Bacteriological study of Escherichia coli and its pathological role in urinary tract infection

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Abstract

Background: One of the most prevalent bacterial infections in both adults and children around the world is urinary tract infections (UTIs). Bacteria cause more than (95%) of infections, including the majority of UTIs. The most frequent cause of urinary tract infections is Escherichia coli (E.coli).

Aims: to detect E.coli using culture media, as well as biochemical assays, and to analyze antibiotic resistance using the disc diffusion method, VITEK 2 system, and Biofilm from urine samples of patients with UTI.

Methods: A total of 471 midstream urine samples from UTI suspects were taken and tested for the presence of E.coli.

Results: The percentage of infection with E.coli was higher in females than in males, 39 (78%) and 11 (22%), respectively. The highest rate of bacterial infection was within the age group 21- 30 years and its rate was 51.28%, followed by the age group 41-50 years and its rate was 12.89% for females. All bacterial isolates were multi-drug resistant (MDR) used in the current study. The results showed that 48 (96%) bacterial isolates were highly resistant to Cefixime and Ceftriaxone, and also resistant to each of the ceftazidime (84%), followed by the two antibiotics, Tetracycline (72%) and Gentamicin (70%). The isolates showed a high ability to form biofilms.

Conclusion: The current investigation found that E.coli had developed resistance to numerous antibiotics.

Keywords: *E.coli, multi-drug resistant, urinary tract infections.*

INTRODUCTION

Escherichia coli (E.coli) are a gram-negative bacterium, it has a coccobacillus shape, it is motile or non-motile, it ferments lactose, and most strains have the ability to ferment other sugars such as rhamnose. Ramnose and sorbitol. It is a normal flora found in the intestines of humans and animals (Al-Saadi and Abdullah, 2019). It belongs to the family Enterobacteriaceae, within the class Gammaproteobacteria, grows rapidly under optimal growth conditions at approximately 37 °C and pH 4.4, and doubles in about 20 minutes

(Jang et al., 2017). These bacteria can survive for long periods outside the intestine and multiply in soil and sand in tropical, subtropical, and temperate regions (Ishii and Sadowsky, 2008; Jawetz et al., 2016). The growth and survival of E.coli in natural media can be affected by biotic and abiotic factors (Rochelle-Newall et al., 2015). Biotic factors include the presence of other microorganisms with the ability to obtain nutrients, compete with other microorganisms, and form biofilms in natural environments. Abiotic factors include temperature, availability of water and nutrients, pH, sunlight, and others (Jang et al., 2017). E.coli causes many diseases in humans such as diarrhoea, neonatal meningitis, sepsis, bacteremia, and urinary tract infections (UTIs) (Al-Saadi and Abdullah, 2019).

Urinary tract infection is one of the most common bacterial diseases worldwide, and the most common genitourinary disease in children. Most urinary tract infections are caused by bacteria, which are the main cause and responsible for more than (95%) of infections (Lee et al., 2016). E.coli is the most common cause of urinary tract infections, including acute cystitis, pyelonephritis, and urosepsis, three common and clinically distinct syndromes (Hadi et al., 2014). It causes 80 to 90% of community-acquired UTs and more than 30% of nosocomial-acquired UTIs. These bacteria can invade and multiply inside the bladder, forming intracellular bacterial communities (IBCs) resembling biofilms. E.coli bacteria can also form cell membranes capable of attaching to the urinary epithelium, which helps stabilize the biofilm, promote host infection and increase antibiotic resistance (Khoshbakht et al., 2013; Sarowska et al., 2019). This study aimed to isolate E.coli bacteria from urine samples of patients with urinary tract infections, detect E.coli bacteria using enriched and selective culture media, as well as biochemical tests and study antibiotic resistance using the disc diffusion method and VITEK 2 system, minimum inhibitory concentration (MIC) and Biofilm.

