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Bacterial Pathogens of Patients with Urinary Tract Infections and Antibiotics Susceptibility in Taiz Governorate–Yemen

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Abstract

Urinary tract infections (UTIs) are one of the most prevalent bacterial infections that can affect any individual at any age. UTIs have become difficult to treat because the appearance of bacterial pathogens resistance to antibiotics. So, the current study aimed to detect the types of bacterial pathogens that caused UTIs and antibiotic sensitivity patterns of the bacterial strains in Taiz Governorate-Yemen. This cohort study included One hundred and ten (110) patients with clinical symptoms of UTIs. A clean-catch midstream urine from all patients were cultured for isolation and identification of bacterial agents that caused UTIs. Out of 110: 64 (58.2%) of urine samples showed a positive growth culture: { [37(33.6%)] were females and [27(24.6%)] were males} and 46 (41.8%) were non-growth. The most predominant bacteria were *Escherichia coli* (32.7%), followed by *Klebsiella pneumoniae* were (9.1%), *Staphylococcus aureus* were (6.4%), *Proteus mirabilis* were (5.5%) and *Pseudomonas aeruginosa* were (4.5%). The prevalence rate of UTIs is high among patients with age between 16-30 years [23(20.9%)]. Also, UTIs were more prevalent among the rural population [55(50%)] than the population residence in the city center [9(8.2%)] and the bacterial pathogens were more prevalent among illiterate patients [49 (44.6%)], compared to literate patients were [15(13.6%)]. Finally, our findings showed that the susceptibility test of the bacterial pathogens to appropriate antibiotics: All bacterial pathogens were more sensitive to Ciprofloxacin, while, all bacterial pathogens showed a very high rate of resistance against Amoxicillin, Ampicillin and Penicillin G.

Keywords: Urinary tract infections, Bacterial pathogens, Antibiotics Susceptibility, Taiz Governorate, Yemen.

الخلاصة

تعد الاصابات البكتيرية من أهم المسببات لالتهابات المسالك البولية والتي تصيب جميع أفراد المجتمع في مختلف الاعمار وتصبح هذه الالتهابات صعبة المعالجة بسبب ظهور حالات المقاومة لهذه الممرضات البكتيرية للمضادات الحيوية. لذلك صممت هذه الدراسة لتحري عن الممرضات البكتيرية المسببة لالتهابات المسالك البولية ومدى حساسيتها للمضادات الحيوية في محافظة تعز- اليمن. وحيث شملت هذه الدراسة (110) مريض يشكو من أعراض التهابات المسالك البولية وتم لأجل ذلك جمع عينات المرضى من وسط الادرار بأوعية معقمة وتمت زراعتها على الاوساط الزراعية الملائمة واجريت عليها الاختبارات البكتريولوجية القياسية للتعرف على انواع المسببات البكتيرية المرضية لهذه الالتهابات. وقد اظهرت النتائج أن عدد 64 من العينات بنسبة (58.2%) كانت موجبة للزرع البكتريولوجي، وكان منها عدد 37 مريض اناث بنسبة (33.6%) وعدد 27 مريض ذكور بنسبة (24.6%)، بينما ظهر عدد العينات السالبة للزرع الميكروبي 46 بنسبة (41.8%). وكذلك اظهرت النتائج ان بكتيريا *Escherichia coli* كانت الأكثر شيوعا، اذ تم عزلها بنسبة (32.7%) يليها بكتيريا *Klebsiella pneumonia* بنسبة (9.1%) وبكتيريا *Staphylococcus aureus* بنسبة (6.4%) وبكتيريا *Proteus mirabilis* بنسبة (5.5%) وفي حين تم عزل بكتيريا *Pseudomonas aeruginosa* بنسبة (4.5%). وكانت نسبة الاصابة عالية لدى هذه الشريحة من مرضى التهابات المسالك البولية بين الفئات العمرية (16-30) سنة بعدد 23 بنسبة (20.9%). كما بينت الدراسة الحالية انتشار التهابات المسالك البولية للمراجعين من ريف المدينة بعدد 55 مريض بنسبة (50%) مقارنة بعدد 9 مريض بنسبة (8.2%) للمراجعين من مركز المدينة، كما ان الاصابة كانت أكثر انتشار بين المرضى الاميين بعدد 49 بنسبة (44.5%) مقارنة بالمرضى المتعلمين كان العدد 15 بنسبة (13.6%). واخيراً اكدت نتائج دراستنا والخاصة بحساسية الانواع المختلفة من الممرضات البكتيرية تجاه المضادات الحيوية شائعة الاستعمال والملائمة للمعالجة في مثل هذه الحالات أن كل الممرضات البكتيرية اظهرت حساسية واضحة للمضاد الحيوي Ciprofloxacin، بينما اظهرت جميع الممرضات البكتيرية مقاومة عالية تجاه المضادات الحيوية Amoxicillin, Ampicillin and Pencillin G.

