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ANTIBIOTIC SUSCEPTIBILITY OF ESCHERICHIA COLI ISOLATE FROM URINARY TRACT INFECTION OF THALASSEMIC PATIENTS IN THI-QAR PROVINCE

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ABSTRACT

Escherichia coli (*E. coli*) is the most common type of pathogen that causes Urinary tract infection disease. It can be presented as pathogenic or non-pathogenic strain and found not only in the animal but also in the human intestine. This bacterium can cause opportunistic infection when the human host comprised of thalassemia patients or changes the healthy hemostatic flora. This study aimed to analyze the presence of bacteria in thalassemia patients with urinary tract infection. A total of 303 samples were collected during the period from August 2019 to January 2020 from thalassemia patients who suffered from urinary tract infection. The results showed that there were 6.9% of patients infected with *E. coli*, 2.6% of patients were infected with *S. aureus*, 0.7% with both *Proteus* and *Klebsiella,* while 89.1% of patients had a negative sample for bacteria. Also, the incidence of urinary tract infections in females is higher than in males. Besides, its occurrence in rural areas is higher than in city residents. Moreover, among 16 antibiotics tested to sensitize bacteria to antibiotics, Imipenem showed 100% efficacy on all isolated bacteria. In contrast, Netilmicin showed 80.1% efficacy, Gentamycin 80.1%, and Amikacin 76.2%. Ampicillin, Aztreonam, Amoxicillin-Clavulanic Acid, Tetracycline, and Ticarcillin-Clavulanic Acid, did not show any effectiveness toward the bacteria while other antibiotics showed different activities. Furthermore, the isolated microbes from thalassemia patients were the highest resistance to antibiotics in comparison with other studies, and this antibiotic-resistant may be due to the weakening of the patient's immune status and frequent blood taking and the antibodies it contains.

Keywords: *thalassemia, urinary tract infection, bactéria, Escherichia coli, antibiotics.*

1. INTRODUCTION:

The term thalassemia (derived from the Greek —thalassaǁ, which means —the seaǁ— referring to the Mediterranean—and —emiaǁ, meaning —related to bloodǁ) indicates a heterogeneous group of genetic disorders of hemoglobin synthesis characterized by a disturbance of the production of globin chains, leading to anemia, ineffective erythropoiesis, and destruction of erythroblasts in the bone marrow and of erythrocytes in the peripheral blood (Faust *et al*., 2011) In individuals that produce normal hemoglobin, two types of polypeptide chains (α and non-α) pair with each other at a ratio close to 1/1 to form normal hemoglobin molecules. In thalassaemic patients, an excess of the customarily produced type accumulates in the cell as an unstable product, leading to the destruction of the cell (Javad *et al*., 2011). This imbalance is the hallmark of all forms of thalassemia. Types of thalassemia are usually named after the underproduced chain or chains, in patients with β-thalassemia major, the most important cause of mortality and morbidity is organ failure due to deposits of iron (Ricerca *et al*., 2009) Urinary tract infection (UTIs) is one of the most common diseases caused by different types of pathogens. In thalassemia patients, infection of urinary tract infection occurs as a result of organ failure, including the urinary system, as a result of the accumulation of iron resulting from the breakdown of red blood cells (Vento *et al*., 2006). Women are often more likely to be infected than men for physiological and anatomical reasons for the urinary system (Aldudak *et al*., 2000). The infection at urinary tract infection called by a local infection in top of the urinary system is called pyelonephritis, and the lower part of the body called cystitis, in the two cases can be detected through clinical manifestations and laboratory diagnosis (NoorBahr *et al*., 2016).

E .coli is the most common type of

pathogen that causes UTI disease. These bacteria are usually considered non-pathogenic when they are present in the intestines, and they reach the urinary system and become pathogenic (Marrs *et al*., 2015). E. coli has many virulence factors that are gained by the ability to invade the host, which is related to adhesion, toxin, and some works to protect the bacteria from attacking the immune system (Nielsen *et al*., 2014). Several studies demonstrated E. coli's capability to cause opportunistic infections when the changing habitat or immune system compromised in the host and also by change hemostat through using antibiotics. Commensal *E. coli* strains efficiently exchange genetic material with pathogenic *E. coli* strain or other pathogens such as *Salmonella*, *Shigella*, *Yersinia,* and *Vibrio* (Hussein *et al*., 2018)(Shahdoust *et al*., 2016).

This study aimed to analyze the presence of bacteria in thalassemia patients with urinary tract infection.

2. MATERIALS AND METHODS:

2.1. Sample Collection

After taking approval from Thi-Qar University ethical committee, The research was performed in Nasiriya Center for Hereditary blood disorder, where three hundred and seven patients with thalassemia agreed to perform and publish this research. Their ages ranged from 4 to 30 years. Their choice is based on having a urinary tract infection, as evidenced by symptoms, doctor diagnosis, and urine microscopy examination. Urine samples were collected and tested for antimicrobial susceptibility of E. Coli. Samples of urine were taken from patients whose normal texture consistency was without any signs of disease. E. coli commensal, isolated from a healthy person, does not take any antibiotic for an extended period of about three months. Approximately 5 ml of urine from the middle of the urethra was collected from each patient and healthy individuals in sterile containers and transported directly to the laboratory.

