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Comparative Assessment of Pathogenic Bacteria in Urinary Tract Infections and Renal Stones: Occurrence, Risk Factors, Genomic Profiles, and Impact on Renal Function

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ABSTRACT

Background: Urinary tract infection (UTI) is one of the most recurrent causes of urolithiasis. Over the last 20 years, the prevalence of infected stone has changed significantly. Several studies have found the relationship between urinary stones and urinary tract infection.

Methods: This study was carried out in the department of urology, and bacteriology of Al nasrih Hospitals, for six months period after ethical approval. A total of 150 patients who had UTI with urinary stones were included. After taking informed written consent, detailed clinical history and necessary investigations were carried out in each patient. Urinary stone was sent for chemical analysis. Data were collected in separated case-record form and analyzed by SPSS 24.

Results: The mean age ± SD of the entire population (150 patients) was 38.8±14.6, ranging from 13 years old to 68 years old 42 patients (65%) with UTI were male, and 33(44%) were female. On the other hand, among the renal stone population, males constituted 62%, while females made up 37.3%. Among all, 150 patients (35.3%) exhibited positive bacterial growth on culture media, wherein majority were gram negative bacteria (30.19%). E. coli was the most common bacteria followed by Klebsiella (22.64%), p. aeruginusa (16.98%) & s. aureus (15.9%). Most commonly found urinary stone. However renal stone had mild impact on renal function

Conclusion: urinary tract infections are strongly linked to urolithiasis, with Gram-negative bacteria, especially E. coli, being the main Bactria that cause urinary stones to become infected. This emphasizes the necessity of microbiological testing and focused antibiotic therapy in stone-forming patients with UTIs.

KEYWORDS: Renal stone ,UTI, E .coli ,Renal function.

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INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections people experience around the world. They're not just a minor inconvenience they can cause real pain, disrupt daily life, and lead to serious complications, especially for those who are more vulnerable. People with diabetes, older adults, and women in their reproductive years are particularly at risk. For many, UTIs mean missed work, repeated doctor visits, and ongoing discomfort, making them a major health and financial concern for individuals and healthcare systems alike (Bitew et al. 2025). One lesser-known but serious complication of urinary tract infections is their role in the formation of kidney stones specifically a type called struvite stones. These aren't your typical kidney stones; they're directly tied to certain bacteria that cause infection. Organisms like Proteus, Klebsiella, and Pseudomonas produce an enzyme called urease, which alters the chemistry of urine and creates the perfect environment for struvite (magnesium ammonium phosphate) stones to form. Unlike other kidney stones that may develop slowly over time, these infection-related stones can grow rapidly—sometimes filling the entire kidney—and often require both medical and surgical intervention. For patients, this means not only dealing with the pain and urgency of a UTI but also facing the risk of significant kidney damage if the infection and stones aren't treated promptly and thoroughly.(Zhang et al. 2014).(Recent evidence indicates that uropathogens do not merely induce transient inflammation but may actively contribute directly or indirectly to stone formation through biochemical mechanisms (e.g., urinary alkalinization) or by facilitating mineral deposition via biofilm formation on bacterial colonies (Song et al. 2024). Moreover, advances in whole-genome sequencing have revealed substantial genomic diversity in virulence and antimicrobial resistance genes among bacterial isolates derived from UTI cases complicated by renal calculi compared to uncomplicated UTIs(Owaid and Al-Ouqaili 2025).

Despite growing insights into the microbiological and clinical dimensions of this interplay, a critical knowledge gap persists regarding a comprehensive comparative assessment of the epidemiological profiles, shared risk factors, and genomic characteristics of pathogenic bacteria in UTIs with and without concomitant renal stones. Furthermore, the cumulative impact of recurrent or chronic infection on long-term renal function remains insufficiently characterized.

Accordingly, this study aims to conduct a systematic comparative assessment of pathogenic bacteria in UTIs associated versus non-associated with renal stones, encompassing: their prevalence and clinical correlates, identification of common demographic and comorbidity-related risk factors, genomic profiling of bacterial isolates using high-resolution sequencing, and evaluation of their potential impact on renal function—assessed via estimated glomerular filtration rate (eGFR) and proteinuria markers. The findings are expected to inform precision diagnostics, guide targeted antimicrobial therapy, and potentially pave the way for microbiome-informed preventive strategies against infection-related nephrolithiasis.

