

Article

A Study of Antibiotic Resistance in Bacteria Isolated from Urine Samples of Individuals with Urinary Tract Infections in Basrah City

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Abstract: Urinary Tract Infections (UTIs) are a rapidly emerging global health problem, contributing significantly in antimicrobial resistance (AMR). **Aim:** The aim of this study is to investigate the present status of the bacterial species involved with UTIs in Basrah City, to determine sensitivity and resistance profile of these bacteria to commonly used antibiotics and to investigate the demographic and clinical risk factors associated to their antibiotic resistance. **Methodology:** Urine samples were taken between December 2025 to April 2026 from patients in Basrah hospitals using standard microbiological methods. Methods employed were biochemical tests and antibiotic susceptibility testing (AST), by the disc diffusion method and by using Vitek 2 Compact system; through which, the sensitivity of the bacteria to common antibiotics was determined. **Results:** This study found that *Escherichia coli* (*E. coli*) predominate (46%) followed by *Staphylococcus haemolyticus* (18%) and *Klebsiella pneumoniae* (10%). The percentage of antibiotic resistance was high with Ciprofloxacin recording 80% and Gentamicin 66%. Conversely, Colistin had high efficacy (84%) followed by Ceftazidime (78%). Resistance is thought to be due to several mechanisms, such as the production of beta-lactamase by *E. coli*, resistance genes in *Staphylococcus haemolyticus*, formation of biofilm-like structures in *Klebsiella pneumoniae*, inappropriate antibiotic use and environmental factors. **Conclusion:** The results revealed a high rate of antibiotic resistance in Basrah city, so that the guidelines for treatment should be updated and the adoption of correct methods to prevent antibiotic resistance.

Keywords: Urinary Tract Infections, Antibiotic Resistance, *Escherichia coli*, *Staphylococcus haemolyticus*, *Klebsiella pneumoniae*

Introduction

Urinary Tract Infections (UTI) is a worldwide health problem, and is one of the most common infectious diseases in the world [1]. These infections affect millions each year, account for a large proportion of antibiotic use and, therefore, play an important role in the acceleration of the Antimicrobial Resistance (AMR) crisis [2]. In recent years, epidemiological data have shown a significant rise in the incidence of UTIs: The incidence was found to have increased by 60.4% from 1990 onwards [3]. The fact that this is an increasing trend strongly indicates the need for a continuous surveillance programme with proper management strategies. UTIs are microbial infections which

could affect any part of the urinary system and mostly caused by bacteria with little contribution from fungi [4]. The urinary tract is normally sterile, and when microorganisms invade it could result in inflammation of the kidneys, ureters, bladder, or urethra [5]. UTI are also more prevalent in Iraq compared to they are in several countries. A detailed meta-analysis performed on pregnant women in Iraq to determine the prevalence of UTI showed that this prevalence is high and *Escherichia coli* is the most important bacterial species among them [6]. Likewise, in Baghdad, the prevalence of UTI was 26.58% with the commonest organism *E. coli* 33.33%, followed by *Klebsiella pneumoniae* 20.63% then *Staphylococcus aureus* 15.87% [7]. These findings highlight the urgent need for detailed studies on microbial prevalence and susceptibility pattern to establish effective treatment guidelines and controlling the spread of infection.

UTIs have significant economic impact on healthcare systems worldwide, in addition to the associated morbidity. In the USA for example, it costs some 10.5 million outpatient visits and more than 3 million emergency care visits per year and approximately 3.5\$ billion dollars [8]. These statistics highlight the need for strong preventive strategies and interventions to mitigate the economic and health burden of UTIs in both a national and international context. Most cases of UTI are caused by the bacteria with more than 95% of cases being caused by bacteria [9]. Both Gram-negative and Gram-positive bacteria are involved, the most common of which is *E. coli*, which accounts for over 80% of infections [10]. This bacterium still accounts for the majority of community acquired and HCA UTIs. important uropathogens are the various species of *Klebsiella*, *Proteus*, *Citrobacter*, *Enterobacter*, *Enterococcus*, *Pseudomonas*, *Streptococci* and *Staphylococci* [11]. Antibiotic resistance is an increasingly serious threat worldwide and diminishes the efficacy of current antibiotic treatments for many infectious disease conditions as with UTIs [12]. The extensive and sometimes indiscriminate use of antibiotics in human and animal health care are a highly contributing factor to the rise and spread of resistant forms of bacteria [13]. These resistant pathogens are more difficult to treat, resulting in higher hospital stays, costs of health and high death rates [14]. Antibiotic resistance is a significant public health problem in the Middle East/ North Africa, especially in Iraq. Previous studies in Iraq had consistently shown high resistance rates of bacteria causing UTIs to commonly used antibiotics [15].

