

## Assessment of Antimicrobial Resistance among Bacterial Pathogens Associated with Urinary Tract Infections

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### ABSTRACT

Urinary tract infection (UTI) pathogens growing resistance to widely used antibacterial drugs presents serious concerns upon an international scale. Investigating the susceptibility to antibiotics characteristics of pathogenic microbes collected from infections of the bladder was the aim of this investigation. between the June and August of 2025, an additional 65 samples of urine were taken from individuals at Al-Hindiya Teaching Hospital in the Karbala Province region. The findings indicated that *P. aeruginosa* , *S. agalactiae*, *E. coli*, and *Enterobacter spp.* were indicative of a potential link to nosocomial infections. Conversely, *Klebsiella* species were found alone less frequently. Overall, the results show a wide range from , alongside *E. coli* clearly predominating. The ability to respond of the collected organisms to the tested antibiotics varied, alongside noteworthy In general , the results show a wide range of urinary tract pathogens . In order for successfully handling infections of the urinary tract, it is crucial for choosing the right medications and conduct continual monitoring. The identified organisms showed varying susceptibility toward the investigated antibiotics, with substantial resistance observed to frequently prescribed medications.

**Keywords:** Urinary tract , *E. coli* , *S. agalactiae* , *Enterobacter spp.* , *P. aeruginosa* , Susceptibility

## 1.Introduction

Urinary tract infections (UTIs) are among the most common bacterial illnesses in individuals, within hospitals as well as within the general population [1]. *Klebsiella*, *Staphylococci*, *Enterobacter*, *Proteus*, *Pseudomonas*, and *Enterococci* strains are increasingly commonly identified in individuals, as well as *E. coli* and its variants is acknowledged just like the primary cause of infections of the urinary system, contributing to more than ninety percent of incidents globally [2,3]. microorganisms are rarely harmful in the urinary system [3, 4]. Nowadays, acute uncomplicated cystitis is treated empirically rather than alongside a culture of the bladder or susceptibility examination. The small and stable assortment of infectious factors which may lead to this kind of illness lends credence to this assertion [5]. Antimicrobial agents have revolutionized the treatment of infectious diseases, among them urinary tract infections (UTIs). Having the capacity for organisms to generate and spread resistant antibiotics, however, has been highlighted by the existence of resistant gene sequences for hundreds of thousands of years. This is of clinical importance for the surveillance and management of infections. The misuse and excessive use of antimicrobial agents within human healthcare, medicine for animals, and agricultural production has accelerated this process [6]. A prescription for an antibiotic medication for infections within the urinary tract stops bacteria from growing in the bloodstream and within the tissues of the bladder along with may ultimately eradicate them. This is to avert adverse effects like inflammation in the urinary tract, the spread of infection throughout the bloodstream, along with the development of obstructions and tissue scarring that make it impossible to feed the vast majority about the bladder and kidneys to work properly. Prescribed antibiotics and resistance to disease are increasing worldwide, demanding securely and judicious usage of antibiotics across every field of medicine. To stop the rise in antibacterial resistance that comes from using broad-ranging antibiotics the wrong way, it would be best to choose the right medication that works only on the bacteria you want that to. Nonetheless, a regional or local vulnerability information ought to serve as the foundation for the experimental antibacterial agent choice process [8]. Nevertheless the overuse of antimicrobial agents has led to the development of antibiotic-resistant bacteria with different drug-resistance in addition to the growth of multidrug-resistant infections caused by such microbes [9]. Because of their elevated level of susceptibility to several antimicrobial drugs, many uropathogens are

currently classified as organisms that are multidrug resistant (MDROs). In addition to their exponential growth in recent years, the most important problem in the era of antibiotic resistance is the resistance of organisms to many medicine, and this renders controlling diseases brought on by those microbes difficult. The rapid emergence of antibacterial agent resistant organisms as well as the decrease in the emergence of novel medicines necessitate a reevaluation of the use of previous antimicrobial agents [10]. In order for offering precise information for recommending the best course for therapy and lessen antimicrobial resistance to medications, the present investigation intends to examine the geographical distribution of urinary tract-associated organisms and evaluate their vulnerability to antibiotics behaviors in an observational investigation carried out in Al Hindia Teaching hospital in the Karbala region.

