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ESCHERICHIA COLI BACTERIA AND THEIR RESISTANCE PATTERN TO ANTIBIOTICS IN THE MATERNITY AND CHILDREN'S HOSPITAL/BABYLON

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Abstract: This study focused on collecting urine samples from the midstream of urinary tract from patients with urinary tract infection (UTI). It was clear that E. coli isolates dominated the rest of the isolates due to their importance in causing urinary tract infection in women and men. To achieve this goal, 100 midstream urine samples were collected from patients suffering from urinary tract infection in Maternity and Children's Hospital/Babylon from men and women for the period from 11/11/2018 to 1/11/2019. These samples were cultured on eosin medium and solid MacConkey medium to confirm the diagnosis. The disk diffusion method was used to test the sensitivity of the isolates of this bacterium to a number of antibiotics (18 antibiotics) used as treatments. The most important results are summarized as follows: 1. Isolation and diagnosis of 72 isolates carrying the characteristics of E. coli bacteria, with infection predominance in women (60 cases) more than men (19 cases). All E. coli isolates are resistant to antibiotics that affect the cell wall

Keywords: -

Introduction

Bacterial urinary tract infections are common among all individuals and ages, and recent studies have shown the risk of these infections developing into more severe infections, in addition

to the high cost incurred by hospitals or patients. Urinary tract infections are common diseases that affect people of all ages, and are the second most common disease and one of the common sources of bacterial bloodstream infections caused by Gram-negative bacteria, especially in hospitalized patients. Therefore, the current study aims to study their resistance to antibiotics used in the treatment of urinary tract infections.

Classification of urinary tract infections

The various types of urinary tract infections are divided according to the location of infection into upper infections, which include infections that affect the kidneys, and lower infections, which include infections that affect the urinary bladder (cystitis), the urethra (urethritis), and the prostate in males (prostatitis). 1-3: Causes that contribute to the occurrence of urinary tract infections

It may happen that a person gets a urinary tract infection without showing clear symptoms, and there are causes that contribute to its occurrence: -

1. The presence of congenital defects in the urinary tract, including the congenital defect between the bladder and the ureter, which is due to the return of urine to varying degrees to the ureter and then to the kidneys in advanced cases, which causes recurrent urinary tract infections, and may also lead to kidney failure if not treated early.
2. Obstruction of the urinary tract, which leads to recurrent urinary tract infections, which may be followed by kidney atrophy, and then kidney failure. The causes of obstruction are due to the formation of kidney stones, or to the presence of congenital defects in the urinary tract.
3. Pressure of the uterus on the ureter during pregnancy, which may lead to stagnation of urine, and then inflammation, especially bacterial kidney inflammation.
4. Sexual contact with a person infected with the bacteria that causes the inflammation.
5. Diabetes makes patients susceptible to urinary tract infections, whether bacterial or fungal.

Symptoms of Urinary Tract Infection:

Bacterial urinary tract infections have symptoms that indicate them, which are: -

1. Pain in the upper part of the urinary tract, when there is a kidney infection.
2. Burning sensation when urinating, and a feeling of wanting to urinate, but the patient finds it difficult, as the urine comes out in the form of drops.
3. The appearance of blood in the urine, which may be microscopic, or clear to the patient with the naked eye.
4. Pus coming out of the urinary opening.

A person may also be infected with the infection, but there are no symptoms that indicate it, and this condition is called asymptomatic bacterial infection of the urine.

The relationship between urinary tract infection and some other diseases

Regarding the relationship between urinary tract infection and diabetes, it was found that the highest prevalence of urinary tract infection is recorded in patients with diabetes. It was also found that the contributing factor to the predisposition of people with diabetes to urinary tract infection is a dysfunction in the phagocytic cells with a dysfunction in the bladder, which leads to difficulty in emptying, which makes it difficult to sweep bacterial cells out of the body and purify the bladder from them, in addition to the prevalence of urinary tract infection in women with diabetes more than in men.

