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Analysis of genetic diversity of *E.coli* bacteria isolates from UTI after exposure to some biological effects by using RAPD technique

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ABSTRACT

Background and Objective: *Escherichia coli* bacteria are one of the most important bacterial races causing urinary tract infection, which is responsible for 90% of the causes of urinary tract infection and originated in the human intestine, this is Gram- Negative bacteria and facultative aerobic that moves by peripheral flagella and belongs to the bacteria of the *Enterobacteriaceae*.

Materials and methods: The bacteria were isolated from UTI, after they were diagnosed using light microscope and conducting biochemical tests, then exposed to the antibiotic Ciprofloxacin, trimethoprim, and alcoholic extract of *Punica granatum* and *Trigonella foenum graicum*. genomic DNA was extracted for all samples and Random amplified polymorphic DNA - Polymerase chain reaction (RAPD-PCR) marker was carried out using five random primers.

Results: The results of RAPD-PCR profiles shown that exposed to antibiotics (Ciprofloxacin and Trimethoprim) and alcoholic extracts (*Punica granatum* and *Trigonella foenum graicum*) lead to the disappearance or appearance new bands compared with non-exposed samples, and the highest rate of polymorphism for all each treatment and primers in sample 5 was 102.27% where the ratio % GTS for all treatment and primers is 6.04% in the same sample.

Conclusion: All treatments caused genetic changes in the DNA of *E.coli* bacteria cells especially the *Punica granatum* which gave the highest effect than the rest of the treatments, this indicates its efficiency in treating bacterial infections.

Introduction

UTI is one of the most important and most common diseases, and comes second after respiratory diseases, as it occurs as a result of infection with bacteria and their proliferation in the urinary system, which consists of the lower urinary tract (ureter and bladder) and the upper urinary tract (urethra and kidneys), the inflammation usually begins in the lower urinary tract and is called urethritis, which occurs after contamination urine of bacteria, and is without symptoms, and inflammation of the urinary tract associated with catheters. It can develop into the upper urinary tract, causing urethritis, kidney infections and urethritis Cystitis, and the infection of *E.coli* bacteria causing urinary tract infections is the main source of infection bacteremia [1,2] .A UTI may be accompanied by symptoms or asymptomatic and injury may cause serious complications if not Treated [3] .The incidence of infection increases with age. UTI are often due as a result of gram negative bacteria, of the injuries uropathogenic *E.coli* the most frequent pathogens as isolated by (50-60%)[4],followed by other types, *Pseudomonas aeruginosa*, *Enterobacter coleca*, *Klebsiella pneumonia*, *Citrobacter frendii* & *Proteus mirabilis*, in addition to types of gram positive bacteria which include *staph aureus*, *staph saprophyticus* and *Enterococus leutus* [5]. *E.coli* bacteria are the most important types that cause urinary tract infection [6]. Where Its diseases are due to its possession of many virulent factors, including iron chelates Siderophores, cystic necrotizing factor, colisin, possession of surface structures such as flagella, capsule, and lipopolysaccharides (LPS), as well as possession of cilia(Pilli or Fimbreae), which helps it adhere to the

host's tissues, giving it the ability to produce the biofilm [7,8]. UTI are often treated with broad-spectrum antibiotics prior to bacterial culture testing and antibiotic allergy testing, leading to the development of bacterial resistance and multiple antibiotic resistance not only at the local level but also globally [9]. Bacteria acquired the characteristic of resistance either vertically from their ancestors through a number of mutations that occur in chromosome genes, or horizontally through plasmids [10], which is transmitted by bacteriophage, or conjugation or by transformation, and possibly by genetic vectors, and bacterial conjugation is one of the most common methods that contribute to the transmission of resistant plasmids, or conjugation plasmids, such as plasmids R, as the association occurs with a high frequency between strains of the same species or Similar species [11], such as *E.coli* and some enterobactericeae species that possess multiple antibiotic resistance (MDR)traits by following different and varied resistance methods [12]. namely reduced permeability or uptake increase, efflux pump activity, alteration of drug target and loss of enzyme in antibiotic activation [13,14].

The emergence of antibiotic-resistant microbial strains has prompted researchers to find alternative antibacterial substances and separate their active ingredients, as medicinal plants contain special chemicals substances , the most important of which are alkaloids, glycosides, , volatile and aromatic oils, resin, tannin, gum, light fats, phenols, carbohydrates and soaps substances [15].

Sampling Clinical specimens collection:

Samples were collected 100 bacterial isolations from patients with urinary tract infection according to a way [16]. Urine samples were collected in sterile containers of incoming patients at Salah Al-din General Hospital from July 2019 to December 2019, who were confirmed to have urinary tract infection through a first examination, which included the sighting of pus cells and red blood cells in urine.

Cultivation and isolation of bacteria

The samples specimen were directly cultured in a planned manner on the nutrient culture media and selective media such as mac Conky agar, Mannitol salt agar, Eosin methylene blue agar and incubation inverted at a temperature of 37°C for 24 hours. The single colonies were then selected and sub cultured on new Petri dishes and the same medium that they grew to obtain pure colonies of bacteria, and then moved the isolated and pure colonies to the media of the slant nutrient Agar, and after incubation they were preserved at temperature 4°C until they were used for diagnostic tests [17].

Diagnosis of bacterial isolations

Bacterial isolates were initially diagnosed based on their appearance qualities on different culture media and then stained by using a gram stain to observe the response of the cells to the dye and its forms, sizes and method of assembly, then all the isolates were subjected to various biochemical tests such as IMVC test that involve (indol, methyl red, voges proskauer, citrate utilization) and catalase test, oxidase test and urease test [18].