MATERIAL AND METHODES :

Sample collection

Urine samples were collected from 150 patients (75 with UTI and 75 with UTI with kidney stone). Between January 2024 and February 2025, urine samples were collected from patients who visited the Urology department at Al-Nasiriyah Hospitals. Mid-stream urine samples were directly inoculated onto MacConkey and Blood agar, which was subsequently cultivated at 37°C for 24 hours. Bacterial colonies were detected using standard culture and biochemical criteria. and their ability to respond to various antibiotics was assessed using the disk diffusion method. The stones were washed with distilled water, dried, weighed, triturated into a powdered form, and mixed with distilled water to make a 50 ml solution, followed by analysis using Fourier-transform infrared spectroscopy (FT-IR). Ethical clearance was obtained, and informed written consent was secured from each participant. All enrolled patients, both male and female aged 13-68 years, underwent a thorough evaluation, including history-taking, physical examination, and investigations such as urine routine and culture sensitivity, blood sugar, blood urea, serum creatinine, , ultrasonography of the kidney, ureter, and bladder ,

Isolation and Identification of Bacteria

One ml of mid-stream urine was centrifuged at 3000 rpm for 5 min. The sediments were inoculated onto preprepared and sterilized selective and differential media, including MacConkey agar (MAC) and blood agar (BA), followed by incubation at 37°C for 24 h. Phenotypic identification was based on colony morphology, pigmentation, Gram staining, and biochemical tests such as oxidase, catalase, urease, indole, methyl red, Voges-Proskauer (VP), and citrate utilization. All isolates were further confirmed by the automatic system VITEK II (bioMérieux, France).

PCR analysis.

The microarray data were verified by PCR amplification using primers targeting genes located upstream and downstream of pathogenicity island

Statistical analysis

This study employed descriptive statistics. The categorical variables, including age categories, sex, residency, and bacterial isolation, were expressed as percentages (%). Statistical comparison was conducted using the Chi-square test ($\chi 2$) with two-sided. The significance level was 0.05. All statistical analysis was conducted by SPSS version 19.

Ethical approval and considerations

The study protocol was approved by the scientific committee of the College of Al-Shattrah Veterinary Medicine. Written informed consents were obtained from patients by direct interview, after explaining the purpose of the study and assuring the confidentiality of personal information.

RESULT

This study included a total of 150 patients divided into two groups: 75(50%) had urinary tract infections, designated as the UTI group, and 75 (50%) had renal stones or a history of recurrent renal stone, designated as the renal stone former group. The mean age ± SD of the entire study population (150 patients) was 38.8±14.6, ranging from 13 years old to 68 years old. The mean age of the UTI group was 38.9 ±14.1, while the mean age of the renal stone former group was 39.2± 15.6. Regarding gender, 42 patients (65%) with UTI were male, and 33(44%) were female. On the other hand, among the renal stone former group, males constituted 62%, while females made up 37.3%. Concerning residency, the number of patients residing in urban areas was higher than that in rural areas for both groups (UTI and renal stones former). Statistical analysis revealed that no significant differences (P≥0.05) existed between the UTI group and renal stone former group for the three variables (age, gender, and residency).

CHARACTER	GROUPS	UTI (%)	STONE (%)	P-VALUE
AGE: MEAN ± SD		38.9 ±14.1	39.2± 15.6	
GENDER	Male	42 (56%)	47(62.7%)	0.5063
	Females	33(44%)	28(37.3%)	
TOTAL		75	75	
RESIDENCY	Rural	27(36%)	33(44%)	0.4048
	Urban	48(64%)	42(56%)	
TOTAL		75	75	

2 Prevalence of urinary tract infection versus Renal stone among different age groups

Table.2 presents a comparison of the prevalence of UTI and renal stone based on the age groups, both urinary tract infections and renal stones appear to share a similar pattern, as the prevalence among adolescents (11-20 years old) was lower (10.7% in the UTI group and 18.7% in the renal stone former group) than in the other age groups. However, the highest prevalence of UTI was found in the adult age group (36.7%), while the highest prevalence of renal stones was observed in the elderly age group (51-70 years old). Statistical analysis revealed that there was a significant difference (P< 0.05) in the distribution of the urinary tract infection among the UTI group. However, no such significant differences in renal stone prevalence were observed in the renal stone former group
(P>0.05).

