

## Unveiling novel antibacterials through DNA sequence analysis and molecular docking leads to *gyrB* gene ATP binding pocket inhibition of *E.coli* that is isolated from UTI

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### Abstract

**Background:** *Escherichia coli*, which normally lives in the colon, is the most common cause of urinary tract infections (UTI), accounting for 80-85% of community-acquired infections. This research aims to study the three-dimensional shapes of the *gyrB* gene, identify the region of the ATP binding pocket, and discover new antibacterials that can bind to this pocket and inhibit the action of DNA gyrase enzyme in *E.coli* bacteria, due to the development of the bacteria and its resistance to antibiotics.

**Methods:** In hospitals of the Holy Kerbala Governorate (Imam Al-Hussein teaching, Imam Al-Hassan Al-Mujtaba, Obstetrics and Gynecology Hospital, and Imam Al-Hujjah Charitable Hospital) 223 specimens were collected from patients with symptoms of UTI, according to standard procedure, and urine culture was performed. The bacteria were then identified using API 20E test. The *gyrB* gene was then amplified, sequenced and examined using bioinformatics techniques. The region of the enzyme's ATP binding pocket was identified and its three-dimensional shape was examined. Afterward, chemical compounds were proposed by using molecular docking to attach to this pocket.

**Results:** 60 out of the 223 specimens had urinary tract infection symptoms had an *E.coli* infection. The sequence alignment of nitrogenous bases in the *gyrB* gene was 99–100% comparable to the global sequences of the same bacterium, according to molecular analysis. It demonstrated 99–100% similarity to the global sequences for amino acids as well. Additionally, several suggested chemical compounds, such as Dieckol, Robinin, Quercitrin, Vitamin D3, Vitamin D2, Myricitrin, and Daidzin have high docking scores with the ATP binding pocket (Which was determined by previous research working in this field) and some have weak docking scores with the ATP binding pocket such as Scopoline, Crocin, Allicin and Propionic acid. Then the compounds with high docking scores were submitted to Lipinski's rule of five to know the chemical compounds most suitable for oral administration, where it was found that the compounds Daidzin, Dalbergin, Quercitrin, Cyproheptadine, Piroxicam, and others are the most suitable compounds for oral administration and for inhibiting the ATP binding pocket of the *gyrB* gene of *E.coli*.

**Conclusions:** Daidzin, Ketotifen, Cyproheptadin, Dalbergin, Piroxicam, Etodolac, Frangulin A, Chrysin, Quercitrin, Myricetin, Peonidin, Flavan-4-ol, Lutin, Emodine, Aurone, Baicalein, and Phaseollidine They are promising compounds that may inhibit the ATP binding domain in the *gyrB* gene of *E.coli*.

## الكشف عن مضادات بكتيرية جديدة خلال تحليل تسلسل الدنا والرسو الجزيئي الذي يؤدي الى تثبيط جيب الـATP الموجود بجين *gyrB* لبكتريا الاشريكية القولونية المعزولة من التهاب المجاري البولية.

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### الخلاصة

**الخلفية:** تعتبر بكتريا الإشريكية القولونية، التي تعيش عادة في القولون، السبب الأكثر شيوعاً لالتهابات المسالك البولية، حيث تمثل 80-85% من الالتهابات المكتسبة من المجتمع. يهدف هذا البحث إلى دراسة الشكل ثلاثي الأبعاد لجين *gyrB* وتحديد منطقة جيب ربط الطاقة (ATP binding pocket) واكتشاف مضادات بكتيرية جديدة يمكنها الارتباط بهذا الجيب وتثبيط

عمل إنزيم DNA gyrase في بكتريا *E. coli*.

**طرق العمل:** في مستشفيات محافظة كربلاء المقدسة (مدرسة الامام الحسين عليه السلام، مستشفى الامام الحسن المجتبي عليه السلام، مستشفى النساء والولادة، مستشفى الامام الحجة عليه السلام الخيري) تم جمع 223 عينة من المرضى الذين يعانون من أعراض التهاب المسالك البولية، وحسب الإجراء القياسي، وتم زراعة البول، ومن ثم تم تحديد البكتيريا باستخدام اختبار API 20E، ثم تم تضخيم جين *gyrB* ومعرفة تسلسله وفحصه باستخدام تقنيات المعلومات الحيوية، وتم تحديد منطقة جيب ارتباط ATP للإنزيم وفحص شكله الثلاثي الأبعاد، وبعد ذلك تم اقتراح مركبات كيميائية باستخدام الالتحام الجزيئي للارتباط بهذا الجيب.

**النتائج:** أظهرت 60 عينة من أصل 223 عينة أعراض عدوى المسالك البولية وكانت مصابة بعدوى الإشريكية القولونية. وكان اصطفاف تسلسل القواعد النيتروجينية في جين *gyrB* مماثلة بنسبة 99-100% للتسلسلات العالمية لنفس البكتيريا، وفقاً للتحليل الجزيئي. كما أظهرت تشابهاً بنسبة 99-100% مع التسلسلات العالمية للأحماض الأمينية أيضاً. بالإضافة إلى

ذلك، فإن العديد من المركبات الكيميائية المقترحة، مثل Dieckol وRobinin وQuercitrin وVitamin D3 وVitamin D2 وMyricitrin وDaidzin لها درجة إلتحام عالية مع جيب ربط ATP وبعضها له درجة إلتحام ضعيفة مع جيب ربط ATP مثل Scopoline وCrocين وAllicin وPropionic acid. بعدها تم إخضاع المركبات ذات درجة الإلتحام العالية لقاعدة ليبينسكي لمعرفة المركبات الكيميائية الأكثر ملاءمة للإعطاء عن طريق الفم، حيث وجد أن المركبات Daidzin ، Dalbergin ، Quercitrin ، Cyproheptadine وPiroxicam وغيرها هي المركبات الأكثر ملاءمة للإعطاء عن طريق الفم ولتثبيط جيب ربط ATP لجين *gyrB* في *E. coil*.

