# DETECTION the GENETIC EFFECTS of ANTIBIOTICS and PLANT EXTRACTS on E.COLI BACTERIAL ISOLATED FROM UTI PATIENTS USING RAPD MARKERS

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***Abstract**:*
**Background and Objective: *E.coli* bacteria is the common cause of the urinary tract infections as it is responsible for about 90% of cases of urinary tract infections and it is considered one of the main problems in hospital infected. So, this study aimed to assess the genetic effects of ciprofloxacin, trimethoprim, alcoholic *pomegranate Granada* extract and alcoholic *Trigonella foenum* on coliform bacteria.**
**Materials and methods: The bacteria were isolated from urinary tract infections, after they were diagnosed using an optical microscope and conducting biochemical tests, then exposed to the antibiotic Ciprofloxacin, trimethoprim, and alcoholic extract of *pomegranate Granada* and *Trigonellafoenum*. Genomic DNA was extracted for all samples and Random amplified polymorohic DNA - Polymerase chain reaction (RAPD-PCR) marker was carried out using five randomprimers.**
**Results: The results of RAPD-PCR profiles shown thate xposed to antibiotics (ciprofloxacin and trimethoprim) and alcoholic extracts (*pomegranate Granada* and *Trigonellafoenum*) lead to the disappearance or appearance new bands compared with non-exposed samples, and the highest rate of polymorphisim for all each treats and primers in sample 3 was 105.88% where the ratio % GTS for all treats and primers is 10% in the same sample.**
**Conclusion: All treatments caused genetic changes in the DNA of *E.coli* bacteria cells especially the *pomegranate Granada* which gave the highest effect than the rest of the treatments, this indicates its efficiency in treating bacterial infections.**

***Key words: E.coli, RAPD-PCR, pomegranate Granada, Trigonellafoenum, Urinary tract infection.***

## INTRODUCTION

Urinary tract infections UTI is one of the important diseases common in societies of developing and developed countries, where the largest proportion of them constitute bacterial pathogens, especially among women, children, and those with renal impairment, and the number of people with urinary tract infections is estimated at about 250 million injured each year(1). This infection constitutes 80% of cases of chronic prostatitis (Chronic Bacterial Prostatitis) and 90% of cases of pelvic nephritis (Pyelonephritis) and also includes infections of the bladder (Cystis) and urethra (Urethritis). The causative agents of this disease are bacteria and fungi, and parasites and viruses rarely participate in infection (2). And the normal urine is free from any bacterial, fungal or viral contamination of healthy people, and inflammation occurs in the urinary tract when the bacteria of the digestive system in the anus reach the organs of the urinary system near the opening of the urinary tract where they begin to grow and multiply (3). *E.coli* bacteria are the most important types

that cause urinary tract infection.(4). 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(5,6). as such Excel bacteria Coli Having multiple antibiotic resistance (MDR) Multing Resistance (7) By possessing resistance enzymes, including enzymes β-lactamases, and Other enzyme confer resistance to antagonists aminoglycosides and for quinolones. In addition to its possession of other resistance mechanisms such as changing the permeability of the cell membrane, changing the target site, inhibiting the manufacture of proteins, and its possession of efflux pumps, as it gives bacteria resistant to antibiotics such as Microlides, Novobiocin and Rifamcin (8). The emergence of strains Bacterial resistance For antibiotics Urging researchers to find more effective alternatives against microorganisms Like Medicinal plants, as they contain substances that have microbial activity Antimicrobial activity, the most important of which are alkaloides, glycosides, essential oils, resin and tannins, Gum, Phenoles, Lighter Fats, and Carbohydrates (9).

## MATERIALS AND METHODS

Collection of samples

Collected (100) a urine sample from patients suffering from symptoms of urinary tract infection, as this study was conducted in the city of Tikrit / Salah al-Din General Hospital in the microbiology laboratory and for the period from 7/1/2019 to 12/3/2019, and urine samples were collected according to the method (10), as I used sterile plastic Countainers to collect urine samples and it was recommended that the collection be from the middle of the morning urine (Clean-Catch-Midstream Urine).

