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# Isolation, identification, and antibiotic sensitivity testing of bacterial species causing urinary tract infection in different zones of Libya's Brega city region

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#### **Abstract:**

The present study aims to investigate the distribution of Gram-positive and Gram-negative bacteria responsible for urinary tract infections (UTIs) among different age groups in Brega, Libya. A total of 500 mid-stream urine (MSU) samples were collected from both "inpatients and outpatients at Brega Qarawi Hospital" (Zone III), the Family Clinic (Zone I), and the Industrial Clinic (Zone I). Participants included 285 males and 215 females, with ages ranging from 15 to 65 years. Findings revealed that the overall prevalence of UTIs in Brega was approximately 45%, with a slightly higher occurrence among males (46%) compared to females (44%). Adults represented the most affected group (72.4%), "followed by the elderly (16.4%)" and adolescents (11.1%). Outpatients accounted for the majority of cases (62.2%), while inpatients represented 37.7%. Regarding bacterial etiology, "Escherichia coli was the predominant pathogen, responsible for nearly half of the infections" (49.7%). Klebsiella pneumoniae and Pseudomonas aeruginosa were identified in 20.8% and 10.2% of the cases, respectively. Gram-positive bacteria were less frequently detected, with Staphylococcus aureus accounting for 5.3% and Staphylococcus saprophyticus for 2.6%.Antimicrobial susceptibility testing indicated that Imipenem (IMI) demonstrated the highest efficacy among the antibiotics evaluated in this study.

Keywords: bacterial species, urinary tract infection, Libya.

# عزل وتشخيص واختبار الحساسية للمضادات الحيوية لأنواع البكتيريا المسببة لعدوى المسالك البولية في مناطق مختلفة من مدينة البريقة \_ ليبيا

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الملخص

(37.7%).

تهدف هذه الدراسة إلى تحليل توزيع البكتيريا موجبة وسالبة الجرام المسببة لعدوى المسالك البولية (UTIs) بين الفئات العمرية المختلفة في مدينة البريقة، ليبيا. تم جمع 500عينة بول منتصف المجرى (MSU) من المرضى الداخليين والخارجيين في مستشفى البريقة القروي (المنطقة الثالثة) والعيادة العائلية (المنطقة الأولى) والعيادة الصناعية (المنطقة الأولى) والعيادة الصناعية (المنطقة الأولى). شملت الدراسة 258ذكرًا و 215أنثى تتراوح أعمارهم بين 15و65 سنة. أظهرت النتائج أن معدل انتشار عدوى المسالك البولية في البريقة بلغ نحو %45، وكان انتشارها أعلى قليلاً بين الذكور (%46)مقارنة بالإناث .(%44) مثلت فئة البالغين النسبة الأكبر من المصابين(%72.4) ، تلتها فئة كبار السن (%46.4) ثم فئة المراهقين .(%11.1) شكل المرضى الخارجيون النسبة الأعلى من الحالات (%62.2) مقارنة بالمرضى الداخليين

من حيث المسببات البكتيرية، كانت الإشريكية القولونية (Escherichia coli) هي الأكثر شيوعًا بنسبة %49.7 الحالات، تأتها الكلبسيلة الرئوية (Klebsiella pneumoniae) بنسبة %20.8 والزائفة الزنجارية Serudomonas) بنسبة %10.2 المكورة العنقودية الذهبية (Staphylococcus saprophyticus) نسبة %5.3 والمكورة العنقودية الصفراء (Staphylococcus saprophyticus) نسبة %5.3 والمكورة العنقودية الصفراء (Staphylococcus saprophyticus) نسبة %5.3 والمكورة العنقودية الصفراء (Staphylococcus saprophyticus)

أظهر اختبار الحساسية للمضادات الحيوية أن الإيميبينيم (Imipenem) كان الأكثر فعالية بين المضادات الحيوية التي تم تقييمها في هذه الدراسة. تؤكد النتائج أهمية المتابعة الدورية لأنماط مقاومة المضادات الحيوية ودعم الاستخدام الرشيد للأدوية في علاج عدوى المسالك البولية في منطقة البريقة.