Prepration of alcoholic extracts

The alcoholic extract for *Punica granatum* and *Trigonella foenum graicum* was prepared by washed with distilled water and then Sodium Hypochloride solution is 1% concentration and washed again with distillated water. The 20g of Pomegranante peel was weighted and placed in the electric mixer (Food perocessor) has been put it 100 ml from the ethanol alcohol with a concentration ot 95% and a mixture for 2-3 minutes and left the throat on the shaker vibrator for 24 hours to dissolve in ethanol alcohol and then nominated by several layers of gauze to get rid of suspended plant parts and remaning fibers and then nominated once again by using exact filtration unit (Millipore) 0.45Mm to prevent germans from extract. After that, the mixture is placed in an electric oven of 940m to evaporate the entire alcohol. Then babe dry extract which has become burning in a sterile glass closed and sterilized in the refrigerator 4c until they are used in experiments [19].

As for the *Trigonella* seeds we have been weighted 20g and grinded with an electric mill and added to it 100ml of ethanol alcohol with a concentration of 95% mixed with shaker vibrator for 24 hours and after the same steps to prepare the alcoholic pomegranante peel extract [20]. And then added 1g of powder as alcoholic extracts to 5ml of ethanol alcohol to obtain the storage solution at 200mg/ml and mixtime in the bidding process pasteurization, The rest of the detainee was then prepared 100%,75%,50%,25% from stock solution, and that these steps were carried out under sterile conditions according to the mitigation law. The Minimal Inhibitory Concentration (MIC) has been determined it's the less concentration of the extract led to the prevention of bacteria growth under study.The extract was confirmed not polluted by planning 0.1ml from which it is on the nutrient agar plate and incubated at temperature 37c for 24 hours [21].

Testing the inhibition effectiveness of extracts

The Agar-well diffusion method was used to test the inhibition effectiveness of the extractors on the growth of microbiology, by pouring 20-25 ml of the agar media per petri dish and after hardening the media the dishes were incubated for 24 hours at a level of 37°C to ensure that the dens are not contaminated. The middle was inoculated by using a sterile cotton scanner where a swab of the bacterial strand was taken and evenly spread on the Muller-Hinton Agar and then left for 15 minutes at room temperature for the purpose of absorbing the inoculum, then 5 holes were made in each dish with the fella drill per hole representing a certain concentration where 50 microliters of each concentration were added and placed in the hole assigned to it and at the same time added 50

microliters of distilled water to one of the pits instead of the plant extracted Control sample for comparison with the inhibition effectiveness of each extract, then the dishes were incubated for 24 hours at a temprature of 37°C, after which the results were read using the ruler by measuring the rates of inhibition zone diameter per concentration, which represents the area of no bacterial growth around each hole [22].

Exposure of bacteria to antibiotics and plant extracts

We Selected 6 samples of the most antibiotic-resistant *E.coli* bacteria and exposed to ciprofloxacin, trimethoprem and pomegranate peel extracts and fenugreek seeds, This was done by preparing several dilution (0.5-32) mg/ml for each antibiotic and (100-25) g/ml for each extract, exposing them to bacteria and identifying the minimum inhibitory concentration (MIC) of each of them, which was relied upon in [23,24].

Genomic DNA isolation

The genomic DNA was isolated based on method [25], and determined the concentration and purity of DNA by using Nanodrop deviece and migrating on agarose gel 1%concentration dyed with red safe.

Preparing RABD interactions

The reactions of the RAPD-PCR technique were performed using five random primers as shown in the Table (1):

Table 1: Primers Used in this Study [26]

No.	Primer code	Nucleotide sequence (5' → 3')
1	OP D-18	GAGAGCCAAC
2	OP E-03	CCAGATGCAC
3	OP E-11	GAGTCTCAGG
4	OP F-20	GGTCTAGAGG
5	OP H-08	GAAACACCCC

RAPD interactions were conducted using accupower PCR premix equipped by Bioneer Of Korea in accordance with the instructions attached to it.

1- Add 2ML of each of the random prefixes of each Eppendorf tube with a concentration of 10 picomol

2- Add 2ML of DNA templet to the mixture.

3. Add 16ML of sterile distilled water per Tube. Mix the mixture well using the Miropipette and then transferred the tubes to the centrifuge to make a quick spin blend of the components of the reaction, and then moved the tubes and put in the thermocycler and performed the double reaction by adjusting the device according to the program dedicated to the interaction.

4- After the end of the program time, the tubes are removed from the thermal for the purpose of electrically relaying them to the agarose gel at a concentration of 1.5% for 55 minutes and by 5v/cm.

5- Draw 5ML from the single Eppendorf and place inside the hole formed by gel and the process of electrical relay is carried out after connecting the power processor and adjust the current, voltage and time.

6- Gently lift the gel from the tub so that the gel is broken and placed inside the UV imaging device to observe the DNA packets formed with their partial sizes[27].

Table 2: Programs of PCR Thermocycling Conditions

Step	Program	
1	Only one cycle for 4 minutes at 94°C temperature for the initial denaturation DNA template	
2	40 cycle included:	
	Denaturation	30second at temperature92°C for double tape dirty.
	Annealing	45 second at a temperature of 36°C to connect the primers to the DNA template.
	Extension	45 second at a temperature72°C for elongation.
3	One cycle for 7 minutes at a temperature of 72°C to complete the final elongation.	

Statistical analysis

The results were recorded And that is by writing the size of the gained and lost bands for each isolate in special tables for both antibiotic and extract and the Genetic template stability GTS% was measured by using the law GTS% = (1-a / n) x 100 [28].that a= number of polymorphic bands detected in each treatment sample, n = number of total bands of the non-exposed control group. The percentage of polymorphisms was measured using the following relationship [29]. Polymorphism value = (total No. of a + b)/(total No .of observed bands of control)×100.

