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Investigation of papA, papC, and papG virulence genes revealed their association with antimicrobial resistance among uropathogenic Escherichia coli strains isolated from patients in Thi-Qar province, Iraq

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Abstract.

Infection of the urinary tract is one of the more common infections in the people of the world, which is more common in women due to the anatomical structure of the woman's body. The main cause of this infection is uropathogenic E. coli, followed by Klebsiella pneumoniae. This disease is controlled and treated by antibiotics, but one of the most important concerns in today's world is the spread of antibiotic resistance in different strains of bacteria. This resistance to antibiotics is caused by specific genes that increase the number of these identified genes every day. This study aims to find the frequency of three coding genes related to p fimbriae, namely papA, papC, and papG, from E. coli strains extracted from patients with urinary tract infections. Also, in this research, by conducting tests related to the detection of the antimicrobial resistance of the extracted strains and using statistical analysis, an attempt has been made to find a significant relationship between the presence or absence of a gene and resistance to antibacterials. At first, 110 urine samples were collected from patients with UTI in the Dhi Qar province of Iraq. In this research, bacterial culture methods in McConkey agar and advanced biochemical tests were used for primary confirmation of E. coli contamination, and Vitek2 and API E20 systems were used for secondary confirmation of bacterial contamination in suspected samples. Finally, the result of these tests was that all 110 samples were infected with E. coli. Then, samples resistant to the antibiotics Ampicillin, Tetracycline, Ciprofloxacin, Nitrofurantoin, and Cotrimoxazole, respectively, were detected by culturing in Mueller-Hinton agar medium and disc diffusion using the CLSI 2021 protocol. 36.36, 24.54, 29.09, 2.72, and 20% of the samples were resistant to these antibiotics, respectively. PCR was used to amplify gene fragments, and agarose gel electrophoresis was used to determine the presence of genes. The result was that the percentages of papG, papC, and papA genes were 61.81, 38.18, and 28.18, respectively. Finally, by performing a chi-square test in SPSS software, it was found that there is a significant relationship between the presence or absence of papA and papC genes with ampicillin and a significant relationship between papG and co-trimoxazole. These findings and the findings of other researchers can help us choose the right treatment and design new treatments by determining the antibiotic resistance factor.

***Keywords*:** urinary tract infection, uropathogenic *E. coli*, papA, papC, papG

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1.1 Introduction

Both outpatient and inpatient UTIs are prevalent. Asymptomatic bacteriuria (ASB), acute uncomplicated cystitis, recurrent cystitis, catheter-associated ASB, CAUTI, prostatitis, and pyelonephritis are all considered "UTI." The further division of these categories includes genitourinary history, including stones, stents, or catheters, sex, concurrent diseases, and urinary system symptoms. Dysuria, urine frequency, and urgency are typical non-catheterized UTI symptoms [1].

UTI incidence and prevalence depend on region, medical specialisation, gender, and concurrent diseases. US women self-reported 12.6% cystitis in 2000, while males reported 3.0%. In 2000, US pyelonephritis hospitalisation rates were 11.7 per 10,000 women and 2.4 per 10,000 men. UTIs affect morbidity, mortality, long-term consequences, antibiotic usage, resistance, and costs. [2]. The most common UTI in all populations is caused by UPEC, a Gramme-negative, facultatively anaerobic, uropathogenic Escherichia coli. E. coli causes 80% of UTIs in healthy women (18–39). UPEC strains of E. coli are better at living in the urinary system and avoiding the host's immune response than stomach strains. UPEC is affected by biofilms and urothelial cell invasion. No diagnostics exist for UPEC, which is extremely variable if an E. coli strain is isolated from urine. Yeast, streptococci, and staphylococci may cause Enterobacteriaceae UTIs. [3]. Over 80% of community-acquired UTIs are caused by UPEC. UPEC cystitis and pyelonephritis are virulent due to adhesives, siderophores, toxins, capsules, and other mechanisms [4].

Symptomatic UTIs are often treated with antibiotics, which may alter the vaginal and gastrointestinal tract microbiota and create multidrug-resistant pathogens. Vacant microbiota niches may boost multidrug-resistant uropathogenic colonisation. The 'golden period' of antibiotics is ending, requiring sensible alternatives. Recently, RNA sequencing was used to evaluate symptomatic UTI pee uropathogens. These studies, foundational research, and better animal models have helped us understand how uropathogens attach, colonise, adapt to the nutritionally constrained bladder environment, elude immune surveillance, and survive and propagate throughout the urinary system [5].

Due to increased antibiotic use in humans and animals, antibiotic resistance is growing. Resistance results from mutations, chromosomal mutations, mobile genetic elements, or both. Plasmids and transposons let bacteria quickly transmit resistance. All antibiotic resistance routes are positive-selected. Inactivation, concentration, or target alterations may occur from antibiotic resistance [6]. First-line treatments for uncomplicated cystitis include nitrofurantoin and fosfomycin, which are seldom resistant in UPEC. Resistance to trimethoprim-sulfamethoxazole, a first-line antibiotic for uncomplicated UTIs in many countries, has increased. Resistance to this antibiotic ranges from 14.6% to 60% in Europe. Drug resistance has increased because of the increasing outpatient use of fluoroquinolones (FQs) like ciprofloxacin. UPEC resistance to FQs is much higher in poorer countries.

