

Molecular Detection Of Virulence Genes For Escherichia Coli And Staphylococcus Aureus Isolates Associated With Urinary Tract Infection.

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Abstract:

Background: Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide, affecting millions annually. They occur when pathogens colonize the urinary system, leading to symptoms like painful urination, frequent urges, and sometimes fever or flank pain. Antimicrobial therapy must be guided by local rather than international guidelines to minimize the inappropriate and overuse of antibiotics.

Patients and Methods: Between October 2024 to February 2025, a total of 150 midstream urine samples were collected from hospitals, health centers, and clinics in the south of Iraq. Urine samples were cultured on blood and MacConkey agars, followed by macro and microscopic identification and biochemical tests. The main bacterial isolates were examined for the presence of virulence genes by polymerase chain reaction.

Results: Only 43 samples showed positive bacterial cultures (28.7%), with females (60.5%) being more susceptible than men (39.5%). The predominant uropathogen was *E. coli* 15 (34.9%), followed by *K. pneumoniae* 11 (25.6%), *P. aeruginosa* 8 (18.6%), and *P. mirabilis* 5 (11.6%). The least isolated pathogen was *S. aureus*, which accounted for 4 (9.3%). More than 60% of *E. coli* isolates were resistant to tetracycline (66.7%), trimethoprim (73.3%), and Trimethoprim/sulfamethoxazole (66.7%). *S. aureus* exhibited significant resistance rates to Tetracycline (75%), Trimethoprim (75%), and Trimethoprim/sulfamethoxazole (75%). The resistance rate of *S. aureus* to Ciprofloxacin was 50%. *FimH* and *KpsMT II* genes for *E. coli* were detected, with high prevalence at 86.7% and 80%, respectively. The prevalence of *mdeA* and *Hla* genes in *S. aureus* isolates was 50% for each, with only 25% of the isolates harboring both of them. **Conclusion:** The study concludes that resistance to commonly used antibiotics is increasing among urinary pathogens, highlighting an increasing challenge in UTI treatment.

Keywords: Urinary tract infection; *E.coli*; *S. aureus*; Virulence factor; antibiotic susceptibility test

INTRODUCTION:

Urinary tract infections are a major health concern that can be caused by a variety of organisms. Annually, over 150 million people worldwide receive a UTI diagnosis [1]. UTI can be classified into upper and lower tract infections based on the anatomy of the infected part of the urinary tract [2]. Lower urinary tract infections are called bladder infections or cystitis, while upper urinary tract infections are referred to as kidney infections or pyelonephritis [3]. More than 95% of UTI cases are caused by bacteria, even though a variety of pathogens, including viruses and fungi, can cause UTI [4]. Based on underlying host factors and underlying uropathogens, UTIs can be either complicated or uncomplicated [5]. Early diagnosis, appropriate antibiotic use, and preventive measures are essential for effective UTI management and reducing the risk of complications [6]. Urinary tract infections with single bacterial species can occur in the majority of elderly people who are not catheterized. Isolating many species in the urine culture is common, nevertheless, when structural abnormalities and catheterization are present [7]. Urinary tract infections represent a major source

of illness in male infants, elderly men, and women across all age groups. Potential severe complications include recurrent infections, pyelonephritis progressing to sepsis, childhood renal scarring, adverse pregnancy outcomes, and antibiotic-related complications are associated with UTIs [8]. Asymptomatic bacteriuria occurs when there is a positive urine culture with 10^5 CFU/mL but no symptoms. Alternatively, these infections can manifest as symptomatic infections, which cause dysuria with or without pain, urgency, and frequency [9]. The increasing resistance to empiric antibiotic therapy in urinary tract infections (UTIs) is a growing concern globally. Thus, this study investigates the rising antibiotic resistance among urinary pathogens to standard empirical therapies. The study also aimed to determine the prevalence of some virulence genes for *E.coli* and *S.aureus* isolates associated with UTI.

PATIENTS AND METHODS:

Study design and Inclusion/ exclusion criteria

A cross-sectional study was carried out on patients with UTIs in Thi-Qar province- Iraq, from October 2024 to February 2025. One hundred and fifty clean-catch midstream fresh urine samples were collected from patients of both sexes who were aged between 11-80 years old, in hospitals, health clinics, and centers. People with history of burning sensation, frequent urination, complaining of Suprapubic pain, Pyuria and others symptoms of UTIs were included. The study exclude any patient showed overlapping symptoms caused by other conditions or diseases such as vaginal infection.