2.2. Microscopic Examination

All specimens were pre-examined by microscopy to identify the presence of pus cell, epithelial cell, red blood cell, throat mucus, bacterial cells, and other substances (Lloyd *et al*., 2009).

2.3. Culture of samples

Selected samples were labeled and cultivated on blood agar and MacConkey agar for urine sample grown on MacConkey agar only incubated overnight at 37°C in an incubator under aerobic conditions. Any *E. coli* suspected from urine sample was submitted to subculture on blood agar and MacConkey agar. All specimens lactose fermenter colonies on MacConkey, because they are fermented lactose sugar, which leads to the formation of lactic acid, and with the presence of a neutral red pigment as an indicator appears the pink color. Depending on morphological features of the colonies and microscopically examination with Gram's stain, the pure cultures were prepared for biochemical tests to distinguish E. coli from other Enterobacteriaceae (Yamamoto, 2007).

3. RESULTS AND DISCUSSION:

3.1. Distribution of *E. coli* among Urinary Tract Infection

A total of 303 urine samples were collected during the period from September 2019 to March 2020 from thalassemia patients who suffered from urinary tract infection. The results showed that there were 6.9% of patients infected with *E. coli*, 2.6% of patients were infected with *S. aureus*, 0.7% with both *Proteus* and *Klebsiella,* while 89.1% of patients had a negative sample for bacteria (Figure 1).

3.2. The Percentage of Infection with *E. coli* Compared to other Isolated Bacteria

A total of 33 infectious bacteria were isolated from thalassemia patients by urine. There were 64% of patients infected with *E. coli*, 36% with other infectious bacteria as *S. aureus*, and 0.7% of patients infected with both *Proteus* and *Klebsiella* (Figure 2).

3.3. Distribution of the Thalassemia patients Infected with *E. coli* According to Age Group

The results showed that the higher infection was in second age groups (38.1%), followed by the third age group (33.3%). The lowest infection was in the first age (28.6%) (Figure 3 and Table 1)

3.4. Distribution of the Thalassemia patients Infected with E*. Coli* According to Gender

The results showed that the higher infection was in females was 61.9%, while the lowest infection was in the males (38.1%) (Figure 4 and Table 2).

3.5. Distribution of the Thalassemia patients Infected with *E. coli* According to Habitation

The results showed that the higher infection was in rural was 61.9%, while the lowest infection was in the urban (38.1%) (Figure 5 and Table 3).

3.6. Identification of *E. coli* Bacterium

3.6.1. Morphological Properties

E. coli colonies appear smooth colonies with distinct edges and pink colonies on the MacConkey agar during incubation at 37ºC overnight. *E. coli* usually lactose ferments. *E. coli* colonies on the blood agar Milky to white color with or without zone of hemolysis, or most UPEC appearance β- hemolytic. While the commensal non-hemolytic (Figure 6).

3.6.2. E. coli Bacterium on CLED agar

CLED agar (cysteine–lactose–electrolyte-deficient agar or medium) is a valuable non-inhibitory growth medium used to isolate and differentiate urinary infectious microbes. It contains cysteine and lactose, and it is electrolyte deficient; the latter trait prevents the swarming of Proteus species. Cysteine promotes the formation of cysteine-dependent dwarf colonies (Figure 7). Bromothymol blue is the indicator used in the agar, and it changes to yellow in case of acid production during fermentation of lactose or changes to deep blue in case of alkalization. Lactose-positive bacteria build yellow colonies. Bacteria which decarboxylate L-Cysteine cause an alkaline reaction and build deep blue colonies. *E. coli* appear on medium opaque yellow colonies with a slightly deeper yellow center.

3.7. Antibiotic Susceptibility

The antimicrobial susceptibility test for 31 isolates of *E. coli* was performed on Muller Hinton agar by the modified Kirby Bauer disc diffusion method. All *E. coli* isolates that included 31 UPEC, 46, and 20 controls healthy, were screened against 16 antimicrobial agents (Figure 8).

3.7.1. Antibiotics Susceptibility Pattern

The results of the current study showed that there were among 16 antibiotics used only Imipenem had activity against all isolated *E. coli*. While the Ampicillin, AT, Streptomycin, Ciprofloxacin, Ticarcillin-clavulanic acid, Amoxicillin- clavulanic acid, and TI had no activity against isolated *E. coli*. Other antibiotics like Amikacin, Piperacillin, Tetracycline, and Netilmicin recorded only one intermediate activity in addition to the other activities (Table 4).

