# Evaluation of the Efficacy of Selected Antibiotics Against Bacteria Isolated from Urinary Infection Tract Samples at Misurata Medical Center, Libya

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**Article information** **Abstract**

**Key words**

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There has been a recent increase in the incidence of urinary tract infections (UTIs) among individuals and a rise in bacterial resistance to antibiotics, leading to numerous complications. This study aimed to examine 86 urine samples from the Urology Department at Misurata Medical Center. The samples were cultured on different media, and diagnostic tests were conducted to identify the isolated bacterial species. The disk diffusion method was employed to test the susceptibility of these isolates to several antibiotics. The results revealed the isolation of several bacterial species in varying proportions: *Staphylococcus aureus* (36%), followed by *Escherichia coli* (23%), *Klebsiella pneumoniae* (11%), Klebsiella spp. (7%), Streptococcus spp. (5%), with *Streptococcus viridans*, *Streptococcus pyogenes*, and *Proteus mirabilis* each at 4%, and *Pseudomonas aeruginosa*, *Enterobacter*, and *Bacillus* sp. each at 2%. Furthermore, the findings indicated that among the Gram-negative bacteria, Pseudomonas aeruginosa exhibited the highest resistance to antibiotics (67%), followed by *Klebsiella pneumoniae*, Escherichia coli, and Proteus mirabilis (42%). The most effective antibiotics against these Gram-negative isolates were Gentamicin, Amikacin, and Cefotaxime. Regarding Gram-positive bacteria, the most resistant species were *Streptococcus* spp. and *Streptococcus viridans* (58%, 50% respectively), followed by *Bacillus* sp. (42%). The most effective antibiotics against the Gram-positive isolates were Vancomycin, Gentamicin, Ciprofloxacin and Amikacin. In conclusion, the results of this study demonstrated that the isolated bacterial species exhibited resistance to the several tested antibiotics. This contributes to the increased pathogenicity of these bacteria and exacerbates the problem of UTIs.



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## 1. Introduction

Urinary tract infections (UTIs) are considered one of the health problems affecting a large percentage of the human population, estimated at millions annually. They are also the primary cause of healthcare-associated infections (HAIs), reaching a percentage of 35%. *Staphylococci* are among the most important causes of these infections, as they are opportunistic pathogens for hospital-acquired UTIs [1]. The hospital environment plays an important role in determining the species involved in UTIs. These infections are among the most widespread bacterial infections, affecting approximately 150 million people annually worldwide. In 2007, more than 10 million medical visits in the United States were attributed to these bacteria [2].
A urinary tract infection is a bacterial infection that affects part of the urinary tract. When it involves the lower urinary tract, it is referred to as cystitis. (Bladder infection). In contrast, when it affects the upper urinary tract, it is known as pyelonephritis (kidney infection). Symptoms of lower UTI include painful urination accompanied by frequent urination or the urge to urinate (or both). Symptoms of pyelonephritis include fever and flank pain, in addition to lower UTI symptoms. Symptoms may be vague or non-specific in the elderly or young children [3]. This infection can affect any part of the urinary system and is often associated with a frequent or urgent desire to urinate [4].
UTIs are the most common healthcare-associated infections, especially in developed and high-income countries [5]. They are considered the second most frequent infection after respiratory tract infections, often occurring as subclinical cases in the form of hypertension, diabetes, and kidney disorders. This results from the doubling of bacterial growth every day, sometimes without clear clinical signs until urine or semen culture analysis is performed [6]. Infections often result from the migration of intestinal bacteria upward into the bladder or kidneys, especially in females due to their shorter and wider urethra [7]. This problem affects various age groups; however, the probability of occurrence is influenced by several factors such as race, genetics, age, gender, sexual activity, nocturnal enuresis, and circumcision status in males [8].
An epidemiological study on UTIs revealed a significant disparity between genders, with women being significantly affected due to anatomical factors such as a short urethra and its proximity to the anus. It is estimated that approximately 50% of all women will experience at least one UTI during their lifetime, and about 20-30% of these will suffer from recurrent infections (rUTIs), defined as three or more infections per year [9]. The study by White Brett in the United States explained that the prevalence of UTIs was more common in children and in females more than in males [10]. In another study, the prevalence of UTIs among samples reached 58.3% of the total samples, and *E. coli* was the main cause of these infections, accounting for 45.7% of total bacterial isolates. Results showed that females recorded the highest infection rate (77.8%) compared to males (22.2%), which was the lowest, reflecting the known relationship between anatomical and hormonal factors and women's increased susceptibility to UTIs [11]. A study conducted by Vasudevan in India showed that the most important bacterial species causing it are: *E. coli*, *Staphylococcus* spp, *Pseudomonas*, *Klebsiella*, *Enterococcus* spp, and *Proteus* [3]. Ronald's study in Canada also confirmed that the most common types of bacteria as causes of UTIs are *E. coli*, followed by *Staphylococcus saprophyticus*, then *Proteus* spp and *Enterococci*. Gram-positive Group B Streptococci were also isolated among the infection-causing isolates [12].

