



Antibiotic Susceptibility Patterns of Uropathogens Isolated from Female Patients with Urinary Tract Infection in Duhok Province, Iraq

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Abstract

Background: Urinary Tract Infections (UTIs) are one of the most common bacterial infections worldwide. The study of bacterial uropathogens in a local area and their susceptibility to antimicrobial agents is required to determine empirical therapy.

Objectives: This study aimed to assess the profile and antibiotic resistance patterns of bacteria, causing urinary infections isolated from female patients in Duhok province, Iraq.

Methods: A total of 530 urine samples were collected from females clinically suspected of UTIs over three years between January 2017 and February 2020. The samples were inoculated directly on MacConkey and Blood agar media and then incubated aerobically for 24 h at 37°C. Samples that gave up colony counts of $\geq 10^5$ CFU/mL were considered as positive growth. Purified colonies were identified through standard bacteriological tests, and their susceptibility to different antibiotics was determined using the Vitek-2 system.

Results: Out of 530 urine samples, 450 (84.9%) contained Gram-negative bacteria, while the other 80 (15.1%) harbored Gram-positive bacteria. *Escherichia coli* was the most common uropathogenic isolate (58.5%), followed by *K. pneumoniae* (14.3%), *Staphylococcus* spp. (8.9%), *P. mirabilis* (6.6%), *E. faecalis* (3.2%), and *S. agalactiae* (3.02%). The majority of Gram-negative uropathogens were resistant to ampicillin, aztreonam, ceftriaxone, and cefepime and around 95% were sensitive to ertapenem and imipenem. Most Gram-positive isolates showed high resistance to benzylpenicillin, oxacillin, gentamicin, and erythromycin, and high susceptibility to linezolid, tigecycline, and nitrofurantoin.

Conclusions: It was concluded from this study that *E. coli* is the predominant pathogen causing UTIs in female patients in Duhok province, Iraq. There were increasing antibiotic resistance rates, particularly to ampicillin, aztreonam, ceftriaxone, benzylpenicillin, and erythromycin. Therefore, empirical antibiotic therapy should be based on local sensitivity patterns rather than international guidelines.

Keywords: UTIs, Antimicrobial Sensitivity, Uropathogens, Multidrug Resistance Bacteria, Duhok, Iraq

1. Background

Urinary Tract Infections (UTIs) are one of the most common acquired bacterial infections. It has been estimated that approximately 150 million people worldwide develop UTIs annually, with high costs, including medical and hospitalization expenses (1). Recent studies conducted in Iraq found that *E. coli* was the most common uropathogen among Gram-negative isolates and *Staphylococcus* species among Gram-positive bacteria causing UTIs (2, 3).

The prevalence rate of uropathogens varies depending on the age and gender of patients, hospitalization, catheterization, and previous uses of antibiotics (4-6). Besides, UTIs occur more commonly in the female gender than in the male gender mainly due to the shorter

urethra that allows pathogens to travel more easily into the bladder and sexual activity that increases the risk of uropathogens (7). It is worthwhile to mention that persistent symptoms for at least one week could increase the risk of recurrent infections because of the occult kidney infection, particularly among females with low socioeconomic status (7).

The emergence of antibiotic resistance is a serious public health concern. Overuse of antimicrobial agents in the community without medical prescription and their low costs are thought to have contributed to the emergence and proliferation of antimicrobial resistance (8). It was previously observed that the increasing resistance rate to commonly used antimicrobial drugs could also play a ma-

major role in the occurrence of complicated and recurrent infections (9). Furthermore, differences in antibiotic resistance rates were documented in different geographical and regional locations (2, 10). Therefore, the periodic evaluation of the frequency of pathogens with their antibiotic resistance patterns is recommended in different countries for therapeutic advice and antimicrobial resistance prevention (11). Additionally, investigating antimicrobial susceptibility patterns could help allocate UTI empirical treatment guidelines in different regions.

2. Objectives

The current study aimed to investigate the most common bacterial uropathogens and their antibiotic sensitivity patterns among females suffering from UTIs in Duhok province, Iraq.

