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ORIGINAL ARTICLE

RISK FACTORS AND ANTIBIOTIC RESISTANCE SPREAD AMONG PATIENTS WITH URINARY TRACT INFECTIONS IN KERBALA, IRAQ

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ABSTRACT

Background: The most frequent infection that requires hospitalization is a urinary tract infection (UTI), which is frequently linked to gram-negative multidrug-resistant organisms (MDROs). This study aims to identify the kind of urine pathogens isolated from patients as well as their pattern of antibiotic resistance and relationship with the risk factors. During the period extending between October 2023 to January 2024.

Materials & Methods: Urine samples from 30 healthy controls and 50 probable UTI patients, ranging in age from 9 to 69, were collected. Sampling was done in the private clinic and Al-Kafeel hospital. The Vitek system was utilized to identify the bacterial growth, and before taking the sample, a questionnaire was taken from the patient, including age, gender, place of residence, whether or not he suffers from diseases related the urinary system ,and family history of the diseases.

Results: The findings indicated that the percentage of infection in females (56%, n=28) was higher than that in males (40%, n=20). In addition, in the 20-29 age group, there was the highest rate of infection, while the 60-69 age group had the lowest rate of infection. Out of 50 urine samples, the results also showed that 2 (4%) had no bacterial growth and 48 (96%) had considerable bacterial growth; only these samples were used in the current study. The bacterial isolates included 19/48 (38%) *Escherichia coli*, 11/48 (22%) *Klebsiella pneumoniae*, 8/48 (16%) *Enterococcus faecalis*, 6/48 (12%) *Staphylococcus aureus*, 4/48 (8%) *Pseudomonas aeruginosa*.

Conclusion: In gram-negative bacteria, meropenem was mostly effective antibiotic, followed by doxycycline. However, in Gram-positive bacteria, linezolid is most effective antibiotic. Among risk factors for the UTIs and MDR-UTIs spread included a previous UTI (n=31, 62%), sexual activity (n=47, 94%), older age (n=29, 58%) and recent pregnancy (n=26, 52%). Determination of risk factors of UTIs is important needing prompt control strategies.

KEY WORDS: Urinary tract infections; Risk factors; antibiotic resistance; Kerbala; Iraq.

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INTRODUCTION

The urinary system is commonly affected by bacterial infections and certain parts of the urinary tract are a target for bacteria which lead to these infections.[1] One of the most prevalent bacterial infections in humans are urinary tract infections (UTIs), which can be classified as a community disease or a health issue.[2] Untreated lower UTIs (caused by pathogenic bacteria growing in the urethra and colonizing the urinary tract) have the potential to heal and spread to the upper urinary tract. UTIs are ascending infections. In clinical terms, pain and frequent urination are indicative of a UTI. Urinary tract symptoms can manifest even in the absence of a positive urine culture, which is important to note.[3] The organs that store and release urine from the body are the kidney, ureter, bladder, and urethra; these organs together make up the urinary system.[4] Based on anatomy, UTIs can be categorized as either upper UTIs or lower UTIs.

Uropathogens that cause inflammation in the bladder through the urethra enter the bladder through the intestine or periurethral muco-

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sa and initiate the development of cystitis.[5] Simple healthy individuals have any hidden urinary tract defects, indwelling catheters or other dysfunctions are susceptible to cystitis. An infection of the upper urinary tract, also called pyelonephritis, is far more dangerous than a lower UTI and frequently results in sepsis (urosepsis).[6] The symptoms of pyelonephritis, which include inflammation of the renal pelvis and parenchyma of one or both kidneys, fever, chills, and nausea, are systemic and can cause localized flank or back pain. Pyelonephritis is caused by a UTI ascends from the bladder to the kidneys and their collecting systems.[7] Uncomplicated pyelonephritis and cystitis are caused by *Escherichia coli (E. coli)*, followed by Gram-positive bacteria like *Staphylococcus saprophyticus* and *Enterococcus faecalis*, and other species of *Enterobacteriaceae* like *Klebsiella pneumoniae* and *Proteus mirabilis.*[8] Common antibiotics for the UTIs and pyelonephritis include trimethoprim-sulfamethoxazole, fosfomycin, trometamol, pivmecillinam, nitrofurantoin monohydrate and fluoroquinolones according to international guidelines.[9] Due to the widespread and indiscriminate use of antibiotics, bacteria have developed resistance to the ones that are currently available on the market.[10] The production of various enzymes such as extended-spectrum beta-lactamases (ESBLs) and carbapenemases has complicated gram-negative infections elimination.[11] The indiscriminate antibiotics usage more frequently results in risk of developing MDR pathogens.[12]

