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Detection of Antibiotic Resistance among Gram-Negative Bacteria Isolated from Urinary Tract Infections

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***Abstract*— This study showed all isolates of bacteria are identified by many testes , such as morphological, biochemical, and API20E testes, as, the results confirmed that the Gram-negative bacteria in Thi-Qar government during the period of June and July 2023-2024. A total of 60 non-duplicate urine samples from patients are collected and examined for detection of Gram-negative bacteria. The results show that out of 60 urine samples 35(58.33%) samples are positive for bacterial growth of Gram negative. The results of bacterial diagnosis depending on testing showed that the frequency of bacterial pathogens that ause UTI as the following 15 (42.85%) are *Escherichia coli*,7 (20.0%) *Enterobacter cloacae*, 5 (14.28%) *Klebsiella pneumonia*, 3 (8.57 %) are from each of Proteus *mirabilis* and *Pseudomonas aeruginosa*, and 2 (4.76%) are Citrobacter spp. Antibiotics were divided into three categories that work on (nucleic acid, protein inhibition ,and bacterial cell wall synthesis) and the three groups were compared with each other .It was concluded which of them was more effective and performed better in eliminating bacteria and the results appeared as follows: Gram negative bacteria showed 91.42 % resistance to ampicillin 85.7% resistance to amikacin, 65.71% resistance to levofloxacin, 45.71% resistance to Norfloxacin, 31.42 % resistance to azithromycin and 13.37% resistance to ciprofloxacin.**

***Keywords*— UTI, Gram-negative bacteria, antibiotic resistance.**

I. INTRODUCTION

Urinary tract infections (UTIs) are prevalent—, particularly for primary care physicians in daily clinical practice. However, in hospital-based settings, UTIs pose a serious health risk [1] [2] . Females are the demographic group most common for urinary tract infections-because the urethra of females is shorter and closer to the rectum, so bacteria can enter the urinary tract more easily. (Sexual activity, pregnancy, age, poor hygiene, and previous urinary tract infection) are additional factors that increase the risk of urinary tract infections [3] [4] Gram-negative and Gram-positive bacteria are the main etiological factors of a UTI, while fungal or viral infections of the urinary tract are uncommon and generally occur in immunosuppressed patients [5] . *Escherichia coli* is one of the most significant pathogens for the development of urinary tract infections, the most important of which are pathogenic *E. coli* (UPEC) strains, and it is considered the main factor in both uncomplicated and complicated urinary tract infection episodes. In addition to the presence of other pathogenic types of Gram-negative microorganisms such as *Proteus mirabilis*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. While *Staphylococcus saprophytes*, *Enterococcus faecalis*, and *Staphylococcus aureus* are among the Gram-positive bacteria that cause urinary tract infections [6].

The most concerning thing about these germs is that they are spreading, particularly in situations related to healthcare. Pregnant patients who are hospitalized in prenatal units are more likely to get infections, particularly UTIs [7]. Finding ESBL-producing Gram-negative bacteria in a lab can aid patients in receiving efficient care and lessen the likelihood of UTI issues. The spread of Gram-negative bacteria that produce ESBLs and cause urinary tract infections in pregnant women was so intriguing to find [8].

. Antimicrobial drug resistance is a direct threat represented by decreased discovery and development of new antibiotics ([9]. This decline has resulted from a number of factors, such as the increasing difficulty in finding new compounds to investigate, the increased capital costs and long time required for drug development, the increasing complexity in planning and executing downstream clinical trials, and the increasing complexity in planning and executing downstream clinical trials. Concern that the emergence of resistance will shorten the duration of drug use [8].

However, antibiotic resistance among the microorganisms causing UTI is rising and is a significant health concern in the treatment of UTI [10], as it is with

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many community-acquired illnesses. Antimicrobial resistance is becoming a global concern, especially with regard to *E. coli*, which is the primary cause of UTIs in expectant mothers [11]. Gram negative bacteria are organisms that have developed antibiotic drug resistance mechanisms through the acquisition of genes, particularly when antibiotic selection pressure is present. They frequently use different methods to combat the same antibiotic or one mechanism to combat several medicines. Gram negative bacteria are capable of using seven different resistance mechanisms, some of which are mediated by mobile plasmids [12]. These mechanisms include loss of porin, thus reducing drug movement across the cell membrane; The presence of βLactamase in the periplasm, which leads to β-lactam hydrolysis; increased expression of the transmembrane efflux pump [13], which flushes the drug from the bacteria before it has an effect; The presence of antibiotic-modifying enzymes, which prevent the interaction of the antibiotic with its target; the occurrence of mutations in the target site, which makes the antibiotic unable to bind to its site of action; Mutations or ribosomal modifications, which prevent the antibiotic from binding and inhibit protein synthesis; metabolic bypass mechanisms, which use an alternative resistance enzyme to bypass the inhibitory effect of antibiotics; and mutation in lipopolysaccharides, rendering the polymyxin class of antibiotics unable to bind this target [8].

