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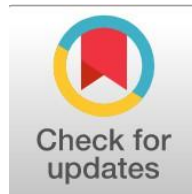
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Prevalence and Antibiotic Susceptibility of Gram-Positive, Gram-Negative Bacteria and Candida in UTI Clinical Samples

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Abstract

General Background: Urinary tract infections (UTIs) are among the most common infections affecting the urinary system, including the urethra, kidneys, and bladder. **Specific Background:** In Iraq, the spectrum of UTI pathogens and their antibiotic resistance patterns remain insufficiently characterized, particularly in Al-Najaf province. **Knowledge Gap:** Limited data exist on the distribution of gram-positive, gram-negative, and fungal uropathogens alongside their antibiotic susceptibility profiles in both male and female outpatients in this region. **Aims:** This study aimed to identify the pathogen spectrum of UTIs, determine the distribution in women and men, and evaluate antibiotic resistance levels of isolated strains. **Results:** Among 618 outpatients, the most clinically significant microorganisms were *Staphylococcus aureus* (27.3%), *Escherichia coli* (25.4%), *Staphylococcus epidermidis* (17.0%), *Streptococcus* spp. (16.3%), and *Candida albicans* (6.1%). Women accounted for 89.8% of infections; *C. albicans* was detected only in women (6.1%). Highest resistance was observed to cefotaxime (up to 100% in *Pseudomonas aeruginosa*). Vancomycin (2% resistance) and amikacin (6-39% resistance) were the most effective antibiotics, with amikacin particularly effective against gram-negative bacteria. **Novelty:** This study provides the first comprehensive UTI pathogen and resistance profile from Al-Sajjad Hospital, Najaf, revealing *S. aureus* as the predominant pathogen over *E. coli*, contrasting with global trends. **Implications:** Empirical UTI therapy in this region should consider vancomycin and amikacin as potential options while avoiding cefotaxime, and clinicians should recognize the high female predominance and unique pathogen hierarchy.

Highlights :

- *Staphylococcus aureus* (27.3%) outnumbered *Escherichia coli* (25.4%) as the leading UTI pathogen in Al-Najaf, Iraq.
- *Candida albicans* (6.1%) was detected exclusively in female patients with UTIs.
- Cefotaxime resistance reached 100% in *Pseudomonas aeruginosa*, while vancomycin showed only 2% resistance across gram-positive cocci.

Keywords: Urinary Tract Infections, Antibiotic Resistance, *Staphylococcus Aureus*, *Escherichia Coli*, Iraq

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Introduction

According to a May 2015 study published in Nature Reviews Microbiology [1], about 150 million people develop a urinary tract infection in a year, and about 10.5 million of them live in the United States. Urinary tract infections (UTIs) are among the most common infections both inpatient and outpatient of the hospital. Urinary tract infections encompass a diverse range of clinical manifestations. Acute complicated cystitis, recurrent cystitis, complex UTI, prostatitis, pyelonephritis, catheter-associated asymptomatic bacteriuria, and catheter-associated UTI (CAUTI) belong to the clinical disorders covered by the word "UTI."

Appropriate classification of UTI syndrome is critical for optimal diagnosis and treatment. The different groups of UTIs differ in the presence or absence of urinary tract symptoms; gender of the patient and concomitant diseases; and genitourinary history, including the presence of stones or stents [2]. The majority of clinical investigations on UTI have been done on adult women since acute cystitis is the most typical UTI presentation and typically affects women.

The kidneys, ureters, bladder, and urethra make up the urinary tract. The kidneys create urine [3]. It then travels through the ureters and into the bladder, where it is held until the urethra allows it to leave the body. Although urine is normally a sterile liquid, infections can contain microorganisms. UTIs, or urinary tract infections, are exceedingly prevalent, particularly in women. They are referred to as recurrent UTIs when they repeatedly occur. You might be sent to a urologist, a medical professional with specific expertise in the diagnosis and treatment of urinary tract disorders, if your primary care physician determines that you have a recurring UTI [4].

Additionally, One of the more common bacterial diseases obtained in hospitals are urinary tract infections (UTIs). When UTIs occur in people without structural or functional abnormalities, they typically go away on their own but frequently return [5].

Because they can produce toxins, siderophores, and adhesions, uropathogens are specialized microorganisms that can infiltrate and colonize the body. UTIs can be transmitted between people and through personal contact. Antibiotic therapy for urinary tract infections (UTIs) tends to promote the growth of resistant uropathogens and commensal bacteria, adversely affects the gut and vaginal microbiota, and expedites the cure of symptoms and likelihood of bacteriuria. It might be time to look into alternate UTI treatment options as uropathogens get more resistant to the antibiotics that are currently on the market [6].

