Journal of Communicable Diseases
Volume 57, Issue 3 - 2025, Pg. No. 103-110
Peer Reviewed & Open Access Journal

Research Article

Isolation and Identification of Bacteria Causing Urinary Tract Infections in Kidney Stone Patients in Baghdad, Iraq

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DOI: https://doi.org/10.24321/0019.5138.202574

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How to cite this article:
Hussein A U, Abdulhussein A J, Al-lami R B Q, Ahmed N, Shakir M N, Gati M A, Sahib A A, Al-Jassani M J. Isolation and Identification of Bacteria Causing Urinary Tract Infections in Kidney Stone Patients in Baghdad, Iraq. J Commun Dis. 2025;57(3):103-110.

Date of Submission: 2024-09-18
Date of Acceptance: 2025-08-18

A B S T R A C T

Introduction: Urinary tract infections (UTIs) represent one of the most common bacterial infections globally and are particularly prevalent among hospitalised patients. The presence of urinary tract stones often increases susceptibility to UTIs, with *Escherichia coli* being the leading causative agent.

Methods: One hundred UTI patients with kidney stones (60 females and 40 males) had urine samples taken. Each sample was cultivated on MacConkey agar medium with blood. Using the Vitek-2 Advanced Expert System, 30 bacterial isolates—24 gram-negative and 6 gram-positive strains—were identified during incubation. The disc diffusion technique was used to investigate the antibacterial susceptibility of ten commonly used medications.

Results: Of the 30 isolates, 20% (6/30) were from males, while 80% (24/30) were from females. *Proteus mirabilis* (33.3%), *Staphylococcus* spp. (6.6%), *Salmonella* spp. (6.6%), and *Klebsiella* spp. (3.3%) were the most common isolates, after *E. coli* (50%). Trimethoprim was ineffective against gram-negative isolates, while ciprofloxacin demonstrated the highest inhibition rate (75%). Ciprofloxacin was 100% effective against gram-positive isolates, whereas penicillin had no inhibitory activity (0%). The findings show that *E. coli* is the most common uropathogen in patients with urinary stones and that UTIs are more common in women.

Conclusion: Antibiotic susceptibility patterns reveal resistance to regularly used drugs. Ciprofloxacin continues to be the most effective antibiotic against both gram-positive and gram-negative uropathogens. In order to effectively manage UTI treatment, the study emphasises the significance of continuous surveillance and antibiotic stewardship.

Keywords: UTI, Kidney Stone, Vitek 2, *E. coli*, *Staphylococcus*, *Proteus mirabilis*

Journal of Communicable Diseases (P-ISSN: 0019-5138 & E-ISSN: 2581-351X)

Introduction

The urinary system, consisting of the kidneys, bladder, ureters, and urethra, is an important system in the human body due to the nature of the work it performs in purifying the blood of harmful and excess substances and disposing of them in the form of urine. Urine's properties, contents, and indications reflect both normal and abnormal physiological states.[1] Every year, millions of individuals suffer from urinary tract infections (UTIs), a serious health issue and one of the most prevalent infections acquired in hospitals. UTI is the most common bacterial infection, affecting people of both sexes at some point in their lives. It encompasses a wide range of clinical conditions, including bladder infections.[2] Prostate infections range from infections of the prostate gland to those of the renal pelvis. Infections in young males are rare and complicated, often due to urinary tract abnormalities. Uncomplicated infections are often the result of unprotected sexual contact with infected individuals.[3,4] Most sources indicate that there is an agreement among various researchers that about 80% to 90% of all UTIs are caused by gram-negative bacteria, with *Escherichia coli* (*E. coli)* being the most common organism.[4,5] UTIs are more common than upper respiratory tract infections, but they also have a higher death rate.

Females are more susceptible to UTIs than men due to anatomical differences that increase the chance of urinary tract pathogens ascending into the urinary tract, thus causing infection. The widespread and ill-advised use of antibiotics has increased the problems of UTIs.[6] This is because the continued use of these antibiotics over long periods has led to the emergence of resistance in bacteria, and consequently, the emergence of strains with high tolerance to antibiotics. This emergence of antibiotic resistance is a major problem faced by the medical community, as well as a major threat to public health.[7] gram-negative bacteria, such as E. coli, mirabilis bacteria Mirabilibacterium, and other species cause Most UTIs. A study conducted by Pitout and Laupland indicated that *mirabilis* bacteria are responsible for 11.85% of UTIs.[8] They also showed that the percentage of these bacteria isolated from women is higher than that of males.