Sample collection

The samples were collected in sterile containers and then transported to the laboratory for further analysis. Each participant's clinical data and demographic details were obtained using a standardized questionnaire. Laboratory urinalysis such as microscopic and macroscopic examinations as well as dipstick tests were used to asses UTI symptoms. Informed consent was obtained from all participants involved in the study. The research was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Shatrah University, college of veterinary medicine (No 494, date: 10-2024).

Culturing on appropriate media:

The urine specimens were handled according to routine laboratory protocols and cultured on blood and MacConkey agar. The culture media was incubated for 18-24 hours at 35-37°C [10].

Isolation and identification of pathogens:

The samples showing positive growth were further analyzed based on colony morphology and Gram-staining for microscopic features. Subsequently, biochemical profiling was performed using standard biochemical tests (IMViC, Oxidase, and catalase) [11]. Uropathogenic *E. coli* (UPEC) were examined for ability to produce indole, lactose fermentation on MaCconkey agar, reaction to Voges-Proskauer, and yielding negative results to citrate utilization and positive ones for methyl red test. Differential media such as Chromogenic UTI agar (HiCrome™) and mannitol salt agar (HiMedia, Maharashtra, India) were used for further identification. Identification of *S. aureus* was based on Gram stain, microscopic morphology, Catalase test, hemolysis on Blood agar, and yellow colonies on mannitol salt agar.

Antibiotic susceptibility test:

The antimicrobial susceptibility test was performed by the standardized Kirby-Bauer disc diffusion method using Muller-Hinton agar, according to the guidelines recommended by CLSI (2024) [12]. The antimicrobials tested were Amikacin (30 µg), Gentamicin (10 µg), Netilmecin (30 µg), Tobramycin (10 µg), Ciprofloxacin (5 µg), Trimethoprim (5 µg), Trimethoprim / Sulfamethoxazole (25 µg), Tetracycline (30 µg), Cefotaxime (30µg), Cefoxitin (30 µg), Piperacillin (100 µg), Nitrofurantoin (300 µg), Imipenem (10 µg), Chloramphenicol (30 µg), Azithromycin (15 µg), Clindamycin (2 µg), Vancomycin (30 µg) and Erythromycin (15 µg).

Molecular detection of virulence genes of *E. coli* and *S. aureus*:

The most frequent pathogen among Gram negative bacteria (*Escherichia coli*) and the most common Gram positive bacteria (*S.aureus*) associated with UTIs were examined for identification of particular virulence genes (*fimH* and *KpsMT II* for *E.coli* and *mdeA* and *hla* for *S.aureus*) by PCR using the primers listed in **Table 1**. DNA extraction was performed by Geneaid™ DNA Isolation Kit (New Taipei City, Taiwan) according to the manufacturer's instructions. . 1-2 ml of cells from overnight culture on BHI broth was harvested by centrifuging at 14,000 rpm, followed by vortexing, cell lysis, DNA Binding, washing and elution. The reaction mixture for polymerase chain reaction (PCR) assay was 50 µl that was prepared as follows: 25 µl of GoTaq® G2 Green Master Mix (Promega ,USA), 13 µl of sterile double distilled water, 4 µl deoxynucleoside triphosphates (dNTPs) mixture, and 4 µl of each forward and reverse primer . On 1.5% agarose, genomic DNA was isolated and stained by 0.5 µg/ml of RedSafe nucleic acid staining solution (Intron, Seongnam-si, South Korea). A comb was placed close to one gel edge. Once the gel had solidified, the tape and comb were carefully taken off. The gel tank was filled with 1X Tris-Borate Ethylenediaminetetraacetic acid buffer (TBE) (Promega corporation, Wisconsin, USA) prior to the slab being positioned horizontally in the electrophoresis tank. After loading the samples and the Safe-Green 100bp Opti-DNA Marker (Abm®, Richmond, Canada) the electrophoresis apparatus was programmed with a constant 80 volt current for 70 minutes.

