram-positive), 96.5% Enterobacteriaceae and 3.42% *Pseudomonas*.

Biochemical and morphological characteristics: Based on the above morphological characteristics including Gram staining, motility, colony types, aeration or oxygen requirement and biochemical characteristics such as IMVIC Test, kind of fermentation of carbohydrate and amino acids and presence or absence of some enzymes. It was possible to classify bacteria of this study which were responsible for the urinary tract infections into the following groups:

Group 1: They are gram negative bacteria, straight rods, motile by peritrichous flagella, or nonmotile, nonsporulating, faculatative anaerobes, producing acid from glucose, sodium either required or not, catalase positive, oxidase negative, they usually reduce nitrite to nitrate (not to N_2) and related to the family Enterobacteriaceae (Table 1). Group 2: They are gram positive bacteria catalase positive, oxidase negative, coagulase positive which have the ability to ferment of the mannitole, ornithin, lysine and lactose but had no ability to ferment arganine. They have the ability to produce DNAase enzyme and can tolerate high concentration of sodium chloride. These biochemical identities belong to the group of *Staphylococcus aureus* (Table 1). Group 3: They are gram positive bacteria characterized by their catalase positive, oxidase negative properties. Also they have no ability to ferment mannitole and lactose, coagulase negative, non-hemolytic. They

Table 1: Bacterial groups isolated from the patients with urinary tract infections

Bacterium
Gram-positive bacteria
Staphyllococcus aureus
Staphyllococcus epidermidis
Staphylococcus saprophyticus
Enterococcus faecalis
Gram-negative bacteria
E. coli
Enterobacter clocae
Enterobacter aerogenes
Klebsiella sp.
Proteus vulgaris
Proteus mirabilis
Proteus morganii
Citrobacter sp.
Serratia marcesens
Pseudomonas aerugenosa

Table 2: Percentage of urinary tract infections in the married (M) and non-married (NM) males and females

Gender	Percentage of urinary tract infections
M female	55
NM female	26
M male	10
NM male	9

Table 3: *Escherichia* Percentage ratio of urinary tract infection with *Escherichia coli* O157 and *E. coli* H 7

Serotype	% of *E. coli* serotype causing UTI
H7	44
O157	8

have the ability to ferment glucose, maltose, arginine, lysine, ornithin but have no ability to produce DNAase enzyme this group include two species of *Staphylococcus* (*epidermidis* and *saprophyticus*) (Table 1). Furthermore it was possible to distinguish between these two genus where the *Staphylococcus saprophyticus* is novobiocin resistant. Group 4, are gram-positive bacteria arranged in chains and can be distinguished from the genus *Staphylocccus* by their catalase negative property (Table 1). They are oxidase negative, homofermentative in which the end product of glucose fermentation is the lactic acid. They have different types of blood hemolysis with different degree. These characteristics revealed that this group belong to *Enterococcus* sp. Group 5, they are Gram-negative bacteria, oxidase positive, have no ability to ferment of lactose, glucose, sucrose and maltose, but they were able to produce pigments (Table 1). These characteristics exclusive to *Pseudomonas aeruginosa.*

During the course of these experiments, it was found that some urine samples obtained from UTI patients gave no detection of bacteria when using usual detection procedures. However, when filter papers were used instead for detection UTI-causing bacteria, the outcome indicated the presence of low bacteria number in such samples.

Table 4: Percentages effectiveness of different antibiotics against gram-positive and gram-negative UTI isolates

Antibiotic	Gram-positive (%)	Gram-negative (%)
Ciprofloxacin	72.88	76.96
Nalidixic acid	68.51	29.32
Vancomycin	62.78	52.05
Ceftazidime	40.57	39.32
Cephalothin	40.05	22.78
Cefuroxime	49.41	48.51
Cefoperazone	62.42	49.05
Amoxicillin	38.7	5.2
Oxacillin	4.32	11.52
Ampicillin	37.6	4.1
Chlomphenicol	75.67	59.07

Table 5: Percentages effectiveness of different antibiotics among urinary tract infections isolates

