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Detection of Bacterial Species Causing Urinary Tract Infections in Brega City Region, Isolation, Identification, and Antibiotic Sensitivity Testing

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The purpose of the study is to ascertain the prevalence of Gram-positive and Gram-negative bacteria that cause urinary tract infections in patients in Brega of various Libyan ages. Inpatients and outpatients at Brega Qarawi Hospital (third zone), Family Clinic (first zone), and Industrialized Clinic (first zone) provided 500 mid-stream urine (MSU) specimens. These people ranged in age from 15 to 65 and included 285 men and 215 women.

According to this study, UTI was 45% common in Brega City, with males having a higher frequency than females (46% vs. 44%). Adults (72.4%) were the age group most likely to have UTIs, followed by the elderly (16.4%) and teenagers (11.1%). The majority of those with UTI were outpatients (62.2%) and inpatients (37.7%). With 49.7% of infections caused by it, *Escherichia coli* was the most prevalent uropathogen. *Pseudomonas aerogenosa* and *Klebsiella pneumoniae* were each in charge of 10.2% and 20.8% of the cases, respectively. UTIs were less commonly caused by the gram-positive bacteria *Staphylococcus aureus* (5.3%) and *Staphylococcus saprophyticus* (2.6%). With the highest sensitivity of the antibiotics examined in this study, Imipenem is the one to use.

1. Introduction

The urinary system is made up of many bodily parts that produce, store, and excrete urine. There is always a chance that bacteria, in particular, will invade these organs. Urinary tract infections (UTIs) are a group of clinical conditions characterized by microbial invasion of the tissues lining the urinary system, which extends from the renal cortex of the kidneys to the urethral meatus. The invasive microorganism may affect the whole tract, or it may be limited to the upper area of the kidneys (pyelonephritis), the lower region, where the invaded organs are the bladder (cystitis), prostate (prostatitis), and urethra (urethritis), or it may simply affect the urine (Tenover, 2006).

The study's goal is to Goals in general particular goals discovery and isolation of the bacterial species responsible for urinary tract infections in the Brega region.

figuring out how common the germs that cause urinary tract infections are in Brega city among patients of various ages. the evaluation of isolated bacteria's susceptibility to antibiotics.

Urinary tract infections continue to be among the most prevalent infectious illnesses due to their vast spectrum of clinical symptoms and impacted host groups, but they are also among the most difficult and little understood. The endogenous micro flora, which also includes Gram-

positive bacteria like Enterococci and Gram-negative bacteria like *Escherichia coli*, frequently causes mono infections such urinary tract infections (Heisig., 2010).

Although though fatality rates are normally not high, community-acquired urinary tract infection is one of the most prevalent infectious illnesses and a frequent reason for out-patient treatment presentations. Gram-negative bacteria, mostly *Escherichia coli* and *Klebsiella pneumoniae*, but also *Acinetobacter* and *Enterobacter* spp., are responsible for UTIs (Heisig., 2010; Drekonja and Johanson., 2008).

Bacteriological reports are becoming more crucial for private practice physicians as well since it is only through an understanding of the resistance patterns of the causative organisms that treatment failures and their repercussions may be avoided (Blieblo and Baiu, 1999; Inglis, 1996; Greenwood *et al.*, 1997).

Escherichia coli's Ur pathogenicity cannot be solely attributed to its serotype. A crucial procedure known as Pilus-mediated adherence of *E. coli* to uroepithelial cells contributes to the pathogenicity of urinary tract infection (Kisielius *et al.*, 1989).

Klebsiella pneumoniae is the major pathogen in the genus *Klebsiella*, while *K. oxytoca* can also cause bacteriuria. The frequent isolation of *K. pneumoniae* biotypes 16 and 17 from haemodialysis fluid encouraged researchers to examine strains seen in regular samples from nephrological patients (Kolmos, 1984).

Staphylococcus aureus, a rare urinary isolate, was responsible for about (0.5–6%) of all positive urine cultures. The most important predisposing factors in the urinary system included indwelling catheters (63%) followed by obstruction (56%) and surgery (43%) (Arpi and Rennerberg 1980).

Although (Makii and Tambyah., 2010) discovered that microbes could enter the urinary tract through lymphatic or haematogenous spread, there is a wealth of clinical and experimental data demonstrating that the ascent of microbes from the urethra is the most frequent pathway leading to a UTI, particularly for organisms with enteric origin (i.e., *Escherichia coli* and other *Enterobacteriaceae*).