Among the most prevalent infections that affect people are urinary tract infections. In healthy women, simple infections are more common, but uropathogenic bacteria typically *Escherichia coli* can ascend from the perineum into the bladder and subdue the host's natural defenses [7]. Patients with urinary tract structural or functional abnormalities are susceptible to complicated infections. The basis for diagnosis is symptoms, and urine testing increases the precision of the diagnosis. Urine cultures are crucial when diagnosing illnesses that are severe, difficult, recurrent, or unclear—such as in cases involving children or the elderly. Urinary tract imaging, functional testing, and cystoscopy should be investigated in children, men, and patients with recurrent or severe infection in order to rule out underlying pathology. However, most women whose symptoms go away quickly don't need additional evaluation. For simple cystitis, empirical antibiotic therapy started based on symptoms and guided by urinalysis is suitable; however, for more serious infections, the course of treatment should be adjusted based on culture results [8]. For women with simple cystitis, three days of antibiotic therapy is typically enough. When nonantibiotic measures have failed to treat a patient's recurrent infection, long-term or postcoital antibacterial therapy is a useful treatment option.

Thus, the purpose of this work was to study the spectrum of pathogens of urinary tract diseases and the resistance of pathogens to antibiotics using the example of Al-Sajjad Hospital in the Najaf region, the Republic of Iraq. To achieve this goal, the following tasks were identified: determine the spectrum of pathogens of urinary tract infections, and the spectrum of pathogens in both men and women. In addition identify clinically significant types of fungi and their distribution in women and men, and the level of antibiotic resistance of the isolated strains.

2. Research methods

The work was carried out at the Al-Sajjad Hospital, Najaf, Iraq, between September 2023 and September 2025, where the number of patients was 618 outpatients.

According to the gender composition of the patients, there were both women and men, but not in equal numbers: there were more women than men. The material for the study was taken before the start of antibiotic treatment. Samples were collected in sterile transport containers designed to collect and transport a specific type of material. Urine samples were taken in the middle of the flow, placed in sterile containers, and delivered to the laboratory. Samples were delivered to the laboratory as soon as possible - within (0.5-1 hour) from the moment of collection, in a sealed container.

2. 1. Preparation of media, solutions and buffers

The following reagents and solutions were utilized in this investigation; those that needed to be sterilized were autoclaved for 15 to 20 minutes at 121°C. One M HCl or one M NaOH was added to the solution to change its pH. The reagents were produced following MacFaddin's instructions (2000).

The oxidase reagent was used freshly prepared. The solution was prepared by dissolving about 0.01 g of

tetramethylpphenylenediamine dihydrochloride (TMPD) in 10 ml of distal water in a dark bottle.

2. 2. Growth on blood agar

There are three types of hemolysis in blood agar, a differential medium that separates bacterial species according to their capacity to lyse red blood cells (beta hemolytic organisms completely lyse red blood cells, alpha hemolytic organisms partially lyse red blood cells). Red blood cells are not lysed by gamma-hemolytic organisms, and the medium is unaltered.

2. 3. Growth on MacConkey agar

MacConkey agar has been used, in particular, to differentiate gram-negative lactose-fermenting bacteria (pink colonies) from non-lactose-fermenting bacteria (colorless) [9].

2. 4. Mobility test

The purpose of this test is to determine the mobility of bacteria. This test was carried out by taking fresh and clean colonies from a plate, then the colonies were dipped into the center of the tube and after 2-3 days of incubation, if the growth of the colonies spread throughout the tube, the result was considered positive, and if there was no growth around the prick, the result was considered negative [10].

2. 5. Methods for identifying gram-positive isolates

Identification of bacteria after 24 hours of incubation at 37°C to species level may depend on Gram stain, morphological characteristics (colony size, colony shape, presence of hemolysis) on blood agar. Biochemical tests were also performed (catalase test, coagulase test). The Gram stain method is used to determine the location and shape of bacterial cells under a light microscope. For each bacterial isolate, a bacterial smear was prepared from a pure young culture [11].