الكلمات المفتاحية: الأنواع البكتيرية، عدوى المسالك البولية، ليبيا.

#### Introduction

Urinary tract infections remain one of the most frequently encountered infectious conditions worldwide, affecting diverse host groups and presenting with a broad spectrum of clinical manifestations. Despite their prevalence, these infections are still considered complex and not fully understood. Typically, UTIs result from the host's own endogenous microflora, with Gram-negative organisms such as Escherichia coli and Gram-positive bacteria including enterococci being the primary causative agents (Heisig, 2010).

Community-acquired urinary tract infections represent one of the most prevalent infectious conditions and are a leading cause of outpatient visits, despite their generally low mortality rates. These infections are predominantly attributed to Gram-negative bacteria, with Escherichia coli and Klebsiella pneumoniae being the primary culprits, while Acinetobacter and Enterobacter species are also implicated (Heisig, 2010; Drekonja & Johanson, 2008). For clinicians in private practice, bacteriological analysis has become increasingly significant, as successful management and the prevention of treatment failures rely heavily on understanding the antimicrobial resistance profiles of the pathogens involved (Blieblo & Baiu, 1999; Inglis, 1996; Greenwood et al., 1997; Mittermyer et al., 1995).

The pathogenic potential of E. coli in urinary tract infections cannot be attributed solely to its serotype. A crucial factor in its virulence is the pilus-mediated adhesion mechanism, which enables E. coli to firmly attach to uroepithelial cells, thereby facilitating colonization and infection (Kisielius et al., 1989).

Within the genus Klebsiella, the species most commonly associated with human infection is Klebsiella pneumoniae, although K. oxytoca has also been linked to cases of bacteriuria. Interest in isolates from nephrology patients arose after repeated detection of K. pneumoniae biotypes 16 and 17 in hemodialysis fluids (Kolmos, 1984). Among urinary pathogens, Staphylococcus aureus is considered an unusual isolate, accounting for approximately 0.5–6% of positive urine cultures. The presence of indwelling catheters (63%) was identified as the most significant predisposing factor, followed by urinary obstruction (56%) and surgical interventions (43%) (Arpi & Rennerberg, 1980). According to Makii and Tambyah (2010), microorganisms may access the urinary tract through hematogenous dissemination or via lymphatic pathways. However, substantial clinical and experimental evidence demonstrates that the predominant mechanism is the ascending route, whereby bacteria travel from the urethra into the urinary tract. This mode of infection is particularly common for enteric organisms such as Escherichia coli and other members of the Enterobacteriaceae family.

# Materials and Methods Collection of specimens

A total of 500 mid-stream urine (MSU) samples were collected from both inpatients and outpatients at three healthcare facilities in Brega city: Brega Hospital (Zone III), the Family Clinic (Zone I), and the Industrial Clinic (Zone I). The study population consisted of 285 males and 215 females, ranging in age from 15 to 65 years. Sample collection was conducted between February and April 2021. Each specimen was placed in a sterile universal container, clearly labeled with the corresponding patient questionnaire. Participants were also provided with detailed instructions on proper urine collection, including the use of a private collection area and guidelines for careful cleaning prior to sample submission.

# Urine culturing

In accordance with standard laboratory procedures, the collected specimens were immediately inoculated onto Cysteine Lactose Electrolyte-Deficient (CLED) agar, MacConkey agar, and blood agar (OXOID LTD). A calibrated loop capable of transferring 0.01 ml of urine was used for inoculation. Following streaking, the culture plates were left on the workbench briefly to allow proper absorption of the urine into the agar surface The inoculated media plates were then inverted and incubated under aerobic conditions at 37 °C for a period of 24 hours.

#### Sub cultured

Bacterial isolates were further processed by subculturing on Nutrient Agar (Oxoid, England). Whenever growth was observed, colonies were re-streaked to obtain purified isolates.

#### **Gram staining**

All purified colonies were subjected to Gram staining to differentiate between Gram-positive and Gram-negative bacteria. For the Gram-positive isolates, the catalase test was employed to distinguish Staphylococcus species from Streptococcus species. This approach allowed accurate classification of the isolates based on their staining characteristics and enzymatic activity (Gregersen, 1978).