2. Materials and Methods

specimen collection

In-patients and out-patients at the Brega Qarawi Hospital (the third zone), Family Clinic (the first zone), and Industrialized Clinic (the first zone) in Brega city provided 500 Mid-stream urine (MSU) specimens. Their ages varied from 15 to 65 years old, and there were 285 males and 215 girls. These samples were

gathered between the months of March and June 2013. The questionnaire page for the patient was appropriately labeled with the sterile universal container containing the sample, and the patients were given instructions on how to collect the sample (the folded equipped private collection of urine sample and directions on how to clean the area carefully).

Urine cultivation

Samples were first cultivated on Cysteine lactose electrolyte-deficient (CLED), MacConkey, and blood agar medium (OXOID LTD) after being received at the lab, according to standard laboratory protocols.

The loop in use may move 0.01 milliliters of urine sample. The plates were inoculated and then placed on the bench for the urine to soak into the agar media. The plates were then turned over and incubated aerobically for 24 hours at 37°C. Omnipresent container housing the sample

Identification of bacteria and antimicrobial sensitivity test using BD Phoenix 100 system

Principles of the procedure

Many of the tests used in the phoenix ID panels are modifications of the classical methods. These include tests for fermentation, oxidation, degradation and hydrolysis of various substrates. In addition to these, the Phoenix system utilizes chromogenic and fluorogenic substrates as well as single carbon source substrates in the identification of organisms. A maximum of 100 identification and antimicrobial susceptibility tests can be performed in the Phoenix instrument at a time using Phoenix ID/AST combination panels. A sealed and self-inoculating moulded polystyrene tray with 136 micro-wells containing dried reagents, serves as the Phoenix disposable. (National Committee for Clinical Laboratory Standards, 2003).

The combination panel includes an ID side with dried substrates for bacterial identification, an AST side with varying concentrations of antimicrobial agents and growth and fluorescent controls at appropriate wells locations. The Phoenix system utilizes an optimized colorimetric redox indicator for AST, and a fluorometric indicators for ID. The AST broth is cation-adjusted (e.g., Ca⁺⁺ and Mg⁺⁺) to optimize susceptibility testing performance. The Phoenix panel is comprised of a 51 wells ID side and an 85 wells AST side. The ID side

contains 45 wells with dried biochemical substrates and 2 fluorescent control wells.

The AST side contains 84 wells with dried antimicrobial agents and 1 growth control well panels are available as ID only (Phoenix NID panels, Phoenix PID panels), AST only (Phoenix NMIC panels, Phoenix PMIC panels), or ID/AST combination (Phoenix NMIC/ID panels, phoenix PMIC/ID panels). Unused wells are reserved for future Phoenix panels are inoculated with a standardized inoculum. Organism suspensions must be prepared only with the BBL crystal spec or BD Phoenix nephelometer. Once inoculated, panels are into the instrument and continuously incubated at 35°C. the instrument test panels every 20 minutes, on the hour at 20 minutes past the hour and again at 40 minutes past the hour up to 16 hours if necessary. Phoenix panels are read only by the instrument. Phoenix panels cannot be read manually. (National Committee for Clinical Laboratory Standards., 2003).

Bacterial identification

The ID portion of the Phoenix panel utilizes a series of conventional chromogenic, and fluorogenic biochemical tests to determine the identification of the organism. Both growth based and enzymatic substrates are employed to cover the different types of reactivity in the range of taxa. The tests are based on microbial utilization and degradation of specific substrates detected by various indicator system. Acid production is indicated by a change in the phenol red indicator when an isolate is to utilize a carbohydrate substrate. Chromogenic substrates produce a yellow colour upon enzymatic hydrolysis of either p-nitrophenyl or p-nitroanilide compounds. Enzymatic hydrolysis of fluorogenic substrates results in the release of a fluorescent coumarin derivative. Organisms that utilize a specific carbon source reduce the resazurin based indicator. In addition, there are other tests that detect the ability of an organism to hydrolyse, degrade, reduce or otherwise to utilize a substrate. (Bohdima, K, A and Topoli, A, S. 2010).

