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Detection Of Acrab Efflux Pumps Gene, Biofilm Formation In Escherichia Coli And Klebsiella Pneumonia Isolated From Pregnant Women Suffered Of Urinary Tract Infections And Diabetes Mellitus In Basrah City, Iraq

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Abstract

Generally, the incidence of multidrug-resistant of both Escherichia coli and Klebsiellapneumonia has increased among urinary tract infectionsin recent years. The purpose of the present study is to investigate the prevalence of 2 groups of efflux pump genes amongE.coliand K.pneumoniaisolates.E.coli and K.pneumonia were isolated from100 urine samples collected from Pregnant women suffered from UTIs and Diabetes mellitus,conventional bacteriology tests and confirmed by specific 16SrDNA primers.Using polymerase chain reaction efflux pump genes have been investigated. The 96-well polystyrene micro-titer plate method and the tube method were used to investigate biofilm formation. In total, 21(51%) E. coli, 8(20%) K.pneumonia isolates. All isolates were resistant to ampicillin, piperacillin and ticarcillin, expect 3 isolates of E.coli were sensitive to antibiotics susceptibility. 14 (78%) E. coli isolates were multidrug-resistant, 4(50%) K. pneumonia were MDR. On the other hands, AcrA and AcrBefflux pump genes frequency ranges from 78% in E. coli and 50% in K. pneumonia. The results of biofilm formation showed that 15 (83%) of E.coliisolates wereformed biofilm, 3(17%) were non-biofilm forming. 13(86%) was weak biofilm,1 (7%) was moderate, and1(7%) was strong biofilm formation. 4(50%) of K.pneumonia isolates were biofilm formation, 4(50%) of K. pneumonia isolates were non-biofilm formation. 4(100%) K. pneumonia isolates were weak biofilm.Furthermore, a strong correlation was found between the presence of study pumps and the production of biofilm.

Keywords: AcrAB Efflux pumps, Biofilm formation,E. coli, K. Pneumonia, UTIs, Diabetes Mellitus.

1. Introduction

Urinary tract infections are common and widespread in pregnancy period, that consequently led to complications and vitally threat the health of both the pregnant womenand fetus if left untreated [1]. Interestingly, Gram negative bacteria are considered the vital reasonsfor UTIs with approximately75% of the total UTIs in comparison with both Gram-positive bacteria and fungi that recorded less than 25%. Moreover, Enterobacteriaceae are recorded as the predominant type of bacteria that is associated with UTI,where uropathogenicE.coliis responsible for 80-90% of all infections [2, 3].In 2017, hyperglycemia in pregnant women has a dramatic influence that threat the life of both the woman and the newborn together [4]. UTIs in pregnant women with diabetes mellitus are defined as one of the common observed maternalinfections not only because of the physiological and anatomical changes observed in the pregnant rental tractor that predispose them to bacteriuria, but also because of the immune system suppression that linked with diabetes mellitus that consequentlyenhance the development of acute cystitis to acute pyelonephritis and renal abscess [5].

Diabetes during pregnancy isclassified in to Type 1 Diabetes mellitus, Type 2Diabetes mellitus and Gestational diabetes [6, 7]. Antibiotic resistance by bacteria into various levels, where multi-drug resistance (MDR) refers to the bacterial resistance to at least one antibiotic in three or more antibiotic classes[8, 9]. Considerably, Efflux pumpsstand out as a primary pathway contributing significantly to bacterial resistance against antibiotics. Efflux pumps are an integral protein carrier pivotal in the expulsion of various substances outside the bacterial cell, mitigating their harmful impact. these efflux pumps constitute a vital component of drug efflux, a prominent mechanism driving antibiotic resistance in bacteria. Beyond their role in resistance, emerging evidence from numerous studies indicates that efflux pumps may wield influence over a broader spectrum of bacterial behavior. This includes their involvement in biofilm formation, pathogenicity, and virulence, hinting at the multifaceted impact of efflux pump activity on bacterial physiology and adaptation [10].

Within the spectrum of efflux pump families, the Resistance-Nodulation Division (RND) family emerges as a crucial contributor to Gram-negative bacteria's development of antibiotic resistance. Notably, two pivotal members of the RND family, AcrAB, play instrumental roles in this resistance mechanism [11]. The AcrAB-TolC multidrug efflux pump, one of the family members of the Resistance-Nodulation Division (RND) found in E. coli and its homologues, stands at the forefront of multidrug resistance (MDR) in Gram-negative bacteria. Renowned for its pivotal role in expelling a broad spectrum of drugs, AcrAB-TolC pump serves as an invaluable model for comprehending the mechanisms underlying MDR. Its significance is underscored by the availability of detailed information on both its chemical structures and functional attributes [12, 13]. Addressing multidrug resistance in clinically relevant pathogenic bacteria has taken a promising turn through the strategic targeting of AcrAB-TolC using small molecule inhibitors [14]. Many mechanisms are used by biofilm to do this, including diminished permeability of antibiotics through increased expression of efflux pumps[15].