The study by Prasada Rao and others showed that the most famous species causing UTIs are *S. aureus* and *E. coli*. Important primary treatments for UTIs caused by these bacteria include the antibiotics

Ciprofloxacin, Ofloxacin, and Nitrofurantoin. It was also emphasized that treatment is important in avoiding serious complications [13]. Another study showed that other Gram-negative bacteria causing these UTIs are *Pseudomonas*, *Klebsiella*, *Enterobacter*, and *Serratia*, which have an impact on recurrent infections. It also indicated several infections by Gram-positive bacteria; it was found that (10-30%) of UTI cases occur due to *Staphylococcus saprophyticus*, especially in samples isolated from females [14]. On the other hand, the study conducted by Yenli and others concluded that UTIs still represent a prominent health problem, and the causing isolates showed Multi-Drug Resistant (MDR) patterns that limit the effectiveness of many traditional antibiotics. Consequently, they recommended the necessity of relying on Antibiotic Susceptibility Patterns to guide treatment decisions and select the most appropriate antibiotic for each case, rather than relying on general treatment protocols that may not reflect the existing microbial reality [10]. Farrell's study in the United Kingdom indicated that bacterial isolates causing UTIs included: *E. coli*, *Enterococcus faecalis, Klebsiella pneumoniae,* and *Proteus mirabilis*. Results showed that Amoxicillin was ineffective against *E. coli* due to its production of beta-lactamase enzyme; it regained its effectiveness after being combined with clavulanic acid. Cefuroxime showed appropriate effectiveness against most isolates except *Pseudomonas* spp. and *Enterococcus* spp., while Nitrofurantoin was effective only against *E. coli* and *E. faecalis*. The study also showed that Trimethoprim was effective against most isolated species, while Ciprofloxacin showed optimal effectiveness against all bacterial isolates [15]. Management of UTIs faces a great challenge due to the alarming global increase in Antimicrobial Resistance (AMR). The spread of multidrug-resistant (MDR) uropathogens, especially Enterobacteriaceae producing extended-spectrum beta-lactamase (ESBL), has rendered many first-line empirical antibiotics ineffective. This has necessitated a shift toward treatment designed according to local susceptibility charts and non-antimicrobial prevention strategies [16], and the development of new methodologies, including anti-virulence compounds and vaccines to combat this widespread health threat [17]. Given the high and recurrent incidence of these bacterial types for several reasons, primarily bacterial resistance to many antibiotics and the increased severity and danger of resulting infections, this study focused on isolating and diagnosing bacterial species in the urinary tract and conducting antibiotic susceptibility tests on them to obtain results that may be useful in preventing or reducing these problems and complications.

## 2. Methodology

This study was conducted on 86 urine samples collected from patients visiting the Misrata Medical Center laboratory during the period from the 15th of July, 2017 to the 15th of October, 2017. The antibiotics listed in Table (1) were used in the susceptibility tests.