Antimicrobial susceptibility testing

The phoenix AST method is a broth based micro dilution test. The phoenix system utilizes a redox indicator for the detection of organism growth in the presence of an antimicrobial agent. Continuous measurements of change to the indicator as well as

bacterial turbidity are used in the determination of bacterial growth. Each AST panel configuration contains several antimicrobial agents with a range of two-fold doubling dilution concentrations. Organism identification is used the interpretation of the MIC values of each antimicrobial agent producing susceptible, intermediate or resistant (S. I. R.) result classifications.



Figure 1. phoenix 100 system

The components required for testing using the phoenix system include, phoenix panels with panel closures, phoenix ID broth, phoenix AST broth, phoenix AST indicator solution, phoenix inoculation station, phoenix transport caddy, BBL crystal spec or BD phoenix spec Nephelometer, 25ul pipettor and sterile tips, and Miscellaneous lab supplies (listed under materials required but provided). Prior to inoculation , the phoenix panel is placed on the inoculation station with the inoculation ports at the top for filling. Separate inoculum is added manually to the ID and AST ports. The inoculum flow down the panel in serpentine fashion, filling the panel wells as the liquid front progresses toward the pad. The pad absorbs excess inoculums. Closures are manually inserted in the fill ports. An air admittance port is located in the divider area of panel lid to ensure adequate oxygen tension in the panel for the duration of the tests. (Stefaniuk, E. *et al.*, 2003).

Phoenix test results

Organism identification will appear on the Phoenix report form with a probability percentage from the Phoenix database based on the substrate reaction profile. Results from each substrate will appear as +, V or X for each reaction. The MIC result and interpretive Categorical results (S. I. R.) will be shown for the appropriate organism antimicrobial agent combinations. However, the aim of the study isolation and identification of bacterial species causing urinary tract infection in the area of Brega city.

Statistical analysis

The data were subjected to chi-square test using the SPSS computerized software version 11(Chicago, USA). Significance was accepted at $P < 0.05$ levels.

3. Results

Five hundred Mid-stream urine samples from 208 men and 292 women were analyzed between March 2013 and June 2013 for this study. Of these instances, 225 (or 45%) were positive.

Prevalence of UTI among both genders

The prevalence of bacterial urinary tract infections among people of different ages was 225 cases (45%). It was more in males than females, 96 (46%) and 129 (44%), respectively (Fig. 2).

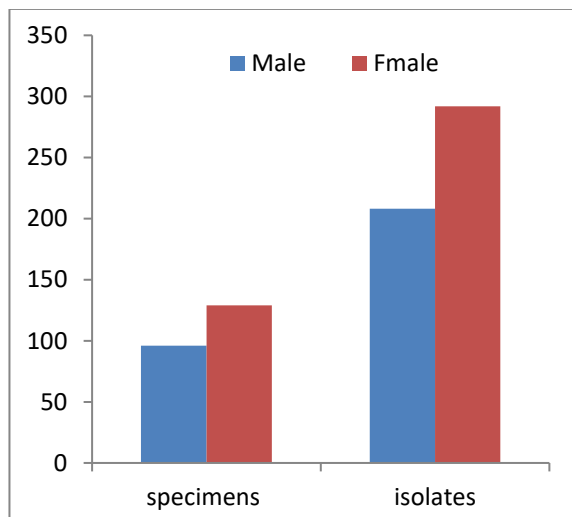


Figure 2. Distribution gender among all collected specimens and isolates.

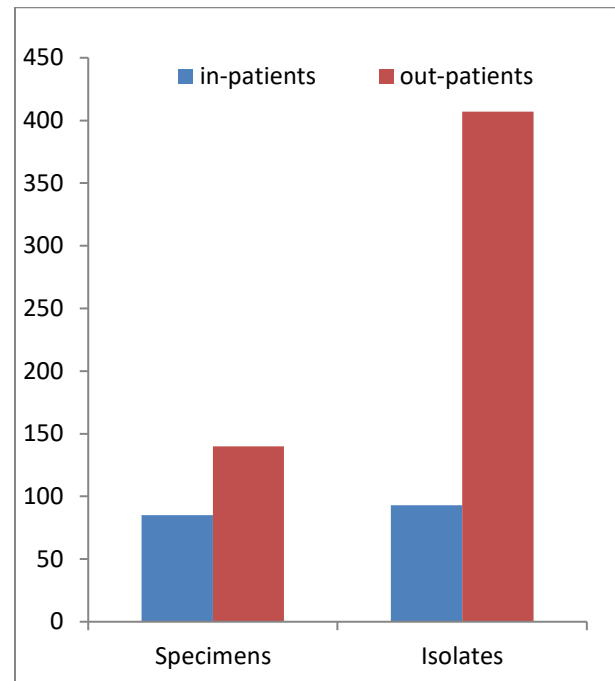


Figure 3. Distribution of the UTI in in-patients and out-patients among all collected specimens and isolates.