Table 1: Primers used in this study

Gene	Primer name	Sequence (5'-3')	PCR size	References
<i>FimH</i>	F	TGCAGAACGGATAAGCCGTG G	508	[13]
	R	GCAGTCACCTGCCCTCCGGT A		
<i>kpsMT II</i>	F	GCGCATTTGCTGATACTGTTG	272	[13]
	R	CATCCAGACGATAAGCATGA GCA		
<i>mdeA</i>	F	GCGAGAGGTGAAACGTTAGC	256	[14]
	R	AGAACAGAGCAGCAGCAACA		
<i>Hla</i>	F	ACCGCCAATTTTCCAGAAT	167	[14]
	R	CCTGGCCTTCAGCATTTAAG		

Statistical Analysis:

"Data analysis was performed using SPSS (Version 2019) to examine the influence of various factors on study outcomes [15]. Percentage comparisons were assessed for statistical significance using chi-square tests at two probability levels ($p < 0.05$ and $p < 0.01$).

RESULTS:

The study revealed that 43 out of 150 urine samples showed positive bacterial cultures (28.7%). After isolation and identification, five types of bacteria were isolated and identified using biochemical reactions and differential media. The predominant Gram-negative bacteria were *E. coli* (34.9%), followed by *Klebsiella pneumoniae* (25.6%), *Pseudomonas aeruginosa* (18.6%), and *Proteus mirabilis* (11.6%). The less common pathogen was *S.aureus* (9.3%) as shown in the **Table 2**.

Table (2): Frequency of isolated pathogens associated with UTIs

Bacterial isolates	Frequency	
	No.	%
<i>Escherichia coli</i>	15	34.9
<i>Klebsiella pneumonia</i>	11	25.6
<i>Pseudomonas aeruginosa</i>	8	18.6
<i>Proteus mirabilis</i>	5	11.6
<i>Staphylococcus aureus</i>	4	9.3
(P≤0.01).		

Regarding sex, this study revealed that the highest prevalence rate of UTI was in females (60.5%) in comparison to males (39.5%). Ten-year age division was used to assess the results.. The prevalence of UTI according to age group was highest in the age group (61- 70) years, with 10 patients accounting for (23.25 %) of the cases, followed by the group aged (21-30), accounting for (20.93%). While the less prevalent groups were the age groups of (11-20), there were two patients with UTIs, representing (4.65%) of overall cases as described in **Table 3**.

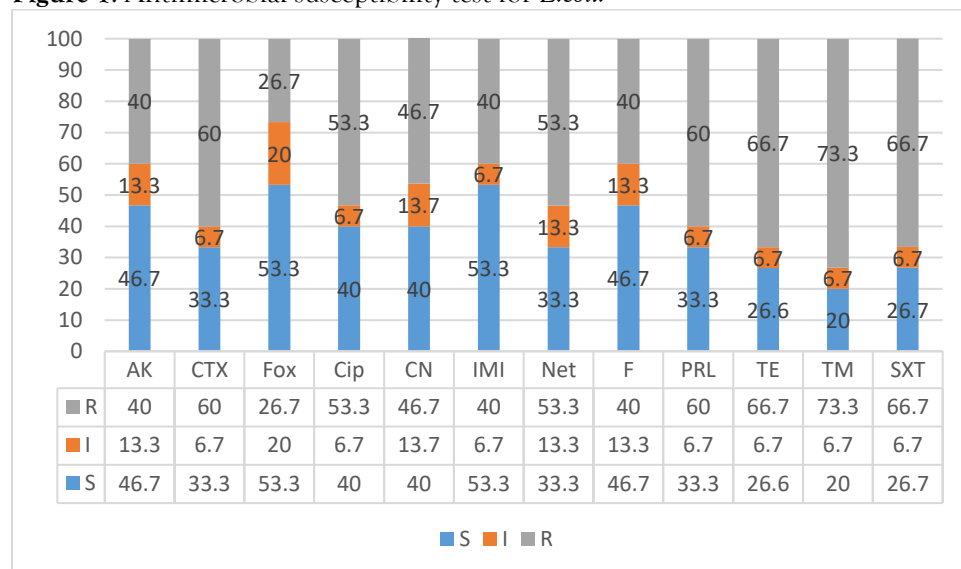
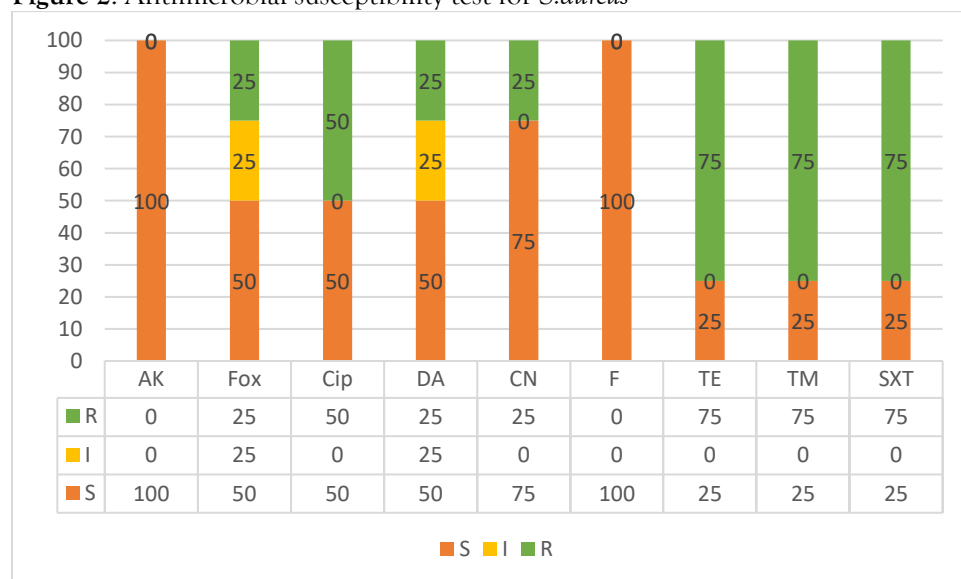
Table 3: Distribution of UTI patients according to age.