## **2. Materials and Methods:**

Approximately 65 specimens from patients with infection of the urinary tract at Al-Hindiya Teaching Hospital in the Karbala province were gathered between the June and August then put in a container that was clean. The specimens were transported into the laboratory within a few minutes after collection, where they were cultivated before they were identified. In addition to modest numbers of *Klebsiella* and *Proteus species*, the samples that were obtained contained *E. Coli*, *Enterobacter*, *S. agalactiae*, and *P. aeruginosa*. The specimens, which comprised mainly hospitalized individuals as well as hospital visitors, were taken among female, children , among geriatric patients who represented different stages of life. Conventional microbiology methods have been employed to identify microorganisms and conduct tests for susceptibility to antibiotics in compliance against the Medical and Laboratories Standardization Institution's (CLSI) recommendations.

### **Bacterial identification**

Bacterial isolates were identified using Bergey's Manual of Systematic Bacteriology [11]. To understand the isolated bacteria, they were cultivated on nutritional and different substrates using the methods outlined in [12] and [13]. Agar made from blood and MacConkey's agar had been used to cultivate urine specimens including cells containing pus in order to examine colony shape and cultural traits. physiological examinations like TSI, superoxide measurement, and IMViC.

### **Antibacterial susceptibility Test ( AST):**

Employing this disk diffusion technique on the Mueller-Hinton agar, the antimicrobial sensitivity of bacteria isolated from 65 urine samples was assessed. A (1.5 ) McFarland turbid guideline was used to create and regulate bacteria cultures. Following homogenous vaccination, antibiotic discs (6.3 mm in diameter) made by Bio analyze subsequently carefully deposited on the culture medium substrate. During sixteen to eighteen hours, the inoculation dishes underwent incubation at 37°C. After treatment, individual disk's area of inhibition width was measured and assessed using the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) recommendations. Isolated bacterial strains were classified as susceptible or resistant to the tested drugs based on the widths of their zones of inhibition. Every kind of bacterium has been evaluated versus a range of medications. Every result have been classified to be either susceptible or resistant because there had been no evidence of moderate susceptibility amongst the isolates that were examined.

The antibiotics used for this study of *E.coli* included Amoxicillin ( AMC) 30µg ,ciprofloxacin (CIP) 30µg ,Amikacin (AK) 30µg , Ticarcillin (TIM) 30µg, Ceftriaxone (CFM) 30µg,Cefotaxime (CTX) 30µg, Aztreonam (ATM)30µg , Tetracycline (TE) 30µg , Vancomycin (VA) 30µg and Fosfomycin (F) 30µg.

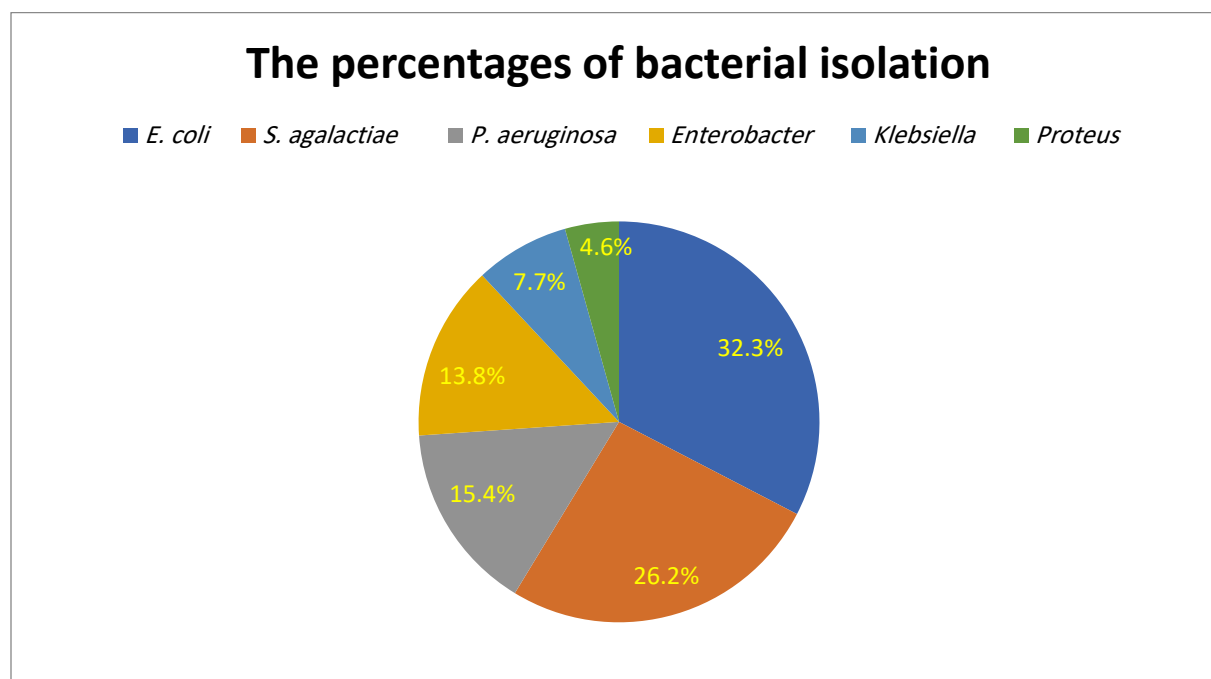
The antibiotics used for antimicrobial susceptibility testing of *Enterobacter ssp.* were as follows: Imipenem (IPM) ) 30µg, Amikacin (AK) 30µg, Ceftriaxone(CFM) 30µg, Ciprofloxacin (CIP) 30µg, Tazobactam(TAB) 30µg, Aztreonam (ATM)30µg, Vancomycin (VA) 30µg, Cefepime (FEP) 30µg, Amoxicillin ( AMC) 30µg, Cefotaxime (CTX) 30µg and Oxacillin (Ox) 30µg.