Introduction

The urinary tract includes organs that collect and store urine and release it from the body and these organs are the kidneys, ureters, bladder, and urethra and accessory structures [1]. Urinary tract infections (UTIs) are one of the most common infectious diseases, and defined as the presence of bacteria in urine together with symptoms or without symptoms [2].

In worldwide, about 150 million people are diagnosed with UTIs each year and affected different age groups of the patients [3, 4]. The main caused of UTIs are Gram negative aerobic bacteria found in the gastrointestinal tract that have colonized the peri-urethral area, known as *Enterobacteriaceae*, included: the *Escherichia coli*, *Klebsiella spp*, *Proteus species*, *Enterobacter*, *Citrobacter* and *Serratia species* [5], the other common pathogens frequently isolated are Gram positive bacteria, which include: *Staphylococcus aureus* and *Enterococcus faecalis*, which presumably result in UTIs following colonization of the vagina or perianal skin and the less common organisms such as: *Gardrenella vaginalis*, *Mycoplasma spp* and *Ureaplasma urealyticum* may infect patients with indwelling catheters [6].

Basically, antibiotics are used by humans for therapeutic purposes, the correlate between intensive use of these agents and development of resistant bacteria is not well-known, however it can be many difficulties in developing countries, the available data are not useful to clinicians, especially where the culture is not done and an empirical treatment can be initiated timely which lead to the increase in the magnitude of antibiotic resistance, therefore, there is a need to a routinely investigation for a specific bacterial pathogen that caused UTIs in every case and perform the antibiotics susceptibility in order to use the effective antibiotics for patients treatment [7].

Aims of the study

In our country, there were a limited study according UTIs and their antibiotics susceptibility, especially in Taiz governorate–

Yemen .So, this cohort study was performed to: Isolate and identify the bacterial pathogens that caused UTIs and determine their antibiotics susceptibility patterns for patients referred to different hospitals and health centers in Taiz governorate–Yemen, as well as explore the relationships between sexes, ages, host environment and education levels with UTIs..

Methodology

This cohort study was conducted with a total of 110 urine samples, which were collected samples from some hospitals and health centers in Taiz governorate -Yemen. These patients included: 65(59.1%) were females and 45 (40.9%) were males and their ages ranged from a one year to ≥ 60 years. The patients were diagnosed by a specialist urologist, which having one or more of the following symptoms: dysuria, frequency, urgency, suprapubic discomfort or flank pain and the information of gender, age, host environments and education level of patients were obtained. A clean catch midstream urine were collected from each patient into a sterile screw capped container, which referred to some hospitals and health centers in Taiz governorate -Yemen, the samples were labeled and directly transferred into laboratory for bacterial isolation and examination of bacterial pathogens through following the standard bacteriological methods described by [8, 9]. A loop full of an centrifuged urine sample was inoculated by spread plate method into MacConkey and blood agar plates, which support growth of most Gram-negative bacilli and Staphylococci and then incubated at 37°C for 24hrs, bacteriuria was defined as culture of a single bacterial species from the urine sample at concentration of more than 105CFU/ml and the confirmed it of by more than 5 white blood cells in each high power field according microscope examination [10]. Bacterial counts less than 105 CFU/ml were considered as negative samples [11]. Identification of bacterial pathogens were done by standard bacteriological methods of culturing and biochemical tests, including: Gram staining, catalase test, coagulase test, oxidase test, and Mannitol (fermentation test), urease test,