Results and Discussion

Isolation : *E.coli* bacteria are the most isolated and more common bacterial species of urinary tract infections, as they were isolated by 35% and were close to a number of studies, including the results of the researcher Al zirjawi [30] in the city of Qadisiyah, where the rate of isolation of this species was 39.06% and the researchers Seifu and Gebissa [31] isolated it by 39.3%, as well as with the results obtained by Almazroo'e [32] isolated by 39% and by isolating it by 30%, while not agreeing with many studies Among them were ponnusamy and Nagappan [33] in India, as well as a study conducted by hegazy [34] in Egypt, where the isolation of *E.coli* bacteria was 54.7% and 53.85% respectively among the types of bacteria causing urinary tract infections, respectively. The high isolation of these bacteria may be the result of the adaptation of bacteria to live in the

environment of the urinary tract and their tolerance to inappropriate environmental conditions moreover is the possession of many virulence factors that enable them to cause infection, including the possession of adhesion factors that help them to stickнce, which is the first step of infection, as well as the production of capsule and toxins that analyze the host tissues, and may be due to the high insulation of these bacteria in urinary tract infections to the transmission of bacteria from the regulatory system (exit opening) which is the environment. Natural to her to the nearby urine hole [35].

Identification

The results of biochemical tests characterized *E.coli* as positive for both the Indol test and the appearance of a red ring above the middle surface as a result of the action of tryptophase enzyme to analyze the tryptophane acid triptophane using kovacs reagent detector and this test is an important test in the distinction between *E.coli* and other enterobacteraceae types. And positive for the test of the methyl red test as a result of the acid consumption and fermentation of glucose and peptose peptose, which leads to the production of acid and decrease dépnotation of the center and then the color of the liquid medium to red, these two qualities are important for its [36], was negative for the voges-proskauer test due to the appearance of yellow-brown in the liquid medium due to the inability of bacteria to convert glucose glucose into a methyl acetylmethyl carbinol (acetoine) Result in the non-interaction of the alphanaphthol (VP1) and the second potassium hydroxide detector (VP2) with acetoine resulting acetoine, and also was negative for testing citrate utilization, resulting in no change in the middle color from green to blue as a result of the lack of citric acid production and no change in PH, and this supports the words of [37] [38].

Sensitivity

Sensitivity was examined for bacterial isolation using 10 most commonly used antibiotics for the treatment of urinary tract infections, including (MEM) Meropenem, (CIP), Ciprofloxacin, (TM), Trimethoprim, (AK), Amikacin and (RA), The results were interpreted in (39)**.**

E.coli bacteria isolates showed 100% absolute resistance to RA and were 88.5% resistant to Ak, F and TE (85.7%), GN (80%), NA (65.7%), CIP (57.2%), TM (51.42%) and C (25.7%) , MEM antibiotic showed highly efficient against *E.coli* bacteria as all isolation was 100% sensitive to it and the results were agreed with[40,41] as they found it 100% sensitive to MEM and was close to the results of [42,43] It was 91.89% and 93.5% sensitive, respectively, and also agreed with the results of [44] as they found it to be 99.9% sensitive toMEM,[45] result was approached by The RA, who found it 93.5% and 94%, respectively, to be resistant to it. Our results on CIP were not 100% sensitive to it and agreed with [46,47] as they found it to be resistant to it by 57.7% and 56.25% respectively and also agreed with the results of [47].

From the foregoing, there is a widespread spread of antibiotic resistance, which can be given by chromosomes or mobile genetic elements such as plasmids, which are given by resistance mechanisms, namely, the reduction of the permeability of the membranes of antibiotics, the destruction of antibiotics, or the escape of antibiotics and the mutation of the cellular targets [48].

The antibacterial character is due to its possession of resistance plasmids and transposable gene Transposones, which can be transferred from chromosome to healthy plasmid [49].

In addition, these antibiotics are commonly used and more available, and because of this they are frequently used by the public without consulting a doctor, it is expected that these antibiotics will be resistant to these antibiotics, especially in developing countries,The resistance of bacteria to antibiotics is often due to the patient's use of various antibiotics or the result of chromosome mutations that encode resistance from external sources[50], as the patient's long-term use of various antibiotics increases the ability of bacteria to develop their resistance mechanisms against many. Betalactam antibiotics is due to the fact that bacteria are resistant to penicillins' antibiotics due to their ability to produce a penicillin enzyme that breaks the betalactam ring that interferes with the manufacture of the peptidoglycan layer of the bacterial cell wall [51], or a change in the processing of the cell wall that sensitive to antibiotic, resulting in increased thickness or decreased accidental links, causing a change in the target location of the antibody, or may produce resistance by reducing the permeability of the outer membrane of negative bacteria and may affect the absorption rate of these antibiotics.

This mechanism is found exclusively in the gram negative bacteria because it contains the outer membrane while it lacks gram positive bacteria, and one of the factors causing bacterial resistance against the amino-glycoside group is the change in the location of target 30S or resistance may be caused by the production of enzyme by the antibody bacteria or the lack of permeability of the external membrane [52].

Antibiotic (C) results in a type of anemia as a result of breaking down red blood cells so it is not used to treat minor infections and resistant bacteria have the potential to produce chloramphenicol acetyltransferase,
which converts it into an inactive compound against bacteria [53].

The increased appearance of rsesistatnce isolates of anti (T) has reduced its use and may be due to the active flow mechanism that reduces the accumulation of this antibiotic within the cell and its ejection [54], this antibiotic works on the 30S unit of ribosum as it prevents the association of tRNA-Amino acyl in site

A site of the 30s ribosome and then stop building protein [55].

(Cip) inhibits the enzyme DNAgyrase, an enzyme responsible for de-wrapping the supercolling of the bacterial cell chromosome and affects unit A of the gyrase enzyme, which consists of two units A, B [56]. While (TM) inhibits the last step of folic acid metabolism by inhibiting the enzyme dihydro folate reductase thus inhibiting or inhibiting the production of Tetrahydrofolate bacteria can eliminate this inhibitory effect of these antibiotics if guanine, Thiamine and Methionin are available in the medium in which they live, and for RF antibiotic, which prevents the transcription of mRNA as it prevents the cloning of the gene in question, meaning that it is associated with unit B and converts DNA to mRNA [57].