**الاستنتاجات:** Frangulin A , Etodolac , Piroxicam , Diadzin , Ketotifen , Cyproheptadin , Dalbergin , Aurone Emodine , Lutin , Flavan-4-ol , Peonidin , Myricetin , Quercitrin , Chrysin , Phaseollidine و Baicalein وهي مركبات واعدة قد تثبط جيب ربط ATP في جين *gyrB* في الإشريكية القولونية

**الكلمات المفتاحية:** عدوى المسالك البولية، الإشريكية القولونية، جين *gyrB* ، جيب رب

## 1. Introduction

Urinary tract infections (UTIs) are the most common bacterial infection, affecting 1 million people worldwide each year. Among hospital infections, UTIs rank second only to lower respiratory tract infections, accounting for 24% of such cases in developing countries(Zhou *et al.*, 2023) . Symptoms of UTIs are variable, ranging from asymptomatic to severely ill patients with fever and, occasionally, secondary bacteremia. UTIs can be divided into three categories: acute pyelonephritis, acute cystitis, and asymptomatic bacteriuria (Tullus and Shaikh, 2020). The Uropathogenic Escherichia coli (UPEC) strain of Escherichia coli is the main cause of this type of infection due to its virulence factors and rapid division during human infections, enabling it to move from the digestive system to the area surrounding the urethra, then the urethra, bladder, ureter, and kidneys, and may reach the bloodstream (Sojo-Dorado *et al.*, 2022). There are strains of *E.coli* that have genes at specific locations in the pathogenic island that range in size from 10 to 200 kb. This pathogenic island can overcome the immune system. Virulence factors include capsule, lipopolysaccharides, adhesion, and enzymes (Forsyth *et al.*, 2018; Dadi *et al.*, 2020). The problem facing doctors is the presence of antibiotic-resistant bacteria, as a result of the misuse of antibiotics. *Escherichia coli* is considered the most antibiotic-resistant bacteria due to the different mechanisms of drug resistance (Najm and Hussein, 2023). A developing medical problem is antibiotic resistance. 2019 had more than a million deaths due to bacterial illnesses that were resistant. If this urgent issue is not addressed, by 2050, there might be 10 million fewer deaths each year (Ranjbar and Alam, 2023). *E.coli* also represents a major reservoir of resistance genes that may be responsible for the failure of current treatments. Large and increasing numbers of resistance genes have been identified in *E.coli* isolates over the past decades. *E.coli* acts as both a donor and a recipient of resistance genes, and can thus acquire resistance genes from other bacteria, and can also transfer its resistance genes to other bacteria. Bacteria can acquire resistance to multiple antibiotics through mobile genetic elements such as plasmids and transposons(Rasheed *et al.*, 2014; Poirel *et al.*, 2018). Targeting DNA gyrase and topoisomerase IV, fluoroquinolones are among the "five highest priority antimicrobials" that are now being used in clinical settings, according to the World Health Organization (WHO)(Organization, 2017). Unfortunately, the high frequency of DNA gyrase and topoisomerase IV mutations (i.e., target-mediated resistance) reduces the practical usefulness of this class of medications. Novel classes of antibacterial medications are being created to address the serious problem of antibiotic resistance(Gibson *et al.*, 2018; Bush *et al.*, 2020). Supercoiling is regulated by DNA Gyrase, which eliminates positive supercoils that build up as a result of these DNA activities before replication forks and replication complexes. It is the sole enzyme capable of unraveling DNA, even negative supercoils. Topoisomerase IV is primarily responsible for separating or removing tangles between daughter chromatids formed during replication and removing knots made during recombination, even though it is also capable of relaxing both positive and negative DNA supercoils(McKie, Neuman and Maxwell, 2021). High levels of enzyme-mediated DNA accumulation are caused by tyrosine residues in the active site forming covalent bonds with the terminal phosphates of cleavage-mediated DNA, which stabilizes cleavage complexes stabilized by fluoroquinolone interactions with DNA gyrase and topoisomerase IV. Fluoroquinolone interactions also prevent gyrase and topoisomerase from participating in their overall catalytic reaction. This deprives the cell of these two enzymes' vital catalytic functions, which can hinder the transcription and replication of DNA and tangle developing chromosomes (Bush *et al.*, 2020; Hirsch and Klostermeier, 2021). Because DNA

gyrase and topoisomerase IV have similar structures, there is a great chance to target both of them with novel antibacterial compounds, which will lessen the likelihood of simultaneous mutations on both of these targets and prevent bacteria from developing target-based resistance. *GyrB* and *ParE* contain an ATP-binding site, where ATP hydrolysis provides the energy for the reaction catalyzed by *GyrA* and *ParC* (Azam, Thathan and Jubie, 2015). Targets for antibacterial drug development include many binding sites on DNA gyrase and physically related topoisomerase IV. A well-known family of fluoroquinolones targets the *GyrA/ParC* component by binding to the DNA gyrase complex and stabilizing it in a cleaved DNA state, which inhibits DNA synthesis (Durcik *et al.*, 2019). Some topoisomerase inhibitors may have a distinct binding mechanism since they do not exhibit any resistance to fluoroquinolones. Apart from fluoroquinolones, there exist ATP-competitive inhibitors that specifically target the DNA gyrase and topoisomerase *GyrB/ParE* component. By blocking the ATP binding site, ATP hydrolysis is stopped and the energy needed for the enzyme's catalytic activity is not supplied. Consequently, one intriguing target for the development of novel DNA gyrase inhibitors is the ATP binding site (Savage, Charrier, Salisbury, Box, *et al.*, 2016; Savage, Charrier, Salisbury, Moyo, *et al.*, 2016). In *E.coli GyrB*, mutations that give resistance to various fluoroquinolones are most frequently found at position Arg136, however Asp73, Gly77, Ile78, and Thr165 have also been identified as additional sites where mutation might result in fluoroquinolone resistance. Like the amino acid residues in *gyrA* with similarly charged side chains, the two quinolone binding sites (Asp426 and Lys7) in the *E.coli gyrB* gene are resistant to medicines that bind to Asp and Lys when Asp is substituted with Asn or Lys with Glu (Finstad, 2022). Fluoroquinolones, which include ciprofloxacin, levofloxacin, and moxifloxacin, are often used in medicine because they are typically effective against infections brought on by both Gram-positive and Gram-negative microorganisms. Nevertheless, despite suggestions to restrict their usage, resistance to these agents has developed and is still spreading (Durcik *et al.*, 2020). Molecular docking is a specialized tool for studying the different interactions between inhibitor molecules and the active sites of target receptor molecules (Saxena, Kumar and Srivastava, 2022). Usually, docking is observed to affect the supercoiling of DNA through the N-terminal domain of the gyrase B subunit targeting ATP binding sites that are supposed to compete with ATP for binding to gyrase B, so it is necessary to make compounds that resemble the action of antibacterial and some bacterial toxins (Saleh *et al.*, 2022). Typically, compounds are sorted according to their docking score, with the best compound chosen in Glide's standard precision (SP) mode for docking. A sampling strategy based on improved and refined growth is employed by the SP mode. Next, for XP (extra precision) docking, the best compounds are chosen based on the docking score in SP docking. Compared to SP mode, XP mode is less forgiving (Alotaibi *et al.*, 2023). In this study, we investigated the binding interactions of the *GyrB* subunit of *Escherichia coli* and compared the antibacterial activity obtained during our study. We evaluated the binding interactions and visualized different interactions such as hydrogen bonding, and salt bridges in three and two dimensions.