3.8. Discussion

3.8.1. Distribution of Thalassemia Patients according to Age, Gender and Habitation

The current study showed a significant increase in thalassemia among the second age group. The high rate of prevalence of thalassemia occurred in the second age group because the syndrome begins in the people who carry it, according to the inherited, it may be within two years or more and the patients who are up to 20 years or more is exposed to many complications, the most important of which is the increase iron overload and this effects on the heart muscle and kidneys and endocrine, in addition to the viral infections, which lead to many deaths status. The results agree with the study of (Al-attar *et al*., 2014), in Irbil, their results designate that major thalassemia phenotype can be diagnosed perfectly within the early months or early days of age because the exhibitions of the disease may perform after a complete switch from fetal to adult Hb synthesis occurs. Typically, this switch is completed by the sixth month after birth. Frequently, milder forms are discovered by chance and at different ages, and many patients whose conditions to be homozygous may show no significant symptoms or anemia for several years (Pourgheysari *et al*., 2016).

According to gender, the results showed that there was a non-significant difference between gender because thalassemia is a genetic disease that is transmitted from parents to offspring and for both sexes equally. The results agree with the study of (Ayyash *et al*., 2018) that evaluated biochemical and hematological parameters in β-thalassemia in Gaza and recorded non-significant differences between thalassemia syndrome according to gender.

According to habitation, the result showed that there was a significant increase in thalassemia syndrome in rural habitats. This is due to some common tribal traditions among the rural population and their mating and lack of openness to other families, especially those who marry cousins. The current results agree with the study of (Kremastinos *et al*., 2010), in North America, they showed that the distribution of thalassemia increases significantly in rural habitat than urban habitat. Although the study disagrees with the (Al-attar *et al*., 2014) study, Irbil showed that there

was no relationship between the distribution of the syndrome and the habitat, the compatibility between the two studies may be due to random sampling of models or all ages without discrimination. The disagreement between the two studies may be due to the number of reasons, such as a large number of rural-urban migrations, especially after the 1990s, the scarcity of rural resources and employment in the city, or the study was limited to the study of family genetic diseases.

3.8.2. Isolation and Identification E. coli Bacterium

E. coli bacterium is composed of pathogenic and non-pathogenic strains, Non-pathogenic strains that may evolve into pathogenic variables, and vice versa. Most extra-intestinal infections associated with *E. coli* are caused by commensal strains that become pathogenic by adaptation stratagems or the acquisition of virulence determinants (Sáez-López *et al*., 2016).

The commensal *E. coli* and Uropathogenic *E. coli* can colonize the gastrointestinal tract and be well adapted to conditions in the large intestine (Bailey *et al*., 2010). Some physiological studies about *E. coli* had demonstrated the ability of these organisms to adapt themselves to their different habitats (Polage *et al*., 2012).

The isolates of *E. coli* from thalassemia patient who suffer from urinary tract infection assigned for the current study and based on patients with UTI were 21 6.9%, out of a total of 303 patients with UTI, and 8 2.6% S. aureus, 2 0.7% for both *Klebsiella* and *Proteus*. They were diagnosed by a physic and microscopic examination of urine. By culture, both isolated commensal and uropathogenic *E. coli* have the same morphological form on MacConkey agar. Still, most uropathogenic *E. coli* β- hemolytic and commensal *E. coli* were non-hemolytic on blood agar.

In the current study, the UPEC isolates were diagnosed as 28.6% isolates in group I from 6 samples, 38.1% isolates in group II from 8 samples, and 33.3% isolates group III from 7 samples. This result corresponds with many studies that focused on the predominant of UPEC among categories patients. The current study agreed with the study of (Abd ALameer *et al.*, 2015), where they study 318 different samples from a burn, vaginal swab, urine, and environment and recorded 25:28% of *E. coli* from a urine sample. Also, a local study performed by (Al Badry, A. *et al*., 2016), in Thi-Qar province, they studied the identification of pathogenic bacteria from thalassemia patient and recorded among 40 patients the *E. coli* recorded 6.6% among other bacteria. The study also agreed with the study of (Shah *et al*., 2019), in Waist province they study virulence factors of uropathogenic Escherichia coli (UPEC) and correlation with antimicrobial resistance and recorded 22: 20% from 105 urine sample infected with *E. coli* bacterium. The study also agreed with the study of (Hosseini *et al*., 2009), in Tehran, they studied the distribution of *E. coli* among patients with urinary tract infection and showed 243: 16.2% from total sample infected with pathogenic E. coli. While the current study disagreed with the study of (Melo *et al*., 2015), in Brazil, they study the diversity of Escherichia coli isolated from humans and foods and recorded among 84 urine samples 48: 57.1% infected with E. coli. The reason behind the current and previous studies may be due to the different location of the study, as well as the immune status of the patient, in addition to the development of this bacterium as well as novel strains of E. Coli, a potential human pathogen, has been attributed to advances in diagnostic capabilities and not to the emergence of a new pathogenic strain (Sullivan *et al*., 2011).

