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Prevalence of Gram-negative Bacterial Pathogens in Populations Suffering from Urinary Tract Infections (UTI) in the City of Sirte.

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Abstract

Background: Urinary tract infections (UTIs) are a prevalent ailment on a global scale. The occurrence of urinary tract infections (UTIs) in both men and women has been associated with unusual bacterial growth. The aim of the present study is to identify and characterise gram-negative bacteria that are capable of causing urinary tract infections.

Methodology: Midstream urine samples were collected from each patient into sterile, screw-capped universal container and inoculated into CLED agar plates subsequently placed in an incubator at 37°C for a period of 24 hours. A series of biochemical tests were conducted using Analytical Profile Index (API) .

Results: a total of 150 samples were isolated and subjected to rigorous investigation. A total Out of 96 samples were isolated and identified as gram-negative bacteria, and the most prevalent isolated bacteria were *Escherichia coli*, with 51 cases (53.1%), followed by *Klebsiella* spp. in 17 cases (17.7%), *Pseudomonas* in 12 cases (12.5%), *Acinetobacter* in 9 cases (9.4%), and *Enterobacter* in 7 cases (7.3%)

Conclusions: The present study revealed a widespread prevalence of urinary tract infections caused by Gram-negative bacterial species, with E. coli being the most virulent, particularly in women and children.

Keywords: Urinary tract infections, Gram negative bacteria, CLED agar, MacConkey agar.

تقييم مسببات الأمراض البكتيرية سالبة الجرام لدى المرضى الذين يعانون من التهابات المسالك البولية في مدينة سرت

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الملخص

المقدمة: تُعدّ التهابات المسالك البولية من الأمراض الشائعة عالميًا. وقد ارتبط حدوث هذه الالتهابات لدى كل من الرجال والنساء بنمو بكتيري غير طبيعي. تهدف هذه الدراسة إلى تحديد وتوصيف البكتيريا سالبة الغرام القادرة على التسبب في التهابات المسالك البولية.

طريقة العمل: جُمعت عينات بول من منتصف مجرى البول من كل مريض في عبوات معقمة ذات غطاء لولبي، ثم زُرعت في أطباق أجار CLED، ووُضعت لاحقًا في حاضنة عند درجة حرارة 37 درجة مئوية لمدة 24 ساعة. أُجريت سلسلة من الاختبارات الكيميائية الحيوية باستخدام مؤشر الملف التحليلي (API).

النتائج: عُزلت 150 عينة وخضعت لفحص دقيق. تم تحديد 96 عزلة بكتيرية سالبة الجرام من بين 150 عينة، وكانت تشمل الاجناس البكتيرية هي (*Escherichia* coli) بواقع 51 حالة بنسبة (53.1%)، تليها جنس (*Klebsiella* spp) في 17 حالة بنسبة (17.7%)، كانت البكتيريا المسببة للعدوى هي *Pseudomonas* في 12 حالة (12.5%)، وبحيث كانت بكتريا Acinetobacter في 9 حالات (9.4%)، و *Enterobacter* في 7 حالات (7.3%). كشفت هذه الدراسة عن انتشار واسع النطاق لالتهابات المسالك البولية الناجمة عن أنواع البكتيريا سالبة الغرام، حيث كان الجنس البكتيري (*Escherichia coli*) الأكثر ضراوة، لا سيما لدى النساء والأطفال.

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الكلمات المفتاحية: (التهابات المسالك البولية، البكتيريا سالبة الجرام، وسط اجار CLED، وسط اجار ماكونكي).