Culture and isolation of *Ecoli* bacteria

The urine samples were cultured on the media of the blood agar, macConkey agar and mannitol salt agar, and then the petri dishes were incubated in aerobic conditions at 37 ° C° C for a period of 24 hours, and after the first culture, the isolates were purified by making a secondary isolation Sub Culturing to Isolate bacteria causing to UTI According (11,12). The isolated and pure colonies were then transferred to the nutrient Agar Slants, after being incubated, were kept in the refrigerator at a temperature of 4 ° C until the diagnostic tests were carried out (13).

Diagnosis of bacterial isolates:

The bacterial isolates were phenotypically diagnosed by observing the cultivation characteristics of the developing colonies and staining them with Cram stain and tested By optical microscope using the oil lens X100 (14,15). These isolates were also diagnosed based on biochemical tests The diagnosis was confirmed using the VITEC-2 device (16).

Antibiotics resistant of *E.coli* bacteria

The resistance of bacteria to antibiotics is one of the most important health and economic problems in the world, as infection with bacteria Resistance drug leads to longer treatment times and increased risk of infection. There are several types of antibiotic resistance, including (MDR (multi-drug resistant), XDR (extensively- drug resistant) and (PDR) pan-drug resistant (MDR) means bacteria are resistant to at least one out of three antibiotics, and XDR resistant means Bacteria are resistant to two or all of the antibiotics taken, whereas PDR means that the bacteria are resistant to all antibiotics (17). This resistance trait is either innate or acquired, either through mutations in the genes. (Chromosomal mutations),or through the transfer of the genetic material from one bacterium to another in several ways, which is either through bacterial conjugation, in which the genetic material is transferred from one cell to another directly, such as plasmids and transposons, or by

transformation, in which the genome bacteria is taken that is released from dead bacteria, or by transduction, it's the transmission of genetic information between bacterial cells by bacteriophages (18).Possesses bacteria Several antibiotic resistance mechanisms emerge after resistance genes are transferred to them These include alteration of the target site, change in the permeability of the cell membrane, production of enzymes, alteration of metabolic pathways (8).

Diagnosis of bacterial isolates:
The bacterial isolates were phenotypically diagnosed by observing the cultivation characteristics of the developing colonies and staining them with Gram stain and tested By optical microscope using the oil lens X100 (14,15). These isolates were also diagnosed based on biochemical tests The diagnosis was confirmed using the VITEC-2 device (16).

Antibiotics resistant of *E.coli* bacteria
The resistance of bacteria to antibiotics is one of the most important health and economic problems in the world, as infection with bacteria Resistance drug leads to longer treatment times and increased risk of infection. There are several types of antibiotic resistance, including (MDR (multi-drug resistant), XDR (extensively- drug resistant) and (PDR) pan-drug resistant (MDR) means bacteria are resistant to at least one out of three antibiotics, and XDR resistant means Bacteria are resistant to two or all of the antibiotics taken, whereas PDR means that the bacteria are resistant to all antibiotics (17). This resistance trait is either innate or acquired, either through mutations in the genes. (Chromosomal mutations),or through the transfer of the genetic material from one bacterium to another in several ways, which is either through bacterial conjugation, in which the genetic material is transferred from one cell to another directly, such as plasmids and transposons, or by transformation, in which the genome bacteria is taken that is released from dead bacteria, or by transduction, its the transmission of genetic information between bacterial cells by bacteriophages (18).Possesses bacteria Several antibiotic resistance mechanisms emerge after resistance genes are transferred to them These include alteration of the target site, change in the permeability of the cell membrane, production of enzymes, alteration of metabolic pathways (8).

Test of the inhibitory effectiveness
The agar diffusion-well method is used,where a swab of the bacterial suspension was taken at the age of 24 hours and the turbidity of the growth was compared with standard turbidity constant solution (McFarland) and spread on the center of Molar Hinton agar. Then 5 holes of 8 mm diameter were made on the surface of the culture medium in the dishes using a sterile cork borer. Different concentrations of each extract will moved (25, 50, 75, 100) by 50 ML in each hole with a control hole made containing sterile distilled water, and the efficacy of the two extracts was measured by measurement Diameter rates of Formed around each hole, estimated in milliliters, after an incubation period of (24) hours, at a temperature of (37) C, and the process was repeated (3) times for each isolation. (19).