E. coli which generally produces from fecal or the per urethral flora and the vaginal serve as a reservoir for it, are responsible most UTIs tapping expands in (2000) (Kawamori *et al*., 2008), the commensal *E. coli* contain specific surface structures that can able induce signal cascades if the organism encountering the appropriate receptor and the ability to acquire and propagate plasmid types can vary between *E. coli* commensal and pathogenic subgroups *E. coli* (Cohen, 2017). However, commensal E. coli that evolved into a pathogen depends not only on the gaining of virulence factor genetic information enabling successful colonization of the host but as well on the presence of functional genes directly contributing to pathogenesis. That is what they explained (Marrs *et al*., 2015); these strains cause disease if the host is compromised immunologically.

The isolation of *E. coli* from thalassemia patients considering them immunocompromised patients in the present study was consistent with most local and international studies, as for the differences in the results, it may be due to: the study was more specialization for a particular category, living conditions and nature of the population, the progress of society or hygiene, ratio of males to females allocated in the study, isolated from the patient without symptomatic urinary tract infection, social status and methods

of insulation and techniques used.

3.8.3. Antibiotic Susceptibility Pattern

Antibiotic susceptibility patterns will help choose the right antibiotic and reduce antibiotics problem, from misuse, antibiotics. This information will be useful to reduce the problem of antibiotic resistance, and it is a global problem, resistance may be natural resistance or acquired resistance. Natural resistance is especially specific to bacteria with certain antibiotic resistance, such as having a cellular wall that prevents the passage of the antibiotic, the acquired resistance is caused by genes inherited by plasmids, transposons, and integrin‘s or conjunction. Also, increased resistance is usually due to the widespread and incorrect use of antibiotics. When talking in general about *E. coli* through sensitivity and resistance to the direction of antibiotics.

The results of the current study showed that there were among 16 antibiotics used only Imipenem had activity against all isolated *E. coli* with 100% sensitivity. While the Ampicillin, Aztreronam, Streptomycin, Ciprofloxacin, Ticarcillin-clavulanic acid, Amoxicillin- clavulanic acid, and Tetracycline had no activity against all isolated *E. coli* with 100% sensitivity. Other antibiotics as Amikacin, Piperacillin, Tetracycline, and Netilmicin recorded only one intermediate activity in addition to the other activities. The *E. coli* showed in the present study resistance for most β-lactamase antibiotics, while the anti-β-lactamase antibiotics act by binding to certain proteins in the cytoplasmic membrane called Binding Proteins Penicillin. These proteins are responsible for some interactions in the peptidoglycan synthesis process. Therefore, the association of anti-β-lactam with these enzymes leads to the disruption of its work and thus stops the process of manufacturing the cell wall to produce a cell wall deficient incoherent, which makes the bacteria sensitive to the pressure of osmosis and then the death of bacteria. The previous study done by (Abd ALameer *et al*., 2015*)*, in Thi-Qar province showed among 46 isolate

E. coli from a patient with urinary tract infection 100% resistant for Penicillin, 96% resistant for Amoxicillin Clavulanic acid, 74% resistant for Ceftriaxone, 87% resistant for Tetracycline and 96% resistant for both Ticarcillin Clavulanic acid and Trimethoprim. While all isolate sensitive for Amikacin and 96% sensitive for Netilmicin. Also, a study performed by (Igbeneghu *et al*., 2014*)*, in Nigeria, they showed that the strains were highly resistant to Cefalothin 100%, Streptomycin 94.0 %, Tetracycline 92.0 %, and Trimethoprim 89.3 % while resistance to the Quinolones was low 3.3 - 14.0 %. A study of (Patricia *et al*., 2011*)*, in Brazil, they studied antimicrobial susceptibility for *E. coli* isolate from diarrheagenic patients and showed the most effective drug were Ceftazidime, Ceftriaxone, Imipenem and Piperacillin-tazobactam, for which no resistance observed. Most recent studies suggested the increase and spread of antibiotics resistance related to horizontal transfer of resistance genes such as integrons among bacterial populations or through conjunction. Transport resistance genes from commensal *E. coli* to pathogenic bacteria represent a potential risk to public health as well as the common and wrong use of antibiotics in addition to taking large doses of antibiotics at an early age, which leads to curbing and weakening the immune system.

4. CONCLUSIONS:

E. coli has the highest incidence among other bacterial in thalassemia patients infected with urinary tract infection, the incidence of urinary tract infection in females is higher than in males, as well as in rural population is higher than in the urban population. The antibiotics have proven to be very effective in treating bacteria are Imipenem, Gentamycin, Amikacin, while the Ampicillin, Aztreonam, Tetracycline, Amoxicillin-Clavulanic acid and Piperacillin are not effective against isolated bacteria, the culture medium CLED and IPE 20E have proved effective in diagnosing isolated bacteria.

