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Antibacterial Susceptibility Pattern of Urine Bacterial Isolates in Diyala, Iraq

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Abstract **Objectives:** UTIs are a common bacterial illness, often treated by doctors. However, antibiotic misuse increases the likelihood of resistant microorganisms. This study aimed to determine the frequency of UTI-causing microorganisms and their susceptibility to antibiotics, as these bacteria are more resistant to antimicrobial medicines. This study aims to identify prevalent uropathogens in Diyala governorate's urine samples, evaluate their resistance against antibiotics, and guide empirical therapy for urinary tract infections. **Materials and Methods:** Between February 2024 and April 2024, 100 urine samples were taken from patients who visited the Teaching Albatool Hospital and the Teaching Ba'aqubah Hospital as part of this prospective study. Vitek Compact 2 devices were used to confirm the bacterial pathogens that were identified using bacteriological culture techniques. The disc diffusion method was used to test for antibacterial susceptibility. **Results:** Seventy of the 100 samples exhibited a positive culture. Gram-negative bacteria, specifically Escherichia coli 24 (34.3%), Klebsiella pneumonia 10 (14.3%), Staphylococcus aureus (13; 18.5%), Aeromonas hydrophila 8 (11.4%), Proteus mirabilis 9 (12.9%), and S. saprophyticus 6 (8.6%), were the most common bacterial pathogens. Antibiotic resistance was elevated in the isolated Uropathogen. Staphylococcus aureus, Klebsiella pneumoniae, and Escherichia coli showed the highest levels of antibiotic resistance. Imipenem, Meropenem, Ceftazidime, Cefepime, Cefotaxime, and Ceftriaxone Both ampicillin and sulbactam Gentamicin, Amikacin, Ciprofloxacin, Levofloxacin, Ticarcillin-clavulanic acid, and Piperacillin-Tazobactam **Conclusions:** The findings emphasised the emergence of bacteria that are highly resistant to most of the tested medications and recommended that physicians adjust their treatment regimens in light of antimicrobial susceptibility findings.

Key Words Antibiotic resistance pattern, Urinary tract infection, Urine culture

INTRODUCTION

UTIs are common bacterial infections affecting various parts of the urinary system, with women being more susceptible due to their anatomy and reproductive function.[1] They are common in outpatients and can be caused by bacteria, viruses, and fungi. Only 10% of UTI infections are caused by gram-positive bacteria, while 90% are caused by gram-negative bacteria [2].

Numerous factors that determine the presence of bacteria (more than 10 mL) in the urine determine the occurrence of UTIs [3]. These bacteria can develop and have serious repercussions for the patient if left untreated [4,5,6]. With 65–90% of UTIs caused by Escherichia coli, the most common isolated uropathogen is followed by Staphylococcus species, which make up about 10-15% [7]. Furthermore, the transmission of infections is not significantly aided by bacterial species such as Klebsiella, Pseudomonas, Proteus, and Enterococcus [1].

Several variables, such as age, parity, gravidity, pregnancy, and the existence of illnesses that worsen the infection, are associated with UTI [1]. Antibiotic treatment for UTIs is typically administered before the microbiology test results are obtained. Treatment failure and antibiotic resistance will ensue from using this medication without a valid prescription [3,8]. The discovery of antibiotics was one of the greatest advances in modern medicine [9].

However, irrational use of antibiotics contributes to the everyday rise in antibiotic resistance, especially in nations like Libya that have unconstrained antibiotic policies and no precise goals [10]. According to the World Health Organisation in 2014, antimicrobial resistance is posing a serious threat to contemporary medicine and is becoming a worldwide public health concern. As a result, all nations

have focused on this issue [11ven though UTIs are common, they can be promptly resolved with the appropriate administration of antibiotics [4]. Antibiotic resistance can be effectively treated by identifying and studying the microorganisms that cause UTIs [12]. This study aims to determine the antibacterial susceptibility patterns of bacterial isolates from urinary tract infections in Diyala, Iraq, with a focus on identifying multidrug-resistant strains and guiding appropriate antibiotic use. Finding the frequency of bacteria linked to UTI cases and their pattern of antibiotic susceptibility was the aim of this investigation.