## **2. Materials and Methods**

### **2.1. Patients**

Range of ages of 4 – 76 years, individuals with urinary tract infections who attended local hospitals in the Holy Governorate of Karbala provided a total of 223 urine specimens, Under the supervision of specialized doctors for the period from 8/3/2023 to 11/25/2023. They were plated directly on the culture media using the technique of (streaking) utilizing (a loop) on the MacConkey medium, and special containers (a plastic tube with a cover) were employed for collection. (Vandepitte, 2003; Carroll, Butel and Morse, 2015) It was then incubated for 24 hours at

37°C. The autoclave was used to sanitize it for 15 minutes at 121°C and 15 pounds per inch of pressure. To prevent infection, it was then poured into plates that had been sterilized. In addition, all glassware spent two hours being sterilized at 180 degrees Celsius in an electric oven (Biswas and Rather, 2019).

## 2.2. Biochemical Diagnosis

Enterobacteriaceae can be identified by the 20 microtubes of dried substrates included in the API 20E strip Fig.1. Use Procedure for API 20E: prepare a bacterial suspension with a turbidity of 0.5 McFarland standard in sterile 0.85% saline then Fill the API 20E microtubes with the bacterial suspension and For 18 to 24 hours, incubate the strip at 35 to 37°C, The chemicals needed for particular tests are added after incubation, and the outcomes are read. The color changes are interpreted using the reading table, and the analytical profile indicator is used to confirm identification. Sixty clinical isolates were diagnosed with *E.coli* by the (API 20E system)(Kusumaningsih, IG and RRR, 2021) .



**Figure 1:** API 20E test result on a clinical isolate to diagnose *E.coli* bacteria after incubating it for (18-24) hours and adding its reagents (The catalog number for API test is 20160).

### 2.2.1. Catalase Test

Using sterile wooden sticks, a colony grown on MacConkey agar was moved onto a dry, sterile glass slide, and one drop of 3% catalase reagent was applied. Once bubbles developed on the glass slide's surface, signifying the formation of catalase enzyme (positive result) (Biswas and Rather, 2019).

### 2.2.2. Oxidase Test

The filter paper was moistened with a few drops of a solution of tetramethyl-*p*-phenylene diamine hydrochloride for this test. Using a sterile wooden stick, a mass of cells was aseptically removed from the slanting growth and spread out over the moistened paper. A negative outcome is indicated if a violet or purple color does not appear within 10 seconds(Hemraj, Diksha and Avneet, 2013).

## 2.3. DNA Extraction

The special kit prepared by the Korean company Geneaid was used to extract the deoxyribonucleic acid, and then the DNA concentration device (DNA Nanodrop) was used to measure the purity and concentration of the DNA. (The DNA concentration was ~ 30 µg/ml).

### 2.3.1. Primers Sequence

The primer sequences used in this study are designed by Prof. Dr. Hassan Mahmood Mousa Abu Almaali University of Karbala/College of Pharmacy as described in the Table 1.

**Table 1: Primer Sequences Used in The Study**

gene	Primer sequence (5' - 3')		Base pair	Source of design
<i>gyr B</i>	ATATCGGCGACACGGATGAC	F	998	Prof.Dr. Hassan Mahmood Mousa Abu Almaali
	TGCCAGCAGTTCGTTTCATCT	R		

**2.3.2. Amplification Reaction Mixture for *GyrB* Genes:**

A polymerase chain reaction kit (PCR Premix kit) was used to amplify the *gyrB* gene. Five microliters of DNA extract, one microliter of each primer (Forward + Reverse) at a concentration of 10 pmol/μl, and 13 microliters of nuclease-free water made up the total reaction volume of 20 microliters. After that, he was placed into a PCR device (Ravi, Baunthiyal and Saxena, 2014) as detailed in the Table 2.

**Table 2: Cycling Parameters of *GyrB* Gene Amplification**

Cycle No.	Stage	Temperature °C	Time
1	Initial denaturation	95	5 min.
35	Denaturation	95	20 sec.
	Annealing	55	20 sec.
	Elongation	72	45 sec.
1	Final extension	72	5 min.

**2.3.3. DNA Sequencing**

Specimen sequencing was carried out by the MacroGen company (Korea). The sequencing outcome was examined with Software called MEGA11 was used to view the sequencing result. (Tamura, Stecher and Kumar, 2021).

**2.4. Alignment Assay and Phylogenetic Tree**

Using the Mega11 software, the alignment test was carried out to establish the existence of mutations in bacterial genes. The nitrogenous base sequences were found and compared with global sequences using the BLASTn tool that targeted the same gene. Then, to find alterations in proteins, the amino acid sequences of the target gene were ascertained and compared to global sequences using BLASTx software. (Tamura, Stecher and Kumar, 2021). To determine the evolutionary relationships between the strains of *E.coli* bacteria and compare them with strains worldwide, as well as the variations in nitrogenous bases and amino acids of the bacteria, the phylogenetic tree was tested using the Mega11 program.

**3. Results****3.1. Population of The Study**

It 60 isolated of *E.coli* samples were diagnosed (out of 223 samples). Their ages ranged from 4-76 years. There were 45 (75%) females and 15 (25%) males as shown in Table 3. (Öztürk and Murt, 2020).

**Table 3:** Age Group of The Patients by Sex

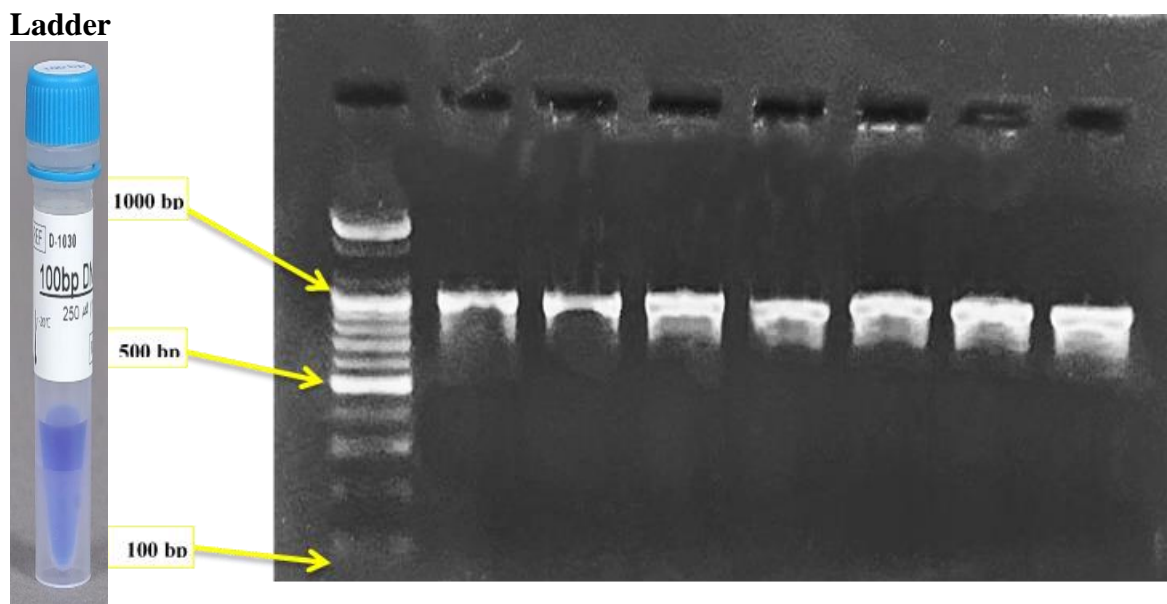
Age category	Sex		
	Female	Male	
≥ 4	5	2	7
11 - 30	12	6	18
31 – 50	19	2	21
51 – 70	6	3	9
≤ 76	3	2	5
Total	<b>45</b>	<b>15</b>	