The first-line treatment for pyelonephritis or complex UTI is amoxicillin-clavulanic acid. The geographic frequency of UPEC amoxicillin-clavulanic acid resistance varies. Antimicrobial use for UTI therapy must be restricted due to rising UPEC resistance. [7].

Extraaintestinalipathogenic E. coli (ExPEC), which includes avian pathogenic E. coli (APEC) and causes a wide range of poultry extra-intestinal disorders, is likely to have Pap or Fimbria as a virulence factor. The pap gene cluster, 111 genes organised into an operon, encodes these fimbriae. The two most upstream genes in the operon, pap I and pap B, encode proteins that control environmental variables and phase variation in P fimbriae. PapA encodes the primary fimbrial shaft, whereas lpapH, papE, papF, papG, and npapK encode the six other components of the fimbriae [8].

1.2 Aims of the study :

The pyelonephritis-associated pilus (P fimbria) is essential to the UPEC bacteria's pathogenicity. In this investigation, we want to determine the prevalence of three genes—papA, papC, and papG—among UTI-causing bacteria in patients' urine. The frequency of these three genes and antimicrobial resistance should also be examined.

1.3 sample collection

One hundred and ten urine samples were collected from patients with urinary tract infections hospitalized in Al-Hussein Teaching Hospital, whose ***E. coli*** infection was present in the patient records. In the sampling process, 10% error was considered, so 10 more samples than what was written in the proposal were taken. After informing the patients about the research, 100 mL of urine samples were collected from

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the patients. The samples were immediately transferred to Al-Hussein Teaching Hospital and the public health laboratory in Nasiriyah to culture and extract bacteria from the samples. Also, the patients' information was collected from their hospital files while maintaining the confidentiality of the information and identity of the patients.

1.4 Bacterial detection

MacConkey agar is a selective and differential bacterial culture medium. It is intended to identify and distinguish gramme-negative and enteric (usually found in the gastrointestinal system) bacteria based on lactosecfermentation. Lactose fermenters become red or pink on MacConkey agar, while nonfermenters do not. With crystal violet and bile salts, the medium suppresses Grammeme-positive organism development, enabling the selection and isolation of Grammeme-negative bacteria. The pH indicator neutral red detects lactose fermentation by intestinal bacteria in the medium.

Based on the instructions [43], MacConkey agar was used for bacterial detection of *E. coli* using advanced biochemical tests of standard tests for indole, citrate, Voges-Proskauer, urease, hydrogen sulphide (H2S) generation, motility, and lysine decarboxylase (LDC).

1.4.1 Identification in culture medium

According to prior research, the most widely utilised urine culture media are blood agar and MacConkey agar, which are selective and differential for Gramme-negative bacteria. We employed MacConkey agar from Merck with the number 146066 in this investigation, which is especially used for the diagnosis of Enterobacteriaceae and *E. coli*.

It should be observed that bacterial colonies will develop pink on MacConkey agar because it is lactose-fermenting bacteria.

1.4.2 Advanced biochemical identification test of common uropathogens

After we have identified enterobacteria using MacConkey agar culture, it is time to more accurately identify *E. coli* utilising a series of biochemical variables and associated assays.

1.4.3 Procedure

1. The ability to grow on MacConkey agar plates was examined.
2. The ability of the glucose-topped MacConkey agar plate to ferment glucose or lactose was investigated.
3. The ability to ferment according to the standard MacConkey agar plate was examined.
4. A gramme-stained, isolated colony was performed.
5. For Gram-negative rods, , the results of a standard-oxidase test were also done.
6. The results of standard tests for indole,acitrate, Voges-Proskauer, urease, hydrogen sulphide (H2S) roduction, motility,,1motility and lysine decarboxylase (LDC) were studied next.
7. The presence or absence,of nitrites from the urine dipstick test was determined. The presence of nitrites indicates a positive nitrate reduction test.

Following tables show the expected results for *E. coli*:

Table 1 Basic biochemical identification

Bacterium	MacConkey	Gram-Stained Bacterial Cell Morphology	glucose fermentation	oxidase
Enterobacterales	Pos	Red or pink rod-shaped	Pos	Neg

Table 1 Advanced biochemical identification

Bacterium	lactose fermentation	indole	citrate utilization	Voges-Proskauer	urease	motility	hydrogen sulfide production	lysine decarboxylase	nitrates reduction
E. coli	Pos	Pos	Neg	Neg	Neg	Pos	Neg	Pos	Pos

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1.4.4 Using the VITEK® 2 Compact system and API E20 to confirm the reliability of the results of some samples

The VITEK system was established in the 1970s as an automated approach for identification and AST, and it has since evolved into the VITEK 2 system, which automatically performs all of the procedures required for identification and AST once a main inoculum has been generated and standardized. This device provides kinetic analysis by reading each test every 15 minutes. By combining multichannel fluorimeter and photometer readings, the optical system records fluorescence, turbidity, and colorimetric data [44].

Due to uncertainty in the findings of culture and biochemical testing, we used the Vitek2 system to confirm the presence of ***E. coli*** bacteria in the samples. As a result, we sent them to a private diagnostic institution that had this equipment.

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Figure 1 Vitek 2 compact system

The API E20 approach was utilised for these samples in addition to sending them to the laboratory for Vitek2. API identification products include test kits that can distinguish between grammeme-positive and grammeme-negative bacteria and yeast. API strips are user-friendly, standardised testing methods that deliver precise identifications based on extensive databases. Each of the 20 microbiochemical tests included in the kits is simple, quick, and safe to perform.