Antibiotic	Disc code	Sensitive	Resistant	% Efficacy
Ciprofloxacin	Cip_5	130	31	80.74
Nalidixic acid	Na_{30}	124	37	77.01
Vancomycin	Va_{18}	125	36	77.63
Ceftazidime	Cef_{30}	80	81	49.68
Cephalothin	Cep_{75}	74	87	45.96
Cefuroxime	Ceu_{30}	104	57	64.59
Cefoperazone	Ceo_{75}	115	46	71.42
Amoxicillin	Am_{75}	65	96	40.37
Oxacillin	Oxa_1	25	136	15.52
Ampicillin	Amp_2	65	96	40.37
Chloramphenicol	C_{30}	127	34	78.88

Table 6: Age versus number of patients for each bacterial type of infections

Group number	Age (years)	Bacterium	No. of patients for each bacterial type of infection
1	15	*Escherichia coli*	4
		Enterococcus faecalis	3
		Staphyllococcus saprophyticus	3
		Staphyllococcus aureus	1
2	16-50	*Escherichia coli*	58
		Enterococcus faecalis	24
		Staphyllococcus saprophyticus	13
		Staphyllococcus aureus	20
		Enterobacter	6
		Proteus	24
		Citrobacter sp.	3
		Serratia marcescescens	1
		Pseudomonas aeruginosa	4
3	> 50	*Escherichia coli*	1
		Enterococcus faecalis	1
		Staphyllococcus epidermidis	1

Most of the UTI incidence was discovered in married female (55%) of the total UTIs cases where as half of the percentage observed on single females (Table 2). On the contrary both single and married males showed almost equal percentage of incidence (9 and 10%). Generally, females showed 81% of total UTI incidence compared to that in males (Table 2).

In case of UTI-causing *Escherichia coli* serotype O157 appeared to be in higher incidence (84.71%) than the serotype H7 (Table 3). Each UTI-causing bacterial species that have been detected in this study showed different incidence percentage. *Escherichia coli* was the highest (53.24%) and *Serratia marcescence* (0.8%) was the lowest. In Table 4 and 5 the data shows the resistance

level versus commonly used antibiotics in urinary tract infection. Generally most of the isolates were found to be sensitive toward the range of antibiotics being used.

It is important to notice that children and older patients (> 50 years) infected with limited types of bacteria including E. coli, *Enterococcus faecalis* and *Staphyllococcus* sp. In the contrary, patients have ages between (16-50) are infected with wider range of different bacterial species (Table 6).

DISCUSSION

Microbiologically, UTI occurs only when positively pathogenic bacteria are indicated in urinary tract[7,15]. Such infections were considered significant and require treatment when 10^5 bacterial cells mL^{-1} of urine specimens were present in properly collected samples. In our study the sex distribution of UTI infected patients was consistent with that of other reported studies showing significant prevailing of females of UTI (81%). The elevated occurrence of infection among females particularly at their twenties age is related to differences between the male and female genitourinary systems in anatomy and microflora[6,7,16]. Moreover there seams to be a proportional relationship between the hormonal concentration and the occurrence of UTI in females. Many studies indicated Previously that the incidence of UTI differs markedly with sex and age[17].

A total of 170 patient's urine samples were subjected to bacteriological detection testes, but only 41 samples showed positive monomicrobial cultures. In this study different types of bacteria were found to be responsible for the urinary tract infection. Proportionally, different gram negative and gram-positive isolates is shown in Table 1 and 6. The frequency of UTI-causing gram negative is less than the gram positive bacteria. These detected bacteria belong to the families/groups including Enteriobacteriaceae, Micrococcaceae, *Streptococcus* and *Pseudomonas*. A finding consistent with that reported by many investigators[18]. An exception was the linkage of UTI with the existence of *Staphylococcus* and *Streptococcus* in urine samples. It is known that local drinking water used for domestic consumption contains high amounts of salt particularly in summer time. Therefore, it is possible that salt resistant bacteria like *Staphylococcus* and *Streptococcus* might prevail over other bacterial groups in UTI[19]. Additionally, *Staphylococcus* is normal flora of the skin and the mucous membrane which might enter the body by different means such as the use of catheter in order to cause UTI[20].

E. coli is known to have higher incidence than other UTI-causing organisms[21-23]. Also, it was established that the host factors play an important role in the infection by these bacteria particularly in women. For example a clinical correlation has been made between increased cholesterol in serum and the higher incidence of UTI's[22,20].