MATERIALS AND METHODS

Study Setting

Patients visiting Al-Batoul Teaching Hospital and Baqubah Teaching Hospital in Diyala, Iraq, constituted the study population. This study, which ran from February 2025 to March 2025 and was approved by the Ethics Board of the Department of Biology, University of Diyala, included 100 untreated outpatients with various clinical presentations of urinary tract infections. Patients who had taken antibiotics within 3 days of sample collection were excluded.

This cross-sectional study aims to investigate the prevalence and antimicrobial susceptibility patterns of bacterial pathogens isolated from urine samples of patients in Diyala Province, Iraq, in order to identify the dominant Uropathogen and assess the extent of antibiotic resistance to support effective treatment strategies. describe the percentage of E. coli that are resistant to ciprofloxacin.

Inclusion and Exclusion Criteria

This study included patients aged 20 years or older who had a history of recurrent UTIs or who had symptoms of suspected UTIs (pyelonephritis or cystitis). Otherwise, patients who had a nephrostomy tube, urinary stent, or catheter and asymptomatic bacteriuria were not included.

Sample Collection and Sampling Technique

The sterile plastic disposable container was used to collect midstream urine samples in the early morning, and the samples were promptly transferred to the laboratory for analysis after each patient was given the proper sampling instructions [13]. The urine specimens were then inoculated on four different types of media: Blood agar, Mannitol salt agar, MacConkey agar, and Cystine lactose electrolyte deficient agar CLED plates [14]. All inoculated plates were then incubated at 37° C for 24 to 48 hours to observe growth. Urine samples with a colony count of over 105 cfu/mL were considered pathogenic. Bacterial colonies were identified using gram staining and Vitek 2 compact system. Antimicrobial susceptibility tests were performed using the modified Kirby-Bauer disc diffusion method. Antibiotics used included Ceftazidime, Cefepime, Cefotaxime, Ceftriaxone, Imipenem, Meropenem, Ampicillin-sulbactam, Piperacillin-Tazobactam, Ticarcillin-clavulanic acid, Gentamicin, Amikacin, Ciprofloxacin, and Levofloxacin. Isolates were classified as sensitive, intermediate, and resistant according to the standardized table.

Mueller Hinton agar plates were streaked using a sterilized cotton swab. Using an aseptic method, filter paper disks with a certain concentration of the antimicrobial medications were positioned on the agar surface. The zone of inhibition's diameter was measured and compared to the antibiotic sensitivity table in order to understand the antibiotic results. More than 18 mm was regarded as a sensitive zone, 13–18 mm as an intermediate zone, and less than 13 mm as a resistant zone [14]. Strains that demonstrated resistance to three or more of the tested drugs were classified as multi-drug resistant (MDR).

Statistical Analysis

Data were presented as frequency and percentages. Statistical analysis was performed using Package of Social Sciences (SPSS) version 26.

RESULTS

Of the 100 urine samples that were included, 60 were from females and 40 were from males between the ages of 20 and 70. However, only 70 (70.00%) of the total samples tested positive for bacterial growth, with 30 (30.00%) samples showing no bacterial growth and 28 (40.00%) and 42 (60.00%) samples being male and female, respectively. Of the entire culture, 42 (60.00%) were female and 28 (40.00%) were male.

The prevalence of Gram-negative bacteria was higher than that of Gram-positive bacteria. The most frequent Gram-negative isolates organisms were *Escherichia coli* 24(34.3%), the second most prevalent isolate was followed by *Staphylococcus aureus* 13 (18.5%), Klebsiella pneumoniae 10(14.3%), *Proteus mirabilis* 9(12.9%) and *Aeromonas hydrophila* 8(11.4%). Meanwhile, and *S. saprophyticus* 6 (8.6%) were the most often isolated gram-positive bacteria. Additionally, our study found that the prevalence of UTI was higher in women than in men, 42 (60.00%) and 28 (40.00%), respectively. These results are consistent with other studies that found that women experience UTIs more frequently than men [13]. Additionally, this outcome is consistent with the findings of Mahmoud and colleagues' 2016 paper. Similar results have also been reported by numerous other researchers [2,8,20] (Table 1).

Bacterial isolates were subjected to antibiotic susceptibility testing for 12 antibiotics using the disk diffusion method. The antibiotics' diameters of inhibition were measured and contrasted with the CLSI (2020) diameters of inhibition. as displayed in Figure 1 and Table 2.

