Dinucleotide detection of *fimH* gene, and its predicted antagonist agent in uropathogenic *Escherichia coli*

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Acknowledgments

Dedication

TO ....

The sky, I can fly in it, My father who

 Gave me the power and made me better.

The oasis, I can shade in it, my mother,

 Who has filled me with the confidence.

My wife, whose support and

 Encouragement have enabled me to

 Complete this work.

My kids, Zainab, Mohammed, Ali and

Maryam,

 Who filled my life with untold blessings.

Alaa Al-nuaimi

2022

Summary:

Urinary tract infections (UTIs) are one of the most common pathological conditions in both community and hospital settings. It has been estimated that about 150 million people worldwide develop UTI each year, with high social costs in terms of hospitalizations and medical expenses. Among the common uropathogens associated to UTIs development, UroPathogenic *Escherichia coli* (UPEC) were the primary cause. UPEC strains possess a plethora of both structural (as fimbriae, pili, flagella) and secreted (toxins, iron-acquisition systems) virulence factors that contribute to their capacity to cause disease, although the ability to adhere to host epithelial cells in the urinary tract represents the most important determinant of pathogenicity.

In this study, a total of 100 samples were obtained from patients suffering from urinary tract infections who attended to Al-Husain medical city in Kerbala province and Al mussaib general hospital in Babylon province for a period ranging from February 2021 to October 2021. The patient's ages ranged from (15years - to 78 years).

Out of the 89 uropathogens, only 63 (70.79%) were found to be *Escherichia coli* and 26 (29.21%) other organisms.

The isolates were subjected to a susceptibility test to antibiotics by a modified Kirby - Bauer disc diffusion method. The results showed that all of these isolates are completely sensitive (97%) to the imipenem and 94% to the chloramphenicol. Isolates also showed sensitivity to a lesser extent for each of the norfloxacin(72%), and gentamicin(45%). the study also showed that the isolates were resistant to Amikacin(11%),

and Piperacillin / Tazobactam(15%), Co-Trimoxazole(13%), and Amoxyclav(15%). In contrast, the isolates showed resistance to a lesser extent for each of the Erythromycin (35%), tetracycline (45%), Tobramycin (41%), and Ampicillin (28%).

Molecular detection for *fimh* genes was done by using a specific PCR primer to investigate the presence of these genes in all isolates. In the current study, DNA sequencing was tested on 43 clinical samples that were positive for *Escherichia coli*. The Sequencing reactions were performed on the *fimh* PCR products.

The ability to determine how dinucleotide bases organized in a bit of DNA was provided by DNA sequencing. The range of dinucleotide signature value (0.06-0.14) for most *fimh* gene of strains of *Escherichia coli*. Our results showed the uniformity or similarity of signatures throughout the genome, these mean that all isolates have genetic homogeneity and had unique species-specific patterns. The range of G+C% of all samples is nearly similar (7.44% - 8.15%), which a high relationship among isolates.

In our study, DNA sequencing tested on 43clinical isolates, which covered all positive *Fimh* samples and 32 international samples. In conclusion, *Fimh* analysis together with PCR phylogrouping seems to be a simple strain typing tool for epidemiological studies of *Escherichia coli* isolates. The sequence analysis involves a single small DNA fragment (*Fimh*) of clinical and international samples.

The result showed, one clinical isolate with an incomplete match (99%), Indicated replacement in one nitrogen base in this study (Query) comparing with global isolates (Sbjct) guanine by thymine, Consequently,

resulting in missense mutation which is carrying change in DNA sequencing that leading to altering in amino acid at transcription level.

The results also showed that the frequency of GC and CG was the largest one among isolates than other dinucleotide signatures, and the frequency of AG and TC is the smallest one among isolates respectively.

By using the docking approach on many chemicals, supplement, drugs, and other useful material, to find suitable *fimh* inhibitor, the ligands were docked to the Apigenin and *fimh* proteins, as reported by our study, and given a high docking score(-9.4), so, Apigenin can provide protection from UTI in rats by 75% when it's taken as prophylaxis, and protect about 50% of rats after getting an infection, so in our study, we recommended using Apigenin that found in chamomile as a natural herbal product in adjuvant therapy with other drugs or products in UTI treatment.

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List of abbreviations

Abbreviation	Meaning
BLIS	Bactericin-like inhibitory substances
mchC	Chatechol microcin
Col	Colicin
CFU	Colony Forming Units
^{o}C	Degrees Celsius
D.S	Dinucleotide signature
EAEC	Enteroaggregative E.coli
EHEC	Enterohemorrhagic E.coli
EIEC	Enteroinvasive E.coli
EPEC	Enteropathogenic E.coli
ETEC	Enterotoxogenic E.coli
EDTA	Ethylene diamine tetra acetic acid
ESBLs	Extended-spectrum B-lactamase
Hly	Hemolysin
HUS	Hemolytic Uremic Syndrom
KDa	Killodalton
μg	Micro gram
μl	Micro liter
MSA	Multiple sequence alignment
Omp	Outer membrane protein
PCR	Polymerase chain reaction
PAP	Pyelonephritis –associated pili
ROS	Reactive oxygen species
TCA	Tri-chloro acetic acid
TBE buffer	Tris Borate- EDTA- buffer
TE buffer	Tris - EDTA buffer
UTI	Urinary Tract Infection
UPEC	Uropathogenic E.coli

1.1 Introduction :

Urinary tract infections (UTIs) are one of the health problems achieved by various microorganisms, including uropathogenic *Escherichia coli* (UPEC) (Maria E *et.al* 2017). UPEC strains are the most frequent pathogens responsible for 85% and a 50% of community and hospital acquired UTIs, respectively. UPEC strains have special virulence factors, including type 1 fimbriae, which can result in worsening of UTIs. (Chhaya *et.al* 2019).

The essential step in the infection process is the colonization to the urinary tract. Ordinarily, without binding to epithelium cells, bacteria can be washed out by urination process. (Soman& Yuxuan, 2015). UPEC have many different mechanisms for adherence to the uroepithelium, such as fimbriae, which is appendages with rod-like shape arises from the bacterial cell surface enthroning in a tip adhesion; fimbrillae, flexible, extended conformations with adhesions present throughout the structure (i.e. not only at the tip), and a fimbrial adhesions such as autotransporters. Moreover, extensive regulatory systems coordinate expression of these different adhesions (Snyder *et al.*, 2005).

Type 1 fimbriae are coded in the genome of most almost 95% *E. coli* strains (Alexi *et al.*, 2021). Which is compromised from FimA, the really underlying subunit, a few minor subunits, and *Fimh*, the grip which is found at the tip of fimbriae (Qiangde *et al.*, 2017).

Analysis of DNA composition at several length scales constitutes the bulk of many early studies aimed at unravelling the complexity of the organization and functionality of genomes. (Konstantinos *et.al*,2017). Dinucleotide relative abundances are considered an idiosyncratic feature

of genomes, regarded as a 'genomic signature'. The nucleotide composition of the sequence had been filtered out by dividing the observed frequency of a given dinucleotide by its expected frequency (expected on the basis of mono-nucleotide composition).

Dinucleotide signature: the set of dinucleotide biases constitutes a 'genomic signature' that can discriminate sequences from different organisms. Set of dinucleotide relative abundance values (dinucleotide biases) is a remarkably stable property of the DNA of an organism in single strand of DNA (Robert &

Hanseok;2008).

The dinucleotide usage profiles or genome signatures are similar for sequence samples taken from the same genome. The dinucleotide biases appear to reflect species-specific properties of DNA stacking energies, modification, replication, and repair mechanisms (Ratna *et.al*, 2014).

A sequence alignment is a way of arranging the sequences of DNA, RNA, or protein to identify regions of similarity that may be a consequence of functional, structural, or evolutionary relationships between the sequences. Aligned sequences of nucleotide or amino acid residues are typically represented as rows within a matrix (Troy *et.al*,2001).

Computational approaches to sequence alignment generally fall into two categories:

- Global alignments: Calculating a global alignment is a form of global optimization that "forces" the alignment to span the entire length of all query sequences (Agrawal *et.al* 2013).

- Local alignments : identify regions of similarity within long sequences that are often widely divergent overall. Local alignments are often preferable, but can be more difficult to calculate because of the additional challenge of identifying the regions of similarity (Agrawal *et.al* 2013)..

Protein sequences conversion to 3- dimensional structure. a statistical method for template-based protein modeling that improves alignment accuracy by exploiting structural information in a single or multiple templates(Meier,2015).

A suitable Inhibitor to fim-H was found by Lipinski's rule of five, and bioinformatics approach to analysis and the prediction of inhibitors. Lipinski's rule of five, also known as Pfizer's rule of five or simply the rule of five (RO5), is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997(Syed *et.al*, 2019).

Aims of study:-

Objective to a chive aims of current study are:

Detection of dinucleotide signature among clinical isolates of UPEC, and to find local DNA and proteins *Fim-H* sequences leading to find it is novel inhibitors, to treat UTI.

To achieve the above aim, the following objectives were suggested:

1- Isolation of *E. coli* from clinical sources.

2-Detcetion of *fimh* by PCR techniques

3-Detection and Study of dinucleotide signature among *fimh* gene,by Bioinformatics approach is done for calculating the dinucleotide signature in *E. coli* isolates.

4- DNA sequencing for *Fimh* amplicons.

5- Protein sequences alignment.

6- Protein sequences conversion to 3- dimensional structure.

7- Finding a suitable Inhibitor to *fimh* and Lipinski's rule of five, by Bioinformatics approach to analysis and the prediction of inhibitors.

8- Testing the Inhibitors in lab animals (Test and control).

1.2 Literature review:-

1.2.1 *Escherichia coli*:

Escherichia coli is Gram-negative, facultative anaerobic and non-sporulation. Cells are regular bar formed, and are around 2.0 microns (μm) long and 0.5 μm in distance across, with a cell volume of 0. 6–0.7(μm) , (Barák, 2018). *Escherichia coli* , can live on a wide assortment of substrates. *E. coli* utilizes blended corrosive aging in anaerobic conditions, creating lactate, succinate, ethanol, acetic acid derivation and carbon dioxide. Since numerous pathways in blended corrosive aging produce hydrogen gas, these pathways require the degrees of hydrogen to be low, just like the situation when *E. coli* lives respectively with hydrogen-devouring life forms, for example, methanogens or sulfate-lessening microbes (Million *et al*.,2013).

Escherichia coli were first depicted as a Bacterium coli cooperative by the German pediatrician Theodore Escherich in 1885. *E. coli* species are an individual from the family Enterobacteriaceae inside the phylum Proteobacteria. It is gram-negative, pole molded, non-spore framing, and normally motile (Darnton *et al*., 2007).

Escherichia coli is facultative anaerobes, oxidase-negative, glucose, lactose and sucrose aging, with an ideal development pH of 6-7,and Optimal temperature development of *E. coli* happens at 37 °C yet some research facility strains can increase temperatures of up to 49 °C (Fotadar *et al*., 2005). Development can be driven by high-impact or anaerobic breath, utilizing an enormous assortment of redox sets, including the oxidation of pyruvic corrosive, formic corrosive, hydrogen and amino

acids, and the reduction of substrates like oxygen, nitrate, fumarate, dimethyl sulfoxide and trimethylamine N-oxide Ingle dew (Ralf *et al.,* 2006).

The flagella have a peritrichous course of action (Darnton *et al*., 2007). *E. coli* and related microscopic organisms have the capacity to move DNA by means of formation, transduction or change, which permits hereditary material to spread evenly through a current populace. This cycle prompted the spread of the quality encoding shiga toxin from Shigella to *E. coli* O157:H7, conveyed by a bacteriophage (Muniesa *et al*., 2012).

E. coli can deliver extracellular hemolysin, which is answerable for extra intestinal contaminations, and is additionally a significant destructiveness factor , *E. coli* is a commensal of the digestive tract of human and different warm blooded creatures. It comprises the most predominant facultative anaerobic species with 107 cell forming units (CFU) per gram waste substance (Leimbach *et al*., 2013).

Gastrointestinal or extra-digestive *E. coli* diseases are brought about by strains holding onto various destructiveness factors situated on plasmids, bacteriophages, or the bacterial chromosomes. A few investigations have shown that pathogenic *E. coli* strain might be inferred from commensal strains by the acquisition of chromosomal or extra-chromosomal virulence operons (Alkandari *et al*., 2017).

Some strains, called enteropathogenic *Escherichia coli* can cause various diseases: intestinal infections, urinary tract infections, meningitis, peritonitis, mastitis, blood infection and kidney failure. Risk group are

small children, the elderly and people with weakened immunity (Sora, *et al.,* 2021).

The particularly dangerous strain of *Escherichia coli* O157: H7. This strain also produces toxins and causes deterioration of food (most often leads to spoilage and cheese meat). This may lead to poisoning of food (Zhu, *et al.*, 2013).

On the other hand, strain of *E. coli* Nissle1917) O6: K5: H1) is used as a remedy for intestinal diseases (Franco *et.al*, 2016).

1.2.2 Uropathogenic *Escherichia coli* and its virulence:

Uropathogenic *Escherichia coli* (UPEC) is the main cause of community-acquired UTIs about 80– 90% (Flores-Mireles *et al.*, 2015). Four main UPEC phylogroups (A, B1, B2, and D) have been identified on the basis of the occurrence of genomic Pathogenicity Islands (PAI) and the expression of virulence factors, such as adhesins, toxins, surface polysaccharides, flagella, and iron-acquisition systems (Bien *et al.*, 2012). Usually, many of these virulence factors are required for UPEC to cause UTI . However, besides UPEC, UTI can be caused by *Klebsiella pneumoniae* (about 7%), *Proteus mirabilis* (about 5%), and *Pseudomonas aeruginosa, Enterococcus faecalis, Enterobacter cloacae, Streptococcus bovis,* and the fungus *Candida albicans* for the remaining percentage (Litster, *et al.*, 2007).

During UTIs, UPEC pathogenesis includes:

(a) UPEC colonization of the periurethral and vaginal areas with colonization of the urethra (Maria, *et.al*,2017).

(b) Ascending into the bladder lumen and growth as plantktonic cells in urine; (Maria, *et.al*,2017).

(c) Adherence to- the surface and interaction with the bladder epithelium defense system ((Lene, *et.al*,2020));

(d) Biofilm formation(Lene, *et.al*,2020);

(e) Invasion and replication by forming bladder Intracellular Bacterial Communities (IBCs) where quiescent intracellular reservoirs (QIRs) form and reside in the underlying urothelium; (John, *et.al*,2020).

(f) Kidney colonization and host tissue damage with increased risk for bacteremia/septicemia. Replication of bacteria in the IBC can easily reach as many as 105 bacteria per cell; furthermore, bacteria in the IBC undergo morphological changes, flux out of the infected cell, and go onto infect neighboring cells (Flores-Mireles *et al*., 2015).

The flushing of urine removes most of the invading bacteria, along with UPEC-filled exfoliated bladder epithelium cells (Kaper *et al*., 2004). UPEC colonize the bladder using a variety of virulence factors that therefore play critical roles in UTI pathogenesis. These include surface structural components, such as lipopolysaccharide (LPS), polysaccharide capsule, flagella, outer-membrane vesicles, pili, curli, non-pilus adhesins, outermembrane proteins (OMPs), as well as secreted toxins, secretion systems, and TonB-dependent iron-uptake receptors, including siderophore receptors. All of these components are attractive candidates for the development of new drugs and vaccines (Werneburg *et al*., 2015).

1.2.3. Uropathogenic agent – type 1 fimbria (pilus)

Type 1 fimbriae (pili) are expressed by a large number of *E. coli* strains, and are found in more than 95% of *E. coli* isolates from intestinal and extra-intestinal infections. Pili act as highly efficient adhesion tools for bacterial inhabiting in diverse environments, including biotic and abiotic surfaces. On the surface of UPEC, type 1 pili are uniformly distributed (Caitlin *et.al*,2018), commonly 100 to 400 per cell. Structurally, type 1 pili are 7 nm wide and several micrometers long rod-like fibers. The pili rods are composed of immunoglobulin like (Ig) FimA pilin subunits that are anchored into a chain, and the chain is further coiled into a helix. FimA helix is joined to a short 3 nm thick distal tip fibrillum that consists of two adaptor proteins, FimF and FimG. At the tip of each pilus is a single mannose-specific adhesin – *Fimh*. The pilus rod is assembled by the chaperone/usher pathway and in their mature form the Ig fold of each subunit is completed by an amino-terminal extension from a neighboring subunit in a process termed "donor-strand exchange"(Gustavo, *et.al*; 2013).

1.2.4 The bacterial adhesin *Fimh*

The adhesin *Fimh* (M.W. 29 KDa), located at the tip of type 1 pilus, consists two Ig-like domains: the lectin domain (residues 1-156) at the N-terminus, which contains the carbohydrate recognition domain (CRD), and the pilin domain (residues 160-279), which connects *Fimh* to the pilus rod and regulates the switch between highand low-affinity states of the lectin domain (Mei-an *et.al*,2001). While the lectin domain alone is stable, the full-length *Fimh* in solution is only stable in presence of FimC. The first structure of FimC-*Fimh* complex was solved in 1999, and three

years later Hung and his coworkers reported the first co-crystallized structure of FimC-*Fimh* with a mannose ligand , which gave important insight into the binding site. Later, numerous structures of the *Fimh* lectin domain alone or in complex with diverse mannoside ligands were published, greatly facilitating the discovery of high affinity *Fimh* antagonists for the treatment of UTIs (Isolde Le, *et. al*,2010).

1.2.5 *Fimh* catch bonds

The term "catch bonds", first proposed in 1988, has been defined as a stronger or longer lived molecular interaction under tensile mechanical forces.

Catch bonds were observed with two types of adhesive proteins, selectins and *Fimh* (Evgeni, *et.al*,2008).

Further flow chamber assays and atomic force microscopy (AFM) studies 64 experimentally supported the allosteric catch bond for *Fimh*. However, the structural mechanism of *Fimh* catch bond behavior was just a putative model until recently the crystal structure of native full-length *Fimh* was published (Thomas, 2007).

The crystal structure elucidates that the pilin domain of *Fimh*, which anchors the lectin to the fimbrial shaft, interacts with the lectin domain such that a twist in its β-sandwich fold is caused . The twisted β-sheet loosens the mannose-binding pocket of *Fimh*, which is located on the opposite end of the lectin domain, thus leading to a low affinity state of the lectin. When tensile forces are applied, the pilin and lectin domains separate, and the lectin domain untwists and elongates, resulting in a tight mannosebinding pocket therefore a high-affinity state of *Fimh* (Veronika, *et.al*, 2011).

1.2.6 *Fimh* binding site

The receptor-binding site of the *Fimh* consists of a highly specific mannose-binding pocket, with a tyrosine gate (Tyr48, Ile52 and Tyr137) at one extension and a hydrophobic ridge (Ile13, Phe1, Phe142) at the other edge lining its entry.(Touaibia, *et.al*,2017).

