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Distribution of *dfrA1* and *cat1* antibiotic resistance genes in uropathogenic *Escherichia coli* isolated from teens pregnant women in Iraq

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Abstract

The present study aims to detect the distribution of *dfrA1* and *cat1* antibiotic resistance genes among uropathogenic Escherichia coli (UPEC) in pregnant teens women and determine their susceptibility to common antibiotic uses. We collected urine (116) samples from patients in hospitals in Baghdad, Iraq. Isolation and identification of bacteria (culturing, biochemical test, and genetically by *16S rRNA* gene), antibiotic susceptibility tests (eight antibiotics), and detection of the *dfrA1* and *cat1* resistance genes, and used SPSS program for statistically analyzing the results. The distributed UPEC in patients most than another causative agent in percentage (50%). It was highly resistant to Trimethoprim (82%) and Cefotaxime (82%) antibiotics. And they highly distributed frequency for *dfrA1*-gene (Trimethoprim resistance gene) (74%) than *cat1*-gene (Chloramphenicol resistance gene) (38%).

Keywords: Urinary Tract Infections; Uropathogenic *Escherichia coli*; Teens pregnant women; *dfrA1* and *cat1* genes.

انتشار جينات مقاومة المضادات الحيوية *dfrA1* و*cat1* في *Escherichia coli* الممرضة البولية المعزولة من المراهقات الحوامل في العراق

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الخلاصة

تهدف الدراسة الحالية إلى الكشف عن انتشار الجينات المقاومة للمضادات الحيوية *dfrA1* و*cat1* في *Escherichia coli* الممرضة البولية من النساء المراهقات الحوامل وتحديد مدى حساسيتها للمضادات الحيوية وانتشار. جمعت (116) عينة ادرار من مرضى في مستشفيات بغداد، العراق. تم عزل وتشخيص البكتريا بواسطة (الكشف الزرعي على الاطباق، والاختبار البيوكيميائية والوراثي بواسطة جين *16S rRNA*)، واختبارات الحساسية للمضادات الحيوية (ثماني مضادات حيوية)، والكشف عن الجينات المقاومة *dfrA1* و cat1، واستخدام برنامج SPSS لتحليل النتائج إحصائيًا. كان انتشار UPEC في المرضى أكثر من العامل الممرضة المسببة الآخر بنسبة (50٪). وكانت شديدة المقاومة للمضادات الحيوية Trimethoprim (82٪)

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وCefotaxime (82٪). وقد كان جين *dfrA1* (جين المقاومة لل Trimethoprim) (74٪) أكثر انتشارا فيها من جين *cat1* (جين المقاومة لل Chloramphenicol) (38٪).

Introduction

Urinary tract infections (UTIs) in community and hospital settings are among the most common pathological situations. UTIs have been developed worldwide each year, with high public costs regarding medical expenses and hospitalizations. During pregnancy, UTIs are common and can be related to a negative consequences for both the mother and fetus [1]. UTIs are mainly the second most common infections bacterial after respiratory tract infections, seen in primary care. UTIs is a bacterial invasion-induced inflammatory reactions of the urothelium related to bacteriuria and pyuria [2].

Anatomically, divided of UTIs are into two portions, pyelonephritis is an upper urinary tract portion (ureters, renal pelvis, and kidneys), and cystitis is a lower urinary tract portion (urethra and urinary bladder) [3].

UTIs In both pregnant and non-pregnant women, it is the most frequent bacterial infection. UTIs with in pregnant women is are higher among non-pregnant women. Moreover, it has previously been observed that pregnant women incline to develop frequent UTIs. Physiological changes and hormonal in the urinary tract of pregnant women, an infection may be facilitated by changes in bladder volume and ureteral dilation [4, 5]. The most common kind of bacterial infection among pregnant women is uropathogens. Uropathogens involve Gram-negative (E. coli, Pseudomonas aeruginosa, Klebsiella oxytoca, K. pneumonia, and Proteus mirabilis), Gram-positive bacteria (Enterococcus facecalis and Staphylococcus saprophyticus). Among the joint causative agent focus on UPEC and associated with UTIs progress, which is predominantly and most frequent causative agents responsible for 80 %–90 % of infection during pregnancy [6-8].

Antibiotic resistance has become a worldwide health threat that will need a concerted effort from various stakeholders to combat. Broad antibiotics for resistant bacteria may result in illness in humans less responsive to treatment with conventional antibiotics [9]. The situations of deficiency directly related to pregnancy commonly prescribed drugs, such as supplementation with drugs like iron, folic acid, calcium, and vitamins in the management of anemia, or for the treatment of conditions secondary to pregnancy such as urinary tract infection, eclampsia, and many more [10].

Trimethoprim is a common antibiotic used to treat UTIs in all countries of the world [11]. Treatment failure and increased burden in primary health care and first-line therapy result from the widespread use of Trimethoprim antibiotics [12]. Mutations in a dihydrofolate reductase (dfr) gene lead to trimethoprim resistance [13].

Chloramphenicol is a wide-spectrum antibiotic used extensively in medicine until worry over its toxicity emerges [14]. Resistance to Chloramphenicol may be mediated either enzymatically (chemical inactivation of the drug) or non-enzymatically (efflux). The enzymatic Chloramphenicol resistance through Chloramphenicol acetyltransferase encoded by cat1-genes, by acetylation of the 3-OH of Chloramphenicol [15].