### 2.1 Isolation and Identification of Bacteria

After collecting the samples, they were first cultured in Nutrient broth and incubated for 24 hours at 37°C to ensure initial bacterial growth. This was followed by subculturing on a set of diagnostic culture media, including blood agar, Chocolate agar, and MacConkey agar. After incubation for 24 hours at 37°C, the morphological characteristics of the growing bacterial isolates were observed, including colony shape, color, texture, and edge nature, as well as their ability to ferment lactose, hemolyze red blood cells, and produce characteristic pigments. Culture results were supported by morphological observations through direct microscopic examination after performing gram stain to determine cell shape, arrangement, and type (Gram-positive or Gram-negative).

### 2.2 Antibiotic Sensitivity Test

The susceptibility of bacterial isolates to a group of antibiotics was tested using the Disk Diffusion Method on Petri dishes, following the methods used in the studies by Bauer [18], and Pundir and Jain [19]. Culture was performed on Mueller-Hinton agar using cotton swabs moistened with bacterial suspensions, adjusted to a 0.5 McFarland standard to ensure uniform bacterial density. After allowing the surface to dry, antibiotic disks were placed on the culture plates, which were then incubated at 37°C for 24 hours. Following the incubation period, the diameters of the inhibition zones around the disks were measured by Vernier calipers equipment. The susceptibility of each bacterial isolate to the tested antibiotics was determined using standard reference tables from the Clinical and Laboratory Standards Institute guidelines [20].

Table (1) The used antibiotics susceptibility tests

| Antibiotic | Abbreviation | Concentration (mcg) |
|---|---|---|
| Amikacin | AK | 30 |
| Amoxycillin/Clavulanic acid | AMC | 30 |
| Ceftriaxone | CRO | 30 |
| Cefuroxime | CXM | 30 |
| Cefotaxime | CTX | 30 |
| Ciprofloxacin | CIP | 5 |
| Erythromycin | E | 15 |
| Gentamicin | CN | 10 |
| Nitrofurantoin | F | 300 |
| Co-Trimoxazole (Bactrim) | SXT | 25 |
| Vancomycin | VA | 30 |
| Cephalexin | CL | 30 |
| Nalidixic acid | NA | 30 |
| Piperacillin | PRL | 100 |

## 3. Results and Discussion

### 3.1 Samples and Bacterial Growth

86 urine samples were collected from various cases, including 29 samples from males (34%) and 57 samples from females (66%). Ages ranged between 2-60 years. After culturing these samples on various media and incubating at 37°C for 24 hours, results showed that 55 samples had bacterial growth (64%), while 31 samples (36%) showed no bacterial growth, as shown in Figure (1).