motility test, [Indole, Methyl red, Voges-Proskauer, Citrate utilization (IMVIC) tests [12, 13]. Kirby-Bauer disc diffusion technique was done for sensitivity of the bacteria to various antibiotics according [14]. These antibiotics include: Penicillin G (PG) 10µg, Amoxicillin (AMX) 25µg, Ampicillin (AMP) 10µg, Ceftriaxone (CRO) 30µg, Cefotaxime (CTX) 30µg, Cephalothin (CF) 30µg, Gentamycin (GN) 10µg, Rifampicin (RA) 5µg, Erythromycin (E) 15µg, Amikacin (AK) 30µg, Ciprofloxacin (CIP) 5µg. Bacterial cultures suspension were spread on Muller-Hinton agar plates using sterile swabs and incubated aerobically at 37°C for 24hrs, the inhibition zones diameter around antibiotic disks were measured and the results were expressed as sensitive or resistant according to the criteria recommended by the clinical laboratory and standard institute guidelines CLSI [15]. Statistical analyses were performed using Chi-square to compare between different variables, P-value less than 0.05 were considered a significant.

Results

A total of one hundred and ten patients with clinical symptoms of UTIs were investigated in this study, sixty five [65/110 (59.1%)] of them were females and Forty five [(45/110 (40.9%)] were males. Also, sixty four [64(58.2%)] of cases were showed a significant bacterial growth, while 46 forty four [46 (41.8%)] have no significant bacterial growth, as showing in Figure 1-A.

The prevalence of Gram negative bacteria were [57/64 (89.1%)] and Gram positive were [7/64(10.9%)], respectively, as showing in Figure 1-B.

Our results were showed that the types of bacterial pathogens that were isolated and identified of Gram negative bacteria as *Escherichia coli* with prevalence rate (32.7%), *Klebsiella pneumoniae* were (9.1%) , *Proteus mirabilis* were (5.5%) and *Pseudomonas aeruginosa* were (4.5%) and Gram positive bacteria, the main bacterial pathogens

identified was *Staphylococcus aureus* (6.4%), as showing in Figure 2.

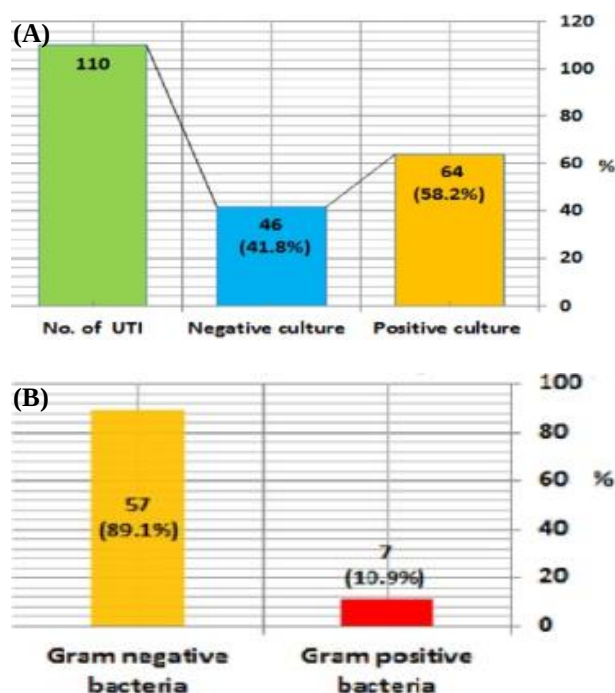


Figure 1: A) Pattern of growth in urine samples, B) Distribution of bacteria in culture positive sample.

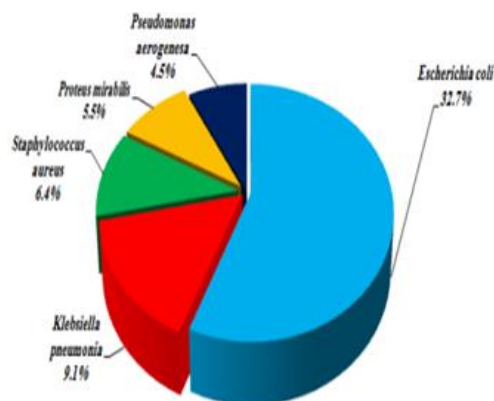


Figure 2: Distribution of the bacteria isolated from patients with UTI.

A distribution of bacterial isolates were observed between females and males as in Gram negative bacteria: *Escherichia coli* [20 (31.3%)] in females and [16 (25%)] in males , *Klebsiella pneumoniae* were [6 (9.4%)] in females and [4 (6.3%)] in males, *Proteus mirabilis* were [4 (6.3%)] in females and [2 (3.1%)] in males, *Pseudomonas aeruginosa* were [2 (3.1%)] in females and [3 (4.7%)] from

males patients. While, Gram-positive bacteria: *Staphylococcus aureus* were more frequently responsible for UTIs in females than in males [(7.8%) vs (3.1 %), respectively)]. However, no significant differences ($P>0.05$) were found in the most bacterial isolates in relation to the gender of patients, as showing in Figure 3.