3. Methods

3.1. Sample Collection and Processing

A cross-sectional study was conducted in Duhok province, Kurdistan Region, Iraq, from January 2017 to February 2020. A total of 530 urine samples were collected from females referring to private clinical health centers. The age of the patients ranged from 10 to 65-years-old. Clean catch midstream urine (4 - 5 ml) was obtained in sterile disposable containers from patients to avoid contamination, and the containers were transferred immediately to the Duhok private clinical laboratory.

A loopful of urine sample was cultured on MacConkey and Blood agar (Oxoid Ltd., Basingstoke, Hampshire, UK), incubated overnight at 37°C and inspected for the presence of bacterial growth. Plates without any colony at the end of the 24 h incubation period were further extended for 48 h. Agar plates with a colony count of equal to or more than 10⁵ CFU/ml of urine indicated significant bacteriuria and evidence of UTIs. The inclusion criteria included female gender, positive microbiological evidence of UTIs, and agreement to be recruited in the study.

3.2. Bacterial Identification and Antimicrobial Sensitivity

Purified colonies were initially classified using Gram staining and then identified based on standard microbiological cultures and biochemical characteristic reactions according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (12, 13). The Vitek-2 system (bioMérieux, US) was used for antimicrobial susceptibility testing. The antimicrobial susceptibility pattern of the isolates was performed by using the Vitek-2 system (bioMérieux, US).

3.3. Ethics Statement

The study and the protocol of attaining consent were approved by the scientific committee at the College of Medicine, University of Zakho, Kurdistan, Iraq. Formal consent was obtained from the patients recruited in this study. Only participants who gave their consent before sample collection were included in the study.

4. Results

In this study, 530 female subjects clinically infected with UTIs who met the inclusion criteria were recruited in the study. Of them, 450 (84.9%) were positive for Gram-negative bacteria and 80 (15.1%) for Gram-positive bacteria (Table 1). The results showed that *E. coli* was the most common Gram-negative uropathogen (n = 310, 58.5%), followed by *K. pneumoniae* (14.3%), while *M. morgani* recorded the lowest rate (1.3%) (Table 1). On the other hand, the most common Gram-positive uropathogen was *S. haemolyticus* (3.8%), followed by *E. faecalis* (3.2%), while *S. aureus* showed the lowest rate (0.94%) (Table 1).

Table 1. Distribution of Bacterial Profiles in Urine Samples in this Study (n = 530)

Variable	No. (%) ^a
Gram-negative isolates	
<i>Escherichia coli</i>	310 (58.5)
<i>Klebsiella pneumoniae</i>	76 (14.3)
<i>Proteus mirabilis</i>	35 (6.6)
<i>Acinetobacter baumannii</i>	13 (2.5)
<i>Pseudomonas aeruginosa</i>	9 (1.7)
<i>Morganella morgani</i>	7 (1.3)
Total	450 (84.9)
Gram-positive isolates	
<i>Staphylococcus haemolyticus</i>	20 (3.8)
<i>Enterococcus faecalis</i>	17 (3.2)
<i>Staphylococcus epidermidis</i>	16 (3.02)
<i>Streptococcus agalactiae</i>	16 (3.02)
<i>Staphylococcus lentus</i>	6 (1.12)
<i>Staphylococcus aureus</i>	5 (0.94)
Total	80 (15.1)

^aData are reported as numbers of isolates and percentages (within brackets) of total.

Regarding antibiotic susceptibility patterns, *E. coli* isolates were the predominant uropathogens and showed high resistance to ampicillin (84.8%), aztreonam (58.7%), ceftriaxone (58.1%), and cefepime (57.4%) and high sensitivity to ertapenem (98.7%), imipenem (97.7%), and amikacin

(92.9%) (Table 2). Besides, *K. pneumoniae* and *P. mirabilis* displayed a similar resistance pattern as for *E. coli* and showed high susceptibility to ertapenem and imipenem and high resistance to ampicillin, aztreonam, ceftriaxone, and cefepime (Table 2). In terms of the sensitivity pattern of *A. baumannii* isolates, it was revealed that 92% of the isolates were resistant to ampicillin, amoxicillin/clavulanic acid, cefazolin, and cefoxitin and highly sensitive to trimethoprim/sulfamethoxazole (92.3%) (Table 2). About 78% of the *P. aeruginosa* isolated were resistant to ampicillin and nitrofurantoin, while they were highly susceptible to ertapenem (77.8%) (Table 2). Additionally, *M. morgani* isolates were absolutely sensitive to cefepime, aztreonam, ertapenem, imipenem, amikacin, ciprofloxacin, levofloxacin, and nitrofurantoin (100%) while expressed very high resistance to ampicillin (100%) (Table 2).