2. Materials and Methods

One hundred urine specimens from pregnant women suffered from UTIs and DM were included in the studybetween September 2022 to February 2023from both of Al-Fayhaa General Teaching Hospital and Al-Basrah General Teaching Hospital for Women and Children, in addition to Outpatient clinics. Sterile containers were used to collect the clean catch mid-stream urine sample. all urine samples were cultivated on MacConkey agar and blood agar plates. Morphological diagnosis, microscopically, biochemical tests and cultural were used for the primary identification of the bacteria isolates. Specific 16SrDNA primers were used for confirmation identification of Escherichia coli and Klebsiella pneumonia.

2.1 Profiling of bacterial isolates

2.1.1 Detection of E.coli using specific primer

E.coli isolates were identified using specific 16S rDNA primers[16, 17], using Forward primer: (GAC CTC GGT TTA CTT CAC AGA), Reverse primer:(CAC ACG CTG ACG CTG ACC). PCR tube with 25µl total volume of reagents included: DNA:1µI, Forward primer:1 µI, Reverseprimer:1 µl, Go Taq Green Master Mix:12.5 µI and Nuclease- free water:9.5 µI. The program of PCR amplification is carried out based on the program described in table (1).

Table (1): PCR amplification program for E.coli isolates using specific 16SrDNA primers.

Steps	Temperature °C	Time	No. of cycles
Initial denaturation	94	4min	1
Denaturation	94	90 sec	30
Annealing	62	90 sec	
Extension	72	2min	
Final extension	72	7min	1

.1.2 Detection of K. pneumonia isolates using specific primer

K. pneumonia isolates were identified using specific 16SrDNA primer [18],Forwardprimer:(ATTTGAAGTTGCAAACGAT),
Reverse primer:(TTCACTCTGAATTTTCTTGTGTTC).
PCR tube of 20 µItotal volume included: DNA:2µI, Forwardprimer:1µI, Reverse primer:1 µI, Go Taq Green Master Mix:5 µIandNuclease- free water:11 µI. The program of PCR was described in table (2).

Table (2):PCR amplification program for K. pneumonia isolates using specific 16SrDNAprimers.

Steps	Temperature °C	Time	No. of cycles
Initial denaturation	95	5min	1
Denaturation	94	30 sec	35
Annealing	58	90 sec	
Extension	72	90 sec	
Final extension	72	10min	1

2.2 Phenotypic Detection of efflux pumps

2.2.1 Efflux pumps activity

Cartwheel EtBr-agar (EtBrCW) is considered as one of powerful technique for identifying elevated efflux activity in big batches of clinical isolates from different types of bacteria. This technique facilitates the comparison of isolates according to their capacity to extrude EtBr. After 18-24 h. incubation, the bacterial isolates were adjusted to 0.5 of a McFarland standard after being cultivated for 24 hours in a shaker with nutritional broth. Radial lines were used to divide the nutrient agar plates into a cartwheel design. The bacteria were then swabbed onto nutrient agar plates with increased concentrations of EtBr using a sterilized cotton swab, and they were cultured for 16hours at 37°C. The culture on the nutrient agar plates was examined using a UV transilluminator. The isolates exhibiting fluorescence at elevated concentration of Ethidium Bromide were.

2.2.2 Genotypic detection of Efflux pumps genes

2.2.2.1 Detection of AcrA gene

AcrA gene was detected in bacterial isolates using specific primers [19]. The detection of AcrA gene in E.coli and K. pneumonia were conducted based on specific primers that are:
Forward primer:(ATCACCTTTCGCACTGTCGT),
Reverse primer:(CGACAAACAGGCCCAACAAG).PCR tube of 25 µl total volume of reagents included: DNA:5µI, Reverse primer:2 µI, Forwardprimer:2 µI, Go Taq Green Master Mix:12 µIandNuclease- free water:4 µI. The program of PCR was described in table (3).

Table (3):PCR amplification program used to amplify AcrA gene in both E.coli and K. pneumonia.

Steps	Temperature °C	Time	No. of cycles
Initial denaturation	95°C	5 min	1
Denaturation	95°C	30 sec	35
Annealing	58.3°C	30 sec	
Extension	72°C	1 min	
Final extension	72°C	5min	1

2.2.2.2 Detection of AcrB gene

The detection of AcrB gene is targeted through the utilization of specific primer[19]. Polymerase chain reaction (PCR)was utilized toConfirm the existence of Escherichia coli and K.pneumonia using specific primers from the AcrB gene:
Forward primer:(CATAAACACGCCCTGGTCCT),
Reverse primer:(GCTACCCGTAAGTCGATGGG) PCR tube of 25 total volume of reagents included: DNA:5µI, Reverse primer:2 µI, Forwardprimer:2 µI, Go Taq Green Master Mix:12 µIandNuclease- free water:4 µI. The program of PCR was described in table (4).

Table (4): PCR amplification program used to amplify AcrB gene in both E.coli and K. pneumonia.