2. 6. Methods for identifying gram-negative isolates

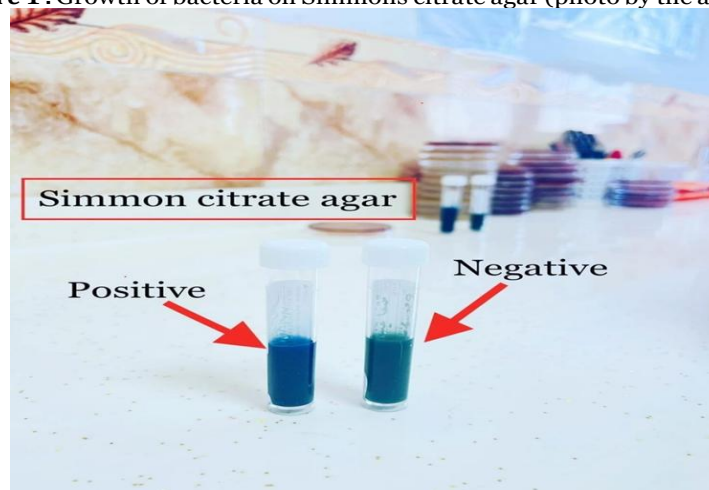
Identification of bacteria after 24 hours of incubation at 37°C to species level may depend on Gram stain, morphological characteristics (colony size, colony shape, lactose fermentation) on MacConkey agar. Biochemical tests (growth on Simmons citrate agar, oxidase test, and motility test) were also used to identify Gram-negative isolates.

2. 7. Biochemical tests

2. 7.1. Citrate utilization test (Simmons agar)

This test, used to differentiate Enterobacteriaceae, depends on the use of citrate as the sole carbon source. This test was performed by inoculating fresh and clean colonies onto an agar slant and incubating for 24-48 hours. If the color changed to blue [10], it was considered a positive result (Figure 1).

Figure 1 . Growth of bacteria on Simmons citrate agar (photo by the author).



2.7.2. Catalase test

The catalase test is used to detect the presence of the catalase enzyme by decomposing hydrogen peroxide to release oxygen and water.

The presence of catalase is manifested in the rapid formation of bubbles. There are variations of the catalase test method, including the glass slide method, the test tube or Petri dish method, or the agar slant method [10]. In our work, the test was carried out on a glass object (Figure 2).

Figure 2. Catalase test (photo by the author).



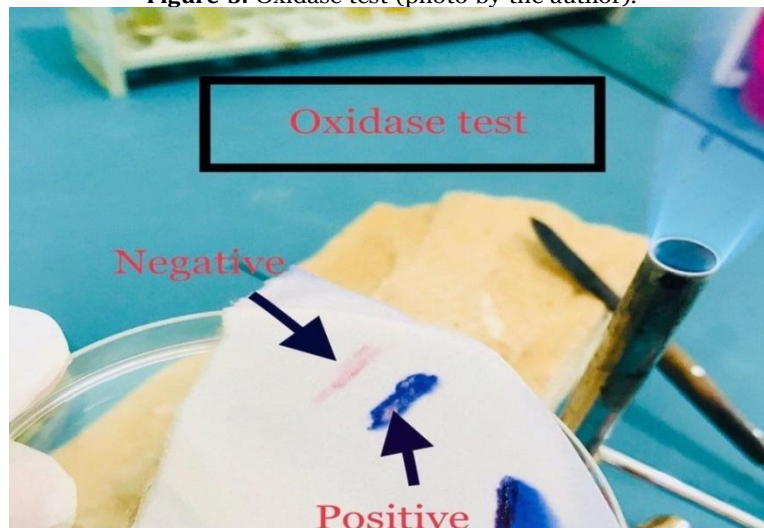
2. 7.3. Coagulase test

To conduct the tube coagulase test, bacterial cells and a substantial amount of plasma are combined inside a tiny tube. Bacteria release plasmacoagulase when they grow in the plasma. This enzyme activates prothrombin, which starts blood clotting [12].

2. 7.4. Oxidase test

The purpose of this test is to test the ability of bacteria to produce cytochrome C oxidase. This test was carried out by taking the test colonies and then placing them on filter paper soaked in the substrate - N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (TMPD). Then, if the color changed from purple to dark blue (Figure 3) within 20 seconds, it was considered a positive result [10].

Figure 3. Oxidase test (photo by the author).



2.8 Storage methods for isolates

2.8.1 Short-term storage

Nutrient agar was used to preserve bacterial isolates for a short time. For this process, one pure colony was taken, then streaked onto a nutrient agar slant, the tube was kept at 37°C for 18-24 hours and then cooled at 4°C. for two months [10].