Table (2). Prevalence of urinary tract infection versus Renal stone in different age groups of the two study groups

AGE GROUPS	UTI	RENAL STONE	TOTAL
11-20	8(10.7%)	14(18.7)	22(14.6)
21- 35	24(32%)	18(24)	42(28)
36-50	23(30.7%)	20(26.6)	43(28.7)
51-70	20(26.6)	23(30.7)	43(28.7)
TOTAL	75	75	150
P-VALUE	**0.03**	0.516	
X^2	8.680	2.280	
DF	3	3	

4.3 Bacterial Species Diversity and Recovery Rate in the Former UTI and Renal Stone Groups

The bacterial isolation showed that out of 150 urine samples from the two groups, only 53 (35.3%) exhibited positive bacterial growth on culture media. The bacterial recovery rate in the UTI group was 38.7%, slightly higher than the 32% in the renal stone former group. However, statistical analysis showed no significant difference (P>0.05) in the overall bacterial recovery rates. The recovery rate of *E coli* and *K. pneumoniae* was higher in the UTI group than in the renal stone former group; on the other hand, the isolation rate of *P. mirabilis*, *P. aeruginosa,* and *S. aureus* was higher in the renal stone former group than in the UTI group.

Table(3). The isolation rate and distribution of various bacterial species isolated from UTI and renal stone populations.

Bacterial Species	**No. isolates**	**UTI n(%)**	**Renal stone**	**P-value**
E. coli	16(30.2)	10(34.5)	6(25)	0.332
K. pneumonia	12(22.6)	9(31)	3(12.5)	0.146
P. mirabilis	5(9.4)	0(0)	5(20.8)	----
p. aeruginosa	9(16.2)	4(13.8)	5(20.8)	0.999
S. aureus	8(15.1)	3(10.3)	5(20.8)	0.726
E. faecalis	3(5.7)	3(10.3)	0(0)	------
Total	53(35.3)	29(38.7)	24(32)	0.4946

Risk factors of UTI and Renal stone

This study found that 59.3% of patients in both populations have no risk factors, while 40.7% were found to have several risk factors, including dehydration and hypertension, at 16.7% each. Diabetes mellitus was present in 8%, smoking in 15.3%, and only two patients had urinary catheterization. Univariate analysis showed no significant differences in each risk factor with the outcome (UTI or renal stone). Similarly, multivariable logistic regression analysis showed no significant differences (P>0.05)

Table (4): univariate and multivariate analysis of the risks factors of UTI and renal stone populations

Predisposing factor	No. (%)	UTI	STONE	p-value	
				Uni	Multi
Dehydration	25(16.7)	13(17.3)	12(16)		.805
Hypertension	24(16)	15(20)	9(12)		.459
DM	12(8)	8(10.7)	4(5.3)		.402
Smoking	23(15.3)	11(14.7)	12(16)		.773
Urinary catheterization	2(1.3)	1(.13)	1(1.3)		.957
Hyperthyroidism	3(2)	1(1.3)	2(2.7)		.548
No predisposing factors	61(40.7)	26(34.7)	35(46.7)		.842
Total	150	75(50%)	75 (50%)		