1.2.7 Culturing of Bacterial *E. coli*

The identification of *E. coli* depends mainly on the cultural and biochemical characteristics and also microscopic patterns. During the present investigation of samples which was processed to isolate *E. coli* morphologically typical colonies were verified by Gram staining, to determine the size, shape and arrangement of bacteria. The organisms revealed gram negative, pink colored with rod shaped appearance and arranged in single or in pair were suspected as *E. coli*. A series of biochemical tests were performed which included catalase are positive, oxidase test are negative, TSI test was performed by incubation at 37ºC for 24 hours, the tubes showing acid butt (yellow), acid slant (yellow), with gas production and no H2S production were interpreted to be positive for E. coli, this give rise to be consider the *E. coli* is an acid resistant food borne pathogen that survives in the acidic environment of stomach and colonize the gastrointestinal tract (Baylis, 2009). Furthermore, it also increases the survival of *E. coli* for extended periods, especially at refrigeration temperature (Farrokh et al., 2012). *E. coli* can be differentiated from other members of the Enterobacteriaceae on the basis of a number of sugar fermentation and other biochemical tests. Classically an important group of tests used for this purpose are known by the acronym IMVIC. These tested for the ability to produce indole from

tryptophan; the test based on the ability of bacteria to decompose amino acid tryptophan to pyruvic acid, ammonia and indole, appearance of red ring indicated a positive reaction for E. coli, sufficient acid to reduce the medium pH below 4.4, the break point of the indicator methyl red is positive; but V-P and Citrate utilization test were negative, which are in agreement with the reports of Adams and Moss, (2008). Most of the isolates of *E. coli* showed fermented, glucose, lactose and sucrose with the production of both acid and gas. Samples were placed in enrichment by inoculated into MacConkey's at 37ºC for 18-24 hours followed by selective plating. Pink colonies from MacConkey agar occurred due to utilizing the lactose available in the medium with surrounding areas of precipitated bile salts, these colonies were inoculated onto Eosin Methylene Blue (EMB) agar contained lactose and the dyes eosin and methylene blue that permitted differentiation between enteric lactose fermenters and non-fermenters as well as for selection and isolation purposes, and considered as a rapid and accurate method of distinguishing *E. coli* from other gram-negative pathogens (Shuvra., 2017). Colonies were small, dark centered with greenish metallic sheen caused by the large quantities of acid that was produced and precipitated out the dyes onto the growth surface on EMB. The organisms showing characteristic colony morphology of *E. coli* was repeatedly sub cultured onto EMB agar until the pure culture with homogenous colonies were obtained. The well separated pure colonies were picked up on nutrient agar as pure culture and used for standard morphological and biochemical tests. However, some other characteristic of *E. coli* should be considered to confirm the

identification of these bacteria through using specific markers via PCR techniques (Müller, *et. al*.,2007).

1.3 Dinucleotide signature

The set of dinucleotide biases constitutes a 'genomic signature' that can discriminate sequences from different organisms Set of dinucleotide relative abundance values (dinucleotide biases) is a remarkably stable property of the DNA of an organism in single strand of DNA(Andrew & Samuel,2001). The dinucleotide usage profiles or genome signatures are similar for sequence samples taken from the same genome. The dinucleotide biases appear to reflect species-specific properties of DNA stacking energies, modification, replication, and repair mechanisms (S. Karlin,1998).

1.3.1 Uses of dinucleotide signature

- The genomic signature is useful for detecting pathogenicity islands (PAIs) in bacterial genomes.
- Genome signatures have been used to study several organisms including viruses.
- Genome signatures assume greater importance in the case of host–pathogen interactions.

1.3.2 Principle of dinucleotide signature

Advances in sequencing technologies have led to an exponential growth in gene and genome sequences deposited in public databases.

Statistical analysis of the nucleotide sequences has been carried out to study the nature of information stored at the molecular level.

Certain dinucleotide patterns were also found to be common in large group of organisms, like the dinucleotide TA is underrepresented in most organisms, and CG is known to be underrepresented in vertebrates (Wang, *et.al*.2019).

 Dinucleotide compositions have been shown to be consistent throughout a genome as opposed to G+C content this led to the concept of (genome signature)(Brierley, *et.al*,2021).

Genomic signatures have since then been used to study relationships between several organisms

 There are two approaches to calculate the genome signatures:

1.3.2.1 Chaos game representation (CGR) approach

The CGR was proposed as a new way to map the main sequence of DNA on a two-dimensional plot using iterative functions in 1990, and it is an uncommon approach that will be used (Jonas,2009).

(CGR) $i = 0.5 \times (CGR)i-1 + gi$

i=number of nucleotide, g=represent the four nucleotide bases

1.3.2.2. Dinucleotide relative abundance profile (DRAP)

 In the post genomic era, access to complete genome sequence data for numerous diverse species has opened multiple ways for examining and comparing primary DNA sequence organization of entire genomes. Previously, the concept of a genomic signature was introduced with the observation of species-type specific Dinucleotide Relative Abundance Profiles (DRAPs); dinucleotides were identified as the subsequences with the greatest bias in representation in a majority of genomes (Yingwei,

et.al,2005). Yingwei, 2005 demonstrate that DRAP is one particular genomic signature contained within a broader spectrum of signatures. DRAP was defined as the odds of occurrence of each dinucleotide (FXY, where X and Y are the single nucleotides) in a sequence, divided by the product of the odds of appearance of the corresponding single nucleotides (FX, FY). After DNA sequencing, dinucleotide frequency calculating by "odds ratio": $\rho XY = [XY]/([X][Y])$, with $X,Y \in \{A,C,G,T\}$,(Yingwei, *et.al*,2005). These can easily be done by python Module 3.9.

1.4 Bioinformatics approaches to find inhibitors

Bioinformatics is conceptualizing biology in form of molecules with the sense of physical chemistry and applying informatics techniques derived from scopes such as applied math, computer science and statistics to understand and systemize information associated with these molecules on large scale moreover, bioinformatics is an administration information system for molecular biology and has many practical applications (Luscombe *et al.*, 2001). However, another expression that often closes with bioinformatics or match together is computational processes related to molecular biology, and as well as computational systems biology in latest years, or computational biology such as a more broad term. Moreover, People occasionally use this expression for different meaning things, but from time to time use them in interchangeable protocols (Hogeweg, *et.al*,2011).

computational biology is an expansive term, which gives idea about all struggles of scientific investigations in biological feature or associated with biology that include computation and mathematics. Computation processes of molecular biology, on the other hand, focuses on the

molecular sides of biology in computational biology, which therefore has more or fewer the similar meaning with bioinformatics. (Jiang *et al*.,2016).

Three scopes of usage bioinformatics involving:

First, bioinformatics regulates data in way that permits researchers to reach finding information and to introduce new entries as they are produced e. g the protein Data Bank for 3D macromolecular structures. In addition, data-curation is an essential task, the information stored in these databases is essentially useless until analyzed. Therefore, the purpose of bioinformatics outreached much further (Faria *et al*., 2018).

The second aim is to evolve devices and resources that assisted in analysis of data, such as sequenced a particular protein having benefit to compare it with previously characterized sequences. For this aim needs more than a simple text based search, while formula such as FASTA and Position-Specific Iterative Basic Local Alignment Search Tool (PSI-BLAST) necessary to configures what biologically significant match, evolution of such resources requires experience in computational theory, as well as a thorough understanding of biology concepts (Healy.,2007).

The third aim is to use these devices to analyze the data and explain results in biologically meaningful manner. Imitatively, biological studies checked individual systems in detail, and compared them with little that are related, while in bioinformatics we can now conduct global analyze of all the available data with the objective of uncovering common principles that apply over many system and highlight novel features. (Luscombe *et al*., 2001).

1.5 Initial Discovery of *Fimh* ligands based on α-D-mannose

The first mention of mannose as a possible inhibitor of *E. coli* attachment to intestinal epithelial cells was reported back in 1957 (Dadi,*et.al*,2020), but remained unnoticed until the seminal work by Nathan Sharon in 1977 (Mojaz-Dalfardi, *et.al*,2020). In this paper, they presented the first solid data on the mannose-mediated attachment of *E. coli* K12 and B strains to human epithelial cells, and reported the presence of a "lectin-like" substance on the surface of *E.coli*, which would eventually become known as *Fimh*. Through competitive inhibition experiments, it was found that both α-d-mannose and methyl α-d-mannopyranoside (αMM) (Fig. 1.5)(Azarkan, *et.al*,2018) could both prevent *E. coli* from binding to epithelial cells, and displace pre-attached *E. coli* from epithelial cells. In 1979, the first mouse study was reported by Baldiris-Avila, *et.al*, (2020), wherein αMM was used as a prophylactic to prevent the colonization of *E. coli* within the urinary tract. From this proof-of-concept study, the *Fimh* lectin emerged as a promising therapeutic target to combat UTIs. Throughout the 1980s, several groups (Spurbeck, *et.al*,2012) began efforts aimed at designing more effective *Fimh* inhibitors. During this period, a variety of synthetic mannosides and naturally occurring oligosaccharides isolates, were further identified as inhibitors of the *Fimh* lectin. This pioneering research laid the foundation in which the future generations of rationally designed, potent and orally bioavailable, small-molecule *Fimh* mannoside antagonists would be built upon (De La Cadena, *et.al*,2020).
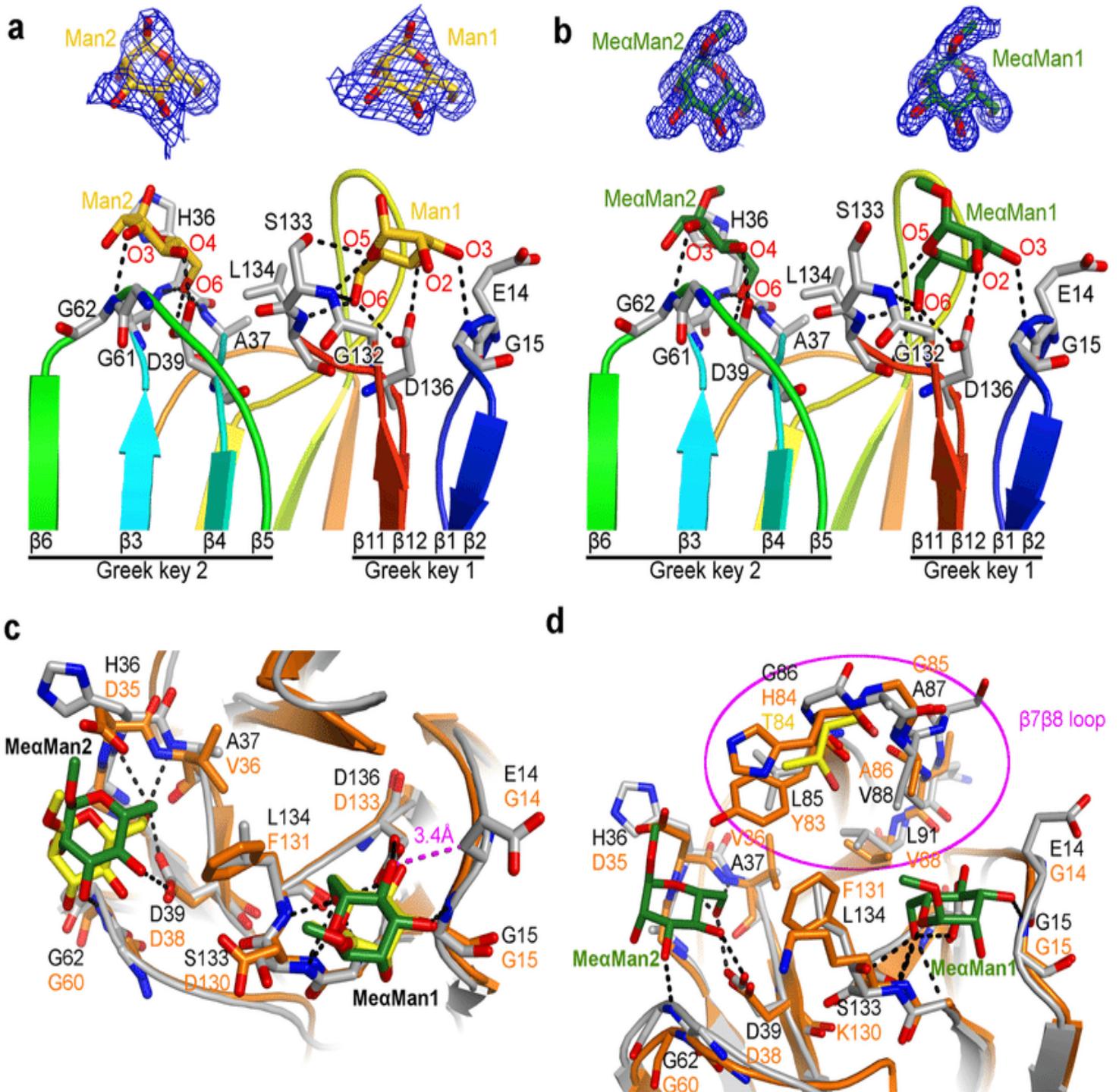
Figure 1.1 Interactions of the α-D-mannose and methyl-α-D-mannopyranoside in the carbohydratebinding sites of AcmJRL and comparison with BanLec. (a) Monomer A of the AcmJRL:Man complex is shown in cartoon representation with rainbow coloring from the blue N-terminus to the red C-terminus. The D-mannose molecules are shown as yellow sticks, and the amino acids involved in their binding as grey sticks. The D-mannose binding site 1 and 2 are formed by the loops of the Greek key

1 and 2 respectively. Two inserts highlight the electron density of the feature-enhanced map at the 1σ level around the two D-mannose molecules. (b) Same as A for the AcmJRL:MeαMan complex, the methyl-mannose molecules being displayed in green. (c) Superposition of AcmJRL and BanLec in complex with methyl-α-D-mannopyranoside. AcmJRL is represented in grey cartoon and sticks and BanLec in orange. MeαMan molecules in complex with AcmJRL and BanLec are shown as green and yellow sticks respectively. The close contact between MeαMan1 and Glu14 in AcmJRL is highlighted in magenta. (d) Comparison of the β7β8 loops of AcmJRL and BanLec with the same representation style as (c). Thr84 of the BanLec His84Thr mutant is shown in yellow sticks.(Azarkan, *et.al,*2018).

1.6 *Fimh*–Oligomannose-3 Complex

The urinary tract infection are one of the most prevalent infections for humans. Almost half of all women will experience at least one UTI in their lifetime. More problematic is the evolution of acute UTIs into chronic infections, with recurrence of the symptoms two or more times within months of a primary infection (Singaravelu,*et.al*,2014). Modifications in the glycosylation of *Fimh* receptor proteins on eukaryotic cells may alter the host sensitivity to UTI causing strains. For example, diabetic patients and elderly women show increased bladder cell binding by *Fimh* (Tomašiˇc, *et.al*, 2021) and this is further correlated with an increased frequency of asymptomatic bacteriuria (Ardila-Leal, *et.al*,2021). Free Manα1,3Manβ1,4GlcNAc oligosaccharide can be isolated in abnormally high amounts from urine of patients with mannosidosis (Dumych,*et.al*,2018). For mannosidosis patients the abundance of this high-affinity *Fimh* epitope in the urine may act as a natural inhibitor for urinary tract infections, although a decreased risk of

UTIs in patients with α-mannosidase deficiency has not been described(Wellens, *et. al*.,2008).

The high frequency of recurrent infections and the increasing antibiotic resistances of UPECs (Bouckaert, *et.al*,2006) highlight the need for alternative treatments using carbohydrate-derived molecules as potential anti-adhesives. Their non-bactericidal effect makes the selection of strains resistant to such agents much more unlikely than those resistant to antibiotics (Johnson, *et.al*, 2005). Biochemical and docking studies predicted that the enhanced binding of *fimh* and bases on the fact that the potential of ligand-based design of antagonists of UTIs appears to be ruled by structural mimicry of specific spots on mannosylated receptors. This anti-adhesive ability extends into blocking of bacterial invasion, intracellular growth and capacity to fluxing and recurrence of the infection (Wellens, et. al., 2008).

1.7 *Fimh* inhibitor agents

The urinary tract infection present high morbidity due to bacterial antibiotic resistance, which has increased over time due to antibiotic overuse. Therefore, it is necessary to look for new non-antibiotic alternatives to mitigate these infections (Alfredo, 2021). The pathogenicity mechanism of UPEC begins with cellular adhesion through the participation of fimbrial adhesins, and other virulence genes present can aid the process .The *fimh* gene encodes a protein of approximately 300 amino acids (*Fimh*), which participates in the regulation and mediation of fimbriae. It is composed of two domains: a pilin domain that allows polymerization (Mark, *et.al*,2000) and a lectin domain, which allows the binding to the host cells through mannosylated proteins. These

proteins are present in the bladder epithelium and bind to *Fimh* based on the rearrangement of the host actin cytoskeleton (Alfredo, *et.al*,2021). Due to their role in the virulence of UPEC, several therapeutic strategies have focused on *Fimh*, including vaccines, mannosides (as competitive compounds of the *Fimh* binding pocket), and molecules which inhibit the assembly of *Fimh*. Studies have shown that *Fimh* inhibitors can also increase the susceptibility of UPEC to antimicrobials even in resistant bacteria, making them a prospective non-antibiotic strategy for UPEC management and treatment (Cusumano, *et.al*,2011).

Currently, different contributions have been reported that show that several inhibitory molecules function as potential candidates with the ability to bind or couple to *Fimh* in different states of low, medium, and high affinity (Wellens, *et.al*,2012).

These contributions have also focused on the development of bioavailable mannosides molecules with an anti-virulence behavior, suggesting that the compounds derived from D-mannose (fig.1-7) show a high efficacy both in reducing symptoms and in the rate of recurrence of urinary infections (Montes-Robledo, *et.al*,2021). In addition, Sarks *et al*. (Starks, *et.al*,2021) suggest a bioavailable vaccine, which can induce functional antibodies over patients with recurrent urinary tract infections, contributing to the prevention of urinary infections. However, more accurate, reproducible, and standardized tests are needed to explore the effectiveness of D-mannose-derived antagonists in *Fimh*.

Fimh has been reported to bind to several glycosylation-dependent receptors in the urinary tract, among which are uroplakin Ia (UPIa) (Ren,*et.al*,2016), Tamm-Horsfall glycoprotein (THP) (Toscano,

et.al,2020) and β1 and α3 integrins (Mathers,*et.al*,2015). Many pathogens gain entry into target host cells by binding integrins either directly or indirectly via the recognition of extracellular matrix proteins. So, in our study suggests the best *fimh* inhibitor is a plant extract called "Apigenine" that extract from Chamomile plant (fig 1.2&1.3).

Type 1 fimbriae mediate hostpathogen interactions critical in pathogenesis. The *Fimh* adhesin, located at the tip of type 1 fimbriae, binds to mannosylated glycoproteins on human and mouse bladder epithelial cells and facilitates UPEC colonization and invasion of the bladder epithelium (urothelium) (Klein & Hultgren,2020). After invasion, UPEC can escape the endocytic vesicle and rapidly replicate within the urothelial cell cytoplasm, forming intracellular bacterial communities (IBCs) that resemble biofilms (Hardison, *et.al*; 2018). IBC formation occurs primarily during acute bladder infection and allows bacteria to rapidly expand in numbers and establish infection in a host niche that is protected from neutrophil attack and antibiotics (Sharma, *et.al*; 2021).   The IBC pathogenic cascade has been extensively characterized in a murine model of cystitis (McLellan, *et.al* 2016), and exfoliated bladder epithelial cells containing IBCs have been significantly observed in urine from women with recurrent UTI but not from healthy controls (De Nisco,*et.al*; 2019).

Animal models have defined 2 distinct chronic outcomes to experimental UPEC infection of the bladder in immunocompetent hosts: spontaneous resolution of bacteriuria that is often accompanied by a persistent latent intracellular infection (ie, a quiescent intracellular reservoir [QIR]) (Klein,*et.al*; 2020) or by chronic cystitis . UPEC within

QIRs can reemerge months later to seed a recurrent infection (Hibbing, *et.al* 2020). On the other hand, the development of chronic cystitis can sensitize mice to recurrent UTI when they are challenged with a new bacterial strain after clearance of infection following antibiotic treatment (Klein,*et.al*; 2020).Thus, UPEC can effectively colonize the host bladder and establish acute, chronic, or recurrent infections. On the basis of murine models that mimic aspects of human disease, *Fimh* is critical for UPEC pathogenesis. *Fimh* is also under positive selection in human clinical isolates of UPEC, further supporting its role in human disease (Hibbing,*et.al*; 2020). Inhibiting *Fimh* function may thus represent a therapeutic strategy for the treatment and prevention of UTI. In this respect, novel biaryl mannoside *Fimh* inhibitors, including compound ZFH-04269(4′-[a-D-Mannopyranosyloxy]-N,3′-dimethylbiphenyl-3-carboxamide),were recently shown to attenuate UPEC virulence in mice by impeding *Fimh* binding to the bladder epithelium, thereby preventing bacterial invasion and IBC formation and resulting in significantly reduced bladder bacterial titers during both acute and chronic cystitis (Jones,*et.al*; 2021).

Recently, a clone of UPEC belonging to serotype O25b:H4 and sequence type 131 (*E. coli* ST131) has emerged as a leading multidrug-resistant pathogen causing urinary tract and bloodstream infections in hospitals and the community.