The results showed that all *E. coli* isolates were resistant to Imipenem, Ciprofloxacin, Gentamicin, and Amikacin (100%), with the highest sensitivity being to Ticarcillin-clavulanic acid (25.0%). *Klebsiella pneumoniae* isolates also showed resistance to Cefotaxime, Ampicillin-sulbactam,

Table 1: Frequency of bacterial agents isolated from urine specimens according to patient gender

Isolate	Gender No.	Age (years)	No. of isolates
Escherichia coli	Female (18)	20-35	24(34.3%)
	Male (6)		
Klebsiella pneumoniae	Female (6)	43 – 47	10(14.3%)
	Male (4)		
Staphylococcus aureus	Female (8)	50-56	13(18.5%)
	Male (5)		
Aeromonas hydrophila	Female (3)	59-63	8(11.4%)
	Male (5)		
Proteus mirabilis	Female (5)	55- 60	9(12.9%)
	Male (4)		
S. saprophyticus	Female (2)	35-47	6(8.6%)
	Male (4)		
Total	Female	42 (60.00%)	70(70.0%)
	Male	28 (40.00%)	

Table 2: Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards

Group		Antibiotics	Code	Disc (µg)
β–lactam	Pencillines	Piperacillin	PRL	100µg
		Ceftazidime	CAZ	30µg
		Cefepime	FEP	10µg
	Cephems	Cefotaxime	CTX	10µg
		Ceftriaxone	CRO	10µg
	Carbapenems	Imipenem	IMP	10µg
		Meropenem	MEM	10µg
β-lactam combination agents		Ampicillin –sulbactam	AMS	20µg
		Piperacillin –tazobactam	TPZ	110µg
		Ticarcillin –clavulanic acid	TTC	85µg
Aminoglycosides		Gentamicin	CN	10µg
		Amikacin	AK	10µg
Fluoroquinolones		Ciprofloxacin	CIP	10µg
		Levofloxacin	LEV	5 µg

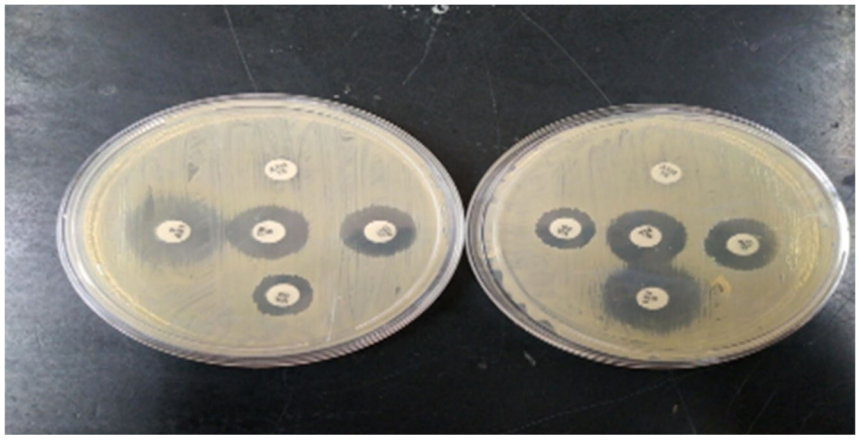

Figure 1: Clinical and Laboratory Standards Institute, the antimicrobial susceptibility test (Antibiotic susceptibility testing)

Gentamicin, and Amikacin (100%). As for *Staphylococcus aureus*, resistance to Ampicillin-sulbactam, Levofloxacin, and Cefotaxime (100%) was demonstrated. Its sensitivity to the antibiotic Piperacillin-Tazobactam was (100%), and it was moderately sensitive (15.4%) to the antibiotic Amikacin, which belongs to the aminoglycosides, and it works through the complete union with the subunits (30s) of the ribosomes (70s) only, without affecting the ribosomes (80s) or their subunits found in higher organisms, as this antibiotic interferes with the functions of the cellular structures of the ribosomes, stopping them from working, which leads to killing the bacteria.