Materials and Methods:

A. Collection of specimens: Four hundred and seventy-one (471) urine samples were collected from patients with urinary tract infections (UTIs) who came to hospitals (Baghdad Teaching, Ghazi Al-Hariri Teaching, Al-Yarmouk Teaching and Teaching Laboratories in the Medical City) in Baghdad, their ages ranged between (18-70) years, and of both sexes, Males and females, samples were collected during the period from 08-28-2022 to 11-28-2022, as sterile containers were used to collect samples from mid-urine in the early morning for all patients and were transferred directly to the laboratory in a refrigerated box for the purpose of diagnosing and isolating bacteria E.coli.

B. Isolation and identification of E.coli:

• Urine analysis: Microscopic and macroscopic examination for urine samples was done. Physical examination (appearance, colour, odour, and specific gravity) was carried out for urine samples. Rapid dipstick urine strips were also used to examine and detect pus cells in the urine by testing leukocyte esterase (LE) in the urine before centrifugation (Harris, 1969; Rodriguez et al., 2011). After placing 10 ml of each urine sample in a sterile centrifuge tube, wet slide smears were prepared, and the apparatus was run at 3000 rpm for 5 minutes. Examination of pus cells for each sample using a 40X high magnification lens. Urine samples containing more than 5 pus cells / microscopic field were considered positive and the patient had pyuria due to infection with pathogenic bacteria (Rodriguez et al., 2011). Urine samples containing 5 cells / microscopic field

were cultured on culture media in order to detect E.coli bacteria.

• Staining of Urine Deposits: The method of work was followed according to (Harris, 1969; Rodriguez et al., 2011). 5 mL of each urine sample was added into a centrifuge tube. And placed in the device that operates at a speed of 3000 rpm for 5 minutes. The supernatant was discarded, 0.1 ml of the precipitate was taken by means of a micropipette and placed on a sterile glass slide, then dry slide smears were prepared and left to dry in the open air and they were passed three times on the flame of a Bunsen burner in order to fix them. Drops of crame stain were added to the glass slide containing the smear, and the result was read. If any type of bacteria appears with a number of 20 bacteria / field when using an oil immersion lens (20 Bacteria\ 100X Fields), the result is considered positive.

• Identification of bacterial isolates: Both blood agar media and McConkey agar were used in order to study the phenotypes of E.coli bacteria, such as the shape, colour, size, and odour of colonies, as well as their ability to lyse blood if present (Baron et al., 2007). For the purpose of microscopic examination of bacteria, a Gram stain was used to detect E.coli isolated on culture media (Baron et al., 2007). As for biochemical tests, the following biochemical tests were carried out to diagnose the bacterial isolates, such as the Catalase Test, the Motility Test (Collee et al., 1996), the Oxidase Test (Prescott and Harley, 2002), and the (IMViC Test) (Collee et al., 1996).

C. Sensitivity of Bacteria to Antibiotics: A modified Kirby-Bauer method (Disk Diffusion Method) according to the reference (Morello et al., 2004) was performed to test the resistance and sensitivity of E.coli isolates in the current study. The results were compared with the standard inhibition zone (CLSI, 2018). Also, the Compact system-VITEK2 was used to determine the minimum inhibitory concentration (MIC) value for 18 antibiotics: Ampicillin/Sulbactam), piperacillin/ Tazobact, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Aztreonam, Ertapenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Levofloxacin, Tetracycline, Tigecycline, Nitrofurantoin, Sulfamethoxazole / Trimethoprim, using Antibiotic Susceptibility Test Card (AST Card)

D. Biofilm Formation: Microtiter plate method was used in order to investigate biofilm-forming E.coli. Antibiotic-resistant bacteria under study were selected (Al-Ouqaili and Al-Kubaisy, 2008). A 0.1% crystal violet dye solution was used. Optical Density was read at 630 nm using an ELISA reader. The readings are based on Table 1.

Table (1): The average optical intensity and the intensity of biofilm formation for bacteria

Biofilm intensity	Mean OD
Non – adherent	OD ≤ ODc*
Weak	2ODc> OD> ODc
Moderate	4ODc> OD> 2ODc
Strong	OD > 4 ODc

*Cut off Value (ODc) = Average OD of Negative Control + (3*Standard Deviations).