Several reports have demonstrated the global distribution of this lineage, indicating that it constitutes a major threat to public health worldwide. The successful dissemination of *E. coli* ST131 is thought to be due to a combination of antibiotic resistance and virulence. *E. coli*

ST131 commonly harbor genes encoding several types of β-lactamases, particularly of the cefotaximases (CTX-M family) of extended-spectrum β-lactamases (ESBLs), and are typically associated with limited treatment options (Jones, *et.al*; 2021). While the *E. coli* ST131 genes conferring resistance to multiple classes of antibiotics have been the focus of many studies, the virulence mechanisms used by this clone are less well understood.

Although *E. coli* ST131 strains are derived from phylogenetic group B2, which includes several characterized pathogenic *E. coli* clonal groups, only a few virulence genes (eg, *fimh*, iutA, and sat) appear to be uniformly encoded in all *E. coli* ST131 strains (Johnson,*et.al*; 2019). Clinical studies have demonstrated transmission of virulent *E. coli* ST131 strains between family members (Toombs-Ruane, *et.al*; 2020), but the factors that contribute to the widespread dissemination of this lineage and the pathogenic mechanisms used during UTI remain poorly defined. We have previously demonstrated that the genome sequence of a representative multidrug-resistant UPEC ST131 isolate, *E. coli* EC958, contains genes encoding a variety of potential virulence factors, including numerous adhesins, autotransporters, and siderophore receptors (Clark,*et.al*; 2021). In this study, we examined the pathogenic lifestyle of *E. coli* during experimental UTI in mice with acute and chronic infection. We demonstrated that *E. coli* is able to invade the bladder epithelium and form IBCs. In addition, we showed that *E. coli* can persist in the bladder and establish chronic infection. We also demonstrated a key role for type 1 fimbriae in the ability of *E. coli* to establish bladder infection, as prophylactic treatment with an oral *Fimh* inhibitor prevented acute

cystitis. Moreover, a single oral dose of the same *Fimh* inhibitor significantly reduced the bacterial load in the bladder of mice chronically infected with *E. coli* (Tamadonfar,*et.al*; 2019). This study revealed the potential of *Fimh* inhibitors as an alternative treatment against multidrug-resistant UPEC strains.
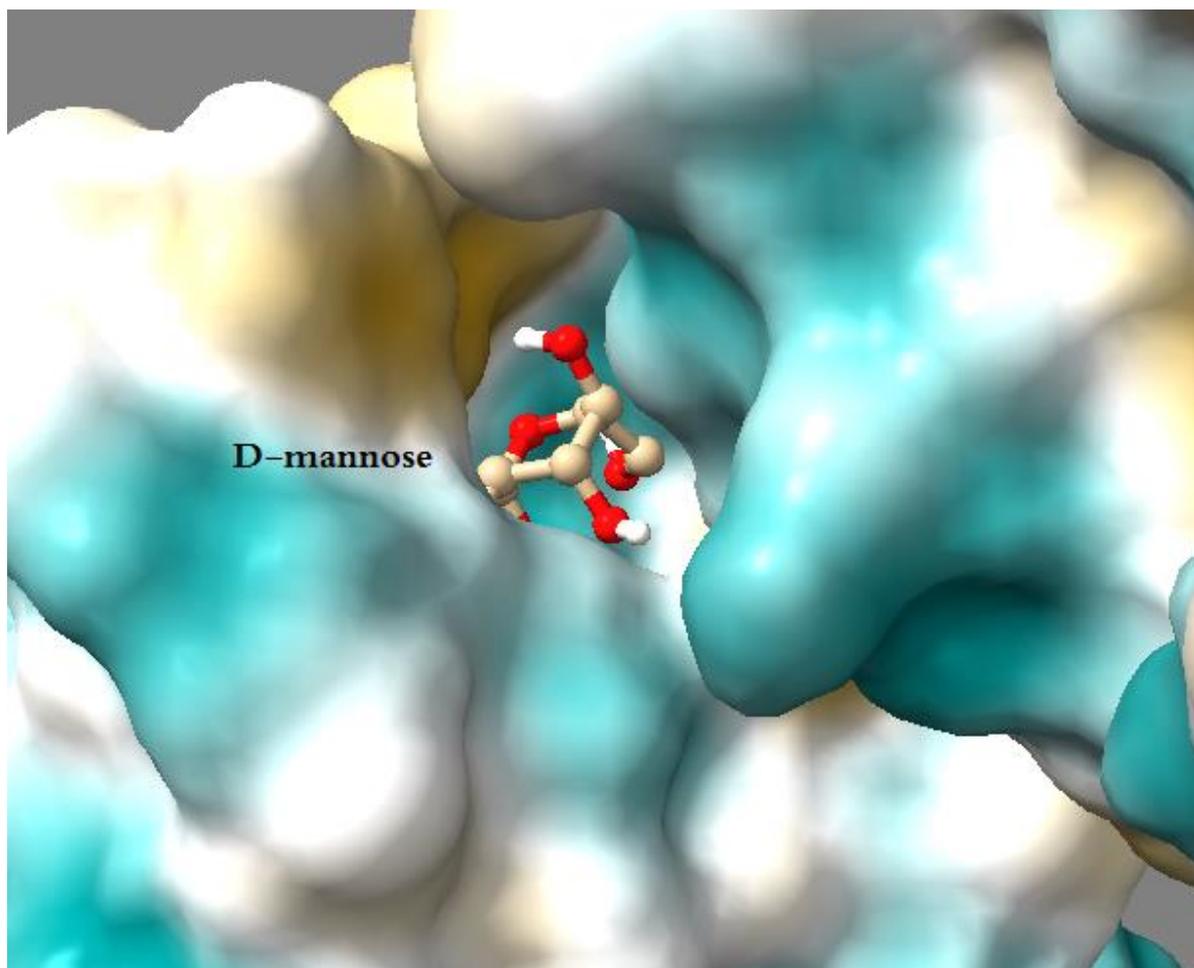
Figure (1-2): Electron density maps, for oligomannose-3 in the *Fimh* receptor-binding site with D-mannose
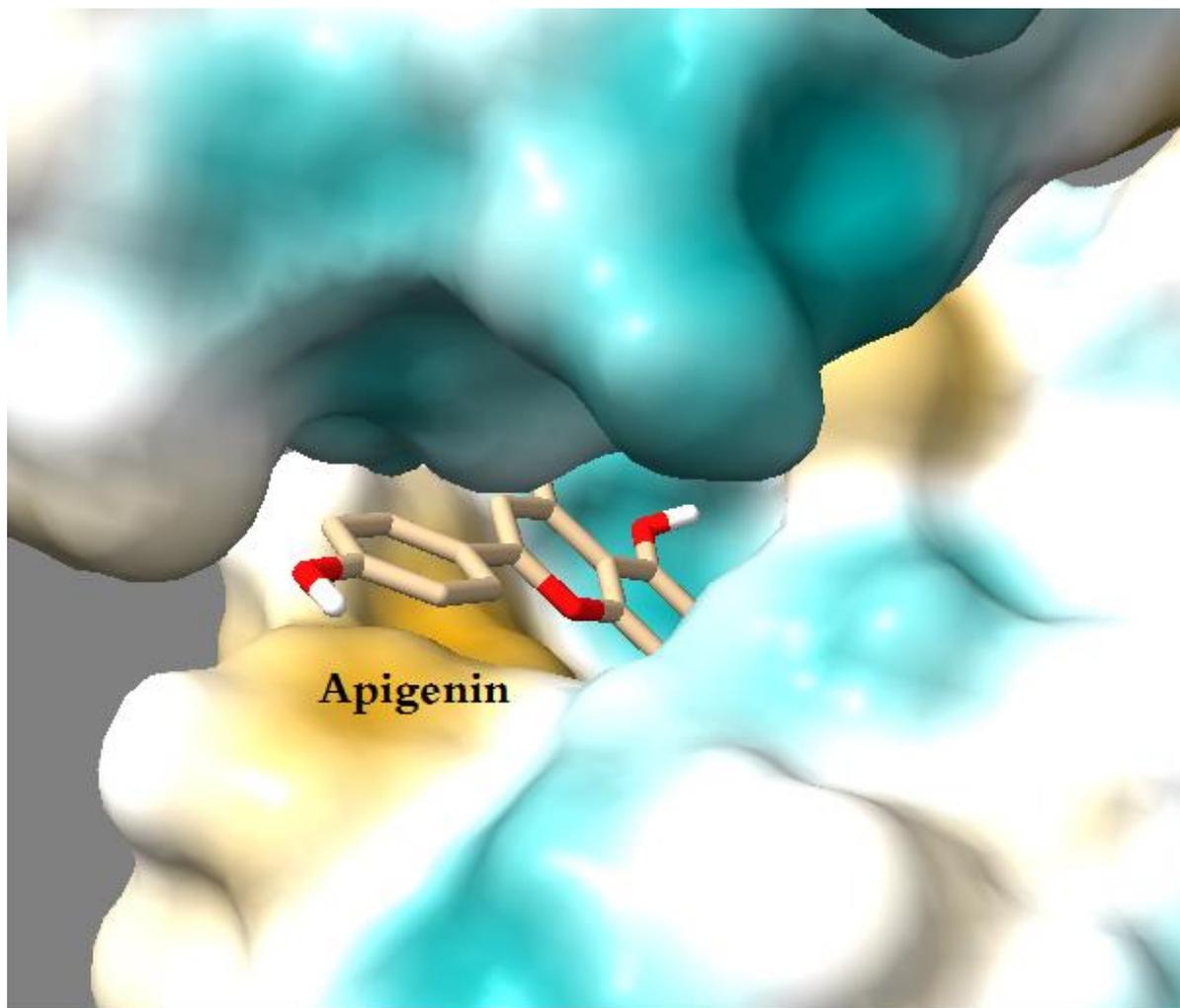
Figure (1-3): Electron density maps, for oligomannose-3 in the *Fimh* receptor-binding site with Apigenine

1.8 *Fimh* inhibitor "Apigenine" that extracted from chamomile

Chamomile, scientifically known as *Matricaria recutita*, is a plant native to Western Europe and North Asia that is characterized by having a herbaceous bearing and flowering for medicinal use. This species has been used, since ancient times, in many European regions due to its medicinal and health-beneficial properties. It began growing in abundance in the Balkans to later be introduced in central Europe and in countries such as Italy, Bulgaria or Russia (Némethy *et al*., 2020).

Chamomile (or chamomile) is an herbaceous, annual and aromatic plant. It has an erect, branchy stem and can reach a height of 50 cm (approximately). Its leaves are pinnate, alternate, segmented and have a light layer of hair. In summer, chamomile develops with an inflorescence formed by numerous yellow flowers that are in turn surrounded by white ligules and a yellow carnelian (Gupta, *et.al*,2010).

A number of other species common names include the word "chamomile". This does not mean they are used in the same manner as the species used in the herbal tea known as "chamomile", of the family Asteraceae.

Some commonly used species include (Leach,*et.al*, 2015):

- Matricaria chamomilla Often called "German chamomile" or "Water of Youth"(Tayel, & El-Tras,2009).

- Chamaemelum nobile, Roman, English or garden chamomile, also frequently used (C. nobile Treneague is normally used to create a chamomile lawn).(McKay,*&* Blumberg,2006).

1.8.1 Benefits and uses of Chamomilla (Apigenine)

Chamomile is one of the most popular to use against internal and external conditions. This medicinal plant is used both to treat digestive problems, nerves or cholesterol, as well as to care for the skin or eyes(Vashist & Sharma, 2017).

Chamomile suggested uses include gas (flatulence), gastrointestinal (GI) inflammatory disorders, GI spasms, inflammation (skin or mucous membrane), insomnia, menstrual cramps, and motion sickness.

Chamomile for topical use suggested uses include for hemorrhoids, and mucositis (chemo-induced)(Aglawe *et al*., 2020).

1.8.2 Side effect of Chamomilla (Apigenine)

Common side effects of chamomile include: Severe allergic reaction (anaphylaxis) Contact dermatitis/skin reactions Eye irritation (when applied near the eyes) Hypersensitivity reactions Vomiting (when taken in large amounts), (Fraunfelder, *et.al*, 2014).

1.8.3 Special Precautions and Warnings

Asthma, Hypersensitivity to Asteraceae/Compositae plants chrysanthemums, daisies, marigolds, ragweed (Mullins.,2004).

Pregnancy and Lactation: Avoid the use of chamomile during pregnancy, Use of chamomile is contraindicated during pregnancy because it may stimulate uterine contractions.(Kapalka, *et.al* ,2010). There is no information available about the use of chamomile while breastfeeding

1.8.4 Dosing

Doses in adults vary widely and range from 900 mg to 1200 mg per day, (15-25) mg per kg. Half-life may be short because it is often recommended to take chamomile three times per day (Kolanos, *et.al*,2021; Kapalka, *et.al* ,2010; Shinomiya, *et.al*,2005).

- Mucositis, chemo-induced: Swish orally and swallow three times daily; use 10-15 drops of 1.2% liquid extract in 100 ml warm water.

- One tea cup orally, three times daily to four times daily; 3 g dried flower heads/150 ml water.

Liquid extract 1-4 ml orally three times daily; 1:1 in 45% alcohols

2.1. Materials
2.1.1. Ethical Approval

1-The study was done and the samples were gathered in the wake of getting the understanding of the patients (verbal acknowledgment).

2- Approval of Babylon medical college and Karbala Pharmacy College.

3-Before beginning the review, consent were taken from Karbala and Babylon health directorate and patients.

2.1.2. Laboratory Apparatuses and Instruments

Listed down in **Tables (2-1)** and **(2-2)**, the main scientific apparatus, and technical instruments with disposable materials respectively, those were employed during the course of this study.

Table (2-1): Scientific Laboratory Apparatus.

Item	Company	Country
Anaerobic incubator	Memmert	Germany
Autoclave	Herayama	Japan
Bacteriological cabinet	Labogene	Denmark
Benson burner	Dolphin	Syria
Candle – jar	Kayagaki irikakocy. Co. LTD.	Japan
Centrifuge	Gemmy	Taiwan
Conventional PCR system	Clever Scientific	UK
Digital camera	Samsung	Japan
Distillator	GFL	Germany
Electrophoresis	Clever Scientific	
Freezer	Aucma	China
Light microscope	Stermite Olympus A &D	Japan
Microcentrifuge	Becman	USA

Micropipettes 5-50 μl ,100-1000 μl, 2-20 μl	Top Dragon	Europe
Nano drop	Avans biotechnology corp	Taiwan
Oven	GS	Taiwan
Platinum wire loop	Himedia	India
Refrigerator	Concord	Italy
Sensitive electric balance	Kern	Germany
UV-transilluminator	Clever Scientific	
Vortex	Gemmy	Taiwan
Water bath	GFL	Germany

Table (2-2): Technical Instruments and Disposable Materials.

Item	Company	Country
96-well flat bottomed polystyrene Microplate	Spektar	Serbia
Anaerogen Gas –bag	Oxoid Ltd.	Basingstoke/UK
EDTA-tubes	Afco	Jordan
Glass slides	Sail brand	China
Gynaecological Cusco speculums	TTN	Iran
Medical gloves	Broche	PRC
Medical cotton	Medicare Hygiene Limited	India
Microscopic Cover slide	Gitoglas	China
Millipore filters (0.45mm)	Sigem	Spain
Parafilm	Bemis	USA
PCR tubes 1.5 ml (Eppendrof)	Biobasic	Canada
PCR tubes 200μl (Eppendrof)	Biobasic	Canada
Pettri dishes	Blastilab	Lebanon
Plastic test tubes 10ml	Dolphin	Syria
Sterile swabs	Sigem	Spain
Syringes	Dolphin	Syria
Tips	Dolphin	Syria
Wooden sticks	Supreme	China

30

2.1.3. Chemical Materials

Itemized down in **Table (2-3)**, the main chemicals utilized in this study.

Table (2-3): Chemical Materials and Reagents.

Item	Company	Country
Carbohydrates (glucose, mannitol, lactose and sucrose)	Fluka chemika	Switzerland
Catalase reagent, Indole Kovacs Reagent, Methyl red indicator, 5% alpha naphthol, 40%KOH, 10%KOH.	Schuchariot	Germany
ChloroPhenol red.	Searle	England
Claradone (povidone-iodine USP 10%. W/V).	Medpharma	UAE
Crystal violet	Sigma	USA
Eosin yellow stain solution 2% W/V.	Central brug house LTD.	India
Ethanol 70%, methanol	Fluka chemika	Switzerland
Ethanol absolute (95%)	GCC	UK
Gentamycin.	Julphar	UAE
Gram stain kit and giemsa stain.	Crescent	KSA
HCL, Glacial acetic acid, Tannic acid Glycerol, Barium chloride, Sulfonic acid	B.D.H	England
Hemin, Vitamin K, L-Cystine.	Cromatest	Spain
Isopropanol absolute	GCC	UK
Kanamycin sulphate.	Gemini bio products	USA
NaCl, Na2HPO4, KH2PO4, NH4CL, MgSO4, CaCl2, FeSO4.	Merk Darmstade	Germany
Normal saline	Pharmaline	Egypt
Oxidase reagent (Gordon-Mcleod reagent)	Himedia	India
Phosphate buffer	Himedia	India

Urea supplement 5%, Pancreatic digest of casein, Papaic digest of soyabean meal, yeast extract, corn extract, glucose.	Himedia	India

2.1.4. Biological Material

Itemized down in **Table (2-4),** the main biological materials utilized in this study.

Table (2-4): Biological Materials.

Item	Company	Country
1-Eosin Methylene blue (EMB) agar, Blood agar medium, MacConkey agar, Nutrient agar, the Brain heart infusion agar, Brain heart infusion broth. Peptone water broth, Muller Hinton agar, MR-VP broth	Himedia	India
2- Urea agar base, Triple sugar iron agar, Simmon's citrate agar, Lysine Decarboxylase broth, ornithine Decarboxylase broth.	Diffco – Michigan	USA

2.1.5. Molecular Materials

Categorized and detailed down in **Table (2-5)**, the materials and kits employed in the molecular study.

Table (2-5): Molecular-Related Materials.

