

Isolation and Identification of Bacteria Causing Urinary Tract Infections and Their Antibiotic Sensitivity Pattern at Mohammad Almoghariaf Hospital in Ajdabiya

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عزل وتعريف البكتيريا المسببة لالتهابات المسالك البولية وانماط حساسيتها للمضادات الحيوية في مستشفى محمد المقريف بأجدابيا

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Received: 11-08-2025; Accepted: 22-10-2025; Published: 14-11-2025

Abstract:

Urinary tract infections represent a significant health concern, leading to elevated morbidity rates in both men and women. The objective of the current study was to isolate and identify various bacterial agents responsible for UTIs. A total number amount to 200 urine samples from at suspected urinary tract infection in the age group 1 year to 105 years old were collected from patients at Mohammed Almoghariaf hospital during August to October 2024. Bacterial isolates identified in this study included *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Pseudomonas spp.*, and *Streptococcus spp.* The results demonstrated a higher prevalence of urinary tract infections (UTIs) among females, with an infection rate of 82.26%, compared to 18.43% in males. Furthermore, the data indicated that the incidence of UTIs was significantly greater in the female population. The study also assessed the antibiotic sensitivity profiles of the isolated bacterial strains, revealing variations in susceptibility to different antibiotics.

Keywords: Isolation, Sensitivity, *Klebsiella Pneumonia*, *Pseudomonas*, *Streptococcus*.

المخلص:

تمثل التهابات المسالك البولية قضية صحية هامة، حيث تؤدي إلى زيادة معدلات المرض في كل من الذكور والإناث. كان الهدف من الدراسة الحالية هو عزل وتحديد العوامل البكتيرية المختلفة المسؤولة عن التهابات المسالك البولية. تم جمع 200 عينة من البول من المرضى المشتبه بإصابتهم بالتهاب المسالك البولية، الذين تتراوح أعمارهم من سنة واحدة إلى 105 سنوات، وذلك من مستشفى محمد المقريف خلال الفترة من أغسطس إلى أكتوبر 2024. أظهرت النتائج ارتفاع معدل انتشار التهابات المسالك البولية (UTIs) بين الإناث، حيث بلغ معدل الإصابة 82.26%، مقارنة بـ 18.43% لدى الذكور. علاوة على ذلك، أشارت البيانات إلى أن حدوث التهابات المسالك البولية كان أعلى بشكل كبير في الفئة النسائية. كما قامت الدراسة بتقييم ملفات حساسية المضادات الحيوية للبكتيريا المعزولة، مما أظهر اختلافات في قابلية هذه البكتيريا للعديد من المضادات الحيوية.

الكلمات المفتاحية: العزل، حساسية، *Streptococcus*، *Pseudomonas*، *Klebsiella Pneumonia*.

1. Introduction:

Urinary tract infection (UTI) refers to a wide range of infectious conditions that impact the urinary system and the kidneys. (Al-Lawati, 2024) UTIs are among the most prevalent infections, and the healthcare expenses related to them are significantly elevated. (Dincer et al., 2023)

The lower urinary tract is often affected by bacteria that primarily originate from the gastrointestinal tract. These bacteria can enter the urethra and proliferate within the bladder, potentially leading to either a singular or recurrent infection. In otherwise healthy individuals, these infections typically remain localized to the urethra and bladder. Annually, approximately 150 million people worldwide are diagnosed with urinary tract infections (UTIs), establishing UTIs as one of the most prevalent bacterial diseases (Abdul-Karem et al., 2023).

The urinary system consists of the kidneys, ureters, bladder, and urethra, with its primary function being the filtration of blood through the removal of waste products and excess water. This system plays a vital role in eliminating metabolic waste from the bloodstream. Furthermore, it contributes to several essential processes, including the regulation of ion and solute concentrations in the blood, as well as the control of blood volume and blood pressure (Mancuso et al., 2023).