Bacterial exposing to antibiotics and plant extracts
We chose 6 samples of bacteria *E.coli* The most resistant to antibiotics and was exposed to ciprofloxacin, trimethorim, and extracts alcohol *pomegranate Granada* and *Trigonellafoenum*. This was done by preparing several dilutions for each antibiotic and each extract, exposing them to the bacteria, and determining the minimum inhibitory concentration (MIC)to all of them were relied upon to extract bacterial DNA after exposure.

Genomic DNA extraction

The genomic DNA was isolated according to the (20). The integrity and purity of the DNA was determined by electrophoresis on a dye-stained agarose gel by Red safe stain.

RAPD-PCR reactions

The reactions of the RAPD-PCR technique were performed using five random Primers as shown in the table 1:

Table (1): Shows names and sequences used random primers

| No. | Primer code | Nucleotide sequence 5 to 3 |
|---|---|---|
| 1 | OP A-01 | CAGGCCCTTC |
| 2 | OP A-06 | GGTCCCTGAC |
| 3 | OP B-14 | TCCGCTCTGG |
| 4 | OP B-20 | GGACCCTTAC |
| 5 | OP D-03 | GTCGCCGTCA |

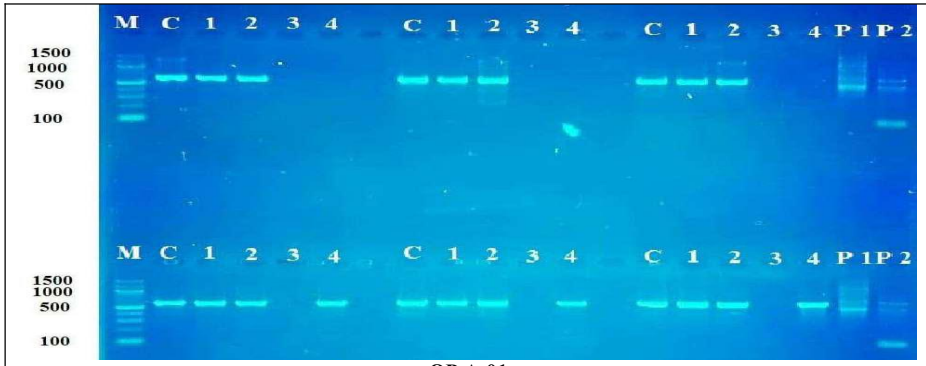
Accupower PCR premix supplied by the Korean company Bioneer. If the reaction was performed by addition 2uL is a random primers for each eppendrof tube at a concentration of 10 picomole. Then added 2uL of the template DNA into the mixture And 16 ML From sterile distilled water to each tube To become total volume 20ML and a light blending is done (Spin) to the components of the reaction, and then the tubes were transferred and placed in the Thermocycler device and the amplified reaction was carried out by adjusting the device according to the program specified for the reaction and the After the end of the program time Lifted The tubes from the thermocycler device for the purpose of electrophoresis on an aerosol gel concentration of 1.5% Then put Inside the UV imaging machine to observe the DNA bands formed with their molecular sizes based on molecular marker 100pb DNA laddar (21).