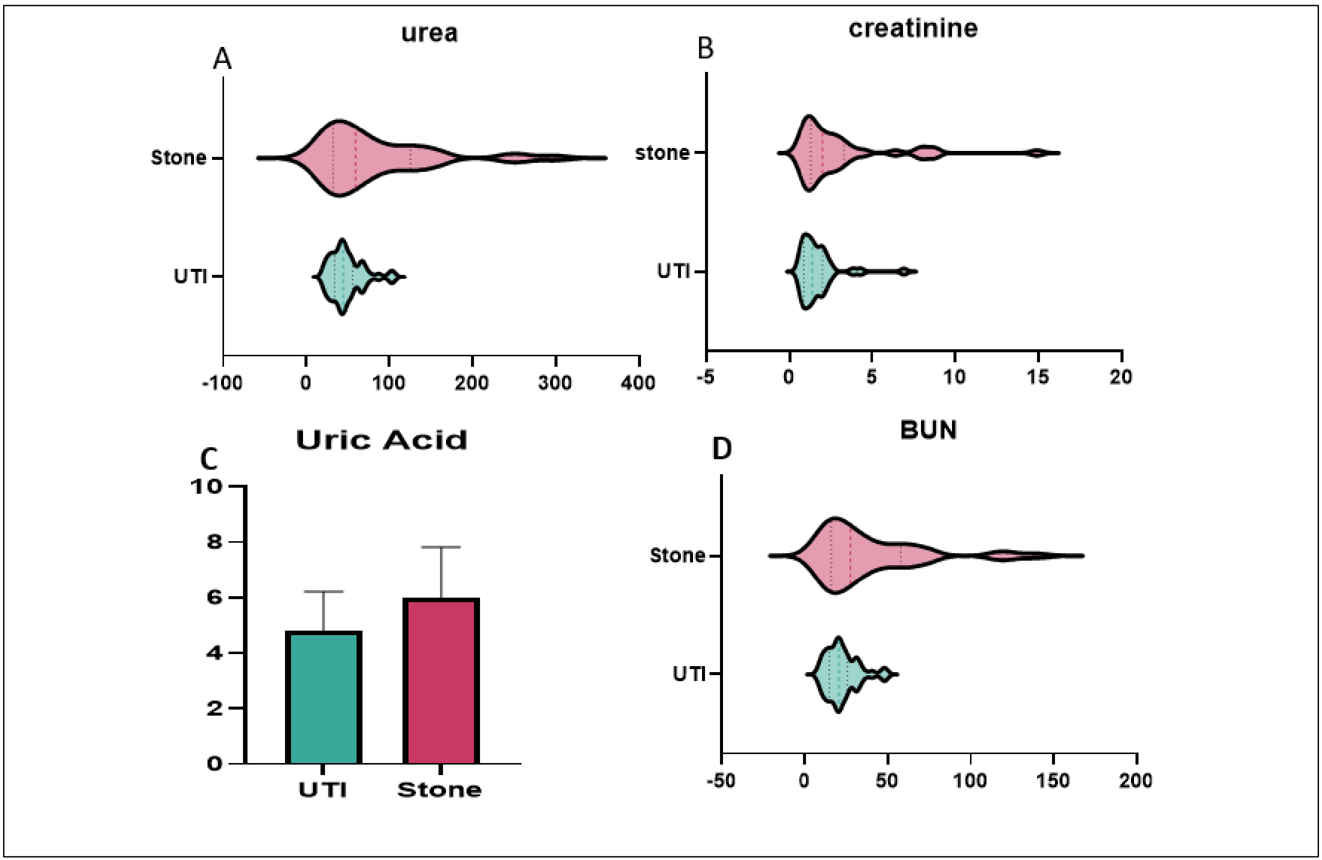
Comparison of renal functions in Renal Stone and UTI populations

The comparison of renal functions of the UTI and renal stone populations illustrated in the Table (4). The median of the blood urea level in the renal stone population, 59, was higher than that recorded in the UTI population, 44.2. The difference was statistically insignificant (P>0.05). Nevertheless, the median of creatinine level in the renal stone population was 2, higher than in the UTI population, 1.4; the statistical analysis revealed that the difference was statistically significant (P<0.05). Similarly, uric acid and blood urea nitrogen (BUN) showed higher levels in the renal stone population than in the UTI population, as the mean of uric acid in the renal stone population was 5.985 compared to 4.823 in the UTI population. In the same context, the median of BUN in the renal stone population, 27.4, was higher than in the UTI population, 20.6. Statistical analysis showed a significant difference (P<0.05) for both uric acid and BUN.

Table (4) comparison of renal functions of the UTI and renal stone former populations

Population	Urea [A]	Creatinine [A]	Uric Acid [B]	Blood-urine nitrogen [A]
UTI	44.2[A]	1.4	4.823	20.6
Renal stone	59	2	5.985	27.4
P-value	0.0617	0.0218	0.0024	0.0342