### 3.2. Identification of Isolates

The isolates were identified as *E.coli* through a combination of morphological and biochemical characteristics. This means that the isolates were characterized by their microscopic appearance and their ability to perform specific biochemical reactions, ultimately leading to the conclusion that they were all *E.coli*.

### 3.3. Detection of *GyrB* Gene In *E.Coli* Isolates:

The *gyrB* gene, a vital component of the DNA gyrase enzyme, was checked in all UPEC *E.coli* isolates. DNA extraction was done using the Geneaid/Korea DNA Extraction Kit. The findings confirmed that the *gyrB* gene was present in every isolate, as shown in Fig.2.

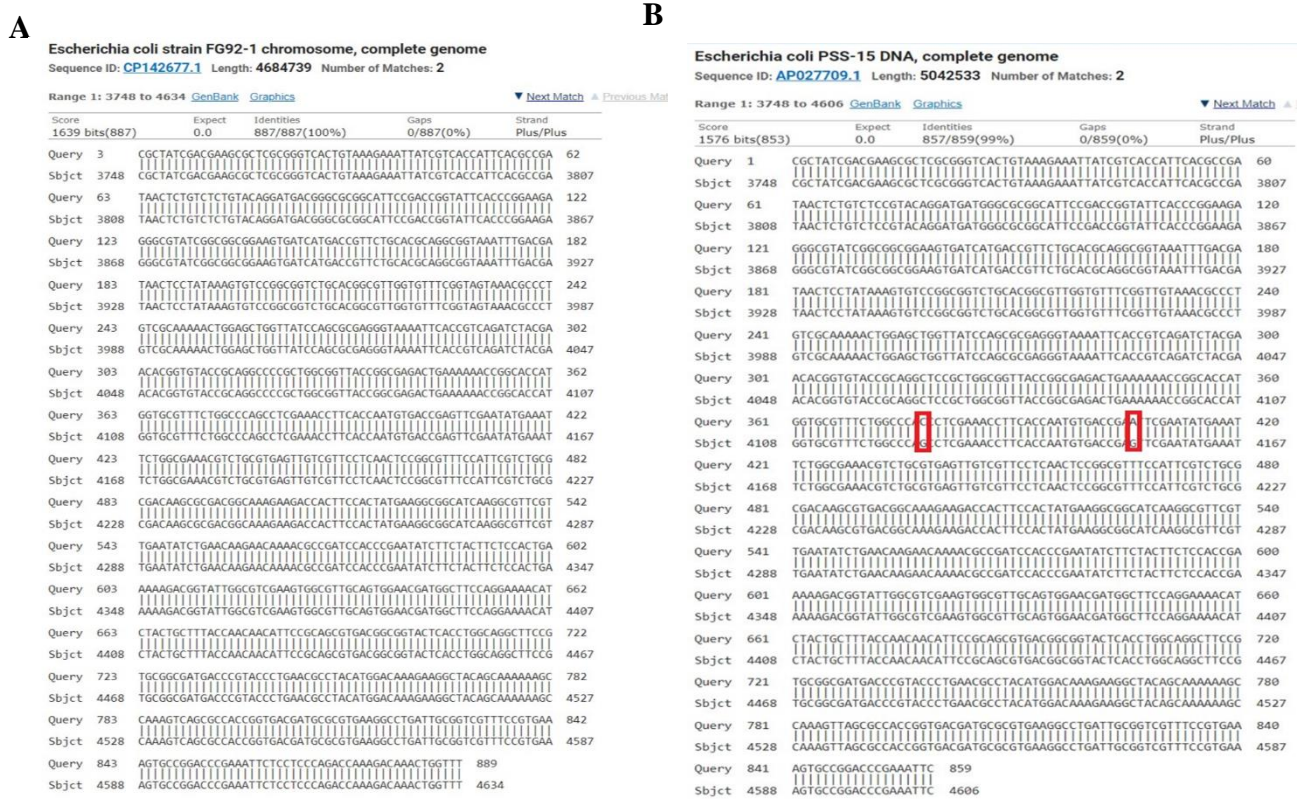


**Figure 2:** PCR Result of The *GyrB* Gene Using Agarose Gel Electrophoresis

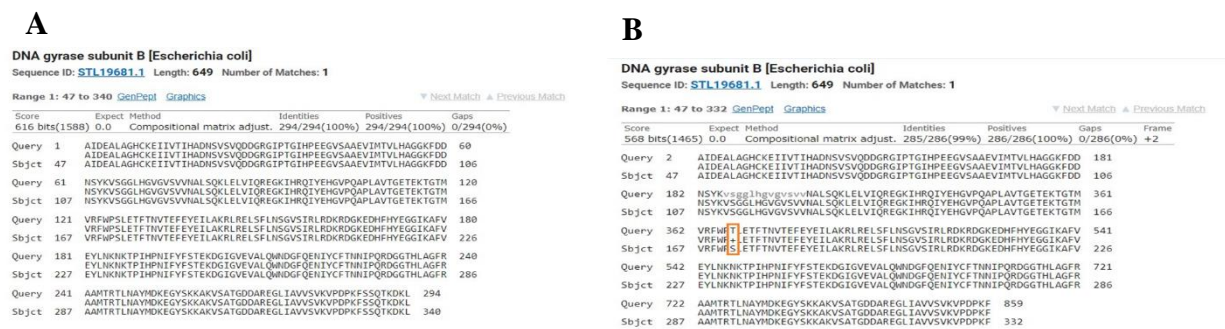
### 3.4. DNA Sequencing

Genetic Characterization and Comparison of UPEC Isolates: The study involved DNA sequencing of the *gyrB* gene for 39 bacterial isolates and a nucleotide alignment analysis using the BLASTn program to compare the local clinical *E.coli* isolates with global isolates from GenBank. The results revealed that most isolates had a complete 100% match, as shown in Fig.3A, while some had a 99% match, as shown in Fig.3B, resulting missense mutation which is carrying change in nucleotides (cytosine instead of Guanine) and (Adenine instead of Guanine) that lead to change in amino acids. Furthermore, the nucleotide sequences were translated into amino acids, and an amino

acid alignment analysis was conducted using the BLASTx program. The majority of the isolates exhibited a 100% match with international isolates, as seen in Fig.4A, while some had a match rate of up to 99%, as shown in Fig.4B.



