Isolation and Diagnosis of Escherichia *Coli* Causing Urinary Tract Infections from People of Different Ages in Mosul City

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Abstract: urine samples of 125 patients with urinary tract infection and 25 uninfected people as control were collected from Al-Salam Teaching hospital of Mosul city from the period from September 2020 to January 2021. The positive results of UTIs caused by microorganisms was 78 state with the ratio of 74.37%, 25.63% for females and males respectively. The highest ratio of infection was 34.63% for the age range between (15-28) years for both females and males, while the lowest ratio (7.69%) of infection for the age range between (1-14) years for both sex. *Escherichia coli* was formed The highest percentage (37.23%) of gram - negative bacteria that cause urinary tract infections, while the other gram -negative bacteria (*Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Serratia ficaria* and *Shigella* spp.) consist of (10.3, 8.97, 7.7, 1.28 and 1.28)% respectively, while the ratio of Gram-positive bacterial species (*Staphylococcus aureus* and *Staphylococcus epidermidis*) were (17.9 and 11.5)% respectively, finally the ratio of *Candida albicans* was (3.84%).The sensitivity of bacterial isolates to a group of antibiotics showed that all isolates were sensitive to the antibiotic meropenem while the highest percentage of bacterial isolates were resistant to ampicillin antibiotic.

Introduction: Urinary tract infections are one of the most common and widespread diseases around the world, as it represent's a global challenge, as millions of people are diagnosed with urinary tract infections annually. 50% of women worldwide suffer from a UTI in their lifetime **(Naber *et al*., 2008)**. Urinary tract infection affects both sexes and it affects females more than males for several reasons, the most important of which is short urethra in females compared to males, in addition to the hormonal activity of females are much more than males, which affects the acidity of the urine and thus provides an appropriate environment for bacterial growth **(Martelli *et al*., 2000).** The highest incidence of urinary tract infections occurs at the age of sexual activity, which ranges between (15-45) years. Likewise, infection occurs in women of childbearing age more than women of other ages, and 27% of pregnant women are affected in the first six months of pregnancy **(Alos, 2005).** The intestinal family represents more than 90% of the isolated bacteria in cases of urinary tract infections, and the intestinal bacteria are natural bacteria in humans, unlike parasites and other bacterial types, especially Gram-positive bacteria, causing about 10% of urinary tract infections **(weeks, 2015),** The type *Escherichia coli*, a member of the intestinal family, is considered one of the most important bacteria that cause urinary tract infections, which is isolated of 50-60% ratio, followed by gram – negative bacteria such as *Klebsiella pneumonia*, *Enterobacter*, *Citrobacter*, *Proteus merabilis*, *Serratia* spp. and *Pseudomonas aeruginosa*, as well as Gram-positive bacteria such as *Staphylococcus*

aureus, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Enterococcus* **(Singh *et al*., 2014).**

Materials and methods:

Isolation and diagnosis of bacterial isolates from UTI : urine samples of 125 patients were collected for the period of September 2020 to January 2021 from people referred to the consultant of Al Salam Teaching Hospital in the Mosul city. A microscopic examination of these samples were performed and then samples containing purulent cells and bacteria were implanted on differential and diagnostic media and incubated at 37 ° C for a period of 24 hours, Then on the second day the phenotypic characteristics of individual colonies were observed and biochemical tests were performed. The phenotypic particles of the bacteria were verified by staining them with Gram stain and performing some tests, such as movement and growth tests, on differential and diagnostic media such as macConkey agar, blood agar and methylene blue medium, in addition to biochemical tests, the fermentation of sugars, as well as IMViC tests was done. The diagnosis was confirmed using API 20 E strips (figure,1).