The Analytical Profile Index (API) 20E is a biochemical panel used to identify and differentiate diverse Enterobacteriaceae strains of bacteria. As a result, it is a tried-and-true method for manually identifying microorganisms down to the species level [45].

The following tests are conducted with the test kit:

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The following tests are conducted with the test kit:

1. **ONPG**: test for β-galactosidase enzyme by hydrolysis of the substrate onitro phenyl-b-D-galactopyranoside
2. **ADH**: the amino acid arginine decarboxylation by arginine dehydratase
3. **LDC**: decarboxylation of the amino acid lysine by lysine decarboxylase
4. **ODC**: decarboxylation of the amino acid ornithine by ornithine decarboxylase
5. **CIT**: citrate utilization as only carbon source
6. **H2S**: production of hydrogen sulfide
7. **URE**: the enzyme urease test
8. **TDA** (Tryptophan deaminase): detection of the enzyme tryptophan deaminase: Reagent- Ferric Chloride.

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9. **IND**: Indole Test-production of indole from tryptophan by the enzyme tryptophanase. Reagent-Indole is detected by addition of Kovac's reagent.
10. **VP:** The Voges-Proskauer test for the detection of acetoin (acetyl methylcarbinol) produced by fermentation of glucose by bacteria utilizing the butylene glycol pathway
11. **GEL**: test for the production of the enzyme gelatinase which liquefies gelatin
12. **GLU**: fermentation of glucose (hexose sugar)
13. **MAN:** fermentation of mannose (hexose sugar)
14. **INO:** fermentation of inositol (cyclic polyalcohol)
15. **SOR:** fermentation of sorbitol (alcohol sugar)
16. **RHA:** fermentation of rhamnose (methyl pentose sugar)
17. **SAC:** fermentation of sucrose (disaccharide)
18. **MEL:** fermentation of melibiose (disaccharide)
19. **AMY:** fermentation of amygdalin (glycoside)
20. **ARA:** fermentation of arabinose (pentose sugar)

The test results for *E. coli* should be as followed:

Table 2 E. coli detection guide with API E20

E. coli ATCC 25922	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	
	+	-	+	+	-	-	-	-	+	-	-	
	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OXI	NO_2	CAT
	+	+	-	+	+	-	+	-	+	-	+	+

Figure 2 E. coli results in API 20 strip

1.5 Antibacterial resistance test

1.5.1 Preparation of culture medium

In the disc diffusion test, Müeller-Hinton agar was used as the culture medium, and it was prepared in the following manner:

After homogenization over a flame, 37 g of factory-prepared material (Merck 103872) was dissolved in one litre of distilled water and sterilised in an autoclave. The air's pH ranges between 7.2 and 7.4. After preparation, the solution was dispersed on 10 cm-diameter plates at a depth of around 4 mm. After that, the substance was refrigerated to allow it to coagulate.

1.5.2 Preparing a standard half McFarland solution

To prepare this solution, the following actions were taken:

1. A two-water barium chloride solution with a weight concentration of 1.175% was made.
2. Sulfuric acid was also synthesised in a one-volume-by-volume solution.
3. 0.5 mL of the first solution was mixed with 99.5 mL of the second solution.

At 625 nm, the optical absorbance of the produced solution should be between 0.08 and 0.1.

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1.5.3 Preparation of microbial suspension equivalent to 0.5 McFarland:

A few immature colonies (18 to 24 hours) are taken from the agar medium and transferred to the tube containing TSB, and the OD is measured to the standard equivalent to reach 0.5 McFarland in the spectrophotometer at 6245 nm every 30 minutes.

1.5.4 Performing sensitivity determination test by disk fusion method

The antibiotic susceptibility pattern of all *E. coli* isolates was determined using the CLSI 2021 agar disc diffusion method for the synthesised antibiotics.

1. From the colonies developed on agar medium, 3–4 colonies were extracted, and a suspension equal to half McFarland was made.
2. The tubes were incubated at 37 °C for 2 to 4 hours before being compared to a 0.5 McFarland
3. A sterile swab was immersed in the bacterial suspension and, after removing surplus fluid from the tube wall, was applied in all directions to the prepared Müller-Hinton agar medium to cover the whole surface of the medium.
4. To remove moisture, the plates are set at room temperature for 3 minutes.
5. Next, 15-mm antibiotic discs are placed on the surface of the plates at a distance of 24 mm.
6. After 16–18 hours, the plates were transferred to a 35-degree incubator, and the results were assessed.

The following antibiotics were chosen for susceptibility testing based on previous research:

Table 3 Antibiotic discs used in susceptibility test based on CLSI 2021

Antibiotic disc	Dose	Inhibition Zone (mm)			Company
		S<	I	R>	
Ampicillin	10 µg	17	14-16	13	Padtan Teb co. (Iran)
Tetracycline	30 µg	15	12-14	11	Padtan Teb co. (Iran)
Ciprofloxacin	5 µg	24	-	23	Padtan Teb co. (Iran)
Nitrofurantoin	300 µg	17	15-16	14	Padtan Teb co. (Iran)
Trimethoprim Sulfamethoxazole	1.25/23.75 µg	16	11-15	10	Padtan Teb co. (Iran)

1.5.5 DNA purification

To perform the PCR test, we must first extract bacterial DNA from the complete bacterial cell. Takara Bio Co.'s NucleoSpin® Microbial DNA kit is used to isolate genomic DNA from *E. coli*.