Age groups	Positive cultures		Total	
	No.	%	No.	%
11-20	2	4.65	37	24.67
21-30	9	20.93	29	19.33
31-40	5	11.63	23	15.33
41-50	6	13.95	16	10.67
51-60	7	16.28	18	12
61-70	10	23.26	19	12.67
> 70	4	9.30	8	5.33
Total	43	28.7	150	100
(P≤0.01).				

Antibiotic susceptibility test:

Thirteen antibiotics with different mechanisms of action were tested against Uropathogenic *E. coli* (UPEC) and *Staphylococcus aureus* isolates associated with UTI. *E.coli* isolates exhibited high resistant to Cefotaxime , Ciprofloxacin , Netilmicin and Piperacillin, accounting for 60%, 53.3% ,53.3%, 60%, respectively. More than 66% of Uropathogenic *E. coli* showed significant resistance to Tetracycline, Trimethoprim/sulfamethoxazole, and Trimethoprim (accounted for 66.7%, 66.7% , and 73.3%, respectively). Susceptibility rates of *E. coli* for Cefoxitin , Gentamicin, Imipenem, Nitrofurantoin, and Amikacin were 53.3%, 40%, 53.3%, 46.7%, and 46.7%, respectively, as described in **Figure 1**.

Most of *S.aureus* isolates exhibited significant resistance against Tetracycline, Trimethoprim, and Trimethoprim / Sulfamethoxazole (75% for each). 50% of *S.aureus* isolates were resistant to Ciprofloxacin. The data indicate that susceptibility rates of *S. aureus* for Amikacin, Cefoxitin, Clindamycin, Gentamicin and Nitrofurantoin were 100%, 50%, 50%, 75%, and 100%, respectively, as shown in **Figure 2**