Analysis of the genetic results

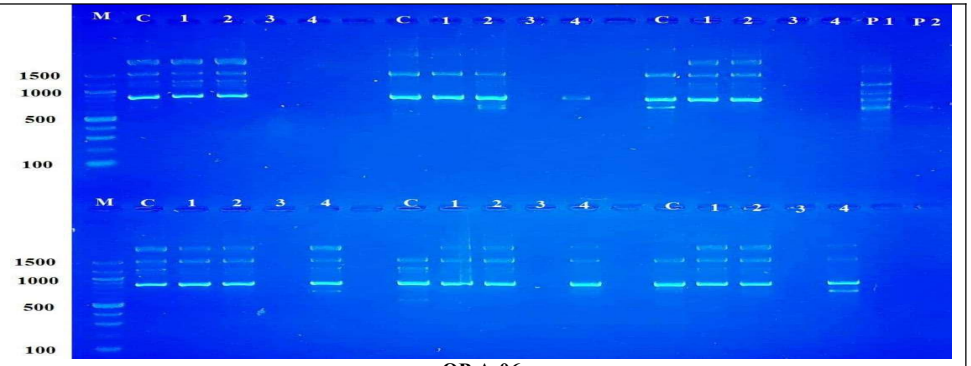
The results were recorded And that is by writing the size of the gained and lost bands for each isolate in special tables for both antibiotic and extract and the Genetic template stability GTS% was measured by using the law GTS% = (1-a / n) x 100 (22).that:-a = number of polymorphic bands detected in each treated sample, n = number of total bands of the non-exposed control group. The percentage of polymorphisms was measured using the following relationship (23). Polymorphism value = (total No.of a+b)/(total No.of observed bands of control)×100.

**RESULTS**

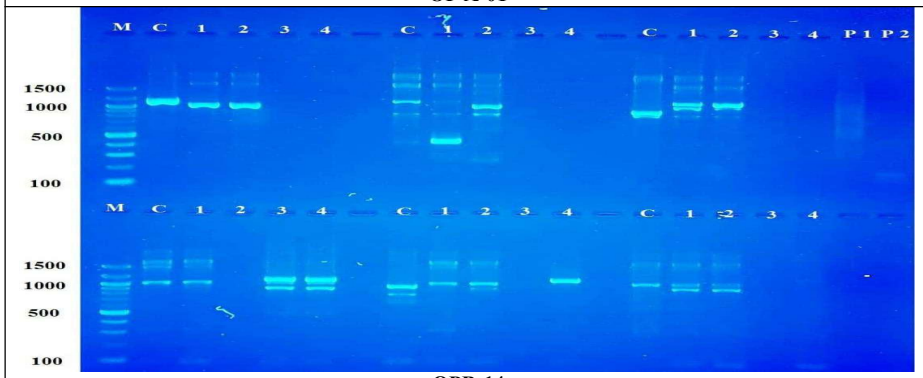
The results of the RAPD-PCR profiles of *E.coli* bacteria using five primers are shown in the figure 1. There is a clear contrast to the sites the link bacterial isolates treated with antibiotics (ciprofloxacin and trimethoprim) and plant extracts alcohol (*pomegranate Granada* and *Trigonellafoenum*) as shown in table (2-6). From the results, we notice the disappearance of bands after treatment with these biological effects due to the inability of the bacteria to resist them than to destroyed DNA Loss of link sites. On the other side, we notice the emergence of new bands In isolates treated with biologics were not present in control isolates because of its ability to repair genetic mater and find new link sites.