1.5.6 Kit components

Contents included in the kit is in the following table:

Table 4 DNA extraction kit component

#	Contents	amount/number
1	Lysis Buffer MG	38 mL
2	Wash Buffer BW	30 mL
3	Wash Buffer B5 (Concentrate)	6 mL
4	Elution Buffer BE	30 mL
5	Liquid Proteinase K	600 µL
6	NucleoSpin® Bead Tubes Type B	50
7	NucleoSpin® Microbial DNA Columns (light green rings)	50
8	Collection Tubes (2 mL)	100
9	User manual	1

NOTE: THIS TABLE IS FOR A 50 PREPS-KIT. 2 OF THIS KIT IS PREPARED.

1.5.7 Protocol

This technique is based on the grammeme-positive and grammeme-negative bacteria user instructions that came with the kit, and we followed them perfectly.

1. Preparing sample:

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We centrifuged the cultured samples in a micro centrifuge tube and removed the supernatant. Then we picked 40 mg of wet-weight microbial cell culture pellet and added 100 µL of elution buffer BE and suspended cells.

2. Then we transferred the cell suspension into the NucleoSpin Bead Tube Type B that was provided in the kit. After that, 40 µL Buffer MG and 10 µL Liquid Proteinase K were added, respectively, and the tube was closed. Agitation was done with the centre of the micro centrifuge device, and the centrifuge was done for 30 seconds at 11,000 x g to clean the lid.
3. We removed the cap, and 600 µL of Buffer MG was added to the tube and mixed. Then we centrifuged at 11,000 g for 30 seconds. Glass beads and cell debris were sedimented.
4. For binding DNA, we transferred approximately 600 µL of supernatant onto the NucleoSpin Microbial DNA Column, placed in a 2 mL collection tube. We then centrifuged for 30 s at 11,000 x g. Then we removed the previous collection tube and put the column into another fresh collection tube.
5. We added 500 µL of buffer BW. Centrifuged for 30 s at 11,000 x g. I removed the flow-through and placed the column back into the collection tube. This step was done twice.
6. In order to dry the silica membrane, we centrifuged the column at 11,000 x g for 30 s.
7. Finally, we placed the column into a 1 mL nuclease-free tube and added 100 µL Buffer BE to the column. Incubate at room temperature for 1 minute. Centrifuge at 11,000 x g for 30 seconds.

1.6 Examining the presence of studied genes

The polymerase chain reaction (PCR) is a widely used method for rapidly making millions to billions of copies (complete or partial) of a given DNA sample, allowing scientists to take a small amount of DNA and amplify it (or a portion of it) to a large enough quantity to analyse in detail.

We employed this approach, as well as agarose gel electrophoresis of the PCR result, to determine if the examined genes were present in the extracted bacterial strain.

1.6.1 Primers

Primers are single-stranded DNA fragments that attach to a specific section of DNA and enable us to amplify that region. Each gene requires two forward and reverse primers.

We chose our primers by crediting and referencing reliable sources, as well as testing them in the NCBI Blast programme.

The following table lists the primers used:

Table 5 Primers of this study

Gene	Primer sequence	Direction	Product size(bp)	Ref.
papA	GCTGCTCCAACTATTCCACA	F	421	[46]
papA	ACTGCAGAAAAAGCACCT	R		
papC	TGTTTACTGGCATGATGGTCA	F	1614	
papC	ACGCCGAAAGACGTATAT	R		
papGII	GGGATGAGCGGGCCTTTGAT	F	801	[47]
papGII	CGGGCCCCCAAGTAACTCG	R		

1.6.2 Primer preparation:

After sending the primer sequences to the supplier, we received solid primers that needed to be converted before usage. The user handbook that came with the product was used for this purpose, and the following procedures were taken:

1. The entire content was spun for 5 seconds.
2. A concentration of 100 µM of the solution was made using sterile water, free of nuclease, and with a pH of 7, according to the product brochure's directions and numbers.
3. Vortex the vial for 5 seconds to thoroughly dissolve the primer.

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4. Under the specified conditions, 10 µl of oligonucleotide (prepared solution) was withdrawn and 90 l of sterile water was added.

1.6.3 PCR solution preparing

To perform PCR, a solution containing extracted bacterial DNA and primer must be prepared. Ingredients in this solution:

Table 6 PCR solution integrations

Integration	Volume
Master Mix: This ready-made mix contains Mg2 +, dNTPs, Taq and buffer	7.5 µL in each microtube
Primer	0.3 µL forward and 0.3 mL reverse
DNA	1.5 µL
H_2O	5.4 µL
Total volume	15 µL

Also, a solution without bacterial DNA was prepared for control, in which water was added instead of DNA to a volume of 15 µL

1.6.4 PCR time table

One of the main components in performing PCR is the timing according to which the double-stranded DNA is opened, the replication is done from the specified region and the fragment is separated from the DNA. PCR was done based on the following timetable:

Table 7 PCR time-table

Steps	Temperature (°C)	Time (mm:ss)	Purpose
1	95	3:00	Initial Denaturation
25 cycles of Steps 2 to 4:			
2	95	00:30	Denaturation
3	*papA*: 60 papB: 60 *papG*: 59	00:30	Annealing
4	72	00:30	Extension
5	72	5:00	Final Extension
6	4	∞	Storage

1.6.5 Agarose gel preparation

By combining 0.6 grammes of agarose powder with 30 millilitres of TAE buffer, a 2 percent agarose gel was created. The mixture was microwaved three times for ten seconds each time and then mixed by moving the dish between each time. Before pouring the liquid into the container, 0.9 µl of harmless dye was added to allow DNA to be visible under UV light once the mixture had warmed up. Finally, the liquid is placed in an appropriate container and left to gel for 20 minutes in a dark, out-of-the way location..