According to the CDC, urinary tract infections (UTIs) represent the most prevalent bacterial infection necessitating medical attention, leading to 8.6 million visits to ambulatory care facilities in 2007, with 23% of these occurring in the emergency department (ED) (CDC, 2011).

Between 2006 and 2009, over 10.8 million individuals in the United States sought treatment for UTIs in the ED, and 1.8 million of these patients (16.7%) were subsequently admitted to acute care hospitals (Sammon, 2014).

The economic consequences of employing the emergency department (ED) for the treatment of urinary tract infections (UTIs) are projected to be approximately \$2 billion annually. Additionally, UTIs are recognized as the most common infection leading to an antibiotic prescription after a physician's consultation (Abdo, 2014).

Among these, 80-90% of urinary tract infections (UTIs) are attributed to *E. coli* (H.S. Rushton, 1997). In cases involving ambulatory patients and nosocomial infections, the most commonly isolated organisms include *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Enterococcus faecalis* (Ouno et al., 2013).

The kidney medulla is characterized by a hypertonic environment, which results from its low pH, the presence of urea, various metabolic byproducts, and numerous enzymes. This hypertonicity ensures that only a small number of organisms can survive in the lower urinary tract, which is regularly cleared of potential pathogens by urine and some mucus approximately 4-5 times a day.

In males, the anatomical length of the urethra (20 cm) serves as a physical barrier, effectively preventing microorganisms from entering the urinary bladder (Lamma et al., 2019). In contrast, the shorter urethra (5 cm) in females is more easily penetrated by microorganisms, which explains the significantly higher incidence of urinary tract infections in females, being 14 times more common than in males (Ouno et al., 2013). Urinary tract infections result from the invasion of pathogenic microorganisms into the urinary system. The morbidity rates for pediatric UTIs are 1.1% in boys and 3% in girls, while the prevalence of UTIs in children under 6 years old is 1.8% for males and 6.6% for females. The urinary tract is categorized into three types: acute pyelonephritis, lower UTI, and asymptomatic bacteriuria (Shahab et al., 2017).

2. Materials and Methods:

2.1 Isolation of bacteria:

A total of 200 urine samples were collected between August and October 2024. Fresh urine samples were aseptically obtained from the midstream portion of the urethra and placed in sterile scientific containers. The samples were transported to the laboratory within 30 minutes of collection. A loopful of each urine sample was then streaked onto a CLED agar plate and incubated at 37°C for 24 hours.

The following day, specific colonies were chosen and recognized based on their morphological, cultural, and biochemical traits. (Priyadharsini et al., 2014).

2.2 Identification of bacterial isolates

Bacterial isolates were identified using standard biochemical tests, **including** microscopic observations, the form, diameter, color, edge, and elevation of colonies, Gram staining, and biochemical tests.

2.3 Antimicrobial Sensitivity test

An antibiotic sensitivity pattern for the microbial isolates was determined using the Kirby-Bauer disc diffusion method. From an overnight culture plate, 4 to 5 colonies of the bacterial isolate were selected with a sterilized inoculating loop and emulsified in 5 mL of sterile normal saline until the turbidity matched approximately that of the McFarland No. 0.5 turbidity standard.

The surface of a Mueller-Hinton agar plate was inoculated with the bacterial isolate as follows: a sterile swab was used to streak the entire surface of the plate. After the first streak, the plate was rotated 45 degrees and streaked again across the surface, followed by a final 90-degree rotation and another streaking.

Using sterile forceps, antimicrobial discs were carefully placed onto the surface of the inoculated agar plate, ensuring full contact with the agar by gently pressing each disc. A total of five antimicrobial discs were applied to each plate. This step was repeated for all antimicrobial discs being tested, ensuring that they were spaced evenly apart.