Figure (1) Diagnosis of *E*. *coli* bacteria by API 20 E strip

Sensitivity test: The susceptibility test for isolated bacteria to 13 antibiotics was performed using the tablet diffusion method **(Vandepitte *et al*.,2003).** 3-5 colonies of young bacteria growing on the culture medium at 24 hours age were transferred to a sterile tube containing 5 mL of physiological solution and the turbidity of the bacterial suspension was controlled with the turbidity of the McFarland tube. The swab was immersed in the tube of the bacterial suspension and then pressed against the inner wall to remove the excess inoculum and then spread the inoculum onto trays containing Muller-Hunton medium to obtain homogeneous growth. The antibiotic tablets to be tested for bacterial sensitivity were placed by sterile forceps on the surface of the Muller-Hunton medium and the dishes were incubated for 24 hours at a temperature of 37 C. The results were read by measuring the diameters of the inhibition areas around the discs with inhibitory activity by means of a ruler measured in millimeter units, and the results were compared with the standard tables **(CLSI, 2017)** (table,1)**.**

Table (1) Antibiotics for sensitivity testing for *Escherichia coli*

Antibiotics	code	Concentration (mcg)	the manufacture company
Amikacin	AK	10 mcg	
Ampicillin	AMP	10 mcg	
Cefixime	CFM	5 mcg	
Ceftazidime	CAZ	30 mcg	
Ceftriaxone	CRO	30 mcg	
Levofloxacin	LVX	5 mcg	
Meropenem	MEM	10 mcg	Bioanalyse (Turkey)
Nitrofurantoin	NIT	100 mcg	
Piperacillin	PRL	100 mcg	
Tetracycline	TE	30 mcg	
Trimethoprim	TMP	5 mcg	
Ciprofloxacin	CIP	10 mcg	
Gentamicin	CN	10 mcg	

Results and discussion:

urine samples of 125 patients were studied from September 2020 to January 2021. 78 Gram -positive and negative bacteria were isolate. Gram-negative bacteria consist the largest proportion (66.7%) of the bacteria that isolated, while the percentage of Gram-positive bacteria was (33.3%). Most of the negative bacterial isolates were *Escherichia coli* (37.23%), followed by the other Gram-negative bacteria (*Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Serratia ficaria* and *Shigella* spp.) With percentages (10.3, 8.97, 7.7, 1.28 and 1.28)% respectively, while the Gram-positive bacterial species (*Staphylococcus aureus* and *Staphylococcus epidermidis*) at ratio of (17.9 and 11.5)% respectively and *Candida albicans* formed a ratio of (3.84%).

These results was agreed with (**Nihad *et al*., 2017** ; **Elie *et al*., 2020**) in their study conducted in Baghdad and Lebanon respectively, when the percentage of *Escherichia coli* bacteria was (28.5%) and *Klebsiella pneumoniae* (10%), and the results of the current study were similar to the study conducted by (**Martin *et al*., 2019**) in Uganda, where the percentage of *Escherichia coli* was (41.9%), while the percentage of *Klebsiella pneumoniae* (11.6%) and the percentage of *Proteus mirabilis* (6.7%) and the percentage of *Pseudomonas aeruginosa* (7.7%) came close to (**Bitsori *et al*., 2012**) in his study conducted at Heraklion University Hospital in Greece, When he indicated that the percentage of these bacteria that were isolated from urinary tract infections was (6.7%). While the percentage of *Serratia* spp. (1.28%) was close to what was mentioned in the (**Márió & Edit, 2019**) study, as it indicated that the percentage of *Serratia* spp. isolated from urinary tract infections was (3.8%). The percentage of *Shigella* spp. was close to what (**Maria *et al*., 2003**) in her study that she conducted at the Venizillion General Hospital in Greece. While the percentage of Gram positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) was (17.9 and 11.5)% respectively, and this agreed with (**Seifu & Gebissa, 2018**) whom indicated in their study as the ratio of *Staphylococcus epidermidis* was 12.32% and also agred with (**Nosfor *et al*., 2016**) who found that the percentage of *Staphylococcus aureus* (20.7%) in his study in Nigeria

Table(2) Number and percentage of bacteria isolated from people with urinary tract infections

Types of bacteria	The number of isolates	Percentage
Escherichia coli	29	37.23
Klebsiella pneumonia	8	10.3
Proteus mirabilis	7	8.97
Pseudomonas aeruginosa	6	7.7
Serratia ficaria	1	1.28
Shigella spp.	1	1.28
Staphylococcus aureus	14	17.9
Staphylococcus epidermidis	9	11.5
Candida albicans	3	3.84
Total	78	100