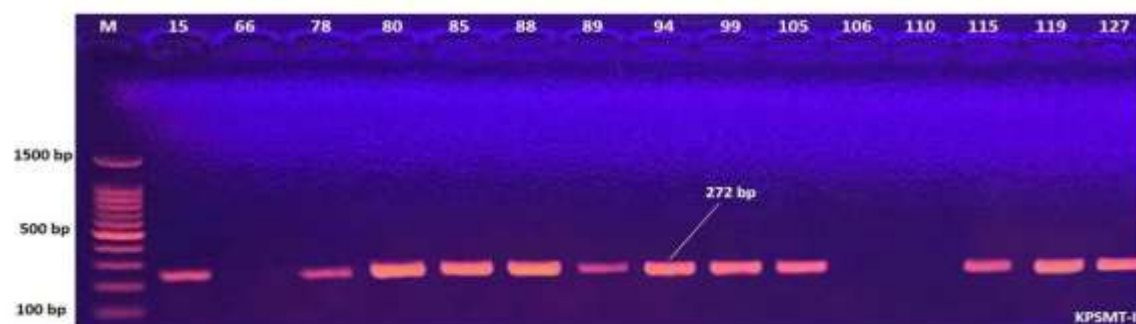
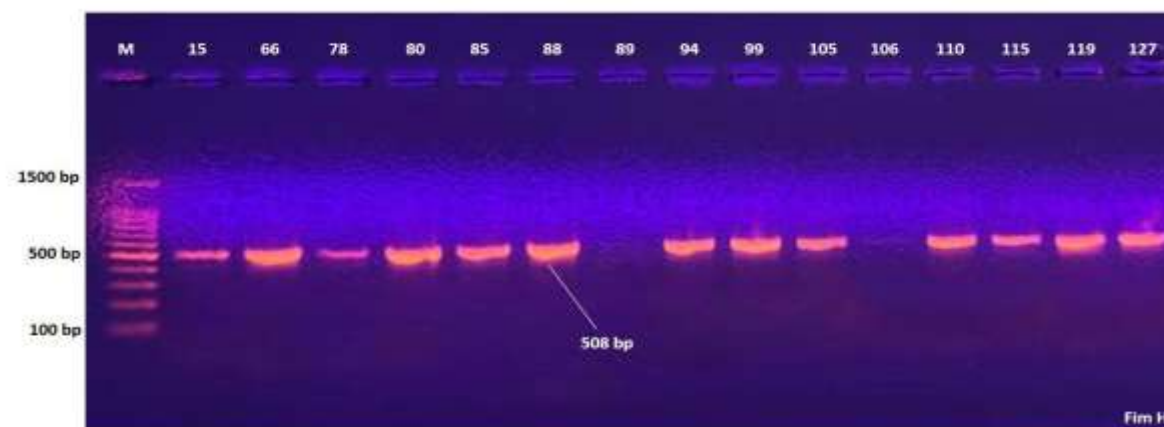
Figure 1: Antimicrobial susceptibility test for *E.coli*.**Figure 2:** Antimicrobial susceptibility test for *S.aureus***Molecular detection of virulence genes:**

This study investigated the presence of virulence genes of leading causative agent among Gram negative rods (*FimH* and *KpsMT II* genes for *E. coli*) and the virulence genes of the most common Gram Positive bacteria linked to urinary tract infections (*mdeA* and *hla* genes for *S. aureus*) by Polymerase chain reaction (PCR), as described in **Table 4**.

Table 4: prevalence of virulence genes of UPEC and *S.aureus*.

Bacteria	Gene	Positive results		Total	
		No.	%	No.	%
<i>E.coli</i>	<i>FimH</i>	13	86.7	15	100
<i>E.coli</i>	<i>KpsMT II</i>	12	80	15	100
<i>S. aureus</i>	<i>mdeA</i>	2	50	4	100
<i>S. aureus</i>	<i>Hla</i>	2	50	4	100
(P≤0.01).					

Out of the 15 *E. coli* isolates, the bacteria were identified using biochemical tests. All isolates of *E. coli* were examined by PCR amplification via *FimH*, and only 13 isolates (86.67%) were positive for molecular detection of the *fimH* gene (**Figure 3**). All 15 isolates of *E. coli* were tested by PCR amplification via *KpsMT II*, and the gene was found in only 12 isolates (80%), as shown in **Figure 4**. PCR assay revealed that The combination of *FimH* and *KpsMT II* genes was found in eleven isolates (73.3%), and only one isolate showed absence of both at the same time (6.7%).