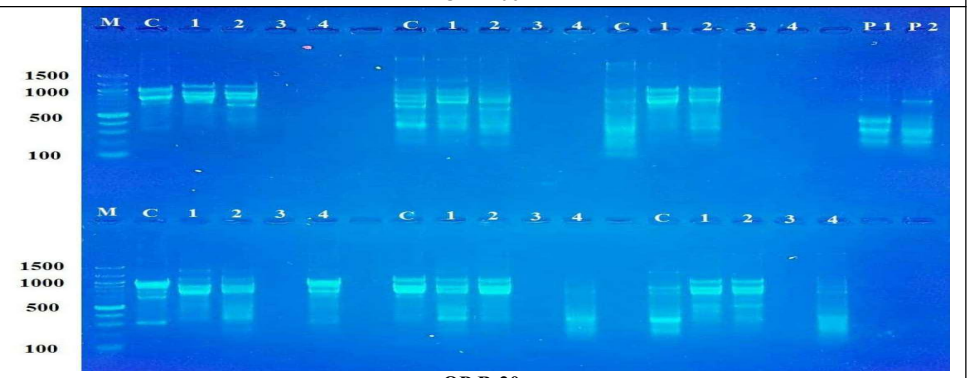

**OP A-01**



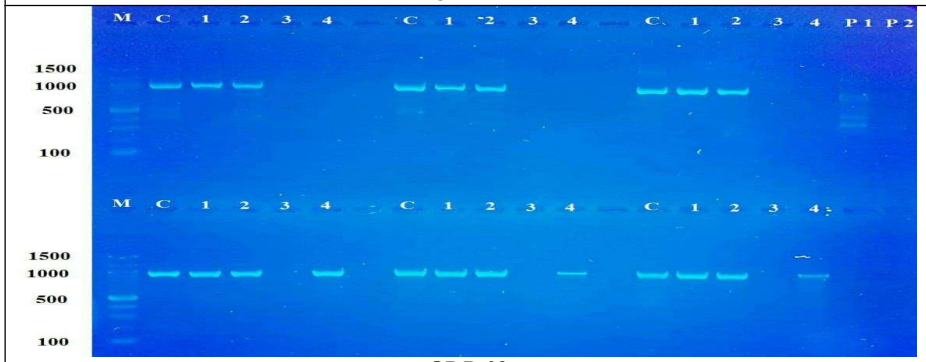

**OP A-06**




**OPB-14**




**OP B-20**




**OP D-03**

Figure 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 primers
OP A-01,OP A-06, OPB-14,OPB-20 and OPD-03.

Table (2): Shown total number bands and number control, polymorphisim bands for primer OP A-01 to samples that treatments with ciprofloxacin, Trimethoprim, *pomegranate Granada* and *Trigonellafoenum.*

| Samples | ControlBands | 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 | 2 | - | 1300 | - | 1300 | - | 1300,650 | - | 1300,650 | 4 | 6 | 66.66% |
| 2 | 1 | - | - | 1300,500 | - | - | 650 | - | 650 | 5 | 4 | 80% |
| 3 | 1 | - | - | 1300 | - | - | 650 | - | 650 | 4 | 3 | 75% |
| 4 | 1 | - | - | - | - | - | 650 | - | - | 4 | 1 | 25% |
| 5 | 1 | - | - | - | - | - | 650 | - | - | 4 | 1 | 25% |
| 6 | 1 | - | - | - | - | - | 650 | - | - | 4 | 1 | 25% |
| Total | 7 | 0 | 1 | 3 | 1 | - | 7 | - | | 25 | 16(64%) | 49.44% |
| a+b | | 1 | | 4 | | 7 | | | | - | | |
| Polymorphism % | | 14.28% | | 57.14% | | 100% | | 57.14% | | - | | |
| ∑Polymorphism % | | 57.14% | | | | | | | | - | | |

**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 (3): Shown total number bands and number control , polymorphisim bands for primer OP D-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 | 1 | - | - | - | - | - | 950 | - | 950 | 3 | 2 | 66.66% |
| 2 | 1 | - | - | 1600 | - | - | 950 | - | 950 | 4 | 3 | 75% |
| 3 | 2 | - | 1550 | - | 1500 | - | 1550,950 | - | 1550,950 | 4 | 5 | 150% |
| 4 | 1 | - | - | - | - | - | 950 | - | - | 4 | 1 | 25% |
| 5 | 1 | - | - | - | - | - | 950 | - | - | 4 | 1 | 25% |
| 6 | 1 | - | - | - | - | - | 950 | - | - | 4 | 1 | 25% |
| Total | 7 | - | 1 | 1 | 1 | - | 7 | - | 4 | 23 | 14(60.865) | 61.11% |
| a+b | | 1 | | 2 | | 7 | | 4 | | - | | |
| Polymorphism % | | 14.28% | | 28.57% | | 100% | | 57.14% | | - | | |
| ∑Polymorphism % | | 49.99 % | | | | | | | | - | | |