Because of the importance and riskiness of E. coli, the increased incidence of infection and the possibility of the epidemic of infection as well as the riskiness of the disease and the lack of treatment have led to the focus of researches in the world on improving the efficiency of molecular detection using advanced technologies and reduce time and effort, the best diagnostic methods, such as PCR technique, are characterized by the technique of specificity and high speed in the detection of the genes encoding for the virulence and antibiotic resistance factors in isolates of E.coli isolated from the clinical samples [16].

Materials and Methods

Collection of urine samples

The urine samples from patients who were admitted with UTIs were collected from Al-Alwaiya Maternity educational Hospital, and Abu-Ghraib hospital in sterile containers, the containers transfer immediately to the laboratory for microbiological analysis. Through During the period from 1st December 2019 to 1st March 2020, samples were collected from pregnant women in an aged less than 18 years old (teens).

Isolation and identification of bacterial isolates

The urine samples were cultured on different media Blood agar and MacConkey agar and incubated at 37 ˚C for overnight. The E. coli isolates was identified using conventional methods and molecular method by detection detecting the 16R rRNA gene at the microbiology research laboratory / Department of the Biotechnology/ College of Science/ University of Baghdad.

Antibiotic susceptibility tests

The E. coli isolates were tested to for the susceptibility of antibiotics by disk diffusion (Kirby-Bauer) method, by cultured culturing the bacterial colony on Muller-Hinton (MH) agar. Eight antibiotics (Mastdisc, U.K.) used in these studies: Aztreonam (ATM, 30µg), Chloramphenicol (C, 30µg), Cefotaxime (CTX, 30µg), Gentamicin (GM, 10µg), Imipenem (IMI, 10µg), Nitrofurantoin (NI, 300µg), Norfloxacin (NOR, 10µg), and Trimethoprim (TM, 5µg), the diameter of inhibition zone was measured and compared to the chart provided by National Committee for Clinical Laboratory Standard Institute (CLSI, 2017). Calculated the Multidrug resistance (MDR) when isolate which showed resistance to 3 classes of antibiotics or more.

Molecular Assay

The E coli Genomic was extracted using Geneaid™ DNA Isolation Kit. The extracted DNA is used as a templet template for polymerase chain reaction (PCR) amplification. PCR analysis was performed for the detection gene associated with the identification of E. coli (16S rRNA), Trimethoprim resistant (dfrA1) gene, and Chloramphenicol resistant (cat1) gene, sequence of primers and size of the product are described in Table 1.

Table 1- The primers sequence and the size of the amplicon of genes.

Gene		Primer	Sequence of primer (5'-3')	Product size (bp)	Reference
Antibiotic resistance genes	***dfrA1***	F: cat1 R: cat1	GGAGTGCCAAAGGTGAACAGC GAGGCGAAGTCTTGGGTAAAAAC	367	[18]
	cat1	F: dfrA1 R: dfrA1	AGTTGCTCAATGTACCTATAACC TTGTAATTCATTAAGCATTCTGCC	547	[19]
Identification gene	***16S rRNA***	F:16S rRNA R:16S rRNA	GGAAGAAGCTTGCTTCTTTGCTG GAGCCCGGGGATTTCACAT	546	[20]

* *cat1*: Chloramphenicol resistance gene, *dfrA1*: Trimethoprim resistance gene, bp: base pair.

The total reaction volume for monoplex and diplex PCR reaction were was up to 25µl. For monoplex, 12.5µl of Go Taq®Green Master mix (2X), 0.5µl for forward and reverse primers (10 pmol), 2µl of DNA concentration, 9.5µl of nuclease-free water. For diplex (dfrA1, and cat1 genes) 12.5µl of Go Taq®Green Master mix (2X), 0.5µl for two forward and two reverse primers (10 pmol), 2µl of DNA concentration, 8.5µl of nuclease-free water. Used PCR thermal cycler (MultiGene, Labnet, U.S.A.) to carry out the amplification reaction as follows: for 16S rRNA gene, Initial denaturation 5 mins at 95ºC, followed by 40 cycles of denaturation at 94ºC for 1 minute, annealing for 90 secs at 55ºC, and extension at 72ºC for 1 minutes, followed by a final extension 10 mins. at 72ºC, for dfrA1 gene, Initial Denaturation one cycle 5 mins at 94ºC, 30 cycles each cycle of 1 mins at 95ºC, 70 secs. at 55ºC, and 2 mins at 72ºC, followed by a final extension of 8 mins at 72ºC, for cat1 gene, Initial Denaturation one cycle 8 min. at 94ºC, 30 cycles each cycle of 45 secs at 95ºC, 1 mins at 59ºC, and 1 mins at 72ºC,

followed by a final extension 8 mins at 72ºC, for diplex (dfrA1, and cat1 genes) Initial Denaturation one cycle 8 mins at 94ºC, 35 cycles each cycle of 45 secs at 94ºC, 45 secs at 60ºC, and 45 secs at 72ºC, followed by a final extension 10 mins at 72ºC.

The PCR products were identified using electrophoresis through an agarose gel (1.5%) to determine the size of the amplified fragment; after staining ethidium bromide (0.5g/ml) dye, then the visualization was done using a UV-transilluminator.