This is because the antibiotic resistance profile is constantly changing and region-specific, so continual antibiotic surveillance at the local level is essential to formulating empirical treatment plans and maximize outcomes for patients. Being a major urban center in the southern part of Iraq, Basrah City is a very important place to study these patterns because of the large population and the possible high rate of antibiotic usage in case of widespread use. Although many studies had been conducted on antibiotic resistant in Iraqi, there was a shortage on comprehensive information about antibiotic resistant pattern of bacterial isolates from UTI patients in Basrah city. Older literature might not contain up-to-date epidemiological data, given the dynamic nature of antimicrobial resistance. In addition, many studies do not report in detail the factors associated with resistance, and many studies do not cover all the widely used antibiotics for the region. This is an unmet need for up-to-date local information on the epidemiology of antibiotic resistance. The purpose of this study was to evaluate the present prevalence of the bacterial species causing UTIs in Basrah City and to evaluate the sensitivity and resistance characteristics of the collected bacteria of the infection to the most frequently used antibiotics. It also aims to examine demographic and clinical risk factors associated with antibiotic resistance and share these updated data with clinicians to guide treatment, and to inform antibiotic resistance prevention strategies in the local community.

Materials and Method

Urine samples were collected from patients visiting Abi Al-Khasib General Hospital and Basrah Teaching Hospital under the supervision of the Microbiology Laboratory Unit, starting from 1/12/2025 until 1/4/2026. The urine samples were then cultured on Blood agar, MacConkey's agar, Nutrient agar, and Eosin-Methylene Blue Agar media for the purpose of studying bacterial colonies. These media were prepared according to the manufacturer's recommendations.

Patients were enrolled in the study if they had signs and symptoms of a urinary tract infection (UTI), provided a urinary sample for microscopy in the midstream, and had a urinary culture with a significant bacterial count (e.g., $\geq 10^5$ colony forming units (CFU)/mL of a single pathogen).

Patients were excluded if they had received antibiotics in the 72 hours before sampling, had an indwelling urinary catheter, were pregnant or had a history of underlying conditions associated with difficulties in diagnosing a UTI (e.g. kidney failure or serious immunocompromise). Samples having bacteria in a mixed growth (> two bacteria) were also excluded for clarity of results.

A portion of the culture medium, incubated on Nutrient Agar for 18–24 hours, is transferred to filter paper. Two drops of oxidase reagent are added. If the colony turns from colorless to purple, the result is positive [16].

A portion of the isolated bacterial colony is transferred to the bottom of a tube (anaerobic section). Diagonal lines are drawn towards the aerobic section of the tube, and it is left for 24 hours at 37°C. The tube should be loosely sealed to allow air in, allowing for differentiation between enteric bacteria based on their fermentation of glucose, lactose, and sucrose, and the production of hydrogen gas, carbon dioxide, and hydrogen sulfide [17].

A portion of the culture medium, incubated on Nutrient Agar for 18–24 hours, is transferred to a sterile glass slide. A drop of 3% hydrogen peroxide is added, and the results are observed. If air bubbles appear, this indicates the production of the catalase enzyme and the subsequent release of oxygen gas [18].

Citrate consumption as the sole carbon source is detected by growing bacterial isolates in Simmons citrate agar medium. The medium tubes are then inoculated with the bacterial culture for each isolate at an age of (18-24) hours and incubated at a temperature of 37°C for (24-48) hours. If the color of the medium changes from green to blue, this is evidence of a positive result and the presence of growth [19].

Using the Disc Diffusion Method of antibiotics susceptibility test, the antibacterial susceptibility of the 50 samples of bacteria isolated was investigated following standard method. Oxoid manufactured discs with diameters of 6.3 mm, which were used with Muller-Hinton Agar. Results were confirmed using the Vitek 2 Compact System in order to improve the accuracy of analysis and bacterial identification and antibiotic susceptibility assessment [20]. This system has specific test kits for Gram-negative bacteria, Gram-positive bacteria, and a susceptibility test kit. The French company BioMérieux recommended that each test kit have 64 wells with dried medium and a colorimetric indicator. This is where biochemical tests are given and the device reads the colour changes caused by the growing of the bacteria. A kit contains 18-20 antibiotics arranged in the wells with different doses/concentrations of the antibiotics to ensure MIC finding (Minimum Inhibitory Concentration). The antibiotics listed in the following (**Table 1**), which are the most commonly used in treating bacterial infections, were used, as per the manufacturer's (BioMérieux) recommendations.