The results of the current study showed that the highest incidence of urinary tract infections among Al-Salam Hospital consultation visitors in the Mosul city was within the age group (15-28) years for both sexes, at a rate of (34.63%). While the incidence rate was in the age group (1-14) years, at a rate of (7.69%), which is the lowest rate among the age groups, as shown in Table (3). The results of the current study are in agreement with **(Martin *et al*., 2019)** in his study of people attending the Bushni District Hospital in Uganda, where the highest incidence of urinary tract infections was in the age group (20-29) years, at a rate of (32%) while the lowest incidence of urinary tract infections was in the age group (1-9) years, at a rate of (1.2%) and the current study also agrees with what **Al-Mukhtar (2018)** mentioned in his study that he conducted in the city of Kirkuk, when he indicated that most of the patients with urinary tract infections were from the age group (21-30).

The current study was agreed with the study conducted by **(Santhosh & Sumitha, 2018)** in India, when the highest incidence rate was in the age group (26-35) years, at a rate (20.9%), while the lowest incidence of urinary tract infections was in the age group (5-15) years, at a rate (9.9%) and this is what we had confirmed. The current study showed that the lowest incidence of urinary tract infections was in the age group (1-14) years, at a rate (7.69%). The reason for the increase in the incidence of urinary tract infections is due to the age groups of more than 20 years because of sexual activity increasing, which increases the risk of developing a urinary tract infection **(Martin *et al*. 2019).**

Table (3) The number of males and females with urinary tract infections caused by Escherichia coli and the percentage of infection for both sexes distributed according to age groups

Age groups	Male	Female	percentage
1-14	0	2	6.89
15-28	2	10	41.40
29-42	0	5	17.24
43-56	2	5	24.13
57-70	1	2	10.34
Total	5	24	100

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The rate of infection among females was (82.76%), while the rate of infection in males was (17.24%), as shown in the figure (2) and this was also confirmed by **(Martin *et al*., 2019)** In his study conducted in Uganda, his study reviewed that the proportion of patients with urinary tract infections was (22.0%) in the Bushni Hospital.The study by **(Stefaniuk *et al*., 2016)** In Poland indicated that the incidence of urinary tract infections among females were (72.5%) and (27.5%) for males. Our study is also agreed with **(Ke *et al*., 2018)** of his study conducted in China, when he founnd that the incidence of urinary tract infections among females was 67.3%, which is higher than the males.

Urinary tract infection affects both sexes, but it affects females more than males for several reasons, the most important of which is short urethra in females compared to males in addition to hormonal activity in females, which is much more than males, which affects the acidity of the urine and thus provides an appropriate environment for bacterial growth **(Martelli *et al*., 2000)**, The high prevalence of *E. coli* in the female gender could be due to the close proximity of the anus to the vagina, This high possibility of UTIs in females is due to the inherent virulence of *E. coli* for urinary tract colonization such as its abilities to adhere to the urinary tract and also association with other microorganisms moving from the perineum areas contaminated with fecal microbes to the moist warmth environment of the female genitalia **(Minardi *et al*., 2011).**
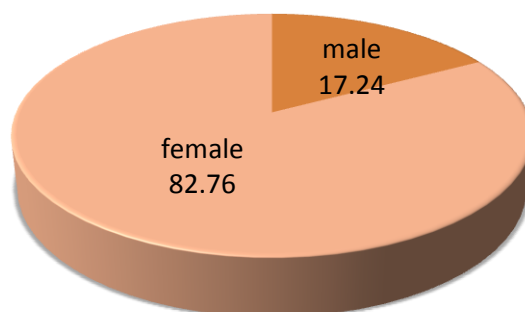

Figure (2) the incidence of urinary tract anemia in diets and females

The present study showed that, the number of pregnant women with urinary tract infections caused by *Escherichia coli* was 15 women with the rate of 62.5% of the 24 women with urinary tract infections caused by this bacteria, and the incidence of urinary tract infections resulting from this bacteria was concentrated among pregnant women of the age groups (15-28) and (29 -42) years old. According to the trimesters of pregnancy, the present study showed that 20% of the infected women were in their first trimester, 20% in their second trimester and 60% in their third trimester (Table,4).