UTI among people of different ages

The study showed a distribution of urinary tract infections of patients according to age (Table 1). Positive specimens were different in ages. The results showed that the positive specimens of adolescent (15 years to 21 years) were 25 cases (11.1%), of them 11 males and 14 females, and in adults aged (22 years to 60 years), 163 cases (72.4%), 48 males and 115 females, and in elderly more than (60 years), 37 all affected patients were male (16%), however there were more cases in females than males. (Buzayan, M and Baiu, S., 1998).

Table 1: Bacterial urinary tract infections according to age

Gender	# of pos cases	Adolescents		Adults		Elderly	
		15-21 Year		22-60 Year		Over 60 Year	
		No.	%	No.	%	No.	%
Male	96	11	11	48	50	37	39
Female	129	14	11	115	89	0	0
Total	225	25	11.1	163	72.4	37	16

Table 2: The Frequency of Bactrial Uropatogens

Bacterial Isolates	Frequency	%
<i>Escherichia coli</i>	112	49.7
<i>Klebsiella pneumoniae</i>	47	20.8
<i>Pseudomonas aeruginosa</i>	23	10.2
<i>Proteus mirabilis</i>	16	7.1
<i>Staphylococcus aureus</i>	12	5.3
<i>Staphylococcus saprophyticus</i>	6	2.6
<i>Enterobacter aerogenes</i>	5	2.2
<i>Enterobacter cloacae</i>	4	1.7
	225	100

Distribution of Gram-positive and Gram negative bacteria among Ur pathogens

Gram-negative bacteria were the most common of Ur pathogens responsible for UTI with (92%), in

comparison to (8%) for Gram-positive bacteria as shown in (Table 3) and (Fig.4).

Table 3: Distribution of Gram-positive and Gram-negative bacteria among Ur pathogens

Gram negative bacteria	Total 91.7%	Gram positive bacteria	total % 7.9
<i>Escherichia coli</i>	49.7	<i>Staphylococcus aureus</i>	5.3
<i>Klebsiella pneumoniae</i>	20.8	<i>Staphylococcus saprophyticus</i>	2.6
<i>Pseudomonas aeruginosa</i>	10.2		
<i>Proteus mirabilis</i>	7.1		
<i>Enterobacter cloacae</i>	1.7		
<i>Enterobacter aerogenes</i>	2.2		

Antibacterial Sensitivity Testing *Escherichia coli* isolates from UTI were resistant to Trimethoprim-Sulfamethoxazole, Ampicillin, Augmentin, Ciprofloxacin, Gentamicin, Nitrofurantoin, Ceftazidime and Cefuroxime ; 57%, 55 %, 41 %, 37 %, 34 %, 32 %, 31 % , and 24 % respectively, while it was 80 % sensitive to Imipenem. *Klebsiella pneumoniae* was found to be resistant to Ampicillin, Ceftazidime, Cefuroxime, Trimethoprim-Sulfamethoxazole, Ciprofloxacin, Gentamicin, Augmentin and Nitrofurantoin; 100 % ,51%, 47 %, 43 %, 38 %, 36 %,

30 % and 28.5 respectively. While it was 100% sensitive to Imipenem.

Pseudomonas aeruginosa was found to be resistant to Cefuroxime, Ampicillin, Nitrofurantoin, Trimethoprim-Sulfamethoxazole, Augmentin, Gentamicin, Ciprofloxacin and Ceftazidime; 100 %, 78 %, 65 % , 61 % , 57 % , 26 % , 17 % and 13 % respectively . While it was 100 % sensitive to Imipenem. *Proteus mirabilis* was found to be resistant to Ampicillin, Augmentin, Gentamicin, Nitrofurantoin, Cefuroxime, Trimethoprim.

