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Original Article

Prevalence and Antibiotic Resistance Pattern of Pathogenic Bacteria Isolated From Urinary Tract Infections in Qal'at Saleh Hospital, Iraq

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Abstract

Background: Antibiotic resistance emerged in the pathogens causing urinary tract infections (UTIs) and became widespread. Moreover, increasing drug resistance has highlighted the need to evaluate the antibiotic resistance pattern to improve experimental treatment. The purpose of this study was to evaluate the bacteria causing UTIs and their susceptibility patterns based on the geographical area.

Methods: The present study was conducted on outpatients referred to Qal'at Saleh Hospital in Iraq from January 2018 to January 2019. The pathogenic bacteria were detected using API 20E kit. The antimicrobial susceptibility testing was conducted using the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Results: Of 216 isolates, 87.9% contained gram-negative bacteria and 12.03% contained gram-positive bacteria. In this study, *Escherichia coli* was identified as the main cause of UTIs. Of all the isolates, 73.61% were resistant to three or more classes of antibiotics. The antibiotic susceptibility and resistance patterns of all isolates showed that amikacin and ciprofloxacin had the highest activity against gram-negative bacteria and vancomycin, amikacin, and levofloxacin had the highest activity against gram-positive bacteria.

Conclusions: Due to the widespread resistance to drugs used in the treatment of UTIs, it is difficult to select the appropriate drugs for treating UTIs. UTI affects different age groups; therefore, sufficient knowledge should be transferred to the community to prevent these infections. If urine culture is unavailable, or it is impossible to wait for antibiotic susceptibility testing, Amikacin and Vancomycin might be the best candidates for UTI treatment.

Keywords: Urinary tract infections, Drug resistance, Anti-bacterial agents, Multidrug resistance

Background

Urinary tract infections (UTIs) are among the most common infections, affecting 150 million people worldwide each year (1). Most UTIs are caused by the transmission of bacteria from the fecal flora and through the urethra (2). Some human physiological and anatomical factors, incomplete bladder emptying, and vesicoureteral reflux, especially in the elderly and pregnant women, play major roles in increasing the prevalence of UTI. UTIs are mainly caused by bacteria, and sometimes by viruses, fungi, and parasites (3, 4). Among bacteria, gram-negative bacteria are the most common causes of UTIs, including *Escherichia coli*, *Klebsiella spp.*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., and *Serratia* spp. *E. coli* is the most prevalent agent that has been isolated from the urine samples of 70% to 90% of infected people (5). Only 10% of reported UTI cases are caused by gram-positive bacteria, including *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Enterococcus faecalis* (6).

The most appropriate treatment for bacterial infections is the selection of an antibiotic with high efficiency. Due to the acquisition of antibiotic resistance genes by bacteria over time and the changing antimicrobial resistance pattern of bacteria, choosing the right antibiotic for treatment has become a challenge, mostly based on information obtained from their antimicrobial resistance pattern in the area (7-9). Researchers believe that the causes of UTIs and drug susceptibility patterns can vary depending on geographical, social, and biological conditions (10-12). In many infectious diseases, including UTI, the physician needs to begin treatment before identifying the cause of the infection and its antibiotic susceptibility, which requires sufficient knowledge of the possible cause of the infection and its antibiotic susceptibility pattern in order to prescribe the appropriate medication (13).

The emergence of antibiotic resistance in UTIs is a serious public health problem, especially in developing countries, where there is a high level of ignorance, poverty, and poor hygiene practices (14). Over the past few decades, reports of antibiotic resistance in UTI-causing bacteria have increased dramatically worldwide. Rapid detection and culture facilities are not available in many areas in developing countries, which may lead to misdiagnosis or self-medication, resulting in increased antibiotic resistance among urinary tract pathogens (13). Therefore, the selection of appropriate antibiotics for the treatment of UTI should be based on antibiotic susceptibility profiles of different bacteria causing the infection.

In developing countries, including Iraq, UTIs occur every year, and the administration of inappropriate antibiotics, due to lack of information about pathogens and their resistance profiles, increases the duration of treatment and drug resistance in other bacteria. The aim of this study was to evaluate the bacterial species isolated from outpatients with UTI and to determine their antibiotic susceptibility pattern at Qal'at Saleh Hospital in Maysan Governorate, Iraq.

Materials and Methods

Uropathogenic Isolates

This study was performed from January 2018 to January 2019 at Qal'at Saleh Hospital in Maysan Governorate, southern Iraq. A total of 830 patients with clinical symptoms of UTI were referred to the bacteriology laboratory, including 460 (55.4%) females and 370 (44.5%) males.