Table 1. Shows the types of antibiotics used in diagnosis and their concentrations

Concentration mg/tablet	Antibiotic	Concentration mg/tablet	Antibiotic
Tigecycline	30	Sulfamethoxazole	30
Tetracycline	30	Piperacilin	20
Colistin	25	Teicoplanin	10
Ceftazidime	25	Clindamycin	30
Erythromycin	15	Moxifloxacin	25
Ciprofloxacin	5	Ciprofloxacin	30
Cefoxitin screen	25	Oxacillin	20
Benzylpenicillin	20	Gentamicin	30
Ampicillin	10	Fusidic Acid	25

Result

The study showed that the highest percentage of bacteria causing urinary tract infections in patients was *E. coli* (46%), followed by *Staphylococcus haemolyticus* (18%), and *Klebsiella pneumoniae* (10%), as shown in the (Table 2).

Table 2. Shows the number and types of bacterial isolates

Bacterial isolates	Number	Staining gram	%Percentage
<i>Escherichia coli</i>	23	-	46
<i>Klebsiella pneumoniae</i>	6	-	12
<i>Pseudomonas aeruginosa</i>	2	-	4
<i>Proteus mirabilis</i>	1	-	2
<i>Staphylococcus hemolytic</i>	9	+	18
<i>Streptococcus agalactiae</i>	3	+	6
<i>Staphylococcus aureus</i>	3	+	6
<i>Enterococcus faecalis</i>	2	+	4
<i>Enterococcus cloacae</i>	1	+	2

The (Table 2) shows that both Gram-positive and Gram-negative bacteria infect the urinary tract, often causing acute infections. Gram-negative bacteria are characterized by a structurally dynamic cell envelope, granting them multi-resistance capabilities. They also possess purines, efflux pumps, and biofilms composed of complex proteins, sugars, and extracellular DNA [21]. When bacteria reside within these biofilms, they are protected from the host's immune system and the effects of antimicrobials. Gram-positive bacteria, on the other hand, are characterized by their ability to induce enzymatic inactivation, modify the target of treatment, and reduce drug absorption [22].

The study results also revealed that *Escherichia coli* accounted for the highest infection rate, at 46%, among the bacterial isolates. The high prevalence of this type of bacteria, particularly among women (up to 82% of infected individuals), is attributed to the proximity of the anus to the vaginal opening and urethra. *E. coli* leaves its original habitat (the large intestine) and enters the urinary tract during bowel movements from back to front. In women, the urethra allows bacteria to reach the bladder more quickly, and sexual activity can also push these bacteria into the bladder. Another reason for the spread of *E. coli* bacteria in women, especially pregnant women, is the weakened immune system during this period [23]. The ability of this type of bacteria to cause infection is also attributed to its secretion of the beta-lactamase enzyme [24]. There are several types of beta-lactamase enzymes due to genetic mutations in the bacteria, leading to antibiotic resistance [25]. As for *S. haemolyticus*, it is characterized by three antibiotic resistance genes. The first two genes, *blaZ* and *norA*, are responsible for resistance to β -lactams and quinolones. The third gene, *msr(A)*, mediates cross-resistance to various antimicrobial agents such as macrolides, lincosamides, and streptogamine [26]. The risk is exacerbated the presence of multidrug-resistant strains, especially in hospital environments, and their spread in these environments poses a significant risk. It is possible that these bacteria can store resistance genes and transfer them to other species [27]. *Klebsiella pneumoniae*, on the other hand, can resist antibiotics by forming biofilms and undergoing genetic mutations. In some cases, treatment failure and exposure to a wide range of antibiotics can lead to the emergence of multidrug resistance [28].

Furthermore, its resistance can be exacerbated by the presence of a giant polysaccharide capsule surrounding the bacterial cell, which acts as a barrier to protect the bacteria from phagocytosis [29]. It should also be noted that the environment plays a significant role in increasing AMR rates. The use of antibiotics to accelerate animal growth or prevent disease increases bacterial exposure to antibiotics, leading to the emergence of new resistance genes. This is because the residues of these drugs are released into the environment through sewage or animal manure, creating a suitable environment for bacterial proliferation and gene exchange. There is also a relationship between rising local temperatures and increased antibiotic resistance. Some studies have shown that a 10 °C increase in

temperature correlates with elevated resistance levels in bacteria [30], particularly in *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*.