The plates were incubated at 37°C for 18-24 hours. After incubation, the plates were inspected for the presence of an inhibition zone (clearings) surrounding the antimicrobial discs. If no inhibition zone was observed, the organism was classified as resistant to the antimicrobial agent associated with that disc. If an inhibition zone was present, its diameter was measured in millimeters and compared to the values listed in a standard chart (Shahab et al., 2017).

3. Results

In this study, we collected 200 urine samples, 141 Show positive urine cultures, 116 (82.3%) females and 26 (18.4%) males in patients with urine positive culture on the CLED agar plate, the bacteria were isolated and identified age group of [1] – [105] as Table 1.

Table 1: Distribution of Urinary Tract Infections by Age Group.

Sl. No	Age-group (year)	Count of Male Participants	Count of Female Participants
1	1-10	6	18
2	11-20	3	7
3	21-30	3	29
4	31-40	11	37
5	41-50	12	20
6	51-60	3	13
7	61-70	8	7
8	71-80	1	5
9	81-90	4	7
10	91-100	0	4
11	101-110	0	2

Female patients exhibited more severe cases of urinary tract infections compared to males. The bacteria isolated from the urine samples included *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus* spp. Among these, *Staphylococcus aureus* was the most commonly isolated bacterium, while *Streptococcus* spp. accounted for the fewest isolates. Furthermore, Gram-positive bacteria were found to be more prevalent (56%) than Gram-negative bacteria in the urine samples examined.

51 % of the bacterial isolates were identified, with the most commonly isolated bacteria being *Staphylococcus aureus* (62 isolates, 43.9%), *Escherichia coli* (55 isolates, 39%), *Staphylococcus epidermidis* (13 isolates, 9.2%), *Klebsiella* spp. (11 isolates, 7.8%), *Pseudomonas* spp. (6 isolates, 4.2%), and *Streptococcus* spp. (4 isolates, 2.8%) (Table 2, Figure 1).

Table 2: Percentage of Gram-positive and Gram-negative Bacteria isolated from UTI patients.

Baterial isolates	Colony morphology	Total	Total % of UTI isolates
Gram (-) ^{ve}	-	72	51 %
<i>E.coli</i>	Small circular, slight % raised smooth	55	39.01%
<i>K.pneumoniae</i>	Circular, mucoid convex, small colonies	11	7.80%
<i>P.aeruginosa</i>	Small, rough colony flat edges	6	4.25%
Gram (+) ^{ve}	-	79	56 %
<i>S.aureus</i>	Circular pinheadad colonies, convex with entire margins	62	43.97%
<i>S.epidermidis</i>	Whitish circular pinheadad	13	9.21%
<i>Streptococcus SP.</i>	Circular with entire margin often, raised depressed centres	4	2.83%