Steps	Temperature °C	Time	No. of cycles
Initial denaturation	95	5 min	1
Denaturation	95	30 sec	35
Annealing	60.3	30 sec	
Extension	72	1 min	
Final extension	72	5min	1

2.3 Biofilm formation

2.3.1Tube method for detection biofilm formation

A qualitative testing of biofilm development capability was detected. Brain heart infusion broth (2ml) was inoculated with loop-full of culture growth and incubated at 37°C for 24 hours. All tubes were emptied and Left to dried for 15 minutes after inverted, then stained with crystal violet, then let tubes sit for 10 minutes, and tubes were washed away with phosphate buffer saline that, tubes were then dried out through inverted and biofilm growth was examined. It was considered positive for biofilm development when an observable film wrinkled the tube's walls.

2.3.2Biofilm formation by using Micro-titer plate (MTP) method

Biofilm formation was detected using sterile micro-titer plate method, It has (96) wells,All isolates were grown over night for 24 hours at 37°C. Bacterial colonies were inoculated in 5ml Brain heart infusion broth. The bacterial suspension was incubatedfor 24 hours at 37°C, then shaking by vortex. 200 µI of Brain heart infusion broth were transferred into wells of the first column of the Micro-titer plate that served as a negative control. In the first three wells of the second column, 200 µI from first bacterial suspension were placed in three replications, after which the same method was followed for each isolate. The plates were incubated at 37°C for 18-24 hours. The Contents of each well was discarded, then washed gently with phosphate buffer saline solution. The plates were stained with 200 µI of crystal violet dye for 45 minutes, then the dye was eliminated by giving it athorough wash in sterile distilled water and leaving plate to dry at room temperature for 45 minutes. 200 µI of absolute ethanol was added. The optical density (OD) was measured using an ELISA device at 630nm. The efficiency of isolates in biofilm production was determined by comparing the readings according to the following equations:

A. The isolation is considered by non-biofilm producers if the optical density rate is the control greater than or aquel the average optical density of isolation ($OD \leq ODc$).
B. The isolate is weakly biofilm producers if the optical density is high for isolation, the average optical density of the control is greater than equal to, or less than twice the optical density to control ($ODc<OD\leq 2xODc$).
C. The isolate is considered moderately biofilm producers if the average optical density of the isolate is greater than twice the optical density of control or equal to or less than four-fold control ($2xODc<OD\leq 4xODc$).

D. The isolate is a strong biofilm producer if the optical density is high isolate was more than four times the rate of control (4xODc<OD).

3. Results

Among 100 pregnant women with diabetes mellitus and UTI: 41 (41%) of urine samples showed positive bacterial growth. Gram negative bacteria were predominant among all isolates accounting for 40 (98%) of isolates, while only 1 (2%)isolate ofGram-positive bacteriaappeared, according to biochemical testingfollowing a general urine screening forurinesamples, as sen in table (5).

Table (5): Number of bacterial growths inUTIs

Samples type			Total	
Positive Culture	G-ve	40 (98%)	No.	%
	G+ve	1 (12%)	41	41.0
Negative Culture			59	59.0

Based on the outcomes of the present investigation's microscopically, biochemical tests and cultural, 40 distinct gram-negative bacterial isolates were discovered from 100 urine samples of pregnant women with UTIs and diabetes mellitus. These isolates constituted 21 isolates (51 %) of Escherichia coli, 8 isolates (20%) were K.pneumonia, table (6).

Table (6): Distribution of pregnant women infected with UTIs and diabetes mellitus.

G-ve bacteria	Isolated bacteria	Total	
		No.	%
	E. coli	21	51.0
	K. pneumonia	8	20.0

Many bacterial species isolates included (n=18) E.coli, (n=8) K.pneumoniain pregnant women with UTIs and diabetes mellitus were shown to be multiple drug resistant (MDR), meaning they were resistant to at least one antimicrobial drug in three or more than three categories of antibiotics. while 3 isolates of ofE. coli showed sensitivity to most antibiotics. out of 18 isolates of E.coli14 isolates were MDR (78%), K. pneumonia 4/8 (50%) MDR isolates, as in table (7).

Table (7): Multiple - drug resistant of E.coli and K. pneumoniaisolates.

Bacterial species	**Total number of isolates**	**Multiple-drug resistance**	**%MDR**
***E.* coli**	18	14	78
K.pneumonia	8	4	50

3.1 Genetic profiling of bacterial isolates

3.1.1 Detection of E.coli using specific primer

The Extracted DNA from 21 E.coli isolates was subjected to PCR for characterized in (585bp), figure (1).

