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RESEARCH ARTICLE

Role of Few Gram Positive and Gram Negative Bacteria Isolated from UTI Patients

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ABSTRACT

This study was conducted from 10/1/2021 to 25/4/2021 at Al-Husseini Teaching Hospital. This study aimed to know the role of gram-negative and gram-positive bacteria in urinary tract infections and the sensitivity and resistance of these bacteria to antibiotics. During this period (60) specimens were collected for different age groups between (more than 60) years and for both sexes. The specimens were cultured on different culture media in regular conditions and diagnosed laboratory. Bacterial cultures were positive in 83.3%, while 16.6% of patients revealed negative in culture.

Results of the study showed 29 (58%) isolates of G+ve bacteria were obtained, which included *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus agalactiae*, while 21 (42%) isolates of G-ve bacteria were obtained, which is included *E. coli*, *Pseudomonas aeruginosae,* and *Proteus mirabilis*.

The results of the bacterial isolation showed a predominance of *S. aureus* bacteria by 36%, followed by *E. coli* by 32%, followed by *E. faecalis* by 20%, *P. aeruginosa* by 6%, *P. mirabilis* by 4%, and finally, *S. agalactiae* by 2%.

The study showed that the rate of infection of females is more compared to males, where the percentage of females was 56%, while males were 44%, and the highest percentage of infection was in the age group (1–35) years; where the infection rate among younger age groups was higher than with the older groups.

Sensitivity tests were conducted for the most important bacterial isolates that cause the disease towards some antibiotics. In this study G+ve bacteria, including *S. aureus* showed high rate of sensitivity to amikacin (100%), levofloxacin (77.8%), gentamycin (38.9%), and cefixime (5.6%). *E. faecalis* was sensitive to levofloxacin (30%), amikacin (50%), cefotaxime (10%) and gentamycin (40%). *S. agalactiae* showed sensitivity to levofloxacin, amikacin and gentamycin (100%). In comparison, G-ve bacteria, including *E. coli* showed high rate of sensitivity for azithromycin (75%), cefotaxime (62.5%), amikacin (37.5%), levofloxacin with percentage (25%), ceftriaxone (6.25%) and gentamycin (18.75%). *P. aeruginosa* showed a high sensitivity rate to azithromycin (100%), and levofloxacin (33.3%). While *P. mirabilis* showed a high sensitivity rate to azithromycin (100%), amikacin and cefotaxime (50%).

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INTRODUCTION

Infections of the urethra, bladder, ureters, or kidneys, which comprise the urinary tract, are called urinary tract infections. *E. coli* bacteria bring on the majority of UTIs, although numerous other bacteria, fungi, and parasites can also result in UTIs.[1]

Due to their architecture, females are more likely to develop UTIs than most males; other risk factors for UTIs include any condition that could obstruct urine flow, such as an enlarged prostate, congenital anomalies of the urinary system, and inflammation.[2]

UTIs are more likely to occur in people who use catheters, have urinary surgery, have enlarged prostates in men, or have any of these conditions.[3] In addition to age, sex, and the part of the infected urinary tract, there are certain differences in the symptoms and signs of UTI that rely on the infecting agent.[4] Urinary tract infections are often diagnosed by isolating and identifying the urine pathogen from the patient. Urinary tract infections can have a wide range of complications, including dehydration, sepsis, kidney failure, and even death. Most individuals with a UTI have a favorable prognosis if they receive prompt and effective treatment.[5]

A bacterial infection of the urinary tract is known as a UTI. An infection happens when bacteria from the rectal area enter the urinary tract through the urethra and bladder and grow in the urine.[6] Bacteria frequently enter the body through the urethra initially. Infections can develop as germs grow. Urethritis refers to an infection that only affects the urethra. Cystitis is a bladder infection that develops when bacteria travel to the bladder and multiply.[7,8] Pyelonephritis, a kidney infection that can spread up the ureters if the illness is not treated quickly, may then develop.[9]

The kidneys, ureters, bladder, and urethra make up the urinary tract.[10] A urinary tract infection (UTI) is an infection brought on by pathogenic organisms in any of the urinary tract's constituent parts, such as bacteria, fungus, or parasites. This is a general definition of urinary tract infections, though; many authors prefer to use more precise terms that isolate the infection to the main structural segment involved, such as urethritis (urethral infection), cystitis (bladder infection), ureter infection, and pyelonephritis (kidney infection).[1]

Dysuria (pain and/or burning when peeing), organ damage, and even death are among the potential consequences of UTIs. The active organs that generate 1.5 quarts of urine every day are the kidneys. They assist in the clearance of waste products (urea), maintain the balance of electrolytes and fluids (such as potassium, sodium, and water), and create a hormone that promotes the production of red blood cells. These essential activities may be lost or compromised if an infection damages or destroys the kidneys.[2,3]