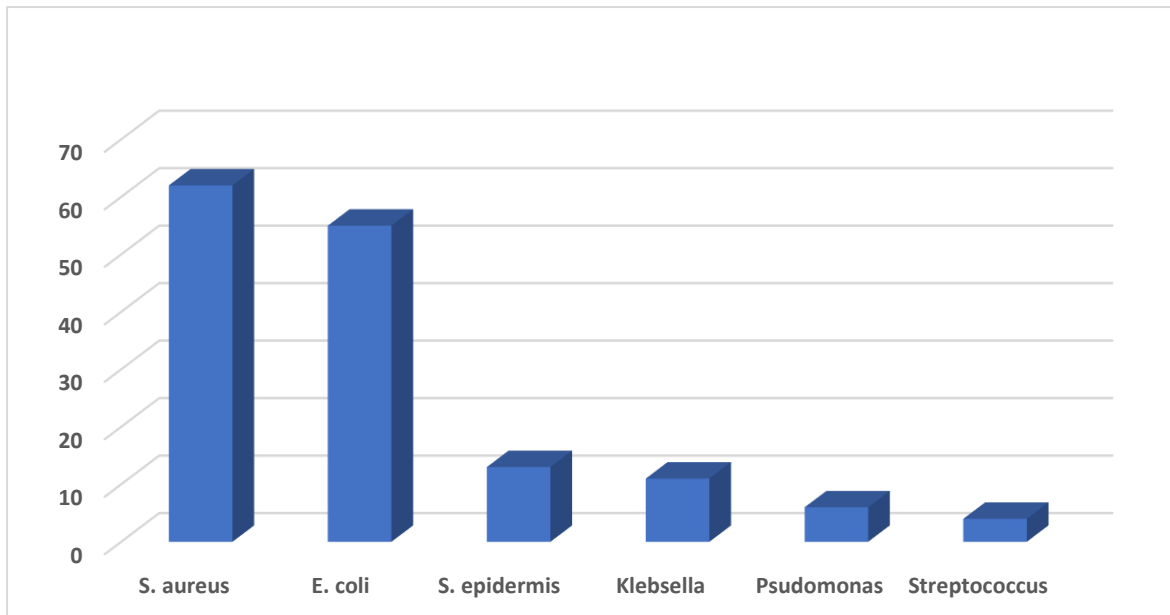


Figure 1: Distribution of bacteria isolated from urine.

The following types of bacteria have been isolated from patients in facted with UTI and identified by biochemical tests as the following figures.

1. *Staphylococcus aureus* as shown in the picture.



Figure 2: *Staphylococcus aureus* on CLED agar

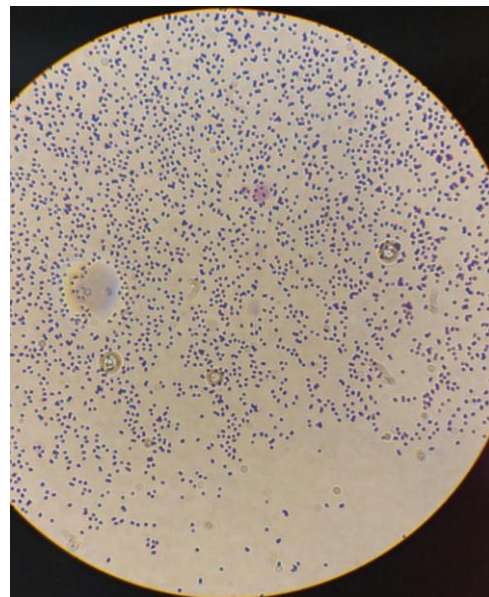


Figure 3: *Staphylococcus aureus* staining by gram stain

2. *Escherichia coli* As shown in the picture.