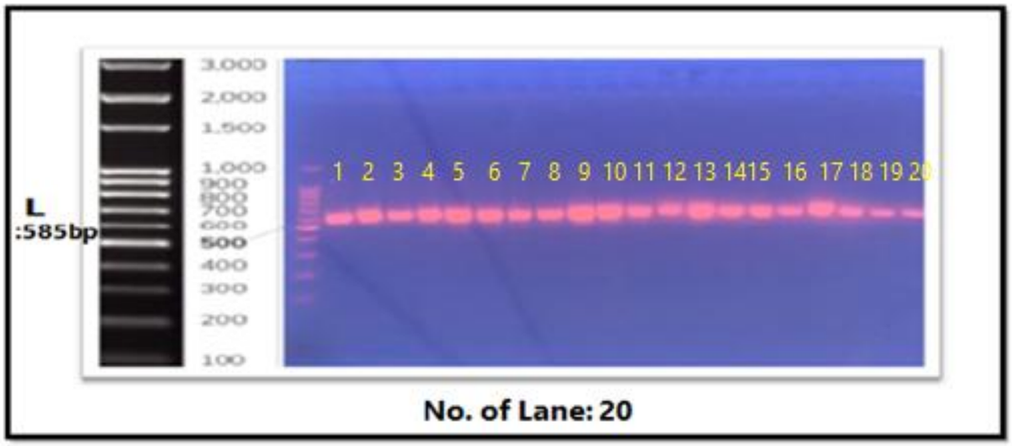
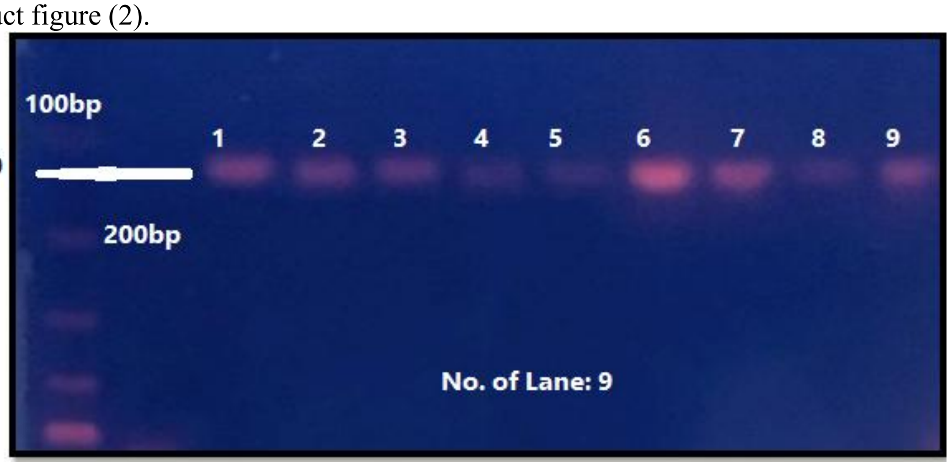


Figure (3-1): Detection of E.coli using Agarose electrophoresis patterns show PCR amplified products, Lane L:(3000 bp DNA ladder), Lane:(No =20) usingspecific primer band of E.coli isolates. using 1.5% agarose gel, 70V, 45minutes

3.1.2 Detection of K.pneumonia using specific primer

The Extracted DNA from 8 K.pneumonia isolates was subjected to PCR for characterized (130bp) PCR product figure (2).

L:130 bp



Figure(2): Detection of K.pneumonia using Agarose electrophoresis patterns show PCR amplified products, Lane L:(3000 bp DNA ladder), Lane:(no=8) specific primer band of K. pneumonia isolates. using 1.5% agarose gel, 70V, 45minutes.

3.2 Biofilm formation

It was found that 19 isolates from 26 isolates (73%)produced biofilm while 7 isolates (27%) did not generate biofilm.Distributions of 7 isolates are (3) E.coli, (4) K.pneumonia.Two different techniques were used to evaluate the isolated bacteria's capacity to form biofilm:

3.2.1Tube method (TM)

Using the tube method to examine isolates' capacity to produce biofilm, It was discovered that 19 isolates from 26 isolates (73%) produce biofilm, while 7 isolates (27%) did not produce biofilm. Distributions of 7 isolates are (3) E.coli, (4) K.pneumonia, as seen in figure (3).

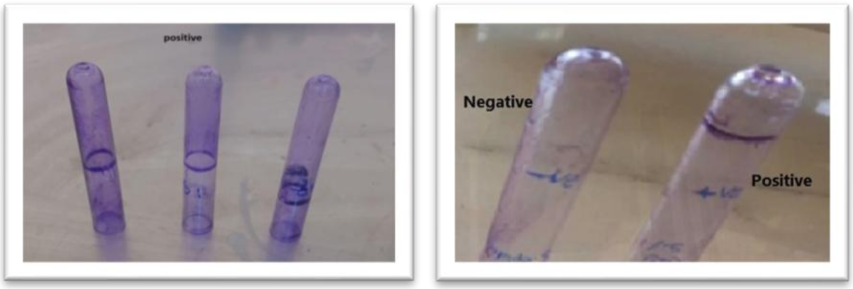


Figure (3): Screening test for biofilm formation by tube method.

3.2.2Micro titer plate (MTP) method

Utilizing a micro titer plate (MTP) to measure isolates' ability to produce biofilm, it was discovered that themajority of isolates were to do so, 19 isolates from 26 isolates were biofilm producers,13 (86%) of Escherichia coli isolates were weak biofilm producers, 1 of E.coliisolates (7%) produce strong biofilm and 1of Escherichia coli isolates (7%) produce moderate biofilm, While 4 of K.pneumonia isolates were weak biofilm producers. The results obtained are presentedin figure (4) andtable (8).