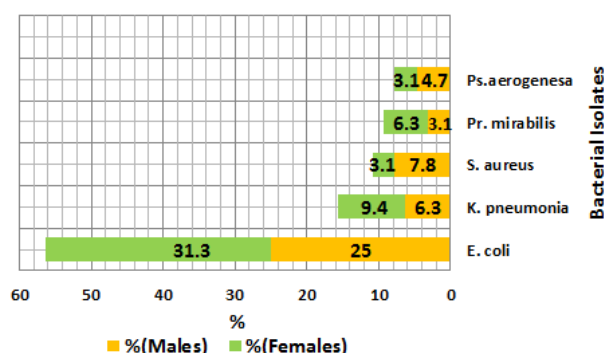


Figure 3: Distribution of positive bacteria isolates according gender of patients with UTI.

Importantly, the total prevalence of UTIs are a high significant among females (33.6%) than males (24.5%) in all positive cases, as showing in Figure 4.

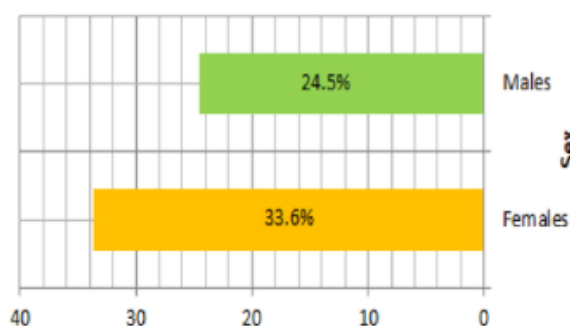


Figure 4: Prevalence of UTIs per sex distributions.

The highest percentages of UTIs were discovered among the age groups : 16- 30 years as (20.9%), (46-60) years as (13.6%) years , (31-45) years as (10.9%), ≥ 60 years as (7.3 %) and (1-15) years as (5.5%) years, as showing in Figure 5.

Also, our findings were demonstrated that the rate of infection was more prevalent among the rural area population (50%) than the population of the city center (8.2%), as showing in Figure 6.



Figure 5: Prevalence of UTIs per age distributions.

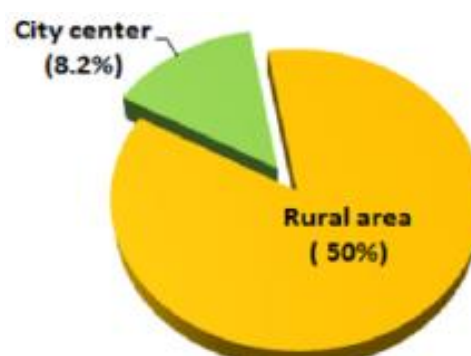


Figure 6: Prevalence of UTIs according host location.

Additionally, our findings showed that the prevalence of UTIs and the educational levels among the illiterate population (no read and write) were (44.5%), compared to literate population (read and write), which had a rate of infection (13.6%), as showing in Figure 7.

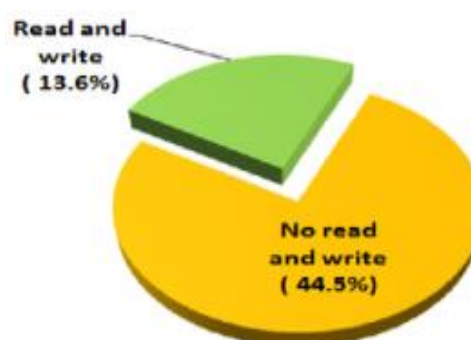


Figure 7: Prevalence of UTIs according educational level.