The relationship between the number of bacterial cells and urinary tract infection is usually associated with huge numbers of pathogenic bacterial cells, which are found in the urine, and many studies in this field indicate that the occurrence of urinary tract infection is associated when

the number of bacterial cells reaches 105 colony-forming units or more/ml of urine. The presence of purulent urine disease with asymptomatic bacterial urine disease in elderly women occurs as a result of the ability of bacterial cells to invade or cause tissue injury in the urinary tract. The results of microscopic examination of urine also showed the appearance of white blood cells associated with cases of urinary tract infection. White blood cells associated with cases of urinary tract infection.

The most important types of pathogens causing urinary tract infections
Proteus mirabilis,
Enterobacter ssp.,
Klebsiella,
Escherichia coli,
Staphylococcus saprophyticus,
Streptococcus faecalis,
Pseudomonas aeruginosa,
Providencia ssp.,
Alcaligenes ssp.,
Enterococci,
Candida albicans,
Vaginalis haemophiles

The causes of urinary tract infections are different types of Gram-negative and Gram-positive microorganisms. More than 90% of urinary tract infections are caused by one type of microorganism, and most urinary tract infections are caused by E. coli bacteria, which causes more than 80% of acute infections, especially cystitis in females. Other types of negative bacteria that cause this disease are Pseudomonas, Klebsiella, Enterobacter, and Serratia, which have an effect on recurrent infections. Especially in females, it was found that pregnant women are more susceptible to urinary tract infections, and the incidence curve begins from the 6th week to the 24th week of pregnancy, due to the increase in bladder size with less ureteral tension, in addition to the fact that the increase in plasma volume from pregnancy reduces the concentration of urine. It was found that more than 70% of pregnant women suffer from glycosuria, which encourages bacterial growth in urine, and the increase in the hormones Progestins and Estrogens in urine may lead to a decrease in the ability of the lower urinary tract to resist bacterial invasion, which allows the selective growth of some strains of bacteria.

Since E. coli is the most important microorganism responsible for pathogenic urinary tract infections, it is called Uropathogenic E. coli (UPEC).

UPEC bacteria differ from non-pathogenic E. coli, as well as from other pathogenic E. coli species, by producing specific virulence factors, which include two main types of factors: factors present on the surface of the bacterial cell, and factors inside the cell, which in turn are an important source of pathogenicity. In addition, the presence of adhesion factors, toxins, bacterial envelope and capsules, and other bacterial products contribute to the severity of the pathogenicity of bacterial strains.

Among the most important factors present on the cell surface are different types of appendages called fimbriae, which play a role in adhesion to the surface of the host cell, in addition to that they may have other additional functions, including invading the host tissue. Another factor in pathogenicity is the production by E. coli of bacteriocins called colicins, which were previously and are still thought to play an indirect role in bacterial pathogenesis.

Bacterial sensitivity to antibiotics

In the field of studying bacterial sensitivity to antibiotics: -

The mechanism of the antibiotic's effect on the bacterial cell, and then its efficiency

The antibiotics used in the study were divided in terms of the mechanism of the antibiotic's effect on the bacterial cell, as mentioned, into: -

1. A group of antibiotics that interfere with the construction of the cell wall Cell Wall Synthesis. Penicillin interferes with the vital processes of building the wall by affecting the assembly of the components of the cell walls of bacterial cells, thus preventing their association and participation in the cell wall, while cephalosporins inhibit the perpendicular bonds in the peptidoglycan layer that forms the bacterial cell wall.
2. Antibiotics that inhibit protein synthesis. These antibiotics inhibit the 30S ribosomal and 50S ribosomal structural units.
3. Antibiotics that inhibit a metabolic pathway. The antibiotic works on one of the stages of nucleic acid formation, which leads to a fatal effect on bacterial cells.
4. Antibiotics that interfere with the formation of nucleic acids.
5. Antibiotics that cause disruption of bacterial membrane structure. This is a group that disrupts the construction of the cell's phospholipids.