The (Table 3) that bacterial isolates showed high resistant rates to both ciprofloxacin and gentamicin, similar to the results in McCarty et al., study [31]. This is due to its frequent prescription by physicians, as it was once considered an effective treatment against bacterial infections [22]. A common mistake made by patients is using this medication when they are infected with viral diseases such as influenza, which creates an opportunity for genetic mutations in bacteria, leading to the emergence of new, more resistant strains [32]. Furthermore, all bacteria that secrete the ESBL enzyme are resistant to all types of penicillin and gentamicin [31]. These enzymes attack the β -lactam ring and break down the amide chain, converting the antibiotic into an inactive compound. These enzymes are expressed via plasmids or transposons [33]. It should also be noted that *Escherichia coli* has shown very high resistance to gentamicin, similar to the results in Abu Alba study [34]. It is worth noting that in the year In 2012, a study Vidaillac et al., reported that bacterial isolates from children with urinary tract infections were more sensitive to gentamicin, but it is certain that the misuse of this drug has led to the emergence of new, more resistant strains [35].

Table 3. Shows the effectiveness of antibiotics according to bacterial sensitivity

Antibiotic	Sensitivity	Resistance	%For resistance
Tigecycline	33	27	54%
Tetracycline	30	20	40%
Colistin	42	8	16%
Ceftazidime	39	11	22%
Erythromycin	34	26	52%
Ciprofloxacin	10	40	80%
Cefoxitin screen	34	16	40%
Benzylpenicillin	32	28	56%
Ampicillin	14	36	72%
Vancomycin	34	26	52%
Piperacilin	28	22	44%
Clindamycin	33	27	54%
Moxifloxacin	39	11	22%
Amikacin	35	15	38%
Oxacillin	34	16	40%
Gentamicin	27	33	66%
Fusidic Acid	32	28	56%
Lmipenem	27	23	46%

This study also showed that Colistin was the most effective antibiotic against bacteria, with an efficacy rate of 84%. It exhibits broad-spectrum activity against Gram-negative MDR bacteria, including *Enterobacteriaceae*, *Klebsiella*, and *Escherichia coli* [36]. Colistin works by disrupting the cell membrane through its lipophilic and hydrophobic components. Upon binding to lipopolysaccharide (LPS) in the cell membrane, it creates pores, leading to the leakage of cell contents and ultimately cell death [37]. Ceftazidime was also effective against bacterial isolates, with a efficacy rate of 78%. This is attributed to its effect on cell wall synthesis, as it binds to PBP enzymes responsible for polymerizing peptidoglycan, the primary component of the bacterial cell wall [36]. Moxifloxacin was also effective, with a 78% efficacy rate against bacterial isolates, but it is rarely prescribed for urinary tract infections because it is not. It is excreted in the urine only in a small percentage not exceeding 20% of its concentration, making it difficult to eliminate bacteria inside the bladder [37].

Discussion

The present study demonstrates that urinary tract infections in Basrah City are caused by a diverse group of Gram-negative and Gram-positive bacteria, with *Escherichia coli* representing the predominant isolate. According to Table 2, *E. coli* accounted for 46% of the 50 bacterial isolates, followed by *Staphylococcus haemolyticus* at 18% and *Klebsiella pneumoniae* at 12%. The predominance of *E. coli* is consistent with previous reports identifying this organism as the major uropathogen in both community- and hospital-associated urinary tract infections [9], [10]. Its predominance may be associated with its ability to colonize the intestinal tract and subsequently reach the urinary system, particularly because of anatomical and behavioral factors that facilitate ascending infection. Moreover, several virulence characteristics of uropathogenic *E. coli*, including adhesion, biofilm formation, and β -lactamase production, may contribute to both its pathogenicity and persistence during infection [12], [24]. These findings reinforce the importance of *E. coli* as the principal target when developing empirical treatment strategies for UTIs in Basrah.

The relatively high proportion of *S. haemolyticus* observed in this study is also clinically important. Although coagulase-negative staphylococci have historically been considered less prominent causes of UTIs, *S. haemolyticus* has increasingly been recognized as an opportunistic pathogen capable of causing difficult-to-treat healthcare-associated infections. Its clinical relevance is enhanced by its ability to harbor multiple antimicrobial-resistance determinants, including genes associated with resistance to β -lactams, quinolones, and other antimicrobial classes [25], [26]. The presence of this organism among 18% of the isolates therefore suggests that Gram-positive uropathogens should not be overlooked when selecting antimicrobial therapy. Likewise, the isolation of *K. pneumoniae* highlights the importance of multidrug-resistant Enterobacteriaceae in UTIs. Its resistance potential is associated with multiple mechanisms, including capsule formation, biofilm production, β -lactamase activity, and acquisition of resistance genes [27], [28]. Consequently, the bacterial distribution identified in this study reflects the complexity of antimicrobial management for UTIs in the local population.