Finally, the results of antibiotics sensitivity tests showed against different bacterial isolates: *E. coli* were sensitive (100%) for Ciprofloxacin ,but , Erythromycin was reported as the most resistant antibiotics for *E. coli* (77.8%).While *Klebsiella pneumonia* showed the highest sensitivity to Ciprofloxacin and Amikacin with the same percentage (80%), followed by

Gentamycin (70%) and the highest resistance to Erythromycin (100%). Rifampicin, Cephalothin, Amoxicillin, Penicillin G with the same percentage of resistance (90%). *Proteus mirabilis* had the highest sensitivity to Ciprofloxacin (100%), followed by Amikacin, Cefotaxime with the same percentage (83.3%) and the highest resistance to Erythromycin, Rifampicin, Cephalothin with the same percentage (100%) and Penicillin G, Amoxicillin, Ampicillin [(83.3%), (66.7%), (66.7%)], respectively. *Pseudomonas aerogenes* showed there were

sensitive to Ciprofloxacin and Gentamycin with the same percentage (80%), it was resistant to the other antibiotic: Rifampicin, Amoxicillin, Penicillin G (100%), Erythromycin, Cephalothin (80%), Ampicillin (60%). But *S. aureus* bacteria were sensitive to Ciprofloxacin (85.7%), Erythromycin, Amikacin with the same percentage (71.4%), also there were resistant to Penicillin G(100%), Ampicillin, Amoxicillin with the same percentage (85.7%), Ceftriaxone, Cefotaxime [(71.4%) , (57.1%) , respectively], as showing in Figure 8.

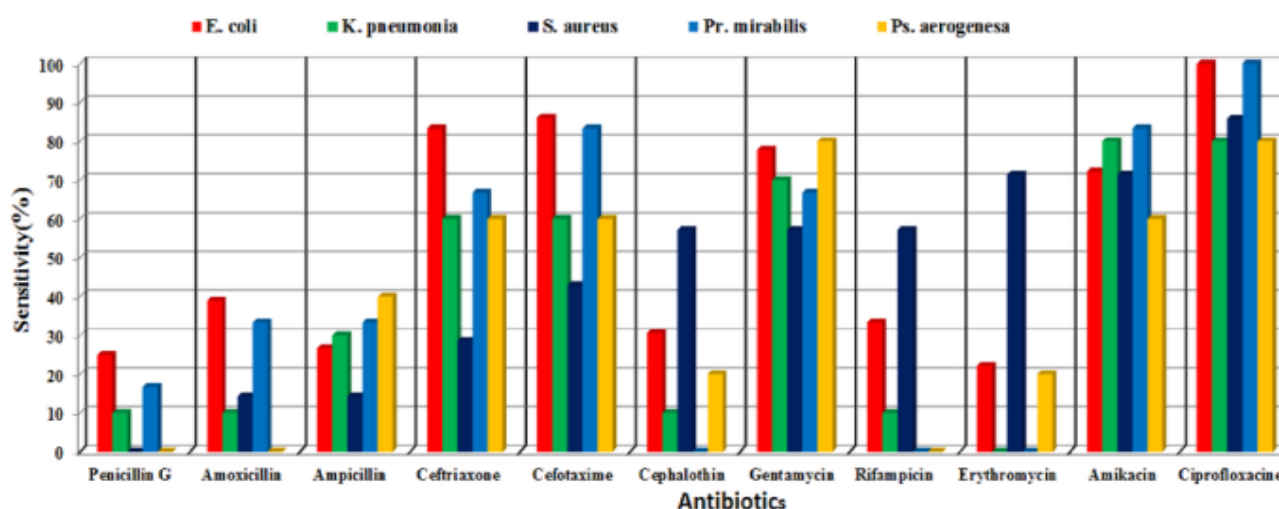


Figure 8: Antibiotics susceptibility percentage among the bacteria isolates.

Discussion

UTIs are one of the common disorders in the worldwide and the pattern of antimicrobial resistance varies in different regions. Out of (110) samples of patients with UTIs: 64 (58.2%) were bacterial growth and there were no bacterial growth as 46 (41.8%) after 24 - 48hrs incubation at 37°C, as in Figure 1 (A, B), these results were similar to other reports which reported (85.2%) [16], the reason for the non-bacterial growth among a number of the patients may be due to the fact that some of the patients have antibiotic therapy before attending to the hospital or laboratory, these antibiotics may have inhibited bacterial growth and the use of midstream urine was aimed for reducing and eliminating the influence of normal flora and other contaminants [1, 17].