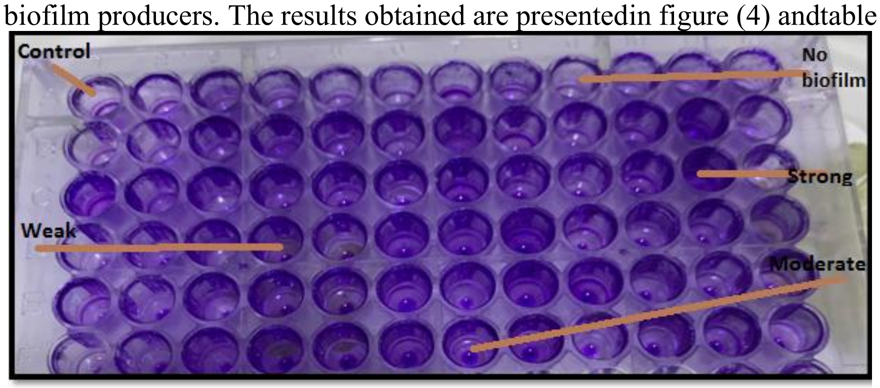


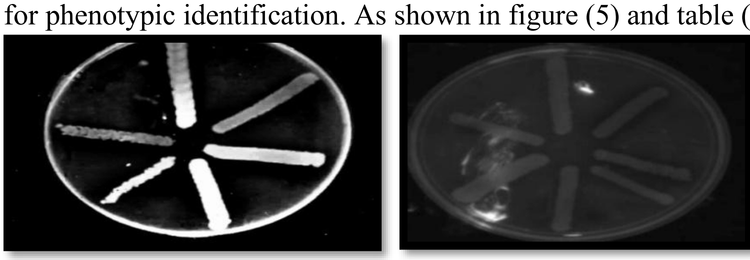
Figure (4): Biofilm formation of bacterial isolate by Micro titer plate method.

Table (8): Biofilm formation in Escherichia coli and Klebsiellapneumoniafrom pregnant women with UTIs and diabetes mellitus.

Bacterial isolates	Number of biofilm	Total number of isolates	Biofilm formation			% of biofilm formation
			Strong %	Moderate %	Weak %	
E. coli	15	15	1(7)	1(7)	13(86)	100
K. pneumonia	4	4	0	0	4(100)	100

3.3 Phenotypic detection of efflux pumps

The efflux pumps were detected in 18E. coliisolates, 8 isolates of K.pneumonia using the ethidium bromide-agar cartwheel method (EtBr CW), relying on the ethidium bromide dye at different concentrations as a guide for phenotypic identification. The results showed the E. coli and K.pneumonia isolates were positive for phenotypic identification. As shown in figure (5) and table (9).



A- Positive efflux pumps B-Negative efflux pumps

Figure (5): Phenotypic detection of efflux pumps in 18 of E.coli isolates, 8 of K.pneumonia isolates using ethidium bromide- agar cartwheel method (EtBr CW), relying on the ethidium bromide dye, A-Positive efflux pumps, B- Negative efflux pumps.

Table (9): Number of phenotypic detections of efflux pumps in E.coli and K.pneumonia

Bacterial isolates	No. of isolates	Phenotypic detection of efflux pumps	% Phenotypic detection of efflux pumps
E. coli	18	14	78
K. pneumonia	8	4	50

3.4 Genotypic detection of efflux pumps genes

3.4.1 Detection of AcrA efflux pumps gene

The Extracted DNA from 26 E. coli, K. pneumoniawas subjected to PCR for characterized of AcrA positive results showed (256 bp) PCR product band, figure (6) and table (10).

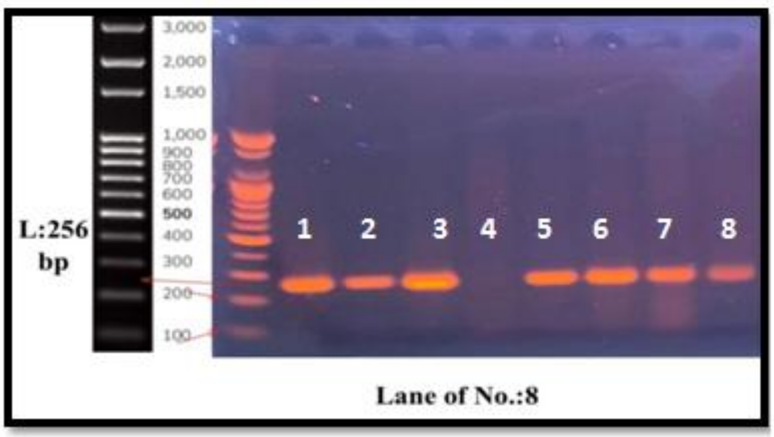


Figure (6):Detection of AcrA efflux pumps gene using agarose electrophoresis patterns showed PCR amplified products of the AcrA gene. Lane L: (3000bp DNA ladder), Lane: (No.:8) AcrA gene bands of bacterial isolates using 0.8% agarose gel, 70 V, 60 minutes.