Results and discussion

A total of 471 urine samples were collected from patients suffering from urinary tract infections from Al-Yarmouk Teaching Hospital, Baghdad Teaching Hospital, Ghazi Al-Hariri Teaching Hospital, and educational laboratories in the Medical City and Private Laboratories in the city of Baghdad. Their ages ranged between (14-67) years, from both sexes

(male and female). Conducting phenotypic, microscopic, and biochemical tests, 50 isolates of E.coli were obtained at a rate of (10.61%), as shown in Table 2. The percentage of infection with E.coli was higher in females than in males, 39 (78%) and 11 (22%), respectively, as shown in Table 3. The highest rate of bacterial infection was within the age group 21- 30 years and its rate was 51.28%, followed by the age group 41-50 years and its rate was 12.89% for females. As for males, the percentages were almost equal in the age groups (21- 30), (41-50) and (51-60) with a rate of 27.27%, as shown in Table (4).

Table (2): Percentages of E.coli isolates isolated from patients with urinary tract infections using selective and enrichment culture media.

(%) Total		The number of (%) negative samples		Number of positive samples containing *E.coli* isolates (%)	
(%)	No.	(%)	No.	(%)	No.
(100)	471	(89.38)	421	(10.61)	50

Table (3): Percentage of E.coli isolated in both males and females who suffer from urinary tract infection

(%)	The number of positive cases of *E.coli*	Sex
22	11	male
78	39	female
100	50	Total

Table (4): Distribution of E.coli bacteria by age groups in patients with urinary tract infection

(%) Total		The number of bacterial Isolates *E.coli*				age group (year)
		(%) female		(%) male		
(%)	No.	(%)	No.	(%)	No.	
(8)	4	(7.69)	3	(9.09)	1	20-11
(46)	23	(51.28)	20	(27.27)	3	30-21
(18)	9	(15.38)	6	(27.27)	3	40-31
(12)	6	(12.82)	5	(9.09)	1	50-41
(14)	7	(10.25)	4	(27.27)	3	60-51
(2)	1	(2.56)	1	(0)	0	70-61
(100)	50	(100)	39	(100)	11	(%) total

In the current study, urine samples were cultured on selective and special culture media to isolate E.coli after conducting a General Urine Examination (GUE). Urine samples were selected for culture, which showed a positive result for Leukocyte esterase-LE when using Rapid Urine dipsticks, and the number of Pus cells (Dead White Blood cells) was higher than 5 cells/ microscopic field using a high-magnification lens. High Power (X40). Examination results of positive urine samples for the LE test and pus cells in them higher than 5 cells/microscopic field gave positive results when implanting the urine, and this is consistent with many international studies.

The results of the current study were consistent with what was reached by (Ibrahim et al., 2020) in Dohuk, as the results of his study showed that (56.2%) of the cases had bacterial pathogens, with E.coli being the most common with (24.7%). In the urine samples of patients who were diagnosed with urinary tract infection, it was also found that the samples that gave a positive result for bacterial culture contained pus cells and bacteria, and that the highest isolation rate appeared in females (66.5%), while among males the isolation rate was (33.5%) (Ibrahim et al., 2020). E.coli is the main cause of nearly (90%) of all urinary tract diseases because of the virulence factors it possesses, such as hemolysin production and the presence of pili. The results of the current study were also close to the results of the researcher (AL-Saadi, 2019) that was able to isolate and diagnose E.coli from patients coming to Baghdad hospitals, and the isolation rate is (50%). The results documented by many researchers in Iraq revealed that UPEC isolates were able to be one of the main causative agents of urinary tract infection, especially in females, which are considered normal inhabitant bacteria that inhabit the intestines and vagina, so these sites can serve as potential reservoirs for urinary tract infection urinary tract (Jabbar, 2013; Tawfeeq, 2014; Assafi et al., 2022). In a similar study of an outbreak of bacteria that cause urinary tract infections in the Brazilian city of São Paulo, State of Brazil, the study was based in 2018 that the percentage of the presence of UPEC bacteria was (90.2%) among females with ages ranging from (18-65) years who were clinically diagnosed as having, they suffer from Cystitis and pyelonephritis, in addition to the presence of gram-negative bacteria and pus cells in the urine when examining the urine sample microscopically (Tanabe et al., 2022). A possible explanation for this could be that most of these patients were females who had a short urethra, as well as the proximity of the urethra to the anal canal and vagina (Zagaglia et al., 2022).