1.6.6 Conduct electrophoresis

Following gel preparation, electrophoresis was carried out for 30 minutes at 110V and 60A. In the first well, a ladder was placed. In the second and third wells, the negative control and PCR production were inserted, respectively.

1.7 Analysis

For this reason, IMB SPSS® version 26 was used. In this study, chi square and Fisher's exact were used for statistical analysis of the obtained data and to check their significance.

1.8 Results

1.8.1 Description of the samples

The samples were taken from 110 patients with UTIs admitted to the hospital. All of these samples were found to be infected with E. coli by culture, biochemical, and Vitek2 tests. The following table includes information extracted from the files of these patients.:

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Table 9 Description of collected samples and patients' information from their files

Num	Sample ID	Sex	Age	Days of hospitalization	Appearance	Treatment
1	UTI-001	Male	37	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
2	UTI-002	Female	42	3	Yellow and clear	Co-Trimoxazole
3	UTI-003	Female	75	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
4	UTI-004	Female	25	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
5	UTI-005	Female	59	4	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
6	UTI-006	Male	64	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
7	UTI-007	Male	65	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
8	UTI-008	Female	45	3	Yellow and clear	Co-Trimoxazole
9	UTI-009	Female	32	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
10	UTI-010	Male	39	4	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
11	UTI-011	Female	50	3	Yellow and clear	Co-Trimoxazole
12	UTI-012	Male	34	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
13	UTI-013	Female	25	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
14	UTI-014	Male	26	3	Yellow and clear	Co-Trimoxazole
15	UTI-015	Male	45	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
16	UTI-016	Male	72	4	Yellow and clear	Co-Trimoxazole
17	UTI-017	Male	27	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
18	UTI-018	Female	68	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
19	UTI-019	Female	28	3	Yellow and clear	Co-Trimoxazole
20	UTI-020	Male	54	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
21	UTI-021	Female	32	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
22	UTI-022	Male	59	4	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
23	UTI-023	Female	42	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
24	UTI-	Female	27	4	Yellow and	Co-Trimoxazole

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	024				clear	
25	UTI-025	Female	72	3	Yellow and clear	Co-Trimoxazole
26	UTI-026	Female	29	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
27	UTI-027	Male	25	3	Yellow and clear	Co-Trimoxazole
28	UTI-028	Male	35	4	Yellow and clear	Co-Trimoxazole
29	UTI-029	Female	51	3	Yellow and clear	Co-Trimoxazole
30	UTI-030	Female	27	4	Yellow and clear	Co-Trimoxazole
31	UTI-031	Female	33	3	Yellow and clear	Co-Trimoxazole
32	UTI-032	Female	69	4	Yellow and clear	Co-Trimoxazole
33	UTI-033	Female	67	4	Yellow and clear	Co-Trimoxazole
34	UTI-034	Female	35	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
35	UTI-035	Male	28	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
36	UTI-036	Female	55	4	Yellow and clear	Co-Trimoxazole
37	UTI-037	Female	68	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
38	UTI-038	Male	45	3	Yellow and clear	Co-Trimoxazole
39	UTI-039	Male	33	3	Yellow and clear	Co-Trimoxazole
40	UTI-040	Female	27	3	Yellow and clear	Co-Trimoxazole
41	UTI-041	Female	44	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
42	UTI-042	Male	27	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
43	UTI-043	Female	37	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
44	UTI-044	Female	40	4	Yellow and clear	Co-Trimoxazole
45	UTI-045	Female	63	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
46	UTI-046	Female	39	4	Yellow and clear	Co-Trimoxazole
47	UTI-047	Female	30	3	Yellow and clear	Co-Trimoxazole
48	UTI-048	Female	58	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
49	UTI-049	Female	54	4	Yellow and clear	Co-Trimoxazole

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50	UTI-050	Male	45	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
51	UTI-051	Female	57	4	Yellow and clear	Co-Trimoxazole
52	UTI-052	Female	74	3	Yellow and clear	Co-Trimoxazole
53	UTI-053	Female	55	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
54	UTI-054	Female	62	3	Yellow and clear	Co-Trimoxazole
55	UTI-055	Male	25	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
56	UTI-056	Female	33	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
57	UTI-057	Female	59	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
58	UTI-058	Male	29	4	Yellow and clear	Co-Trimoxazole
59	UTI-059	Female	51	4	Yellow and clear	Co-Trimoxazole
60	UTI-060	Female	40	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
61	UTI-061	Female	52	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
62	UTI-062	Female	27	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
63	UTI-063	Female	52	3	Yellow and clear	Co-Trimoxazole
64	UTI-064	Female	53	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
65	UTI-065	Female	24	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
66	UTI-066	Male	32	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
67	UTI-067	Female	57	4	Yellow and clear	Co-Trimoxazole
68	UTI-068	Female	45	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
69	UTI-069	Male	72	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
70	UTI-070	Female	70	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
71	UTI-071	Female	21	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
72	UTI-072	Female	40	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
73	UTI-073	Female	73	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
74	UTI-074	Male	39	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
75	UTI-	Female	75	7	Dark and	Co-Trimoxazole and