Statistical analysis

Data were subjected to analysis using SPSS. The differences among proportions were determined used using the ANOVA one-way and Chi-square tests. P-value≤0.05 is considered statistically significant.

Result and Discussion

Isolation of Bacteria

A total of 116 samples of urine were collected from patients in two hospitals, Al-Alwaiya Maternity educational Hospital and Abu-Ghraib Hospital, in Baghdad from 1st December 2019 to 1st March 2020. Results revealed that out of 116 samples, only 100 samples (86.2%) showed growth. Twenty-five isolates (25%) gave Gram-positive bacteria, and 75 isolates (75%) gave Gram-negative bacteria growth. The E. coli isolates were more distributed in patients in a percentage of 50% (50 isolates) than other bacterial isolates according to typical morphological characteristics, biochemical tests, and PCR detection by the 16S rRNA identification gene (Figure 1).

Table 2 shows E. coli isolates distribution according to age, pregnant frequency, and pregnant month of patients. It is statistically significant (P<0.05) according to both age and pregnant months. Regarding the pregnant frequency, the different distribution of E. coli isolates was nonsignificant (P > 0.05).

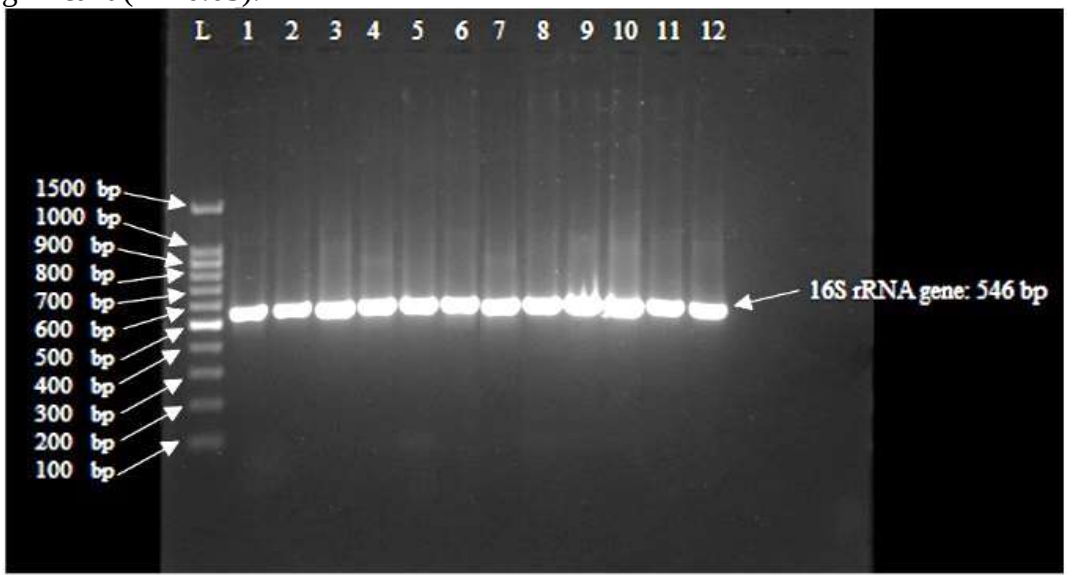

Figure 1-PCR detection for *E. coli* by *16s rRNA* identification gene. Agarose 1%, 5 V/cm for 80 mins, stained with ethidium bromide dye then the visualization was done using a UV-transilluminator. *L Lane: DNA ladder, 1-12 Lanes: the No. of *E. coli* isolates from 1-12. Other Lanes: Negative control (had all PCR mixture with the substitution of water for DNA template).

Table 2- The distribution of E. coli isolates according to ages, pregnant frequency, and pregnant month.

Patients study group		No. of Isolates (%)	Chi *value*	*P-value*
Age	15 **years**	9 (18%)	8.9	0.01*
	16 **years**	15 (30%)		
	17 **years**	26 (52%)		
Pregnant Months	First trimester	13 (26%)	12	0.002*
	Second trimester	28 (56%)		
	Third trimester	9 (18%)		
Pregnant Frequency	First	11 (22%)	5.3	0.07^{NS}
	Second	24 (48%)		
	Third	15 (30%)		

* Statistically significant at *p-value* ≤ 0.05, First-trimester: (1-3pregnant month), Second-trimester: (4-6pregnant month), Third-trimester: (7-9pregnant month).

During pregnancy, UTIs with a high frequency are commonly agreeable because a the human body undergoes physiological changes in a pregnant situation [21]. The risk factors for UTIs during pregnancy beside that it could be explained to by the presence of unique structures in gram-negative bacteria which help attachment to the uroepithelial cells and prevent bacteria from urinary lavage, allowed allowing multiplication and tissue invasion, resulting in invasive infection and pyelonephritis in pregnancy as a complicated state [1].

The E. coli isolates showed at age 17 years were higher than other (15, and 16) years, and in the second pregnant were higher than other (first, and third) pregnant frequency, also in the second trimester of pregnancy was higher than a first or third trimester. E. coli has a high frequency with of UTIs in this study and other studies [22, 23]. An increase in the lactose and amino acid levels during pregnancy promotes E. coli growth. There is a diversity of the pathogens which are responsible for the urinary tract infection and that would be due to the differences in host susceptibility to pathogens as a result of both biological and environmental factors which encourage biodiversity in host, pathogens, vectors, and social factors such as people's efforts in controlling disease [24]. Many virulence factors that enable infection with UTIs either secreted or surface virulence factors explain a high distribution incidence of E. coli isolates. The secreted virulence factors such as α-haemolysin (the most important secreted virulence factor) is a pore forming toxin gain enhance access to host nutrient and iron stores, and damage effector immune cells [25]. Surface virulence factors such as fimbria (adhesive molecules), these types of organelles different ways to in virulence like promoting bacterial invasion, directly triggering host and bacterial cell signaling pathways, and facilitating the delivery of other bacterial products [26, 27].