Bacterial isolates were identified using enriched, special and selective culture media. E.coli colonies appeared on Differential and selective MacConkey agar in pink colour, as in Figure (A-1). The bacteria isolates produced shiny metallic green colonies on (EMB) Eosin Methylene Blue agar (Fig. B-1). This feature is one of the distinguishing features of E.coli from other members of the Enterobacteriaceae family, as a result of the medium containing Eosin and Methylene dyes. Methylene blue, which is deposited in the acidic media after their association with each other, encourage their association with the bacteria and then their absorption by them, giving a green metallic, which indicates that the bacteria produced organic acids as a result of their fermentation of the sugars lactose and sucrose. As for the chromagar agar, E.coli bacteria produced intense blue colonies on it (Figure C-1).

Figure (1): A- Bacterial isolates on MacConkey agar medium. B- Bacterial isolates on Eosin Methylene Blue agar (EMB) medium. C-Bacterial isolates on Chromagar medium

A

B

C

In the current study, E.coli colonies appeared on blood agar medium, large, circular, grey to white, and moist. Twenty isolates (40%) gave hemolysis type β-hemolysis and 30 isolates (60%) gave hemolysis type γ-hemolysis, which indicates that the isolates that analyzed blood are hemolysin-producing isolates. The current study came close in its results to a study conducted in 2017 (Abdul-Ghaffar, 2017), where beta-type hemolysis was observed in (25.58%) of E.coli isolates. Bacteria were stained using a Gram stain, and examined under a normal light microscope, as Bacilli and other coccobacilli, short, Gram-negative, single or double arranged, were assumed to be E.coli bacteria, and these results were consistent with what was previously mentioned (Zagaglia et al, 2022).

Several biochemical tests were performed on all bacterial isolates, which gave positive results for the catalase test, Indole test and Methyl red test. As for the Voges-Proskauer test and the citrate utilization test, the bacterial isolates gave negative results for these two tests. E.coli strains were negative for the Oxidase test and Urease test, and this is consistent with what came in the results of previous local and international studies. (Brown and Smith, 2017; Dahwash et al., and AL-Tamemi 2021). The motility test also showed a positive result. An indication that E.coli are mobile because they possess Peritrichous multiple flagella, as proven by previous researchers in this field (Riley et al., 2018; Kasew et al., 2022).

All UPEC isolates were tested for antibiotic susceptibility. All bacterial isolates were multi-drug resistant (MDR) used in the current study. The results showed that 48 (96%) bacterial isolates were highly resistant to Cefixime and Ceftriaxone, and also resistant to each of the ceftazidime (84%), followed by the two

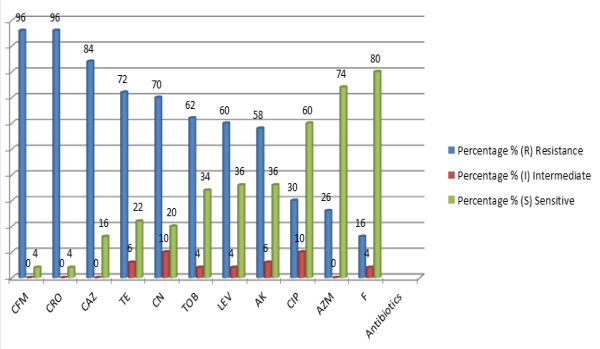
antibiotics, Tetracycline (72%) and Gentamicin (70%). As for the percentage of resistance against Tobromycin, Levofloxacin, Amikacin and Ciprofloxacin, they were (30%, 58%, 60%, and 62%) respectively. As for Azithromycin and Nitrofurantoin, bacterial isolates recorded the least resistance to it, as they were (26%) and (16%), respectively. While UPEC bacterial isolates recorded the highest sensitivity to Azithromycin (74%) and Ciprofloxacin (60%), Table (5) and Figure (2).