As for *A. hydrophila*, it was resistant to the antibiotics Ceftazidime, Amikacin, and Levofloxacin (100%), semi-sensitive to Cefotaxime (25.0%), and sensitive to Meropenem (100%). *Proteus mirabilis* was 100% resistant to

Table 3: Prevalence and antibacterial resistance of bacterial agents isolated from urine species

Antibiotic	*E. coli*	*K. pneumonia*	*S. aureus*	*A. hydrophila*	*P. mirabilis*	*S. saprophyticus*
	N=24	N= 10	*N=13*	N= 8	N=9	N=6
CAZ (30µg)	17(70.8%)	(70%)7	11(84.6%)	8(100.0%)	9(100.0%)	6(100.0%)
FEP (10 µg)	22(91.7%)	(90%)9	12(92.3%)	5(62.5%)	4(44.4%)	1(16.7%)
CTX (10 µg)	16(66.7%)	10(100.0%)	13(100.0%)	2(25.0%)	9(100.0%)	3(50.0%)
IMP (10µg)	24(100.0%)	8(80%)	10(76.9%)	7(87.5%)	5(55.6%)	5(83.3%)
MEM (10µg)	18(75.0%)	5(50%)	7(53.8%)	0(00.0%)	3(33.3%)	0(00.0%)
AMS (20µg)	22(91.7%)	10(100.0%)	13(100.0%)	3(37.5%)	0(00.0%)	6(100.0%)
TPZ (110µg)	(%83.3)20	4(40%)	(% 00.0)0	6(75%)	8(88.9%)	5(83.3%)
TTC (85µg)	(%25.0)6	0(0%)	9(69.2%)	4(50%)	7(77.7%)	4(66.7%)
CN (10µg)	24(100.0%)	10(100.0%)	11(84.6%)	5(62.5%)	6(66.7%)	3(50.0%)
LEV (5 µg)	(%41.7)10	3(30%)	13(100.0%)	8(100.0%)	9(100.0%)	0(00.0%)
CIP (10µg)	24(100.0%)	9(90.0%)	12(92.3%)	4(50.0%)	7(77.8%)	6(100.0%)
AK (10µg)	(%100.0)24	10(100.0%)	(%15.4) 2	8(100.0%)	7(77.8%)	5(83.3%)

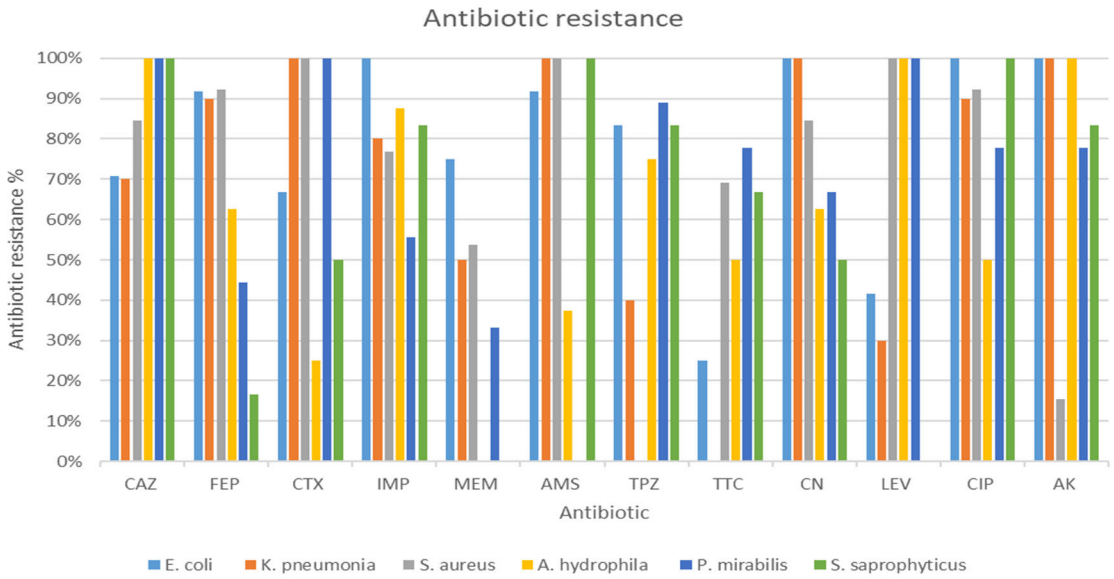

Figure 2: Prevalence and antibacterial resistance of bacterial agents isolated from urine species

all antibiotics, including ceftazidime, levofloxacin, and ampicillin-sulbactam. *S. saprophyticus* was 100% resistant to all antibiotics, including ceftazidime, ciprofloxacin, and ampicillin-sulbactam. *S. saprophyticus* was 100% resistant to all antibiotics, including ceftazidime, ciprofloxacin, and ampicillin-sulbactam. It was 16.7% semi-sensitive to cefepime. As shown in Table 3 and Figure 2.