Antibiotic Susceptibility Test Results

The antibiotic susceptibility results for UPEC isolates of patients with UTIs, which showed varying resistance levels (Figure 2). E. coli isolates showed the highest resistance against Trimethoprim, Cefotaxime, Aztreonam, and Norfloxacin in percentages of 82%, 82%, 78%, and 70%, respectively. In contrast, the isolates were showed low resistance against Gentamicin, Nitrofurantoin, Chloramphenicol, and Imipenem in percentages of 40%, 16%,12%, and 12% respectively. Distribution of antibiotic resistance with age, gestation period, and pregnant frequency shows in Table 3. The statically analysis by used using One-way ANOVA only shows statically significant according to gestation period, at p-value ≤ 0.05.

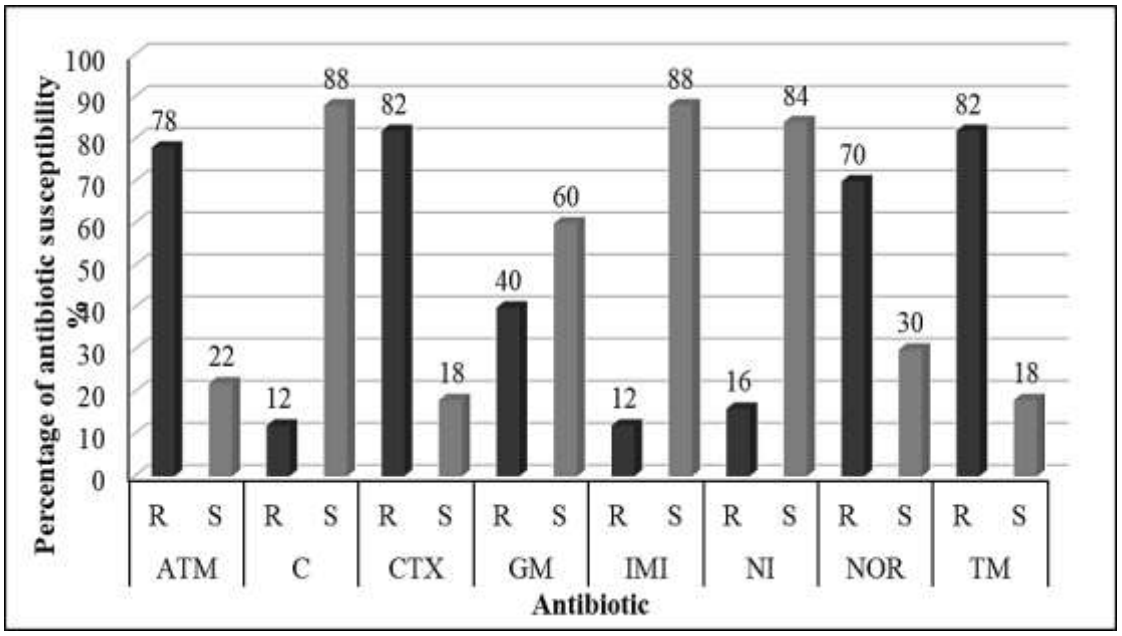

Figure 2-Antibiotic susceptibility results for UPEC isolates of patients with UTI.

* ATM.: Aztreonam, C: Chloramphenicol, CTX.: Cefotaxime, GM: Gentamicin, IMI.: Imipenem, NI.: Nitrofurantoin, NOR: Norfloxacin, TM; Trimethoprim antibiotics, R: resistance, S: sensitive.

Table 3- The distribution of antibiotic resistance according to ages, pregnant pregnancy frequency, and pregnant pregnancy month.

Group / Antibiotic resistance	Age (year)			Gestation period			Pregnant Frequency		
	15	16	17	First trimester	Second trimester	Third trimester	First	Second	Third
ATM	8	14	17	11	22	6	10	19	10
C	0	2	4	0	4	2	3	2	1
CTX	8	15	18	12	22	7	10	20	11
GM	5	9	6	5	12	3	8	7	5
IMI	2	1	3	3	3	0	3	2	1
NI	0	2	6	1	3	4	2	4	2
NOR	7	12	16	11	17	7	9	16	10
TM	8	10	23	11	23	7	8	22	11
Mean	4.8	8.1	11.6	6.8	13.3	4.5	6.6	11.5	6.4
S.E.	1.3	2	2.7	1.8	3.2	0.9	1.2	3	1.6
One-way ANOVA		0.08			0.025*			0.2	

*Statistically significant at *p-value*≤0.05.

Multidrug resistance

Grouping isolates to obtain a pattern of resistance is essential for clearing the view of their infectivity behavior. Accordingly, the results shown in Figure 3 indicate that forty E. coli isolates (80%) showed multiple resistance to various types of antibiotics used in this study. The results of antibiotics show that most isolates of E. coli are resistance frequency to four and five antibiotics that are more than others antibiotic antibiotics.