1. Introduction

Urinary tract infections (UTIs) are a common disease worldwide. Abnormal bacterial growth causes urinary tract infections in both men and women (Ozturk & Murt, 2020). Factors contributing to the spread of urinary tract infections include poor personal hygiene, hospitalization status, sex, vaginal infections, diabetes, pregnancy, and poor secretion of antibacterial agents in the prostatic fluid (Foxman, 2014). The most bacterial pathogens belong to gram negative group which cause UTI, for example, *E. coli*, *P. aeruginosa*, *K. pneumoniae.*, *Staphylococcus.*, *Proteus* spp, and *Enterococcus* spp (Bashir, et al.,, 2008). Antibiotics are commonly utilized to inhibit and treat microbial growth in the urinary tract. It is one of the most common bacterial infections seen in doctors' offices, especially in developing countries. It can lead to a lot of illness and is expensive to treat. Poor hygiene and urinary system problems are some of the main things that can cause UTIs. The germs that cause UTIs are different in each place, and they can also change how sensitive they are and how they develop resistance (Goli Angoti, et al., 2016). There are different types of bacteria that can cause UTIs. Prevalent excessive utilize of antibiotics has supported bacterial cells to develop resistance (Ruppe, Woerther & Barbier, 2015). It has been established that the aforementioned mechanisms act as a barrier to the binding of antibiotics to bacteria. The majority of antibiotics are required to traverse the outer membrane to access their targets within microbial cells. Therefore, the plasma membrane of bacterial cells, particularly Gram-negative ones, serves as the primary mechanism of resistance to a considerable number of antibiotics, including beta-lactams, colistin, and others (Valenza, et al., 2014). Gram-negative bacteria have some mechanisms that they might use to resist antibiotics. These mechanisms involve secreting enzymes to destroy antibiotics and mutations in bacterial cells, such as antibiotic-binding proteins, ribosomes, and the outer membrane, which modify antibiotic uptake (Maechler, et al., 2015). Most urinary tract infections have revealed high resistance to many commonly used antibiotics. The objective of this study is to identify and characterise gram-negative bacteria that are capable of causing urinary tract infections.

2. Materials and Methods

2.1. Study Design

The study was designed as a descriptive cross-sectional study. It was conducted at the Hospital of Basina in Sirte City with the objective of investigating the prevalence and distribution pattern of Gram-negative bacteria obtained from urine samples. The study encompassed the analysis of 150 urine specimens obtained from clinical sources. These specimens were processed through standard microbiological methodologies, employing techniques that facilitate the identification of bacterial contaminants. The isolates of the gram-negative variety were categorised by species and stratified according to patient group (women, men, and children) in order to evaluate distribution trends and potential demographic associations .

2.2. Study Area

Clean-catch urine specimens were collected from patients at Besina Hospital in Sirte, Libya, for microbial examination. The study ran for 3 months, from 1 September 2025 to 30 December 2025. The study was done in the hospital's microbiology laboratory.

Sample size: Total participants were 150 samples were isolated and subjected to rigorous investigation. A total of 96 isolates were identified as gram-negative bacteria, with 90 of these isolates belonging to female subjects and 60 to male subjects.

2.3. Sample Collection

The data were collected through the meticulous documentation of the microbiological findings of urine samples that were processed using standard laboratory procedures.

The sample collection approach employed was the clean catch method, which was implemented to minimize contamination. Midstream urine samples were collected from each patient and stored in a 20-mL calibrated, sterile, screw-capped universal container. These samples were initially distributed to the patients for analysis. The specimen was appropriately labeled, transported to the laboratory, and analyzed within two hours after collection. Prior to sample collection, all patients were meticulously instructed on how to collect the urine sample in an aseptic manner to avert contamination.

2.4. Sample Processing and Identification of Organisms

Urine samples were collected from patients in sterile containers. Subsequently, the bacterial culture process was performed on positive urine culture (≥100 CFU/mL) under sterile conditions. All urine samples were subjected to a process of culturing, which entailed their inoculation into CLED agar plates. These plates were then placed in an incubator maintained at a temperature of 37°C for 24 hours. Individual colonies were isolated and cultivated on MacConkey agar plates to gain pure cultures for each colony. The cultivation of microbial communities on a differential media facilitates the observation of their morphological characteristics. This approach also enables the discernment of alterations in the nutritional medium resulting from the metabolic activity of these microorganisms. A series of biochemical tests were conducted with the objective of identifying the bacterial sample using Analytical Profile Index (API). The bacterial specimens that were identified were preserved in nutrient agar slants at 37°C for 24 hours, and were sub-cultured periodically.

2.5. Statistical analysis

The distribution of the entire sample set is as follows: the total number of samples was 150, of which 96 were gram-negative isolates. The ratio of gram-negative isolates was thus 96/150 x 100 = 64%. The most prevalent isolate was Escherichia coli, accounting for 53.1% of the total.

2.6. Ethical consideration

Ethical approval was obtained from the institutional research ethics committees of the University of Sirte and the Basina Hospital in Sirte City prior to the initiation of the study. The urine samples included in this study were collected as part of routine clinical diagnostic procedures for suspected urinary tract infections. Patient confidentiality and privacy were strictly maintained throughout the study. Prior to analysis, all data were anonymised and coded, thereby ensuring that no personally identifiable information was accessible to the research team. Participation in the study did not expose patients to any additional risks beyond those associated with standard diagnostic care.