MATERIALS AND METHODS

Sample collection: Samples for this study were collected from October 2023 to January 2024 at the Al-Kafeel Super Specialty Hospital in the province of Karbala. Thirty healthy controls and fifty probable UTI patients had urine samples taken from, before taking the sample, a questionnaire was taken from the patient, including age, gender, place of residence, whether or not he suffers from diseases related the urinary system and family history of the diseases. Participants were selected based on their health status concerning urinary tract infections. After that, using a sterile standard loop (1 mL) a culture on nutritional agar, blood agar, and MacConkey agar plates was performed, and then incubated for 24 hours at 37°C. An antimicrobial susceptibility test for the isolated pathogens was performed using the Kirby-Bauer disk diffusion method.[14]

Morphological and Biochemical Identification of Isolated Bacteria: Based on fundamental criteria such as the colony shape, color, and appearance on general culture media such as blood agar medium, nutrient agar medium, and some media differentiation such as MacConkey agar media, which aids in the growth and isolation of only Gram-negative bacteria, the initial diagnosis of the isolated bacterial species was made. Biochemical tests were also used. The results of biochemical identification of isolated bacteria was confirmed using VITEK 2 diagnosis system.

Antimicrobial susceptibility: In UTIs, most of patients use fluroquinolones as the empirical medication of choice. One colony of the organism was tested for turbidity using 0.5 McFarland standards after it was emulsified in 1 mL of sterile normal saline and properly mixed. The sample was inoculated onto Mueller Hinton agar plates using a sterilized cotton swab. Antibiotic susceptibility was assessed using this medium in compliance with CLSI. As stated by Turnidge and Jorgensen, it has low concentrations of trimethoprim, sulfonamide, and tetracycline inhibitors and grows most non-fastidious pathogens satisfactorily. To prepare this medium, the solid ingredients were dissolved in 1 L of purified water. To ensure sterilization, the mixture was then autoclaved for fifteen minutes at 121°C. Gram-negative bacteria are used to classify antibiotics according to the type of microorganisms they target: Meropenem, Amikacin, Levofloxacin, Gentamycin, Ofloxacin, Ciprofloxacin, Piperacillin – Tazobactam, Nitrofurantoin, Cefepime, Amoxicillin-Clavulanic acid, Ceftazidime, Tetracycline, Norfloxacin, Cefixime, Colistin. Also, use the following antibiotics with Gram-positive: (Tetracycline, Gentamycin, Amikacin, Levofloxacin, Gentamycin, Cefepime, Ofloxacin, Vancomycin, Teicoplanin).

The antibiotic selection was according to the CLSI directive of 2023. The clear zone was measured in millimeters, and its interpretation was based on comparison with the CLSI standards (CLSI, 2023). For each strain, the reporting was completed by designating it as sensitive, intermediate, or resistant.

RESULTS

Risk factors: Among risk factors for the UTIs and MDR-UTIs spread, gender, a previous UTI (n=31, 62%), sexual activity (n=47, 94%), older age (n=29, 58%), recent pregnancy (n=26, 52%), rural residence (n=30, 60%), recurrent infection (n=31, 62%), familial history (n=21, 42%) and renal stones (n=27, 54%) were considerable. Determination of risk factors of UTIs is important needing prompt control strategies. (Table 1) indicates that there was a statistically significant variation in patient characteristics based on sex ($p<0.05$). There, 28 (56%) of the females and 22 (44%) of the males had urinary tract infections, respectively. The same table indicates that the age range between 20 and 29 accounted for 36% of the total, followed by 30 to 59, with the age group between 60 and 69 having the lowest percentage. The results given in the table 1 highlight that significant differences were found between UTIs and place of residence. Where the number of people with UTIs in rural areas 30 (60%) was relatively higher than in urban areas 20(40%).

The study's findings also demonstrated a substantial association between the family history of disease and urinary tract infections (UTI) among cases and

Table 1: Patient and control groups are divided based on sex and other risk factors

Characteristic	Control (n = 30)	Patient (n= 50)	Statistic test (p value)
Mean ±SD Range	34.66 ±12.33	35.40 ±14.79	1.283 0.092
Age			
< 20 years, n (%)	2 (6.67%)	5 (10%)	
20 - 29 years, n (%)	10 (33.33%)	18 (36%)	
30 - 39 years, n (%)	9 (30%)	9 (18%)	
40 - 49 years, n (%)	6 (20%)	9 (18%)	
50 - 59 years, n (%)	2(6.67%)	6 (12%)	
60 - 69 years, n (%)	1 (3.33%)	3 (6%)	
Sex			
Male, n (%)	11 (37%)	22 (44%)	3.779 0.027
Female, n (%)	19 (63%)	28 (56%)	
Residence			
Urban	10 (33.3%)	20 (40%)	<0.001
Rural	20 (66.7%)	30 (60%)	
Recurrent infection	0.00	31 (62%)	<0.001
Familial history	0.00	21 (42%)	<0.001
Renal stones	0.00	27 (54%)	<0.001