DISCUSSION

The overuse of antibiotics to treat diseases has detrimental effects on public health organizations, the economy, and the development of drug resistance in the bacteria that cause the infections. Therefore, the study's primary objective was to regularly evaluate the level of antibiotic resistance in a population, particularly about UTIs.

High antibiotic resistance was shown by the isolates from UTIs in the current investigation. The majority of bacteria exhibited resistance to three or more antibiotics. In impoverished nations like Iraq, where medications are widely accessible without a prescription, antibiotic resistance is a prevalent occurrence.

The primary Uropathogen responsible for the current study is *E. coli*, which also demonstrated resistance to IMP, CN, CIP, and AK and a resistance pattern like Klebsiella's. This conclusion contradicts the findings of earlier studies in Diyala that found lower rates of resistance to the antibiotics in question [17,18,19]. In contrast to the findings of earlier research in Diyala, we also discovered that the majority of Uropathogen showed strong sensitivity to Cefixime, followed by FEP [17,18]. According to Najim Abbas *et al.* (2018), in Baqubah, Diyala study, Most frequent pathogen: *E. coli* (31.8%), *Proteus mirabilis* (18.2%), *K. pneumoniae* (11.9%), *P. aeruginosa* (12.7%). Observed high resistance (>95%) to aztreonam, cefotaxime, and co-trimoxazole; moderate resistance (70–95%) to tetracycline and gentamicin; better sensitivity to amikacin (45%), ciprofloxacin (50%), and tobramycin (80%) [19].

Both Gram-positive and Gram-negative bacteria investigated in this investigation have different medication susceptibility profiles. For example, it has been demonstrated that bacteria are more resistant to ciprofloxacin. This investigation contradicts the findings of Elayah *et al.* [20], who found that the most efficient

antibacterial agent was ciprofloxacin. Mazin S. Salman (2022), in Basra Found most frequent: *E. coli* (28.3%), *Staphylococcus spp.* (19.3%). And noted high UTI susceptibility in females; widespread resistance to commonly used antibiotics.[21] As a result, we found that *E. coli* 24 had a greater frequency of ciprofloxacin resistance (100.0%) than *K. pneumoniae* 10 (100.0%).in Duhok (2023)result study shown that *S. aureus*: 100% resistant to vancomycin and rifampin; lowest resistance to imipenem (11.8%). *E. coli*: 100% resistant to vancomycin and rifampin; most sensitive to meropenem (87.5%) and imipenem (68.8%) [22].

In the meantime, the isolated strain of *S. aureus* also showed greater resistance to ciprofloxacin 13 (100.0%). Chromosome mutations that change DNA gyrase and topoisomerase IV, overexpress efflux pumps, alter the quantity of porins, and transfer resistance to the plasmids' genes are also linked to specific ciprofloxacin resistance [17,20].

Furthermore, as has been reported in other research across the nation, the majority of the Gram-negative bacteria isolates in this investigation were multi-drug resistant, meaning they were resistant to three or more medications [18,19]. This illustrates how multi-drug resistance is becoming into a significant problem in Libya's uropathogen treatment. To stop the spread of drug-resistant bacteria in the area, this highlights the necessity of rigorous adherence to antibiotic policy, national antimicrobial surveillance, and in-vitro susceptibility testing programs.

CONCLUSIONS

In conclusion, we evidenced that *E. coli* was the most prevalent isolate followed by *Klebsiella pneumoniae*. These two organisms were highly resistant to the commonly used antibiotics. In addition, these organisms exhibit resistance too many first-line drugs used for UTI infection. To prevent resistance to antibiotics, appropriate therapy as per bacterial sensitivity pattern needs to be initiated.

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