**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 B-14 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 | 1 | 1600,1550, 1050 | 1100 | 1600,1550, 1050 | 100 | - | 1100 | - | 1100 | 7 | 10 | 142.85% |
| 2 | 6 | 1500,450, 300 | 1750, 430 | 1500,1400,1000 600,300,280 | 1750, 430 | - | 1750,1700,1600, 1150,800,430 | - | 1750,1700,1600, 1150,800,430 | 20 | 25 | 125% |
| 3 | 3 | 1600,1550, | 900 | 1600,1550, | 900 | - | 1700,900,850 | - | 1700,900,850 | 15 | 16 | 106.66% |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1100,1500 | | 1100,1500 | | | | | | | | |
| **4** | **5** | - | - | - | 1700,1500, 350,1550, 1000 | 950, 1150, 650 | 1700,1500,350 | 1150, 950, 650 | 1700,1500,350 | **20** | **17** | **85%** |
| **5** | **4** | 1550,1590, 1150,350 | 950,850, 650 | 1550,1590, 1150,350 | 950,850, 650 | - | 1700,950, 850,650 | 1100 | 1700,950, 850,650 | **15** | **23** | **155.33%** |
| **6** | **6** | 950 | 600,350 | 950 | 600,350 | - | 1650,1590, 1500,1100, 600,350 | 1200 | 1650,1590, 1500,1100, 600,350 | **18** | **18** | **153.33%** |
| **Total** | **25** | **15** | **9** | **18** | **14** | **3** | **23** | **5** | **22** | **59** | **109(114.74%)** | **120.39%** |
| **a+b** | | **24** | | **32** | | **26** | | **27** | | - | | |
| **Polymorphism %** | | **96%** | | **128%** | | **104%** | | **108%** | | - | | |
| **∑Polymorphism %** | | **109%** | | | | | | | | - | | |

**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 A-06 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** | **6** | - | 680 | - | 680 | - | 1700,1550,1300, 1180,850,680 | - | 1700,1550,1300, 1180,850,680 | **16** | **14** | **87.5%** |
| **2** | **4** | - | 650 | 1200,800,750 | 1700,650 | - | 1700,1550,850,680 | - | 1700,1550,680 | **14** | **13** | **92.85%** |
| **3** | **4** | 1700, 1300, 1180 | 1800, 750 | 1700,1300, 1180,1750 | 1800, 1750 | - | 1800,1550,850,750 | - | 1800,1550, 850,750 | **15** | **19** | **126.66%** |
| **4** | **5** | - | - | - | - | - | 1700,1550,1300, 1180,850 | 750 | - | **21** | **6** | **28.57%** |
| **5** | **5** | 1700 | 600 | 1700 | 600 | - | 1550,1300,1180, 850,600 | 1700, 750 | 1300,1180 | **19** | **13** | **68.42%** |
| **6** | **2** | 1700, 1300, 1180 | - | 1700,1300,1180 | - | - | 1550,850 | 1700, 750 | - | **16** | **10** | **62.5%** |
| **Total** | **26** | **7** | **5** | **11** | **6** | **-** | **26** | **5** | **15** | **101** | **75(74.25%)** | **77.75%** |
| **a+b** | | **12** | | **17** | | **26** | | **20** | | - | | |
| **Polymorphism %** | | **46.15%** | | **65.38%** | | **100%** | | **76.92%** | | - | | |
| **∑Polymorphism %** | | **72.11%** | | | | | | | | - | | |