N: number of cases, **SD**: standard deviation, [NS]: Non-significant difference between groups (p value >0.05)
T: independent sample T test, **C**: chi squared test [S]: Significant association between groups (p value <= 0.05)

Table 2: The susceptibility of antimicrobial rate to the isolated Gram-negative bacteria

Gram-negative bacteria / Antibiotics	*E. coli* n=19(38%)	*K. pneumoniae* n=11 (22%)	*P. aeruginosa* n=4 (8%)
Meropenem	n=16 (84%)	n=5 (45%)	n=0
Gentamicin	n=10 (52,6%)	n=0	n=0
Doxycycline	n=10 (52,6%)	n=4 (36%)	n=0
Cefepime	n=10 (52,6)	n=0	n=0
Tetracycline	n=8 (42%)	n=2 (18%)	n=0
Norfloxacin	n=8 (42%)	n=4 (36%)	n=0
Cefixime	n=4 (21%)	n=0	n=0
Amoxicillin/Clavulanic acid	n=1 (5%)	n=0	n=0
Ofloxacin	n=0	n=4 (36%)	n=0
Colistin	n=1 (5%)	n=0	n=1(25%)
Ciprofloxacin	n=4 (21%)	n=0	n=0
Ceftriaxone	n=4 (21%)	n=0	n=0
Levofloxacin	n=6 (31%)	n=0	n=2(50%)
Ceftazidime	n=1 (5%)	n=0	n=0
Cefotaxime	n=1 (5%)	n=0	n=0
Amikacin	n=0	n=0	n=0
Piperacillin-Tazobactam	n=0	n=0	n=0
Nitrofurantoin	n=0	n=0	n=0
Trimethoprim/sulfamethoxazole	n=0	n=0	n=0

controls. The study's findings indicated a significant difference ($p < 0.001$) between the prevalence of kidney stones and UTI infections.

Table 3: The Susceptibility of Antimicrobial rate to the isolated Gram-positive bacteria

Gram-positive bacteria / Antibiotics	*Staphylococcus* spp N=6(12%)	*Enterococcus* spp N=8(16%)
Meropenem	n=0	n=0
Gentamicin	n=2(33%)	n=0
Doxycycline	n=2(33%)	n=0
Penicillin	n=0	n=0
Tetracycline	n=2 (33%)	n=0
Norfloxacin	n=0	n=0
Erythromycin	n=0	n=0
Amoxicillin/ Clavulanic acid	n=0	n=0
Ofloxacin	n=0	n=0
Colistin	n=0	n=0
Trimethoprim/ sulfamethoxazole	n=2(33%)	n=0
Ceftriaxone	n=0	n=0
Levofloxacin	n=0	n=0
Clindamycin	n=2(33%)	n=0
Linezolid	n=1(16%)	n=2(25%)
Amikacin	n=0	n=0
Teicoplanin	n=0	n=3(37%)
Nitrofurantoin	n=1(16%)	n=0
Vancomycin	n=0	n=2(25%)

Identification, isolation and susceptibility to antibiotics: Based on the type of gram stain used, bacteria can be identified as either Gram-positive or Gram-negative. In order to identify gram-positive bacteria, the VITEK 2 system was utilized in conjunction with the catalase and tube coagulase tests. Tryptophan deaminase activity, fermentation of glucose and cellobiose, generation of H_2S, and finally production of indole are the five biochemical techniques used in the CTIA tube to identify gram-negative bacteria. The VITEK 2 system was used to confirm the identification. Thirty controls did not harbor any pathogenic bacteria, whereas fifty patients had bacteria which caused UTIs. The most frequent isolated Gram-negative bacteria were *Escherichia coli* (38%), *Klebsiella pneumoniae* (22 %), and *Pseudomonas aeruginosa* (16%). *Staphylococcus* spp. (12%) and *Enterococcus faecalis* (16%) among the Gram-positive bacteria. Among the tested antibiotics, meropenem (84%) proved to be more effective against *E. coli* isolates, which are the most common isolates for UTIs. Gentamycin, doxycycline and cefepime (52%), tetracycline and norfloxacin (8%) showed lower effectiveness, while there was no effect of amikacin, piperacillin-tazobactam, nitrofurantoin, trimethoprim/sulfamethoxazole, ofloxacin on *E. coli*, as shown in Table 2. While *Enterococcus* species as Gram-positive bacteria were the most common bacteria with resistance to every antibiotic listed in (Table 3), with the exception of teicoplanin (37%), which makes it more potent than linezolid and vancomycin (25%). Nevertheless, *Staphylococcus* spp. sensitive to 33 percent of clindamycin, doxycycline, tetracycline, trimethoprim/sulfamethoxazole, linezolid, and less effective (16 percent) nitrofurantoin.