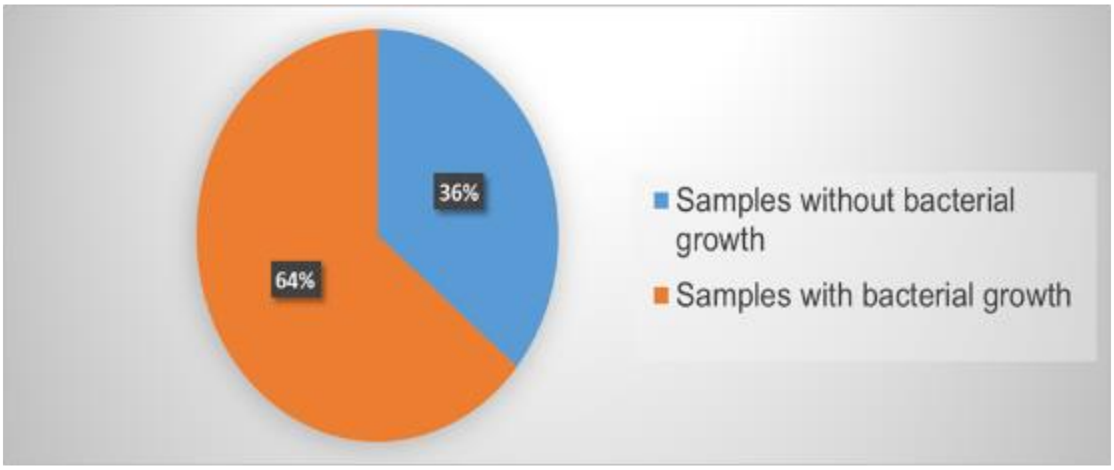


Figure (1) The percentage of bacterial growth in the isolated samples

### 3.2 Isolated Bacteria Types

Through microscopic examinations, biochemical tests, and identification procedures, several bacterial species were isolated and identified. The percentages of Gram-positive bacteria (51%) and Gram-negative bacteria (49%) indicated that the amount of Gram-positive bacteria is greater than that of Gram-negative bacteria. *Staphylococcus aureus* was the most present and frequent species at (36%), followed by *Escherichia coli* (23%), *Klebsiella pneumoniae* (11%), *Klebsiella* spp. (7%), and *Streptococcus* spp. (5%). *Streptococcus viridans*, *Streptococcus pyogenes*, and *Proteus mirabilis* each accounted for (4%). *Pseudomonas aeruginosa*, *Enterobacter*, and *Bacillus* sp. each accounted for (2%), as shown in Table (2) and Figure (2).

Table (2) Isolated bacterial species and their frequency percentages

| No. | Bacterial Species | Frequency | Percentage (%) |
|---|---|---|---|
| 1 | *Staphylococcus aureus* | 20 | 36% |
| 2 | *Escherichia coli* | 13 | 23% |
| 3 | *Klebsiella pneumoniae* | 6 | 11% |
| 4 | *Klebsiella* spp | 4 | 7% |
| 5 | *Streptococcus* spp | 3 | 5% |
| 6 | *Streptococcus viridans* | 2 | 4% |
| 7 | *Streptococcus pyogenes* | 2 | 4% |
| 8 | *Proteus mirabilis* | 2 | 4% |
| 9 | *Pseudomonas aeruginosa* | 1 | 2% |
| 10 | *Enterobacter* | 1 | 2% |
| 11 | *Bacillus* sp | 1 | 2% |

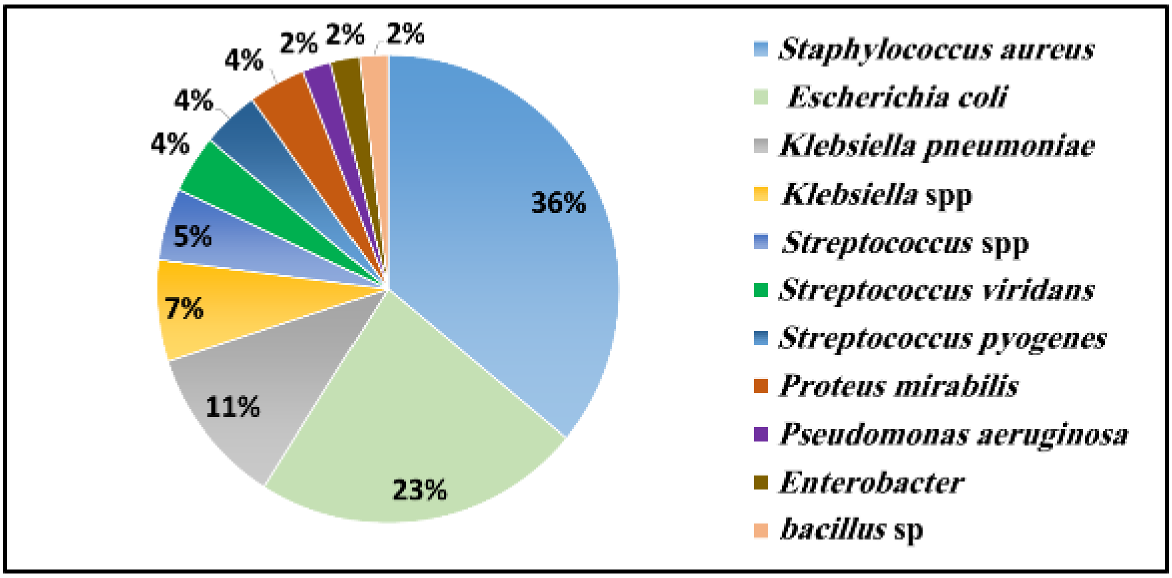


Figure (2) Isolated bacterial species and their percentages

### 3.3 Effect of Antibiotics on Gram-Positive Bacteria

Table (3) and Figure (3) illustrate the varying effects of antibiotics on Gram-positive bacteria isolated from urine samples. All these species showed sensitivity to Vancomycin, Ciprofloxacin, Gentamicin, and Amikacin, with inhibition zone diameters ranging from 16 mm to 28.3 mm, and also to Nitrofurantoin, except *Bacillus* sp. On the other hand, all of these types were resistant to Bactrim and cefuroxime, except for *Streptococcus pyogenes*.