The panel of antibiotics used for *P. aeruginosa* consisted of: Imipenem (IPM) ) 30µg, Amikacin (AK) 30µg,Amoxicillin ( AMC) 30µg, Cefepime (FEP) 30µg, Ciprofloxacin (CIP) 30µg and Ceftriaxone (CFM) 30µg .

While the antibiotics used in the *S .agalactiae* sensitivity test were Ticarcillin (TIM) 30µg,Amikacin (AK) 30µg ,Amoxicillin ( AMC) 30µg,Imipenem (IPM) ) 30µg, Cefotaxime (CTX) 30µg , Prednisolone( PRL) 30µg and Ciprofloxacin (CIP) 30µg .

### 3. Result and Discussion

The percentage that consists of *E. coli*, *S. agalactiae*, and *P. aeruginosa* was 32.3% (about 21 isolates), 26.2% (about 17 isolates), *P. aeruginosa* 15.4% (about 10 isolates) , *Enterobacter* 13.8% (about 9 isolates), *Klebsiella* 7.7% (about 5 isolates), and *Proteus* 4.6% (about 3 isolates) , respectively, after the specimens collected were diagnosed. according to the graphic in Figure 1 below.



**Figure1. The percentages of bacterial isolation.**

Bacterial isolates were grown on selective and differential media for identification purposes. Gram staining along with various biochemical tests was then conducted, and the findings are presented in the table 1 below.

**Table 1 . The characteristics and the diagnostic Tests of Urinary Tract Bacterial Isolates**

Bacteria	Gram Stain	Culture Media	TSI	Gas	H <sub>2</sub> S	Indole	MR	VP	Citrate	CAMP	Characteristics
<i>E. coli</i>	-ve	MacConkey, Blood Agar	A/ A	+	-	+	+	-	-	N/A	Lactose fermenter , typical IMVIC pattern
<i>Enterobacter</i>	-ve	Blood Agar , MacConkey,	A/ A	+	-	-	-	+	+	N/A	Ferments glucose and lactose /sucrose with gas
<i>Klebsiella</i>	-ve	MacConkey, Blood Agar	A/ A	+	-	-	-	+	+	N/A	Lactose fermenter with gas , Mucoid colonies , Capsule present
<i>Proteus</i>	-ve	Blood Agar	K/ A	±	+	+	+	-	±	N/A	Ferment glucose only with H <sub>2</sub> S producer ,
<i>P. aeruginosa</i>	-ve	MaConckey ,Blood Agar	K/ K	-	-	-	-	-	+	N/A	Non – fermenter , Oxidase +ve
<i>S. agalactiae</i>	+ve	Blood Agar	N/ A	-	-	N/A	N/A	N/A	N/A	+	B - hemolysis