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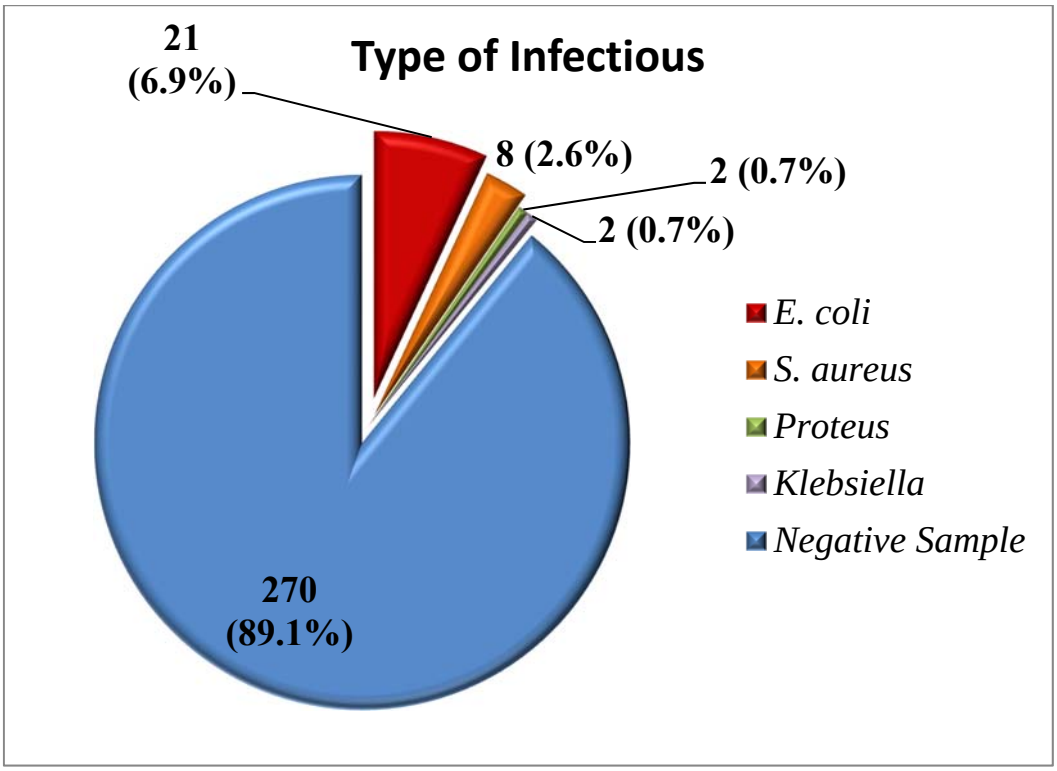

Figure 1. *The percent of Escherichia coli isolated from urine samples of thalassemia patients with UTI*

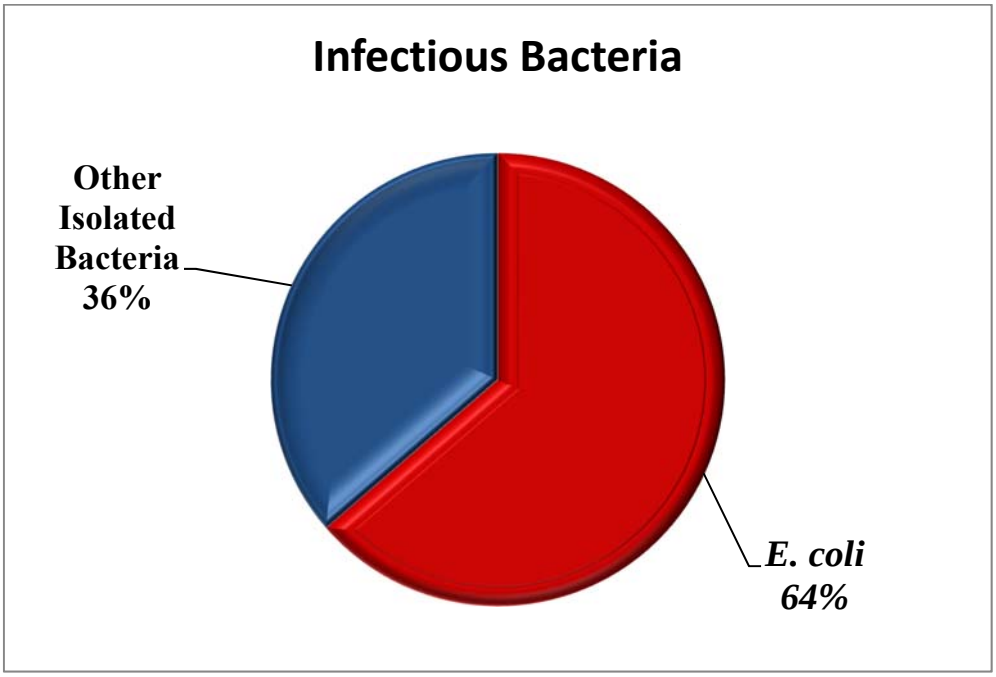

Figure 2. *Percentage of Infection with E. coli Compared to other Isolated Bacteria*