Also, our results showed that Gram negative bacteria were more prevalent (89.1%) than Gram positive bacteria (10.9%), as in Figure 2. These results similar to other studies [18], *Enterobacteriaceae* were the most common bacteria , which were isolated from UTIs in present study accounting (89.1%) of total isolated bacteria ,these results were agreement to many previous findings [19,20,21]. Particularly, *E. coli* is the most bacterial pathogen in patients with UTIs as (32.7%), which similar to other studies [22, 23]. *Klebsiella pneumoniae* being the second bacterial pathogens as (9.1%),but, these results disagreement with others, who reported *Klebsiella* species to be more prevalent than *E. coli* in UTIs, followed by *Proteus mirabilis* ,as showing in Figure 3 [1, 24]. *E. coli* have

several factors responsible for their attachment and colonization to the urogenital mucosa with adhesion, pilli, fimbriae and P1-blood group phenotype receptor [25]. Gram-positive cocci were less isolates in our study; this is similar to other studies in different countries [26, 27]. Whereas, the results of Gram positive cocci, *Staphylococcus aureus* (6.4%) were the most common bacteria isolated from urine samples which agreement with others [28], while in contradiction with some of studies that showed the *Staphylococcus* as etiologic agents of infections, especially with infections associated with medical devices and in immunocompromised patients as UTIs [29-32]. The incidence of UTIs and its clinical impact are very different for both sexes at different stages of life. In the present study, it has been shown the higher prevalence of UTIs in females (33.6%) compared to males (24.5 %), as in Figure 4. These observations is supported by other works, which illustrated to the high incidence of UTIs among the females than the males is due to anatomic and physical factors related to females (the urethra of females is much shorter and closer to the anus than in males, and it also lacks the bacteriostatic properties of prostatic secretions). Also, the higher incidence in females observed in this work, may be as a result of the unhygienic habits of not cleaning the vagina from front to the back after urination but rather from back to front, thus resulting for causing autoinfection [33, 34,35]. The UTIs in our study showed that the highest rates in the age groups 16 - 30 years in both sexes as (20.9%), as showing in Figure 5. This may be as a result of increased sexual activity within these age groups which predisposes them to UTIs; this observation is supported by reports of other workers [36]. In the present study, UTIs were more prevalent in Taiz rural area population as (50%) than the population in the Taiz City center (8.2%), as showing in Figure 6. Also, (44.5%) of patients with UTIs were illiterate (no reading and writing) than (13.6%) of them were literate (reading and writing), as in Figure 7. These result agreements well with other researcher who found that the distribution of UTIs may result from geographical variability: host

factors and practices of people such as healthcare and education programmers, socioeconomic standards, hygiene practices in different geographic regions and countries and with regards to the fact that most bacteria causing UTIs are commensals of perianal and vaginal regions, therefore personal hygiene may be important in reducing the incidence of UTIs [37-40]. Finally, our findings showed that UTIs were resistance to certain antibiotics as in Figure 8. All bacterial pathogens showed a variable resistance to beta (β) -lactam antibiotics such as, Ampicillin, Amoxicillin and Penicillin G. These agreements, with many earlier studies, reported a high degree of these drug resistances [41]. The Gram negative bacilli also were resistant to Cephalothin, Rifampicin and Erythromycin that reported the high prevalence of resistance to the commonly used antibiotics such as Ampicillin and Cephalothin [42]. *Staphylococcus aureus* was resistance to Ceftriaxone as (71.4%) and Cefotaxime as (57.1)%, these results are supported by the other work [43,44], Ciprofloxacin showed a strong activity against of all bacterial isolated and were very active against *E.coli* and *Proteus mirabilis*, however it has a less activity against *Staphylococcus aureus* , *K. pneumonia* and *Pseudomonas aeruginosa*. Ciprofloxacin had previously been reported to be very active against UTIs [45-47], while, other researcher reported that the most effective antimicrobial agents were amikacin and ciprofloxacin for Gram negative bacilli (81%-100%) [48]. Based on the results, it was revealed that the susceptibility test of bacteria to Ciprofloxacin and other antibiotics, which were similar to many studies [49, 50]. These patterns of antimicrobial resistance to bacterial pathogens causing UTIs vary from place to place and from time to time [51, 52]. In order to prevent or decrease resistance to antibiotics, the use of appropriate antibiotics should be kept under supervision, should be given in an optimal doses for a limited period of time [53, 54].

Conclusions

Our finding indicated that UTIs were more prevalent in females than males. The most

common bacteria pathogens was *E. coli* and the most effective antimicrobial agents were Ciprofloxacin, Amikacin, Gentamycin and Cefotaxime, while Ceftriaxone against Gram-negative bacilli and the most effective antibiotics against Gram-positive cocci were Erythromycin, Rifampicin and Cephalothin.

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