We note from the foregoing that increased resistance to bacterial isolation of used antibiotics may be due to the frequent use of antibiotics or the acquisition of genetic factors by multiple resistance by the conjugation [58].

Inhibition effect of alcoholic extracts on *E.coli* bacteria

The results showed that the highest inhibition of *E.coli* bacteria was when the concentration was 100% for alcohol *Punica granatum* peel extract with a inhibition diameter of 25 mm, while for concentrations 75%, 50% and 25%, the inhibition countries were 22mm, 20mm, 14mm, respectively.

E.coli was less sensitive to alcohol *Trigonella foenum* seeds extract , with inhibition diameters of 18mm at 100 mm and 16mm at 75% concentration, while no sensitivity was shown at 50% and 25% concentration ‹This is consistent with the researchers' study [59].Their study showed that the minimum inhibitory concentration (MIC) of pomegranate peel was 100% when the concentration.

Results of the effect of genetically bioactive factors

The random amplified polymorphic DNA index (RAPD-PCR) was used to assess the effect of two types of antibiotics (Trimethoprim & Ciprofloxacin) and two types of alcoholic medical plant extracts (*Punica granatum* peels & *Trigonell* seeds) on the genetic material of *E.coli* bacteria isolated from urinary tract infections and compared them with the same isolations prior to treatment.

In this study, 5 random primers were used after which the samples were relayed on the agarose gel with a concentration of 1.5% and the gel was photographed by the UV imaging device and then analyzed the results of RABD-PCR for each primers based on the appearance of the bands and their disappearance as a result of the multiplication of certain parts of the genome of the pathogen samples and compared with the control group [60] as shown in the tables below.

Table 3: Shown total number bands and number control bands , polymorphisim bands for primer OP D-18 to samples that treatment with ciprofloxacin, Trimethoprim, *pomegranate Granada* and *Trigonellafoenum*

Samples	Control Bands	Treated 1		Treated 2		Treated 3		Treated 4		Total Bands	Polymorphic bands	Polymorphic of each primer
		A	B	A	B	A	B	A	B			
1	9	1650,1300, 620,530,450	1600,1520,1400 1000,620,500, 280	620,450	1600,1520,1400 1000,650,500,280	-	1700,1600,1520,1400 1000,780,650,500,280	-	1700,1600,1520,1400 1000,780,650,500,280	20	39	195%
2	8	-	1700,1600 900,400	1520,780,600 500,450,350	900,400	-	1700,1600,1300 1100,1000,900 720,400	-	1700,1600,1300 1100,1000,900 720,400	23	28	121.73%
3	5	1700,1300,1100 , 650,580	1680,1500	1759,1400,600 450,400,300	1680,1500 1200,550	-	1600,1500,1200 1000,550	-	1600,1500,1200 1000,550	19	27	142.10%
4	6	1650,1520,1150 650,580	1600	1650,1520,115 0 650,580	1600	-	1700,1600,1400 1050,780,500	-	1700,1600,1400 1050,780,500	36	24	66.66%
5	6	1700,1150,1000 800,750,500	1750,1680,1110 0	1000,1150,750	1750,1400 1680,1100	-	1750,1680,1500 1400,1100,600	1520,1000	1750,1680,1500 1400,1100,600	22	30	136.36%
6	7	1650,1520,600	650	1520,1300 1650,600	650	-	1700,1400,1200 1050,750,700 650	1550,1650, 1500 1300,600 450,350	750,650	30	25	83.33%
Total	41	24	18	26	19	-	41	14	31	150	173(115.33%)	124.24%
a+b		42		45		41		45		-		
Polymorphism %		102.44%		109.76%		100%		109.76%		-		
∑Polymorphism %		105.49%								-		

A= presence new bands. **B**=absence bands. **Treated 1**= treated with ciprofloxacin. **Treated 2**= treated with trimethoprim **Treated 3**= treated with pomegranate Granada. **Treated 4**=treated with *Trigonellafoenum.*

Table 4: Shown total number bands and number control, polymorphisim bands for primer OP E-03 to samples that treatments with ciprofloxacin, Trimethoprim, *pomegranate Granada* and *Trigonellafoenum*

Samples	Control Bands	Treated 1		Treated 2		Treated 3		Treated 4		Total Bands	Polymorphic bands	Polymorphic of each primer
		A	B	A	B	A	B	A	B			
1	12	950,380	1000,550	1750,1520 950,380	1000,550 430,150	-	1700,1500,1300 1100,1000,800,650,550, 430,350,250,150	-	1700,1500,1300 1100,1000,800,650,550, 430,350,250,150	36	36	100%
2	13	1300,700,470	1800,1780,1700 500,250,150	1550,700 470,400	1800,1780,1700 ,250,150	-	1800,1780,1700 1500,1100,1000 850,550,500,350 280,250,150	470	1800,1780,1700 1500,1100,1000 850,550,500,350 280,250,150	35	45	128.57%
3	9	1780,1700,1300 650,600	1750,1600,450	1700,1300 650,600	1750,1600	-	1750,1600,1400,1100 1000,850,450,350,250	-	1750,1600,1400,1100 1000,850,450,350,250	31	32	103.22%
4	10	1750,1600,1300 950,650	1800,1700 450,200	1300,950 700,300	1800,1700 450,200	-	1800,1700,1400,1100 800,550,450,350,250,200	1600,1500,1300 950,700	1800,1700,200	42	35	83.33%
5	8	1000	1750,1700,1650	1000,800 450	1750,1700	1300	1750,1700,1650,1100 850,600,350,250	1400,1000 700,450	1750,1700,1650,600	29	26	89.65%
6	8	1400,1300 700,500	750,650	500	1500,1200 750,650	-	1500,1200,1000,750 650,600,350,300	-	1500,1200,1000,750 650,600,350,300	23	27	128.57%
Total	60	20	20	20	22	1	60	10	48			
a+b		40		42		61		58				
Polymorphism %		66.66%		70%		101.66%		96.66%				
∑Polymorphism %		83.75%										