**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 B-20 to samples that treatmeants 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** | **5** | 1200 | 1100,350 | 1200,650, 450 | 1100,700, 350 | - | 1100,950, 800,700,350 | - | 1100,950, 800,700,350 | **14** | **19** | **135.71%** |
| **2** | **9** | - | 700 | 650,450, 300 | 700,380 | - | 1750,1400,1000 900,800,700, 500,400,380 | - | 1750,1400,1000 900,800,700, 500,400,380 | **25** | **24** | **96%** |
| **3** | **7** | 1200,900 | 1700,1100, 500,350,150 | 1200,900, 300 | 1700,1100, 800,150 | - | 1730,1700, 1100500, 800,350,150 | - | 1730,1700, 1100500, 800,350,150 | **17** | **28** | **164.70%** |
| **4** | **7** | 1100,900, 500,350 | 1800,1650, 1000,650,300 | 1100,900,500 450,350,250 | 1800,1650, 1000,650,300 | - | 1800,1650, 1400,1000, 800,650,300 | 900,850, 550,350 | 1800,1650, 800,300 | **82** | **35** | **125%** |
| **5** | **6** | 1400,250 | 750 | 1400 | 750 | - | 1100,1200,850 750,500,350 | 300,230 | 750 | **62** | **14** | **53.84%** |
| **6** | **6** | 1700,1100, 450,400 | 1400,350,250 | 1700,1100, 400 | 1400,350,250 | - | 1400,900,800 500,350,250 | 1700,1100 | 1400,500 | **25** | **23** | **92%** |
| **Total** | **40** | **13** | **17** | **19** | **18** | **-** | **40** | **8** | **28** | **135** | **143(105.93%)** | **111.21%** |
| **a+b** | | **30** | | **37** | | **40** | | **36** | | - | | |
| **Polymorphism %** | | **75%** | | **92.5%** | | **100%** | | **90%** | | - | | |
| **∑Polymorphism %** | | **89.38%** | | | | | | | | - | | |

**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*.

Calculate of the Genomic Template Stability (GTS %)

| Primer | Control | Treated 1 | Treated 2 | Treated 3 | Treated 4 | GTS% of each primer |
|---|---|---|---|---|---|---|
| **OP A-01** | 1 | 1 | 1 | 0 | 1 | 75% |
| **OP A- 06** | 1 | 0 | 0 | 0 | 0 | 0 |
| **OP B- 14** | 1 | 1 | 0 | 0 | 0 | 25% |
| **OP B-20** | 1 | 0 | 0 | 0 | 0 | 8.33% |
| **OP D-03** | 1 | 1 | 1 | 0 | 1 | 75% |
| **Total** | **100%** | **50%** | **50%** | **0** | **46.66%** | **36.67%** |
| **Total GTS % for all treatments** | **36.67%** | | | | | |

the result of treatment with antibiotics and plant extracts is valuable GTS% They vary between a high and a low value, and that a high value for the gene stability ratio means that the genome is less susceptible to the resulting damage, while its low value means that it is more exposuer to harm. as shown in the tables below:

Table (7): 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 | 50 | 50 | 0 | 0 | 25% |
| **OP A- 06** | 100 | 83.33 | 83.33 | 0 | 0 | 41.66% |
| **OP B- 14** | 100 | 0 | 0 | 0 | 0 | 0 |
| **OP B-20** | 100 | 40 | 0 | 0 | 0 | 10% |
| **OP D-03** | 100 | 100 | 100 | 0 | 0 | 50% |
| **Average** | **100** | **54.66%** | **46.66%** | **0** | **0** | **25.33 %** |
| **Total GTS % for all treatments** | | **25.33%** | | | | |

Table (8): Shown ratio GTS% for sample 2 for all treatments and primers.

Table (9): shown ratio GTS% for sample 3 for all treatments and primers.

Table (10): Shown ratio GTS% for sample 4 for all treatments and primers.

Table (11): Shown ratio GTS% for sample 5 for all treatments and primers.

Table (12): Shown ratio GTS% for sample 6 for all treatments and primers.

Table (13): 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 | 51 | 85% | 25.33% |
| **2** | 69 | 82.14% | 22.50% |
| **3** | 72 | 105.88% | 10% |
| **4** | 60 | 78.95% | 49% |
| **5** | 52 | 76.47% | 45.33% |
| **6** | 53 | 82.81% | 36.67% |
| **Total** | **357** | **85.21%** | **23.31%** |