Item	Company	Country
100 bp Ladder, consists of: 11 double-stranded DNA fragments ranging in sizes from 100 to 1500 bp with 100 bp increments. The 500, 1000 and 1500 bp bands are double to triple of the intensity of other fragments and brighter, for easier identification and comparison of molecular weight. While all other fragments seem with equal intensity on gel.	Bioneer Promega a	Korea USA
Agarose.	Promega	USA
Blue/Orange Loading Dye, 6X containing: 1-0.4% orange G. 2-0.03% bromophenol blue. 3-0.03% xylene cyanol. 4-15% Ficoll. 5-10mM Tris-HCl (pH 7.5). 6-50mM EDTA (pH 8.0). It is used for loading DNA samples into wells and tracking migration during gel electrophoresis.	Biobasic	Canada
Ethidium Bromide Solution, (10mg/ml).	Biobasic	Canada
Geneaid Genomic DNA Isolation Kit	Geneaid	UK
Green master mix 2X Kit, consist of: 1-Taq DNA polymerase. 2- dNTPs, 400µM for each. 3-Tris-HCl (pH 8.5-9.0), 10 mM. 4-KCl, 30 mM 5-MgCl$_2$, 3mM. 6-eppendroffs of Nuclease free water 7-Stabilizer and tracking dye	Promega	USA
Nuclease free water.	Bioneer	Korea
Primer pairs	Bioneer	Korea
TBE Buffer (Tris-Borate-EDTA), 10X (pH 8.3) Composition: 890mM Tris-borate, 890mM boric acid, 20mM EDTA.	Promega	USA

TE Buffer, 1X (pH 8.0) Composed of 10mM Tris-HCl containing 1mM EDTA•Na2.		Bioneer	Korea

2.1.7. Antibiotics Disks:

Table (2-6): Antibiotics disks. (Bioanalyze / Turkey)

No.	*Groups*	*Antibiotics*	*Potency (µg per disk)*
1.	**B- lactam**	**Amoxicillin**	25
2.		**Pipracillin**	100
3.		**Cefazolin**	30
4.		**Cefuroxime**	30
5.		**Ceftriaxone**	30
6.		**Cefotaxime**	30
7.		**Ceftazidime**	30
8.		**Meropenem**	10
9.		**Aztreonam**	30
10.		**PolymyxinE**	30
11.	**Aminoglycoside**	**Gentamycin**	30
12.		**Amikacin**	30
13.		**Streptomycin**	10
14.	**Macrolide**	**Azthromycin**	15
15.		**Clarithromycin**	15
16.		**Erythromycin**	15
17.	**Fluroquinolons**	**Nalidixic acid**	30
18.		**Ciprofloxacin**	5
19.		**Levofloxacin**	5
20.		**Gatifloxacin**	5

2.1.8 Study design

This study involved (100) specimens were collected from patients with UTI, the samples collected from both sexes and different ages, who attended to hospitals of karbalaa Province: al-Hussain medical city and Pediatrics Hospital, and general Hospital of Al- Musayyib in Babylon, during the period from February to October 2021.The age of patients ranged from 15 to 78 years (figure 2.1).
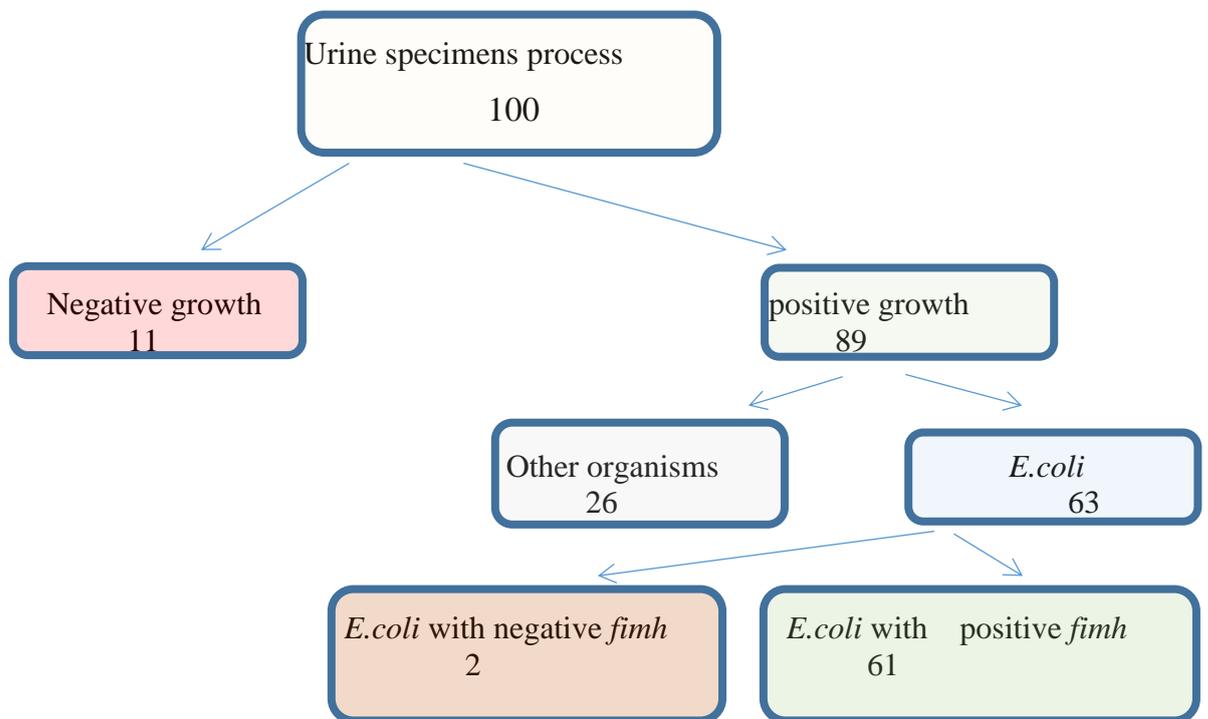
Figure (2.1): scheme of study design

2.2. Methods:
2.2.1. Reagents, Solutions and media:

Table (2-7): Reagents, Solutions and media that used in our study

Reagent, solution and culture media	Methods of preperation	References
Agarose Gel		Sambrook and Russel, 2001
Blood Agar Medium		Forbes *et al*., 2007
Brain Heart Infusion (BHI) Broth – Glycerol Medium		Forbes *et al*., 2007
Brain Heart Infusion Agar		MacFadden, 2000
Brain Heart Infusion Broth		MacFadden, 2000
Catalase Reagent		Forbes *et al*., 2007
Eosin Methylene Blue (EMB) medium	Prepare as a company instructions	Murray *et al.,* 2003
Kovacs reagent		MacFaddin, 2000
MacConkey Agar Medium		Winn *et al*., 2006
McFarland (0.5) turbidity standard		CLSI, 2012
Methyl red reagent		MacFaddin, 2000
Methyl red Vogues – Proskauer medium		MacFadden, 2000
Muller-Hinton Agar Medium		MacFadden, 2000
Normal Saline Solution		MacFaddin, 2000
Nutrient Agar Medium		MacFaddin, 2000
Oxidase Reagent		Forbes *et al*., 2007
Peptone Water medium		MacFaddin, 2000
Simmon's Citrate Agar		Forbes *et al*., 2007
Triple sugar Iron Agar Medium		MacFaddin, 2000
Urea Agar Medium		MacFadden, 2000

Urea solution		MacFaddin, 2000
Vogues – Proskauer reagent (Barrett's		MacFaddin, 2000

2.3 Preparation of Molecular Materials

2.3.1 Preparation of 1X TBE Buffer

The preparation of 1X TBE buffer was performed by dilution of a concentrated 10X TBE buffer, this dilution was accomplished as 1:10 (v/v); 1 volume of 10X TBE: 9 volumes of distilled water. This solution was used to prepare agarose gel and as a transmission buffer in electrophoresis process. Thus each 100ml of 10X TBE added to 900ml of sterile distal water to produce final concentration,1X TBE (Sambrook and Russel, 2001).

2.3.1.1 Preparation of Agarose Gel

This gel was prepared by adding agarose powder in 1X TBE buffer to be dissolved by boiling, and then it was left to cool to 50ºC. The dissolved amount of agarose powder is depending upon the aim for which agarose is used.

For DNA profile (visualization of the DNA after extraction), 1% agarose is used. While for visualization of PCR product (amplicon), 1.5% of agarose was employed and for single nucleotide polymorphism detection, 3% agarose is used.

Ethidium Bromide stock solution with a concentration 10mg/ml was used. Only 5μl of this stock solution were supplemented to 100ml of melted agarose gel to get final concentration 0.5μg/ml (Sambrook and Russel, 2001). Then after the addition of ethidium bromide, mixed well and dispensed to the tray of gel electrophoresis.

2.3.1.2 Rehydration of Primers

Lyophilized primer pairs were rehydrated by DNA rehydration solution 1X (pH 8.0) Tris- EDTA buffer (TE-buffer). Initially, primer storage-stock tube prepared and then the working solution would prepare from primer stock tube. Consistent with the instructions of the producer (Bioneer/Korea), TE buffer was added to produce 100 picomole/microliter concentration of primer stock solution. The working solution prepared from stock as 1:10 (v/v) by dilution with TE buffer to get 10 picomole/ microliter.

2.4 Collection of Specimens:

The proper specimens collected for bacteriological analysis are described below. Those specimens were collected in proper ways to avoid any possible contamination (Forbes *et al.*, 2007).

2.4.1 Urine samples

The specimens were generally collected from patients suffering from UTIs. Mid-stream urine samples were collected in sterilized screw-cap containers.

2.5 Laboratory Diagnosis:

2.5.1 Bacterial Identification Assays:-

According to the diagnostic procedures recommended by Forbes *et al*, (2007), the isolation and identification of *E. coli* associated with patients under study were performed as follows:

2.5.1.1. Colonial Morphology and Microscopic Examination:

A single colony was taken from each primary positive culture. Its identification depends on the morphology properties (colony size, shape, color, translucency, edge, and elevation of texture). The colonies were

then investigated by gram stain to observe bacterial cells. Specific biochemical tests were done to reach the final identification.

The *E coli* appeared with circular shape, entire margin, raised, small size, smooth texture, green shiny appearance, on culture media (EMB).

2.6 Biochemical Tests

Table (2-8): biochemical test of *E.coli*

Characteristics	Test result of *E.coli*
Acetate Utilization	Positive (+ve)
Acid Phosphatase	–
Alkaline Phosphatase	–
Amidase	–
Arabinose	Positive (+ve)
Arginine dehydrolase	Variable
Arylsulphatase	–
Beta Lactamase	–
Bile Solubility	–
CAMP	–
Capsule (Capsulated/Non-Capsulated)	Variable
Catalase	Positive (+ve)
Citrate	Negative (-ve)
Coagulase	–
DNase	Negative (-ve)
Enzymatic Reactions	
Fermentation of	
Flagella (Flagellated/Non-Flagellated)	Flagellated
Fructose	–
Gas	Positive (+ve)
Gelatin	Negative (-ve)
Glucose	Positive (+ve)
Gram Staining	Negative

H2S	Negative (-ve)
Hemolysis (Alfa/Beta/Gamma)	Some Strains shows Hemolysis
Hippurate	–
Hyalurodinase	–
Indole	Positive (+ve)
Inositol	Negative (+ve)
Inulin	–
Lactose	Positive (+ve)
Lecithinase	–
Lipase	–
Lysine	Positive (+ve)
Maltose	–
Mannitol	Positive (+ve)
Mannose	–
Motility (Motile / Non-Motile)	Motile
MR	Positive (+ve)
MyoInositol	–
Nagler	–
Niacin	–
Nitrate Reduction	Positive (+ve)
OF (Oxidative/Fermentative)	Fermentative
Oxidase	Negative (-ve)
Pigment	–
PYR	–
Raffinose	–
Ribose	–
Shape (Cocci/Diplococci/Rods)	Rods
Sorbitol	Positive (+ve)
Spore (Sporing/Non-Sporing)	Non-Sporing
String Test	–
Sucrose	Variable
Urease	Negative (-ve)
VP	Negative (-ve)

2.6.1. Catalase Test:

Catalase is an enzyme that catalyses the release of oxygen from

hydrogen peroxide. Nutrient agar medium was streaked with the selected bacterial colonies and incubated at 37ºC for 24 hrs, then the growth was transferred by the wooden stick and it was put on the surface of a clean slide, a drop of (3% H2O2) was added. Formation of gas bubbles indicated a positive result (Forbes *et al*., 2007).

2.6.2. Oxidase Test:

The test depends on the presence of certain bacterial oxidases that would catalyze the transport of electrons between electron donors in the bacteria and a redox dye (tetramethyl-ρ-phenylene-diaminedihydrochloride), the dye was reduced to a deep purple color.
 A strip of filter paper was soaked with a little freshly made reagent, and the colony to be tested was picked up with a sterile wooden stick and smeared over

The filter paper a positive result was indicated by an intense deep purple color which appeared within 5-10sec. (Forbes *et. al*., 2007).

2.6.3. Indole test:

This test was performed by inoculating peptone water medium with bacterial growth by the loop, and it was inoculated for 24 hour at 37oC. Indole test was done by adding 6-8 drops of Kovac,s reagent (ρ-dimethyl amino Benzaldehyde in amyl alcohol). The positive reaction was characterized by the formation of red color ring at the top of the broth (MacFaddin, 2000).

2.6.4. Methyl-red test:

The tubes of the MR-VP broth were inoculated with selective bacterial

colonies and were incubated at 37oC for 24 hour. Five drops of methyl red reagent were then added to it. The appearance and observation of red color means a positive result and a complete hydrolysis of glucose (MacFaddin, 2000).

2.6.5. Vogues – Proskauer test (VP):

The tubes of the MR-VP broth were inoculated with selected bacterial colonies and were incubated at 37oC for 24 hours. The result was then read by adding 5-12 drops of alpha nephthol (reagent A) and 4 drops of 40% KOH solution (reagent B) the appearance of red color after 15 minutes -1 hrs means a positive result due to the partial hydrolysis of glucose, which produced acetoin or Acetyl - methyl - carbinol (MacFadden, 2000).

2.6.6. Citrate utilization test:

After the sterilization of Simmons Citrate slants by autoclave, the bacterial colonies were inoculated and incubated for 24 hours at 37oC. The change of
color of media from green to blue indicated that the organisms were able to utilize citrate as sole carbon source (Benson, 2001).

2.6.7. Urease test:

This test was carried out by inoculating urea medium with bacterial growth. The tubes were incubated for 24-48 hours at 37oC. The color change of medium into pink indicated a positive result (McFadden, 2000).

2.6.8. Hemolysis test:

Blood agar medium was incubated with bacterial isolate and incubated at 37ºC. After incubation the appearance of clear zone around the colonies referred to complete haemolysis (β-haemolysis) while greenish zone around the colonies referred to partial haemolysis (α-haemolysis) whereas no change in medium referred to non-haemolysis (γ-haemolysis) (Brooks *et al.*, 2010).

2.6.9. Triple sugar Iron Agar Medium:

If lactose (or sucrose) is fermented, a large amount of acid is produced, which turns the phenol red indicator yellow both in butt and in the slant. If lactose was not fermented but only amount of glucose was fermented, the slant remain red while the butt is yellow with acid. If neither lactose/sucrose nor glucose was fermented, both the butt and the slant will be red. The slant can become a deeper red-purple (more alkaline) as a result of production of ammonia from the oxidative deamination of amino acids , if H2S was produced, the black color of Ferrous Sulfide was seen(McFaddin, 2000).

2.7. Rapid Identification System by VITEK-2 Compact System:

E. coli identified with the automated VITEK-2 compact system. This system identifies an organism via a methodology based on the characteristics of the data and knowledge about the organism and reaction being analyzed. That provide the confirmatory tool for biochemical test had been performed according to manufactures' instruction.

The system was prepared with an extended identification data base for

all routine identification test that provides an improved efficiency in microbial diagnosis which reduce the need to perform any additional tests so that will increase safety for both, the test and users.

This VITEK-2 compact system includes a strip consist of 47 biochemical tests. Bacteria was suspended in 3 ml normal saline test tube then inserted into dense check machine for standardization of colony to Mac Farland standard solution (1.5 x 108 cell/ ml). A test tube containing the microorganism suspension was placed into a special rack (cassette) and the identification card is placed in the slot while inserting the transfer tube into the corresponding suspension tube.

The filled cassette was placed manually into a vacuum chamber station. After the vacuum was applied and air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells.

A transmittance spectrophotometer was used to read of test reactions using different wavelengths. During incubation, each test reaction is read every 15 minutes to measure either turbidity of bacterial mass growth or color products of substrate metabolism.

All reagents and equipment's needed for processing supplied by Manufacturer Company. All isolates introduced to the computer before processing and inoculated cards were processed in the instrument within 30 min of inoculation.

The card thus connected to the sample ID number, then the cassette is placed in the filler module, when the card are filled, transferred the cassette to the reader incubator module. All following steps handled by the instruments, the instruments controls the incubation temperature, the optical reading of the cards and continually monitors and transfers test

data to the computer for analysis (Biomerieux, France).

2.8. Antibiotic Susceptibility Testing

2.8.1. Disc diffusion test (DD test):

The Kirby-Bauer method is a standardized system for this test that takes all variables into consideration. It is sanctioned by the United States FDA and the Subcommittee on Antimicrobial Susceptibility Testing of the NCCLS (Benson, 2001).

1. It was performed by using a pure culture of previously identified bacterial organism (107 CFU). The inoculums to be used in grown in this test was prepared by adding growth from 5 isolated colonies on blood agar plates to 5 ml of nutrient broth, this culture was then incubated for 2 hrs. to produce a bacterial suspension of moderate turbidity that compared with turbidity of ready-made (0.5) McFarland tube standard. A sterile swap was used to obtain an inoculums from the standardized culture, this inoculums was then swabbed on Mueller–Hinton plate.

2. The antibiotic discs were placed on the surface of the medium at evenly spared intervals with flamed forceps, and then incubated at 37ºC for a full 18 hrs (Weigelt, 2007).

3. Antibiotics inhibition zones were measured using a transparent ruler. Zone size was compared to standard zones (Matuschek, et. al, 2022) to determine the susceptibility of organism to each antibiotic.

2.9. Genotyping Assays:

2.9.1. DNA Extraction from Gram Negative Bacteria:

DNA was extracted from clinical isolates. One colony of each isolate cultured and inoculated into 5 ml of BHI (Brain Heart Infusion) and grown over night at 37ºC. From these isolate cultures; DNA was

purified from bacterial cells using Genomic DNA kit supplemented by the manufacturing company. DNA obtained were used as templates for all PCR experiments.

The DNA concentration is measure by Nano drops machines at 260/280nm give 1.8 purify DNA. The PCR reactions were carried out in a Thermal Cycler. Before PCR assay, the DNA profile was performed by using bacterial DNA and loading buffer without thermal cycling conditions, and according to the following steps:

First- Sample preparation of Gram Negative Bacteria:

1. Overnight bacterial cells (up to 1 x 109) are transfer to a 1.5 ml micro centrifuge tube.

2. Then tubes centrifuged at 14.000 rpm for 1 min. to pellet the cells. the supernatant was Removed.

3. A total of 180μl of GT Buffer aws added, then re-suspend the cell pellet by vortex or pipette.

4. Added 20μl of Proteinase K (make sure ddH2O was added). Incubate at 60ºC for at least 10 minutes. During incubation, invert the tube every 3 minutes.

Second - Cell Lysis:

5. Added 200μl of GB Buffer to the sample and mix by vortex for 10 seconds. Incubate at 70ºC for at least 10 minutes to ensure the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, pre-heat the required Elution Buffer (200 μl per sample) to 70ºC (for step of DNA Elution).

Third- DNA Binding:

6. Added 200μl of absolute ethanol to the clear lysate and immediately

mixed by shaking vigorously for 10 minutes.

7. GD Column were placed in a 2ml collection tube.

8. All of the mixture was transferred (including any precipitate) to the GD column, centrifuged at 14,000 rpm for 2 minutes.

9. The collection tube was discarded containing the flow through and the GD column was placed in a new collection tube.

Forth - Wash step:

10 - Added 400 μl of W1 buffer to the GD Column. Centrifuged at 14,000 rpm for 30-60 seconds.

11 - The flow-through was discarded and the GD column placed back in the 2ml collection tube.

12 - Added 600 μl of wash buffer (Ethanol added) to the GD column, centrifuged at 14,000rpm for 30 seconds.

13 - The flow-through was discarded and the GD column placed back in the collection tube and then centrifuged again for 3 minutes at 14,000 rpm to dry the column matrix.

Fifth - Elution step:

14 - The dried GD column was transferred to a clean 1.5 ml centrifuge tube.

15 - 100 μl of pre-heated elution buffer or TE was added to the center of the matrix, and let stand for at least 3 minutes to ensure the elution buffer is absorbed by the matrix, centrifuged at 14,000rpm for 30 seconds to elute the purified DNA.

16 - The DNA was stored at -20ºC to avoid degradation.

2.9.2. Polymerase Chain Reaction (PCR):

Polymerase Chain Reaction was performed in a final volume of 25μl as in Table (2-7). Then DNA amplification was carried out with the thermal cycler.

Table (2-9) Contents of the Reaction Mixture

No.	*Contents of reaction mixture*	*Volume*
1.	master mix	12.5μl
2.	Upstream primer	2.5μl
3.	Downstream primer	2.5μl
4.	DNA template	5μl
5.	Nuclease free water	2.5 μl
	Total volume	**25 Ul**

2.9.3 Detection of *E.coli Fimh* by PCR

Nucleic acid (DNA) that extracted from bacterial cells, was used as a template in specific PCR for the detection of virulence genes listed in **Table (2- 8)**. A single reaction mixture contained 2.5μl of upstream primer, 2.5μl of Downstream primer, 5μl of extracted DNA, 12.5μl of master mix and 2.5μl of nuclease free water. The resulting PCR products were run in 1.5% agarose gel.

2.9.4. Preparation of Primers:

The upstream and downstream primers are prepared according to the manufacturing company instructions (Bioneer, Korea) and stored at -20ºC.