In terms of Gram-positive bacteria, *Staphylococcus* spp. were responsible for about 8.9% of UTI cases. Among them, *S. haemolyticus* as the most frequent uropathogen showed high resistance to benzylpenicillin (100%), oxacillin (95%), and erythromycin (95%) and high sensitivity to nitrofurantoin and tigecycline (100%) (Table 3). Similarly, *S. epidermidis* was highly resistant to erythromycin (100%), benzylpenicillin (87.5%), and oxacillin (81.3%), but highly sensitive to moxifloxacin, linezolid, and nitrofurantoin (100%) (Table 3). On the other hand, *E. faecalis* susceptibility to tigecycline and nitrofurantoin was high (100%), but it was highly resistant to erythromycin (100%) and clindamycin (100%). The resistance rates of *S. agalactiae*, *S. lentus*, and *S. aureus* to all the 16 selected antimicrobial agents are summarized in Table 3.

5. Discussion

Urinary tract infection is considered to be one of the most common bacterial infections that affect people in the community and hospitals worldwide (1). In general, females are more likely to get a UTI than males because females have a shorter urethra, so pathogens have a shorter distance to travel to reach the bladder (7). Approximately one in every three females requires antimicrobial treatment for a UTI by the age of 24, and 40 - 50% of females may suffer from UTIs during their lifetime (14).

As UTI is a very common disease in females, its diagnosis and treatment have important implications for patient's health, healthcare costs, and antibiotic resistance development (15). Prevalence studies on local UTI pathogens and their susceptibility patterns to antimicrobial agents are considered useful to guide empirical antibiotic therapy because the prevalence of uropathogens and their features could vary with time and geographical regions (11). Therefore, the present study investigated the

distribution and antimicrobial susceptibility of bacterial uropathogens in patients with UTIs. In agreement with other studies, we found that Gram-negative bacteria were common than others (3, 12, 16).

It is well known that the spectrum of pathogens isolated from patients suffering from a UTI is nearly stable, and *E. coli* remains the most common prevalent etiological agent (2, 17). The current study showed that *E. coli* was the predominant uropathogen in females in our locality. This was in agreement with the results obtained by other investigators in Iraq (2, 3). In the present study, *E. coli* accounted for 58.5% of the Gram-negative isolates, which is well comparable with the rates reported from outpatient studies conducted in Iran as 65.2% (18) and 51.5% (17). In another study conducted in Turkey, recruiting 429 females aged 18 to 65-years-old, *E. coli* (71.3%) was found to be the most common etiological agent of UTIs (16). In addition, *K. pneumoniae* was the second most common Gram-negative uropathogen recorded as 14.3%. In agreement with other studies, a study reported that *Klebsiella* spp. were observed as the second most common agents causing UTIs (26%) (18). Other frequent Gram-negative isolates included *P. mirabilis*, *P. aeruginosa*, *M. morgani*, and *A. baumannii*. This was in agreement with several previous studies that reported that these pathogens caused less frequent infections in females suffering from UTIs (2, 3, 19). The major contributing factor to the higher infection rates of *E. coli* in females could be due to poor genital hygienic practices by them. Because the most infecting sources of uropathogens are commensals of perianal and vaginal regions, an emphasis on personal hygiene, particularly in females, could be essential to reduce the prevalence rate of uropathogens.

Concerning antimicrobial susceptibility testing, it must be noted that significant changes in bacterial susceptibility patterns have been established over the last two decades (20). In several countries, for example, the average resistance rates of uropathogen isolates, especially *E. coli*, for ampicillin have increased by more than 50% (20). We found that *E. coli* was highly resistant to ampicillin (84.8%) and moderately resistant to aztreonam (58.7%), ceftriaxone (58.1%), and cefepime (57.4%). These results were similar to previous studies conducted in the USA (21), Iran (12), Turkey (16), and Iraq (19). On the other hand, most *E. coli* uropathogens were sensitive to ertapenem (98.7%), imipenem (97.7%), and amikacin (92.9%) in the current study.