3.4.2 Detection of AcrB efflux pumps gene

The Extracted DNA from 26 E. coli, K. pneumoniawas subjected to PCR for characterized of AcrB positive results showed (432bp) PCR product band, figure (7) and table (10).

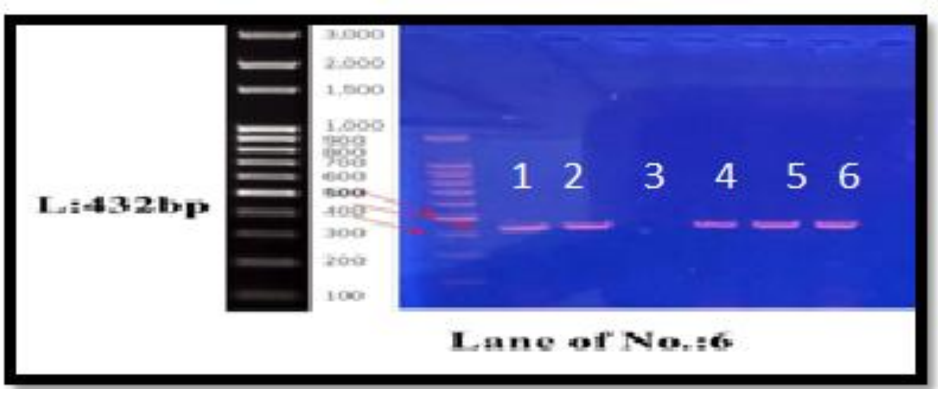


Figure (7): Detection of AcrB efflux pumps gene using agarose electrophoresis patterns show PCR amplified products of the AcrBgene. Lane L: (3000bp DNA ladder), Lane: (No.:6)AcrB gene bands of bacterial isolates using 0.8% agarose gel, 70 V, 60 minutes.

Table (10): Percentage ofmolecular detection ofAcrA and AcrB efflux pumps in E. coli and K. pneumonia

Test results	Molecular detection of AcrA and AcrB efflux pumps gene in E. coli (%)	Molecular detection of AcrA and AcrBefflux pumps gene in Klebsiella pneumonia (%)
Positive results	14 (78)	4 (50)
Negative results	4(22)	4(50)

The relationship between phenotypic detection and molecular detection of efflux pumps and the ability to form biofilm in isolates of 18 E. coli and 8K.pneumonia, as seen in table (11) and table (12).

Table (11): The relationship between phenotypic detection and molecular detection of AcrA and AcrB efflux pumps and the ability to form biofilm of E. coli isolates.

Test results	The relationship between Phenotypic detection of efflux pumps-possessing a biofilm (%)	The relationship between Molecular detection of efflux pumps-possessing a biofilm (%)
Positive results	11 (61)	12 (67)
Negative results	1 (6)	1 (6)

Table (12): The relationship between phenotypic detection and molecular detection of AcrA and AcrB efflux pumps and the ability to form biofilm of K. pneumoniaisolates.

Test results	The relationship between Phenotypic detection of efflux pumps-possesing a biofilm (%)	The relationship between Molecular detection of efflux pumps-possesing a biofilm(%)
Positive results	3 (38)	4 (50)
Negative results	4 (50)	4 (50)