Table (5): The number of UPEC bacterial isolates and the percentage of their resistance and sensitivity to antibiotics

E.coli isolates						Code	Class	Antibiotic Name
(S)		(I)		(R)				
(%)	No.	(%)	No.	(%)	No.			
4	2	0	0	96	48	CFM	Cephalosporins	Cefixime
4	2	0	0	96	48	CRO	Cephalosporins	Ceftriaxone
16	8	0	0	84	42	CAZ	Cephalosporins	Ceftazidime
22	11	6	3	72	36	TE	Tetracyclines	Tetracycline
20	10	10	5	70	35	CN	Aminoglycosides	Gentamicin
34	17	4	2	62	31	TOB	Aminoglycosides	Tobromycin
36	18	4	2	60	30	LEV	Fluoroquinolones	Levofloxacin
36	18	6	3	58	29	AK	Aminoglycosides	Amikacin
60	30	10	5	30	15	CIP	Fluoroquinolones	Ciprofloxacin
74	37	0	0	26	13	AZM	Macrolides	Azitromycin
80	40	4	2	16	8	F	Aminoglycosides	Nitrofurantion

R: Resistance, I: Intermediate, S: Sensitive

Figure (2): Percentages of resistance and sensitivity of E.coli (UPEC) isolates to antibiotics



It was noted that there was a high level of resistance of E.coli isolates to most of the antibiotics under study, and these results are close to the results of some studies conducted by a number of researchers. The results of the current study are consistent with previous studies, including the local results reached by the researcher AL-Tamemi in 2021, where it appeared that E.coli isolated from patients in Kut Governorate was resistant to cefixime (89.36%) and (82.80%) Tetracycline, Ceftriaxone (74.46%) and Tobramycin

(72.34%), while it did not agree with the results of Ceftazidime, which were (59.57%), Ciprofloxacin (46.80%) and Gentamycin (31.91%) (AL-Tamemi, 2021). The reason is that some of the researcher's results do not agree with the results of the current study because the samples belong to children with ages ranging between (6-12) years, and the geographical location (Kut Governorate) is far from the geographical location of the samples that were taken in (Baghdad Governorate). On the other hand, the results of the current study agreed with the results of Abdul-Ghaffar in 2017, which isolated E.coli from the urine of patients with urinary tract infections in Baghdad governorate, as it became clear that all isolates were resistant to antibiotics and to more than three antibiotics, meaning that they fall under Multi-drug resistant bacteria were labelled with resistance to Ceftriaxone (72.09%), Ceftazidime (62.79%), Cipnfloxaein (60.46%) and Gentamicin (46.51%) (Abdul-Chaffar, 2017). With regard to Nitrofurantoin, it was found that it is the best treatment that can be used against E.coli (UPEC), as the current study was compatible with the local studies Al-Tamemi, Al-Saadi, Abdul-Ghaffar with percentages (87.5%, 96%, 100%) on relay (AL-Saadi, 2019; AL-Tamemi, 2021).

The results showed that the percentage of resistance of E.coli isolates to antibiotics and the minimum inhibitory concentration (MIC) was high for most of them when tested using the VITEK2 device, as follows: (100%) for Ampicillin / Sulbactam, (MIC>= 8 µg/ml) and Cefazolin (MIC>=64 µg/ml) and resistance (80%) to ceftazidime (MIC 16 µg/ml) and cefepime (MIC 4 µg/ml) and resistance to Gentamicin (72%) (MIC>=16 µg/ml), and resistance (70%) to Tetracycline (MIC>=16 µg/ml), Trimethoprim/Sulfamethoxazole (MIC>=320 µg/ml) and Ceftriaxone (MIC>=64 µg/ml), respectively, and (64%) resistance to Tetracycline (MIC>=64 µg/ml). MIC<=0.5 µg/ml) and Piperacillin/Tazolin (MIC<=4 µg/ml), resistance (60%) to Tobramycin (MIC<=1 µg/ml), resistance (58%) to Levofloxacin (MIC 1 µg/ml), resistance (56%) to Cefoxitin (MIC<=4 µg/ml), resistance (52%) to Aztreonam (MIC 16 µg/ml), and resistance (50%) to Amikacin (MIC<=2 µg/ml), (30%) for Meropenem (MIC<=0.25µg/ml), and (12%) for Nitrofurantoin (MIC<=16 µg/ml), Table (6).