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	075				cloudy	Nitrofurantoin
76	UTI-076	Male	24	3	Yellow and clear	Co-Trimoxazole
77	UTI-077	Female	25	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
78	UTI-078	Female	37	3	Yellow and clear	Co-Trimoxazole
79	UTI-079	Female	50	3	Yellow and clear	Co-Trimoxazole
80	UTI-080	Female	43	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
81	UTI-081	Female	25	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
82	UTI-082	Female	65	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
83	UTI-083	Male	65	4	Yellow and clear	Co-Trimoxazole
84	UTI-084	Male	63	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
85	UTI-085	Female	25	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
86	UTI-086	Female	75	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
87	UTI-087	Female	64	3	Yellow and clear	Co-Trimoxazole
88	UTI-088	Female	51	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
89	UTI-089	Female	62	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
90	UTI-090	Female	28	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
91	UTI-091	Female	67	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
92	UTI-092	Female	33	3	Yellow and clear	Co-Trimoxazole
93	UTI-093	Male	43	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
94	UTI-094	Male	31	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
95	UTI-095	Male	58	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
96	UTI-096	Female	48	6	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
97	UTI-097	Female	51	4	Yellow and clear	Co-Trimoxazole
98	UTI-098	Female	48	4	Yellow and clear	Co-Trimoxazole
99	UTI-099	Female	72	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
100	UTI-100	Female	35	3	Yellow and clear	Co-Trimoxazole

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101	UTI-101	Female	62	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
102	UTI-102	Female	25	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
103	UTI-103	Female	43	3	Yellow and clear	Co-Trimoxazole
104	UTI-104	Female	30	3	Yellow and clear	Co-Trimoxazole
105	UTI-105	Female	26	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
106	UTI-106	Male	44	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
107	UTI-107	Female	33	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
108	UTI-108	Female	39	7	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
109	UTI-109	Female	25	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin
110	UTI-110	Female	46	5	Dark and cloudy	Co-Trimoxazole and Nitrofurantoin

1.8.2 Bacterial detection result

Bacterial detection was done by bacterial culture methods and biochemical tests, and in cases where it was necessary, the Vitek 2 test method was confirmed. The result of these tests was that all samples were infected with *E. coli* bacteria, and no samples were found to be uninfected or infected with other bacteria.

Figure 3 E. coli bacteria on MacConkey agar

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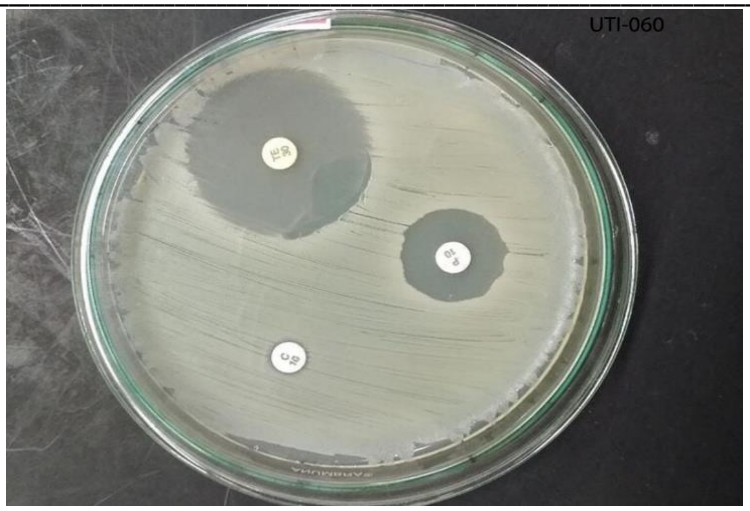

Figure 4 Antibiotic resistance test of sample 070 for Ampicillin, Tetracycline and Ciprofloxacin

1.8.3 PCR and gene detection results

After amplifying the DNA fragments extracted from each sample, the resulting product was loaded into an agarose gel and electrophoresed. In this way, the result of the presence or absence of the gene in the DNA of the strain was determined. The following table shows the prevalence of each gene in each sample.:

Table 10 presence of papA, papC and papG (allele II) genes in samples

Sample ID	*papA*	*papC*	*papG*-II
UTI-001	pos	pos	pos
UTI-002	neg	pos	pos
UTI-003	neg	neg	pos
UTI-004	neg	neg	pos
UTI-005	neg	neg	neg
UTI-006	neg	pos	pos
UTI-007	pos	pos	pos
UTI-008	neg	neg	neg
UTI-009	pos	pos	pos
UTI-010	neg	pos	pos
UTI-011	pos	pos	pos
UTI-012	neg	neg	neg
UTI-013	pos	pos	pos
UTI-014	neg	neg	neg
UTI-015	neg	pos	pos
UTI-016	neg	neg	neg
UTI-017	pos	pos	pos
UTI-018	pos	pos	pos
UTI-019	pos	pos	pos
UTI-020	neg	neg	neg
UTI-021	neg	neg	neg
UTI-022	pos	pos	pos
UTI-023	neg	neg	pos
UTI-024	neg	neg	pos
UTI-025	neg	neg	pos
UTI-026	neg	neg	neg
UTI-027	neg	neg	neg
UTI-028	pos	pos	pos
UTI-029	neg	neg	neg