2.8.2 Long-term storage

Long-term preservation of bacterial isolates was achieved through the use of nutrient broth, this process was carried out by transferring several clean and fresh colonies into the broth with the addition of 15% glycerol to preserve the isolates for a

long time, and then the tube was deep frozen at -40°C [10].

2.9 Statistical Analysis

All statistical analyses were performed using the Microsoft Excel 2016.

3. Results

The study was conducted at Al-Imam Al-Sajjad Hospital in Najaf Governorate during the period from January 2022 to October 2022. The sample included outpatients, and the material for the study was taken before the start of antibiotic treatment. Specimens were collected in sterile shipping containers designed for the collection and transport of a specific type of material.

As a result of the specific and generic identification of microorganisms, the etiological structure of UTIs in the departments of the Imam al-Sajjad Hospital was revealed. At the same time, several genera of bacteria that cause urinary tract infections were isolated (Table 1). The most common pathogen was *Staphylococcus aureus*, the next most common was *E. coli*, then *Streptococcus* spp., and the lowest frequency in the structure of UTIs in the examined sample of patients was observed for *Klebsiella* spp.

In urine samples, the most clinically significant microorganisms were: *Staphylococcus aureus* - 27.3%, *Escherichia coli* - 25.4%, *Staphylococcus epidermidis* - 17.0%, *Streptococcus* spp - 16.3%, *Candida albicans* - 6.1%. *Pseudomonas* spp. 2.6%, *Enterococcus faecalis* 2.1%, *Proteus* spp. 1.8%, *Klebsiella* spp. 1.3% (Figure 4).

Table 1. Total number of strains of microorganisms isolated from outpatients with UTI.

Type of microorganism	Number of isolates	%
<i>S. aureus</i>	169	27.3%
<i>E. coli</i>	157	25.4%
<i>S. epidermidis</i>	105	17.0%
<i>Streptococcus</i> spp.	101	16.3%
<i>Candida albicans</i>	38	6.1%
<i>Pseudomonas</i> spp.	16	2.6%
<i>Enterococcus</i> spp.	13	2.1%
<i>Proteus</i> spp.	11	1.8%
<i>Klebsiella</i> spp.	8	1.3%

The etiological pattern of bacterial UTIs in women and men in (Table 2), with women being more infected than men in all of them.

The above table shows that the most common microbial pathogen affecting women is *Staphylococcus aureus* (24.1% in women and 3.2%), followed by *Escherichia coli* (22.9% and 2.4% in men). The least common cause is microbial *Klebsiella* (women 1.1% and men 0.1%).

Table 2. Etiological structure of bacterial UTIs in female and male patients.

Type of microorganism	Female		Male	
	No	%	No	%
<i>S. aureus</i>	149	24.1%	20	3.2%
<i>E. coli</i>	142	22.9%	15	2.4%

S. epidermidis	98	15.8%	7	1.1%
Streptococcus spp.	89	14.4%	12	1.9%
Candida albicans	38	6.1%	0	0
Pseudomonas spp.	13	2.1%	3	0.4%
Enterococcus spp.	12	1.9%	1	0.2%
Proteus spp.	9	1.4%	2	0.3%
Klebsiella spp.	7	1.1%	1	0.1%

Antibiotic resistance tests were carried out on the isolates, and it was found that the highest percentage of resistance was found in *Staphylococcus aureus* to the antibiotic cefotaxime (50%), and the lowest resistance was noted to vancomycin (2%) (Table 3).

The highest antibiotic resistance in *E. coli* was also observed against cefotaxime (42%). The least resistant *E. coli* is to amikacin (6%), the highest resistance in *Staphylococcus epidermidis* is noted to cefixime (56%), and the lowest to vancomycin (2%).

Table 3. Proportion of antibiotic-resistant strains (%) isolated from patients infected with UTI in Al-Najaf city in Iraq.

Antibiotics	VA	TE	F	TMP	E	CIP	AK	CN	CRO	CTX	CFM	KF
<i>S. aureus</i>	2	45	12	24	41	21	19	24	21	50	38	22
<i>E. coli</i>	-	32	10	24	38	16	6	19	28	42	31	42
<i>S. epidermidis</i>	2	25	12	13	44	20	12	23	40	39	56	17
<i>Streptococcus spp</i>	2	51	24	38	35	21	39	48	38	50	65	35
<i>Candida albicans</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas spp.</i>	-	62	62	18	87	37	18	37	81	100	37	100
<i>Enterococcus spp.</i>	-	30	7	15	23	46	30	23	15	15	23	30
<i>Proteus spp.</i>	-	72	54	27	27	9	18	27	9	36	18	63
<i>Klebsiella spp.</i>	-	12	25	25	62	12	-	-	25	12	12	62