Table (2-10): Cycling parameters of *Fimh* gene amplification

Cycle No.	Stage	Temp.°C	Time	primer	Product size(bp)	Reference
1	Initial denaturation	95	3 min.	***Fimh* forward**: 5'-ATGAAAC GAGTTAT TACCCT-3'		
35	Denaturation	94	40 sec.			
	Annealing	42	45 sec.		903	**Mood,*et al*., 2021**
	Elongation	72	1 min.	***Fimh* reverse**: 5'-TTATTGAT AAACAAA AGTCAC-3′		
1	Final extension	72	5 min.			
1	Final hold	4	------			

2.9.5. Detection of Amplified Products by Agarose Gel Electrophoresis:

Successful PCR amplification was confirmed by agarose gel electrophoresis (Lodish *et al*., 2004). Agarose gel was prepared by dissolving 1.5 gm of agarose powder in 100ml of TBE buffer (pH:8) then the volume completed to 100 ml deionized water, in boiling water bath, allowed to cool to 50°C and Ethidium bromide at the concentration of 2-3µl/ml was added.

The comb was fixed at one end of the tray for making wells used for loading DNA sample. The agarose was powered gently into the tray, and allowed to solidify at room temperature for 30 min. The comb was then removed gently from the tray.

The tray was fixed in an electrophoresis chamber which was filled with TBE buffer covering the surface of the gel, 4µl of DNA sample was transferred into the signed wells in agarose gel, and in one well we put the 5µl DNA ladder.

The electric current was allowed at 70 volt for 45 min. UV trans illuminator was used for the observation of DNA bands, and gel was photographed using a digital camera.

2.9.6 Programs and websites

Programs and websites used in current study are shown in Table (2-10).

Table (2-11): Programs and websites with purpose of usage

Program or website	Purpose of use
Nucleotides BLAST	Determine sequence similarity and phylogenetic distances
BLAST X	Translate polynucleotides to polypeptide
MEGA 7	Alignment of sample sequence
Raptor X	Build 3D structure form of protein
UCSF Chimera	Make protein picture with some actions
Gel Analyzer	Determine size and concentration of DNA fragments in agarose gel
Python 3.9	To calculate dinucleotide signature from DNA sequence

2.9.7 Bioinformatics

Bioinformatics' service technology has been advancing the field of medical and ecological research and diagnostic in next-generation

sequencing (NGS).it is specialized in life science data, mining setting new strands for high level service of data analysis.

2.9.8 Bioinformatics Programs and Websites

2.9.8.1 Molecular Evolutionary Genetic Analysis (MEGA) Program:

A computer program package called MEGA has been developed for estimating evolutionary distances, reconstructing phylogenetic trees and computing basic statistical quantities from molecular data. It is written in C++ and was intended to be used on IBM and IBM-compatible personal computers. In this program, various methods for estimating evolutionary distances from nucleotide and amino acid sequence data, three different methods of phylogenetic inference (UPGMA, neighbor-joining and maximum parsimony) and two statistical tests of topological differences were included. For the maximum parsimony method, new algorithms of branch-and-bound and heuristic searches were implemented. In addition, MEGA computes statistical quantities such as nucleotide and amino acid frequencies, transition/transversion biases, codon frequencies (codon usage tables), and the number of variable sites in specified segments in nucleotide and amino acid sequences. Advanced on-screen sequence data and phylogenetic-tree editors facilitate publication-quality outputs with a wide range of printers. Integrated and interactive designs, on-line context-sensitive helps, and a text-file editor make MEGA easy to use (Kumar, et. al., 1994).

2.9.8.2 Fundamental Local Alignment

Basic local alignment search tool (BLAST) is a succession similitude

Search program which can be utilized through a web interface or as independent instruments to contrast a client's question with an information base of groupings. A few variations of BLAST look at all mixes of protein or nucleotide questions with protein or nucleotide information bases. BLAST is one of the most generally utilized Bioinformatics research instruments it is an aide that discovers short matches between two arrangements and attempt to begin arrangements from these "problem areas". Moreover, BLAST gives factual data about an arrangement this is normal worth or bogus positive rate (Soutis *et al.*, 2011).

2.9.8.3   Raptor X Methods

Raptor X Methods can be useful to determined features of protein structural from sequence of the amino acid only, without expending any information of the template, Raptor X, defined as a web server which calculated character of protein structure uniquely based on sequence of protein or sequence A sketch is imitative from multiple sequence alignment (MSA) taken from homologs of sequence in a protein family. Moreover, this ideal arrests not only structure -intricate sequence relationship, but also ideals the feature marker correlation in the midst of neighboring residues. However, to treat the extreme distribution of some feature label, such as order-disorder structure, which is a upright measure for data of class-imbalanced or extreme. Therefore, the investigational results expresses that Raptor X server significantly outperforms surviving servers in feature of protein structure expectation. Moreover, Raptor X is a three dimensional structure prediction server can be expected much quicker than three dimensional structure using separating them rapidly

react to those customers who only want structure feature expectation and can be make it viable to prepare genome-scale structure feature expectation through Raptor X web server (Wang *et al*., 2019).

2.9.8.4   University of California, San Francisco (UCSF) Chimera

Chimera is created by the Resource for Biocomputing, Visualization, and Informatics (RBVI) at the University of California, San Francisco. Is an extensible program for intuitive representation and investigation of atomic designs and related information, including thickness maps, supramolecular gatherings, arrangements, docking results, directions, and confessional groups. High resolution pictures and movies can be done.

Chimera includes complete documentation and can be downloaded free of charge for noncommercial use.

User to simplify the presentation of some features or accent others . We pronounce an addition to the UCSF Chimera molecular visualization system for the aim of showing and underlining features of nucleic acid, containing a new illustration of sugar crumple, numerous choices for concept of base geometries which emphasize base pairing, stacking, and development of the ribbon backbone to provide accommodations of backbone of the nucleic acid. Moreover, molecules are manipulated and exhibited interactively, permitting the user to adjustment the illustrations as preferred for small molecules, for example nucleic acids and proteins. Moreover, UCSF Chimera software is available as portion of the system of molecular visualization therefore is united with a set of obtainable tools

for graphics of molecular (Meng *et al*., 2006).

2.9.8.5 Docking of molecules

Docking is a strategy which predicts the favored direction of one particle to a subsequent when bound to one another to frame a steady mind boggling. Information on the favored direction thus might be utilized to foresee the strength of affiliation or restricting partiality between two atoms utilizing, for instance, scoring capacities.

Docking of molecules is a computer method that generates a binding model by predicting the interaction between molecules.

Docking is one of the most much of the time utilized techniques in structure-based medication configuration, because of its capacity to foresee the limiting adaptation of little particle ligands to the suitable objective restricting site. Docking is done between a small molecule and a macromolecule in various drug discovery applications, such as protein-ligand docking.

Docking has lately been used to predict the binding mode between two macromolecules, such as protein-protein docking. https://mcule.com/ provided a tool for 1-click docking.

2.9.8.6 Mcule.com

It's a web-based medication revelation stage. It offers an extraordinary answer for pharma and biotech organizations by giving the best available compound information base and molecular demonstrating instruments.

2.9.8.8. Dinucleotide relative abundance profile (DRAP).

The standard odds ratio index also known as DRAP. Is used to remove the single nucleotide frequency bias (Karlin and Burge 1995; Karlin *et al*.1997, 1998). DRAP was defined as the odds of occurrence of each dinucleotide (FXY, where X and Y are the single nucleotides) in a sequence, divided by the product of the odds of appearance of the corresponding single nucleotides (FX, FY). After DNA sequencing, dinucleotide frequency calculating by "odds ratio": $\rho XY = [XY]/ ([X][Y])$, with X,Y $\in$ {A,C,G,T}, These can easily be done by python Module 3.9.

2.10 *Escherichia coli* cell count

Using our Photopette handheld spectrophotometer, we were able to perform a direct cell count of *E.coli* at OD600. We were able to measure *E.coli* directly in the cell culture flask, meaning the measurement could be performed at any location and with no requirements for a lab.

- OD600 is measured by a spectrophotometer with a light source of 600 nm. It measures light scattering, as opposed to light absorption to determine optical density.

- Because of bacterial cells are colorless, the amount of light they absorb is very low. Using a light source at 600 nm, and measuring scattering is a better way to measure cell concentration.

- OD600 is an arbitrary value that helps determine the cell growth stage, when compared to a standard or control reference. By comparing a measured result to a control, it is possible to estimate the growth stage and

the total concentration of cells.

- The reason for measuring optical density at 600 nm is because this is a known wavelength that minimizes cell damage and growth, and is not destructive in nature (Puay, & Dieter,2019).

2.11 Rats Infections with *Escherichia coli*

All procedures were done according to the guidelines of the Babylon medical college and Karbala Pharmacy College for Animal Experimentation under Institutional Committee for Ethics in Animal Research approval.

A groups of rats would be taken, weighted 150-200 gm, and divided into four groups, 1st, not infected, 2nd,3rd, and 4th, were infected by inoculated with 10^8-10^{10} colony forming units (CFU) of UPEC bacteria directly into the bladder by transurethral catheterization(AL_Okhedi *et al*., 2020; Chockalingam *et al*., 2019) ,by using plastic angio-catheter of 24G, diameter=0.7mm, length =19mm(to prevent rats injury), and small injection to push *E. coli* in rats bladder (Gupta *et al*., 2017).

2.14 *Fimh* Inhibitor Studies

One oral dose (200 mg/kg) of pure Apigenine (that extracted from chamomile plant and purified) was administered to rats by oral gavage in one day prior to transurethral inoculation with *E. coli* (prophylactic therapy), And on day **2** after infection (treatment of chronic infection). This study was done by Taking a groups of animals and divided it's into four groups, the 1st group was taken as a controls (healthy animals without infection), the 2nd group, were animals take drug before infections (prophylaxis),the 3rd groups were taking drug after **2** days of infection

(chronic treatment), 4th groups was infected only without treatment.

The result of *fimh* inhibition was examined by general urine examination, urine culture, WBC count, bacterial cell count in urine, and temperature measurement for tested animals.

Fimh inhibitor study by determination of the bacterial contents in rats' urine, after given chamomile as a *Fimh* inhibitor. To collect urine samples in a volume appropriate for routine urinary testing techniques, rats were usually Single-housed in metabolic cages for 16 to 24 hours (Kurien *et al*., 2004). The collected urine was diluted in 0.85% NaCl, plated on nutrient agar supplemented with 0.1% yeast extract and 0.1% glucose, and incubated at 37 $^{\circ}$C for 20 hrs. The colonies formed and then counted. The extent of bacteriuria was graded as follows: no excretion of bacteria ($<10^3$ CFU), occasional excretion (10^2-10^3 CFU), and excretion of bacteria (10^{10} CFU), (Aronson *et al*., 1979).

3.1 Isolation of *Escherichia coli*

In this study, a total of 100 urine *isolates* were obtained from patients suffering from UTI symptoms , who were attended Al-Husain General Teaching Hospital in Kerbala in a period ranging from (February 2021 to August 2021). The patient's age ranged from 15 years to 78 years. A total of 63 isolates showed positive for *E.coli* culture, and these isolates of *E.coli* were subjected to identification**.**

In our study, of the 100 urine specimens of urinary tract infection processed 89 (89%) specimens showed positive culture and the rest 11 (11.0%) were negative (Table 3-1). Among the isolates, 63 aerobic Gram negative *E. coli* (70.79 %) were identified. Fatemeh *et al.* (2008) have reported that microorganisms isolatesd from urine cultures were *E. coli* in 27 (75%), *Enterococcous* in 3 (8.3%), *Staphylococcus epidermidis* in 3 (8.3%), *Kingella spp.* in 1 (2.8%), and *Candida albicans* in 2 (5.6%) patients. According to Sabahat *et al* .(2007), Three hundred and forty five(345) urinary pathogens belonging to 6 different genera of gram negative bacteria isolatesd from urine specimens, as follow: *Escherichia coli* 270 (78.26%), *Klebsiella pneumoniae* (51), *K. ozaenae* (3), *Proteus mirabilis* (5), *Pseudomonas aeruginosa* (10), *Salmonella typhi* (1), *S. paratyphi* A (2), *S. paratyphi* B (1) and *Serratia marcescens* (2), respectively (Sabahat *et al* .,2007).

According to Bazaid, A. S., *et.al,* (2022), UTIs can be caused by (Gram-positive or Gram-negative) bacteria, viruses or fungi, with more than 80% of UTIs being of bacterial origin. Most UTIs (75%) are caused by *Escherichia coli*.

Gowsami *et al*. (2001); Supriya *et al*. (2004) and Kebira *et al*. (2009), have reported that 64.3, 59.8 and 60% respectivily of isolates are identified as *E. coli*

among other uropathogenic organisms. The result of this study showed *E. coli* as the predominant microorganism among the uropathogens.

In this study, out of the 89 uropathogens, only 63 (70.79%) were found to be *E. coli* and 26 (29.21%) other organisms.

Table (3-1): The overall status of the cultural urine specimens

No.	*Isolates*	*N(%)*
1	Urine specimens processed	100(100.0)
2	positive growth	89 (89.0)
3	negative growth	11 (11.0)
4	*E. coli*	63 (70.79)
5	other organisms	26 (29.21)
6	*E.coli* with *Fimh* gene	61(96.82)

3.2 Antibiotics susceptibility pattern of *Escherichia coli*

All the identified *E. coli* isolates from urine *isolates* were subjected to *in vitro* susceptibility test by modified Kirby - Bauer disc diffusion method . Selective antibiotics were used to show their effect on *E. coli* strains isolates.

It has been observed that all isolates are multi-resistant , the highest rate of resistance is seen with Amikacin 89%, Piperacillin/Tazobactam 85%,Co-Trimoxazole 87% and Ampicillin 72% , and are moderately resistant to

Erythromycin 35% ,tobramycin 59%, Tetracycline 55% and Gentamicin 55%, whereas some isolates have shown highest rates of sensetivity to Norfloxacin 72% ,Chloramphenicol 94%,and Imipenem ,were sensitivity of *E.coli* strain to this antibiotics reach up to 97%. All *E. coli* isolates were subjected to antibiotic sensitivity tests (Fig. 3-1), the susceptibility was found to be 94 and 97% for chloramphenicol and imipenem (Fig 3.1). Tetracycline and gentamicin, constitutes the reasonable option for treatment of UTI as 45 and 46% were sensitive to these antibiotics respectively. Imipenem was found to be the drug of choice for treatment of UTI caused by *E. coli* that all strains were sensitive to it.

The Poovendran & Ramanathan (2014), have reported that 54% of the strains were sensitive to gentamicin followed by tobramycin (50%), co-trimoxazole (44%) and ciprofloxacin (44%), where as in the present study, the uropathogenic *E. coli* were less susceptible to the tested antibiotics. Bhargavi *et al*. (2010) have reported that 82 and 79.6% of *E. coli* were resistant to co-trimoxazole, and ampicillin. Similar results were observed in the present study indicating maximum resistance to these drugs. *E. coli* with integrons are significantly more likely to exhibit multi drug resistance (MDR) to gentamicin, chloramphenicol, ampicillin, tetracycline and nalidixic acid(Sin *et al*., 2020).

In this work, it was found that some of *E. coli* isolates were resistant to more than six antibiotics, which mean that an alternative choice of antibiotic is needed to eradicate *E.coli* associated with urinary tract infection(Zalewska-Piątek & Piątek, 2020).

Despite the widespread availability of antibiotics, UTIs remain the most common bacterial infections in human populations (Longhi *et al*., 2022).

E. coli as the commonest cause of UTI exhibiting high antibiotic resistance among the strains, so, this sure that the need for judicious use of antibiotics. In chronic UTI, a slow growing *E. coli* with atypical colony morphology and multiple drug resistance (MDR) strain was reported by Niranjan & Malini (Niranjan & Malini, 2014).

Gram-negative bacteria express various types of resistance. Intrinsic resistance is a result of co-operation of outer membrane barrier and multidrug efflux pumps. Some Gram-negative bacteria possess drug specific efflux pumps, which mediate resistance to certain classes of antibacterial. More recent antibacterial such as fluoroquinolones and broad-spectrum β lactams are likely to select for over production mutants of these pumps and make bacteria resistant to practically all classes of antibacterial agents (Huwaitat *et al*., 2016).

Penicillins, such as ampicillin and amoxicillin, were used previously as front-line therapies for UTIs. Resistance to these agents is mediated by β -lactamases which degrade them, and these enzymes play an important role in antibiotic-refractory UTIs. The production of β-lactamases is the main mechanism of resistance to β-lactam antibiotics in Enterobacteriaceae. The most prevalent enzymes (TEM-1 and SHV-1) are able to inactivate penicillins and narrow-spectrum cephalosporins, but they are susceptible to β-lactamase inhibitors. Two strategies have been developed to thwart the activity of these enzymes: the use of stable β-lactam antibiotics such as oxyimino cephalosporins, and the combination of penicillins and b-lactamase inhibitor.However, oxyimino cephalosporin-hydrolysing TEM and SHV mutants, designated extended spectrum β-lactamases (ESBLs), Likewise, the intensive use of penicillin/β-lactamase inhibitor combinations has been followed by the emergence of inhibitor-resistant TEMs (IRTs) harbouring point mutations conferring resistance to β-lactam-based

inhibitors. In addition, TEM and SHV enzymes combining IRTand ESBL-type substitutions have been discovered since the mid-1990s. These inhibitor-resistant enzymes have been mainly observed in Escherichia coli, which is one of the major bacteria isolatesd in clinical microbiology (Girlich *et al*., 2020).

Despite lower activity of the inhibitors, most IRT- and CMT-producing strains are susceptible to the piperacillin/tazobactam combination in vitro, Di Conza, *et al* (Di Conza *et al*., 2014) have suggested a possible loss of bactericidal activity.

The TEM, SHV and OXA classes of B-lactamases hydrolyze penicillin B-lactam antibiotics (e.g. amoxicillin) and are widely distributed among UPEC (Tooke *et al*., 2019), resulting in 38-48% of these isolates being ampicillin-resistant (Hilbert, 2011). The genes encoding these B-lactamases are usually found on plasmids that are horizontally transferred between bacteria. Penicillin B-lactam resistance can be overcome by combining the penicillin B-lactam with a B-lactamase inhibitor, such as ampicillin-sulbactam, amoxicillin-clavulanic acid or piperacillin-tazobactam. However, inhibitor-resistant TEM B-lactamases have evolved, leading to emerging UPEC resistance(Douafer *et al*., 2019).

Trimethoprim and sulfamethoxazole (co-trimoxazole) inhibit dihydrate folate reductase, and dihydropteroate synthetase, respectively, and resistance to co-trimoxazole can be mediated by horizontal transfer of genes encoding resistant versions of these enzymes. A study of 305 co-trimoxazole -resistant UPEC isolates found that 66% of them encoded a (*dfr*) allele encoding a trimethoprim-resistant dihydrate folate reductase, and 96% of them had a (*sul*) gene encoding a sulfamethoxazole-resistant dihydropteroate synthetase. The presence of these genes on integrons and plasmids facilitates their spread among bacterial populations (Kamng'ona, 2014).

Fluoroquinolones, such as ciprofloxacin and norfloxacin, target bacterial DNA gyrase and topoisomerases, enzymes responsible for DNA unwinding during DNA replication. They are currently recommended for use as second-line agents for uncomplicated UTIs, and front-line therapy for nosocomial UTIs and pyelonephritis (Bush *et al*., 2020). Resistance to these agents is largely due to mutations in the gyrA gene encoding the gyrase enzyme(Jaktaji & Mohiti, 2010).

Indeed, 25% of UPEC from catheter-associated UTIs are fluoroquinolone-resistant (Hidron *et al*., 2008). A comparison of 2073 nosocomial UPEC isolates from 1990-1994 to 3112 isolates from 2000-2004 found that resistance to ciprofloxacin increased from 0.9% to 9.8% (Jaktaji & Mohiti, 2010).

The resistance to amikacin, as well as to tobramycin, observed in the isolates analysed in the present study may be related to the joint presence of *aacA4* and *aacA7*, which both encode aminoglycoside-modifying enzymes capable of inactivating amikacin. Interestingly, previous use of tobramycin to treat the patient may have been important in selecting resistance from the patient's endogenous flora, although it is clearly not possible to exclude an exogenous source for this amikacin-resistant *E. coli* strain (Ruiz *et al*., 2005).