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UTI-030	neg	pos	pos
UTI-031	neg	neg	neg
UTI-032	neg	neg	pos
UTI-033	neg	neg	pos
UTI-034	neg	neg	neg
UTI-035	pos	pos	pos
UTI-036	neg	neg	neg
UTI-037	neg	neg	pos
UTI-038	neg	neg	neg
UTI-039	neg	neg	pos
UTI-040	neg	neg	pos
UTI-041	neg	neg	neg
UTI-042	neg	neg	neg
UTI-043	neg	neg	pos
UTI-044	neg	neg	pos
UTI-045	neg	neg	neg
UTI-046	pos	pos	pos
UTI-047	neg	pos	pos
UTI-048	pos	pos	pos
UTI-049	neg	neg	pos
UTI-050	neg	neg	neg
UTI-051	neg	neg	pos
UTI-052	neg	neg	pos
UTI-053	pos	pos	pos
UTI-054	neg	neg	neg
UTI-055	neg	neg	pos
UTI-056	neg	neg	neg
UTI-057	pos	pos	pos
UTI-058	neg	pos	pos
UTI-059	neg	neg	pos
UTI-060	neg	neg	neg
UTI-061	neg	neg	neg
UTI-062	pos	pos	pos
UTI-063	pos	pos	pos
UTI-064	pos	pos	pos
UTI-065	pos	pos	pos
UTI-066	neg	neg	pos
UTI-067	pos	pos	pos
UTI-068	neg	neg	neg
UTI-069	pos	pos	pos
UTI-070	pos	pos	pos
UTI-071	neg	neg	neg
UTI-072	neg	neg	neg
UTI-073	pos	pos	pos
UTI-074	neg	neg	neg
UTI-075	neg	neg	neg
UTI-076	pos	pos	neg
UTI-077	neg	neg	pos
UTI-078	neg	neg	pos
UTI-079	neg	pos	pos

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UTI-080	neg	pos	pos
UTI-081	neg	neg	neg
UTI-082	neg	neg	pos
UTI-083	neg	neg	pos
UTI-084	pos	pos	pos
UTI-085	neg	neg	neg
UTI-086	neg	neg	neg
UTI-087	neg	neg	neg
UTI-088	pos	pos	pos
UTI-089	neg	neg	neg
UTI-090	pos	pos	neg
UTI-091	pos	pos	pos
UTI-092	neg	neg	neg
UTI-093	neg	neg	pos
UTI-094	neg	neg	neg
UTI-095	neg	neg	pos
UTI-096	neg	neg	pos
UTI-097	neg	neg	pos
UTI-098	neg	neg	pos
UTI-099	neg	neg	neg
UTI-100	neg	neg	neg
UTI-101	neg	neg	pos
UTI-102	neg	neg	neg
UTI-103	pos	pos	pos
UTI-104	neg	neg	neg
UTI-105	pos	pos	pos
UTI-106	neg	pos	pos
UTI-107	neg	neg	neg
UTI-108	neg	neg	neg
UTI-109	pos	pos	pos
UTI-110	neg	pos	pos

And the following table shows the number and percentage of prevalence of each gene:

Table 11 prevalence of different genes

Status	*papA*	*papC*	*papG*
Positive	31	42	68
Negative	79	68	42
Percentage	28.18	38.18	61.81

In the image below, which is the image of the electrophoresis gel, one of the strains that has all three genes is shown:

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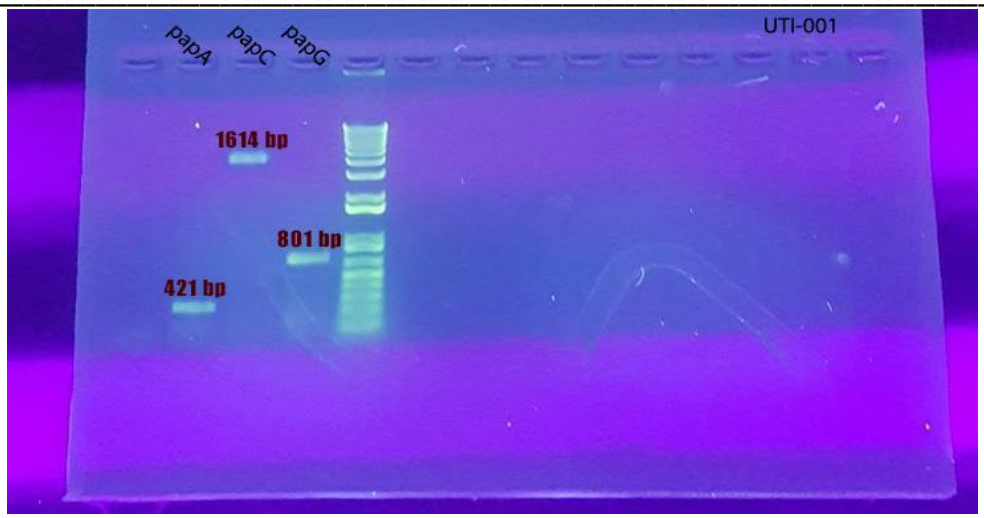

Figure 5 Gel electrophoresis of sample 001: first well = DNA-free control, second to fourth well = tested genes, fifth well = ladder

1.8.4 The results of the analysis

Antibiotic resistance and PCR test data were loaded into SPSS software for statistical analysis. Strains with moderate resistance were designated antibacterially resistant in the submitted data. The chi-square and Fischer's exact tests were used for statistical analysis.

1.8.5 The *papA* gene effect on antibiotic resistance

The presence of the papA gene in the extracted strains shows a significant relationship with ampicillin antibiotic resistance. But there is no such relationship in the case of other antibiotics..