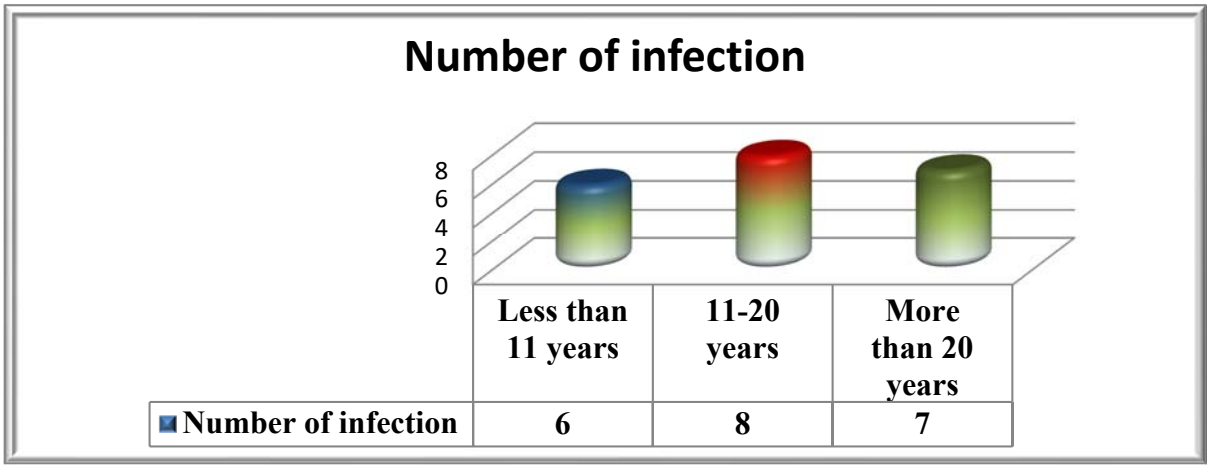

Figure 3. *Distribution of E. coli According to Age Group.*

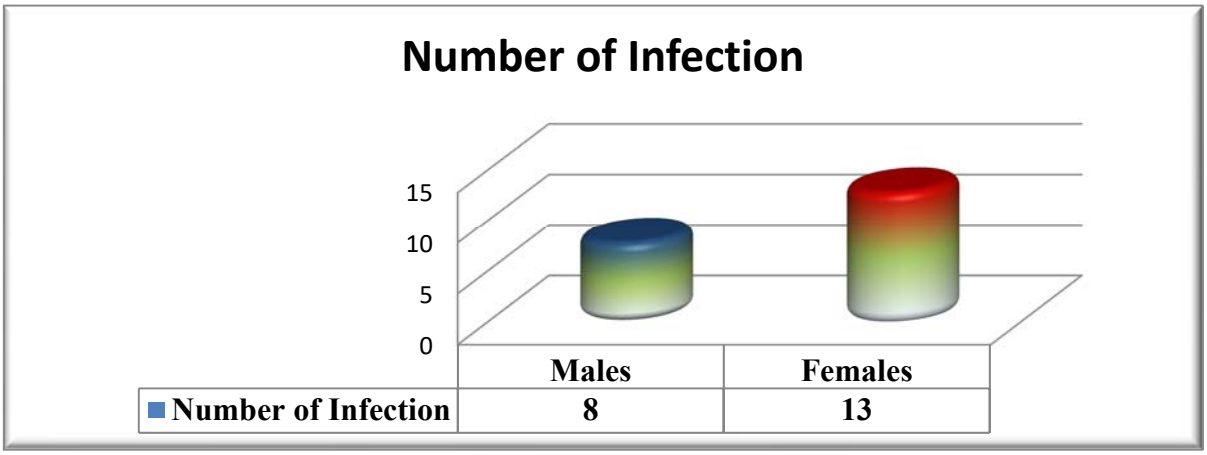

Figure 4. *Distribution of E. coli According to Gender.*

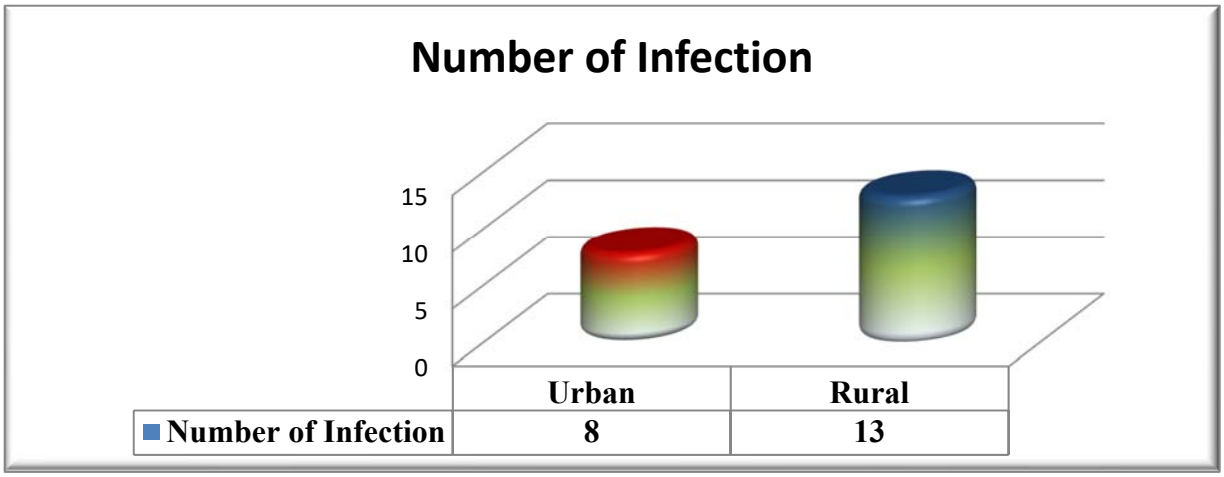

Figure 5. *Distribution of E. coli According to Habitation.*