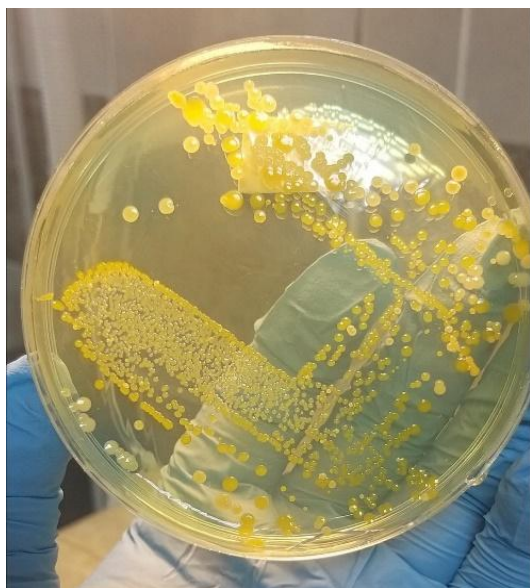


Figure 4: *Escherichia.coli* on CLED agar

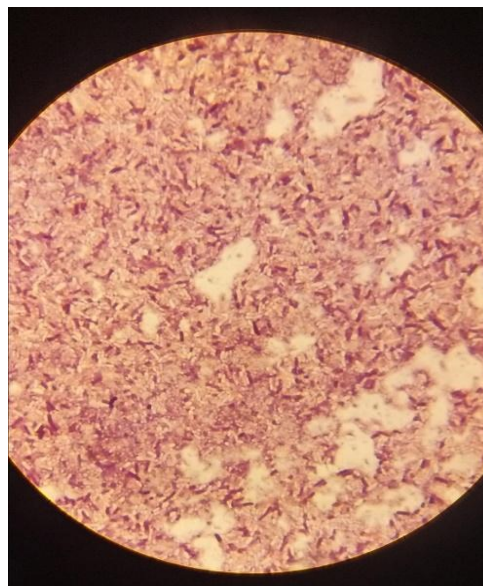


Figure 5: *Escherichia. coli* staining by gram stain

3. *Staphylococcus epidermis* As shown in the picture:



Figure 6: *Staphylococcus eipdermias* on CLED agar

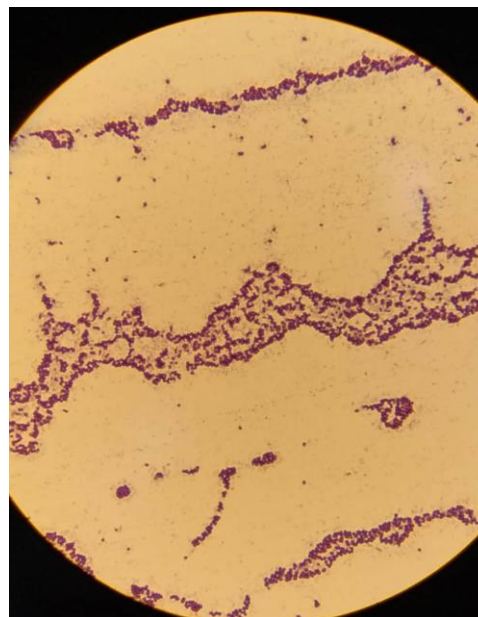


Figure 7: *Staphylococcus epidermias* staining by gram stain

4. *klebsilla Spp* shown in the picture.



Figure 8: *Klebsilla Spp* on CLED agar

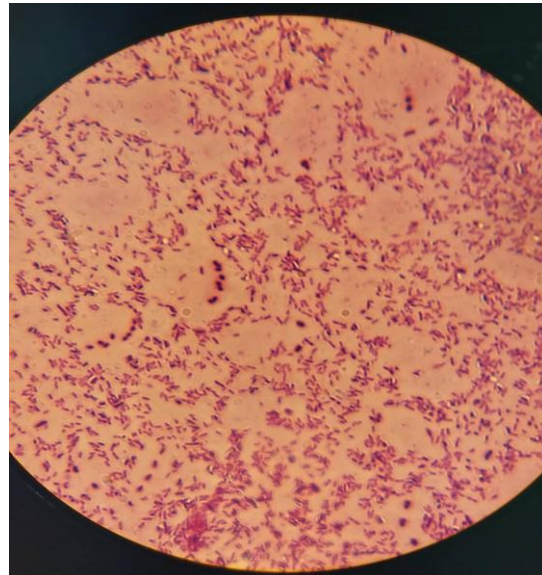


Figure 9: *Klebsilla Spp* staining by gram stain

5. *Pseudomonas* on CLED agar:

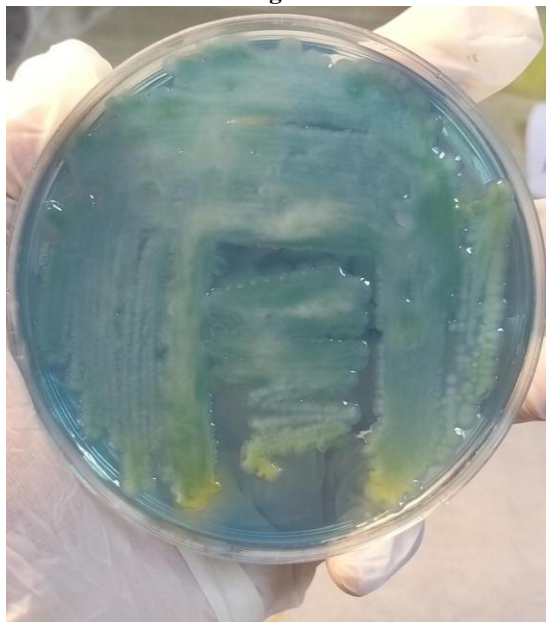


Figure 10: *pseudomonas* on CLED agar

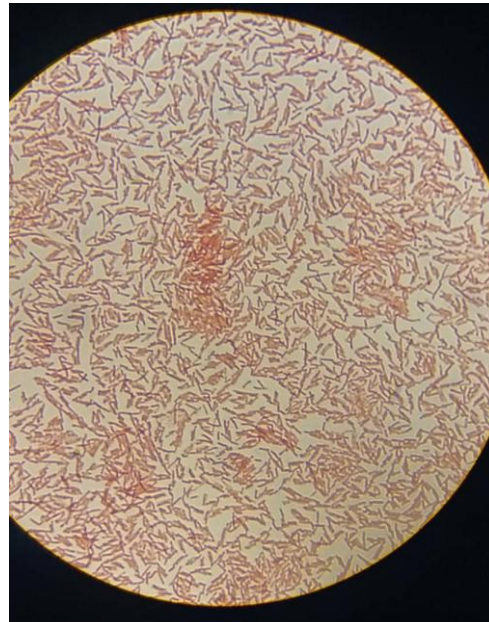


Figure 11: *Pseudomonus* staining by gram stain

6. *Streptococcus* As shown in the picture:Figure 12: *Streptococcus* on CLED agarFigure 13: *Streptococcus.SP* staining by gram stain**Table 3:** Biochemical tests of gram-positive bacteria isolated from samples of urine and some attributes appearance and microscopic.

Biochemical tests	<i>Staphylococcus aureus</i>	Coagulase negative <i>staphylococcus</i>
1. Coagulase	+	-
2. Haemolysis	+	-
3. Catalase	+	+
4. Oxidase	-	-

Table 4: Biochemical Characteristics and Microscopic Attributes of Gram-Negative Bacteria Isolated from Urine Samples.

Type of bacteria / test	<i>E.coli</i>	<i>Klebsiella, spp</i>	<i>psudomonas</i>
Oxidase	-	-	+
Catales	-	-	-
Indole	+	-	-
Methyl red	+	-	-
Urease	-	-	-
Citrate	-	+	-

The following bacterial species were identified by biochemical tests:

1. *Escherichia coli* It was identified by the indole test:

Figure 14: Indole rang test of *Escherichia coli*.

2. *Klbseilla Spp* pneumoniae was identified by citrate test:



Figure 15: Citrate test of *Klbseilla Spp*

3. *Psadomonas auroginosa*:

The test was identified as Oxidase.