Assessing the vulnerability characteristics of strains of bacteria obtained from infections of the urinary tract is the goal of our investigation. An important medical concern in the management of infection of the urinary tract (UTIs) is the investigation of resistance to antibiotics in microbial strains derived from these diseases. Because of its capacity to stick to the cilia covering of the urinary system as well as its presence of infectious components (adhesions and fimbriae), *E. coli* is the most common cause of UTIs globally, and responsible for the greatest number of infections in urinary tract infection (UTI) patients, according to several research. For example, a large Saudi study of positive urine samples found that *E. coli* accounted for almost 29.8% of infections. The proportion in this investigation was 32.3%, which is in line with the mean percentage seen in studies on strains of bacteria found in the urine [14]. The substantial amount of *E. coli* amongst samples involving infections of the urinary tract corresponds that previous longitudinal investigations that show *E. coli* to be the most common uropathies in community-acquired infections of the urinary tract. According to several studies, *E. coli* is most strongly commonly recovered bacteria, accounting for a major fraction of infections and usually exhibiting notable characteristics related to resistance to antibiotics even if prevalence numbers differ by population and geography [15,16]. Gram-positive cocci, such *S. agalactiae*, and other can also be detected in samples of urine, especially in older patients or women in specific situations, even though the majority of research focuses on gram-negative bacteria in infections of the urinary tract. The significant frequency seen in our investigation had additional backing by a 2023 Egyptian investigation that reported a 26 percent frequency of these particular microorganisms [17]. Infections contracted in hospitals frequently involve *P. aeruginosa*, especially in individuals who have catheters for the urinary tract. It can be found at levels which render it a serious infectious agent, while being less common than *E. coli*. Flores-Mirelles *et al.* conducted a study [18]. According to our research, the overall incidence of *P. aeruginosa* in UTIs was 15.4%, which is greater compared to the two percent observed in 2016 [16] as well as line with the seven to ten percent predicted in 2023 [19]. In comparison to *E. coli*, *Klebsiella* and *Proteus* continue to be comparatively low-risk causes of UTIs in studies that have been published globally. For instance, comparable to the seven percent and 4% percentages in our analysis, *Klebsiella* accounted for almost 10% and *Proteus* for roughly 3.7 percent of urine isolated in a Lebanese investigation [19]. In a similar vein, *Proteus* was found in 4.6% and

*Klebsiella* in 7.7% of the instances of people with urinary tract infections in a Ugandan investigation [20]. Every organism was investigated for antibiotics that had specific following tests of sensitivity on the bacteria that were isolated, and the findings remained as follows.

The results of the antibiotic susceptibility testing of *E.coli* bacteria were as follows:

**Table 2 . The Susceptible (S) and The Resistant (R) patterns of *E. coli* from UTI**

Antibiotic	Sensitive (S) %	Resistance (R)%
Ticarcillin (TIM)	67	33
Amoxicillin (AMC)	43	57
ciprofloxacin (CIP)	24	76
Amikacin (AK)	57	43
Ceftriaxone (CFM)	34	66
Cefotaxime (CTX)	10	90
Aztreonam (ATM)	48	52
Tetracycline (TE)	48	52
Vancomycin (VA)	43	57
Fosfomycin (F)	71	29

1- In Table 2 The level of sensitivity tests conducted (n=21) *E. Coli* samples from infections of the urinary tract show an increasing trend of resistance to popular antimicrobial agents, which falls in line with findings from a number of research investigations conducted globally. These findings show that a strain of *E. coli* has developed an immunity to frequently used antibiotics such as fluoroquinolones, which is consistent with the rising frequency of susceptibility in urine isolates. This is in line alongside multiple studies which have shown that *E. coli* samples that cause infections in the urinary system have high oral cephalosporins , and fluoroquinolones in particular. One research found that *E. coli* resistance to various fluoroquinolones, including ciprofloxacin, varied between (70 – 80) % in many extremely strains [21]. According to recent studies, antibiotic resistance prevalence of *E. coli* isolated from UTIs to amoxicillin-containing combinations of therapies (AMCs) range. Approximate to current results of susceptibility against this antimicrobial in the larger context of the present investigation, including susceptibility documented at around 36.9% in one study [22]. Although this varies based on where it is used ,