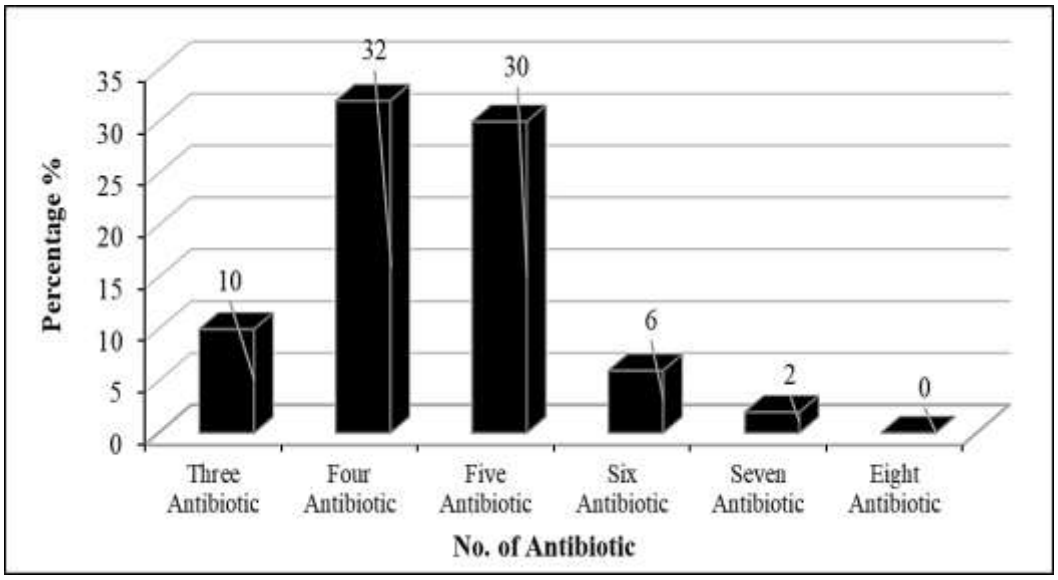

Figure 3-Multi-Antibiotic Resistance results for UPEC isolates of patients with UTI.

Lately used trimethoprim_sulfamethoxazole as the criterion antibiotic for UTIs therapy and because of UPEC strains increasing resistance to this antibiotics class. Therefore, using broad-spectrum antibiotic agents fluoroquinolones with rising recurrence in complicated and uncomplicated UTIs [28], but emerged resistant to fluoroquinolones after a short time [29]. In contrast, the results showed the nitrofurantoin is a choice drug due to its lowest antibiotic resistance 16% in UPEC strains. The previous study reported that 8% to 15% of UPEC strains were resistant to nitrofurantoin [30]. Our results showed that resistance to chloramphenicol in percentage 12%, but this is a forbidden antibiotic because of having a high degree of mutation that increases bacterial resistance to this antibiotic. The Momtaz et al., 2013 study indicates that the irregular and unauthorized use in drug therapy [31].

The resistance of E. coli against the B-lactam group is the highest to cefotaxime (3rd generation cephalosporins) at 82%, and the aztreonam (monobactam antibiotic) resistance at 78%. The resistance of E. coli against β-lactam group is because of many reasons the most important ability of E. coli is the producing production of β-lactamases enzyme that lysis the β-lactam ring [32-34]. Resistance of bacteria to Trimethoprim often results from overproduction of the enzyme (dihydrofolate reductase, DHFR) targeted promoter for the antibiotic by promoter mutation [35]. The resistance of E. coli to Norfloxacin (fluoroquinolone) due to mutations in the enzyme DNA gyrase (decrease the binding ability of the antibiotic), the target for this antibiotic and in the topoisomerase IV the target for Norfloxacin, can render bacterial cells resistant to those antibiotics [36].

Gram-negative bacteria have developed resistance to numerous antibiotics, posing a therapeutic challenge in hospitals and communities [37]. Therefore, it is essential to raise awareness about the non-use of antibiotics only after the made of tests that reveal the sensitivity of antibiotics and describe at being by doctors in this specialty. The antibiotic resistance of bacteria could be due to transferable plasmids carrying resistant genes that are transferred among pathogenic bacteria such as pBS13, pBS12, pB2, and pMS4 plasmids [38, 39]. In addition to that, a particular mutation could occur due to random use and overuse of antibiotics [40]. In recent years, the recurrence of antibiotic resistances among human E. coli clinical isolates rose substantially, posing a severe challenge in treating these illnesses. This high rate of antibiotic resistance might be attributed to the overuse of antibiotics, which has resulted in a selection of novel antibiotic-resistant bacterium strains. In the case of UPEC strains, this event is apparent, antibiotics readily available over the counter without a prescription of a registered medical practitioner, inadequate doses of antibiotics intake, lack of

dependency on laboratory guidance, comparatively cheaper antibiotics intake, and self-prescription policy. The treatment of an infections that are causing caused by E. coli has become more challenging, and it can even rising a morbidity or mortality of a UTIs simple [41].