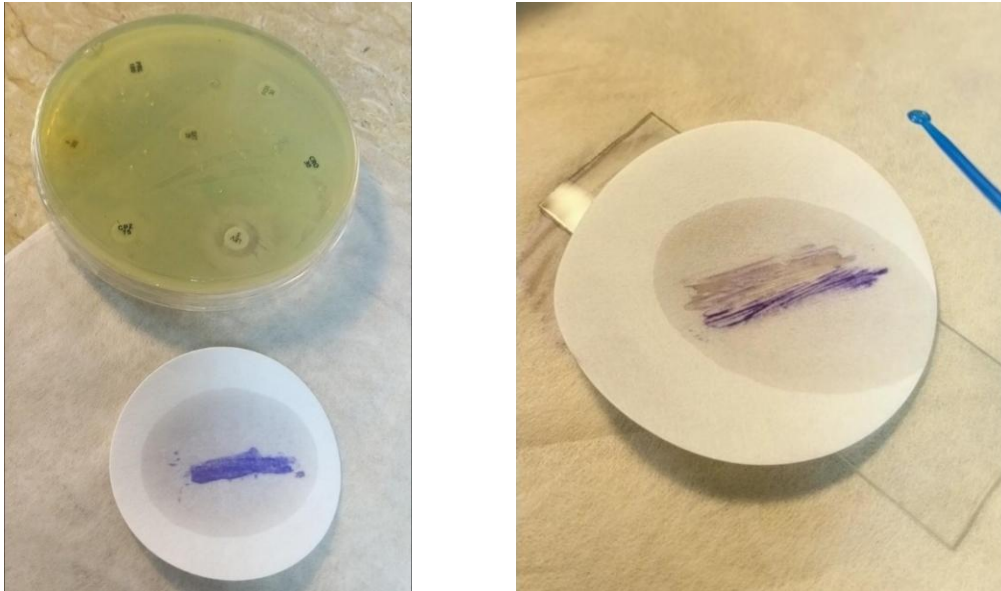


Figure 16: Oxidase test of *Psadomonas*.

4. Identify the following two types of bacteria using the catalase test. In case of a positive catalase test is *Staphylococcus*. In the event of a negative catalase test is *Strptococcus*.



Figure 17: Catalase test of *Stahpylococcus*.

5. In the case of *Staphylococcus*, we use a coagulase test that is positive, causing a clot known as *Staph aureus*.

Bacterial isolates	Do	LEV	SXT	FOX	CRO	PRL	CIP	CN	F	AK
<i>E.coli</i>	(25)	(30)	(29)	(20)	(23)	(23)	(22)	(17)	-	(18)
<i>Klbseiliila Spp</i>	-	(28)	(20)	-	-	-	(29)	-	-	-
<i>Pseudomonas</i>	(15)	(27)	-	-	(23)	-	(21)	-	-	-
<i>S.aureus</i>	(20)	(20)	-	(20)	(23)	(25)	-	(15)	(18)	-
<i>S.epidermis</i>	-	(20)	-	-	(23)	-	(21)	-	(19)	-
<i>S.saprophylic</i>	(25)	(19)	(20)	-	-	-	(21)	(17)	-	-
<i>Stratpoygens</i>	-	(28)	-	-	-	-	-	-	-	-