1.8.6 The *papC* gene effect on antibiotic resistance

In the case of the PapC gene, just like the PapA gene, there was a significant relationship between the presence of the gene in the genome of the strain extracted from the sample and resistance to ampicillin, but no such relationship is seen in the case of other antibiotics..

1.8.7 The *papG* gene effect on antibiotic resistance

In the case of this gene, there is a significant relationship between cotrimoxazole antibiotic resistance and the presence or absence of the gene, but not in the case of others.

Table 12 association between antimicrobial resistance and genes

Genes Antibiotics	*papA* (n=31)	*papC* (n=42)	*papG* (n=68)
Ampicillin N (%)	16 (40)	21(52.5)	24(60)
Ciprofloxacin N (%)	7 (21.9)	11(34.4)	16(50)
Tetracycline N (%)	6 (22.2)	8(29.6)	14(51.9)
Cotrimoxazole N (%)	7 (31.8)	7(31.8)	9(40.9)
Nitrofuranation N (%)	1 (33.3)	2(66.7)	3(100)

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1.9 Discussion

1.9.1 General Discussion

This research was done to determine the prevalence of the three genes papA, papC, and papG (allele II) and also to measure the resistance to common antibacterials for the treatment of urinary tract infections caused by E. coli bacteria. This bacterium is the normal flora of the body, but under special conditions, it can leave its usual place in the body and cause disease in other organs of the body, such as the organs of the urinary system..

In addition to these cases, the relationship between the presence of these genes and resistance to antibiotics was also measured using statistical analysis.

In order to conduct this research, urine samples collected from 110 patients with urinary tract infections who were admitted to Al-Hussein Teaching Hospital in Dhi Qar Province, Iraq, were tested. After collecting these samples, they were immediately transferred to the laboratory so that other stages of research could be done on them.

At first, it was necessary to ensure that the samples contained E. coli bacteria. Therefore, bacterial culture methods, biochemical tests, and the Vitek 2 system were used. The result of this test showed that all the samples were infected with E. coli bacteria..

Then the samples were cultured in Mueller-Hinton agar to test antibiotic sensitivity using the disc diffusion method. In this test, which is based on the CLSI 2021 guide and the rate of bacterial growth in the presence of antibiotics, bacterial strains are divided into three categories: sensitive, intermediate, and resistant, by measuring the radius of growth of bacteria around the antibiogram discs. The results of this test show that 36.36, 24.54, 29.09, 2.72, and 20% of our strains are insensitive to the antibiotics ampicillin, tetracycline, ciprofloxacin, nitrofurantoin, and cotrimoxazole, respectively.

The polymerase chain reaction (PCR) method was used to amplify fragments of DNA that are part of the gene. In this way, after loading the resulting product into an agarose gel and electrophoresis, it was determined how many percent of the strains had the studied genes. In this way, it has been determined that the highest prevalence of the gene among these three is related to papG, which is 61.81%. After that, papC and papA are placed at 38.18 and 28.18%, respectively.

Finally, by entering the data from the experiments into SPSS software and performing the chi-square and Fisher's exact tests as well as phi and Cramer’s V, it was found that there is a significant relationship between the presence or absence of papA and papC genes with ampicillin and a significant relationship between papG and co-trimoxazole.

Hemagglutinins that are mannose-resistant are called P fimbriae. They are mostly found on the cell surface of E. coli strains that are linked to urinary tract infections (UTIs) in humans. These fimbriae adhere to the kidney vascular endothelium and are related to upper urinary tract colonisation and pyelonephritis. P-related fimbriae are also linked to urinary tract infections in dogs and septicemia in pigs. The adhesin component PapG is found at the tip of the P fimbriae and facilitates bacterial adhesion to a-D-galactosyl-(1-4)-b-galactopyranose (Gal-Gal)-containing receptors found in host tissues [48]. The P pili encoded by the pap operon are heteropolymers consisting of about 1,000 major pilin subunits, which are called PapA and PapC genes, producing PapC protein, which is required for polymerization [49].

In a study by Biggel et al., in comparison to 59.8% of papGII-negative isolates, 85.2% of papGII+ isolates were predicted to be resistant to both ciprofloxacin (mediated by QRDR mutations or aac(6')-Ib-cr) and 3rd generation cephalosporins (mediated by ESBL) (P 0.001 [Fisher's exact test], OR = 3.9 [95% CI 3.0-5.0]). Predictions of resistance to ciprofloxacin and third-generation cephalosporins were 72.4% for papGII+ isolates and 26.1% for papGII-negative isolates in the three isolate collections that were not initially pre-selected for ESBL-producing E. coli (P 0.001 [Fisher's exact test], OR 7.4 [95% CI 4.2–13.0]) [50]. This report is in contrast with our research.

Carriage of papC is a robust predictor of resistance to any antibiotic, and papC was also shown to be an independent predictor of resistance to ampicillin [51]. This information from Karami et al. and the emphasis of Yazdanpour et al. [52] support our findings.

No sufficient information or research was found about the relationship between papA and antibacterial resistance, but this research [53] suggests that there might be a relationship between amoxicillin resistance and papA..

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1.9.2 Suggestions

This study was associated with limitations, including the number of samples,the method and number of studied genes, the distribution of collected samples, etc.
It is recommended to other researchers to perform this test with a large number of samples and a greater dispersion of the sample, as well as with a large number of antibacterials.

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