4. Discussion

Among 100 Urine samples collected in the current study 41samples (41%) showed positive bacterial growth, as shown in table (5). E. coli and K. pneumonia were the predominant types of bacteria that were responsible for UTIs with (51%) and (20%), respectively as illustrated in table (6). Generally, E.coli is recorded as the most prevalence bacteria in UTIs. this result in agreement with previous studies byJoni, [20], in Iraq who observed the prevalence of E. coli in pregnant women with urinary tract infection and diabetes mellitus were 50%. Interestingly, the results in the current study are similar with the results carried out by Khoshaba et al. [21], in Iraq who found the prevalence of K.pneumonia in pregnant women with urinary tract infection and diabetes mellitus was 17.27%.the results in the current study are similar with the results carried out by Naher et al. [22], in Bangladesh who found the prevalence of Escherichia coli, Klebsiella pneumonia in pregnant women with urinary tract infection and Diabetes mellitus was (57.9%), (21.1%), respectively. E. coli bacteria are the main cause of urinary tract infections (UTIs) infecting about 90% of patients suffering from urinary tract infections in the world [23]. The current study is consistent with many local and international studies that reported that one of the primary causes of urinary tract infections was Escherichia coli. The reason of the link between pregnant women with diabetes and urinary tract infection is considerably referred to mitigation of neutrophil response and decrease of cytokines in urine and decrease of leukocyte concentration Joni, [20], it was found that the invasion of microbes to the epithelial cells lining the urinary tract is easier in comparison with to non pregnancy is associated with diabetes due to the lack of local cytokines secretion [24].The difference in the infection rate is due to the difference in geographic and healthy conditions and the number of samples, as well as the use of antibiotics before taking the sample [25]. The reason for the high percentage of E. coli bacteria may be due to the bacteria's adaptation to live in urinary tract environment and its tolerance of unsuitable environmental conditions, in addition to its possession of many powerful virulence factors that increases its ability to cause infection. The most important reason for the high percentage of these bacteria in urinary tract infections is the transfer of bacteria from the outlet opening, which is their natural environment to urinary opening [26]. The diagnosis of E. coli was conducted using specific primer 16SrDNA, which is known for being a stable gene and exhibiting some heterogeneity over an extended period in the bacterial type, was used to perform molecular diagnostics on all E. coli isolates using PCR technology. The results indicated that the diagnostic gene sequence for E. coli bacteria was present in all isolates at a percentage of (100%), as shown in figure (1). The results in the present study agree with the results of the other study reached by Maleki et al., [27], in Iran and the study by Lai et al. [28], in Malaysia, also the results in this study agree with the results of the study conducted by Jenkins et al. [29] and the study by Hussein and Naser, [30] in Iraq. This method is a rapid and precise way to detect bacteria and has high sensitivity for bacterial diagnosis. This gene has stable regions that coincide with variable regions that help identify the bacterial genus and species. It is mildly diverse or changeable. The genetic sequence changes very little over time [31, 32].The diagnosis of K. pneumonia was carried out using specific primer 16SrDNA, which is known for being a stable gene and exhibiting some heterogeneity over an extended period in the bacteria. The results indicated that the diagnostic gene sequence for K. pneumonia bacteria was present in all isolates at a percentage of (100%). The results in the present study agree with the results of the other study reaches by Osman et al. [18], in Egypt and Mozan and Al-Amara, [33] in Iraq. Genotyping is important to identify cases or outbreaks due to K. pneumoniae and to further track the source and spreading of infections [34]. Phenotypic methods are less reliable than genotypic methods for characterizing bacteria because phenotypes can vary depending on environmental factors, such as temperature, pH, and growth conditions. Therefore, using specific primer 16SrDNA for diagnosis is preferable to using biochemical and phenotypic methods, since it has several benefits: the gene is universal among bacteria and does not mutate frequently [35]. Tatusova et al. [36], suggested that NCBI is introducing a novel way to present microbial genomes and a new non-redundant protein data model. The results in the current study showed that 14 (78%) of E. coli, 4 (50%) of K.pneumonia in pregnant women with UTI and DM were MDR. The results in the current study are consistent with the results reached by (Khalafet al. [3], in Iraq and Al-Daamy, [37], in Iraq found that (63.6%), (73.3%) of E.coli isolates were MDR. The results in the current study are consistent with the results reached by (Khalafet al. [3], in Iraq found that (38.8%) of K.pneumonia isolates were MDR. Our findings indicated that the bacterial isolates were multidrug resistant (MDR), which may have resulted from a mutation or the acquisition of a plasmid that gave the cell a high level of resistance. Multi-drug resistant E. coli has also become increasingly common because of the extensive and inappropriate usage of broad-spectrum antibiotics [38]. Since E. coli is the primary cause of UTIs, one of the largest health concerns in the world, this high level of antibiotic resistance is regarded as one of the most significant health challenges [39]. The development of novel antibiotics may boost efforts to combat multi-drug resistance (MDR) in bacteria, but, in cases where resistance is mediated by modifications to cell wall permeability, innovative antibiotics might not be enough to counteract widespread resistance [40]. Drugs that actively move out of cells through efflux pumps may be the source of reduced permeability, which lowers the intracellular concentration of antibiotics [41, 42]. A rising number of pathogenic isolates are resistance to antibiotics, particularly multi-resistance, which makes it more difficult to select the best course of action and raises the mortality rate [43, 44]. Antibiotics resistant bacteria pose a major threat to human health because there is currently no effective treatment for them. Research is required to determine whether new antibiotics are effective against these bacteria [45].The results showed that (100%) of Escherichia coli isolates from pregnant women with UTI and DM produced biofilm to different degrees, compared to the negative control, so it was 1 isolates (7%) of which have the ability to produce a strong biofilm adherent strong, 1(7%) isolates bacteria with the ability to produce moderately adherent biofilm and 13 (86%) isolation bacteria have the ability to produce an adherent weakly biofilm and the results showed that 4 (100%) of K.pneumonia isolates from pregnant women with UTI and DM have the ability to produce biofilm to different degrees, compared to the negative control, so it was 4 isolates (100%) of which have the ability to produce a weak biofilm, as shown in table (8). The results of the current study regarding the ability of bacteria to form biofilm are similar with the results obtained by Zhao et al. [46], in China who showed that the percentage (87.9%) of Escherichia coli isolates produced biofilm to different degrees. Also, the results conducted by Gawadet al.