A= presence new bands. **B**=absence bands. **Treated 1**= treated with ciprofloxacin. **Treated 2**= treated with trimethoprim **Treated 3**= treated with pomegranate Granada. **Treated 4**=treated with *Trigonellafoenum.*

Table 5: Shown total number bands and number control, polymorphisim bands for primer OP E-11 to samples that treatments with ciprofloxacin, Trimethoprim, *pomegranate Granada* and *Trigonellafoenum*

Samples	Control Bands	Treated 1		Treated 2		Treated 3		Treated 4		Total Bands	Polymorphic bands	Polymorphic of each primer
		A	B	A	B	A	B	A	B			
1	3	1800,1530,690	1550	1800,650	1550	-	1550,850,300	-	1550,850,300	12	13	108,33%
2	2	-	-	1850,650	-	-	850,300	-	850,300	8	6	75%
3	3	1850,850,650	600	1100	600	-	600,300	-	600,300	8	10	125%
4	3	1100,650	-	1100,650,250	-	-	750,450,290	650	-	18	9	50%
5	4	1100,800,650	1850,750	1100,800,650	1850,750	-	1850,750,450,300	650	1850,750,450	16	18	118,75%
6	2	650	-	650	-	-	850,300	650	850	10	6	60%
Total	17	12	4	12	4	-	16	3	11	72	62(86.1%)	90%
a+b		16		16		16		14		-		
Polymorphism %		94.11%		94.11%		94.11%		82.24%		-		
∑Polymorphism %		91.64%								-		

A= presence new bands. **B**=absence bands. **Treated 1**= treated with ciprofloxacin. **Treated 2**= treated with trimethoprim **Treated 3**= treated with pomegranate Granada. **Treated 4**=treated with *Trigonellafoenum*.

Table 6: Shown total number bands and number control, polymorphisim bands for primer OP F-20 to samples that treatments with ciprofloxacin, Trimethoprim, *pomegranate Granada and Trigonellafoenum*

Samples	Control Bands	Treated 1		Treated 2		Treated 3		Treated 4		Total Bands	Polymorphic bands	Polymorphic of each primer
		A	B	A	B	A	B	A	B			
1	9	1300,1100	300	1300,1100 480,250	500,300	-	1700,`600,1400,950 700,650,500,380,300	-	1700,`600,1400,950 700,650,500,380,300	30	27	90%
2	11	-	-	450	800,550,430	850	1700,1650,1600,1400,1100 800,650,550,430,300,350	900,600	1700,1650,1600 800,550,430	39	24	61.53%
3	9	1000,700 500,250	1650,1600,900	1000,700 500,250	1650,1600,900	-	1800,1700,1650,1600 1200,900,650,400	-	1800,1700,1650,1600 1200,900,650,400	28	30	107.14%
4	4	1100,480	500	1100	500	-	1000,750,500,300	1100,480	500	18	12	116.66%
5	9	1700,1100 ,300	1750,1650,1600,1190, 900,700,500	1700,1100, 300	1750,1650,1600,1190, 900,700,500	-	1750,1650,1600,1500 1190,900,750,700,500	1150,400,300	1750,1650,1600 1190,900,500	26	38	146.15%
6	5	800	-	800,600,400	650	-	1700,1400,1190 650,300	950,600 400,350	650	26	15	57.69%
Total	47	12	12	16	17	1	46	11	31	167	146(87.42 %)	96.52%
a+b		24		33		47		42		-		
Polymorphism %		51.06%		70.21%		100%		89.36%		-		
∑Polymorphism %		77.66%								-		

A= presence new bands. **B**=absence bands. **Treated 1**= treated with ciprofloxacin. **Treated 2**= treated with trimethoprim **Treated 3**= treated with pomegranate Granada. **Treated 4**=treated with *Trigonellafoenum*.

Table 7: Shown total number bands and number control, polymorphisim bands for primer OP H-08 to samples that treatments with ciprofloxacin, Trimethoprim, *pomegranate Granada* and *Trigonellafoenum*

Samples	Control Bands	Treated 1		Treated 2		Treated 3		Treated 4		Total Bands	Polymorphic bands	Polymorphic of each primer
		A	B	A	B	A	B	A	B			
1	8	380	230	1000,800,650	1520,230	-	1520,750,550,480 430,300,280,230	-	1520,750,550,480 430,300,280,230	25	23	92%
2	8	-	1750,1700,1000	650,280	1750	-	1750,1700,1500,1000, 750,480,430,300	280	1750,1700,1500,1000, 750,480,430,300	22	23	104.54%
3	9	1550,1000 550,300	1800,1600,1520 1100,650,500	1000,550,300	1800,1600,1520 1100,800,650,500	-	1800,1600,1520,1100 800,650,500,450,280	-	1800,1600,1520,1100 800,650,500,450,280	21	38	180.95%
4	7	950,420	1520,1200,730 620,450,320	950,420	1520,1200,730 620,450,320	-	1520,1200,730 620,450,320,300	1500,420 280	1520,1200,730 620,450,320,300	18	31	172.22%
5	6	1000	1550,650 500,250	1000	1550,650 500,250	-	1550,650,500 450,300,250	-	1550,650,500 450,300,250	12	22	183.33%
6	4	-	1800	-	1800	-	1800,900 450,300	-	1800,900	12	8	66.66%
Total	41	8	21	11	21	-	42	4	38	110	145(131.82%)	133.28%
a+b		29		32			42		42		-	
Polymorphism %		70.73%		78.05%			102.44%		102.44%		-	
∑Polymorphism %		88.42%									-	