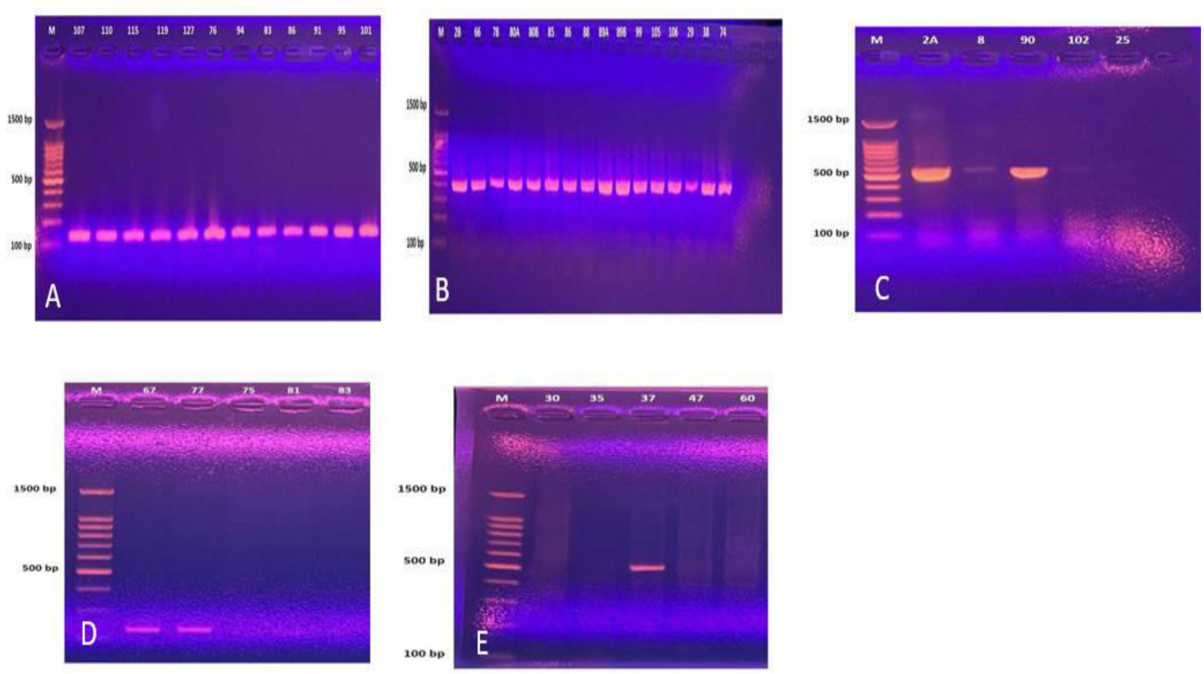
A- Mann-Whitney U for Urea 573.5, Creatinine,532, BUN,549
B- T-value = 3.144, DF=76, F-value =1.745



DNA Extraction: genomic DNA of bacterial samples was extracted following the direction of manufacturing company kit)G-**spin (Intron, Korea**)) with the following steps:
Step 1: bacterial Lysis
Step 2: Cell Lysis
Step 3: DNA Binding
Step 4: Column Washing
Step 5: Elution
Primer Design:
The following Oligoprimer sets (Table) were made as per Snap primer design program and imported to)Macrogen Company (South Korea) were used in the detection of the corresponding SNPs (USD patients by use of conventional Polymerase Chain Reaction (PCR.(

Gene	Sequence (5'-3')	Annealing temperature	Product size	Reference
16srDNA E.coli	CCCCCTGGACGAAGACTGAC ACCGCTGGCAACAAAGGATA	**58°C**	**401 bp**	)Abrar et al., 2019(
16srDNA S. aureus	AACCTACCTATAAGACTGGG CATTTCACCGCTACACATGG	**54°C**	**. 570 bp**	)Kiran Kumar et al., 2020(
16srDNA Klebsiella	ATTTGAAGAGGTTGCAAACGAT TTCACTCTGAAGTTTTCTTGTGTTC	**57°C**	**130bp**	)Abbasi & Ghaznavi-rad, 2023(
Urease proteus	GGTGAGATTTGTATTAATGG ATAATCTGGAAGATGACGAG	**58°C**	**225bp**	)Kamil & Jarjes, 2021(
OprL Pseudomonas	ATGGAAATGCTGAAATTCTCTGCT CTTCTTCAGCTCGACGCGACG	**55°C**	**504b**p	)Chand et al., 2021(

PCR products of the amplification of different identification genes of the isolated bacterial species; A, 16S rRNA of *k pneumonia* (130bp); B, 16S rRNA of E. Coli (401bp); C, 16srDNA *S. aureus* (570bp); D,*p. mirablis* ,Urease proteus gene, (225bp); E, OprL *Pseudomonas* gene 504bp