**Figure 3:** A) Alignment test for nucleotides of the *gyrB* gene for local isolates and comparison with global isolates: Alignment Match rate 100% by using BLASTx. B) Alignment test for nucleotides of the *gyrB* gene for local isolates (Query) and comparison with global isolates(sbjct): Alignment Match rate 99% by using BLASTx



**Figure 4:** Alignment test for peptides of the *gyrB* gene for local isolates (Query) and comparison with global isolates(sbjct) by BLASTx, A) Alignment Match rate 100%. B) Alignment Match rate 99%. (Therionine instead of Serine)

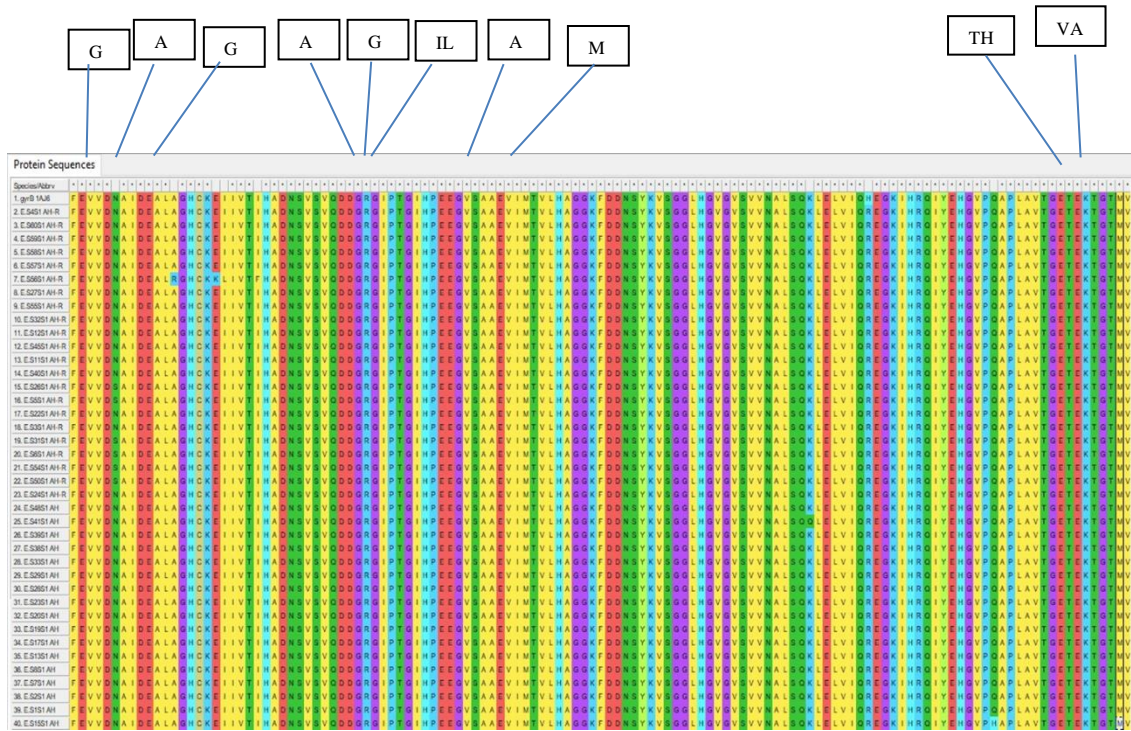
### 3.5. Gyrb Multiple Sequence Alignment and Determination Gyrb Gene ATP Binding Pocket

Alignment testing was performed for all specimens using MEGA11 software and using the ClustalW option. Thus, 47 specimens included local isolates and were matched with the corresponding global sequences

belonging to different isolates. Some isolates were completely identical, while some isolates differed in one or more nitrogenous bases. In addition, all DNA sequences of local clinical isolates were translated into amino acids, and their amino acid sequences were matched using the BLASTx program. Some sequences were completely identical to the global isolates, while some isolates differed in one or more amino acids. As shown in Fig.5 and Fig.6. Determine the *gyrB* gene ATP-binding pocket This pocket contains many amino acid residues that are necessary for enzyme catalysis in both vitro and in vivo settings. Particularly, amino acid residues that comprise the ATP-binding pocket in the *E.coli* gene *gyrB* (D73, G77, I78, E42, N46, E50, R76, P79, K165, V167, V47, and M95) are essential for enzyme activity(Gross *et al.*, 2003).

Protein Sequences	
Species/Abbrev	Protein Sequence
25. E.S2351 AH	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
26. E.S2201 AH+R	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
27. E.S2051 AH	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
28. E.S1951 AH	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
29. E.S1751 AH	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
30. E.S1551 AH	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
31. E.S1351 AH	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
32. E.S1251 AH+R	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
33. E.S1151 AH+R	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
34. E.S851 AH	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
35. E.coli Japan s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
36. E.coli Germany s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
37. E.coli France s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
38. E.coli France s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
39. E.coli Czech RepubH	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
40. E.coli China2 s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
41. E.coli China2 s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
42. E.coli Canada2 s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
43. E.coli Canada s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
44. E.coli Brazil s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
45. E.coli Belgium s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
46. E.coli Australia s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
47. E.coli USA s1(2)	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
48. E.coli USA s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
49. E.coli United KingdomH	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
50. E.coli United KingdomH	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
51. E.coli Taiwan s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
52. E.coli Switzerland s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
53. E.coli Sudan s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
54. E.coli South KoreaH	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
55. E.coli Saudi ArabiaH	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
56. E.coli Pakistan s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
57. E.coli Norway s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
58. E.coli Mexico s1	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF
59. E.coli Kazakhstan	HADNSVSVQDDRRGIP TGIHPEEGVSAAEVIMTVLHAGGKFDONSYYKSGGLHGVSVVNALSOKLELVLDREGKIHRRQIYEHGVPQAPLAYTGETEKTGTWVRFNPSLETF