One of the most frequent nosocomial illnesses is urinary tract infections.[11] Numerous gram-positive and gram-negative bacteria commonly cause UTIs. *Staphylococcus* sp., *Streptococcus* sp., and *Enterococcus* sp. are examples of gram-positive bacteria. Numerous aerobic bacteria, including *Escherichia* sp., *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp., *Proteus* sp., *Serratia* sp., *Salmonella* sp., and *Pseudomonas* sp., are classified as gram-negative.[12] Among these, *E. coli* is responsible for 80 to 90% of UTIs in ambulatory patients and nosocomial infections, with the most common isolates being *K. pneumoniae, P. mirabilis, S. aureus*, and *E. faecalis*.

A complicated interaction between an organism, its environment, and a prospective host occurs during the development of UTIs. Age and location affect a person›s symptoms of urinary tract infection.[13] Urinary tract infections, both acute and chronic, can cause high blood pressure, renal damage, and even death. Acute and chronic pyelonephritis (a disease process brought on by infection of the kidney's parenchyma and pelvis), cystitis, renal carbuncles, urethritis, and prostatitis are chronic symptoms of UTIs.

MATERIALS AND METHODS

Study groups: Urine samples were taken from patients who were hospitalized to Al-Husseini Teaching Hospital in Kerbala city between 10/1/2021 and 25/4/2021 and who had signs and symptoms of UTI, such as fever and vomiting, and who came from various geographic locations of Kerbala city.They were made up of (60) urine samples taken from patients who had (60) UTIs.

Collection of samples: A total of (60) samples were taken from people with UTIs in all different age categories, ranging from (1 to over 60). To ensure that the samples were collected in dry, clean containers, information about each patient's name, age, and gender was recorded.

Culture the samples

The streaking method was used to inoculate the culture media, nutritional agar, blood agar, and McConkey agar, and these media were then incubated at 37°C for 18 to 24 hours. The physical properties of the colonies were first used to diagnose them.

Antimicrobial sensitivity testing (Kirby-Bauer method)

The most used method for evaluating an antibiotic's susceptibility is the Kirby-Bauer test, often known as the disk-diffusion method. It helps doctors choose the best antibiotic to use while treating an infection. The success of this procedure depends on the measurement of bacterial growth inhibition under controlled circumstances. For this test, Mueller-Hinton agar is used as the culture medium. It is evenly and aseptically injected with the test organism before filter paper discs that have been impregnated with a particular antibiotic are placed on the medium. The bacteria will continue to multiply on the agar plate as the antibiotic "works" to prevent it. No growth will occur around the antibiotic-containing disc if the organism is susceptible to it, according to the zone of inhibition.

RESULTS

Bacterial growth of urine sample: According to the doctor's diagnosis, 60 specimens were thought to be UTI patients;

of them, 50 (83.3%) had positive urine cultures confirming bacterial illness, whereas 10 (16.6%) had negative urine cultures confirming bacterial infection as summarized in Table 1.

Types of isolated bacteria among the study group

In the current investigation, 50 gram-positive and negative bacteria isolates were found in urine samples from the study groups, 29 (58%) of which were gram-positive bacteria and 21 (42%) of which were gram-negative bacteria as summarized in Table 2.

Distribution of isolated bacteria among study groups

S. aureus had the highest prevalence of a particular type of bacteria among all UTI patients (36%) while *E. coli, E. faecalis, P. aeruginosae, P. mirabilis,* and *S. agalactiae* had the lowest percentages (32, 20, 6, 4, and 2%), respectively as summarized in Table 3.

Distribution of positive UTI patients according to age groups and gender

The patients in the current study ranged in age from 1 to over 60. According to Table 4, women are more likely than men to get a urinary tract infection, with women being infected at a rate of 56% compared to men's rate of 44%. According to the study, the age group from 1 to 35 years old has the highest infection rate compared to other age groups. In this age group, women comprised 12 samples, or 42.9% of the total, while men comprised 11 samples or 50%. Men had 6 samples and a percentage of 27.3 for those between the ages of 40 and 60, while women had 10 samples and a rate of 35.7. Men made up 5 of the samples among those over 60, with a rate of 22.7%, while women made up 6 samples at 21.4%. Studies by and our results from this investigation were in agreement.

Antibiotics sensitivity for G+ve and G-ve bacteria isolated from UTI patients

The results of antibiotics sensitivity for G+ve and G-ve bacteria isolate from UTI patients are depicted in Table 5.