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3. Results

A total of 150 bacterial isolates were analysed, of which 96 (64%) were Gram-negative bacteria. In the Gram-negative isolates, *E. coli* was the predominant pathogen (53.1%), followed by *Klebsiella* spp. (17.7%), *Pseudomonas* spp. (12.5%), *Acinetobacter* spp. (9.4%) and *Enterobacter* spp. (7.3%) (See Table 1 and Figure 1).

Table 1: Distribution of gram negative bacterial isolates (N = 96).

Bacterial isolates	Number (n)	Percentage
E. coli	**51**	**53.1%**
Klebsiella spp	**17**	**17.7%**
Pseudomonas spp	**12**	**12.5%**
Acinetobacter spp	**9**	**9.4%**
Enterobacter spp	**7**	**7.3%**
Total	**96**	**64%**

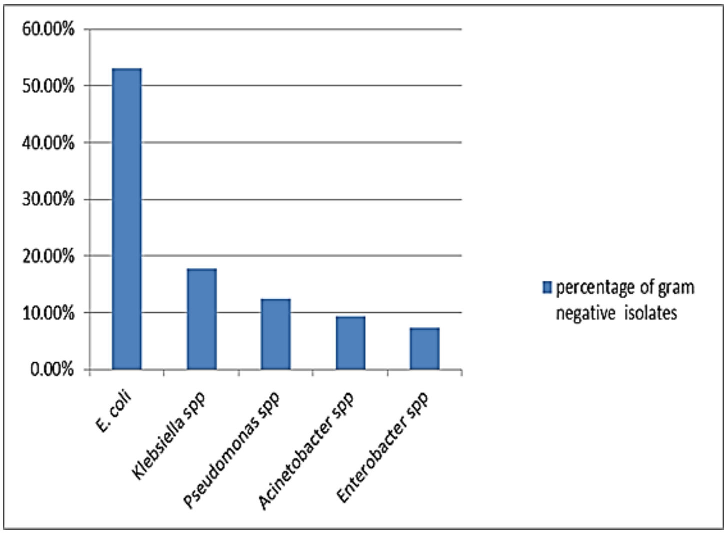

Figure 1: Distribution the total percentage of bacterial isolates.

With regard to gender distribution, the preponderance of infections was observed to be predominantly female across the majority of bacterial genera. The prevalence of *E. coli* infections was found to be higher in women (54.9%) compared to men (27.5%) and children (17.6%). In a similar manner, *Klebsiella* spp. demonstrate a higher prevalence among the female population (76.5%). As demonstrated in Table 2 and Figure 2, in addition to *Pseudomonas* spp., *Acinetobacter* spp. and *Enterobacter* spp. infections were more

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prevalent in women (66.7, 66.7 and 57.4, respectively) than in men and children. The chi-squared test yielded a p-value of 0.39, which is deemed to be statistically insignificant given that p-values > 0.05 are considered not to be statistically significant (see Table 3).

Table 2: Distribution the genera of gram negative isolates according to gender.

Bacterial isolates	Female n (%)	Male n (%)	Children n (%)
E. coli	**28 (54.9)**	**14 (27.5)**	**9 (17.6)**
Klebsiella spp	**13 (76.5)**	**2 (11.8)**	**2 (11.8)**
Pseudomonas spp	**8 (66.7)**	**3 (25.0)**	**1 (8.3)**
Acinetobacter spp	**6 (66.7)**	**2 (22.2)**	**1 (11.1)**
Enterobacter spp	**4 (57.4)**	**3 (42.9)**	**0 (0.0)**
Total n (%)	**59 (61.5%)**	**24 (25.0%)**	**13 (13.5%)**

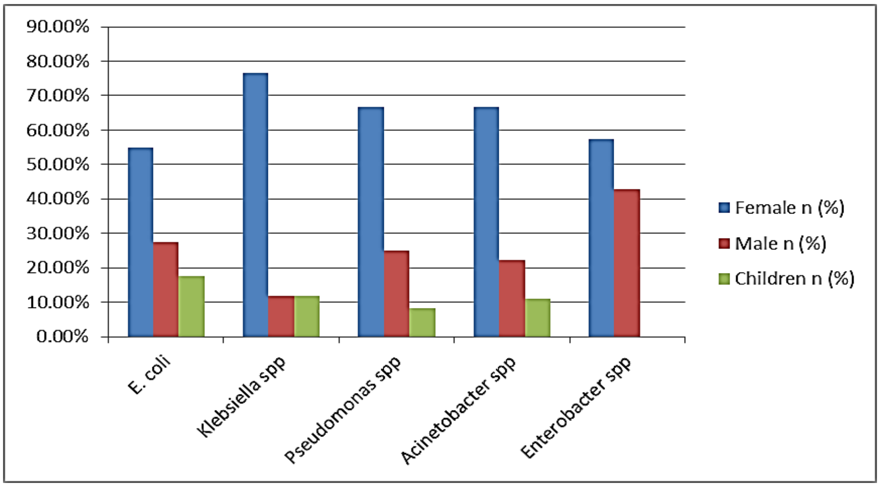

Figure 2: Distribution the genera of gram negative isolates according to gender.