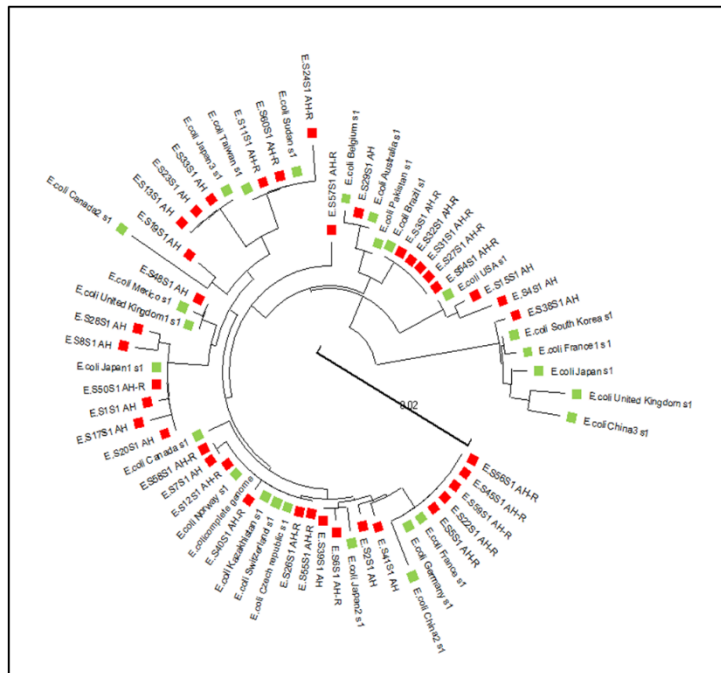
**Figure 5:** Alignment of the amino acids of the *gyrB* gene sequences of local isolates and comparing them with sequences of global isolates



**Figure 6:** Alignment of *GyrB* Gene Sequences for Local Isolates Shown Amino Acid That Component of The ATP Binding Pocket.

### 3.6. Phylogenetic Analysis of *GyrB* Gene

Using the neighbor joining approach, a genetic tree analysis was carried out for the local samples and compared with the global isolates to determine the link between them. The tree was drawn on a scale (Scale length = 0.02), Fig.7, which illustrates the relationships between the local samples, which include clinical isolates and the global isolates. Some of the few local isolates are far apart, indicating significant differences in genetic characteristics and amino acid sequences, and the rest of the isolates are distributed approximately with the global samples.



**Figure 7:** Genetic tree of *E. coli* bacteria : Neighbor joining method for local and some of global isolates, the red color represents local isolates and green color represents global isolates, with scale length (0.02).

### 3.7. Molecular Docking

The three-dimensional shape of the gene was constructed using the (phyre2) program and using the (1-click docking) program, several proposed chemical compounds were docked to bind to the ATP-binding pocket to inhibit the *gyrB* subunit of DNA gyrase enzyme and thus inhibit *E.coli* bacterial replication as shown in the Table 4.

**Table 4:** Shows Some of The Chemical Compounds Proposed to Bind to the ATP-Binding Pocket of The *Gyrb* Gene.

NO.	Chemical compound (Drug)	International Chemical Identifier (InChIKey)	Docking score Kcal / Mol
1	Dieckol	DRZQFGYIIYNNEC-UHFFFAOYSA-N	-9.9
2	Robinin	PEFASEPMJYRQBW-HKWQTAEVSA-N	-9.6
3	Quercitrin	OXGUCUVFOIWWQJ-HQBVPOQASA-N	-8.9
4	Vitamin D3	QYSXJUFSXHHAJI-YRZJJWOYSA-N	-8.8
5	Vitamin D2	MECHNRXZTMCUDQ-RKHKHRCZSA-N	-8.7
6	Myricitrin	DCYOADKBABEMIQ-OWMUPTOHSAN	-8.7
7	Daidzin	KYQZWONCHDNPDP-QNDFHXLGSA-N	-8.7
8	Rutin	IKGXIBQEEMLURG-NVPNHPEKSA-N	-8.6
9	naringin	DFPMSGMNTNDNHN-ZPHOTFPESA-N	-8.6
10	ketotifen	ZCVMWBYGMWKGHF-UHFFFAOYSA-N	-8.6
11	Rutoside	IKGXIBQEEMLURG-NVPNHPEKSA-N	-8.6
12	Cyproheptadine	JJCFRYNCJDLXIK-UHFFFAOYSA-N	-8.5
13	Xanthorhamnin	NMGVHLDIHNFGQB-OTCPXFHUSA-N	-8.5
14	Dalbergin	AZELSOYQOIUPBZ-UHFFFAOYSA-N	-8.5
15	Diosmin	GZSOSUNBTXMUFQ-YFAPSIMESA-N	-8.4
16	Afzelin	SOSLMHZOJATCCP-AEIZVZFYSA-N	-8.4
17	Cascaroside	MNAYRSRTNMVAPR-UHFFFAOYSA-N	-8.4
18	Hesperidin	QUQPHWDTPGMPEX-QJBIFVCTSA-N	-8.3
19	Baicalin	IKIIZLYTISPENI-ZFORQUDYSA-N	-8.3
20	Hyperion	OVSQVDMCBVZWGM-DTGCRPNFSA-N	-8.3
21	Genistin	ZCOLJUOHXJRHDI-CMWLGVBASA-N	-8.2
22	Piroxicam	QYSPLQLAKJAUJT-UHFFFAOYSA-N	-8.2
23	Carmine	DGQLVPJXFOQEV-UHFFFAOYSA-N	-8.2
24	Clorobiocin	FJAQNRBDVKIHK-LFLQOBSNSA-N	-8.1
25	Amygdalin	XUCIJNAGGSZNQT-JHSLDZJXSA-N	-8.1
26	Etodolac	NNYBQONXHNTVIJ-UHFFFAOYSA-N	-8.1
27	Frangulin A	DTTVUKLWJFJOHO-FUCRAMRQSA-N	-8.1
28	Cyanidin	VEVZSMAEJFVWIL-UHFFFAOYSA-O	-8
29	Phaseollidin	OFWYIUYPVHYPQNX-JXFKEZNVSA-N	-8
30	Chrysin	RTIXKCRFFJGDFG-UHFFFAOYSA-N	-7.9
31	Diacerein	TYNLGDBUJLVSMA-UHFFFAOYSA-N	-7.9
32	Quercetin	REFJWTPEDVJJIY-UHFFFAOYSA-N	-7.9
33	Baicalein	FXNFHKRTJBSTCS-UHFFFAOYSA-N	-7.9
34	Myricetin	IKMDFBPHZJCSN-UHFFFAOYSA-N	-7.9
35	Peonidin	XFDQJKDGGOEYPI-UHFFFAOYSA-O	-7.9
36	Flavan-4-ol	YTMFRMLVZQOBDR-WUJWULDRSA-N	-7.9
37	Sophoraflavonolside	LKZDFKLGDSGEO-UJECXLDQSA-N	-7.9
38	Eckol	PCZZRBGISTUIOA-UHFFFAOYSA-N	-7.9
39	Lutin	RJKFOVLPORLFTN-LEKSSAKUSA-N	-7.9
40	Emodine	YDQWDHRMZQUTBA-UHFFFAOYSA-N	-7.9
41	Aurone	OMUOMODZGKSORV-UVTDQMKNNSA-N	-7.9
42	Luteolin	IQPNAANSBPBGFQ-UHFFFAOYSA-N	-7.8
43	Neohesperidin	ARGKVCXINMKCAZ-UZRWAPQLSA-N	-7.8