Table 1: Bacterial growth of urine samples

Result of urine culture	*Patients with UTI*	
	No.	*%*
Bacterial infection with positive bacterial culture	50	83.3
Bacterial infection with negative bacterial culture	10	16.6
Total	60	100

Table 2: Types of isolated bacteria among study groups

Results of urine culture	*Patients with UTI*	
	No.	*%*
Gram Positive Bacteria	29	58
Gram Negative Bacteria	21	42
Total	50	100

Table 3: Distribution of isolated bacteria among study groups

Isolated Bacteria	*Positive UTI cases*	
	Patients with UTI	
	No.	*%*
S. aureus	18	36
E. coli	16	32
E. faecalis	10	20
P. aeruginosae	3	6
P. mirabilis	2	4
S. agalactiae	1	2
Total	50	100

Table 4: Distribution of UTI patients according to age group and gender

Age groups	*Male*		*Female*		
	Positive NO.	*%*	*Positive NO.*	*%*	
1-35	11	50	12	42.9	
40-60	6	27.3	10	35.7	
Over 60	5	22.7	6	21.4	
Total	22	44%	100	28	56% 100

Distribution of antibiotics sensitivity of gram-positive bacteria isolated from UTI patients

In this study *S. aureus* showed high rate of sensitivity to amikacin (100%), levofloxacin (77.8%), gentamycin (38.9%), and cefixime (5.6%). *E. faecalis* was sensitive to levofloxacin (30%), amikacin (50%), Cefotaxime (10%) and gentamycin (40%). *S. agalactiae* showed sensitivity to levofloxacin, amikacin and gentamycin (100%).[60] Our results were in agreement with the studies.

Distribution of antibiotics sensitivity of gram-negative bacteria isolated from UTI patients

Escherichia coli showed high rate of sensitivity for Azithromycin (75%), cefotaxime (62.5%)**,** Amikacin (37.5%), levofloxacin with percentage (25%), ceftriaxone (6.25%) and gentamycin (18.75%). *P. aeroginosae* showed high sensitivity rate to azithromycin (100%), and levofloxacin (33.3%). While *P. mirabilis* was showed high sensitivity rate to azithromycin (100%), amikacin and cefotaxime (50%). Our results obtained in this study agreed with studies as shown in Table 6, the antibiotic amikacin could inhibit all types of bacteria except for *Pseudomonas*, and thus it is the best antibiotic used.

In Table 5, show the sensitivity of the bacterial species that appeared to a group of antibiotics amoxicillin, levofloxacin, azithromycin, cefixime, amikacin, ceftriaxone, cefotaxime, and gentamycin.

Biochemical tests for G+ve and G-ve bacteria isolated from UTI patients

The results of biochemical tests for G+ve and G-ve bacteria isolated from UTI patients are depicted in Table 7.

Table 5: The sensitivity of G+ ve and G- ve bacterial species isolated from UTI patients to different antibiotics

Types of bacteria		*amoxicillin*		*Levofloxacin*		*Azithromycin*		*Cefixime*	
	NO. Isolated	*NO.*	*%*	*NO.*	*%*	*NO.*	*%*	*NO.*	*%*
E. coli	16	_	_	4	25	12	75	_	_
E. faecalis	10	_	_	3	30	_		_	
P. mirabilis	2	_	_	_	_	2	100	_	_
S. aureus	18	_	_	14	77.8	_	_	1	5.6
P. aeruginosae	3	_	_	1	33.3	3	100	_	_
S. agalactiae	1	_	_	1	100	_	_	_	_

Table 6: The sensitivity of G+ ve and G- ve bacterial species isolated from UTI patients to different antibiotics

Types of bacteria		*Amikacin*		*Ceftriaxone*		*Cefotaxim*		*Gentamycin*	
	NO. Isolated	*NO.*	*%*	*NO.*	*%*	*NO.*	*%*	*NO.*	*%*
E. coli	16	6	37.5	1	6.25	10	62.5	3	18.75
E. faecalis	10	5	50	_	_	1	10	4	40
P. mirabilis	2	1	50	_	_	1	50	_	_
S. aureus	18	18	100	_	_	_	_	7	38.9
P. aeruginosae	3	_	_		_	_	_	_	_
S. agalactiae	1	1	100	_	_	_	_	1	100

Table 7: Biochemical tests for G+ve and G-ve bacteria isolated from UTI patients

Type of bacteria	*Biochemical tests*						
	Gram stain	*Catalase*	*Coagulase*	*Indole*	*Motility*	*Oxidase*	*Hemolysis*
E. coli	-ve	+ve	-ve	+ve	+ve	-ve	Beta, Alpha
Enterococcus faecalis	+ve	-ve	-ve	-ve	-ve	-ve	Beta
P. mirabilis	-ve	+ve	-ve	-ve	+ve	-ve	Alpha, Beta
S. aureus	+ve	+ve	+ve	-ve	-ve	-ve	Beta
P. aeruginosa	-ve	+ve	-ve	-ve	+ve	+ve	Beta
S. agalactiae	+ve	-ve	-ve	-ve	-ve	+ve	Beta