Bacterial resistance to antibiotics:

On the other hand, bacteria may show resistance to antibiotics, which means that the antibiotic is not successful in treating the patient's infection, and bacteria become resistant to antibiotics through several mechanisms, including:

1. Causing a change in the antibiotic, such as breaking down β-lactam in the penicillin molecule by the β-lactamase enzyme, thus making the molecule ineffective. The main way bacteria resist penicillins is by secreting penicillinase or β-lactamase enzymes when exposed to the antibiotic, and most common urinary tract pathogenic bacteria have mechanisms of resistance to antibiotics, or acquire these mechanisms, especially those involving the production of β-lactamase enzymes, through genetic restructuring.
2. Causing a change in the internal bacterial structure, which is any change in the chromosomal genes, known as chromosomal abnormalities, and thus these genes acquire an acquired genetic trait that resists the antibiotic, or by acquiring resistance genes from other microbes, which may qualify the bacterial cells either to produce enzymes that destroy the antimicrobial, or change the permeability systems, which prevents the antibiotic from reaching its target inside the bacterial cell, or produce an alternative metabolic pathway, which spares the bacterial cell the effect of the antibiotic. 3. Inhibiting the receptors to which the antibiotic will bind, such as building a new cell membrane from a different protein that does not have antibiotic receptors. 1-9: Materials and Methods

**Nutrient media

1- Eosin Methylene Blue
2- MacConkey Agar
3- Nutrient Agar

Methods

Sample collection:

Samples were collected from patients who visited the Maternity and Children's Hospital in Babylon after being referred by the specialist doctor for the period from 11/11/2018 to 11/1/2019. A questionnaire was organized to collect information from patients, including (patient name, age, gender).

* Microscopic examination of urine:

Urine samples were taken and placed in test tubes in a centrifuge at a speed of (5000 rpm) for (15) minutes. After that, the filtrate was neglected and the precipitate was taken Deposit. A drop of it was placed on a clean glass slide and the slide cover was placed over it and examined under a microscope at a magnification power of (40x) to detect pus cells.

*Cultivation on media

Urine samples were taken and cultured on suitable culture media, namely Eosin Methylene Blue Agar and MacConkey Agar. All urine samples were cultured on the aforementioned culture media for the purpose of isolating the bacteria causing urinary tract infection, and then they were diagnosed using a number of traditional tests.

*Isolation and identification of bacteria:

The bacteria were identified by culture on Eosin methylene agar medium based on the growth of colonies on MacConkey agar medium based on the ability or inability of the bacteria to ferment lactose.

*Materials needed for resistance testing

- Bacterial cultures aged (24-48) hours
- Petri dishes containing sterile culture medium
- Antibiotic tablets

*Method of work for resistance testing:

1. Using sterile forceps, take one of the tablets containing antibiotics and place it in one of the corners of the dish containing the bacterial culture and press it slightly to obtain good contact between the tablet and the medium
2. We repeat the above with the rest of the tablets, so that the number of tablets is within (4) in each dish.

We incubate the dishes upside down at a temperature of (37) degrees Celsius for (24-48) hours, then we record the observations about the inhibition zones surrounding the discs by measuring the diameter of the zone, estimated in millimetres, and comparing them with the values listed in the attached table and extracting whether the bacteria are resistant, intermediate resistant, or sensitive

Result and Discussion

Bacteria were identified according to the rules followed to identify samples infected with urinary tract infection, and morphological, physiological and biochemical tests were followed, in the microbiology laboratory at the Maternity and Children's Hospital/Babylon.