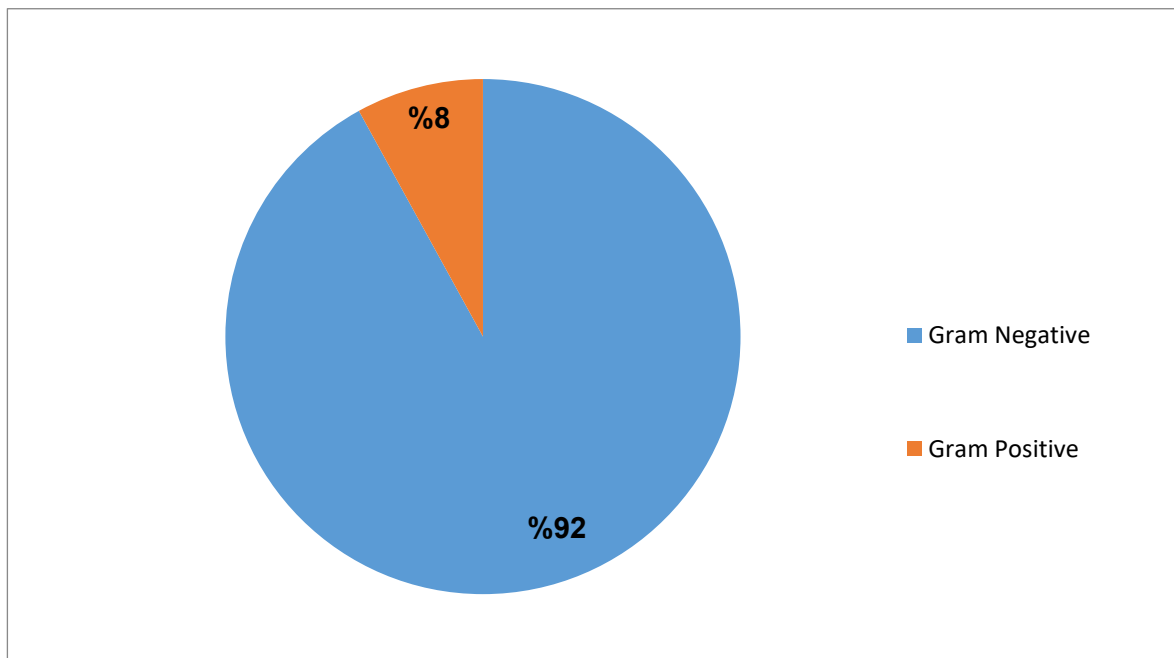


Figure 4. Distribution of Gram-positive and Gram-negative bacteria among Ur pathogens

Table 4: Antibiotic susceptibility patterns of bacterial isolates

Bacterial Isolates									
Antibiotics		<i>Staph.aureus</i> (12 isolates)		<i>Staph. saprophyticus</i> (6 isolates)		<i>Enterobacter aerogenes</i> (5 isolates)		<i>Enterobacter cloacae</i> (4 isolates)	
GN	S	8	67%	5	83%	3	60%	4	100%
	R	4	33%	1	17%	2	30%	0	0%
IMI	S	7	58%	5	83%	5	100%	4	100%
	R	5	42%	1	17%	0	0%	0	0%
XIME	S	5	42%	0	0%	3	60%	4	100%
	R	7	58%	6	100%	2	40%	0	0%
CAZ	S	4	33%	6	100%	5	100%	4	100%
	R	8	67%	0	0%	0	0%	0	0%
AMP	S	3	25%	0	0%	0	0%	0	0%
	R	9	75%	6	100%	5	100%	4	100%
AUG	S	5	42%	6	100%	5	100%	2	50%
	R	7	58%	0	0%	0	0%	2	50%
SXT	S	6	50%	6	100%	4	80%	3	75%
	R	6	50%	0	0%	1	20%	1	25%
CIP	S	10	83%	6	100%	4	80%	3	75%
	R	2	17%	0	0%	1	20%	1	25%
NIT	S	10	83%	6	100%	3	60%	1	25%
	R	2	17%	0	0%	2	40%	3	75%

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

AMP= Ampicillin. SXT= Trimethoprim-Sulfamethoxazole.

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

Females had a greater susceptibility to ascending infection, as female's urethra is short and vaginal introitus may become contaminated with fecal organisms. Generally, it is assumed that the short urethra in women accounts for its increased susceptibility to urinary tract infections compared to men. Urinary flow characteristics are important in the initiation of bladder infection (Bohdima, K, and Topoli, A, 2010). Backflow of urine in the females urethra has been observed during micturition. This process will facilitate the spread of colonizing bacteria into the bladder (Noufal and Baiu, 2012).