A particularly concerning finding was the high level of resistance to several frequently used antimicrobial agents. Ciprofloxacin demonstrated the highest resistance rate at 80%, followed by ampicillin at 72% and gentamicin at 66%, as reported in Table 3. These findings indicate that commonly prescribed antibiotics may no longer provide reliable empirical coverage for many UTI cases in Basrah. High ciprofloxacin resistance is especially important because fluoroquinolones have historically been widely prescribed for urinary infections, and repeated exposure may create selective pressure favoring resistant bacterial populations [30], [31]. Similarly, resistance to gentamicin may be related to the circulation of multidrug-resistant organisms and transferable resistance determinants. The observed resistance to ampicillin may additionally reflect β -lactamase-mediated mechanisms, particularly among Enterobacteriaceae. Therefore, continued empirical use of these antibiotics without susceptibility testing could contribute to treatment failure and further expansion of antimicrobial resistance.

In contrast, colistin showed the highest overall antimicrobial activity, with 84% of the tested isolates categorized as sensitive, while ceftazidime and moxifloxacin each demonstrated 78% sensitivity. The high activity of colistin may be related to its ability to disrupt the outer membrane of susceptible Gram-negative organisms through interactions with lipopolysaccharides. Previous investigations have demonstrated its potential activity against multidrug-resistant *K. pneumoniae*, *P. aeruginosa*, and other Gram-negative pathogens [35]. However, the high susceptibility observed in the present study should not be interpreted as support for unrestricted use of colistin, because this drug is generally regarded as an important therapeutic option for selected multidrug-resistant infections. Ceftazidime also demonstrated considerable activity, supporting the continued potential role of selected cephalosporins when susceptibility has been established [36]. Moxifloxacin showed similar laboratory activity, although its pharmacokinetic characteristics and relatively limited urinary excretion may restrict its routine application for UTIs [37]. These findings emphasize that laboratory susceptibility must be interpreted together with pharmacological characteristics and clinical indications.

The resistance patterns observed in this study can also be interpreted within the broader problem of antimicrobial use and environmental selection pressure. Inappropriate antibiotic exposure in humans and animals contributes to the selection and persistence of resistant bacteria, while antimicrobial residues released through wastewater and animal waste may facilitate the spread of resistance genes in the environment. The study also discusses temperature as a potentially relevant environmental factor, since previous research has demonstrated an association between higher local temperatures and increased bacterial resistance [29]. This issue may be particularly relevant in Basrah because environmental and healthcare-related factors can interact to influence antimicrobial resistance patterns. Moreover, horizontal transfer of resistance genes through plasmids and other mobile genetic elements can accelerate the dissemination of resistance between bacterial species. These mechanisms may partly explain why resistance is observed across both Gram-negative and Gram-positive isolates. Thus, antimicrobial resistance in UTIs should be addressed through an integrated approach involving appropriate prescribing, infection control, environmental monitoring, and antimicrobial stewardship.

Overall, the findings indicate that antimicrobial resistance among urinary isolates in Basrah City represents an important clinical challenge. The predominance of *E. coli*, together with substantial contributions from *S. haemolyticus* and *K. pneumoniae*, demonstrates that empirical treatment should consider the local distribution of uropathogens rather than relying exclusively on generalized treatment patterns. At the same time, the very high resistance to ciprofloxacin, gentamicin, and ampicillin suggests that routine empirical use of these agents should be approached cautiously and, whenever possible, guided by culture and antimicrobial susceptibility testing. The comparatively greater activity of colistin and ceftazidime provides useful local susceptibility information but should not replace individualized clinical assessment. Regular surveillance is therefore necessary because resistance profiles can change rapidly over time and differ considerably between geographical areas and healthcare institutions [11], [13]. Periodic updating of local antibiograms and treatment guidelines may help improve therapeutic outcomes while reducing unnecessary antibiotic exposure and limiting the further development of antimicrobial resistance.

Conclusion

The study noted that there is widespread resistance to antibiotics such as ciprofloxacin and gentamicin as these are widely used and often used when not medically indicated. In contrast, colistin has proven the most effective (84%) *in vivo*, with multidrug resistant Gram-negative bacteria isolated from the tissues. These studies highlight the importance of ongoing monitoring of antibiotic resistance trends and implementing effective local treatment strategies to address this public health challenge. These sorts of initiatives are essential to help direct clinical action and provide input into local prevention strategies.

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