Furthermore, *K. pneumoniae* demonstrated similar resistance patterns to *E. coli* and showed high sensitivity to ertapenem and imipenem and high resistance to ampicillin, aztreonam, ceftriaxone, and cefepime. Additionally, the sensitivity of *P. mirabilis* isolates to ampicillin, ceftriaxone, cefepime, aztreonam, and nitrofurantoin was between 60

Table 2. Antimicrobial Susceptibility Patterns of Gram-Negative Uropathogens

Antibiotics	Number of Isolates (Resistance Percentages)					
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. mirabilis</i>	<i>A. baumannii</i>	<i>P. aeruginosa</i>	<i>M. morganii</i>
Ampicillin	263 (84.8)	72 (94.7)	29 (80.6)	12 (92.3)	7 (77.8)	7 (100)
Amoxicillin/Clavulanic acid	126 (40.6)	30 (39.5)	10 (27.8)	12 (92.3)	6 (66.7)	6 (85.7)
Piperacillin/tazobactam	118 (38.1)	16 (21.1)	8 (22.2)	7 (53.8)	3 (33.3)	3 (42.8)
Cefazolin	113 (36.5)	38 (50)	18 (50)	12 (92.3)	6 (66.7)	4 (57.1)
Cefoxitin	142 (45.8)	28 (36.8)	13 (36.1)	12 (92.3)	5 (55.6)	3 (42.9)
Ceftriaxone	180 (58.1)	48 (63.2)	26 (72.2)	8 (61.5)	6 (66.7)	1 (14.3)
Cefepime	178 (57.4)	46 (60.5)	26 (72.2)	9 (69.2)	4 (44.4)	0 (0)
Aztreonam	182 (58.7)	55 (72.4)	32 (88.9)	10 (76.9)	6 (66.7)	0 (0)
Ertapenem	4 (1.3)	2 (2.6)	1 (2.8)	4 (30.8)	2 (22.2)	0 (0)
Imipenem	7 (2.3)	3 (3.9)	1 (2.8)	6 (46.2)	3 (33.3)	0 (0)
Amikacin	22 (7.1)	20 (26.3)	6 (16.7)	4 (30.8)	2 (22.2)	0 (0)
Gentamicin	68 (21.9)	12 (15.8)	7 (19.4)	7 (53.8)	3 (33.3)	1 (14.3)
Tobramycin	85 (27.4)	14 (18.4)	9 (25)	6 (46.2)	3 (33.3)	1 (14.3)
Ciprofloxacin	120 (38.7)	15 (19.7)	10 (27.8)	10 (76.9)	6 (66.7)	0 (0)
Levofloxacin	126 (40.6)	14 (18.4)	9 (25)	8 (61.5)	5 (55.6)	0 (0)
Nitrofurantoin	56 (18.1)	20 (26.3)	22 (61.1)	11 (84.6)	7 (77.8)	0 (0)
Trimethoprim/Sulfamethoxazole	163 (52.6)	37 (48.7)	21 (58.3)	1 (7.7)	4 (44.4)	6 (85.7)