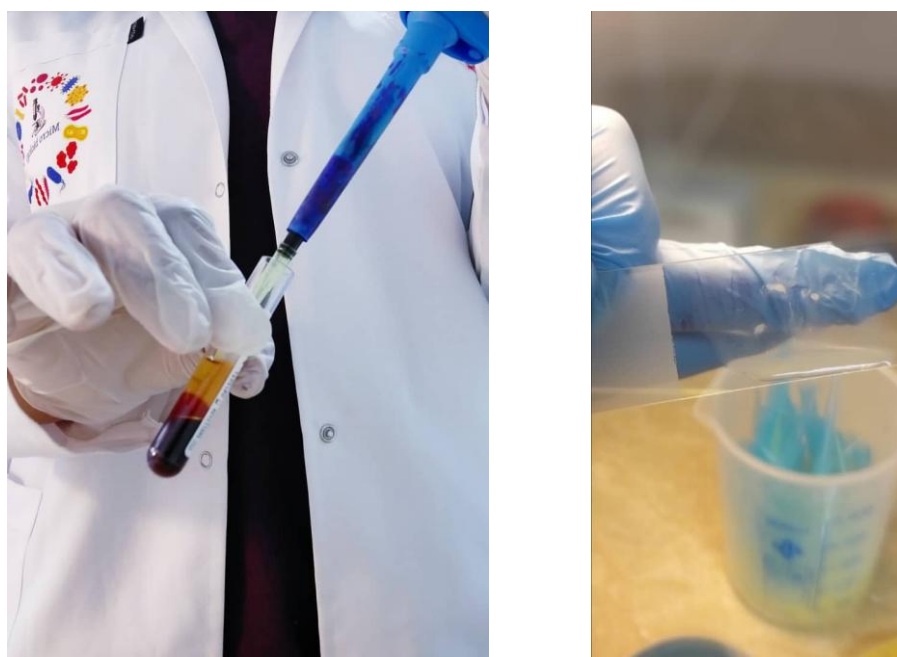


Figure 18: Coagulase test of *Staph aureus*.

Discussion:

Higher results were observed in other studies, which may pertain to the precision in identifying the signs of patients suffering from urinary tract infections (UTI). The prevalence of UTI among females is notably high, with 116 cases (82%). This research aimed to assess the distribution of uropathogen species isolated from UTI patients at Muhammad Al-Maqrif Hospital in Libya, along with their antibiotic resistance profiles. Additionally, it explores the correlation between sex and the pathogens isolated. UTI represents one of the most prevalent infections globally, impacting the urinary system. In this investigation, 141 samples (70.5%) out of 200 collected exhibited positive urine cultures, with a higher incidence of 2% among females compared to 26 males (18.4%), attributed to anatomical and physiological factors associated with females (Al Sweih et al., 2005). The increased infection rates in females are linked to the anatomical and microflora differences between male and female genitourinary systems. The uropathogens identified in our research align with those found in numerous other studies conducted both regionally and internationally; however, varying results have also been documented.

The similarities and discrepancies in the types and distribution of uropathogens may stem from diverse environmental conditions, host factors, and practices such as healthcare and educational programs, socioeconomic standards, and hygiene practices prevalent in each country (Amin et al., 2009). This study revealed that over 43.97% of urinary tract infections are attributed to a single bacterial species, *Staphylococcus aureus*, followed by *E. coli* (39.01%), *Staphylococcus epidermidis* (9.21%), *Klebsiella spp.* (7.80%), *Pseudomonas* (4.25%), and *Streptococcus* (2.83%), which are recognized as the leading UTI pathogens (Murshidi & Farah, 2002; Orrett, 2003; Al-Mardeni et al., 2009; Alshara, 2011).

The present study showed that the *Staphylococcus aureus* isolate possessed a high level of resistance to trimethoprim-sulfamethoxazole, ciprofloxacin, and amikacin. The *E. coli* isolate exhibited high resistance to nitrofurantoin. The *Staphylococcus epidermidis* isolate showed high resistance to doxycycline, trimethoprim-sulfamethoxazole, cefoxitin, piperacillin, gentamicin, and amikacin. The *Klebsiella* isolate showed high resistance to doxycycline, cefoxitin, ceftriaxone, piperacillin, gentamicin, nitrofurantoin, and amikacin. The *Pseudomonas* isolate showed high resistance to trimethoprim-sulfamethoxazole, cefoxitin, piperacillin, gentamicin, nitrofurantoin, and amikacin. The *Streptococcus* isolate showed high resistance to doxycycline, trimethoprim-sulfamethoxazole, cefoxitin, ceftriaxone, piperacillin, ciprofloxacin, gentamicin, nitrofurantoin, and amikacin.

In this study, *Staphylococcus aureus* showed the highest sensitivity to piperacillin, *E. coli* showed the highest sensitivity to levofloxacin, *Staphylococcus epidermidis* showed the highest susceptibility to ciprofloxacin, *Klebsiella* showed the highest susceptibility to ciprofloxacin, *Pseudomonas* showed the highest susceptibility to levofloxacin, and *Streptococcus* showed the highest susceptibility to levofloxacin. This finding differs from the results reported by (Munjanath et al. 2011). The differences in antibiotic resistance patterns among the isolated pathogens may be related to various factors, such as molecular and physiological differences in the pathogens.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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