The relationship between the disease and the presence of E. coli bacteria

Given the dominance of E. coli bacteria in urinary tract infection infections, as it is one of the most isolated pathogens during the study, and is associated with most disease cases, the relationship between these bacteria and the disease was studied from several aspects, namely:

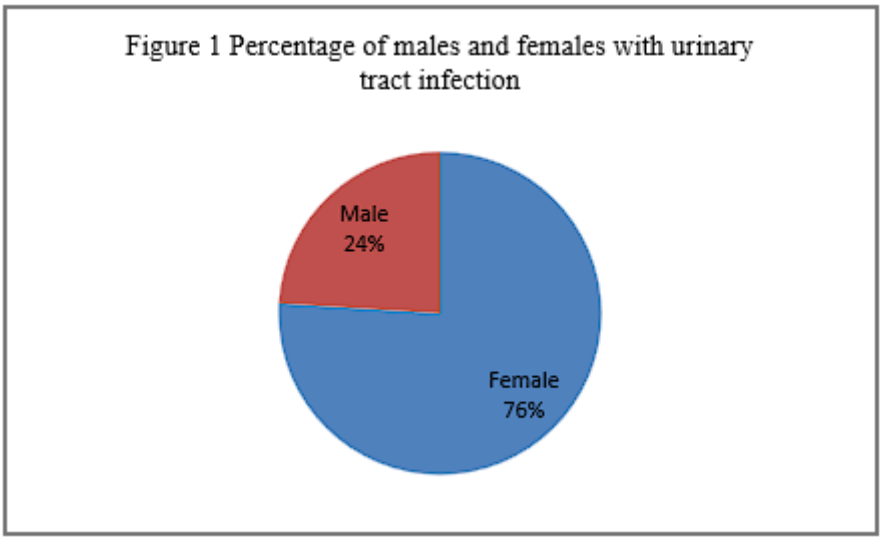
1. Gender

The results of the study show that the study sample was infected with 79 E. coli isolates. The results (Table 1) indicate that females were more infected with E. coli than males, as it was estimated at 60 isolates in females at a rate of 76%, while it was estimated at 19 isolates in males at a rate of 24%, meaning that the infection rate in females was equivalent to 3 times the infection rate in males Figure (1).

This study recorded that the infection rate differed according to gender, as it was doubled in females compared to males. All available research agreed that the pattern of infection with urinary tract infections in females is more frequent than in males. Some research explained the reasons for females being more frequently infected than males to the anatomical structure of the male and female urinary and reproductive systems. The nature of the urethra in males leads to its distance from fecal contaminants in addition to the prostate secretions with antibacterial properties that establish good protection against bacterial invasion.

Table (1) Estimated number of male and female patients with urinary tract infection and percentage of infection

Total		**Sex**			
		Male		**Female**	
%	**No.**	**%**	**No.**	**%**	**No.**
100	**79**	**24**	**19**	**76**	**60**



1. Age groups

When studying the relationship of E. coli bacteria with age groups, samples were taken from 79 disease cases where E. coli bacteria was associated with them. When studying this sample, the results showed the emergence of disease cases (23 cases) in the children category (6-10 years) and they were females. This category had the highest number of infections compared to the rest of the age groups. In the children category (0-5 years), 22 cases appeared out of 31 cases, 8 cases were males compared to 23 cases of females. While 7 cases appeared in the adolescent category (11-15 years), including 2 males and 5 females. The adult category (16-20 years) constituted the age groups in the percentage of infection with E. coli bacteria, as it included 10 cases of females (3.8:1) (Table 2).

In the study of infection according to age groups, the results showed that there is an effect of age groups, and that the most affected groups are children (6-10 years), followed by children (0-5 years), then came the age group (16-20 years). The results of infection rates varied with the current study, but the general trend is consistent with the results of the current study, which showed that the infection rate among women is higher than among males in all age groups. The results also showed an increase in the probability of infection with age.