certain research investigations have found relatively low resistance percentages to amikacin (AK), indicating that it frequently maintains significant effectiveness towards urinary *E. coli*. The moderate resistance produces this investigation is in keeping with a comprehensive evaluation that found bladder *E. coli* samples to have relatively high susceptibility (up to 57%) to amikacin (AK) in many publications [23]. Local investigations have additionally discovered intermediate to substantial levels of Aztreonam (ATM) and Tetracycline (TE) susceptibility, such as those carried out in Iraq, which indicated about 58.7% resistance among *E. coli* [24]. Because of its Gram-negative cell wall construction, *E. coli* has an innate durability against vancomycin [24]. A drug called Ticarcillin (TIM) and Fosfomycin (F) had the least susceptibility percentages at 33 % and 29%, respectively. These results are significant because Ticarcillin and Fosfomycin often demonstrate good activity against resistant isolates, particularly in uncomplicated urinary tract infections. Several recent studies support the efficacy of Fosfomycin as an effective antimicrobial against resistant *E. coli*, while the efficacy of other antimicrobials remains declining[25]. Studies have shown that ESBL-producing isolates are associated with high resistance to cephalosporins and fluoroquinolones, and that alternative treatments such as fosfomycin are often still effective[26]. The results of the antibiotic susceptibility testing of *Enterobacter* bacteria were as follows:

**Table 3 .The susceptible (S) and The resistant (R) patterns of *Enterobacter* from UTI**

Antibiotic	Sensitive (S) %	Resistance (R)%
Imipenem (IPM)	100	0
Amikacin (AK)	70	30
Ceftriaxone ( CFM)	0	100
Ciprofloxacin (CIP)	70	30
Tazobactam (TAB)	56	44
Vancomycin (VA)	67	33
Aztreonam (ATM)	44	56
Cefepime (FEP)	44	56
Amoxicillin ( AMC)	70	30
Cefotaxime (CTX)	30	70
Oxacillin (Ox)	0	100

2- Antibiotic susceptibility testing of *Enterobacter* isolates (n =9) from urine samples In table 3 showed varying patterns of resistance, reflecting the growing challenge in treating urinary tract infections caused by this bacterium, which is a member of the Enterobacteriaceae family known for its high ability to acquire multiple resistance mechanisms[27]. Effectiveness of Carbapenem: Imipenem is (IPM) showed no resistance, indicating that it was highly effective in combating enteric bacteria isolates in this investigation. This result is in line with several studies showing that carbohydrates continue to be amongst the most successful antibiotics versus *Enterobacter* bacteria , especially strains which produce AmpC  $\beta$ -lactamase enzymes, since they are very stable versus the majority of beta-lactamases [28]. It should be noted that Enterobacter's oxacillin susceptibility is a predicted indigenous susceptibility because an antibiotic has no therapeutic value along with is inefficient versus Gram-negative bacteria because of its cellular composition [29, 30]. The isolates shown moderate amounts of resistance to: thirty percent amikacin (AK) Amoxicillin (AMC): 30%, Ciprofloxacin (CIP): 30% Tazobactam (TAB): 44% , Aztreonam (ATM): 56% Cefepime (FEP) 56% and Vancomycin (VA): 33%, as shown in Table 3. The results of this study demonstrate the samples' varying susceptibilities, with ciprofloxacin and amikacin additionally maintaining an intermediate level of effectiveness. This result is in accordance with research suggesting that, notwithstanding rising resistance rates worldwide, aminoglycosides and fluoroquinolones may still be somewhat effective against *Enterobacter* [31]. AmpC activity of the enzyme or additional mechanisms like lower permeability or displacement pumped activity are responsible for the resistance of aztreonam and cefepime [32]. Since vancomycin is an inefficient antibacterial versus gram-negative organisms given that it cannot pass through the membrane that surrounds it, *Enterobacter* resistance to it is an anticipated developing resistance [33]. The results of the antibiotic susceptibility testing of *P. aeruginosa* bacteria were as follows:

**Table 4. The susceptible (S) and the resistant (R) patterns of *P. aeruginosa* from UTI**

Antibiotic	Sensitive (S) %	Resistance (R)%
Imipenem (IPM)	100	0
Ceftriaxone (CFM)	30	70
Ciprofloxacin(CIP)	40	60
Cefepime (FEP)	80	20