Table (3) Effect of antibiotics on Gram-positive bacteria

| Antibiotic / Bacteria | *Staph. aureus* | *Streptococcus* spp | *Strep. viridans* | *Strep. pyogenes* | *Bacillus* sp |
|---|---|---|---|---|---|
| VA | S | S | S | S | S |
| CIP | S | S | S | S | I |
| E | I | R | R | R | I |
| CN | S | S | S | S | S |
| CTX | S | R | R | R | R |
| CL | S | R | R | S | I |
| CRO | I | R | R | S | S |
| F | I | S | S | S | R |
| AK | S | I | I | S | S |
| AMC | I | R | I | I | R |
| SXT | R | R | R | I | R |
| CXM | R | R | R | S | R |

*(S = Sensitive, I = Intermediate, R = Resistant)*

*S. aureus* bacteria also showed high sensitivity to cefotaxime and cephalexin, with inhibition zone diameters of 26 and 20 mm, respectively, and moderate sensitivity to erythromycin (17 mm) and amoxicillin/clavulanic acid (15 mm). *S. pyogenes* has shown sensitivity to both cephalexin and ceftriaxone, and it demonstrates moderate sensitivity to amoxicillin/clavulanic acid, (Figure 3).

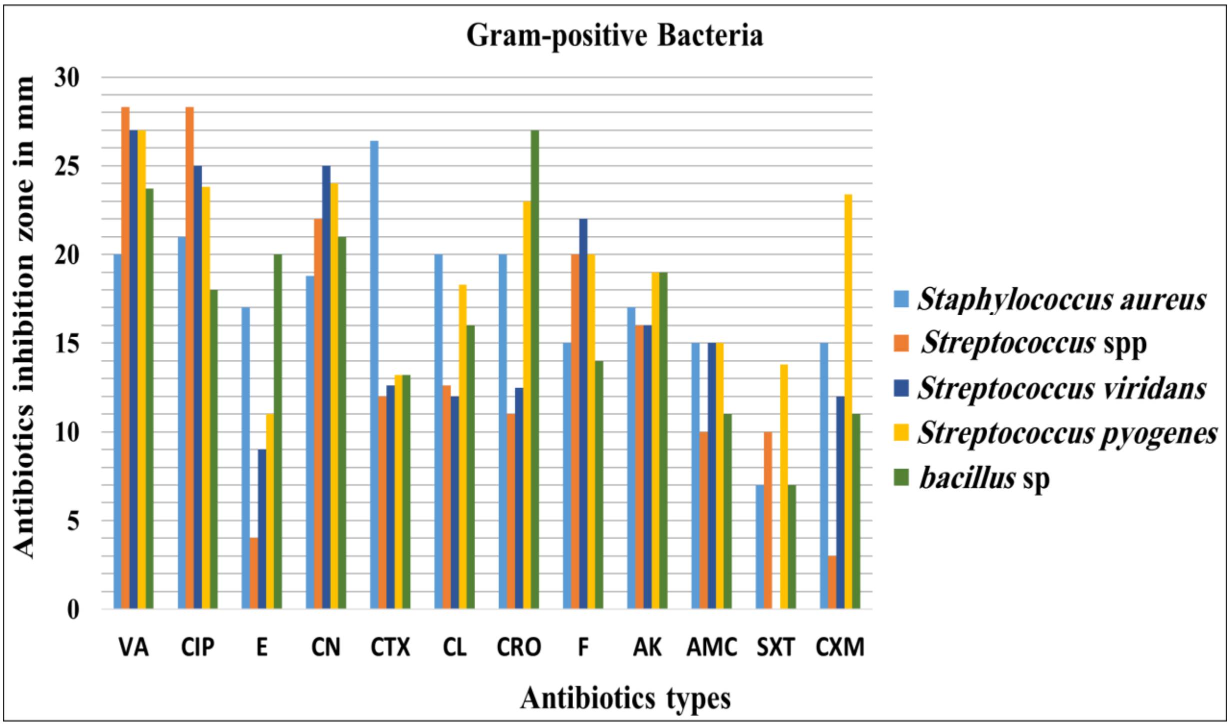


Figure (3) The inhibition zone diameter in millimeters for the effect of antibiotics on Gram-positive bacteria.