DISCUSSION

In this study Table 1 indicates that there was a statistically significant variation in patient characteristics based on sex ($p<0.05$). There, 28 (56%) of the females and 22 (44%) of the males had urinary tract infections, respectively. This research was similar to other studies demonstrating that there are more women than men in many nations, such as the Baghdad Teaching Hospital study, which found that the frequency of women is higher (62 percent) than that of men (37 percent), despite the lack of statistical evidence to support a gender difference.[13,14] The same table indicates that there was a non-significant difference in the age distribution, which was heterogeneous. The age range between 20 and 29 accounted for 36% of the total, followed by 30 to 59, with the age group between 60 and 69 having the lowest percentage. The results given in the table-1 highlight that significant differences were found between UTIs and place of residence. Where the number of people with UTIs in rural areas 30 (60%) was relatively higher than in urban areas 20 (40%). This study agreed with a study conducted in Basra, where the highest percentage of infections was found in rural areas than urban areas.[15]

The study's findings also demonstrated a substantial distinction between the history of the family and urinary tract infections. Given that uropathogens have a strong genetic predisposition to colonize vaginally[16], and this tendency appears to run in families. His research demonstrated the relationship between family history and UTI through genetic predisposition. Perhaps due to increased E expression in vaginal epithelial cells. More Coli receptors than usual allow the bacteria to adhere to the epithelium more effectively.[17] The study's findings indicated a significant difference ($p < 0.001$) between the prevalence of kidney stones and UTI infections. In their investigation, the reference[18] discovered that patients with renal stones had

a high rate of UTI infection (p=0.001). This finding supported the theory that the urine stone cannot pass easily and that an infection could quickly result from an inflammatory ureteric stricture or injuries the stone causes during its descent.

According to the bacterial distribution, the most frequent isolated Gram-negative bacteria were Escherichia coli (38 percent), Klebsiella pneumoniae (22 percent), and Pseudomonas aeruginosa (16 percent) and Enterococcus faecalis (12 percent) among the Gram-positive bacteria. These findings corroborated those of other researches as mention in the reference[19,20] of the tested antibiotics, meropenem (84%) proved to be more effective against E. coli isolates, which are the most common isolates for UTIs. Gentamycin, doxycycline and cefepime (52%), tetracycline and norfloxacin (8%) showed lower effectiveness, while there was no effect of amikacin, piperacillin-tazobactam, nitrofurantoin, trimethoprim/sulfamethoxazole, ofloxacin on E. coli, as shown in Table 2. While Enterococcus species as Gram-positive bacteria were the most common bacteria with resistance to every antibiotic listed in (Table 3) with the exception of teicoplanin (37%), which makes it more potent than linezolid and vancomycin (25%). Nevertheless, Staphylococcus spp. sensitive to 33 percent of clindamycin, doxycycline, tetracycline, trimethoprim/sulfamethoxazole, linezolid, and less effective (16 percent) nitrofurantoin. To ensure that treatment recommendations for UTIs are effective, it is important to monitor the resistance rates of common uropathogens regularly. These rates are on the rise as a result of the overuse of commonly prescribed antimicrobial agents.[21]

CONCLUSION

The findings indicated that the percentage of infection in females (56%, n=28) was higher than that in males (40%, n=20). In addition, in the(20-29)age group, there was the highest rate of infection, while the 60-69 age group had the lowest rate of infection. Out of 50 urine samples, The bacterial isolates included 19/48 (38%) *Escherichia coli* , was the more common bacterial infection in UTIs .In gram-negative bacteria, The antibiotic meropenem was mostly effective, followed by doxycycline, therefore, we recommend using this antibiotics against gram-negative bacteria. However, in Gram-positive bacteria, linezolid is most effective antibiotic. Among risk factors for the UTIs and MDR-UTIs spread included a previous UTI (n=31, 62%), sexual activity (n=47, 94%), older age (n=29, 58%) and recent pregnancy (n=26, 52%). Determination of risk factors of UTIs is important needing prompt control strategies.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	ZHA, SAR
Acquisition, Analysis or Interpretation of Data:	ZHA, SAR, FHZ, HAF, MAA
Manuscript Writing & Approval:	ZHA, SAR, FHZ, HAF, MAA

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.