The preponderance of Gram-negative bacteria, generally Enterobacteriaceae, and notably E. coli, has been shown in several studies to restrict the regional diversity of pathogen incidence in UTIs. The resistance patterns of these organisms might differ substantially between hospitals, countries, and continents in different parts of the world [42]. Because of rising resistance to common antibiotic uses, clinicians have very few antibiotic choices for the UTIs treatment [43]. A transmission phenomenon of antibiotic resistance from bacteria to others spreads worldwide, and the results are dangerous. The selection of optimum antibiotics for treatment is essential to limit this phenomenon and form economic loss and human health. Clearly from the previous, that the choice of suitable antibiotic for treatment is not random. Still, it depends on the antibiotic suitability tests against the microbial isolate to find the appropriate antibiotic to eliminate them. To stop the emergence of resistance and obtain high efficiency in the treatment, choosing an effective antibiotic against the infectious microbial and determining its value and dose [44].

Distribution of UPEC Antibiotic Resistance Gene

The distribution of E. coli resistance gene of patients with UTI for 50 DNA extraction of isolates that identification as E. coli in this study. The results of electrophoresis appearance in 1.5% agarose gel at 70 volts for 80 mins shown in Figure 4 and Figure 5, were dfrA1 (Trimethoprim resistance) gene detected in 37 isolates (74%) of E. coli, cat1 (Chloramphenicol resistance) gene detected in 19 isolates (38%).

In order to confirm the result of the uniplex presence of dfrA1 and cat1 genes in E. coli isolates, several attempts were made to standardize the reaction conditions for genes under study for Diplex PCR. Results of gel electrophoresis showed in Figure 6.

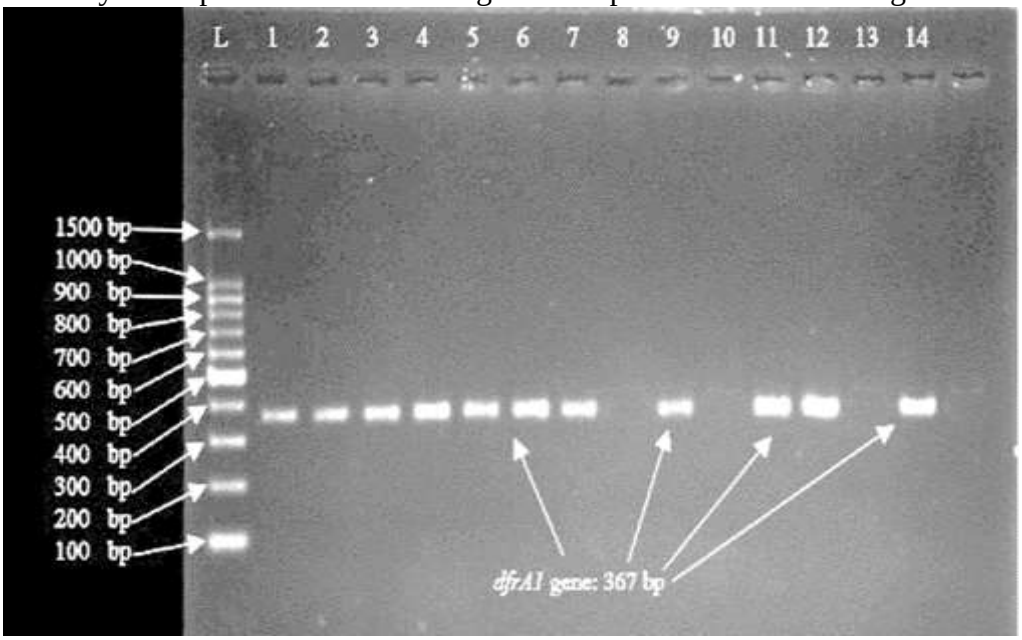

Figure 4-Uuniplex dfrA1 antibiotic resistance gene in UPEC isolates. Agarose 1.5%, 70 volts for 80 mins, stained with ethidium bromide dye then the visualization was done using a UV-transilluminator. *L Lane: DNA ladder, 1-12 Lanes: the No. of E. coli isolates from 11 to 24. Other Lanes: Negative control (had all PCR mixture with the substitution of water for DNA template).