Bacterial Culture

The inclusion criteria in this study included not taking specific antibiotics, absence of any infectious disease, and not being hospitalized for two weeks before referring to the laboratory. The samples were collected from the mid-stream urine, cultured on blood agar and MacConkey agar media (Salucea, New Zealand), and incubated at 37°C for 24 hours. The samples with a colony count of $\geq 10^5$ CFU/mL were considered positive UTI samples (15). The isolates were identified based on their morphology and using Api20 kit (BioMérieux, France) and divided into two groups of gram-negative and gram-positive bacteria.

Antibiotic Susceptibility Test

The antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Müller-Hinton agar medium (Merck Co., Germany) according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2017) (16). Twenty-four commercial antibiotic discs (Bioanalyse Co., Turkey) were used including amikacin (AK, 30 µg), vancomycin (VA, 10 µg), nalidixic acid (NA, 30 µg), augmentin (AMC, 30 µg), gentamicin (GEN, 10 µg), norfloxacin (NOR, 10 µg), ticarcillin (TI, 75 µg), piperacillin (PI, 100 µg), doxycycline (Do, 30 µg), ceftazidime (CAZ, 30 µg), cefixime (CFM, 5 µg), nitrofurantoin (NIT, 100 µg), imipenem (IMP,10 µg), aztreonam (AZT,30 µg), ciprofloxacin (CIP, 5 µg), trimethoprim (TMP, 5 µg), ceftriaxone (CTR, 30 µg), ampicillin (AMP, 25 µg), cefoxitin (CX, 30 µg), linezolid (L, 10 µg), clindamycin (CD, 2 µg), co-trimethoprim (COT, 30 µg), erythromycin (E, 10 µg), and levofloxacin (Lev,5 µg).

Statistical Analysis

Data were analyzed using IBM SPSS version 22.0 (IBM SPSS Inc., USA). Fisher's exact test was used for statistical analysis at a significance level of $P < 0.05$.

Results

Etiological Characteristics of UTIs

In this study, 830 patients with clinical symptoms of UTI were referred to the laboratory; out of 216 positive urine culture samples, 63.89% were female (n=138) and 36.11% were male (n=78). According to the chi-square test, there was a significant relationship between female gender and UTI ($P < 0.05$). The patient's ages ranged from 8 months to 87 years. Among all isolates, 190 isolates (87.97%) were gram-negative bacteria and 26 isolates (12.03%) were gram-positive bacteria. No sample was found to contain both gram-negative and gram-positive bacteria. in this study, *E. coli*, *P. mirabilis*, *E. aerogenesis*, *S. aureus*, and *E. faecalis* were isolated from UTI samples. Among gram-negative uropathogens, *E. coli* and *P. mirabilis* were the most frequently isolated bacteria, with prevalence rates of 47.2% and 29.62%, respectively. The most common gram-positive uropathogen was *S. aureus,* with a prevalence of 9.7% (Figure 1). The overall prevalence of bacteria was higher among women than among men and *E. faecalis* was not observed among male patients (Table 1). The highest prevalence was observed in the age group of 0-5 years (32.4%), while the lowest number of isolates was seen in the age group of 50-84 years (3.7%), all of which were *E. coli*. The number of UTI cases decreased with increasing age (Figure 2).

Drug Resistance Pattern

The antibiotics used in this study were selected according to the guidelines of the Iraqi Ministry of Health for culture

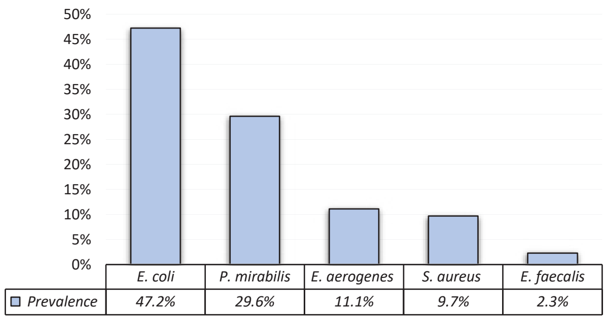

Figure 1. Prevalence of Bacteria Isolated from Urine Samples of Outpatients.