Among *E. coli* strains isolated the serotype O157 represented 54% while serotype H7 gave 9% of total UTI-causing *E. coli*. Generally wild type *E. coli* and *E. coli* O157:H7 strains have considerably different surface charges, reflecting differences in surface characteristics[24,13]. Such differences belong to variations in the type of interactions between these bacteria and their environment. For example increasing the ionic strength had a strong impact on the electrostatic features of wild type but little effects on those of *E. coli* O157:H7 serotype strains.

Pseudomonas aeruginosa represented 3.42% of patients suffering from urinary tract infections. It was the most wide range antibiotic-resistant bacteria. Some people may acquire the UTI's by *Pseudomonas aeruginosa* through staying in hospitals since such bacteria are highly resistant to antiseptic conditions[25].

Proteus sp. represented 19.5% of the infected urine sample with UTI. It is shown that the infection by this bacterium prevailed in males than in females (data not shown). In contrast, the same genus represented 12.5% of UTI-causing bacteria in Gaza strip[7]. Previous works also indicated that the incidence of UTI by *Proteus* sp. is much higher in males than in females[16]. Such bacteria are more common inhabitants of the preputial sac than of the vulva. Moreover, *Proteus* is known to be in swimming and active motile[26]. However, other UTI-causing organisms are more readily traverse the longer urethra in boys.

Identification of the causative agent and its susceptibility to antimicrobial agents is important; in selecting suitable drugs for treating the patient in early stage of the UTI developments[27]. All the bacteria which isolates were then checked for antibiotic sensitivity profile by disc-diffusion procedure with commercially available samples of Ciprofloxacin, Nalidixic acid, Vancomycin, Ceftazidime, Cephalothin, Cefuroxime, Cefoperazone, Amoxicillin, Oxacillin, Ampicillin and Chloramphenicol. In Table 2 and 3 the data shows the resistance level versus commonly used antibiotics in urinary tract infection. Generally most of the isolates were found to be sensitive toward the range of antibiotics being used. These results were not parallel with that of other studies[24-28]. Resistance to aminoglycosides and chloramphenicol in gram-negative *Bacilli* is usually mediated by b-lactamases that

are unaffected by the using of potential drugs[26]. *Pseudomonas aeruginosa* was the most frequent resistant to the antibiotics used in this study. Only Ciprofloxacin (5 μg disk^{-1}), Vancomycin (30 μg disk^{-1}) and Chloromphenicol (30 μg disk^{-1}) were found to be effective against all the bacteria isolated from UTI patients. It has been reported that there is a direct correlation between the antibiotic and the frequency as well as the type of antibiotics-resistant strains isolated from human beings[26-28]. It is known that resistance to antimicrobial agents can easily be interchanged between bacteria by transmissible elements or plasmids[5]. Ciprofloxacin is highly effective toward UTI pathogens[26]. Because of the antibiotic resistance and narrow antibacterial activity of antibiotics some strains of disease causing bacteria in this study escape treatment such as Oxacillin-resistance UTI-causing in this study escape treatment. Also ampicillin, Cephalothin and Cefuroxime showed less than 50% sensitivity toward UTI pathogens.

In this study it is shown that UTI isolates have low susceptibility to most of the antibiotics being used for the treatment of UTI[26,27]. It is very important to take this in consideration in order to develop a new antimicrobial and therapeutic agents which are highly effective with least side effects and more available. From this study one can conclude the following remarks; the family Enteriobacteriaceae, Micrococcaceae and *Streptococcus* are responsible for the major cases of urinary tract infections in south Jordan. *Escherichia coli* is responsible for the major cases of urinary tract infections in females, while *Proteus* sp. is responsible for the major cases of urinary tract infections in males. Ciprofloxacin (5 μg disk^{-1}) was the most effective antimicrobial agents against wide range of bacteria isolated from patients with urinary tract infections, while Oxacillin (1 μg disk^{-1}) was the lowest one. In contrast to other detecting methods filter papers can be used to diagnose urinary tract infections in specimens which contain low numbers of bacteria.