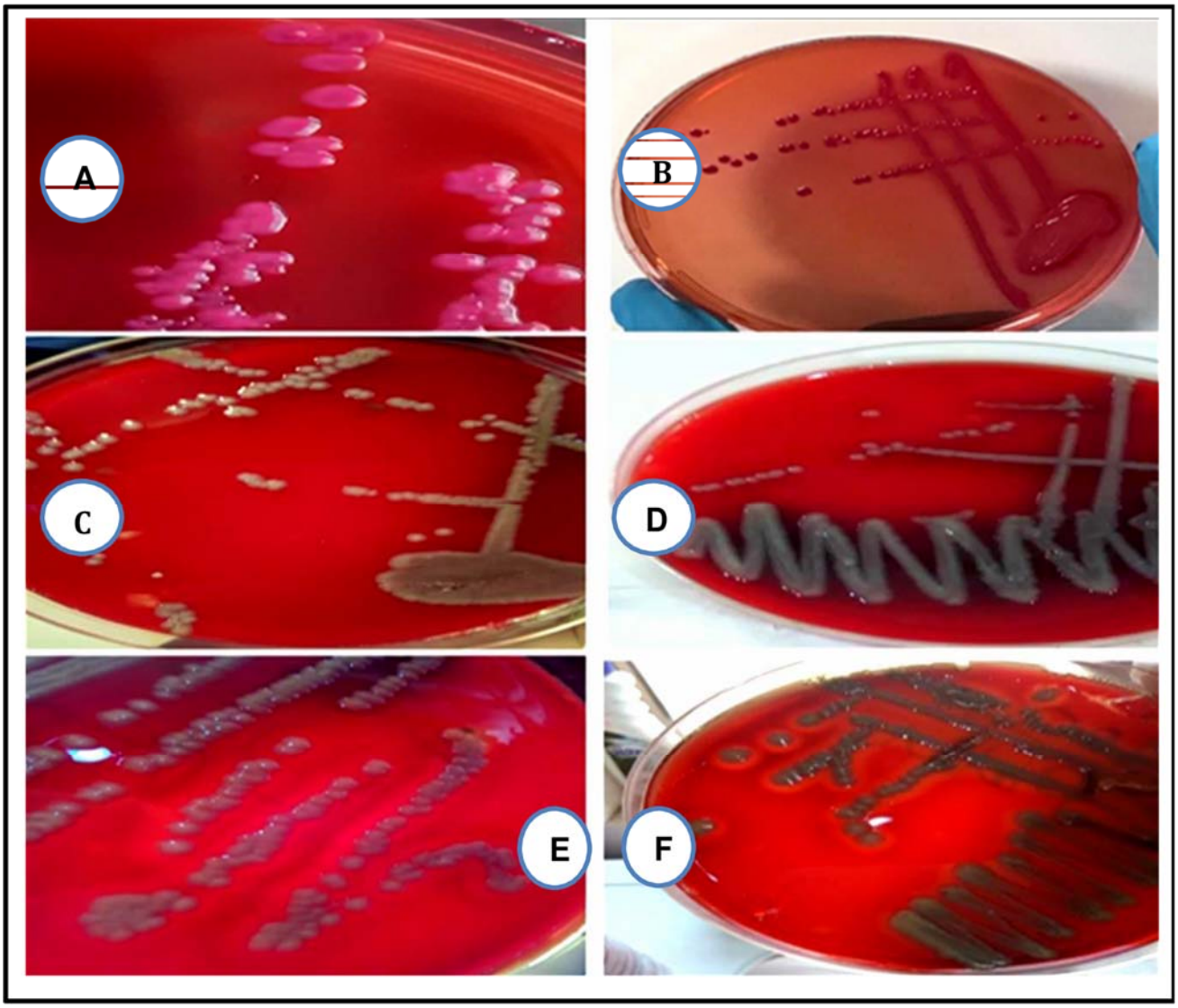

Figure 6. *Morphological of E. coli colony A and B: E. coli colony on MacConkey agar lactos ferment, C: commensal E. coli colony on blood agar non-hemolytic, D: UPEC colony α-hemolytic, E and F: UPEC β-hemolytic E. coli.*