II. MATERIALS AND METHODS

Collection of Samples

Sixty samples were collected from urinary tract infections in the Thi-Qar government during June and July 2023. Data which collected from patients included: age, and marital status,before specimens collections , consent was obtained from patients for sampling. The necessary measures were taken to ensure the safety of the participants while taking it. The samples were put immediately in a sterile tube , and transmitted to the laboratory with a cooling box. The urine isolates were cultured after being centrifuged. Then, the sediment was added under sterile conditions to the medium for isolation and diagnosis.

A. Isolation and Identification of Bacteria

All urine samples collected from patients were cultured on MacConkey agar and Nutrient agar (a loop full of urine sediment was streaked on both media) .The plates were incubated for 24 hr at 37 ^{0}C. Isolation of bacterial species was done according to morphological and biochemical test , including the IMViC test and API 20 E Kit, shown in Figure (1).

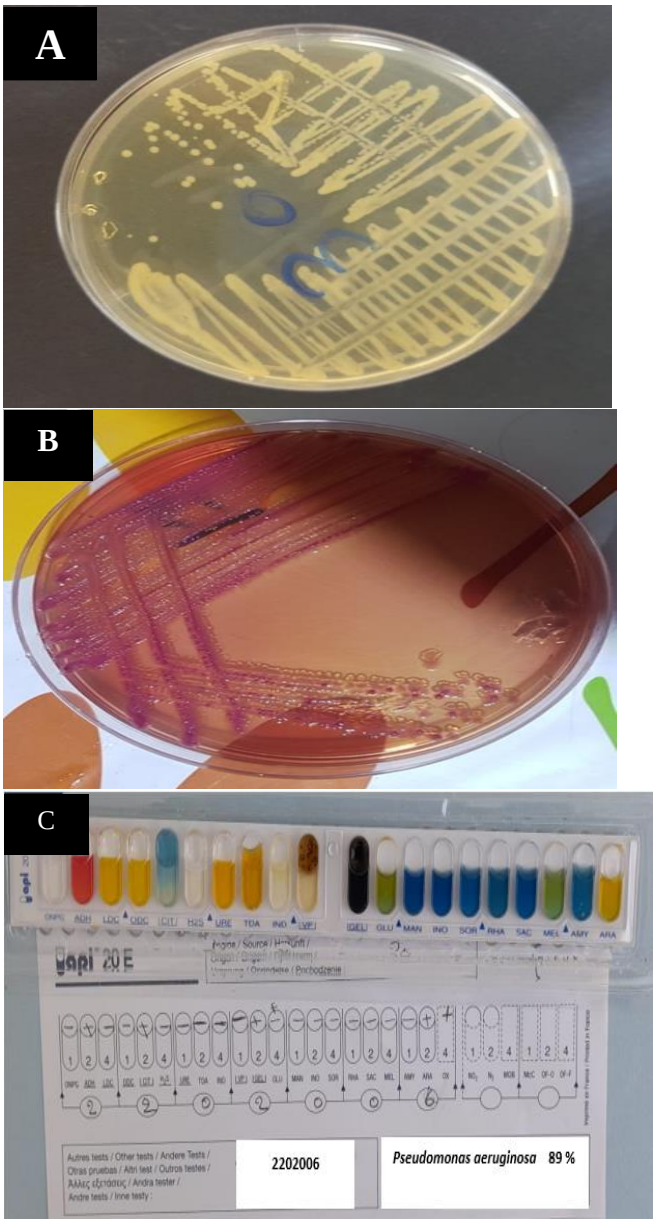

Figure (1): Nutrient agar (A), fermentation on MacConkey agar (B) and API 20 E test (C)

B. Bacterial Storage:

The bacterial isolates were preserved in oblique brain heart infusion agar at a temperature of 4 degrees Celsius, and they were reactivated every month during the study by cultivating them in new culture media to preserve them for long periods.

C. Statistical Analysis

Data were analyzed using by Excel-software for windows 2010. Descriptive statistics were applied to analyze all data and to calculate Chi. square for assess the significant differences (p-value<0.05) between different parameters used in this study.

III. RESULTS AND DISCUSSION

Out of 60 non-duplicate urine samples from patients with urinary tract infections, 35(58.33%) specimens were positive for the growth of Gram-negative bacteria. The frequency of bacterial pathogens as shown in Figure (2), demonstrate the prevalence of 15 (42.85%) are *E.coli* ,7 (20.00%) *E.clocae* ,5 (14.28%)*K. pneumoniae* , 3 (8.57 %) are for each of *P. mirabilis* and *P. aeroginosa*, and 2 (5.71%) are *Citrobacter spp*.