Figure 3: PCR products of the amplification of partial region of gene *FimH* of *E.coli***Figure 4:** PCR products of the amplification of partial region of gene *KpsMT II* of *E.coli*

Only 2 out of 4 isolates (50%) of *S. aureus* in the current investigation were positive for the *mdeA* gene, as shown in the **Figure 5**. All four isolates of *S. aureus* were examined for the *hla* gene by PCR, and only two isolates (50%) were positive for this gene (**Figure 6**). Only one isolate out of four harbored both of these genes at the same time, accounting for 25%

Figure 5: PCR products of the amplification of partial region of gene *mdeA* in the isolates of *S. aureus*

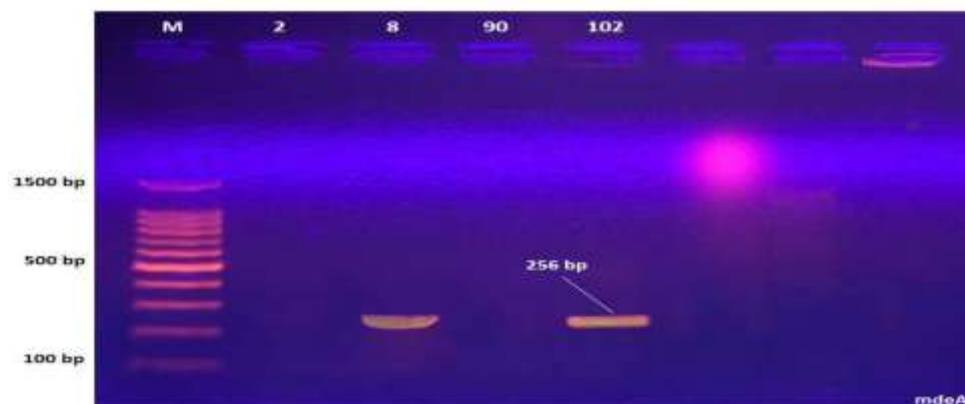
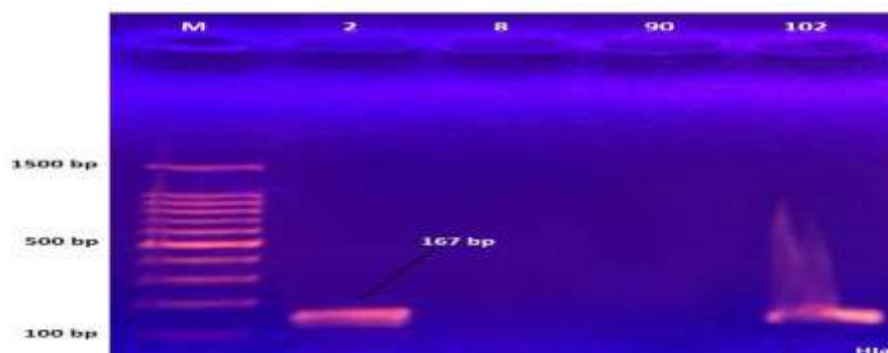


Figure 6: PCR products of the amplification of partial region of gene *hla* in the isolates of *S. aureus*.



DISCUSSION :

In the current study, 150 patients who showed symptoms of urinary tract infection were included. However, only 43 patients (28.7%) exhibited positive bacterial cultures on Blood and MacConkey agar. These results align with previous studies. *E. coli* was the predominant pathogen among Gram-negative bacteria associated with Urinary tract infection, followed by *K. pneumoniae*, *P. aeruginosa*, and *P. mirabilis* (accounting for 34.9%, 25.6%, 18.6%, and 11.6%, respectively). The less common pathogen linked to UTIs was *S. aureus* (9.3%). The results agree with the data reported by Salwa Muhsin Hasan and Khalid S. Ibrahim [16], who found that *E. coli* was the leading cause of UTIs (35.25%). A study conducted in Lebanon stated that the predominant pathogen associated with UTI was *E. coli* (60.53–73.98%), followed by *K. pneumoniae* (5.32–8.33%) [17]. This study revealed that females, 60.46%, experience more UTIs than males (39.54%). The higher incidence of UTI in female compared to males may be due to anatomical and physiological differences, including a shorter urethra and proximity to the anus [18]. According to Abou Heidar et al., women are twice as likely to develop urinary tract infections, compared to men and the frequency of these infections rises with age [19]. The frequency of *S. aureus* linked to UTI in Turkey was 6.2% [20], in Egypt 34% [21], in Saudi