#### The BD Phoenix 100 system is used for bacterial identification and antibiotic sensitivity testing

#### **Fundamentals of the process**

The Phoenix ID panels use a variety of tests that are modifications of conventional microbiological techniques, such as evaluations of oxidation, fermentation, and the hydrolysis or breakdown of different substrates. To improve the precision of bacterial identification, the technique also uses single-carbon source substrates, chromogenic substrates, and fluorogenic substrates. Using the ID/AST combo panels, the Phoenix instrument may conduct up to 100 identification and antimicrobial susceptibility tests concurrently. A sealed, self-inoculating moulded polystyrene tray with 136 micro-wells already loaded with dried reagents makes up each disposable Phoenix panel (National Committee for Clinical Laboratory Standards, 2003). The combination panel is separated into two parts: the ID part, which has dried substrates for identifying bacteria, and the AST part, which has wells with different antimicrobial agent concentrations as well as growth and fluorescent controls placed in the right places. The system uses fluorometric indicators to identify bacteria and an optimised colorimetric redox indicator for AST. To guarantee accurate susceptibility testing, the AST broth is cationadjusted (for example, with Ca2+ and Mg2+). The Phoenix panel is made up of 85 wells on the AST side and 51 wells on the ID side. In particular, the ID portion has two wells that act as fluorescent controls and forty-five wells that contain dry biological substrates.

The Phoenix panel's AST part consists of 84 wells that contain dry antimicrobial agents and one well that serves as a growth control. There are three types of Phoenix panels: combination ID/AST panels (Phoenix NMIC/ID and Phoenix PMIC/ID), AST-only panels (Phoenix NMIC and Phoenix PMIC), and ID-only panels (Phoenix NID and Phoenix PID). Wells that are not being used are set aside for possible future usage. A standardised inoculum is used to inoculate all panels, and the BD Phoenix nephelometer or the BBL CrystalSpec system must be used only to prepare organism suspensions. After being inoculated, the panels are placed inside the device and kept at 35 °C for continuous incubation. At 20 minutes past the hour, on the hour, and again at 40 minutes past the hour, the device automatically takes readings of the test panels. If necessary, this process can continue for up to 16 hours. It is crucial to remember that Phoenix panels cannot be manually evaluated; they are read exclusively by the device (National Committee for Clinical Laboratory Standards, 2003).

#### **Bacterial identification**

To accurately identify bacteria, the ID component of the Phoenix panel uses a range of conventional, chromogenic, and fluorogenic biochemical assays. Both growth-dependent and enzymatic substrate tests are used in these assays to capture the various reactivity patterns found in various microbial species. The basis for identification is the microorganism's capacity to use or break down particular substrates, which can be found using a variety of indicator systems. For instance, a change in the colour of the phenol red indicator indicates the formation of acid from the metabolism of carbohydrates. Enzymatic hydrolysis of chromogenic substrates, which usually involves p-nitrophenyl or p-nitroanilide molecules, produces a yellow colour. A fluorescent coumarin derivative is released upon hydrolysis of fluorogenic substrates. Additionally, a resazurin-based indication is reduced to detect the use of particular carbon sources. The organism's ability to hydrolyse, degrade, decrease, or otherwise metabolise specific substrates is assessed by other assays in the panel (Bohdima & Topoli, 2010).

#### Antimicrobial susceptibility testing

The Phoenix AST technique is a broth-based microdilution assay. It relies on a redox-sensitive indicator to monitor and detect microbial growth within the system. The presence of an antimicrobial compound in the broth is monitored by continuously assessing



.Fig 1. phoenix 100 system.

changes in the redox indicator along with bacterial turbidity. These measurements collectively allow the system to determine microbial growth. Each AST panel is configured to evaluate specific antimicrobial agents under controlled conditions. Each AST panel includes multiple antimicrobial agents, each presented at a series of two-fold serial dilutions. The microorganism's identification is then used to interpret the minimum inhibitory concentration (MIC) for each agent, allowing classification of the isolate as susceptible, intermediate, or resistant (SIR)