Females adult groups were the majority of infected cases in this study, they represented (57.3 %) of the total positive cases. This might be interpreted as in this age female genital tract anatomy is different from males and more susceptible to contamination with bacteria from stool (Abu-Daia *et al.*, 2000). In addition to this, prostatic secretions in males can protect bacterial infections (Qunibi.,1982).

In a study on urinary tract infections in sirt area done by Noufal and Baiu in 2003, (19 %) of the patients visiting Ibn-sina Hospital, complaining of urinary tract infections. Females with these infections were more than males , the percentage of occurrence of urinary tract infection in female and male infection, were (4%) and (5%) respectively. In our study urinary tract infections were common in females than males (Bohdima, K, and Tripoli, A, 2010).

4. Discussion

One of the most typical illnesses is a urinary tract infection, particularly in young children. It can lead to consequences including hypertension, renal failure, and permanent kidney damage if it is not treated with the proper antibiotics, and it is still a major health concern

(Steven *et al.*, 2006). In this study, female adult groups made up most of the positive cases (57.3%), making up the bulk of infected patients. This might mean that at this age, female genital tract anatomy differs from male genital system anatomy and is more vulnerable to bacterial contamination from feces (Abu-Daia *et al.*, 2000). Male prostatic secretions can also prevent bacterial infections, according to research (Qunibi.,1982).

According to our research, Gram-negative bacteria account for 48 percent of all UTIs. This was also discovered in a 2007 Saudi research by Wajeha and a 2013 study by Nicolle et al, in which it was shown that

Gram-negative bacteria were to blame for (75.5%), and (88%), respectively, of UTI.

In 2003, Noufal and Baiu, conducted research on urinary tract infections in the Sirt region. Among the patients who visited Ibn-Sina Hospital, 19% reported having an infection. The prevalence of urinary tract infections in males and females was (4%) and (5%), respectively, with females having more of these illnesses than males. In our study, urinary tract infections affected more women than men.

Regarding antibiotic susceptibility of isolated bacteria, the majority of the isolates in our study were extremely susceptible to imipenem, ciprofloxacin, gentamycin, and nitrofurantoin, but resistant to ampicillin. This was supported by the findings of a study carried out at the Mohammad Al-Mgariaf Hospital in the city of Ejdabia by Bohdima and Topoli in 2010, which found that all isolates were ampicillin-resistant and that the most effective antibiotics were imipenem, ceftazidime, nitrofurantoin, and ciprofloxacin. Imipenem, Nitrofurantoin, Gentamicin, and Ciprofloxacin were the most effective antibiotics in El Gotrani and Topoli's, 2011 investigation, which was conducted in the pediatric hospital in Benghazi, although the bacteria were resistant to

Multiple antimicrobial resistance among Gram-negative organisms has been a long-term and well-recognized problem with urinary tract infections. Resistance has been observed in multiple genera, including *Escherichia coli*, *Enterobacter*, *Klebsiella*, *Proteus*, *Serratia*, and *Pseudomonas*. (Noor et al., 2004).

5. Conclusions

1. Our study found that the prevalence of UTI in Brega city was 45% and males were more than affected females 46 %, 44% respectively.

2.The most affected age group with UTI belonged to the adult age group (72.4%), and who were elderly (16.4%), and the adolescents were (11.1%). Most of those suffering from UTI were out-patients (62.2%) and in-patients with (37.7 %).

3.*Escherichia coli* was the most predominant Ur pathogen responsible for (49.7%) of infections. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* caused (20.8%) and (10.2%) respectively. Also, to less extent the Gram-positive bacteria *Staphylococcus aureus*, *Staphylococcus saprophyticus* that caused UTIs were (5.3%) and (2.6%) respectively.

4.Imipenem is the antibiotic of choice, where it resulted in the highest sensitivity among different antibiotics

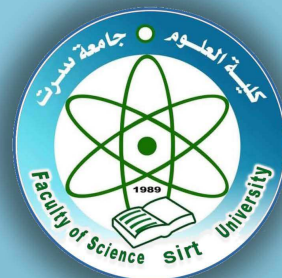
used in this study. On the other hand, Ampicillin was resisted by most bacteria.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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