### 3.4 Effect of Antibiotics on Gram-Negative Bacteria

Antibiotic susceptibility tests on Gram-negative bacteria showed varying effects. All isolated Gram-negative bacteria were sensitive to Amikacin, and Gentamicin, with inhibition zone diameters ranging from 13 mm to 30 mm, and sensitive to Cefotaxime (from 26 to 28,4 mm) except for *E. coli* and *Pseudomonas aeruginosa*, which showed resistance this antibiotic. Most Gram-negative isolates were resistant to Augmentin, Ceftriaxone, Bactrim, Piperacillin, Cefuroxime and Cephalexin. Enterobacter bacteria showed sensitivity to Bactrim and Piperacillin with inhibition zone diameters of 21 mm, 24 mm, and 29 mm, respectively. Similarly, *Klebsiella* spp. demonstrated sensitivity to Cephalexin, exhibiting a zone of inhibition of 20 mm, and to Nalidixic acid, with an inhibition zone of 28 mm. Nalidixic acid had a significant effect on *Klebsiella pneumoniae*, which showed an inhibition zone of 27 mm, while it had a moderate effect on *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Enterobacter*, with respective inhibition zone diameters of 15 mm, 13 mm, and 15 mm, as shown in Table (4) and Figure (4).

Table (4) Effect of antibiotics on Gram-negative bacteria

| Antibiotic / Bacteria | *E. coli* | *Klebsiella spp* | *K. pneumoniae* | *Proteus mirabilis* | *Ps. aeruginosa* | *Enterobacter* |
|---|---|---|---|---|---|---|
| AK | S | S | S | S | S | S |
| CN | I | S | S | S | I | S |
| CTX | R | S | S | S | R | S |
| AMC | R | R | R | R | R | R |
| CRO | R | R | R | S | R | R |
| SXT | S | R | R | R | R | S |
| CL | R | S | R | R | I | S |
| NA | R | S | S | I | R | I |
| PRL | R | R | R | R | R | S |
| CXM | I | R | R | R | R | R |