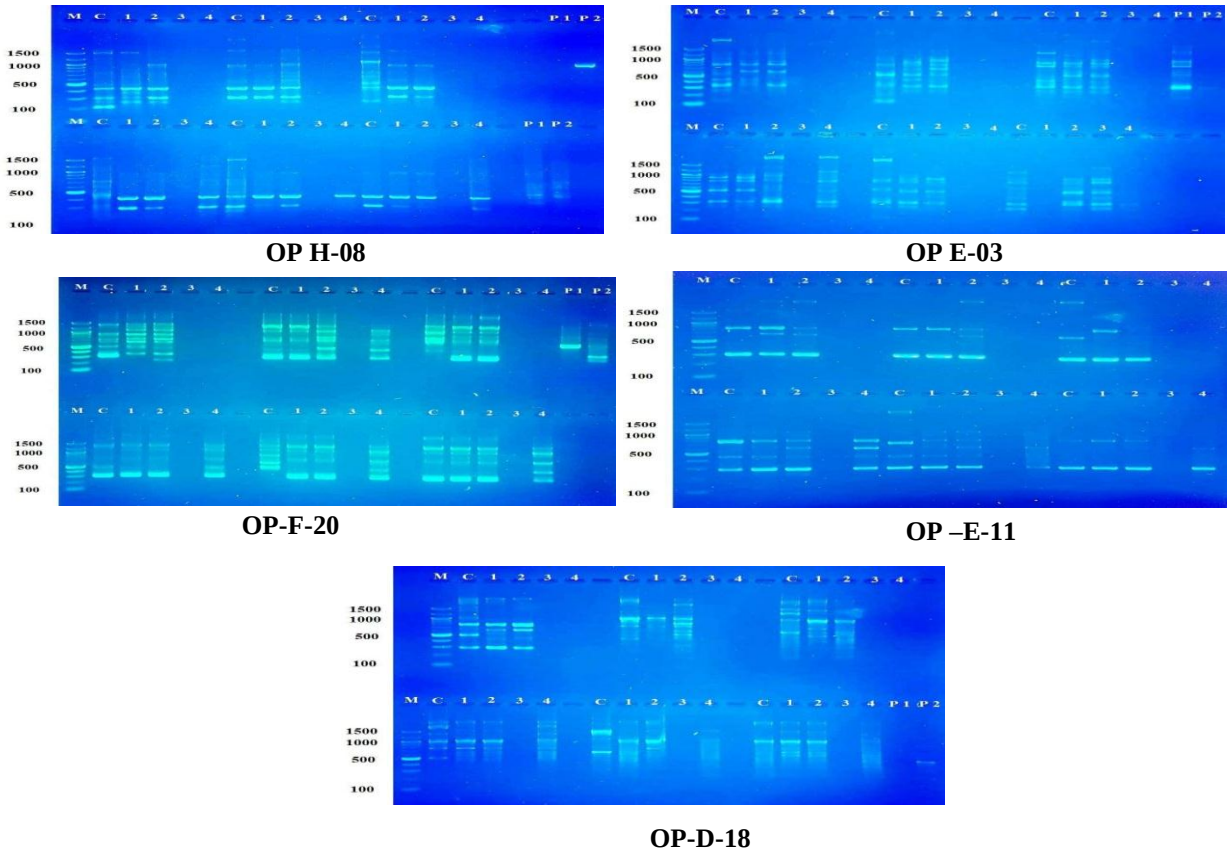

Fig. 1 : Shwon RAPD-PCR profile of *E.coli* genomic DNA after exposed to antibiotics (ciprofloxacin & Trimethoprim) and plant extracts (*pomegranate Granada, Trigonella Foenum*)compared with control samples by using primersOP H-08,OP E-03, OP F-20,OP E-11 and OP D-18.

M=marker 1- treated with ciprofloxacin
C=control 2- treated with trimethoprim
3- treated with *pomegranate Granada*
4- treated with *Trigonella foenum*

- **Genomic Template Stability GTS%**

GTS is a quantitative measure that reflects the changes in the (RABD) and as a result of the treatment of bacterial isolates with alcoholic plant extracts and antibiotics, the value of GTS % ranges from high to low as a result of the action of biological effects based on the law genetic stability as shown in the tables below.

Table (8) shows that the value of the GTS for DNA isolates of the first sample has decreased very significantly compared to the isolations of control, and this decrease increased clearly when treated with the extract of the *Trigonella foenum* seeds of the alcoholic ring by a percentage of zero and this means that the probability of The damage done to the genome by this treatment was greater at this transaction, but the highest value of GTS was at the time of treatment with antibiotic Ciprofloxacin, which amounted to 41.66%, which means that the exposure of DNA to this isolation at this treatment was lower, The GTS was valued at 17.29% as a result of the treatment with four treatments for all prefixes.

Table 8: Shown ratio GTS% for sample 1 for all treatments and primers.

Primer	Control	Treated 1	Treated 2	Treated 3	Treated 4	GTS% of each primer
OP A-01	100	0	0	0	0	0
OP A- 06	100	66.66	33.33	0	0	24.99%
OP B- 14	100	0	0	0	0	0
OP B-20	100	66.66	33.33	33.33	0	33.33%
OP D-03	100	75	37.5	0	0	28.13%
Average	**100**	**41.66%**	**20.83%**	**6.66%**	**0**	**17.29%**
Total GTS % for all treatments		**17.29%**				

As for the second sample isolates, the lowest value of GTS% when treated with pomegranate extract was zero, which means that the bio-effect of *pomegranate Granada* extract on bacterial DNA for this isolation was higher at this treatment, causing DNA destruction. While the highest value for GTS when treated with Ciprofloxacin was 68.65% this indicates that the exposure of DNA as a result of this transaction was lower than the rest of the transactions as in the table(9).

Table 9: Shown ratio GTS% for sample 2 for all treatments and primers.