There are complicated UTIs caused by gram-negative bacteria. Such infections are often associated with structural abnormalities in the urinary tract, obstructions, and congenital malformations,[9] and are difficult to treat with antibiotics due to the presence of bacteria within the casts[10]. Lower urinary tract infections (LUTIs) include both bladder infections and urethral infections.

Materials and Methods

An experimental study was performed to identify bacteria of interest, test their prevalence, antibiotic sensitivity and biochemical characteristics.

Sample Collection

Urine samples were collected from 100 patients withkidney stone(s), who visited the kidney disease unit at the Medical City in Baghdad from June to the end of September 2024, based on the specialist physician's diagnosis. Urine samples were collected in sterile, single-use containers. The samples were collected after discarding the first few drops of urine, and then the containers were transferred directly to the laboratory for culture.

Sample Culture

Samples were cultured immediately on blood agar. They were then incubated at 37°C for 24 hours. Then, different isolated colonies were cultured on Enriched and differential culture media, including MacConkey agar, Mannitol salt agar (MSA) and Eosin-methylene blue agar (EMB) agar. MSA was used for the diagnosis of *Staphylococcus aureus* (*S. aureus*) bacteria, as well as for biochemical tests.

Identification of Isolated Bacteria

The growing colonies on the above mentioned agar media were identified based on phenotypic characteristics, including their shape, colour, surface, and the presence of a distinctive odour, consistency, and lactose fermentation on the surface.[10]

The bacteria were gram stained and the arrangement of the bacterial cells, their shapes, and their reaction to the stain were observed.[11]

Potassium Hydroxide Test (KOH test)

This test was performed by mixing a complete loop of colonies growing on solid blood agar with 3% potassium hydroxide (KOH) on a clean glass slide. A gelatinous substance formed within 60 seconds in this test indicates a positive result and confirms that the bacteria are gram-negative.[12]

Biochemical Tests

A set of tests was conducted to identify the bacteria under study, such as the catalase test, oxidase test, gas and fermentation sugar test, and IMViC tests.[13]

Diagnosis by Vitek 2 Advanced Expert System (bioMérieux, Marcy L'Etoile, France)

All isolates were diagnosed by Vitek 2.

Diagnosis using the API 20E Ready-Made Kit

After obtaining results using biochemical tests, further identification was done using the API 20E kit (Analytical Profile Index, 20 tests for *Enterobacteriaceae* and related gram-negative bacteria). This strip contains 20 samples specifically for biochemical tests.

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202574

The method includes the following:[14]

- **Preparation of bacterial suspension:** A single isolated colony (from a pure culture) was picked and a suspension of it was made in sterile distilled water.
- **API 20E Strip inoculation:** Using a clean, dry syringe, the holes of the squirrels were inoculated. The holes were filled with bacterial suspension according to the manufacturer instructions.. The holes were filled with 0.12 mL of suspension, while the amount of inoculum reached 0.28 mol of the Nabiop Gel & Vp & citrate.
- **Incubation at 37°C for 24 hours**.
- **Addition of reagents:** A drop of TDA reagent was Added to the acid deamination test. A drop of James's reagent was Added to the Indo test. A drop of 2VP and 1VP reagents were Added, respectively, to the Proskauer-Vogas test and a drop of 2Nit&1Nit reagent was added to the glucose test.

Antibiotic Susceptibility Test

Drug sensitivity testing was done for bacterial isolates using the disc diffusion method for ten antibiotics: amikacin 30 µg, ceftriaxone 30 µg, cefepime 30 µg, chloramphenicol 30 µg, ciprofloxacin 5 mg, nitrofurantoin 300 µg, gentamicin 10 µg, piperacillin 100 µg, penicillin 10 units, and trimethoprim 5 µg. The study involved transferring 2–4 colonies of bacteria to a sterile test tube containing 5 mL of tryptone soybean nutrient broth and incubating at 37°C for 8 hours. The resulting growth was reduced using normal saline, and the growth was compared with a standard 0.5 McFarland control tube. Bacteria were spread on solid Mueller–Hinton plates. Antibiotic discs were put on the surface of the culture. The plates were incubated at 37°C for 18 hours, and the diameters of the inhibition zone were measured using a vernier calliper.

Ethical Approval

All permissions are taken from the authority of the Ministry of Health. The aim of this study was explained, and only those who agreed by the responsible physician to participate were included in this study. The study was approved by the Department of Microbiology at Al-Karkh University of Science, Baghdad, Iraq.