DISCUSSION

In current study, the overall mean age of entire study population was (38.8) years, ranges from 13 to 68 years old. A similar population parameter to previous studies (Habeeb Al-Athari et al. 2017)(Ranjit and Singh 2020) ,the current study found that most of the patients were 13 to 68 years old, This is mostly connected to the high occurrence of renal stones in the elderly population. (Ismael 2021) ,in other hand the main age of renal stone patient was at 39.2 These results contrast with(Sigurjonsdottir et al. 2015) who found the median age of stone formers patient was 51 (range, 19–70) years , the average ages of the UTI patient at 38.9 this result is disagree with other studies (García-Agudo et al. 2020) However, we did not found any statistical differences of main age of UTI population and renal stones patient population which is in the line with other study (Xie et al. 2020),(García-Agudo et al. 2020) , The outcomes of the present investigation revealed that male patients were affected with renal stones. more than females, with male percentage (62.7%) and female (37.3%with a 1.6:1 ratio) . This result is in covenant with other studies, which showed that urinary tract stones affect men more than women(Kadir, Ibrahim, and Salih 2010) (Shah et al. 2020a) (Shafi et al. 2013); In this study, the percentage of males was also higher than that of females in the UTI group; this finding agreed with (Nicolle 2014),(Alwan et al. 2023)These differences could be attributed to the sample size examined, demographic characteristics, type of diets, and period of study. Finding of this study showed no significant differences between the two groups (UTI and Renal stone) for the thee variables (age, sex, and residence), this finding was in agreement with(Brain et al. 2021)

This study found that both UTI and renal stone occurrence was lower in adolescents age (11-20) (11-20 years), 10.7% and 18.7%

respectively in both groups compared to other age groups, which was similar to finding of (Al-joudi et *al. n.d.*) , also, in this study the high incidence of UTI in adults, 36-50 years, 30.7%, this finding was in agreement with(Ibrahim, Khalid, and Mero 2021) , and in line with(Kamel and Ali 2024),(Alwan et al. 2023) At the same time, it was disagreed with, may due to an increased sexual activity in this age or, it may related to other environmental factors, like region or Socio-economic factors This result is consistent with previous study (Al-Tulaibawi, AL-Nussairawi, and AL-Zuhairy 2024) ,moreover Elderly individuals(51-70)years old are also prone to UTIs, possibly due to immunosenescence and comorbidities that impair bladder function. This result is in line with local study (Mohammed 2025) on other hand the prevalence of kidney stone increased with age from 8.7% in adolescence to 24% in the age class 36-50 years this result is in line with study conducted in Tunisia (Alaya et al. 2012) .wile other researcher performed a study in which is) Jan et al. 2008(Renal stones were most commonly encountered in the third and fourth decades of life. The findings contradict the presnt study, which found that the majority of renal stone patients were between the ages of 51 and 70. However, there are several causes for the differences in the reported prevalence rates in different countries, including the method of conducting epidemiological research, diagnostic criteria of the disease, definition of kidney stone disease, and the disproportionate age and gender groups under observation.(Moftakhar et al. 2022).

In this study, 53 (35.3%) of 150 urine samples showed microbial growth, similar to the result obtained in a previous local study (Mahmood et al. 2024), and in line with the isolation rate, 25.5% reported by (Ranjit and Singh 2020),However, this study's overall isolation rate was much lower than the 78.5% obtained by (Alkadasi et al. 2014) in Yamen and 79% in Pakistan(Jan et al. 2008), this differences could be attributed to geographical variability(Habeeb Al-Athari et al. 2017) The isolation rate in the UTI group was higher than in the renal stone group, 38. 7% vs 32%, although no significant differences were observed. This finding was in line with a local study (Mohammed 2025), and with (Jan et al. 2008) regarding the isolation rate in renal stone, 36.4%, while contradicting the isolation rate of UTI, 50%. This study's finding regarding the renal isolation rate of stone patients disagreed with a local study (Kadir et al. 2010). This discrepancy could be attributed to the differences in the sample type, sample number, and diagnostic procedure. (Habeeb Al-Athari et al. 2017)