In addition, Alshara (2011) showed that over all antibacterial tested in his study, amikacin was the most active agent against *E. coli* with susceptibility rate of 100%. Relatively higher resistant rates (30 and 31%) of *E. coli* to amikacin were reported in literature (Haghi-Ashteiani *et al*., 2007). There are many possible reasons for increasing resistant rate of *E. coli* to common used antimicrobial drugs, including inappropriate and incorrect administration of antimicrobial agents in empiric therapies and lack of appropriate infection control strategies (Shepherd & Pottinger

.,2013).This problem indicates importance of performing antibiotic susceptibility testing before blind antibiotic therapy.

Chloramphenicol treats infections by binding to the 50S subunit of a bacterial ribosome, thus blocking the tRNA-accepting site of the mRNA complex and effectively stopping transpeptidation (Svetlov *et al*., 2019).

Ultimately, chloramphenicol blocks bacterial growth by disabling the formation of peptide bonds between amino acids. Chloramphenicol has this effect on bacteria when present in concentrations between 1 and 10 μg/ml. However, in strains of resistant bacteria it may take up to 1000ug/ml for chloramphenicol to have an effect (Mapatac, 2015).

Another effect of chloramphenicol could be cell lysis, by inhibiting protein synthesis in *E.coli* cells, chloramphenicol effectively stop the formation of new lysosomes. Thus treated *E.coli* cells are no longer able to digest waste products which instead accumulate in the cells. As a result, osmosis causes water molecules to flow enter the cell eventually causing the cell to become hypotonic, cell membranes then burst (Park & Uehara,. 2008).
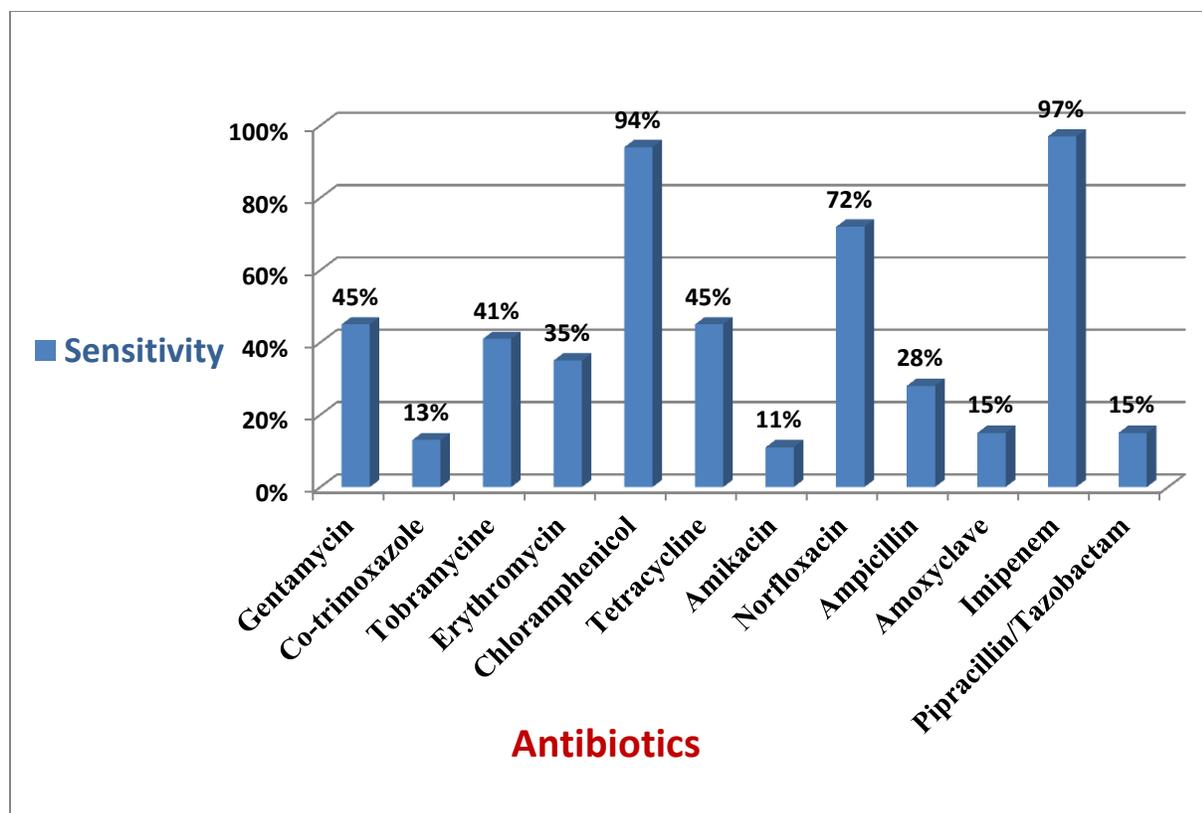
Figure (3-1): Effect of Antibiotics on *E.coli* isolates

3.3 molecular detection of *Fimh* Gene in Uropathogenic *Escherichia coli* Strains Isolates

(Zohreh Hojati *et.al*,2015) reported that UPEC isolates were identified using biochemical tests and were screened by PCR. The *Fimh* gene was amplified using specific primers and showed a band about 164 bp. The *Fimh* gene was found in 130 isolates (92.8%) of the UPEC strains. Of 130 isolates positive for the *Fimh* gene, 62 (47.7%) and 68 (52.3%) belonged to hospitalized patients and outpatients, respectively.

The Tabasi *et al*. (2015) reported that Type 1, or mannose-sensitive, fimbriae are produced by >80% of all Uropathogenic *E. coli*. It is now well established that the

expression of type 1 fimbriae by *E. coli* is a virulence factor for pathogenesis of the urinary tract.

The Meysam Sarshar *et.al*,(2020), have been reported that more than 95% of all *E. coli* isolates express type 1 fimbriae . The type 1 pilus is 2 µm in length and 10 nm in width, and is highly represented in the bacterial surface (100–500 pili per cell) . This pilus is defined as mannose-sensitive, because it is able to interact with the mannosylated receptors expressed by epithelial cells, particularly urothelial cells .This specific function relies on the expression of the adhesion *Fimh* located at the tip of the type 1 pilus (Flament-Simon, *et. al*.,2019)

The Kim, *et al*, 2018, when observing the presence of the adhesin *Fimh* in the total isolates of *Escherichia coli* in urine cultures of *isolates*, also found a relationship between this adhesin and the phylogroups B2 and D. Likewise, Tabasi, *et al*. 2016, found, after studying isolates of adult patients with UTI, that 100% of *Escherichia coli* isolates carried the *Fimh* gene from isolates of patients with UTI in the adult population. Similarly, Rahdar, *et al*., 2015, detected the *Fimh* gene in 95% of UPEC isolates and found no relationship between the presence of the *Fimh* gene and *Escherichia coli* phylogroups. Terlizzi, *et al*(2017), described the mechanism of action of adhesin *Fimh*, which acts and interacts with the uroethelium cells, allowing the entry of UPEC and the formation of intracellular bacterial colonies (CBI) after the first six hours of infection(Terlizzi *et al*., 2017) CBI they are responsible for recurrence, chronicity, and the formation of bacterial reservoirs in the urothelium (Vestby *et al*., 2020) .

The establishment of infection by bacterial pathogens requires adhesion to host cells, colonization of tissues, and in certain cases, cell invasion or persistence. Specific adhesion is mediated by the adhesin *Fimh*, which is found at the distal end

of type 1 pilis and mediates adherence of the bacteria to a number of host glycoproteins and non-glycosylated peptide epitopes, including secretory IgA, glycophosphatidylinositol-bound proteins CD48(Díaz,*et.al*.2016).

Table (3-2):*Fimh* gene distribution among screened *isolates* by PCR

No.	*Isolates*	N (%)
1	*E. coli*	**63 (100.00)**
2	*E.coli* with +ve *Fimh* gene	**61(96.82)**

In this study, table (3-2) and figure (3.2) showed that, The UPEC *isolates* were recognized utilizing biochemical tests and were screened by PCR. The *Fimh* gene was amplified using specific primers and showed a band about 903bp. The *Fimh* quality was found in 63 *isolates* (96.82%) of the UPEC strains, which means that *Fimh*, considered the most important virulence factors in UPEC bacteria to cause UTI(Dadi, *et.al*.,2020).
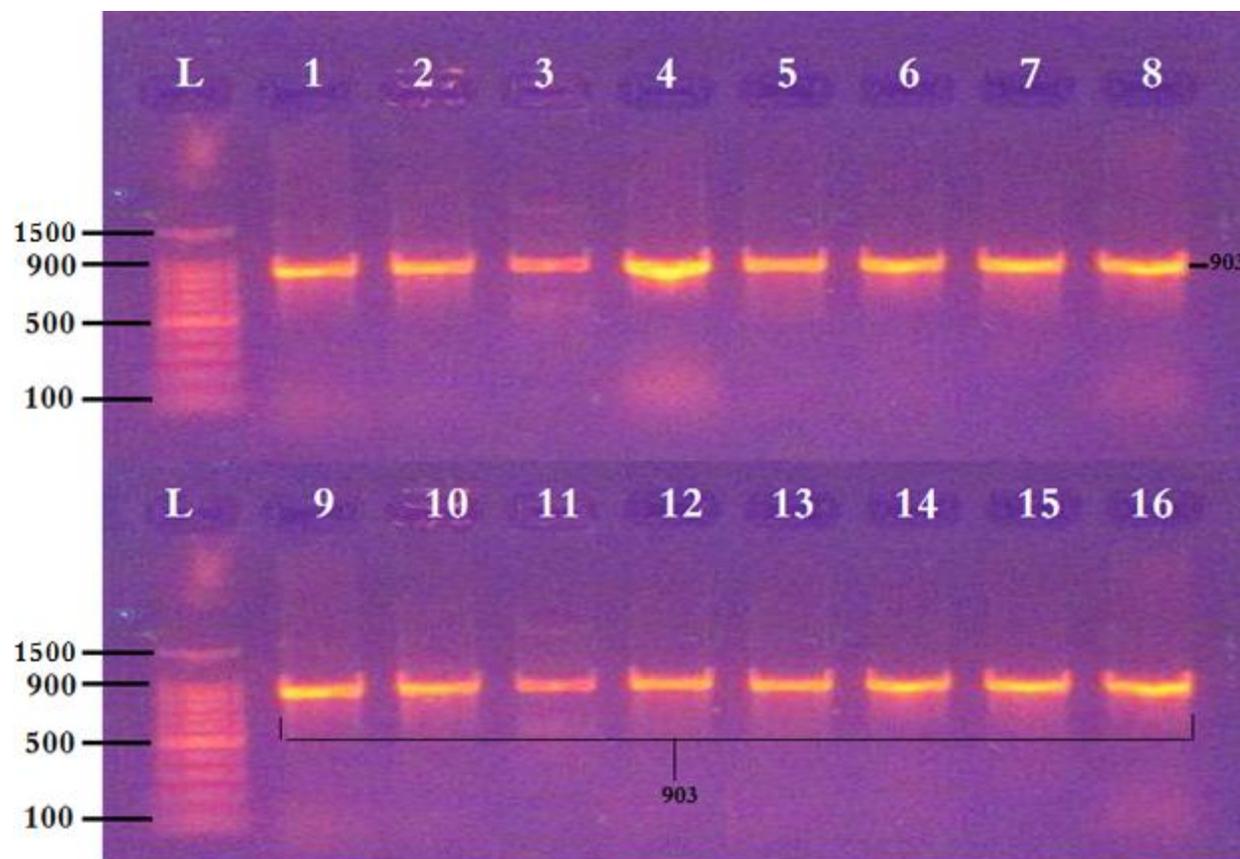
Figure (3.2): 1% Agarose gel electrophoresis at 70 volt for 50 min for *Fimh* PCR products visualized under U.V light at 301 nm after staining with ethidium bromide. L: 1500 bp ladder;lane(1-16) were positive for this gene, the size of product is 903 bp.

3.4 Dinucleotide signature study

3.4.1 Sequence for DNA of *Fimh* Gene

In current study, DNA sequencing tested on 43 clinical *isolates*. The Sequencing reactions were performed on the *Fimh* PCR products.

DNA sequencing provides the means to know how nucleotide bases are arranged in a piece of DNA.

According to this, we will enter the data of this sequence (the sequence of nucleotides) in certain programs (python) in order to obtain numerical data that

had a great importance in research and also for the purpose of comparison with other studies.

Bioinformatics had many benefit by using Data from the Human or microorganism Genome in the development of gene therapy, which is a type of treatment designed to replace defective genes in certain genetic disorders. In addition, it has provided a means to design drugs that can target specific genes that cause disease. (Gonçalves,*et.al*,2017).

3.4.2 Dinucleotide signature and CG% calculations for *Fimh* gene

Advances in sequencing technologies have led to an exponential growth in gene and genome sequences deposited in public databases. Statistical analysis of the nucleotide sequences has been carried out to study the nature of information stored at the molecular level. Several local and global patterns have become apparent by studying the genome sequences, some of which have functional significance (Giani *et al*., 2020).

A genomic pattern that has been extensively studied is the composition of certain 'words', or contiguous arrangement of nucleotides on the DNA sequence of specific length (dinucleotide, trinucleotide, etc.). From the large isochore regions, which are correlated with the number of genes in the region, to repeats found in primate genomes, to simple dinucleotide motifs each has been shown to be a pattern that differs from one organism to another (Fimmel & Strüngmann, 2018).

In table (3-3 & 3-4) shown that the profile generated by taking all dinucleotides together led to unique species-specific patterns .

In table (3-4) and figure (3-5), Dinucleotide compositions have been shown to be consistent throughout a genome as opposed to G+C content(GC%) .

Genomic sequences display heterogeneity on many scales and many authors have emphasized variation in G + C content (e.g. isochore compartments). DNA

primary structure is not physically or thermodynamically homogeneous, nor is it random. Genomic dinucleotide structure is maintained relatively homogeneous and constant in time apparently by genome wide processes of replication, repair, and segregation (Cai *et al*., 2020).

Certain dinucleotide patterns were also found to be common in large group of organisms, like the dinucleotide TA is underrepresented in most organisms, and CG is known to be underrepresented in vertebrates (Herrera *et al*., 2015). This led to the concept of 'genome signature', where the signatures or dinucleotide profiles of sequence *isolates* taken from the same genome were found to be similar compared to the *isolates* taken from other organisms (Krawczyk *et al*., 2018). Moreover, taxonomically close species were seen to have similar dinucleotide profiles compared to distant species. Genomic signatures have since then been used to study relationships between several organisms (Cai *et al*., 2020).

It has been further proposed that the DNA repair machinery plays an important role in maintenance of species-specific dinucleotide bias (Belfield *et al*., 2018).

Phylogenetic analysis was also performed using the genome signatures (Randhawa *et al*., 2020).

In our study, the results showed the dinucleotide usage profiles or genome signatures were similar for sequence *isolates* taken from the similar genome of similar species but were different for taxonomically distant species. Table (3-1) showed the frequency of GC and CG is the largest one among other dinucleotide signatures, and the frequency of AG and TC is the smallest one among another dinucleotide respectively(Dlamini *et al*., 2020). The range of DS value was (**0.06-0.14**), for most *Fimh isolates* of *E.coli*, and when comparing it with the frequency of DS of *Fimh* for salmonella typhimurium for example, so we noted a wide difference between them, can easily be discriminated between them like a

fingerprint. Our results show the uniformity or similarity of signature throughout the genome, these mean that all *isolates* have genetic homogeneity and had unique species-specific patterns.

One important feature of the GC base pair is its higher thermal stability compared with the AT base pair, a feature that arises from the stronger stacking interaction between GC bases and the presence of a triple compared with a double hydrogen bond between the paired bases (Oliveira *et al.*, 2020). In turn, these interactions seem to be important in conferring stability to higher order structures of DNA and RNA transcripts (Skeparnias & Zhang, 2021). In bacteria, for example, an increase in GC content correlates with a higher temperature optimum and a broader tolerance range for a species.

in our study, the range of G+C% (figure 3-5 , table 3-4) of all *isolates* is nearly similar (**7.89%** in A13 and **7.23%** in a8), which give a high relationship and specificity among *isolates*.
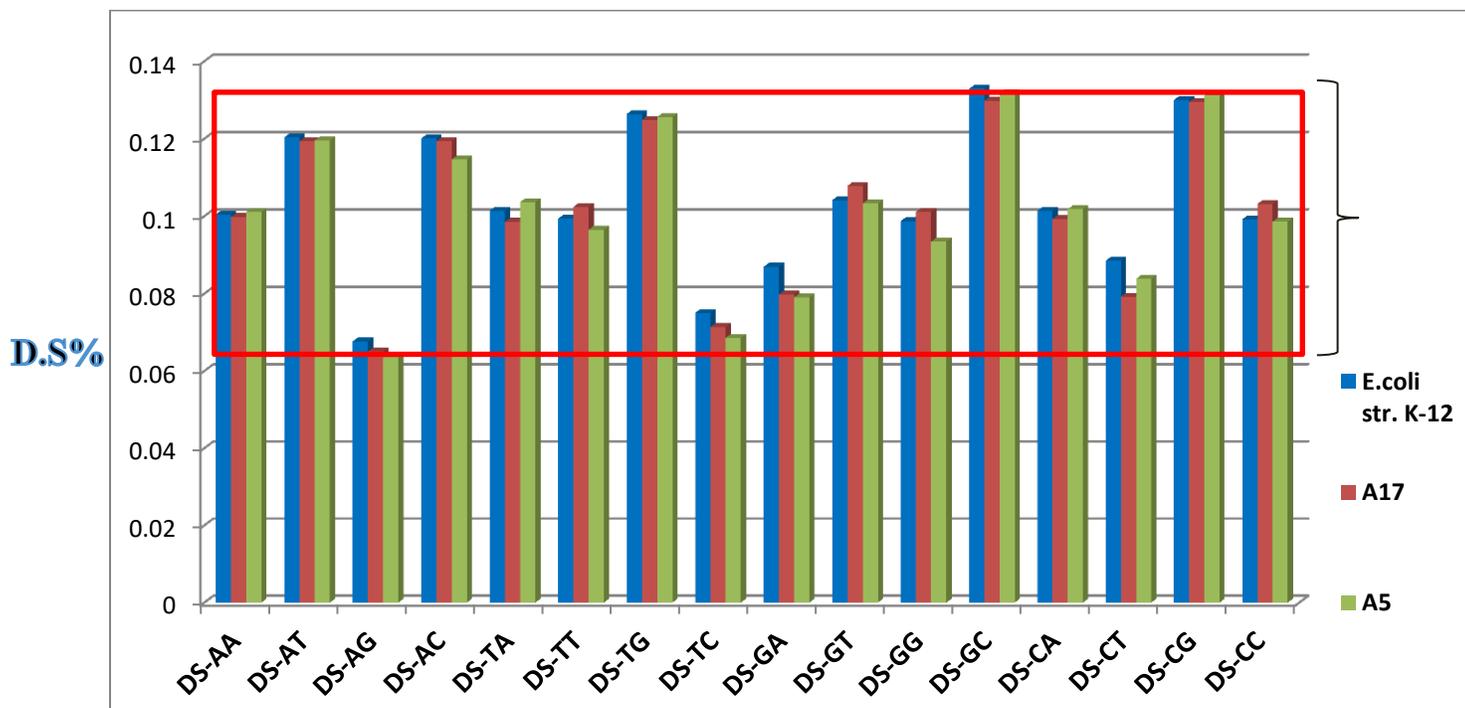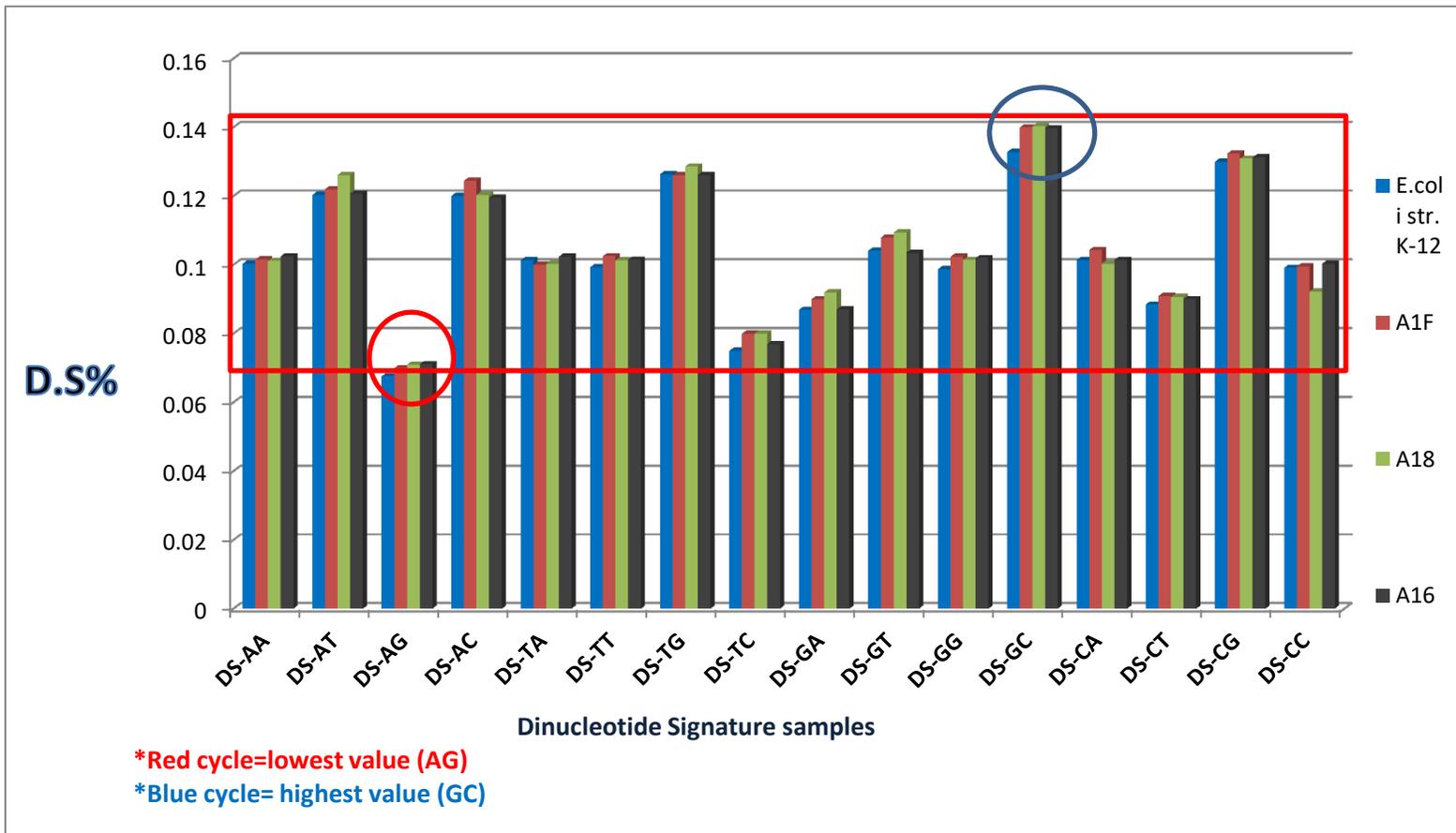