Table 6: MIC values and percentages of resistance and sensitivity to some antibiotics used against 50 E.coli isolates isolated from patients with UTIs using VITEK-2

MIC (Mg/ml)	*E.coli* isolates						Antibitic Name
	Total		(S)		(R)		
	(%)	No.	(%)	No.	(%)	No.	
>=32	(100)	50	(0)	0	(100)	50	Ampicillin Sulbactam
<=4	(100)	50	(36)	18	(64)	32	Piperacillin Tazobactam
>=64	(100)	50	(0)	0	(100)	50	Cefazolin
<=4	(100)	50	(44)	22	(56)	28	Cefoxitin

8-16	**(100)**	**(50)**	**(20)**	**10**	**(80)**	**40**	**Ceftazidime**
4-8	**(100)**	**(50)**	**(20)**	**10**	**(80)**	**40**	**Cefepime**
8-16	**(100)**	**(50)**	**(48)**	**24**	**(52)**	**26**	**Aztreonam**
<=0.5	**(100)**	**(50)**	**(100)**	**(50)**	**(0)**	**0**	**Ertapenem**
<=0.25	**(100)**	**(50)**	**(70)**	**35**	**(30)**	**15**	**Meropenem**
<=2	**(100)**	**(50)**	**(50)**	**25**	**(50)**	**25**	**Amikacin**
>=16	**100**	**50**	**28**	**14**	**72**	**36**	**Gentamicin**
<=1	**100**	**50**	**40**	**20**	**60**	**30**	**Tobramycin**
1-2	**100**	**50**	**42**	**21**	**58**	**29**	**Levofloxacin**
>=16	**100**	**50**	**30**	**15**	**70**	**35**	**Tetracycline**
<=16	**100**	**50**	**88**	**44**	**12**	**6**	**Nitrofurantion**
>=320	**100**	**50**	**30**	**15**	**70**	**35**	**Trimethoprime/ Sulfamethoxazol**
>=64	**100**	**50**	**30**	**15**	**70**	**35**	**Ceftriqxone**
<=0.5	**100**	**50**	**36**	**18**	**64**	**32**	**Tigecycline**

R: Resistance, S: Sensitive MIC: Minimum Inhibitor Concentration

The results of the current study are consistent with many local and international studies, as the researchers (Al-Taai et al., 2018) found that E.coli isolates were resistant to the antibiotics Ampicillin by (93.30%) and (80%) to the antibiotics Cefixime and Ceftazidime. (73.30%) for Trimethoprim/ Sulfamethoxazole, and (46.70%) for Gentamicin. However, the current study did not agree with the researchers' study with regard to Amikacin, as the E.coli isolates recorded a sensitivity rate of (100%), while in the current study, the isolates recorded a sensitivity rate of (50%). As for the MIC values, they were (4-32), (4-128), (<16), and (20-320) µg/mL (Al-Taai et al., 2018).