These results are agreed with **(Al-Jawadi, 2012)** how indicated that the highest incidence of urinary tract infections among pregnant women was in the last trimester of pregnancy and was within the age group ranging between 20-35 years, due to the increased risk factors for urinary

tract infections in women. Which includes sexual intercourse and the presence of infections. Urology at a young age, as well as a mother's history of urinary tract infections.

Table (4) Distribution of UTI according to the three trimesters of pregnancy.

Age group	Trimester of pregnancy		
	First	Second	Third
1-14	-	-	-
15-28	2	3	5
29-42	1	-	4
43-56	-	-	-
57-70	-	-	-
percentage	20	20	60

The results of the current study showed that *Escherichia coli* was sensitive to the antibiotics Amikacin (62.07%), Ceftazidime (72.41%), Ceftriaxone (85.7%), Meropenim (100%), Nitrofurantoin (79.31%) and Gentamicin (55.18%), while these bacteria showed resistance against ampicillin (86.2%), cefixime (41.4), levofloxacin (65.51%), piperacillin (72.41%), tetracycline (79.31%), trimethoprim (82.75%) and ciprofloxacin (65.51%), as shown in the table (5) and figure (3).

Table (5) The percentage of sensitivity of *E. coli* to antibiotics

Bacterial Isolates	sensitivity	Percentage of sensitivity to antibiotics												
		AK	AMP	CFM	CAZ	CRO	LVX	MEM	NIT	PRL	TE	TMP	CIP	CN
Escherichia Coli	S	62.07	6.89	51.72	72.41	85.7	24.14	100	79.31	10.34	13.79	6.89	24.14	55.18
	I	_	6.89	6.89	10.34	3.44	10.34	_	13.79	17.24	6.89	10.34	10.34	6.89
	R	37.93	86.22	41.4	17.24	6.89	65.51	_	6.89	72.41	79.31	82.75	65.51	37.93

(AK): Amikacin, (AMP): Ampicillin, (CFM): cefixime, (CAZ): ceftazidime, (CRO): ceftriaxone, (LVX): Levofloxacin, (MEM): Meropenim, (NIT): nitrofurantoin, (PRL): piperacillin, (TE): tetracycline, (TMP): trimethoprim sulfamethoxazol, (CIP): Ciprofloxacin, (CN): Gentamycin.
S: Sensitive, I: Intermediate, R: Resistant.

Figure (3) sensitivity of *E. coli* to antibiotics

The results of the current study were in agreement with **(Elie *et al*.,2020)** how mentioned in his study that *E. coli* bacteria show complete sensitivity to the antibiotic Meropenem (100%), while these isolates showed moderate sensitivity to the antibiotics Amikacin, Ceftazidime, Ceftriaxone, Gentamicin and nitrofurantoin. Our study agreed with **(Santhosh & Sumitha, 2018)** how confirmed in their study in India, they indicated the high resistance of this bacteria to penicillin antibiotic. And this study also agreed with **(Sumera *et al*., 2014)** whom indicated resistance of *E. coli* bacteria to amoxicillin and penicillin. As the bacterial isolates were resistant to a large degree to the antibiotic Ampicillin and this agreed with **(Bitew *et al*., 2017)** who indicated the resistance of bacterial isolates to a large extent to Ampicillin.

Conclusions and recommendations:

Conclusions The results of the study showed the prevalence of urinary tract infections among the age group (15-28) years, and *E. coli* was the most common bacterial species causing urinary tract infections, Likewise, the incidence of urinary tract infections among women was more than males, and the rate of infection among pregnant women in the last trimester of pregnancy was more than the first and second trimesters and the study also showed the complete sensitivity of this bacteria to the antibiotic meropenem and its resistance to the antibiotic Ampicillin.

recommendations: Increasing health awareness among people about avoiding the use of antibiotics without consulting a specialist doctor, as well as taking care of hygiene, especially genital hygiene and Pregnant women must adhere to regular visits to health care units, which greatly helps reduce the incidence of this disease.

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