Figure (3-3) Frequency of all dinucleotides of *Fimh* gene (blue stander species, Red and green, clinical *isolates*)

Table (3-3): dinucleotide signature for some *E.coli isolates* and wildtype

species (*E.coli* str. K-12 substrate MG1655)

D.S	*E.coli* str. K-12 substr. MG1655	A17	A5	x3-xf	X11-XF	X5-XF	A13
DS-AA	0.10039853	0.099782943	0.101111111	0.10238727	0.1025	0.099117133	0.100752687
DS-AT	0.120348719	0.119358712	0.119537527	0.121998286	0.13215859	0.124860647	0.119875261
DS-AG	0.067679612	0.065048326	0.06362059	0.064158225	0.066666667	0.064913989	0.070240844
DS-AC	0.120068508	0.119358168	0.114638448	0.120243493	0.123786408	0.126960418	0.109903922
DS-TA	0.101411746	0.098550282	0.103658632	0.105098457	0.103348018	0.102740088	0.10034268
DS-TT	0.09938108	0.102411559	0.096530001	0.095935469	0.095092084	0.098298677	0.100331763
DS-TG	0.12636612	0.124855481	0.125624098	0.127236127	0.129221733	0.122913227	0.114470672
DS-TC	0.075040406	0.071396119	0.068587106	0.072567642	0.081262564	0.080202617	0.08195351
DS-GA	0.086951456	0.079784672	0.079043763	0.084037566	0.084444444	0.084388186	0.090122172
DS-GT	0.104166667	0.107824726	0.103297179	0.106346614	0.109642682	0.108237021	0.098681614
DS-GG	0.09875	0.101081706	0.093428838	0.095783676	0.100740741	0.097918781	0.100699537
DS-GC	0.132888889	0.12984098	0.131701454	0.130906565	0.137102481	0.139077985	0.1380683438
DS-CA	0.101394229	0.099278056	0.101820106	0.110223202	0.110004854	0.110513318	0.099497738
DS-CT	0.088509197	0.0792326	0.083828685	0.085015324	0.094093495	0.090755593	0.092980009
DS-CG	0.130032707	0.129501636	0.131204079	0.131065651	0.141709817	0.143377985	0.139940671
DS-CC	0.099186669	0.103171017	0.098593964	0.105308944	0.10604204	0.10604204	0.105061728
No. of nucleotides	903	987	916	859	858	868	852

Isolates D.S	A15	A19	A4F	A11	A1F	A18	A16
DS-AA	0.110331	0.110813	0.114196	0.112367	0.101597	0.101225	0.102506
DS-AT	0.128486	0.125117	0.12624	0.128413	0.122027	0.126151	0.120747
DS-AG	0.070461	0.069612	0.070243	0.067696	0.070018	0.071007	0.071108
DS-AC	0.120762	0.130175	0.130898	0.131209	0.124496	0.120401	0.119551
DS-TA	0.099898	0.105117	0.120218	0.112744	0.100049	0.100328	0.102402
DS-TT	0.108185	0.102324	0.110955	0.100068	0.102567	0.101377	0.101549
DS-TG	0.121857	0.121198	0.127324	0.130281	0.126078	0.128627	0.126145
DS-TC	0.080791	0.072589	0.080174	0.072768	0.080008	0.080077	0.077004
DS-GA	0.080774	0.091378	0.100657	0.101028	0.090015	0.092003	0.087024
DS-GT	0.108671	0.102104	0.113508	0.110214	0.107984	0.109426	0.10352
DS-GG	0.110056	0.100384	0.101732	0.102591	0.102399	0.101489	0.102031
DS-GC	0.138902	0.131016	0.135769	0.130901	0.140028	0.140368	0.139773
DS-CA	0.101076	0.11201	0.11039	0.114363	0.104346	0.100379	0.101463
DS-CT	0.09071	0.095551	0.094713	0.095058	0.090957	0.090671	0.09005
DS-CG	0.136225	0.141175	0.141474	0.136873	0.132464	0.130947	0.131322
DS-CC	0.107769	0.104472	0.104315	0.100346	0.099595	0.09224	0.100347
Length of DNA strand	715	532	641	537	608	565	697

Figure (3-4) Frequency of all dinucleotides of *Fimh* gene. (Blue) wildtype species,

(*E.coli* str. K-12 substrate MG1655), (Red, green and black), clinical *isolates*
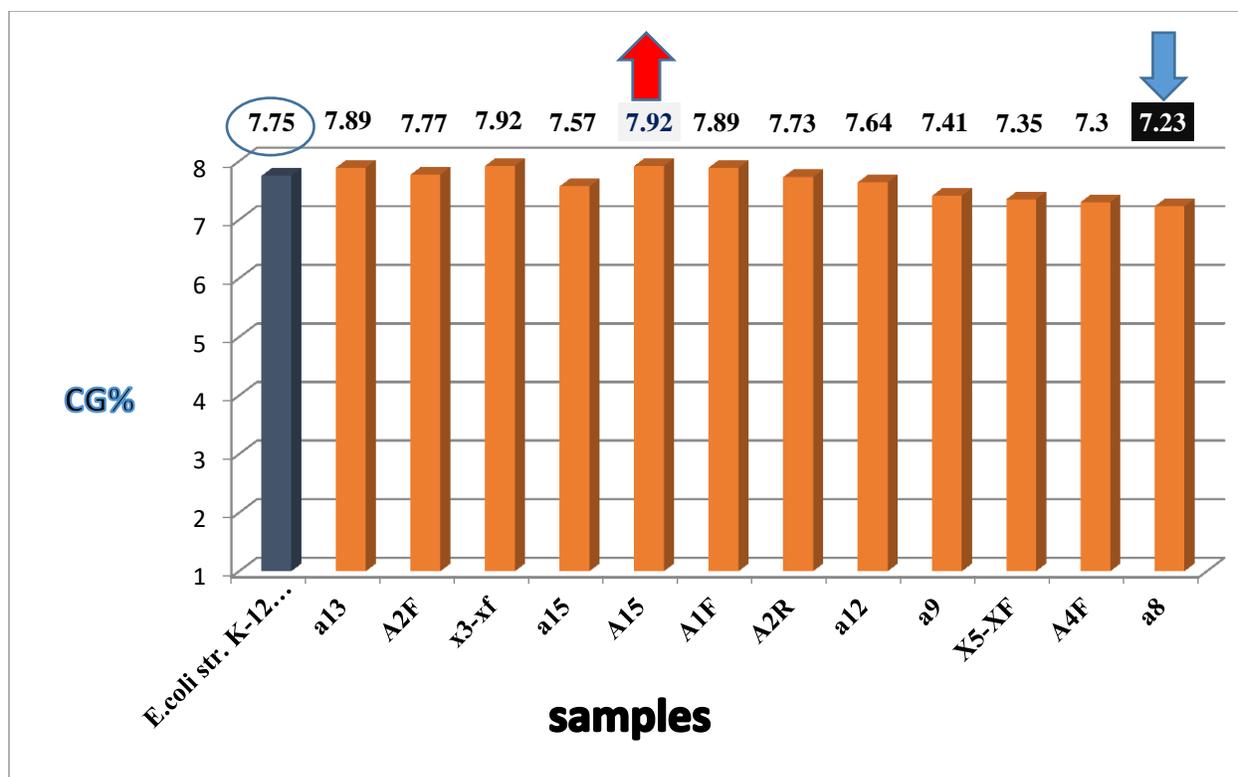
Figure (3-5): CG% for different clinical sample of *Fimh* gene for *E.coli*.

Table (3-4). CG% of *Fimh* gene in some clinical *isolates*

Isolates	CG%
E.coli str. K-12 substr. MG1655	7.75
a13	7.89
A2F	7.77
x3-xf	7.92
a15	7.57
A15	7.92
A1F	7.89
A2R	7.73
a12	7.64
a9	7.41
X5-XF	7.35
A4F	7.30
A8	7.23

3.5 *Fimh* Gene Distribution in clinical Isolates

Detection of *Fimh* gene by PCR applied on 63 isolates taken from different clinical *isolates* to investigation the presence of *Fimh* genes in *E. coli* isolates. In current study, the presence (*Fimh*) in 61(96.82%) from total clinical isolates as showed in table (3-2) & Figure (3-2). These results seem to be similar with study emphasized by Zhi., & *et.al*,2020.

The results of this study indicated that more than 95% of *E. coli* isolates harbored the *Fimh* gene. The high binding ability of *Fimh* could result in the increased pathogenicity of *E. coli*; thus, *Fimh* could be used as a possible diagnostic marker and/or vaccine candidate. Thus, *Fimh* could be used to design inhibitor for prevention of *E. coli* infections by blocking the bacterial attachment and colonization. In addition, *Fimh* could be used as a tool for the extension of rapid detection-based assays.

3.6 Sequences of *Fimh* Gene and its Phylogenic Tree

In current study, figure (3-6), DNA sequencing tested on 43clinical isolates, which covered all positive *Fimh isolates* and 32 international *isolates*. In conclusion, *Fimh* analysis together with PCR phylogrouping seems to be a simple strain typing tool for epidemiological studies of *E. coli* isolates. The sequence analysis involves a single small DNA fragment (*Fimh*) of clinical and international *isolates*. The phylogrouping analysis involves comparison of only up to three bands of known molecular weight. Although this typing tool may not replace Multilocus sequence typing (MLST), this method is suitable for screening large collections of *E. coli* isolates, allowing for the rapid identification.

The branching diagram or a tree showing the evolutionary relationships among

various local and international species or other entities based upon similarities and differences in their physical or genetic characteristics.
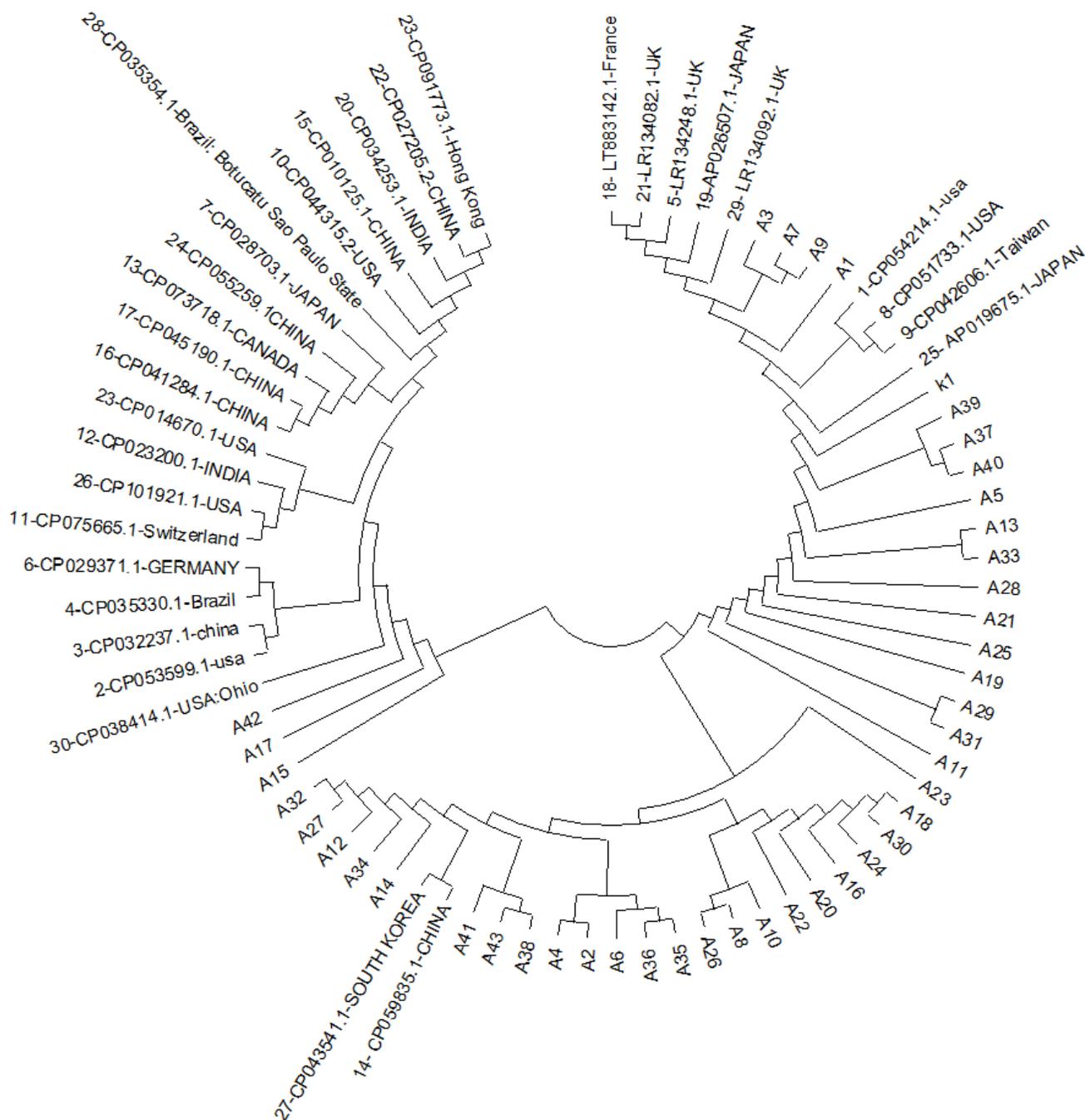
Figure (3-6): Molecular Phylogenetic tree analysis of *Fimh* gene in *Escherichia coli* isolatess (local and international *isolates*)

3.7 Sequence Analysis of *Fimh* Gene of Clinical Isolates

The DNA sequence of *Fimh* gene of *E. coli* clinical isolates were aligned with the reference database records (GenBank) using nucleotides BLAST nucleotide software. As well, one clinical isolates showed an incomplete match (99%) as shown in figure (3-7). Indicated replacement in one nitrogen base in this study (Query) comparing with global isolates (Sbjct) guanine by adenine and cytocin by adenine. Consequently, resulting in missense mutation which is carrying change in DNA sequencing that leading to altering in amino acid at transcription level as showed in Figure (3-7). However, replaced thymine was in forward strand, therefore Adenine base appeared in reverse strand as shown in Figure (3-7).

Figure (3-7): Alignment of *Fimh* gene nucleotide of clinical *Escherichia coli*, sequences by nucleotide BLAST which it showed incomplete match (99%) between sequence of both clinical (UTI) *Escherichia coli* isolates and global isolates, indicated replacement in one nitrogen base (guanine by adenine) in this study (Query) comparing with global isolates (Sbjct).

Figure (3-8): Polypeptide sequence alignment of clinical *Escherichia coli.* Shows incomplete match (99%) with global isolates which indicated change in amino acid sequence Ieucine into isoleucine (Missense mutation) when it translate by BLASTx

If such variations are located at mannose binding pocket, they may be abrogated adhesion to urinary tract epithelial cells (Feenstra *et al*., 2017).

3.8 *Fimh* Multiple Sequence Alignment

All *isolates* were aligned through using MEGA-X software. So, the *isolates* were included locally clinical and international isolates which they were aligned with their corresponding international sequences belong to different isolates tacking from international and clinically isolates involving different host infections as showed in figure (3-10). In addition, some isolates of current study were complete similar of international isolates, whereas some isolates were differed with some international isolates in one or more of nitrogen base. The sequence analysis demonstrated that *Fimh* is highly conserved among avian pathogenic *Escherichia coli* (APEC) isolates (Jørgensen *et al*., 2019). In this study, all *E. coli* isolates showed highly sequence similarity except two nucleotide sub-situation

without effecting on protein active site.

Allelic variants exhibiting homology and encoding proteins differing by as little as a single amino acid substitution confer distinct adhesive phenotypes. This unexpected adhesive diversity within the *Fimh* family broadens the scope of potential receptors for enterobacterial adhesion and may lead to a fundamental change in our understanding of the roles that type 1fimbriae may play in enterobacterial ecology or pathogenesis (Bessaiah *et al*., 2021; Kuźmińska-Bajor *et al*., 2015).

Multiple sequence alignment (MSA) is an important step in various types of comparative studies of biological sequences. MSA is used in phylogenetic inference, conserved region detection, structure prediction of noncoding RNAs and proteins and many other situations. For an easy MSA problem, such as an alignment consisting of a small number sequences with global and high similarity, most of the current programs return a correct MSA, and no special consideration is needed (Bawono *et al*., 2017).

Figure (3-9): *Fimh* gene sequence alignment involving both local and international corresponding sequencing

3.9 *Fimh* Protein Sequence Alignment

All the local DNA sequences including clinical isolates (UTI patients) and international isolates were translated into their corresponding amino acids sequences using BLASTx online software. Then, these sequences were aligned together and with other matching international sequences to identify the differences by using Clustal W option in MEGA-X software. Also, one of clinical sample had one change in its amino acid sequence which contain isoleucine (Il e) instead of Leucine (Leu) as showed in figure (3-11). One international isolates (N1in fig. 3-10), had one single mutation too, but with same amino acids because its mutation was silent type which doesn't change this amino acid.

Moreover, Klein & Hultgren, (Klein, *et.al*,2020), investigated that *Fimh* obtains from UTI *isolates* possess adaptation variants that enhance biofilm formation and suggests a novel role for *Fimh* gene in UTIs infections.

Figure (3-10): Alignment of protein sequence involving both local and international relative sequencing. All the local amino acids sequences belong of *isolates* for UTI patients and international isolate N1 which they were alimented with protein sequence of *Fimh* for international isolates. Sample A1 of current study showed isoleucine instead of Lucien as indicated by red box.