The bacterial strains identified from urine collected from patients with kidney stones, as determined by the responsible physician were the subject of this prospective investigation, which was carried out in the labs of the kidney disease unit at the Medical City in Baghdad, Iraq. One hundred people with urinary tract stones (as identified throughout the study period) had urine samples collected. A bacterial colony was removed from overnight-incubated MHA plates using a loopful of colonies and then combined with suspension medium using the VITEK2 Advanced Expert System and E20api established procedures. After that, the bacterial suspension was transferred to test strip wells and incubated for 24 hours at 37°C.

A total of 30 bacterial isolates—24 Gram-negative and 6 Gram-positive strains—were identified during incubation. The antimicrobial susceptibility of the following drugs was determined by the Kirby-Bauer method: amikacin 30µg., Ceftriaxone 30 µg, cefepim 30µg, Chloramphenicol 30 mcg, Ciprofluxalin 5 mg, Nitrofurantoin 300 mcg, Gentamicin 10 mcg, Pipracillin 100 mcg, Pencillin 10 units, and Trimethoprim 5µg. ciprofloxacin gave the highest inhibition rate, while Trimethoprim and Penicillin were the least effective antibiotics, and the resistant strains were confirmed by minimum inhibitory concentration (MIC). These tests were performed on strains grown on Mueller-Hinton agar plates in triplicate for each isolate, and results were interpreted by more than one independent reader.[15, 16]

Results and Discussion

Identification of Bacteria Isolated from the Urinary Tract

All samples were grown on different media as shown in Table 1. The isolates were identified on the cultures, and their growth characteristics were studied on various media, as shown in Tables 1 and Figures 2. Fifteen *E. coli* isolates, 10 *Proteus mirabilis* isolates from one culture, and two *Salmonella* isolates, two *S. aureus* isolates, and one *Klebsiella* isolate were obtained from another culture (Figure 1).

Staphylococci bacteria appeared as Large, round, convex, shiny, golden-yellow colonies were obtained. They were unable to grow on most MacConkey medium formulations. On the other hand, it appeared microscopically as gram-positive cells with a spherical shape, clustered together, indicating that the bacteria belonged to the genus *Staphylococcus*. This is consistent with the results of Chugh and Jha.[14] When *S. aureus* was grown on this medium, it appeared as relatively large, slightly raised, round colonies with a golden-yellow colour, surrounded by a transparent zone, as a result of its complete haemolysis of the beta type.[17, 18]

Mannitol salt agar is a differential and selective medium due to its high content of sodium chloride (7.5–10%), which inhibits the growth of many microorganisms but can be tolerated by *S. aureus*. It also contains mannitol sugar and phenol red indicator. *S. aureus* bacteria appeared as yellow colonies capable of fermenting mannitol sugar, producing an acid that caused the indicator to turn from pink to yellow (Figure 2).[19]

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202574

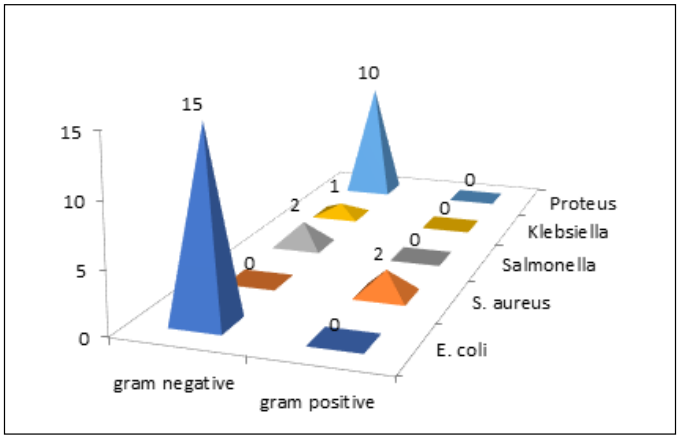

Figure 1.Distribution of Pathogenic Bacteria

Table 1.Culture Characteristics of Pathogenic Bacteria Isolated from UTI Patients

Bacteria	Blood Agar	Mannitol Salt Agar (MSA)	MacConkey Agar	Eosin-methylene blue agar (EMB)
E. coli	Small, round, smooth, may show β-haemolysis or be non-haemolytic	Inhibited by the high salt concentration in MSA	Pink to red colonies (lactose fermenter)	Green metallic sheen colonies (strong lactose fermenter)
Proteus mirabilis	Exhibits a characteristic swarming motility, where it spreads across the agar surface in a thin, film-like layer, often forming concentric rings or waves	Inhibited by the high salt concentration in MSA	Typically appear as colourless or pale, non-lactose fermenting colonies	Typically appear as non-lactose fermenting, pale or colourless colonies, often described as grey
Salmonella	Typically appear as grey/ white, non-haemolytic	Inhibited by the high salt concentration in MSA	Colourless or pale yellow, transparent colonies	Colonies are typically colourless or transparent (weak lactose fermenter)
Klebsiella	Large, mucoid, non-haemolytic colonies	-	Large, mucoid, pink colonies (lactose fermenter)	Large, mucoid, pink to purple colonies, no metallic sheen (weak lactose fermenter)
S. aureus	Small, round, smooth, golden yellow, raised, glistening, haemolytic colonies	Yellow colonies	-	-