REFERENCES

1. Ronald, M., 1989. Microbiology Fundamentals and Applications, 2th Edn., Macmillan Publishing Company, USA., pp: 291-296.
2. Boyd, R.F. and B.G. Hoerl, 1991. Basic Medical Microbiology, 4th Edn., Little Brown and Company, London, 105: 394-477.
3. Schaefferent, A.C., J.A. Steort and T.S. Steven, 2001. Rcepent signals in human cells. Clinical Microbiol., pp: 255-259.
4. Hotoon, T.M. and W.E. Stamm, 2005. Infective disease. Northwestern Medical School, 11: 513-529.
5. Johnson, A. and A. Stell, 2000. *Escherichia coli* is the main cause of urinary tract infections. Medical Microbiol., 47: 97-102.
6. Strom, B.L., 1987. Sexual activity, contraceptive use and other risk factors for symptomatic bacteriuria. A case-control study. Annals of Internal Medicine, 107: 816-23.
7. Astal, Z.Y. and F.A. Sharif, 2002. Relationship between demographic characteristics and community-acquired urinary tract infection. Medical Technology Department, 8: 1-7.
8. Hooton, T.M. and W.E. Stamm, 1991. Management of acute uncomplicated urinary tract infection in adults. Medical Clinics of North America, 75: 339.
9. Sobel, J.M., A.J. Wood and B.A. Hinson, 2000. Screening of fungal infections.Medical Microbiol., 15: 255-259.
10. Feld, L.G. and S.P. Greenfield, 1989. Uriary tract infections in infants and children. Pediatric Review, 11: 71-7.
11. Honkinen, O., O. Russkanen, O.P. Lehtonen and J.C. Mertsola, 1999. Study of the bacterial species causing urinary tract infection and urinary abnormalities in children. Medical Microbiol., 318: 770-771.
12. Bradshaw, L.J., 1963. Laboratory Microbiology. W.B. Saunders Company, Wasington, USA., pp: 49-96.
13. Kunin, C.M. and W.J. Buesching, 2000. Novel screening method for urine cultures using a filter paper dilution system. J. Clin. Microbiol., 38: 1180-1190
14. Thomas, G.B., D.L., Swerdlow and P.M. Griffin, 1995. *Escherichia coli* 0157:H7 and the hemolytic-uremic syndrome. Post Grag. Med., 7: 364-368.
15. Sobel, J.D. and D. Kaye, 1984. Host factors in the pathogenesis of urinary tract infections. Am. Med., 76: 122-30.
16. Cohn, E. and B. Schaeffer, 2005. Urinary tract infections in adults. Northwestern Med. Sch., 75: 495-513.
17. Modarres, S. and N.N. Oskoii, 1997. Bacterial etiologic agents of urinary tract infections in children in the Islamic repulic of Iran. Eastern Mediterranean Health, 3: 290-295.
18. Patton, J.P., D.B. Nash and E. Abrutyn, 1991. Urinary tract infection: Economic considerations. Medical Clinics of North Am., pp: 495-513.
19. Latham, R.H. and W.E. Stamm, 1983.Urinary tract infections in young adult women caused by *Staphylococcus saprophyticus*. Am. Medical., 49: 1051-1052.

20. Smellie, J.M., S.P.A. Ridgen and N.P. Prescod, 1995. Urinary tract infection: A Comparison of a four methods of investigations. Arch. Dist Child., 72: 247-250.
21. Duncan, M.J., L. Guogie, J.S. Shin, J.L. Carson and S.N. Abraham, 2004. Bacterial penetration of bladder epithelium through lipid rafts. J. Biol. Chem., 279: 18944-18951.
22. Svanborg, C. and G. Godaly, 1997. Infective disease. Clinical Northeran of American, 11: 513-529.
23. Mulvey, M.A., J.D. Schilling and S.J. Hultgren, 2001. Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infections. Infect. Immun., 69: 4572-4579.
24. Lytle, G.M., 1999. Serotyping of *Escherichia coli*. American Medical, 3: 212-5.
25. Naylor, G.R., 1984. A 16-month analysis of urinary tract infection in children. Medical Microbial., 17: 13-6.
26. Gul, N., T.Y. Mujahid and S. Ahmad, 2004. Isolation, identification and antibiotic resistance profile of indigenous bacterial isolates from urinary tract infection patients. Pak. J. Biol. Sci., 7: 2051-2054.
27. Kupersztoch, P.Y.M., 1981. Antibiotic Resistance of Gram-negative Bacteria in Mexico: Relationship to Drug Consumption in: Molecular biology, Pathogenicity and Ecology of Bacteria Plasmids (Levey, S.B., R.C. Clowes and E.L. Koening, Eds.). Plenum press, New York, 8: 529-537.
28. Gupta, K.D., Scholes and W.E. Stamm, 1999. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. JAMA., 28: 736-8.24.