The chi-squared test was employed to conduct inferential statistics, with the objective of demonstrating the association between bacterial type and gender.

Table 3: Chi-Squared Test.

Test	X^2	df	p-value
Pearson Chi-Square	**8.42**	**8**	**0.39**

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4. Discussion

The cell wall structure of gram negative bacteria (GNB) are complex which maintenance their species for causing many disease like urinary tract infections (Blair, et al., 2015). The outer membrane involves lipopolysaccharides and phospholipids which preserves the bacterial cell against antimicrobial. The gram negative species are the most frequent infections in both male and female (Rigel, Silhavy. 2012).

In the present study, a 96 (64%) isolates out of 150 samples were isolated as gram negative bacteria and subjected to rigorous investigation. The 96 isolates were divided as 90 of them belonging to female subjects and 60 samples to male subjects. The data demonstrate that women exhibited a higher infection rate (61.5 %) compared to men, who demonstrated a rate of 25.00% for gram-negative infections. However, children exhibited the lowest infection rate, with a percentage of 13.5%, as showed in figure2.

In this study, urinary tract infections caused by *Escherichia coli* were the most common type of infection, accounting for 53.1% of cases. The infection was more prevalent in women and men than in children, representing 54.9%, 27.5% and 17.6% respectively (see Tables 2 and 3 and Figure 2).

A total of 17 cases of *Klebsiella* spp. were identified, accounting for 17.7% of the total. The majority of these cases were female. Specifically, 13 (76.5%) out of the 17 cases were documented in female subjects. This finding indicates that women may possess a heightened vulnerability to bacterial infections in comparison to men and children. However, it is noteworthy that only 2 cases (11.8%) were recorded in male subjects. Furthermore, the analysis revealed 2 (11.8%) cases in children, as demonstrated in Table 2 and Figure 2. The present findings are commensurate with the conclusions of the study by Batool et al. (2020). The investigation revealed that Gram-negative bacteria were the most prevalent pathogens responsible for urinary tract infections in female subjects, constituting 72.3% of cases, which is significantly higher than the 27.7% observed in male subjects. This finding confirmed that Escherichia coli and *Klebsiella pneumoniae* were the most prevalent pathogens, with a percentage of 40.5% and 25.7%, respectively that was consistent over time.

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Pseudomonas aeruginosa was identified in 12 cases (12.50%), while *Acinetobacter* spp. was found in nine cases, constituting 9.4% of the total cases examined. The infection rate was found to be higher among women than men. Genus of *Enterobacter* spp. was isolated from seven cases, which represent 7.29% of the total, as revealed in Table 1 and Figure 1. The current results are comparable to those of a study by Shahbaz et al. (2024), which found a higher number of isolates from *Escherichia coli, Klebsiella, Pseudomonas*, and *Acinetobacter* (40.5%), (26.6%), (12.2%), and (5.6%), respectively. According to Paterson (2006), *Escherichia coli, Klebsiella* spp. and *Enterobacter* spp. are the primary causative agents of urinary tract infections (UTIs). It is evident that these bacteria are all members of the Enterobacteriaceae family.

5. Conclusions

The present study revealed that urinary tract infections caused by Gram-negative bacteria are very common. In the course of the investigation, the most prevalent uropathogens were found to be *E. coli, Klebsiella* spp. and *Pseudomonas* spp. across all age groups. It has been demonstrated that both sexes are susceptible to infection. However, increasing urinary tract infections in female patients appear to be more severely affected than male patients as a result of substandard hygiene practices. It is recommended that future studies focus on the following: antimicrobial susceptibility testing and resistance. This will ensure that empirical treatment guidelines and antimicrobial stewardship programmes are better informed. In addition, the execution of large-scale multicentre research projects across a range of geographical regions is recommended in order to enhance the external validity of the research and facilitate the comparison of epidemiological patterns on an international scale.

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