Table (2) Distribution of male and female patients with urinary tract infection according to age groups

	Sex	**Age**

Total		**Male**		**Female**		**(Year)**
%	No.	%	No.	%	No.	
39.2	**31**	47.4	9	36.67	22	0-5
39.2	**31**	42.1	8	38.33	23	6-10
8.9	**7**	10.5	**2**	8.3	5	11-15
12.7	**10**			16.7	10	16-20
			19		60	Total

E. coli sensitivity to antibiotics:

The study showed that there is a difference in E. coli isolates to antibiotics, between resistance to antibiotics Resistant (R), moderate resistance (M) and sensitive (S) to the effect of antibiotics on them. E. coli isolates were divided into categories according to their compatibility in the effect of different antibiotics on them as follows (Table 3) In the field of studying the sensitivity of bacteria to antibiotics, the study dealt with antibiotics belonging to the Penicillins and Cephalosporins group, which interfere in the construction of the cell wall synthesis and are among the treatment options for urinary tract infections. E. coli isolates showed resistance to cell wall synthesis antibiotics estimated at 49.32% in the current study.

And among the antibiotics whose mechanism of action depends on preventing the construction of the bacterial cell Protein synthesis by inhibiting the 30S and 50S subunits. The current study recorded the resistance of E. coli isolates to Protein synthesis by 25.34%. Whereas E. coli isolates showed little resistance to Gentamicin and Tobramycin.

And from the antibiotics that work to inhibit the metabolic pathway. The current study showed the resistance of E. coli isolates to Trimethoprim by 11.38%.

From the antibiotics belonging to the Quniolones family used in the current study, which are due to their effective action on the synthesis of nucleic acids, it was found that the percentage of E. coli resistance to these antibiotics ranged from 2.05-13.99%. These antibiotics are considered effective for treating patients with urinary tract infections, as its concentration does not reach the effective concentration in the blood, but rather reaches the effective concentration in the urine, and it was found that 40% of it is excreted as it is in the urine. The results of the current study showed that the resistance of E. coli bacteria to Nitrofurantoin was 13.99%.

Table (3) Resistance and sensitivity of E. coli isolates to some antibiotics

Antibiotics							
Antimetabolite Inhibitors		Nucleic Acid Synthesis Inhibitors		Protein Synthesis Inhibitors		Cell wall Synthesis Inhibitors	
No. of Isol. %		No. of Isol. %		No. of Isol. %		No. of Isol. %	
14 (11.38%)	Trimethoprim	3 (2.05%)	Levofloxacin	8 (5.48%)	Azithromycin	15 (10.27%)	Amoxicillin
		20 (13.99 %)	Nitrofurantoin	5 (3.42%)	Doxycycline	6 (4.11%)	Ampicillin
				10 (6.85%)	Gentamicin	3 (2.05%)	Cefotaxime
				3 (2.05%)	Streptomycin	9 (6.16%)	Ceftazidime
				8 (5.48%)	Tetracycline	19 (13.01%)	Ceftriaxone
				3 (2.05%)	Tobramycin	4 (2.74%)	Meropenem
						5 (3.42%)	Oxacillin
						3 (2.05%)	Ticarcillin
						8 (5.48%)	Vancomycin
14 (11.38%)		23 (15.75)		37 (25.34)		72 (49.32)	Total

Recommendations

Negligence in treating urinary tract infections leads to serious consequences, including: the arrival of pathogenic bacteria into the blood, causing bacterial bloodstream poisoning, Bacteremia, as well as the development of the disease from the acute infection stage to chronic infection, followed by the occurrence of renal scars, kidney atrophy, and then ending in renal failure in the case of advanced infections. Based on the above-mentioned seriousness of urinary tract infections, the study recommends the following: -

1. Spreading health awareness to patients, especially with regard to taking a urine sample from the middle of the urinary tract, to avoid incorrect results of the microbial sample analysis.

2. Avoiding the excessive use of broad-spectrum antibiotics, which has led to the emergence of strains of pathogens resistant to many antibiotics.

3. Eliminating the natural microbial flora, which led to colonization by resistant pathogenic microbes, as the natural microbial flora plays a role in protecting the body from diseases by inhibiting the growth of pathogenic microbes through several means, including the death of harmful microbes due to their inability to compete in obtaining nutrients, and their formation of antibiotics and metabolites that are anti-harmful microbes

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