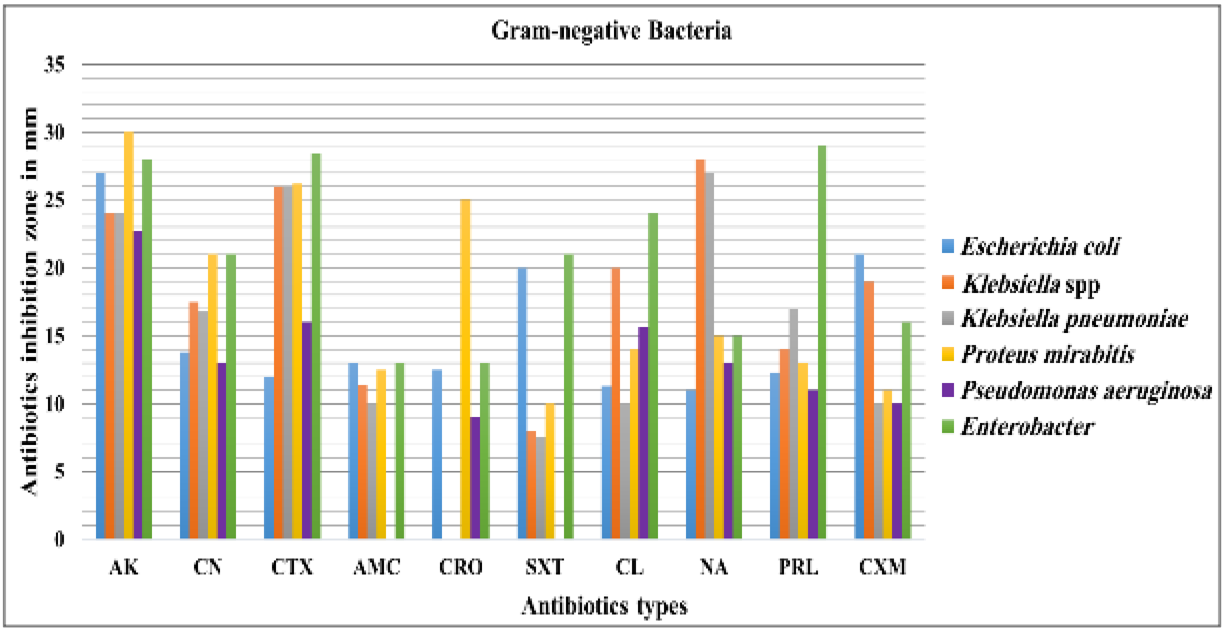


Figure (4) The inhibition zone diameter in millimeters for the effect of antibiotics on Gram-negative bacteria

The results revealed the isolation of several Gram-positive and Gram-negative bacterial species with varying appearance rates. These included *Staphylococcus aureus*, *Streptococcus* spp, *Bacillus* sp., *Klebsiella* spp., *Escherichia coli*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. Most of these types are known for their resistance to many antibiotics, indicating clear UTIs that could lead to other complications such as chronic diseases and infections. These results align with Yenli and others, who also documented significant bacterial growth in urine samples [21]. These results align with the study by Prasada Rao and others, indicating that *S. aureus* and *E. coli* are the most common bacterial species isolated from urinary tract infections [13]. They also agreed with Vasudevan's study in India regarding the isolated types and the higher infection rate in women than in men (66% and 34%, respectively) [3]. The higher infection rate in women also agreed with White Brett's study in the United States [10] and agreed with Aloraibi and others [22].

The findings showed that the most common bacterial species were *S. aureus* (36%), followed by *E. coli* (23%), which somewhat agreed with Aloraibi and others [22]. However, it differed from the study by Arslan and others in Turkey, which showed that *Klebsiella pneumoniae* had the highest percentage among isolated species [1]. The study revealed differences in the sensitivity of isolated Gram-positive and Gram-negative species to several antibiotics. All gram-negative bacterial species were sensitive to Amikacin and Gentamicin. For Gram-positive bacteria, all species were sensitive to Vancomycin, Gentamicin, ciprofloxacin, and Amikacin. These results are consistent with the study by Kebede et al., which confirmed the sensitivity of Gentamicin to Gram-positive bacteria and Gentamicin and Amikacin to Gram-negative bacteria [23]. They are also consistent with Forouzani and others, which confirms the sensitivity of Amikacin against Gram-negative bacteria [24]. Furthermore, the Gram-positive bacterial species were also sensitive to Nitrofurantoin in varying degrees, except for *Bacillus* sp., which was resistant to this antibiotic. Conversely, all species were resistant to Cefotaxime, except for *S. aureus*, which was sensitive. All bacterial species were resistant to Bactrim, except for *Streptococcus pyogenes*, which exhibited intermediate sensitivity to this antibiotic. Moreover, all species were resistant to Ampicillin/ clavulanic acid, with the exception of certain gram-positive species namely, *Staphylococcus aureus*, *Streptococcus viridans*, and *Streptococcus pyogenes*, which demonstrated moderate sensitivity to this antibiotic.

The study concluded that the most resistant Gram-negative bacteria were *Pseudomonas aeruginosa*, which exhibited a resistance rate of 70%. This was followed by *Klebsiella pneumoniae* and *Escherichia coli*, both with a 60% resistance rate. These findings are consistent with the research conducted by Salman and others [25] and Majumder and others [26]. Additionally, *Proteus mirabilis* and *Klebsiella species* showed a resistance rate of 50%. In contrast, the most antibiotic-resistant strains among Gram-positive bacteria were *Streptococcus* spp. (58%), followed by *Streptococcus viridans* (50%), and then *Bacillus* sp. (42%).