As we notice a clear effect on the genetic material of bacteria *E.coli* when exposed to these treatments, which indicates that they have caused damage to the DNA of the bacteria cell, and we find that the highest effect was on the third sample, as the number of disparate sites produced by the primers rise 72 linked sites and that the % of the polymorphism of this group rise 105.88%, and this is evidence of The inability of the bacteria to resist these effects, which lead to damage to the DNA nitrogen base sequences. and that the% GTS for it was 10% evidence that the damage to the genome is very large than the rest of the isolates, on the other side, we find that the isolates least affected by these treatments are the fourth sample isolates, depending on the number of disparate bands as it rise 60 linked sites and that the

ratio of polymorphisms rise 78. 95%, which indicates that the isolates were more resistant to the four effects, meaning that they had a high ability to preserve their binding sites and repair the DNA, while the rate of genetic template stability was 49%, which means that the damage to the genome due to these treatments was less in this isolation than other isolates.

## DISCUSSION

The active role for two antibiotics ciprofloxacin and trimethopim to killing bacteria comes from mechanism of action to these antibiotics against bacterial cells for example ciprofloxacin work against active site in bacterial cell and lead to destroy DNA gyrase enzyme or topoisomerase, these enzymes that very importance during replication cell. It work to reduce supercoiling DNA gyrase that consist of four unit, two called gyr A and the other two called gyr B that coded of two type of genes is gyr A and gyr B, while Topoisomerase consist of two unit called par C and two other unit called par E that coded for par C & par E genes (24). While the action of trimethoprim conjugated with sulphonamide group to inhibition and prevention folic acid for bacteria by inhibition enzyme called dihydrofolatereductase (DHFR) which reduces from configuration middle compound that called dihydrofolate and therefore stopped manufacturing of folic acid so that lead to prevention growth and replication bacteria (25). On the other side increased attention in recent years for study medical plants because its free from side effects compared with antibiotics not be static always (increased resistance bacteria through time) and may be with side effects (26). *Pomegranate Granada* which contain wide range of active secondary metabolic compounds includes (Alkaloides, Glycosides, Essential oils, Resin and Tannins, Gum, Phenols, Flavonoids , and Carbohydrates) and the role of flavonoids against bacteria is to ability to formation complex compound with self cell proteins and ride with cellular wall of bacterial cell, as well as with alkaloids that is works on interacting with DNA bacterial cell while the works of tannins, Phenols to inhibited enzymes and transport proteins, and the works of saponins is interact with sterols cell membrane and lead to lysis of cell bacteria (27), and the role biological effects of *Trigonellafoenum* extract comes from contain variety compound which steroids , nitrogen compounds, phenolic compounds , essential oils amino acids and other (28) the phenolic compounds works to interact with DNA bacterial cell and lead to kill bacteria (29). so that when exposed *E.coli* bacteria to these treatments we notes new bands not present in control sample or disappear bands has presence in control sample. The cause of disappearance in the beams caused by exposure to these biological effects can be due to DNA damage such as the break-up of the single or double DNA chain, or because of changes in the location of the complementary nucleotide sequence ,which may be due to mutations or rearrangements, or due to the occurrence of point mutations and chromosomal rearrangements (30). The disappearance of bands may be a change in the DNA sequence, which leads to a change in the characteristics of the basal pairs caused by these treatments (ciprofloxacin,trimethoprim, *pomegranate Granada* and *Trigonellafoenum*, thereby resulting in different DNA sequences (31). The emergence of new bands may be due to the emergence of new link sites that have become more suitable for the association of primers after exposure to effects biological factors, or because of the deletion of a particular area of DNA (30) or The primer has found complete sites and these sites are in the sequences of DNA control samples genome (32).

Through the above we find that the effect of bacteria by antibiotics and medical plant extracts can be in two forms, the first lead to the absence of the beam when exposed to these treatments 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 treatments or by transposons found within the DNA, where vectors can influence the adjacent genes, resulting in a mutation (33,34). 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 (35) 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 (36). 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 (37).

In conclusion, the expose with biological effects (antibiotics & plant extracts) causes genetic changes in the DNA 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 decreased the gene stability.

So these biological factors can be used in treatment urinay tract infection that causes by bacteria. The RAPD-PCR method could be used to evaluate the biological effects of treatments on bacteria as a biomarker assay.

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