Table 3. Antimicrobial Susceptibility Patterns of Gram-Positive Uropathogens

Antibiotics	Number of Isolates (Resistance Percentages)					
	<i>S. haemolyticus</i>	<i>S. epidermidis</i>	<i>E. faecalis</i>	<i>S. agalactiae</i>	<i>S. aureus</i>	<i>S. lentus</i>
benzylpenicillin	20 (100)	14 (87.5)	13 (76.5)	7 (43.8)	5 (100)	6 (100)
Oxacillin	19 (95)	13 (81.3)	15 (88.2)	8 (50)	3 (60)	5 (83.3)
Gentamicin	4 (20)	2 (12.5)	6 (35.3)	6 (37.5)	0 (0)	2 (33.3)
Tobramycin	4 (20)	6 (37.5)	7 (41.2)	9 (56.3)	1 (20)	2 (33.3)
levofloxacin	5 (25)	5 (31.3)	7 (41.2)	8 (50)	0 (0)	2 (33.3)
Moxifloxacin	1 (5)	0 (0)	9 (52.9)	7 (43.8)	0 (0)	1 (16.7)
Erythromycin	19 (95)	16 (100)	17 (100)	16 (100)	3 (60)	5 (83.3)
Clindamycin	8 (40)	6 (37.5)	17 (100)	12 (75)	4 (80)	3 (50)
Linezolid	1 (5)	0 (0)	2 (11.2)	0 (0)	1 (20)	1 (16.7)
Teicoplanin	3 (15)	3 (18.8)	3 (17.6)	5 (31.3)	1 (20)	2 (33.3)
Vancomycin	5 (25)	3 (18.8)	1 (5.9)	2 (12.5)	1 (20)	2 (33.3)
Tetracycline	12 (60)	7 (43.8)	17 (100)	13 (81.3)	1 (20)	2 (33.3)
Tigecycline	0 (0)	1 (6.3)	0 (0)	1 (6.3)	1 (20)	1 (16.7)
Nitrofurantoin	0 (0)	0 (0)	0 (0)	5 (31.3)	0 (0)	1 (16.7)
Rifampicin	2 (10)	3 (18.8)	9 (52.9)	10 (62.5)	1 (20)	3 (50)
Trimethoprim/Sulfamethoxazole	7 (35)	3 (18.8)	11 (64.7)	4 (25)	2 (40)	1 (16.7)

and 89% in the current study, which was similar to those reported in a study in Taiwan (22). In this study, the antibi-

otic sensitivity patterns of *A. baumannii*, *P. aeruginosa*, and *M. morganii* were comparable with previous studies con-

ducted in Ethiopia (23) and Iraq (2). The sensitivity pattern of *P. aeruginosa* was alarming in this study, and we found that 67-78% of the isolates were resistant to the used antibiotics, including ampicillin, amoxicillin/clavulanic acid, ceftazolin, ceftriaxone, cefepime, ertapenem, ciprofloxacin, and nitrofurantoin. Such high resistance rates to antibiotics in our region can be explained partially by the high rate of antibiotic abuse in the region.

In the present study, 57 (8.9%) isolates of *Staphylococcus* spp. were obtained from UTI cases. Besides, *S. haemolyticus* was the most frequent species (3.8%), followed by *S. epidermidis* (3.02%), *S. lentus* (1.12%), and *S. aureus* (0.94%). In contrast, a recent study conducted in Iraq found that 50% of uropathogens were Gram-positive, and the vast majority of them were *Staphylococcus* spp. (2, 19). Besides, in another study carried out in Iraq on 371 females afflicted with UTIs, *Staphylococcus* spp. were only found in two (0.5%) cases of UTIs (3). Such discrepancies are difficult to explain and could be due to the differences in sample collection, study design, and patient recruitment. Other frequent Gram-positive isolates reported in the current study were *E. faecalis* and *S. agalactiae*, which were responsible for only around 3% of the cases. In the present study, the vast majority of Gram-positive isolates were resistant to benzylpenicillin, oxacillin, clindamycin, erythromycin, and erythromycin accounting for 60% - 100%, while they were highly sensitive to nitrofurantoin, tigecycline, moxifloxacin, and linezolid (100%). Therefore, the use of these antibiotics, especially nitrofurantoin and linezolid, could be considered as empirical treatment for a suspected UTI.

In conclusion, in the present study, *E. coli* was the most frequent Gram-negative uropathogen causing UTIs among female subjects, followed by *K. pneumoniae* and *P. mirabilis*, while *Staphylococcus* spp. were most predominant among Gram-positive isolates. Ertapenem and imipenem showed to be the most appropriate antibiotics for empirical therapy for Gram-negative uropathogens, whereas linezolid, tigecycline, and nitrofurantoin were for Gram-positive isolates. The continuous monitoring of sensitivity patterns is needed to determine the antibiotics of choice for empirical treatment.

Footnotes

Authors' Contribution: We confirm that the manuscript has been contributed, reviewed, and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Conflict of Interests: The authors declare that no competing interest exists.

Ethical Approval: The study and the protocol of attaining consent were approved by the Scientific Committee at the College of Medicine, University of Zakho, Kurdistan, Iraq. Formal consent was obtained from the patients recruited in this study. Only participants who gave their consent before sample collection were included in the study.

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