The greatest resistance is in *Streptococcus spp.* observed to cefixime (65%), the least resistant streptococci are also to vancomycin (2%). In species *p. Pseudomonas* is completely resistant to cephalothin and cefotaxime (100%). The studied strains are less resistant to the antibiotic amikacin (18%). As for enterococci, the greatest resistance is observed to ciprofloxacin (46%), and the least resistance to nitrofurantoin (7%). The greatest resistance of the bacterium *p. Proteus* are resistant to tetracycline (72%), and least resistant to ceftriaxone (9%). The highest resistance of *Klebsiella spp.* have to the antibiotics cephalothin (62%) and erythromycin (62%), and a low level of resistance was noted to the antibiotics cefixime, cefotaxime, ciprofloxacin and tetracycline (12%).

4. Discussion

The present study was conducted on 618 samples from patients with urinary tract infection. The results showed that women were more likely to be infected than men (men = 9.6%; women = 89.8%), these results being reminiscent of previous studies conducted in Al-Manatera, Iraq [13]. Additionally, the observation that more women are infected than men is similar to other studies conducted in many countries around the world Saudi Arabia, [14,15].

Men and women of all ages are susceptible to urinary tract infections, which are a very frequent illness. Women are more prone than men to experience urinary tract infections, which can be attributed to a number of clinical factors such as behavioral patterns, hormonal impacts, and anatomical differences [16,17].

In addition to our results, 62.6% had an increase in gram-positive bacteria, and 30.8% had an increase in gram-negative bacteria. Mushroom growth *Candida* was noted in 6.1% of cases. Therefore, These results are consistent with a previous study in Najaf [18,19].

This is not the case with some studies where higher numbers of *E. coli* bacteria recorded as a cause of UTI [20].

Antimicrobial resistance (AMR) is a global problem that threatens the effectiveness of antibiotics and, as a result, the effectiveness of treatments. Under normal circumstances, AMR occurs spontaneously, but incidence rates may increase due to antibiotic abuse, inappropriate antibiotic prescribing and polypharmacy, and the lack of new treatments by the pharmaceutical industry, which is limited by regulations [21]. One of the objectives of the present study was to evaluate the current activity of various antibiotics against Gram-positive and Gram-negative isolates from patients with UTIs, and to provide up-to-date data on antibiotic resistance in patients with UTIs and to assist the clinician in selecting the appropriate drug. antibiotic. This study used 12 antibiotic discs, therefore, the greatest resistance was observed to cefotaxime (up to 100% in *Pseudomonas aeruginosa*). Among the most effective antibiotics are vancomycin and amikacin.

In this study, it was shown that the infection rate of urinary tract infection among females was higher than that of males, as the infection rate among females reached 71%, while males reached 20%, It agreed with a study where the infection rate was higher among females, reaching 66%, while males reached 34% [22–24]. The reason is due to the fact that the female urethra is structurally different, as it is less effective in preventing the entry of bacteria, meaning that the urethra is shorter and wider [25].

The urinary tract pathogens were determined higher in females in all age groups which is also as expected [26,27]. In general, the level of antimicrobial resistance among gram-negative uropathogens in Al-Najaf is relatively high, which is a warning to prevent unnecessary use of antibiotics. Also in the present study, the ratio of *E. coli* to other pathogens was higher compared to others. Therefore, the prevalence of *E. coli* is increasing compared to previous studies in Erbil [28]. Finally, this study examined a large sample of Gram-positive bacteria from urine cultures. It was divided into two parts: a descriptive study of clinical, epidemiological, and antibiotic susceptibility data collected from adults and children with significant bacteriuria (SB) at our center during the previous five years, and a systematic literature review.

Conclusions

The most clinically significant microorganisms in urine samples were: 27.3% *Staphylococcus aureus* and 25.4% *Escherichia coli*. Therefore, The most common microbial pathogen affecting women is *Staphylococcus aureus* in women and men (24.1% and 3.2%), followed by *Escherichia coli* (22.9% and 2.4%) respectively.

In addition, Among the causative agents of UTIs belonging to fungi, only the species *Candida albicans* was noted, in women this pathogen was found in 6.1% of cases, and in men it was not detected even once. Furthermore, The greatest resistance was observed to cefotaxime (up to 100% in *Pseudomonas aeruginosa*). Among the most effective antibiotics are vancomycin (2%) and amikacin (6-39%), and amikacin was most effective against gram-negative bacteria.

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