In the current study, all 50 isolates have selected the study their ability to form biofilms. 50, 49, 45, 44, 42, 40, 33, 32, 27, 23, 22, 21, 20, 18, 16, 14, 13, 10, 7, 6, 3, 3, 2, 18, 18, 16 have shown high Strong on the formation of biofilms by 46% of the total isolates studied. Eleven isolates (48, 41, 35, 34, 31, 19, 17, 15, 12, 5, 4) showed a moderate ability to form biofilms at a rate of 22%. While 1 isolate (30) Weak had a weak ability to form biofilms. In the current study, all 50 isolates have selected the study of their ability to form biofilms. 50, 49, 45, 44, 42, 40, 33, 32, 27, 23, 22, 21, 20, 18, 16, 14, 13, 10, 7, 6, 3, 2, 1) have shown high affinity. Strong on the formation of biofilms by 46% of the total isolates studied. Eleven isolates (48, 41, 35, 34, 31, 19, 17, 15, 12, 5, 4) showed a moderate ability to form biofilms with a rate of 22%. While 1 isolate (30) had a weak ability to form biofilms. For 15 isolates (47, 46, 43, 39, 38, 37, 36, 29, 28, 26, 25, 24, 11, 9, 8) did not show any ability to form no biofilm at a rate of 30%. Table (7) shows the number of bacterial isolates

and their percentage in their ability to form biofilms.

Figure (3): Isolates of E.coli bacteria (10, 7, 6, 3, 2, 1) that have a high ability to form strong biofilms, while isolates (5, 4) have a moderate ability to form membranes, and isolates (9, 8) does not have the ability to form biofilms. C-: Negative Control, C+: Positive Control.

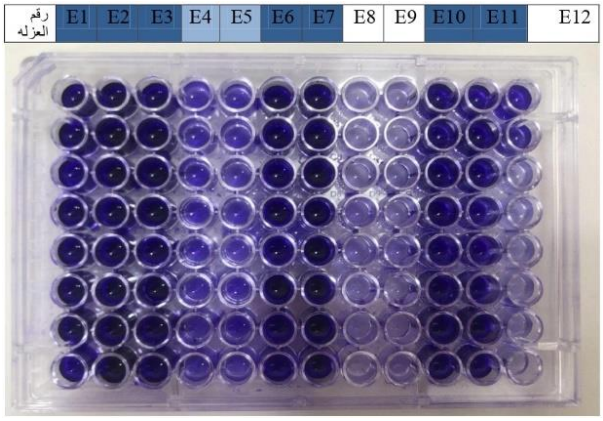


Table (7): Number of E.coli isolates and percentages of their ability to form biofilms

(%)	Of isolates No.	The ability of isolates to form biofilms
46	23	Strong
22	11	Moderate
2	01	Weak
30	15	No Biofilm
100	50	Total

Table (7) shows that the number of total isolates, 35 (70%) of the bacterial isolates, showed the ability to form biofilms at varying rates and that this percentage is consistent with what was reached by Ebraheem and AL-Wendawi in 2015, as a total of 50 isolates were tested. UPEC for biofilm production (100%) by microtiter plate assay. Biofilm production was detected in all bacterial isolates, with different potential capacities for biofilm formation under the same experimental conditions. The tested isolates were restricted to two groups, strong biofilm producers (21 isolates, 42%) and weak producers (29 isolates, 58%). The thickest biofilm ranged from 0.862 to 0.436, while the thinnest biofilm had an O.D of 0.120 (Ebraheem and ALWendawi, 2015). The current results were higher than those obtained in previous studies. The difference in biofilm thickness results from various reasons such as differences in the ability of isolates to form biofilms that depend on many surface determinants such as Fimbriae 1 type, Flagella and F-pilus. The results of (AL-Chalabi et al. in 2010) showed that the thickness of biofilms of UPEC isolates varies according to the nature of bacterial production and multiplication and environmental conditions such as temperature, pH and type of urinary tract infection, where UPEC isolates isolated from prostate give a high ability to produce biofilms with a greater thickness than Isolates causing pyelonephritis and cystitis (AL-Chalabi et al., 2010). The current results were also somewhat close to what was reached by Gawad and others in 2018 in the Arab Republic of Egypt and the results of researchers by Poursinal and others in 2018 also in Iran, where the percentage of UPEC isolates that have the ability to form biofilms reached 76.5% and 80%, respectively, in patients with recurrent UTI (Gawad et al., 2018; Poursinal et al., 2018)

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