[38], in Egypt, and the results reached by Poursinaet al. [47], in India, which bacterial isolates that can produce biofilm in (75.5%) and (80%), respectively. The results of the current study regarding the ability of bacteria to form biofilm are similar with the results obtained by researcher Cruz-Córdovaet al. [48], in Egypt, who showed that the percentage of K.pneumonia (91.7 %). Also, the results of the study conducted by Ammaret al. [49], in Mexico, which the percentage reached bacterial isolates that could produce biofilm (100%). Biofilm-producing bacteria are 1000 times more resistant to antibiotics than non-biofilm bacteria. This is caused by various factors, like: the lack of diffusion of the antibiotic through the viscous interfacial material of the membrane Biogenesis: the transfer of resistance genes within a biofilm environment between bacterial cells, whether mediated by Plasmid or transposons or due to random mutations that lead to resistance antibiotics and toxins, efflux pumps are more expressed in cells components of the biofilm, the biofilm is repaired by a change in the concentration of ions and acetyl ions, pH and finally the presence of presister cells that are also inactive and have a role in preservation on bacterial cells located under the biofilm as a mechanical self-defense mechanism [50]. The isolates were visualized under (UV) source [50]. The results showed that (78%) of E. coli isolates frompregnant women with UTIs and DM detection of efflux pumps. The results of this study converge with the results achieved by Gawadet al. [38], in Egypt who found that (92%) of the isolates revealed positive results for phenotypic detection of efflux pump.Moreover, efflux pumps were responsible for the bacteria acquiring the character of multiple resistance to antibiotics. On the other hands, (50%) of K. pneumoniaisolatesfrom pregnant women with UTI and DMshowed positive phenotypic detection of efflux pumps. The results of this study converge with the results of reached by Patilet al. [52], in India who recorded that (55.32%). of K. pneumonia showed efflux pumps activity. The expression by bacteria of efflux pumps isclosely linked to resistance to antibiotics, Efflux pumps are the main reason for bacteria to acquire the character of multiple resistance to antibiotics, as efflux pumps work to expel large quantities of chemicals,including antibiotics, out of the bacteria by reducing their concentration inside. The cell thus inhibits its function while at the same time giving the bacteria the opportunity to be exposed to random mutations [53]. Molecular detection results for the AcrA gene, which has a size of (256 bp) strands of base, showed (PCR) using a thermocycler, (78%) of E. coliin Pregnant women with UTIs and DM possessed AcrA gene, as seen in figure (6). The results of this study are consistent with the results reached by Malekiet al. [27], in Iran where (82.9%) of the bacterial isolates were multiple antibiotic resistance. Moreover, 50% of K. pneumonia in Pregnant women with UTIs and DM possessed AcrA gene. The results of this study are consistent with the results reached by researcher Razaviet al. [54], in Iran (52.72%). The researcher showed that K. pneumoniawas multiple antibiotic resistance. Molecular detection results for the AcrB gene, (78%) of E. coliin pregnant women with UTIs and DM carried the AcrB gene, as shown in figure (7). The current results are close to the results recorded by Ali and Al-Dahmoshi, [19] in Iraq and Kafilzadeh and Farsimadan, [55], in Iran, where (86%), (75%) of E. coliisolates possess the AcrB gene,respectively. Interestingly, our results found that Escherichia coli isolates possessed both AcrA and AcrB genes together in the same ratio (78%). This is consistent with the results reported by Gawadet al. [38], in Egypt, where (74.84%). of E. coli isolates carried both genes together. Moreover, the current result showed that (50%) of K. pneumoniapossessed the AcrB gene. These results are closely agreed with Razavi et al. [54], in Iran, who recorded that (52.72%) of K. pneumonia isolates carried AcrB gene. It was found that the bacterial isolates possessed the genes AcrA and AcrB together in the same ratio (50%). This is consistent with the results of Razavietal. [54], in Iran who mentioned that (52.72%) of bacterial isolates carried both genes together. The relationship between the susceptibility of E. coli on biofilm formation and its possession of

Efflux pumps goes back to the role of efflux pumps in biofilm formation, as well as encoded genes. The ToIC-AcrAB efflux pump was found to be significantly regulated under the influence of biofilm and during exposure for many antibiotics as shown this researcher stated that the role of the AcrAB efflux pump during biofilm formation is to prevent the accumulation of toxic substances including intracellular antibiotics [56]. The relationship between the susceptibility of K. pneumonia on biofilm formation and its possession of efflux pumps played a vital role in biofilm formation, as well as the encoded genes. Given that the AcrAB pump plays a role in the production of K. pneumoniabiofilms, it is possible that inhibition of the efflux pump system caused the disruption of biofilm formation [57].

5. Conclusion

The results of the current study showed that both E. coli and K. pneumonia were recorded as the predominant types of uropathogenic bacteria that are associated with UTLs. Moreover, the results emphasize the efflux pump pathway that plays crucial role in antibiotic resistance by both Uropathogenic o E. coli and K. pneumonia through the high incidence of both AcrA and AcrB genes. Furthermore, our study suggest the combination of the ability of the isolates to form biofilm with their efflux pump activity.

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