Arabia 1.79% [22], and in Iran 34.6% [23]. The current study revealed that the age group (61-70) exhibited the highest infection rate among patients with urinary tract infection. This may be because elderly people face higher risks for Urinary tract infections due to factors like diabetes, stroke, dementia, kidney stones, and bowel and bladder incontinence [24]. This study disagrees with Isma'il, who reported that the highest bacterial urinary tract infection rate was observed in younger people were between (21-30), followed by the age group 31-40 years [25]. Up to 66.7% of *E. coli* isolates were resistant to Tetracycline, and Trimethoprim/sulfamethoxazole. *E. coli* isolates were highly resistant to Trimethoprim (accounts for 73.3%). This study is in agreement with the data reported by Nuha Abd Al Rheem Saad et al, who found that UPEC isolates were highly resistant to trimethoprim/sulfamethoxazole & tetracycline (65.0% and 57%, respectively) [26]. A study was conducted in Baghdad investigating the disturbance of antibiotic resistance genes of UPEC in pregnant women, stating that the *dfrA1* gene, which is associated with trimethoprim resistance, was found in 74% of UPEC isolates [27]. The present study observed that 75% of *S. aureus* isolates showed significant resistance to Tetracycline, trimethoprim and trimethoprim/sulfamethoxazole. Novel tetracycline resistance pathways in *Staphylococcus aureus* have been discovered by recent investigations. On a multiresistance plasmid, a novel gene called tet(63) was found that encodes an efflux protein and confers tetracycline resistance [28]. Because they produce stronger biofilms than wild-type strains, trimethoprim/sulfamethoxazole-resistant small colony variations (SCVs) provide more difficulties in antimicrobial therapy [29]. The sluggish diffusion of antibiotics through the biofilm matrix may sustain the development of multidrug resistance, maybe through the selection of highly tolerant strains that are momentarily exposed to sub-inhibitory dosages of antimicrobial therapy [30]. Out of 15 isolates of *E. coli* were examined for identification of *fimH* by PCR, we found that only 13 (86.67%) of these isolates were positive. Studies in Iraq have employed PCR-based methods to detect *fimH* and other virulence genes in UPEC isolates. The prevalence of the *fimH* gene in uropathogenic *Escherichia coli* (UPEC) isolates from Iraqi patients is consistently high, ranging from 94.8 % to 100% [31] [32]. This study found that *KpsMT II* was present in 80% of uropathogenic *E. coli*. This observation was close to a study conducted by Umran & Al-Khateeb, 2020), who stated that the prevalence of *KpsMT II* in uropathogenic *E. coli* was (82.3%), 78.5% of these isolates harboring this gene were associated with mild urinary tract infection and 100% of them were linked to urosepsis [33].

In the current study, the prevalence of the *mdeA* gene in isolates of *S. aureus* associated with urinary tract infections was 50%. *S. aureus* isolates, which harbored *mdeA*, were Ciprofloxacin-resistant *S. aureus*. This study agrees with a study conducted in Iran reported that the *mdeA* gene was found in (61.7%) of *S. aureus* isolates, and it had a significant association with ciprofloxacin-resistant *S. aureus*, compared to those with high susceptibility to ciprofloxacin [34]. This study has observed that *hla* gene was expressed by 50% of *S. aureus* isolates. A study carried out in Iran stated that the prevalence of *hla* in *S. aureus* was 93% [35]. According to Ali Jaffar Saleem, the second most prevalent hemolysin gene in *S. aureus* isolated from different sites was *hla* (accounted for 95%) [36].

CONCLUSION:

The study observed that bacterial isolates exhibited variable resistance rate, and many of them had a significant resistance to empiric therapy. These increased resistance rates among *E. coli* and *S. aureus* isolates to commonly prescribed antibiotics such as tetracycline, trimethoprim, and trimethoprim/sulfamethoxazole, suggests that clinicians should consider alternative antibiotics or combination therapies in severe UTI cases.

Conflicts of interest: The authors declare no conflicts of interest.

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Ethical issues: The study followed the ethical standards of Declaration of Helsinki. Therefore, all participants provided written informed consent before undergoing any procedures. This study was approved by the Ethics

Committee of Shatrah University, college of veterinary medicine (No 494, date: 10-2024). Ethical standards include plagiarism, fabricated data, and double publication were observed by the authors.

Authors' Contributions:Karrar Fadhil Hassan: Collection, processing of the material and text writing.

Haydar Khamis Shanan: Concept, design of study and data analysis.

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