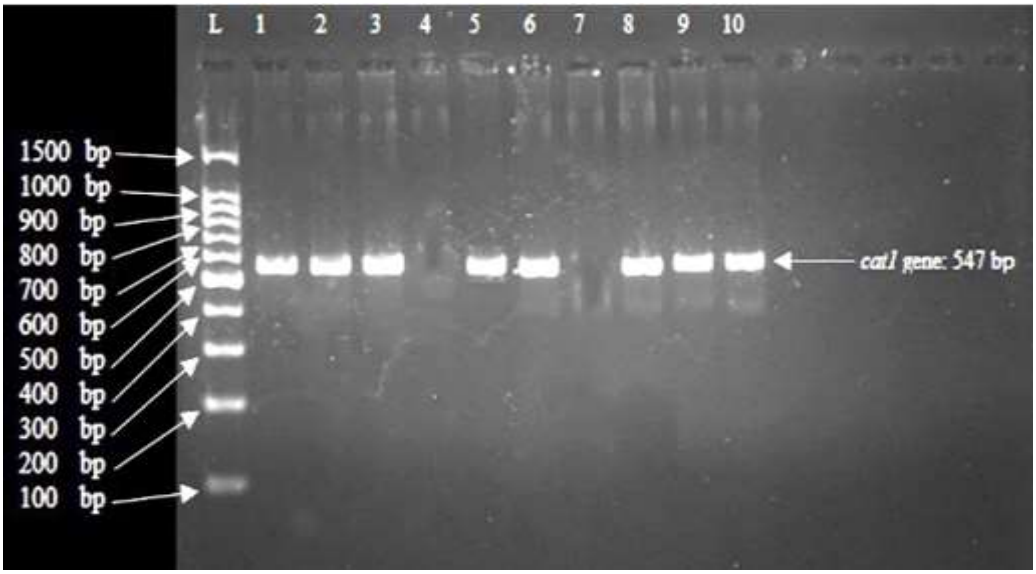

Figure 5- Uuniplex cat1 antibiotic resistance gene in UPEC isolates. Agarose 1.5%, 5 V/cm for 80 mins, stained with ethidium bromide dye then the visualization was done using a UV-transilluminator. *L Lane: DNA ladder, 1-12 Lanes: the No. of E. coli isolates from 41 to 50. Other Lanes: Negative control (had all PCR mixture with the substitution of water for DNA template).

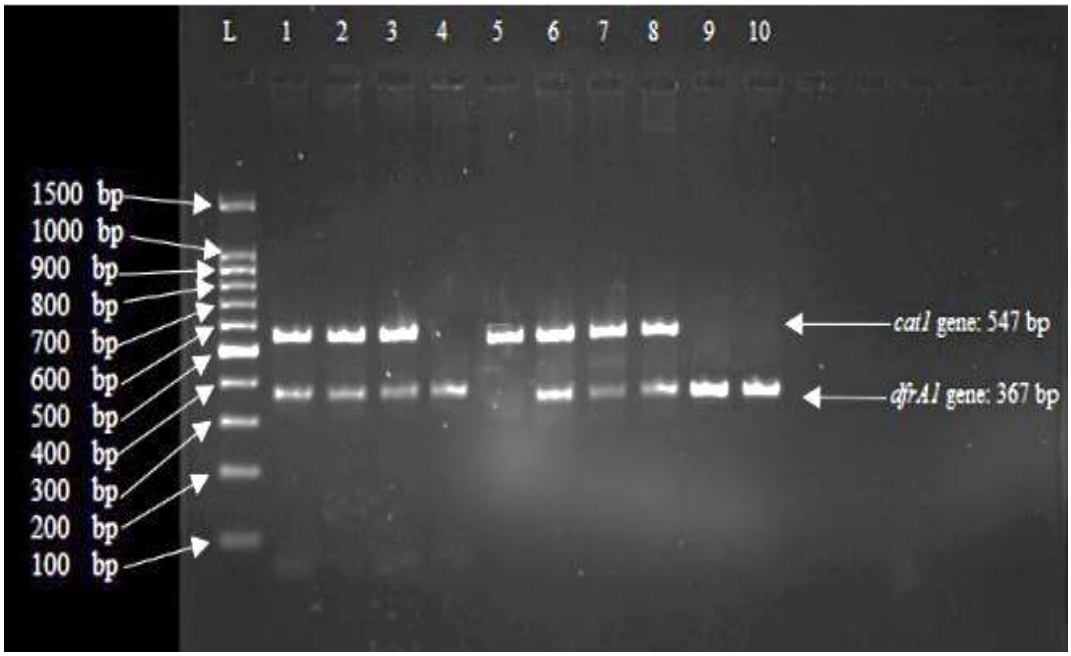

Figure -6 Diplex *cat1* and *dfrA1* antibiotic resistance genes in UPEC isolated**.** Agarose 1.5%, 5 V/cm for 80 mins, stained with ethidium bromide dye then the visualization was done using a UV-transilluminator. *L Lane: DNA ladder, 1-12 Lanes: The No. of *E. coli* isolates from 26 to 29, 32, 33, and 35 to 38. Other Lanes: Negative control (had all PCR mixture with the substitution of water for DNA template).

Cross-tabulation between antibiotic resistance gene and antibiotic susceptibility results of UPEC isolates for pregnant women with UTIs, and Chi-square test. The relationship between Chloramphenicol antibiotic with cat1-Gene (Chloramphenicol resistance gene) is shown in Table 4 appearance the result of the Chi-square test non-significance between them due to a present gene in 17 isolates (34%) in sensitive E. coli isolates. Table 5 showing shows the

relationship between Trimethoprim antibiotics with dfrA1-Gene (Trimethoprim resistance gene). The Chi-square test result is significant between them (P<0.05).

Table 4- The distribution of Chloramphenicol antibiotic resistance with the *cat1*-Gene result and Chi-square test.

		***cat1*-Gene result No. (%)**		Total
		Present Gene	Absent Gene	
Chloramphenicol Antibiotic result	Resist	4 (19%)	2 (6.9%)	6 (12%)
	Sensitive	17 (81%)	27 (93.1%)	44 (88)
Chi-square test		Chloramphenicol Antibiotic with *cat1*-gene	*P-value*	0.192

Table 5- The distribution of Trimethoprim antibiotic resistance with the *dfrA1*-Gene result and Chi-square test.

		***dfrA1*-Gene result No. (%)**		Total
		Present Gene	Absent Gene	
Trimethoprim Antibiotic result	Resist	36 (97.3%)	5 (38.5%)	41 (82%)
	Sensitive	1 (2.7%)	8 (61.5%)	9 (18%)
Chi-square test		Trimethoprim Antibiotic with *dfrA1*-gene	*P value*	< 0.0001*

*Statistically significant at *p-value*≤0.05.