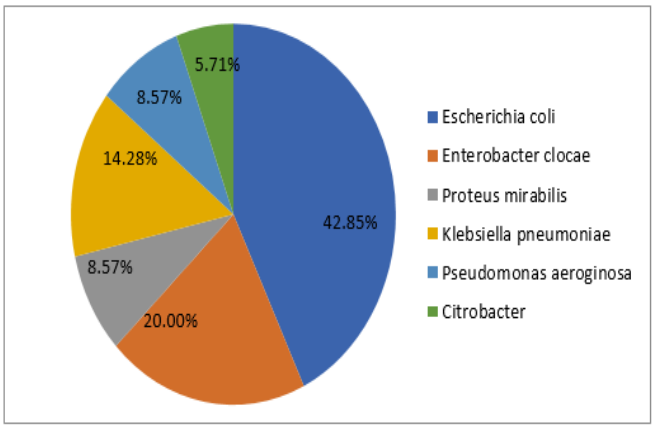

Figure (2) Percentages of bacterial species isolated from UTI infections.

A. A. Identification of Isolated Bacteria according to Age Group

The results showed that the highest percentage of infections were in patients aged 20-35 years18 (51.42%). Other age groups showed infection percentage below 20 years5(14.28%),36-45 years5(14.28%) and Above above45 years7(20.0%). The result also indicated a statistically significant difference between patients infected with isolated bacteria according to age groups and type of infectious bacteria at P. value <0.05.

Table (1): Identification of bacteria according to age group

Age Group / Bacteria	Under 20 decades		20-35 decades		36-45 decades		Over 45 decades		Total	
	No.	%	No.	%	No	%	No	%	No.	%
E. coli	1	6.66	10	66.66	2	13.33	2	13.33	15	42.85
Enterobacter	1	14.28	5	71.42	0	0.0	1	14.28	7	20.0
Klebsiella	2	40.0	2	40.0	0	0.0	1	20.0	5	14.28
Proteus	0	0.0	0	0.0	2	6.66	1	33.33	3	8.57
Pseudomonas	1	33.33	0	0.0	0	0.0	2	6.66	3	8.57
Citrobacter	0	0.0	1	50.0	1	50.0	0	0.0	2	5.71
Total	5	14.28	18	51.42	5	14.28	7	20.0	35	100
$CalX^2 = 22.916$					DF = 15		P. value = < 0.005			

B. Identification of Isolated Bacteria According to Marital Status.

The results of the current study recorded that the Married was a higher rate of infection with isolated bacteria 19(54.28%), while the Singles recorded an infection percentage of 16 (45.71%). The result indicates that there is a non-statistically significant difference between patients (single and married) infected with isolated bacteria at (P< 0.05).

Table (2): Identification of bacteria according to marital status

Marital status / Bacteria	Single		Married		Total	
	No.	%	No.	%	No.	%
E. coli	6	40.0	9	60.0	15	42.85
Enterobacter	2	28.57	5	71.42	7	20.0
Klebsiella	3	60.0	2	40.0	5	14.28
Proteus	3	100.0	0	0.0	3	8.57
Pseudomonas	2	66.66	1	33.33	3	8.57
Citrobacter	0	0.0	2	100.0	2	5.71
Total	16	45.71	19	54.28	35	100
$CalX^2 = 7.215$			DF = 5		P. value = 0.205	

B. C. Antibiotic Susceptibility Pattern

An antibiotic sensitivity test was performed for all of the 35 isolates, including ciprofloxacin, Norfloxacin, ampicillin, levofloxacin, amikacin and azithromycin, to determine the resistance of the bacterial species isolated from UTI. Gram negative isolates showed the highest resistance against ampicillin (91.42%), amikacin (85.7%), levofloxacin (65.71%), and Norfloxacin (45.71%) as shown in Figure (3) and Table (3).

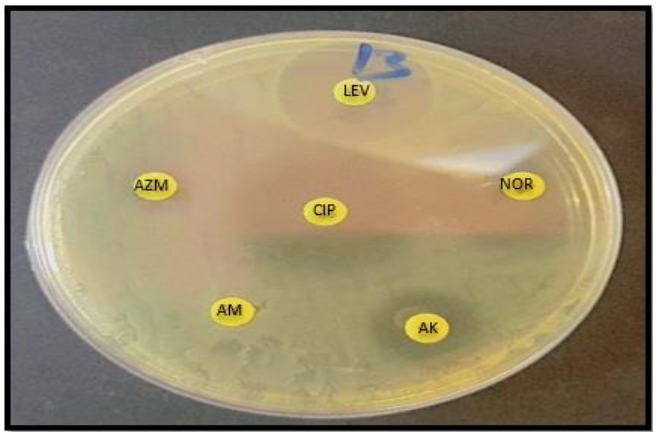

Figure (3): Sensitivity of the isolates to commonly used antibiotics.