Primer	Control	Treated 1	Treated 2	Treated 3	Treated 4	GTS% of each primer
OP A-01	100	50	0	0	0	12.5%
OP A- 06	100	30.76	23.07	0	0	13.45%
OP B- 14	100	100	0	0	0	25%
OP B-20	100	100	63.63	0	27.27	47.72%
OP D-03	100	62.5	62.5	0	0	31.25%
Average	**100**	**68.65%**	**29.84%**	**0**	**5.45%**	**25.98%**
Total GTS % for all treatments		**25.99%**				

The isolations of the third group had the highest effect of bacterial DNA when treated with alcohol *Trigonella foenum* seed extract and alcoholic *pomegranate Granada* extract, based on the lowest value of GTS% at 6.66%, which indicates significant damage to bacterial DNA, while the least effect of the transactions was when treated with trimethimprim, with the value of GTS 17.77%. This means that the DNA affected as a result of this transaction was less affected than the rest of the transactions, while the value of GTS% as a result of the transaction was with four bioactive effects 9.44% for all prefixes as in the table(10).

Table 10: Shown ratio GTS% for sample 3 for all treatments and primers.

Primer	Control	Treated 1	Treated 2	Treated 3	Treated 4	GTS% of each primer
OP A-01	100	0	0	0	0	0
OP A- 06	100	11.11	33.33	0	0	11.11%
OP B- 14	100	0	33.33	33.33	33.33	24.99%
OP B-20	100	22.22	22.22	0	0	11.11%
OP D-03	100	0	0	0	0	0
Average	**100**	**6.66%**	**17.77%**	**6.66%**	**6.66%**	**9.44%**
Total GTS % for all treatments		**9.44%**				

The lowest percentage of GTS for Fourth group isolates is 5 when treated with alcohol ring extract, which indicates the efficacy of the extract in changing the composition of the natrogian base, resulting in significant damage to the blood. While the least affected on the genome when treated with alcoholic *pomegranate Granada* extract was 22.33%GTS%, indicating that this extract affected the DNA of bacteria at a lower rate than other transactions. The GTS% value for all transactions and for all prefixes is 31.75% as in the table(11).

Table 11: Shown ratio GTS% for sample 4 for all treatments and primers.

Primer	Control	Treated 1	Treated 2	Treated 3	Treated 4	GTS% of each primer
OP A-01	100	0	0	0	0	0
OP A- 06	100	10	20	0	20	12.5%
OP B- 14	100	33.33	0	0	66.66	24.99%
OP B-20	100	25	50	25	25	31.25%
OP D-03	100	0	0	0	0	0
Average	**100**	**13.66%**	**14%**	**5%**	**22.33%**	**13.75%**
Total GTS % for all treatments		**13.75%**				

The highest percentage of GTS%, for Fifth group isolates, was 13.33% due to the treatment of Ciprofloxacin, which means that the proportion of damage to the genetic material of bacteria at this treatment was less harmful than the rest of the transactions. The GTS percentage was lower when treated with alcohol *pomegranate Granada* extract and alcohol *Trigonella foenum* extract, which was zero because of its ability to destroy the largest number of associated sites. The overall rate of GTS was 6.04% for all transactions and for all prefixes as in the table(12).

Table 12: Shown ratio GTS% for sample 5 for all treatments and primers.

Primer	Control	Treated 1	Treated 2	Treated 3	Treated 4	GTS% of each primer
OP A-01	100	0	0	0	0	0
OP A- 06	100	50	37.5	0	0	21.87%
OP B- 14	100	0	0	0	0	0
OP B-20	100	0	0	0	0	0
OP D-03	100	16.66	16.66	0	0	8.33%
Average	**100**	**13.33%**	**10.83%**	**0**	**0**	**6.04%**
Total GTS % for all treatments		**6.04%**				

While the lowest value of GTS% for sixth group isolates was zero when treated with alcohol *Trigonella foenum* extract, which is less valuable compared to other transactions, indicating the ability of this extract to affect the genetic material causing damage. The highest value of GTS was found when treated with Ciprofloxacin, which amounted to 57.42% which indicates the ability of bacteria to retain some of the sites of association as a result of their resistance to this antidote and therefore its effect on the genome at this treatment was less than the rest of the transactions. The value of GTS% was 28.11% for all isolations and for all transactions and prefixes and as in the table (13).

Table 13: Shown ratio GTS% for sample 6 for all treatments and primers.

Primer	Control	Treated 1	Treated 2	Treated 3	Treated 4	GTS% of each primer
OP A-01	100	57.14	42.58	0	0	24.93%
OP A- 06	100	25	37.5	0	0	15.63%
OP B- 14	100	50	50	0	0	25%
OP B-20	100	80	20	0	0	25%
OP D-03	100	75	75	0	50	50%
Average	**100**	**57.42%**	**45.01%**	**0**	**10%**	**28.11%**
Total GTS % for all treatments		**28.11%**				

Table 14: Shown a comparison between all bacterial isolates and the treatment with four treatments and for all primers

Samples	No of polymorphism bands	% polymorphism for all each treatments and primers	Rate % GTS for all treatments and primers
1	138	84.14%	17.29%
2	126	75%	25.99%
3	137	97.86%	9.44%
4	91	92.50%	13.75%
5	134	102.27%	6.04%
6	81	75.96%	28.11%
Total	**707**	**87.96%**	**16.77%**

Table No. (14) shows that the total number of bands for all treatments and primers is (707) bands and that the least effect of the samples due to these effects was for sample 6 as the number of polymorphism bands (81) band and the percentage of the number of polymorphism bands was 75.96% while the percentage GTS is 28.11% indicating the portability of DNA to resist these effects, while the high effect of the samples due to these effects was for sample 5 as the number of polymorphism bands was 134 and the percentage of the number of polymorphism bands was 102.27% while the percentage GTS 6.04%,which indicates the inability of the DNA to maintain its genetic material as a result of exposure to these treatments and thus the destruction of many of the link sites of the gene and the decrease of its stability.