In general, the isolated species demonstrated resistance to most of the tested antibiotics. This finding aligns with the study by Shirvani and others [27], which identified a high degree of similarity in the types of bacterial species isolated and their resistance patterns. These results reinforce the conclusions of both studies and confirm the ongoing evolution of bacterial resistance to antibiotics.

## 3. Conclusion

Bacterial UTIs are a major healthcare problem, especially with the increasing resistance of some of these bacterial species to many antibiotics used for treatment. The transmission of such types within hospitals (nosocomial infections) increases the difficulty of combating and controlling them. This was evident from the results of this study and several previous studies, causing significant complications and health problems in and around the urinary system, which increases the costs of hospitalization and treatment. Furthermore, the variation in resistance among cases for the same species confirms genetic changes in these types during each individual infection. This results in resistance to an antibiotic that showed sensitivity in another case, a major problem requiring studies at the genetic level to identify antibiotics resistant to this change. To avoid all this, continuous screening and diagnosis of cases, antibiotic susceptibility testing, and adherence to the basics of infection control, personal and public hygiene, and the use of disinfectants, sterilizers, and medications are necessary.

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# تقييم فعالية مضادات حيوية مختارة ضد البكتيريا المعزولة من عينات التهاب المسالك البولية في المركز الطبي بمصراتة، ليبيا

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2. قسم الصحة العامة، كلية العلوم الصحية، جامعة مصراته

**الملخص**

ازدادت في الفترة الأخيرة التهابات المسالك البولية في كلا الجنسين ومن مختلف الأعمار، والذي يتزامن مع زيادة مقاومة البكتيريا للمضادات الحيوية، مما يسبب العديد من المشاكل والمضاعفات الأخرى، حيث هدفت هذه الدراسة إلى فحص عدد 86 عينة بول من قسم المسالك البولية بمركز مصراته الطبي والتي زرعت على أوساط زراعية مختلفة وتم إجراء الاختبارات التشخيصية لتعريف الأجناس والأنواع البكتيرية المعزولة، وكذلك استخدام طريقة انتشار القرص على أطباق بتري لاختبار حساسية المضادات الحيوية عليها، حيث أظهرت النتائج عزل عدة أنواع بكتيرية بنسب متفاوتة تمثلت في *Staphylococcus aureus* بنسبة (36%)، يليها *Escherichia coli* (24%)، *Klebsiella pneumoniae* (11%)، *Klebsiella* spp (7%)، *Streptococcus* spp (5%)، *Streptococcus viridans* و *Streptococcus pyogenes* و *Proteus mirabilis* (4%) لكل منهما، وكذلك *Pseudomonas aeruginosa* و *Enterobacter* و *Bacillus* sp (2%) لكل منهما، كما بينت النتائج أن أكثر أنواع البكتيريا سالبة الجرام مقاومة للمضادات الحيوية هي *Pseudomonas aeruginosa* بنسبة (67%) ، يليها الأنواع *Klebsiella pneumoniae* و *Escherichia coli* و *Proteus mirabilis* بنسبة (42%) ، وأكثر المضادات الحيوية تأثيرا عليها Gentamicin و Amikacin و Cefotaxime ، أما بالنسبة للبكتيريا موجبة الجرام فكانت أكثر الأنواع مقاومة للمضادات الحيوية هي *Streptococcus* spp و *Streptococcus viridans* بنسبة (58% و50% على التوالي)، يليهما *Bacillus* sp بنسبة (42%)، وأكثر المضادات الحيوية تأثيرا عليها هي Vancomycin وCiprofloxacin و Gentamicin وAmikacin ، وفي الختام خلصت نتائج هذه الدراسة إلى وجود مقاومة من قبل الأنواع البكتيرية المعزولة لعدة مضادات حيوية مستخدمة، مما يسهم في زيادة خطورة هذه البكتيريا وتفاقم مشكلة التهابات المسالك البولية.

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**الكلمات المفتاحية:**

التهابات المسالك البولية، البكتيرية المعزولة،

المضادات الحيوية، مركز مصراته الطبي.