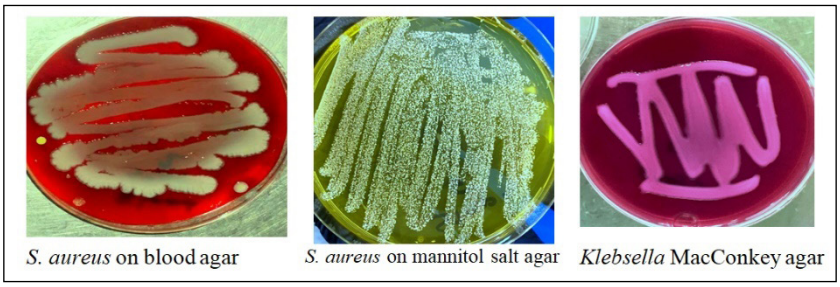

Figure 2.Culture of Pathogenic Bacteria. (a) S. aureus on Blood Agar. (b) S. aureus on Mannitol Salt Agar. (c) Klebsiella on MacConkey Agar

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202574

Biochemical tests are used to identify bacteria based on their metabolic activities and enzyme production. These tests analyze how bacteria utilise different substrates, produce specific end products, and express certain enzymes, allowing for differentiation between bacterial species. Examples include tests for carbohydrate metabolism, protein metabolism, and enzyme activity, like catalase, oxidase, and urease.[20]

Morphological and biochemical tests were conducted on a few gram-negative bacteria that were isolated from UTI patients. *Klebsiella pneumoniae* colonies were big, pale pink, and mucoid on MacConkey medium; 12 isolates exhibited grey, mucoid colonies. They were gram-negative rods under the microscope (Table 2).[21]

MacConkey is a differential medium. It contains crystal violet, which stops gram-positive bacteria from growing. Additionally, it contains bile salt, which prevents the growth of non-enteric gram-negative bacteria. This medium is a differential medium because it separates lactose-fermenting bacteria from non-lactose-fermenting bacteria. Lactose and phenol are present in this medium. Lactose-fermenting colonies produce acid, which lowers the pH and causes the indicator's colour to turn red.[22,23]

The diagnosis of the gram-negative isolates in the study was confirmed using the API 20E kit after they were identified using the results of biochemical tests, morphological characteristics, and microscopic characteristics, as shown in Table 3.

Table 2.Biochemical Tests for Pathogenic Bacteria

Test	E. coli	Proteus mirabilis	Klebsiella	S. aureus	Salmonella
Gram stain	-	-	-	+	-
Catalase	+	+	+	+	+
Oxidase	-	-	-	+	-
Kligler	K/A	K/A	K/A	K/A	K/A
Urease	-	+	+	+	-
Indole	+	-	-	-	-
M-R	+	+	-	+	+
VP	-	-	+	+	-
Citrate	-	+	+	+	+
Vitek-2 (%)	99	99	93	98	95

K/A= alkaline slant and acid butt

Table 3.Gram-Negative Bacteria Diagnosis using the API 20E Diagnostic Kit

	K. pneumoniae	Proteus mirabilis	E. coli	Salmonella
ONPG	+	-	+	+
ADH	-	+	-	-
LDC	-	-	+	+
ODC	-	-	+	+
CIT	V	+	-	+
H2S	-	-	-	-
URE	+	+	-	-
TDA	-	-	-	-
IND	-	-	+	-
VP	V	-	-	+
GEL	V	V	-	-
GLU	+	V	+	+
MAN	+	-	+	+
INO	+	-	-	+
SOR	+	-	+	+
RHA	+	-	+	+

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202574

SAC	+	V	+	+
MEL	+	-	+	+
AMY	+	-	-	+
ARA	+	V	+	+

ONPG: o-nitrophenyl-b-D-galactopyranoside, ADH: arginine dihydrolase, LDC: lysine decarboxylase, ODC: ornithine decarboxylase, CIT: utilization of citrate as only carbon source, H2S: production of hydrogen sulphide, URE: urease, TDA Tryptophan deaminase, IND: Indole, VP: the Voges-Proskauer, GEL: gelatin, GLU: fermentation of glucose (hexose sugar), MAN: fermentation of mannose (hexose sugar), INO: fermentation of inositol (cyclic polyalcohol), SOR: fermentation of sorbitol (alcohol sugar), RHA: fermentation of rhamnose (methyl pentose sugar), SAC: fermentation of sucrose (disaccharide), MEL: fermentation of melibiose (disaccharide), AMY: fermentation of amygdalin (glycoside), ARA: fermentation of arabinose (pentose sugar). V= Variable