Figure 7. *E. coli grow on CLED agar*

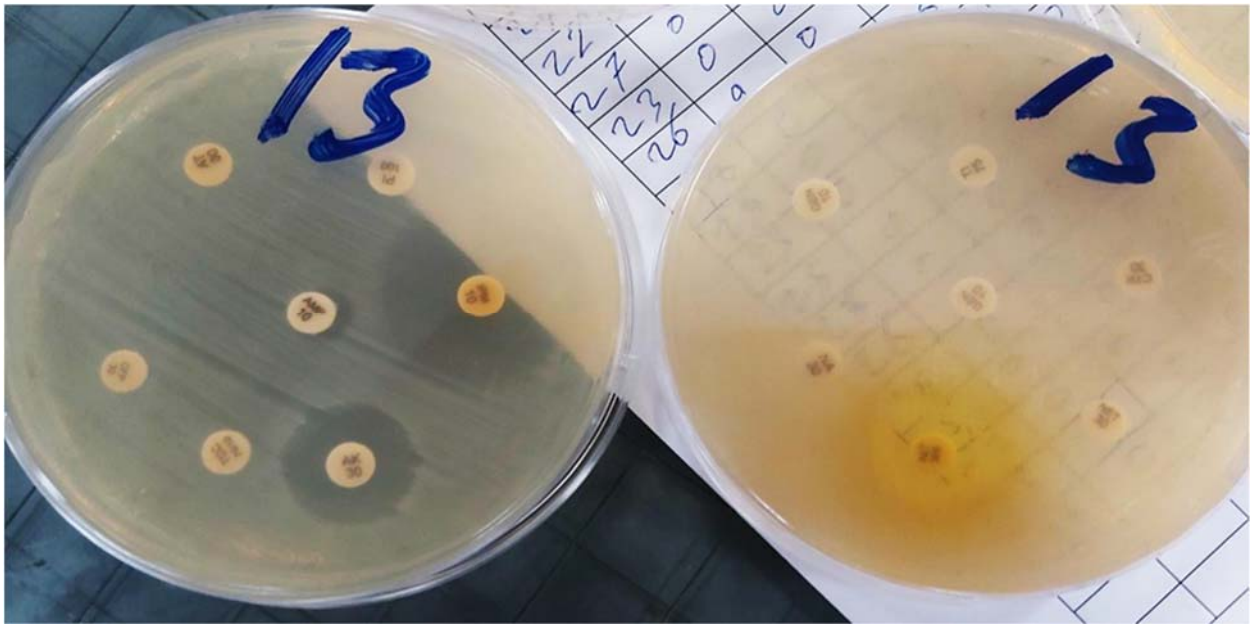

Figure 8. *Antimicrobial susceptibility test; for E. coli isolated.*

Table 1. *Distribution of E. coli According to Age Group*

Pathogen / Groups	*E. coli*		Total	
	No.	%	No.	%
Less than 11 years	6	28.6	6	28.6
11 – 20 years	8	38.1	8	38.1
More than 20 years	7	33.3	7	33.3
Total	21	100	21	100

Table 2. *Distribution of E. coli According to Gender*

Pathogen / Gender	*E. coli*		Total	
	No.	%	No.	%
Male	8	38.1	8	38.1
Female	13	61.9	13	61.9
Total	21	100	21	100

Table 3. *Distribution of E. coli According to Habitation*

Pathogen / Habitation	*E. coli*		Total	
	No.	%	No.	%
Urban	8	38.1	8	38.1
Rural	13	61.9	13	61.9
Total	21	100	21	100

Table 4. *Antibiotic susceptibility of E. coli against isolates 16 types of antibiotics.*

Type of Response / Antibiotics	Antibiotic Susceptibility							
	Resistance		Intermediate		Sensitive		Total	
	No.	%	No.	%	No.	%	No.	%
IPM	0	0.00	0	0.00	21	100	21	100
AMP	21	100	0	0.00	0	0.00	21	100
AT	21	100	0	0.00	0	0.00	21	100
CTR	21	100	0	0.00	0	0.00	21	100
CEP	21	100	0	0.00	0	0.00	21	100
NET	3	14.3	0	0.00	18	85.7	21	100
AK	5	23.8	1	4.8	15	71.4	21	100
TTC	21	100	0	0.00	0	0.00	21	100
PI	20	95.2	1	4.8	0	0.00	21	100
TE	20	95.2	1	4.8	0	0.00	21	100
AMC	21	100	0	0.00	0	0.00	21	100
GEN	4	19.0	0	0.00	17	81.0	21	100
TI	21	100	0	0.00	0	0.00	21	100
NIT	7	33.3	1	4.8	13	61.9	21	100
NA	18	85.7	2	9.5	1	4.8	21	100
CIP	19	90.5	0	0.00	2	9.5	21	100
$CalX^2 = 260.107$	$TabX^2 = 43.77$		DF = 30		P. Value = 0.0001			