Amoxicillin (AMC)	40	60
Amikacin (AK)	70	30

3- *P. aeruginosa* isolates from urine samples as in table 4 show a high tendency toward resistance to multiple drugs, consistent with the pathogen's worldwide increase in infections as well as community-associated urinary tract infections. *P. aeruginosa* learned and inherent systems of resistance make it an extremely challenging infection to treat. The most recent investigation's *P. aeruginosa* samples (n = 10) showed maintained carbapenem action with complete imipenem(IPM) sensitivity. This result implies that important mechanism of resistance, such as carbapenems synthesis, permeability channel expression increase, and OprD porin loss, are absent. Ipenem's sustained therapeutic importance for treating urinary tract infections that are linked to *P. aeruginosa* could be supported by the observed susceptibility, which could be a result of restricted choices and managed carbapenem usage in the medical case under study [34,35]. The significant ciprofloxacin(CIP) resistance in our sample is consistent alongside prior studies showing that *P. aeruginosa* is becoming more resistant to fluoroquinolones due to usage and selection pressure, especially in urine isolates and in hospital settings [36]. Interestingly, these samples' lower resistance to the antibiotic Cefepime (FEP) 20% suggests that the fourth-generation cephalosporins might continue to be therapeutically effective versus a few *P. aeruginosa* bacteria, most likely due to changes in -lactamase production and solubility [37, 38]. Table 4's 30% Amikacin (AK) resistant rate suggests that its aminoglycoside effectiveness toward *P. aeruginosa* has been partially preserved. This is in line with a number of regional studies that show how aminoglycosides may not always be effective but often maintain outstanding activity as compared to other drug classes [39]. Even though Table 4 shows 60% antibiotic susceptibility, this outcome is indicative of *P. aeruginosa*'s inherent resistance because of its gram-negative exterior membrane [40].

The results of the antibiotic susceptibility testing of *S. agalactiae* bacteria were as follows:

**Table 5. The susceptible (S) and the resistant (R) patterns of *S. agalactiae* from UTI**

Antibiotic	Sensitive (S) %	Resistance (R)%
Ticarcillin (TIM)	47	53
Amikacin (AK)	41	59
Amoxicillin (AMC)	71	29
Imipenem (IPM)	94	6
Cefotaxime (CTX)	41	59
Prednisolone (PRL)	24	76
Ciprofloxacin (CIP)	35	65

4- The sensitivity testing outcomes indicated that the sensitivity of the *S. agalactiae* isolate (n = 17) was shown in Table 5. The elevated susceptibility to imipenem (IPM) may be due to changes in the binding proteins to the presence of partially expressed lactamases. Cephalosporins and the carbapenems are among the amino acid antibiotics whose affinity for binding and effectiveness have been found to be decreased by such mechanisms. Similar resistance mechanisms have been extensively investigated in earlier research, where hydrolysis via enzymes and architectural alteration of PBPs were found to be key factors in decreased sensitivity to  $\beta$ -lactams. The antibiotic amoxicillin (AMC) on the other hand, showed better effectiveness, suggesting that  $\beta$ -lactam antibiotics in combination with a  $\beta$ -lactamase inhibitor are still a good therapy choice [41]. The results of our study showed that *S. agalactiae* exhibited moderate susceptibility to Ticarcillin (TIM) (47%). Approximately were reported by previous studies which demonstrated moderate activity of  $\beta$ -lactam antibiotics against some isolates of *S. agalactiae*[42]. In addition, the isolates in the current study showed a sensitivity rate of 41% to both Amikacin (AK) and Cefotaxime (CTX). Comparable results were reported in other studies that observed reduced susceptibility of *S. agalactiae* to aminoglycosides and some cephalosporins, Determining the differences in antimicrobial response across isolates from various areas [43].

### Conclusions

*P. aeruginosa* shown significant antibiotic resistance, although *E. coli* was found to be the bacteria that is most prevalent in urinary tract infections. A single institution research design and an extremely tiny sample number restrict these results. However, the observed variations in

susceptibility amongst bacterial communities emphasize how crucial it is to do susceptibility testing to antibiotics before treatment in order to provide the best possible care and avoid resistant in future infections. The findings highlight the necessity of ongoing monitoring of antibiotic resistance and revising treatment regimens in light of regional information, reflecting the biological variety of susceptibility amongst prevalent urine bacteria.

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