Regarding the species diversity, this study indicated that only six species, namely, *E. coli, K. pneumoniae, P. aeruginosa, S. aureus, P. mirabilis*, and *E. faecalis*, were successfully identified, with E. coli being the most prevalent in both groups. This finding was agreed with many previous studies (Ali and Aljanaby 2023) (Al-Tulaibawi et al. 2024). The high frequency of E. coli as the principal causative agent of UTIs in this study was consistent with previous studies, which support its role as the most frequent bacterium responsible for UTIs globally. In comparing the diversity of bacterial species in the urine of UTI and renal stone, this study revealed almost similar species in both groups (Mhana and Aljanaby 2023)except for *P. mirabilis,* which was found only in the renal stone group (Shah et al. 2020b)Regarding the diversity comparison, the findings of this study were similar to those obtained by(Jan et al. 2008). Many previous studies also support this finding(Golechha and Solanki 2001)(Devraj et al. 2016). The obvious association of P. mirabilis with the urine of stone formers in this study was consistent with many previous studies

This study found that levels of uric acid and blood urea nitrogen (BUN) were elevated in individuals with kidney stones. Specifically, the average uric acid level in this group was 5.985, and the median BUN level was 27.4. These findings contrast with those of another study, which reported that patients with uric acid and struvite stones had significantly lower valued glomerular filtration rates related to those with other types of stone conformations. (Chou et al. 2011)

In this study, there was no statistically significant relationship between Diabetes mellitus and renal stone un like (Tung et al. 2023)

which have found DM type 2 diabetes mellitus, hypertension, cardiovascular diseases are consider risk factor for stone formation and chronic kidney disease also did not show any statistically significant relationship between hypertension and renal stone . Anyway, in this regard, we should consider the role of hypertension as a risk factor for stone formation, yet there was no statistical relationship between smoking and kidney stone this result is agree with(Moftakhar et al. 2022) . However, (Khalili *et al*) in their study introduced smoking as a protective factor against kidney stone, some other studies have identified smoking as an independent risk factor for developing kidney stone

In this investigation, the twelve isolates were first identified as K. pneumoniae using biochemical assays and traditional phenotypic criteria based on physical and biochemical traits.16S rRNA gene sequencing distinguishes between living and dead bacteria by amplifying the V4 variable region of bacterial ribosomal RNA. Genomic DNA (gDNA) was extracted from stone homogenate and bladder urine samples using the Blood and Tissue kit according to standard techniques 16S rRNA gene sequencing was positive for 12 (100%) of UTI patient and renal stone patient t this study is disagree with (Dornbier et al. 2020) ,the result is in line with study conducted in china (He et al. 2016). All 16 isolates of *E. coli* were recognized by biochemical tests, and E. coli bacteria were identified using molecular methods based on the 16S rRNA gene, with the appropriate PCR conditions. Polymerase chain reaction was performed on 16 bacterial isolates, with a gene appearance rate of 100%. Gene appearance rate was 100%. This result agree with local study which is conducted in Baghdad Iraq (Jasim, Ali, and Feisal 2019) &(Al-zubaidi 2025) In this study, five *Pseudomonas* isolates were initially identified based on morphological and biochemical characteristics. However, further confirmation was required using molecular techniques. The presence of the *OprL Pseudomonas* gene was assessed via PCR, and the results showed that only 20% of the isolates tested positive for this gene. This finding contrasts with a local study, which reported that 45 out of 57 isolates (78.9%) were positive for the *oprI* gene , (Alammar 2025),however *p, mirabilis* isolates were initially identified through morphological and biochemical methods, the results showed that 40% of the isolates tested positive for this gene. This finding contrasts with a local study that reported the presence of the ureR gene in 15 P. mirabilis isolates, with 14 of them (93.3%) expressing the ureR gene product.(Al-fahham and Kareem 2022),In this study there is five *S. aureus* isolates that required using molecular techniques. This was achieved through the use of the 16S rDNA gene specific to *S. aureus* ,however there is (40%) of 16S rDNA gene axepretion which is disagree with local study which have revealed that 7/40(17.5%) isolates were S. aureus according to 16S rRNA (Farhood and Hamim 2024)

CONCLUSION

urinary tract infections are strongly linked to urolithiasis, with Gram-negative bacteria, especially *E. coli*, being the main Bactria that cause urinary stones to become infected. This emphasizes the necessity of microbiological testing and focused antibiotic therapy in stone-forming patients with UTIs.

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