#### phoenix 100 system

To use the Phoenix testing system, you'll need a 25-µL pipettor with sterile tips, Phoenix panels with lids, ID and AST broths, AST indicator solution, the Phoenix inoculation station, a transport caddy, the BBL CrystalSpec or BD PhoenixSpec Nephelometer, and other lab supplies listed in the materials section. The Phoenix panel is put on the inoculation station with the ports pointing up so that they can be filled before it is inoculated. Different inocula are added by hand to the ID and AST ports. The inocula run through the panel like a snake, filling each well as it moves towards the absorbent pad, which collects any extra sample. Once the holes are full, closures are put on them by hand. A hole in the lid divider that lets air in makes sure that there is enough oxygen tension during the testing process (Stefaniuk et al., 2003).

## Phoenix test results

The Phoenix system generates a report displaying the identified organism along with a probability percentage derived from the Phoenix database based on the observed substrate reaction profile . Each substrate reaction is indicated using symbols such as +, V, or X. For each organism–antimicrobial combination, the report also presents the minimum inhibitory concentration (MIC) values along with categorical interpretations as susceptible, intermediate, or resistant (SIR). The primary objective of this study is to isolate and accurately identify the bacterial species responsible for urinary tract infections in the Brega city area .

# Stistical analysis

Data analysis was performed using the chi-square test in SPSS software (version 11, Chicago, USA). A p-value of less than 0.05 was considered statistically significant.

#### **Results and Discussion**

A total of 500 mid-stream urine specimens, collected from 208 males and 292 females, were analyzed between February and April 2021. Among these, 225 samples (representing 45%) tested positive for urinary tract infections.

# Prevalence of UTI among both genders

The overall prevalence of bacterial urinary tract infections across different age groups was 225 cases, accounting for 45% of the study population. The infection rate was slightly higher in males, with 96 cases (46%), compared to 129 cases (44%) in females (Fig. 2).

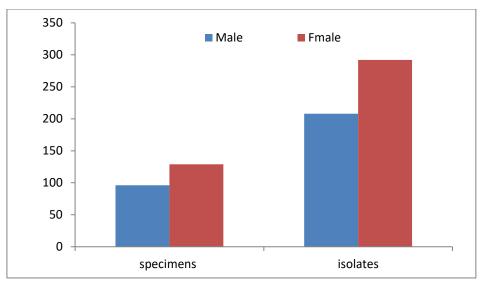
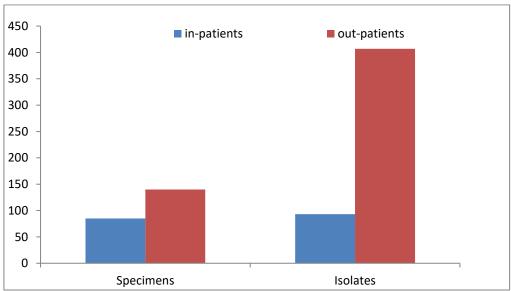


Figure 2. Distribution gender among all collected specimens and isolates.



**Fig 3.** The distribution of UTI among inpatients and outpatients among all collected specimens, as well as the isolation of UTI among persons of various ages .

The study analyzed the distribution of urinary tract infections among patients across different age groups (Table 1). The positive cases varied by age. Among adolescents (15–21 years), 25 cases (11.1%) were identified, including 11 males and 14 females. In the adult group (22–60 years), 163 cases (72.4%) were recorded, comprising 48 males and 115 females. For elderly patients over 60 years, 37 cases (16%) were documented, all of whom were male. Overall, the data indicated a higher number of infections among females compared to males (Buzayan & Baiu, 1998).