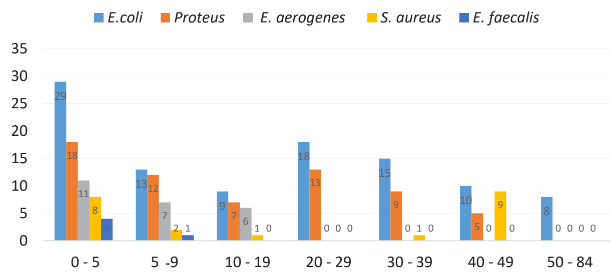

Figure 2. Frequency of Pathogens among Different Age Groups.

of urine samples. The antibiotic resistance and sensitivity of gram-negative and gram-positive bacteria are detailed in Tables 2 and 3. AK and CIP showed the highest activity against *E. coli* and *P. mirabilis*. While AMC and TI showed the lowest activity against *E. coli,* and TMP and NIT showed the lowest activity against *P. mirabilis*. *E. aerogenes* showed the highest sensitivity to AK and CAZ and lowest sensitivity to AMC. In general, the antibiogram profiles of gram-negative bacteria showed that AK and CIP were the most effective drugs. *E. faecalis* showed resistance to AMC, COT, and CTR, while the most effective antibiotics included VA and AK. *S. aureus* showed the highest resistance to AMP and the lowest resistance to VA and LEV. Only one isolate of *P. aeruginosa* was identified, which was highly resistant to most of the antibiotics used; however, IMP (78%) and AK (63%) were the most effective antibiotics against the bacterium. The resistance rates of gram-negative and gram-positive UPEC isolates to different antibiotics are shown in Figures 3 and 4.

Multidrug Resistance

Of all the isolates, 73.61% were resistant to three or more classes of antibiotics. The prevalence of multidrug resistance (MDR) of *E. coli*, *P. mirabilis,* and *E. aerogenes* was reported to be 83.3%, 90.62%, and 87.5%, respectively. All *S. aureus* isolates were MDR and resistant to all classes of antibiotics. In contrast, the lowest MDR rate belonged to *E. faecalis* (40%). The resistance to the highest number of antibiotic classes was observed in *S. aureus* (9 classes) and *P. mirabilis* (8 classes), respectively. The MDR rate of each strain is shown in Figure 5.

Table 1. Comparison of the Distribution of Isolates in Both Males and Females

Gender	*E. coli*	*P. mirabilis*	*E. aerogenes*	*S. aureus*	*E. faecalis*	Total
Female	65	35	19	14	5	138
Man	37	29	5	7	0	78
Total	102	64	24	21	5	216
P value	0.03	0.02	0.001	0.02	0.06	

Table 3. Antibiotic Susceptibility Pattern of Gram-Positive Bacteria Isolated from Urine Samples of Outpatients

E. faecalis = 5				*S. aureus* = 21			
Ab	S (%)	I (%)	R (%)	Ab	S (%)	I (%)	R (%)
VA	100	0	0	VA	100	0	0
AK	76.19	14.28	9.52	LEV	100	0	0
L	52.38	4.76	42.85	CIP	80	20	0
CIP	47.61	4.76	47.61	NOR	80	20	0
CX	42.85	4.76	52.38	DO	40	20	40
NOR	33.33	9.52	57.14	L	40	0	60
GEN	28.57	4.76	66.66	E	20	20	60
CD	28.57	4.76	66.66	NIT	20	20	60
DO	23.8	19.04	57.14	AMP	20	0	80
NIT	9.52	9.52	80.95				
AMP	4.76	4.76	90.47				
AMC	4.76	0	95.23				
COT	4.76	0	95.23				
CTR	0	4.76	95.23				

Ab: antibiotic; R: resistant; I: intermediate; S: susceptibility.

Discussion

The antibiotic *resistance* of *uropathogenic strains* is a significant concern for treating UTIs and it is *increasing day by day.* Improper administration and incomplete course of antibiotics to treat this infection increased the drug resistance of pathogens (17,18). Therefore, there is a need for continuous monitoring of UTI-causing agents and their resistance/sensitivity pattern in a region.

Table 2. Antibiotic Susceptibility Pattern of Gram-Negative Bacteria Isolated from Urine Samples of Outpatients