Medicinal plants produce many pharmaceutical compounds that are used in the treatment of many diseases affecting humans and animals [61] Particularly pomegranate and fenugreek plant, mainly because of its wide range of effective secondary metabolic compounds such as glycosides, tannins, volatile oils, flavonoids, saponies, polyphenols, resins and alkaloids, for example, the effectiveness of flavonoids towards bacteria is attributed to their ability to form a complex compound with endogenous cell proteins and is installed with the cellular wall of the bacterial cell as well as the case with alkalioids. Interference with the DNA of the bacteria cell while tannins and phenolic compounds work to inhibit enzymes and vector proteins, while soaps interfere with cellular membranes, especially with cell membrane steroids and thus work on the analysis of the living cell of bacteria, while phenolic compounds work to inhibit the action of enzymes and proteins conveyors as stated in [62]. The oxidative efficacy of phenol compounds is dispersed from the

α-amylase compound, which is a inhibitor in the extract associated with the active sites of enzymes and those that inhibit their action, inhibiting the growth of bacteria [63].

Alkaloids interfere with cellular membranes, change their permeability, interfere with metabolic processes such as electron transmission chain, nutrient absorption, etc., or inhibit and denaturated cellular enzymes and proteins [64] Causing severe damage to the cell wall, cell membrane, nuclear acids and the formation of ion channels in cellular membranes [65], The study showed a change in genetic material based on differences in RAPD marker pattern as a result of four effects treated bacteria samples compared to unexposed samples where sites that existed prior to treatment disappeared and in turn the emergence of bands that did not exist. The cause of the disappearance in the beams caused by exposure to the biological effects can be due to DNA damage such as the break-up of the single or double-threaded DNA chain, or because of changes in the location of the complementary nucleotide sequencing, which may be due to mutations or rearrangements, or due to the occurrence of point mutations and chromosomal rearrangements [66].

The disappearance of bands may be a change in the DNA sequences, which leads to a change in the characteristics of the basal pairs caused by the exposure with antibiotics and plant extracts , thereby resulting in different DNA sequences [67].The primer has found complete sites and these sites are in the sequences of DNA control samples [68].Through the above we find that the effect of bacteria by the biological effects can be in two forms, the first led to the absence of the beam when exposed to these effects as a result of the influence of the protein within the DNA and therefore the inability of the two chains to wrap around the protein and show the beams. The second form of effect was caused a mutation either because of a change in the sequence of nucleotides caused by these effects stench or by transposons found within the DNA, where vectors can influence the adjacent genes, resulting in a mutation [69].

Genomic template stability (GST%) is a qualitative measure of changes in RAPD profiles used to determine the effect of a biological factors on the genetic material of the bacterial cell and thus reflects the efficiency or ability of the repair and replication system in the organism [66] As a result of these treatments, the value of GTS% is differentiated between high and low value and the high value of genetic stability means that the cell is less susceptible to damage from the biological effect. The low value of the GST means that the treatments has a significant impact on the genome on the DNA of the bacterial cell. The results showed that the value of GTS% has decreased significantly during treatments with antibiotics and plant extracts, which shows that the high proportion of damage to bacterial cell DNA is these treatments [70]. During PCR reaction, when enzyme Taq polymerase meets destroyed DNA, this will lead to the closed of the binding sites, the enzyme's inability to bind, meaning the loss of sites that existed prior to these effects exposure [71].

In conclusion, the expose with biological effects (antibiotics & plant alcoholic extracts) causes genetic changes in the Deoxyribose Nucleic Acid of *E.coli* bacteria, specially the *pomegranate* extract alcohol was the most effective on bacterial isolates from the other treatments, as it destroyed the DNA of bacterial cells and reduced the gene stability. So these biological factors can be used in treatment urinary tract infection that causes by bacteria. The RAPD-PCR method may be used to evaluate the biological effects of treatments on bacteria as a biomarker assay.

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تحليل التباين الوراثي لبكتيريا القولون المعزولة من المصابين بإلتهاب المسالك البولية بعد تعريضها لبعض المؤثرات الحيوية بإستعمال تقنية التفاعل العشوائي متعدد الاشكال

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الملخص

تعد بكتيريا القولون هي احد اهم الاجناس البكتيرية المسببة لإلتهاب المسالك البولية التي تكون مسؤولة عن 90% من مسببات التهاب المسالك البولية ومصدرها امعاء الانسان وهي بكتيريا سالبة لصبغة كرام هوائية اختيارية تتحرك بواسطة اسواط محيطية وتنتمي الى بكتيريا العائلة المعوية.

المواد وطرق العمل: تم عزل البكتيريا من اصابات المسالك البولية بعدها شخصت بإستعمال المجهر الضوئي وإجراء الاختبارات البايوكيميائية، وبعدها عرضت للمضاد الحيوي السبروفلوكساسين والترايميثبريم والمستخلص الكحولي قشور الرمان وبذور الحلبة بعدها تم استخلاص الدنا لجميع العينات وأُستعمل مؤشر التفاعل التضاعفي المتعدد الاشكال بإستعمال 5 بادئات عشوائية.

النتائج: أظهرت النتائج وبإستخدام تقنية التفاعل العشوائي متعدد الاشكال RABD إن بكتريا *E.coli* المعرضة للمضادات الحيوية (السبروفلوكساسين والترايمثبريم) والمستخلصات الكحولية (قشور الرمان وبذور الحلبة) قد أدت الى اختفاء أو ظهور حزم جديدة مقارنة بالعينات غير المعرضة، وان اعلى معدل لتباين الحزم لجميع البادئات كان للعينة 5 إذ بلغ 102.27% بينما بلغ معدل الاستقرار الجيني لهذه العينة ولكافة البادئات والمعاملات 6.04%.

الاستنتاج : ان جميع المعاملات تسببت في حدوث تغييرات جينية في دنا خلايا بكتيريا *E.coli*، لاسيما قشور الرمان الذي اعطى اعلى تأثير من باقي المعاملات مما يدل على كفائته في معالجة الاصابات البكتيرية.