**Table 1.** Bacterial urinary tract infections according to age

Tuble 1. Butterial armary fract infections according to age.							
	# of pos	Adolescents 15-21 Year		Adults 22-60 Year		Elderly	
Gender	cases					Over 60 Year	
		No.	%	No.	%	No.	%
Male	96.0	11.0	11.0	48.0	50.0	37.0	39.0
Female	129.0	14.0	11.0	115.0	89.0	0.0	0.0
Total	225.0	25.0	11.1.0	163.0	72.4	37.0	16.0

**Table 2.** The frequency of uropathogens

Bacterial Isolates	Frequency	%
"Escherichia <mark>coli</mark> "	112.0	49.7
"Klebsiella pneumoniae"	47.0	20.8
"Pseudomonas aeruginosa"	23.0	10.2
"Proteus mirabilis"	16.0	07.1
"Staphylococcus aureus"	12.0	05.3
"Staphylococcus saprophyticus"	6.0	02.6
"Enterobacter aerogenes"	5.0	02.2
"Enterobacter cloacae"	4.0	01.7
	225.0	100

## Distribution of Gram-positive and Gram-negative bacteria among uropathogens

Gram-negative bacteria were the most prevalent uropathogens responsible for UTI, accounting for 92%, compared to 8% for Gram-positive bacteria, as indicated in Table 3 and Figure 4.

Table 3. Distribution of Gram-positive and Gram-negative bacteria among uropathogens

Gram negative bacteria	Total 91.7%	Gram positive bacteria	total % 07.9
""Escherichia coli"	49.7	"Staphylococcus aureus"	05.3
"Klebsiella pneumoniae"	20.8	"Staphylococcus saprophylticus"	02.6
"Pseudomonas aeruginosa"	10.2		
"Proteus mirabilis"	07.1		
"Enterobacter cloacae"	01.7		
"Enterobacter nerogens"	02.2		

Testing for Antibacterial Sensitivity Escherichia coli isolates from urinary tract infections were resistant to Trimethoprim-Sulfamethoxazole, Ampicillin, Augmentin, Ciprofloxacin, Gentamicin, Nitrofurantoin, Ceftazidime, and Cefuroxime, with resistance rates of 57%, 55%, 41%, 37%, 34%, 32%, 31%, and 24%, respectively, while exhibiting 80% sensitivity to Imipenem. Klebsiella pneumoniae was shown to be resistant to Ampicillin, Ceftazidime, Celfuroxime, Trimethoprim-Sulfamethoxazole, Ciprofloxacin, Gentamicin, Augmentine and Nitrofurantoin; 100 % ,51%, 47 % , 43 % , 38 % , 36 % , 30 % and 28 5 correspondingly . It was completely sensitive to Imipenem

Celfuroxime, Ampicillin, Nitrofurantoin, Trimethoprim-Sulfamethoxazole, Augmentine, Gentamicin, Ciprofloxacin, and Ceftazidime were all shown to be resistant to Pseudomonas aeruginosa at rates of 100%, 78%, 65%, 61%, 57%, 26%, 17%, and 13%, respectively. It was completely sensitive to Imipenem. Ampicillin, Augmentine, Gentamicine, Nitrofurantoin, Cefuroxime, and Trimethoprim did not work on Proteus mirabilics.

314 | Albahit Journal of Applied Sciences

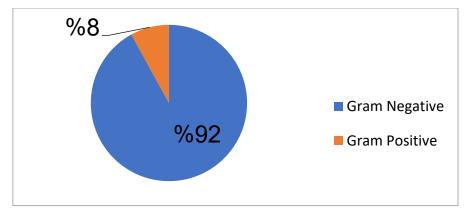


Figure 4. Distribution of Gram-positive and Gram-negative bacteria among uropathogens