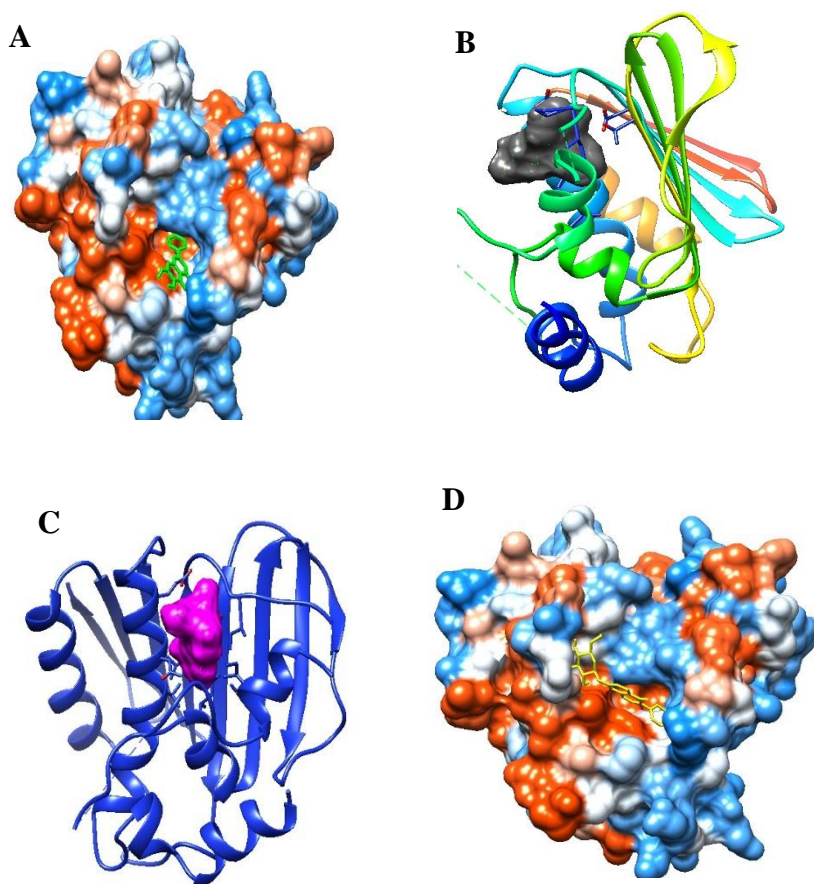
44	Wogonin	XLTFNNCXVBYBSX-UHFFFAOYSA-N	-7.8
45	Spiraeoside	OIUBYZLTFSLSBY-HMGRVEAOSA-N	-7.8
46	Catechin	PFTAWBLQPZVEMU-DZGCQCFKSA-N	-7.8
47	Calophyllolide	PMBLOLOJQZPEND-GIDUJCDVSA-N	-7.8
48	Crocin	SEBIKDIMAPSUBY-RTJKDTQDSA-N	-4.4
49	Allicin	JDLKFOPOAOFWQN-UHFFFAOYSA-N	-4.1
50	Propionic acid	XBDQKXXYIPTUBI-UHFFFAOYSA-N	-3.5

Next, we may use Lipinski's rule of five for oral drug administration to determine the physical and chemical characteristics of compounds crucial to drug transport in the human body by utilizing the property calculator tool on the Mcule platform as shown in the Table 5.

**Table 5:** Shows the Chemical Compounds Most Suitable for Oral Administration.

No.	Name of a chemical compound	H- bond donor	H-bond Acceptors	M.W	Login	No. of rotatable bonds	Total score
1	Daidzin	1	1	1	1	1	5
2	ketotifen	1	1	1	1	1	5
3	Cyproheptadine	1	1	1	1	1	5
4	Dalbergin	1	1	1	1	1	5
5	Piroxicam	1	1	1	1	1	5
6	Etodolac	1	1	1	1	1	5
7	Frangulin A	1	1	1	1	1	5
8	Cyanidin	1	1	1	1	1	5
9	Phaseollidin	1	1	1	1	1	5
10	Chrysin	1	1	1	1	1	5
11	Diacerein	1	1	1	1	1	5
12	Quercetin	1	1	1	1	1	5
13	Baicalein	1	1	1	1	1	5
14	Myricetin	0	1	1	1	1	4
15	Peonidin	1	1	1	1	1	5
16	Flavan-4-ol	1	1	1	1	1	5
17	Sophoraflavonololide	0	0	0	1	1	2
18	Lutin	1	1	1	1	1	5
19	Emodine	1	1	1	1	1	5
20	Aurone	1	1	1	1	1	5

Molecular docking was visualized using UCSF Chimera to provide a clearer picture of the molecular docking to highly docked compounds that were subjected to Lipinski's rule of five as shown in Fig.8A-D.



**Figure (8)** shows the molecular docking of some chemical compounds and the ATP Active pocket in the *gyrB* gene of *E.coli* bacteria: A/ Baicalein - B/ Ketotifen - C/ Etodolac - D/ Diadzin

#### 4. Discussion

Antimicrobial resistance is a growing medical concern. Worldwide, more than 1 million people died from resistant bacterial infections in 2019, and if nothing is done to treat this critical problem, the death will be estimated to rise to 10 million annually by 2050 (Ranjbar and Alam, 2023). Fluoroquinolones, which target DNA gyrase and topoisomerase IV, are listed by the World Health Organization as one of the "top five" antimicrobials currently in clinical use. Unfortunately, the clinical utility of this class of drugs is diminished by the high incidence of mutations in gyrase and/or topoisomerase IV (i.e., target-mediated resistance), and to address this problem, new classes of antibacterial drugs are being developed (Oviatt *et al.*, 2024). Quinolones have demonstrated efficacy against several illnesses, such as TB, chronic bronchitis, sexually transmitted diseases, and urinary tract infections. Although quinolones can target DNA gyrase and topo IV in many bacterial strains, the predominant target depends on the kind of bacteria being treated with quinolone. Their study found that topoisomerase IV is a secondary drug target and DNA gyrase is the major toxic target of quinolones based on an investigation of *E.coli* strains containing drug-resistant mutations in both of these enzymes. Fluoroquinolones seem to prefer to target Topo IV in Gram-positive bacteria, while DNA gyrase is typically their main target in Gram-negative bacteria. (Spencer and Panda, 2023) . Molecules are designed to target the bacterial DNA gyrase of pathogenic bacterial species based on certain

physicochemical properties (especially for orally absorbed drugs), e.g. molecular weight, lipophilicity, water solubility (molecules must be highly soluble), and drug permeability to the intestinal membrane. The charge, mass, and lipophilicity of the molecule also influence its ability to cross through membranes (Kumar *et al.*, 2023).