E. coli was the most common bacterium in this study. This is because its natural habitat is the digestive tract, and because the urinary tract and anus are close together, this type of bacteria can migrate to the urinary tract, causing infections in patients with kidney stones. Kidney stones also scratch the walls of the kidneys and urinary tract, causing blood to leak out. This leaked blood contributes to the increased bacterial growth.[24]

The infection rate was higher in females than in males, as 60 samples were obtained from females suffering from UTIs with kidney stones, while the number of infected males was only 40 (Table 4).

The reason for a higher susceptibility of females to UTIs as compared to men is primarily women's anatomy, with the urinary tract being closer to the anus, making it easier for the bacteria to reach the bladder.[25] The urethra in women is close to the anus and vagina, which increases the chances of bacteria being transferred from these areas to the urinary tract. Though these infections can affect anyone at any age, UTIs are more common in women than in men, particularly in those aged 31 to 50 years, and are mostly brought on by *E. coli* and *K. pneumoniae* [26-27]

Antibiotic Sensitivity Test

Ten antibiotics were tested against gram-negative and gram-positive bacteria. Ciprofloxacin gave the highest inhibition rate of approximately 75% in gram-negative bacteria. It was also the most effective on gram-positive bacteria, with a rate of 100%, while trimethoprim was the least effective antibiotic, with a rate of 0% in gram-negative bacteria. Penicillin was the least effective antibiotic on gram-positive bacteria, with a sensitivity rate of 0% (Table 5).

Antibiotics are of great importance to human life as they reduce the incidence of disease and consequently reduce mortality. When choosing the appropriate type of antibiotic for treatment, several factors must be taken into account, the most important of which are the patient's medical condition and the necessary dosage. Antibiotics are selectively toxic to bacteria, meaning that the antibiotic is harmful to the pathogen without affecting the host cells. They are either bactericidal, causing the pathogen to decompose and thereby killing it, or bacteriostatic, which inhibit bacterial growth and restrict bacterial replication by binding to structures necessary for cell division.[25,28] Antibiotic resistance is encoded by genes responsible for one of the three mechanisms: conjugation, transformation, and delivery to phages.[24] Many antibiotic resistance genes are carried on plasmids, facilitating their separation between bacterial cells.[24,29] This applies to bacteria that carry multiple resistance genes.

Table 4.Rate of Infection on the Basis of Gender of Participants

Sex	No. of Patients	%	No. of Pathogens	%
Female	60	60	24	40
Male	40	40	6	15
Total	100	100	30	30

Table 5.Antibiotic Susceptibility Testing of Bacterial Isolates

Pathogenic Bacteria	K. pneumoniae	Salmonella	S. aureus	E. coli	P. mirabilis
No. of isolates	2	1	2	15	10
Antibiotics	**Sensitivity/ Resistance**				
Amikacin 30 µg	R2	R	R2	R15	R10
Ceftriaxone 30 µg	R2	R	R2	R15	R10

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202574

Cefepime 30 µg	S1\ R1	R	R2	R13\ S2	S1\ R9
Chloramphenicol 30 µg	R1\ S1	R	R2	R14\ S1	R8\ S2
Ciprofloxacin 5 mg	S2	S1	S2	R3\ S12)	R4\ S6
Nitrofurantoin 300 µg	R1\ S1	R	R1\ S1	I14\ R1	S2\ R8
Gentamicin 10 µg	R\ S1	R	R2	R15	R10
Piperacillin 100 µg	R\ S	I	R2	R15	R10
Penicillin 10 units	I\ R	I	R2	I11\ R4	I5\ R5
Trimethoprim 5 µg	R2	R	R2	R15	I7\ R3

R: resistance, S: Sensitive, I: Intermediate

Conclusion

Urinary tract infections, caused by a group of pathogenic bacteria, are usually accompanied by the accumulation of kidney stones. The infection is common in both sexes and all ages. The most common pathogens are *E. coli*, followed by *P. mirabilis*. Some pathogens are resistant to a wide spectrum of antibiotics, while others are sensitive to antibiotics.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: During the preparation of this work, the author(s) used Turnitin to check for AI and plagiarism. The author(s) reviewed and edited the content as necessary and take full responsibility for the publication's content"

Source of Funding: None

Conflict of Interest: None

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ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202574