Biochemical tests of G+ve bacteria

S. aureus showed positive results with gram stain, coagulase, catalase, and β-hemolysis. It showed negative results with oxidase and indole test and motility. However, *E. faecalis* show gram-positive stain and negative for catalase, coagulase indole, motility and oxidase. The hemolysis is beta *S.agalactiae* shows a positive gram stain and positive for oxidase then negative for catalase, coagulase, indole, and motility, the hemolysis is beta.

Biochemical tests of G-ve bacteria

E. coli bacteria showed that it was negative for gram staining and negative for coagulase and oxidase test, while it was positive for catalase and indole and it was analyzed on the plate by two types alpha and beta.

P. aeruginosa show negative gram stain and negative for coagulase and indole but positive catalase, motility, and oxidase results. The hemolysis is beta. *P. mirabilis* show a gram-negative stain and negative for coagulase, indole and oxidase, and show positive for catalase motility and the hemolysis is alpha and beta. Our results obtained in this study were in agreement with the studies.

In Table 7, shows a set of biochemical tests through which the types of isolated bacteria were diagnosed, including gram stain, catalase, coagulase, indol, mortality, oxidase and hemolysis.

DISCUSSION

One of the most prevalent infections in the world is urinary tract infection, which affects people of all ages and is caused in 95% of cases by different types of bacteria. The current search results are displayed in Table 1; they show that 50 (83.3%) of the samples produced a positive bacterial culture growth, while 10 (16.6%) of the samples produced a negative bacterial culture growth. More samples included gram-positive bacteria (58%) than gram-negative bacteria (42%) as shown in Table 2 showing through the diagnostic results, which included tests of species bacterial positive and negative gram stain as shown in Table 3 in the case of infection of urinary tract infection, *S. aureus* accounted for the highest percentage was isolated by (36%) in the case of infection of the urinary tract infection, showing through the diagnostic results, which included tests species bacterial positive and negative gram stain as shown

in Table 3 that the result obtained by came the approval of the findings of a number of studies that have pointed to the sovereignty of *S. aureus* in the cause of infections at the urinary tract, including the study. However, our findings differed from those of who found that *E. coli* accounted for the highest percentage of isolates (35%), and who suggested that the reason for the high prevalence of this bacteria was that it was a natural infection that was most frequently endogenous and present in the human gut.

Results of this study showed a predominance of *S. aureus* bacteria by (36%), followed by *E. coli* by (32%) come in second place, followed by *E. faecalis* by (20%) third place, *P. aeruginosa* by (6%), *P. mirabilis* by (4%), and finally *S. agalactiae* by (2%).

From Table 4, it is clear that UTI was more common first age group (1–35 years) since 12 specimens of female with a percentage 42.9% and 11 specimens for males with percentage 50% contracting the infection annually and 60% of them at some point in their lives. Recurrence is common, with about half of people developing a second infection within one year. Urinary tract infections occur often more in females 56% than in males 44%, our results were agreement with study.

Pyelonephritis occurs 20-30 times less frequently. It is the most common cause of hospital-acquired infections, accounting for about 40%.

Age-related increases in the prevalence of asymptomatic bacteria in urine range from two to seven percent in women of childbearing age to up to 50 in elderly women residing in nursing homes. Our findings were in agreement with those of on the prevalence of asymptomatic bacteria in the urine of men and women over the age of 60 (22.7 and 21.4%, respectively). In Table 4 shows The prevalence of urinary tract infections was higher in women (56%) than in men (44) and was attributed to anatomical differences between the sexes, specifically the shorter length of the urethra in women's bladders, which makes them more susceptible to urinary tract infections.The antibiotic is crucial in the treatment of urinary tract infections, and through sensitivity tests to antibiotics for the purpose of identifying the extent of bacterial sensitivity under study observed that negative and gram-positive bacteria to showed variation in the extent of sensitivity also indicates that in Table 5 as illustrated by this table that the highest rate of sensitivity shown by the gram positive bacteria reached (100%) direction amikacin and gentamicin. While gram negative bacteria had a greater incidence of susceptibility (100%) to azithromycin and cefotaxime, these data indicate that using these antibiotics to treat urinary tract inflammation in cases of infection with such bacteria is effective. On the other hand, can the lack of exposure to the two types of antibiotics lead to low resistance to these bacteria.

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