Table 6 shows cross-tabulation between a *cat1* gene with pregnant months, pregnancy frequency, and sample age. In contrast, Table 7 shows cross-tabulation between the *dfrA1* gene with pregnant months, pregnancy frequency, and sample age.

Table 6- The distribution of Pregnant Months, Pregnant Frequency, and Age of Sample with *cat1*-Gene result Cross tabulation and Chi-square test.

		***cat1*-Gene result No. (%)**		**Total**
		Present Gene	**Absent Gene**	
Pregnant Months	**First trimester**	4 (19%)	9 (31%)	13 (26%)
	Second trimester	12 (57.2%)	16 (55.2%)	28 (56%)
	Third trimester	5 (23.8%)	4 (13.8%)	9 (18%)
Pregnant Frequency	**First**	3 (14.3%)	8 (27.6%)	11 (22%)
	Second	12 (57.1%)	12 (41.4%)	24 (48%)
	Third	6 (28.6%)	9 (31%)	15 (30%)
Age of Sample	**15 years**	3 (14.3%)	6 (20.7%)	9 (18%)
	16 years	4 (19%)	11 (37.9%)	15 (30%)
	17 years	14 (66.7%)	12 (41.4%)	26 (52%)
Chi-square test	Pregnant Months with *cat1*-gene		*P-value*	0.507
	Pregnant frequency with *cat1*-gene		*P-value*	0.442
	Age of Sample with *cat1*-gene		*P-value*	0.2

Table 7- Pregnant Months, Pregnant Frequency, and Age of Sample with *dfrA1*-Gene result Cross tabulation and Chi-square test.

		***dfrA1*-Gene result No. (%)**		**Total**
		Present Gene	**Absent Gene**	
Pregnant Months	**First trimester**	10 (27%)	3 (23.1%)	13 (26%)
	Second trimester	20 (54.1%)	8 (61.5%)	28 (56%)
	Third trimester	7 (18.9%)	2 (15.4%)	9 (18%)
Pregnant Frequency	**First**	6 (16.2%)	5 (38.5%)	11 (22%)
	Second	19 (51.4%)	5 (38.5%)	24 (48%)
	Third	12 (32.4%)	3 (23%)	15 (30%)
Age of Sample	**15 year**	7 (18.9%)	2 (15.4%)	9 (18%)
	16 year	9 (24.3%)	6 (46.2%)	15 (30%)
	17 year	21 (56.8%)	5 (38.5%)	26 (52%)
Chi-square test	Pregnant Months with *cat1*-gene		*P-value*	0.896
	Pregnant frequency with *cat1*-gene		*P-value*	0.249
	Age of Sample with *cat1*-gene		*P-value*	0.331

The results from Table 6 and Table 7 show that these genes are more distributed in the second trimester, second pregnancy, and at 17 years for both genes. The results of the Chi-square test are nonsignificant ($p>0.05$) for all of the relationships.

The dfrA1 (Trimethoprim resistance gene) in the UPEC isolates is more detected than cat1 (Chloramphenicol resistance gene) due to the popular trimethoprim antibiotic in UTIs treatment in Iraq. The comparing results of this study with previous studies for distribution of resistance gene in E. coli isolated from UTIs, shows the dfrA1 gene distribution of this study highest than other previous studies by Momtaz et al., 2013 [31], Mashayekhi et al., 2014 [45], Yahiaoui et al., 2015 [46], ALZirjawi and Hamim, 2016 [47], Paniagua-Contreras et al., 2017 [22] and Osman et al., 2018 [48] found the percentage was 21.95%, 19.6%, 18.2%, 10%, 11.3%, and 58.6% respectively. Still, approximately the distribution with Taheri et al., 2016 [48] was 77.5%. For the cat1 gene the Momtaz et al., 2013 [31] and Paniagua-Contreras et al., 2017 [22] found the percentage was 15.44% and 11.3%, respectively less than for this study and in Siasi et al., 2017 [49] was 92.1% most more than for this study.

Using Diplex PCR in this study aims to reduce time and cost by detecting more than one gene in one reaction compared to the Uniplex PCR. This method was used by researcher Al-Alak, 2012 [50] in a molecular study of the adhesion factors in UPEC and its relationship to fluoroquinolones resistance. Kareem also used this method, 2013 [51] researcher to detect the S.T. and L.T. genes in an E. coli bacteria isolated from urine and stool samples by using multiplex PCR technique. Also used this method by Baqer (2013) [52] investigate method comparative study of E. coli O157:H7 isolates from patients in Baghdad, as well as used by Mohammed and Rasheed (2015) [53] for the detection of fimh, sfa, pap, and afa genes in E. coli bacteria isolated from urine samples used multiplex PCR technique.

Conclusion

The genetic detection for of microbial is more efficient than other methods. The E. coli is the most causative agent causing UTIs in patients in our study. The appearance shows variable resistances to various antibiotics, and the dfrA1 gene for Trimethoprim resistance is more than the cat1 gene for Chloramphenicol resistance.

Ethical Clearance

This research was ethically approved by the Research Ethical Committees of the Ministry of Environmental and Health and the Ministry of Higher Education and Scientific Research, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest

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