Ab	*E. coli* = 102			*P. mirabilis* = 64			*E. aerogenes* = 24			Total			*P* Value
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	
AK	2.94	14.7	82.35	9.37	21.87	68.75	8.33	4.16	87.5	6.88	13.57	79.53	0.014
CIP	36.27	8.82	54.9	37.5	3.12	59.37	25	4.16	66.66	32.92	5.36	60.31	0.001
GEN	29.41	20.58	50	50	10.93	39.06	29.16	4.16	45.83	36.19	11.89	44.96	0.001
NIT	27.45	26.47	46.07	81.25	6.25	12.5	58.33	29.16	12.5	55.67	20.62	23.69	0.018
NOR	40.19	13.72	46.07	40.62	7.81	51.56	29.16	4.16	66.66	36.65	8.56	54.76	0.085
CAZ	48.03	14.7	37.2	53.12	0	46.87	16.66	12.5	70.83	39.27	9.06	51.63	0.03
CTR	55.88	7.84	36.27	53.12	0	46.87	50	0	50	53	2.61	44.38	0.043
AT	62.74	2.94	34.31	73.43	6.25	20.31	62.5	4.16	33.33	66.22	4.45	29.31	0.001
CFM	53.92	13.72	32.35	53.12	1.56	45.31	50	4.16	45.83	52.34	6.48	41.16	0.054
NA	74.5	10.78	14.7	68.75	9.37	21.87	58.33	25	16.66	67.19	15.05	17.74	0.004
TMP	78.43	6.86	14.7	85.93	10.93	3.12	87.5	4.16	8.33	83.95	7.31	8.71	0.241
PI	80.39	6.86	12.74	70.31	0	29.68	83.33	8.33	8.33	78.01	5.06	16.91	0.118
TI	81.37	7.84	10.78	68.75	1.56	29.68	83.33	8.33	8.33	77.81	5.91	16.26	0.006
AMC	81.37	11.76	6.86	70.31	7.81	21.87	83.33	12.5	4.16	78.33	32.07	10.96	0.341

Ab: antibiotic, R: resistant, I: intermediate, S: susceptibility, Total: The amount of susceptibility, intermediate activity, and resistance to each antibiotic in total Gram-negative isolates.

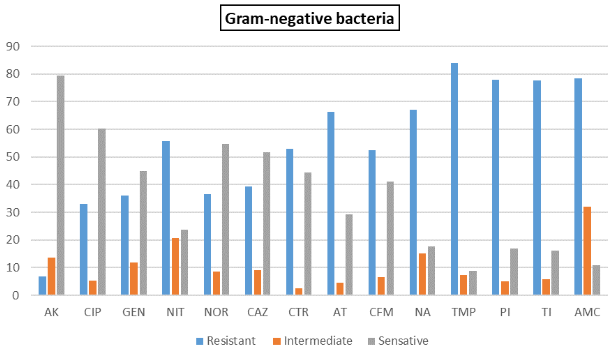

Figure 3. Drug Resistance in Gram-Negative Bacteria.

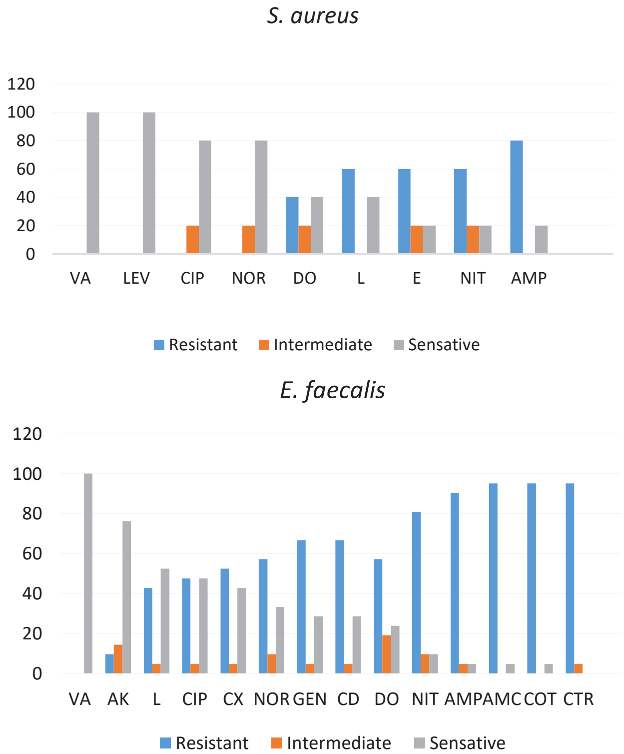

Figure 4. Drug Resistance in *E. faecalis* and *S. aureus*.

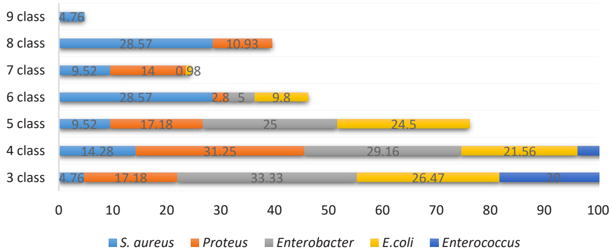

Figure 5. Multidrug Resistance of Bacteria.