Many drugs are resistant to chromosomal mutations and transferable genetic elements like plasmids. Numerous mechanisms are present in antibiotic-resistant bacteria that result in decreased drug entry, decreased intracellular retention and changes to target proteins that lessen the medicines' affinity. Although certain medications have been licensed throughout time that work by blocking this enzyme, bacterial *GyrB* remains a significant therapeutic target (Carpio Arévalo and Amorim, 2021). The need to create new, more potent antibiotics is constant as antibiotic-resistant bacteria become a more serious health concern. The ATP-dependent enzymes Gyrase B (*GyrB*) and Topoisomerase IV (*ParE*), which are essential and highly conserved bacterial type II DNA topoisomerases, control changes in DNA topology and integrity during transcription, recombination, and replication. Both enzymes are also necessary for independent bacterial growth. *GyrB* and *ParE* differ in several structural and functional aspects, but they both catalyze DNA double-strand breaks and the ligation that follows. They also both bind to and hydrolyze ATP to give the energy required for these processes. Quinones' effectiveness is restricted by the ubiquity of target-based resistance (Durcik *et al.*, 2020). Several hydrophobic residues, including VAL43, ALA47, ILE78, PRO79, ILE90, MET91, VAL120, VAL67, ARG136, and ASN46, lining the binding pocket in *gyrB* of *E.coli*. For example, for ATP hydrolysis to occur, ASN46 must coordinate with the Mg<sup>+2</sup> ion present in the ATP binding pocket. Therefore, our docked complexes need to bind to residue ASN46. It is believed that all compounds that exhibit hydrophobic interactions with amino acid residues and form H-bonds with amino acid residues, may be crucial for their potential inhibition of *gyrB* (Kumar *et al.*, 2023). The docking results of our current study are similar to the hydrophobic interactions reported by some studies (Carpio Arévalo and Amorim, 2021; Amorim *et al.*, 2022). Molecular docking was performed according to standard protocols, Figure (4) shows the alignment of amino acids of the *gyrB* gene sequences of local isolates and compared with the sequences of global isolates, and Figure (5) shows the alignment of the *gyrB* gene sequences of local isolates, amino acids that form part of the ATP binding pocket and shows the occurrence of mutations that did not affect the structure of the protein, some mutations appear such as (Alanine) to (Arginine) in site(53), (Glutamic acid) to (Lysine) in site(58), (Lysine) to (Glutamine) in site (129), (Arginine) to (Histidine) in site(136) and (Glutamine) to (Histidine) in site(151). The ATP binding pocket of the *gyrB* gene was identified, this pocket contains many amino acid residues necessary for enzyme catalysis both in vitro and in vivo. In particular, the amino acid residues that form the ATP binding pocket in the *E.coli gyrB* gene were found (D73, G77, I78, E42, N46, E50, R76, P79, K165, V167, V47, and M95), It is essential for enzyme activity. Table 3 shows some of the chemical compounds proposed to bind to the ATP binding pocket of the *gyrB* gene, where the compound Dieckol showed good activity against *E.coli* and gave the highest binding energy of (-9.9) kcal/mol among the compounds tested, while the compound Robinin showed good activity against *E.coli* and gave the highest binding energy of (-9.6) kcal/mol. The compounds from the sequence (3-28) showed less activity and the binding energy ranged from (-8.9) to (-8) kcal/mol. Recently, computational applications have been widely used to predict molecular interactions in biological systems to study molecular recognition or design more effective biologically active compounds. Molecular docking and molecular dynamics are two types of modern computational methods that can provide molecular interactions between receptor proteins and drugs and how to stabilize the interaction. These methods focused on protein-drug complexes (ligands) to study how drugs bind to protein receptors, which is very important

for the discovery or development of new biologically active compounds in combating diseases. Moreover, methods for identifying the fingerprints of the protein-ligand interaction, which shows the strength of the interaction development, are also being developed (Zubair *et al.*, 2021). In our current study, some of the simulated chemical compounds showed suitability for oral administration (after submitted to Lipinski's rule of five by utilizing the property calculator tool on the Mcule platform), The most important one is Daidzin., a natural component of soybeans, is a glycoside form of the flavonoid daidzin. It has also been reported to have anticancer activity in the early stages of prostate cancer development and reduce the risk of postmenopausal breast cancer. It can inhibit telomerase activity by hydrogen bonding with nitrogenous bases as well. Daidzin has lower docking energy on tyrosine kinase receptor proteins, indicating a higher binding affinity for daidzin than other compounds (Wee and Wang, 2017). Daidzein does not show hydrophobic interactions with some amino acids such as LEU 820, LEU694, ALA719, VAL702, and LYS721, while only hydrogen bonding appears between the glycosidic group and amino acid residues of ALA719, LYS721, LEU764, THR830, and ASP831 are located in the phosphate binding region along with the sugar pocket. Moreover, the aglycone portion of daidzein also shows hydrophobic interactions with amino acids LEU820, VAL702, and LEU694 (Fajrin *et al.*, 2018)(Zubair *et al.*, 2021) Daidzein did not show this type of interaction as hydrogen bonding was only found between the glycosidic group and amino acid residues of ALA719, LYS721, LEU764, THR830, and ASP831 located in the phosphate binding region of EGFR along with the sugar pocket. Moreover, the aglycone part of daidzein also showed hydrophobic interaction with amino acids LEU820, VAL702, and LEU694 (Arba and Tjahjono, 2015; Prasasty and Istyastono, 2020).

## 5. Conclusions

It is noted from the current study that the amino acid levels of the Iraqi specimens are identical to the international specimens in the (QRDR) region and the ATP binding pocket. The compounds (Daidzin, Ketotifen, Cyproheptadin, Dalbergin, Piroxicam, Etodolac, Frangulin A, Chrysin, Quercetin, Myricetin, Peonidin, Flavan-4-ol, Lutin, Emodine, Aurone, Baicalein, and Phaseollidine are promising compounds that are likely to be characterized by the inhibitory effect of the ATP binding domain in the *gyrB* gene of *E. coli*, and after further investigation, it is safe to take it orally.

## 6. Recommendations

Given the limited sources available for the amino acid sequences of the *gyrA* gene and the *gyrB* gene in Iraq, it is recommended to study and create a database regarding local isolates of *E. coli* bacterial genes. Study the three-dimensional shape of drug targets and try to devise compounds capable of inhibiting these targets to reach compounds that can work as antibacterial treatments.

## 7. Acknowledgements

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## 8. Ethical Consideration

This study was accepted by the Ethical Committee at the College of Science/ University of Karbala. All subjects enrolled in this work were informed and verbal agreement was obtained from each participant before the collection of specimens, according to facilitating the student's assignment, University of Karbala No. 2686 on 8/2/2023.

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