**Table 4.** Antibiotic susceptipility patterns of bacterial isolates

"Antibiotics"	"Staph." "aureus" (12 "isolates")	"Staph." "saprophyticus" (06 "isolates")	"Enterobacter" "aerogenes" (05 "isolates")	"Enterobacter" "cloacae" (04 "isolates")
"GN"	"S" 08 (67%) "R" 04 (33%)	"S" 05 (83%) "R" 01 (17%)	"S" 03 (60%) "R" 02 (30%)	"S" 04 (100%) "R" 00 (0%)
"IMI"	"S" 07 (58%) "R" 05 (42%)	"S" 05 (83%) "R" 01 (17%)	"S" 05 (100%) "R" 00 (0%)	"S" 04 (100%) "R" 00 (0%)
"XIME"	"S" 05 (42%) "R" 07 (58%)	"S" 06 (100%) "R" 00 (0%)	"S" 03 (60%) "R" 02 (40%)	"S" 04 (100%) "R" 00 (0%)
"CAZ"	"S" 04 (33%) "R" 08 (67%)	"S" 06 (100%) "R" 00 (0%)	"S" 05 (100%) "R" 00 (0%)	"S" 04 (100%) "R" 00 (0%)
"AMP"	"S" 03 (25%) "R" 09 (75%)	"S" 00 (0%) "R" 06 (100%)	"S" 00 (0%) "R" 05 (100%)	"S" 00 (0%) "R" 04 (100%)
"AUG"	"S" 05 (42%) "R" 07 (58%)	"S" 06 (100%) "R" 00 (0%)	"S" 05 (100%) "R" 00 (0%)	"S" 02 (50%) "R" 02 (50%)
"SXT"	"S" 06 (50%) "R" 06 (50%)	"S" 06 (100%) "R" 00 (0%)	"S" 04 (80%) "R" 01 (20%)	"S" 03 (75%) "R" 01 (25%)
"CIP"	"S" 10 (83%) "R" 02 (17%)	"S" 06 (100%) "R" 00 (0%)	"S" 04 (80%) "R" 01 (20%)	"S" 03 (75%) "R" 01 (25%)
"NIT"	"S" 10 (83%) "R" 02 (17%)	"S" 06 (100%) "R" 00 (0%)	"S" 03 (60%) "R" 02 (40%)	"S" 03 (75%) "R" 01 (25%)

GN=Gentamicin. IMI=Imipenem. XIME= Celfuroxime. CAZ= Ceftazidime.

**AMP= Ampicillin. SXT= Trimethoprim-Sulfamethoxazole.** 

NIT= Nitrofurantoin. CIP= Ciprofloxacin. S= Sensitive. R = Resistant

Because females' urethras are shorter and their vaginal introitus might become polluted with faecal germs, they were more vulnerable to ascending infection. Women are often thought to be more susceptible to urinary tract infections than males because of their shorter urethras. According to Bohdima and Topoli (2010), urinary flow parameters have a significant role in the development of bladder infections. During micturition, backflow of urine has been seen in the female urethera. According to Noufal and Baiu (2012), this procedure will make it easier for colonising bacteria to move into the bladder.

The majority of infected individuals in this investigation were female adult groups, accounting for 57.3% of all positive cases. This might be explained by the fact that the structure of the female genital tract differs from that of the male at this age and that it is more vulnerable to bacterial contamination from stool (Abu-Daia et al., 2000). Furthermore, male prostate secretions can prevent bacterial infections (Qunibi, 1982).

According to Noufal and Baiu's 2003 study on urinary tract infections in the Sirit region, 19% of patients who visited Ibn-sina Hospital complained of UTIs. There were more females than men with these illnesses; the percentages of female and male urinary tract infections were 4% and 5%, respectively. According to our research, females experienced urinary tract infections more frequently than males (Bohdima, K. and Topoli, A., 2010).

#### **Conclusions**

Our research revealed that 45% of people in Brega City had a UTI, with men being more likely than women to be afflicted (46% and 44.0%, respectively).

The age group with the highest prevalence of UTIs was adults (72.4%), followed by the elderly (16.4%) and adolescents (11.1%). The majority of those with UTIs were inpatients (37.2%) and outpatients.(%62.2)

- .3 The most common uropathogen, accounting for 49.7% of infections, was Escherichia coli. Pseudomonas aerogenosa and Klebsiella pneumoniae were responsible for 10.2% and 20.8% of the cases, respectively. Additionally, the percentage of Gram-positive bacteria that caused UTIs was 5.3% for Staphylococcus aureus and 2.6% for Staphylococcus saprophylticus.
- .4 Among the several antibiotics employed in this investigation, IMI is the recommended antibiotic since it produced the maximum sensitivity. However, the majority of bacteria were resistant to ampicillin. Conflict of interest: No conflicts of interest are disclosed by the authors.

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# Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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