The present study showed that the prevalence of UTI is higher in women than in men (138 versus 78), which is consistent with studies conducted in Germany (19), France (20), Turkey (21), Iran (22), and India (7). UTIs have been reported in all age groups, and some studies have reported that the prevalence of the disease increases with age (1, 23). However, in this study, the highest prevalence of the disease (32.4%) was observed in the age range of 0-5 years, which is in line with a study conducted by Luty et al in Iraq (18). The high prevalence of infection in childhood may be due to the poor culture and lack of mother's knowledge about child health.

The results showed that gram-negative bacteria are the most common causes of UTI; the highest prevalence of gram-negative and gram-positive bacteria was 87.9% and 12.03%, respectively. Ullah et al in Pakistan (73%) and Kimando et al in Kenya (89.5%) reported a higher prevalence of gram-negative bacteria than gram-positive bacteria, which is consistent with our study (24, 25). Gram-negative bacteria have several unique structures or virulence factors that help them attach to cells in the urinary tract and not be excreted in the urine, which allows them to multiply and invade tissue (26). The present results showed that *E. coli* is the most prevalent gram-negative bacterium (47.2%) among UTI patients, which is consistent with studies conducted in Iraq (18, 27), Iran (22), and India (28). *Enterococcus* has been reported as the second leading cause of UTIs (29), but it had the lowest prevalence (2.3%) in the present study, which is consistent with studies conducted in Iraq (18, 30), India (7), Iran (29), and Uganda (31). Yolbaş et al in Turkey (15) and Vakili et al in Iran (32) showed that 2.7% of UTI cases were caused by *Klebsiella* and 6% were caused by *Streptococcus*, which is inconsistent with the results of the present study. Differences in the prevalence and diversity of uropathogens can be due to differences in cultural traditions, environmental factors, personal hygiene, and healthcare facilities (18).

Antibiotic resistance is a major clinical problem in the treatment of infections, especially UTIs. Drug resistance has increased over time, and the rate of resistance varies from country to country. In general, the isolates from Latin American countries show the lowest susceptibility to all antimicrobial drugs, followed by isolates from Asia-Pacific and European strains. Strains from Canada showed the best global susceptibility testing results (7).

In this study, *E. coli* isolates were susceptible to Ak (82.35%), and the highest resistance was observed against AMC (81.37%), which is consistent with the results of studies conducted by Demirci et al and Shakhatreh et al, respectively (33, 34). *P. mirabilis* showed the highest susceptibility to AK (68.75%) and CIP (59.37%) and the lowest susceptibility to TMP (3.12%) and NIT (12.5%). In a study by Mama et al (2014), *P. mirabilis* was 100% resistant to NIT (100%) and TMP/SMX (100%), but it was sensitive to AK (40%) (35). *E. aerogenes* showed the highest susceptibility to AK (87.5%) and CAZ (70.83%). Prakash et al (2013) reported that the susceptibility to AK and CAZ in *E. aerogenes* were 81.82% and 90.91%, respectively (36). In the present study, the prevalence of *S. aureus* was 9.7% and it showed the lowest sensitivity to AMP, but all isolates were 100% sensitive to VA and LEV, which is in line with the results of some previous studies (31, 37). All isolates of *E. faecalis* were susceptible to VA (100%) and AMP (90.47%). Yolbaş et al reported the highest susceptibility (93.3%) to VA and the highest resistance to AMP (15). *Pseudomonas aeruginosa* was isolated from only one patient, which is inconsistent with a study conducted by Luty et al (18). It was highly resistant to most of the antibiotics used; however, IMP (78%) and AK (63%) were most active against *P. aeruginosa*.

Of all the isolates, 73.61% were resistant to three or more classes of antibiotics, which is consistent with studies conducted by Bajpai et al and Eshetie et al (38, 39). MDR rates are lower in some countries than in Iraq (23); this difference may be due to the use of different MDR detection methods, the number and types of antibiotics used, changes in the pattern of bacterial strains tested, and differences in the social demographic characteristics and lifestyle of the study population (8, 40). The high prevalence of MDR UTI isolates in our study may be related to the increased misuse of antibiotics, leading to the development of organisms carrying resistance genes.

Conclusions

In this study, gram-negative bacteria were the most prevalent among the samples of patients referred to the hospital. Five species of UTI-causing bacteria were isolated from patients, with *E. coli* being the most common. The susceptibility of all bacteria tested in this study to CIP and AK in gram-negative bacteria and to VA, LEV, and AK in gram-positive bacteria was significantly higher than other antibiotics. Due to the increasing use of antibiotics and the spread of antibiotic resistance, it is necessary to control the emergence of antibiotic resistance. One of the most important factors influencing this phenomenon is the improper and incorrect use of antibiotics, and efforts should be made to use antibiotics properly and correctly.

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Conflict of Interests

None.

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