Table (3): Antibiotic susceptibility pattern of different Gram-negative bacilli

Type of antibiotics	S		I		R	
	%	No	%	No	%	No
Azithromycin	13	37.14	11	31.42	11	31.42
Norfloxacin	9	25.71	10	28.57	16	45.71
Levofloxacin	6	17.14	5	14.28	23	65.71
Ciprofloxacin	11	31.42	10	28.57	13	37.14
Amikacin	5	14.28	0	0.0	30	85.71
Ampicillin	2	5.71	1	2.85	32	91.42
$CalX^2 = 48.806$			Df= 10		P. value < 0.001	

The results of the current study with regard to the distribution of patients suffering from UTI infections according to age groups showed that the age group 20-35 years is the highest rate of infection reaching (51.42%), this result corresponded with a study done by Mohammed et al., [14], This result may be due to several reasons, including what is connected to the biological changes that occur in the human body, such as hormonal problems, increased activity of the sebaceous glands [15]. Also, this study showed that the marital status, where the married had the highest infection rate, reached 19(54.28%). Meanwhile, the singles recorded an infection percentage 16 (45.71%). This result may be due to female anatomy, sexual relations, and family history. Sexual activity is the cause of between 75 and 90 percent of bladder infections, with the frequency of sexual activity being correlated with the chance of infection. Bacterial vaginosis can occur at any age. But it is more common in fertile women. The changes that occur in hormones during this period create a fertile environment for the growth of certain types of bacteria [16].

A major medical and health problem in hospitals as well as in society is antimicrobial resistance [17]. Thus, antibiotic susceptibility patterns will be useful for selecting the appropriate antibiotic and hence reducing the length of hospitalization, morbidity, and mortality as well.

In the present study, antibiotics were divided into three groups a group that acts on DNA inhibits (levofloxacin, Norfloxacin , and Ciprofloxacin), inhibits protein (Amikacin and Azithromycin), and inhibits bacterial cell wall synthesis (Ampicillin) in order to compare the three groups with each other and conclude which of them has the most effectiveness and performance in eliminating bacteria, and the results appeared as follows: Gram-negative bacteria is 91.42 %resistance to Ampicillin this result corresponded with a study done by Sharma et al., [18] , 85.7%resistance to Amikacin, this result disagrees with a study done by Mohammed et al. [14], with 65.71%resistance to levofloxacin, Immethodical and unprincipled antibiotic treatment, and self-treatment by strong antibiotics cause such high levels of resistance among bacteria isolated from various infections, particularly UTIs. Nowadays, numerous organizations and programs are working to fight against antibiotic resistance [19]. while 45.71% resistance to Norfloxacin, 31.42% resistance to Azithromycin , and 13.37%resistance to Ciprofloxacin this result corresponded with a study done by Khan et al., [20].

The above results showed that the effect of the antibiotic on the cell wall was very weak compared to the action of the antibiotic on the DNA. Note that there was a difference in the types of antibiotics used to inhibit the protein, as amikacin showed high resistance, while azithromycin was effective against bacterial species. As for the types used against DNA, they have shown great effectiveness in eliminating pathogenic bacteria that cause urinary tract infections, and thus, the effective role of this group has been distinguished.

The cause of antibiotic resistance in bacteria is when bacterial species acquire the ability to tolerate the action of an antibiotic. This process is sometimes referred to as acquired resistance, can happen due to horizontal genetic transfer of foreign DNA coding for antibiotic resistance elements or bacterial chromosome mutation. Horizontal gene transfer is especially troublesome because it Largely contributes to the spread of elements of antibiotic resistance within and even across bacterial organisms. Conversely, though, specific types of bacterial can be naturally resistant to certain antibiotics [21]. The ability of these bacterial species to tolerate the action of antibiotics is due to their inherent structural or functional properties. These properties are encoded in the bacterial genome and ensure that bacteria are intrinsically resistant to some antibiotics [22].

IV. CONCLUSIONS

Identifying the harmful bacterial causes of urinary tract infections is essential. Therefore, this study clarified the Gram-negative bacterial isolates affecting the urinary tract, and determined their resistance characteristics to antibiotics. It is necessary to shed light on this problem and know the extent to which bacterial species have developed resistance to most antibiotics and the extent of their impact on the urinary tract.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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