3.10 Choose suitable *Fimh* blocker by docking approach

The ability of *E.coli* to bind to uroepithelial cells relies mostly on type 1 fimbriae, which are characterized by the presence of an adhesin, *Fimh*, located at the tip of these fimbriae. *Fimh* binds mainly to terminal epitopes of high mannosylated glycans conjugated to uroplakin 1a (UP1a), a receptor specifically expressed on the surface of urothelial cells, known as the catch–bond binding mechanism(Kisiela *et al*., 2021).

The natural sugar d-mannose is considered as an alternative to antibiotics due to its ability to mask the bacterial adhesin *Fimh*, thereby preventing its binding to urothelial cells. Despite its extensive use, the possibility that d-mannose exerts "antibiotic-like" activity by altering bacterial growth and metabolism or selecting *Fimh* variants has not been investigated yet(Scribano *et al*., 2020).

Computational chemistry is a popular tool to predict physicochemical, spectral, and biological properties of newly synthesized chemicals (Bulbul *et al*., 2021). The prime aim of this study attempted to synthesize new biologically potent *Fimh* inhibitor derivatives and investigate the binding affinities and interactions of this inhibitor against the *E. coli* using computational tools to point out a potent inhibitor against bacterial function.

Molecular docking has an important role in drug discovery, assisting in digging out the active or lead compounds from a library of natural compounds (Banik *et al*., 2021). It is one of the most widely used virtual screening tools, particularly when the three-dimensional structure of the target protein is available. Docking enables the prediction of both ligand–target binding affinity and the structure of the protein–ligand complex, which are useful for optimizing the lead (Paggi *et al*., 2021). Prior to the molecular docking study, we checked the physicochemical properties and toxicity potential of *Fimh* antagonists.

In systemic molecular genetics and computer-assisted drug design, molecular docking is an important technique (Gomes et al., 2017). The aim of ligand_protein docking is to expect a ligand's interactions mode with a protein molecules having a defined tri-dimensional shape. Good docking algorithm efficiently explores high dimensional domains and employs a scoring system that scores proposed dockings precisely(Francoeur et al., 2020).

Docking may be used to do virtual scanning in the large libraries of drugs, rank the findings, and supply structural Hypotheses for how the ligand obstruct the targets, all of which is very beneficial in optimization of leads(Qiu et al., 2020). Molecular docking between Fimh and Apigenin give high degree of binding (docking score -9.4), which mean low energy needed for molecule binding (ligand and target) to form stable complex (Fatriansyah et al., 2022).

Table (3-6): docking score for some chemical compound with *Fimh* protein

Scientific name	IUPAC NAME	InChIKey	Docking score
Apigenin	5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one	KZNIFHPLKGYRTM-UHFFFAOYSA-N	-9.4
procyanidins	2-(3,4-dihydroxyphenyl)-2-[[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dihydro-2H-chromen-3-yl]oxy]-3,4-dihydrochromene-3,4,5,7-tetrol	HGVVOUNEGQIPMS-UHFFFAOYSA-N	-8.7
cardomin	(E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-phenylprop-2-en-1-one	NYSZJNUIVUBQMM-BQYQJAHWSA-N	-8.6
Desloratadine	13-chloro-2-piperidin-4-ylidene-4-azatricyclo[9.4.0.03,8]pentadeca-1(11),3(8),4,6,12,14-hexaene	JAUOIFJMECXRGI-UHFFFAOYSA-N	-8.6
Aloe	1,8-dihydroxy-3-(hydroxymethyl)anthracene-9,10-dione	YDQWDHRMZQUTBA-UHFFFAOYSA-N	-8.4
CYPROHEPTADINE	1-methyl-4-(2-tricyclo[9.4.0.03,8]pentadeca-1(15),3,5,7,9,11,13-heptaenylidene)piperidine	JJCFRYNCJDLXIK-UHFFFAOYSA-N	-8.4
green tea extract	[(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl] 3,4,5-trihydroxybenzoate	WMBWREPUVVBILR-WIYYLYMNSA-N	-8.4
beta-carotene	1,3,3-trimethyl-2-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohexen-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohexene	OENHQHLEOONYIE-JLTXGRSLSA-N	-8.3
etoricoxib	5-chloro-2-(6-methylpyridin-3-yl)-3-(4-methylsulfonylphenyl)pyridine	MNJVRJDLRVPLFE-UHFFFAOYSA-N	-8.3
Montelukast	2-[1-[[(1R)-1-[3-[(E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfanylmethyl]cyclopropyl]acetic acid	UCHDWCPVSPXUMX-TZIWLTJVSA-N	-8.2
motilium	6-chloro-3-[1-[3-(2-oxo-3H-benzimidazol-1-yl)propyl]piperidin-4-yl]-1H-benzimidazol-2-one	FGXWKSZFVQUSTL-UHFFFAOYSA-N	-8.2
vit A	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraen-1-ol	FPIPGXGPPPQFEQ-OVSJKPMPSA-N	-8

peanut oil	(2S)-6-amino-2-[[(2S)-2-[[(2R)-2-[[(2S)-2-amino-3-(4-hydroxyphenyl)propanoyl]amino]-5-(diaminomethylideneamino)pentanoyl]amino]-3-phenylpropanoyl]amino]hexanamide	UEVAHGMTRWGMTB-JBXUNAHCSA-N	-8
milk thistle	(2R,3R)-3,5,7-trihydroxy-2-[(2S,3S)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]-2,3-dihydrochromen-4-one	SEBFKMXJBCUCAI-WAABAYLZSA-N	-7.9
Hyperoside	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	OVSQVDMCBVZWGM-DTGCRPNFSA-N	-7.9
melatonin	*N*-[2-(5-methoxy-1*H*-indol-3-yl)ethyl]acetamide	DRLFMBDRBRZALE-UHFFFAOYSA-N	-7.8
phylloquinon	2-methyl-3-[(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione	MBWXNTAXLNYFJB-NKFFZRIASA-N	-7.8
D-mannose	**(3S,4S,5S,6R)-6-(hydroxymethyl)oxane-2,3,4,5-tetrol**	**WQZGKKKJIJFFOK-QTVWNMPRSA-N**	**-6.7**
Biotine	5-[(3*aS*,4*S*,6*aR*)-2-oxo-1,3,3*a*,4,6,6*a*-hexahydrothieno[3,4-d]imidazol-4-yl]pentanoic acid	YBJHBAHKTGYVGT-ZKWXMUAHSA-N	-5.7
garlic oil	3-(prop-2-enyltrisulfanyl)prop-1-ene	UBAXRAHSPKWNCX-UHFFFAOYSA-N	-4.1
Bicarbonate	hydrogen carbonate	UIIMBOGNXHQVGW-UHFFFAOYSA-M	-3.4

3.10.1 Use Apigenin that predicting by molecular docking as a suitable *Fimh* inhibitors

The ligands were docked to the Apigenin (Other names Chamomile; Apigenol; Spigenin; Versulin; C.I. Natural Yellow 1), and *Fimh* proteins using "Mcule web site" by following the protocol described by (Mashraqi *et al*., 2021). To minimize the energy usage of the ligand molecules, a Merck molecular force field (MMFF94) was employed. The ligand atoms were added with Gasteiger partial charges. Docking calculations were done on the target proteins. Essential hydrogen atoms, Kollman united atom type charges and solvation parameters were added by using Auto Dock tools (El-Hachem *et al*., 2017; Huey *et al*., 2012).

Consequently, the binding pocket was added with conserved water molecules to mimic the in vivo environment. An auto grid program was used to generate the affinity (grid) maps sized at $60^\circ \times 60^\circ \times 60^\circ$, the aim of which was to target the grid coordinates in the catalytic site of the target protein (Apigenin and *Fimh*). The x, y, and z coordinate values for the *Fimh* protein targeting the catalytic site were taken as (-16.519, 50.643, and 27.017), respectively.

The initial position, orientation, and torsions of the ligands were set randomly.
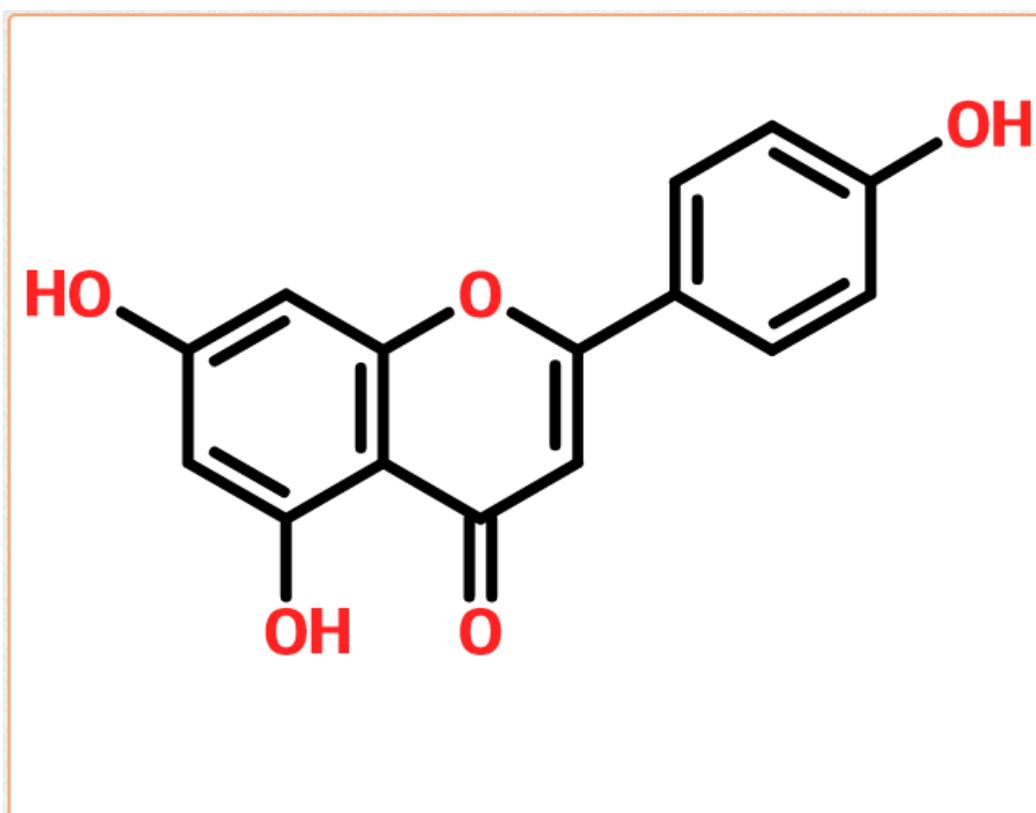
Figure (3-12) chemical structure of Apigenin. with IUPAC name 5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one (Singh, *et.al*,2011).

3.10.2 Apigenin as suitable Inhibitor for _Fimh_ obtained by docking molecule in Mcule web site

About 80-90% of UTIs are caused by _E. coli_ bacteria, and more than 90% of these bacteria contain the _Fimh_, which is present at the end of type 1 pilus and is considered the virulence factor of these bacteria (Soltani, _et.al_,2018).

After choosing some suitable medical compound and docking it by mucule web site, its chosen a medical drugs available in pharmacies and market on the basis of their high safety and appropriate efficacy to treat some diseases that do not harm effect on the body's vital physiology, such as heart diseases, diabetes and neurological diseases (Huang, _et.al_,2020). Rather, choose medicines commonly used to relieve some signs and symptoms of simple diseases such as allergies, colds, antacids, all tested nutritional supplements and various vitamins by docking that do not conflict with chronic diseases or not have a strong side effects on the body. We also make sure that it must reach as a full and effective form to the urinary system and does not destroy in it (Komala & Kumar, 2013; Raheema & Alsaidi;2016; Nyamwamu _et al_., 2020).

Apigenine

Electron density

Maps of

Fimh **protein**

A-

B-
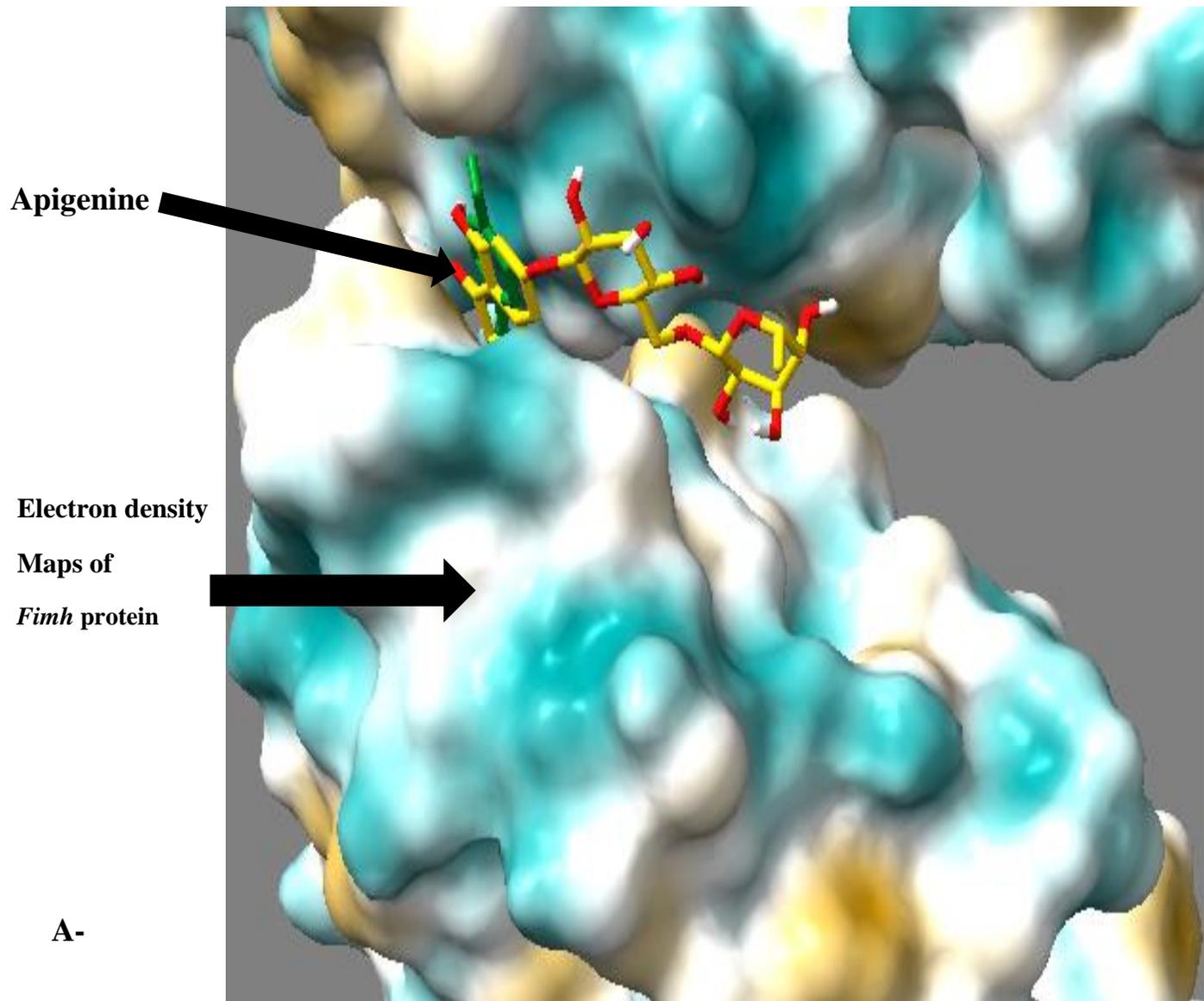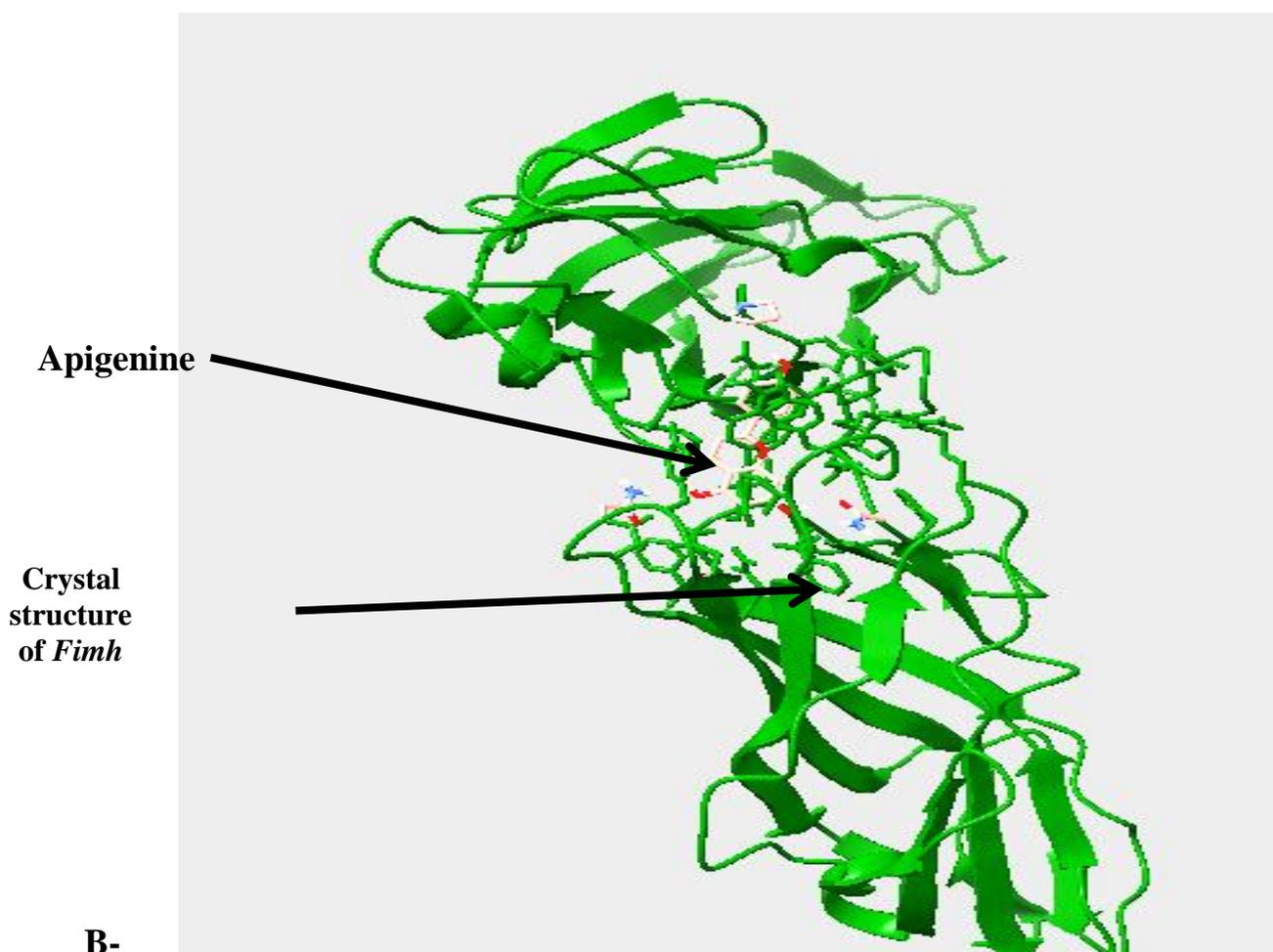
Figure: 3-13 (A) Electron density maps, for oligomannose-3 in the *Fimh* receptor-binding site with Apigenin.(B) (green) in complex with Apigenin

This mechanism of *Fimh* inhibitor is based on the structural similarity between the d-mannose receptor and the mannosylated receptor exposed to the urinary tract epithelium. When ingested in sufficient quantities, D-mannose like substance is rapidly absorbed and then excreted by the urinary tract where it saturates bacterial *Fimh*, thus preventing its binding with uroepithelial cell. Thus, the d-mannose like substance-UPEC reaction facilitates the removal of bacteria that are withdrawn by urine flow (Daniela *et.al*, 2020).

On this basis, we have chosen the Chamomile extract (Apigenine) as a suitable blocker or inhibitor for *Fimh*.

3D structure of binding of *fimh* **protein and** Apigenine
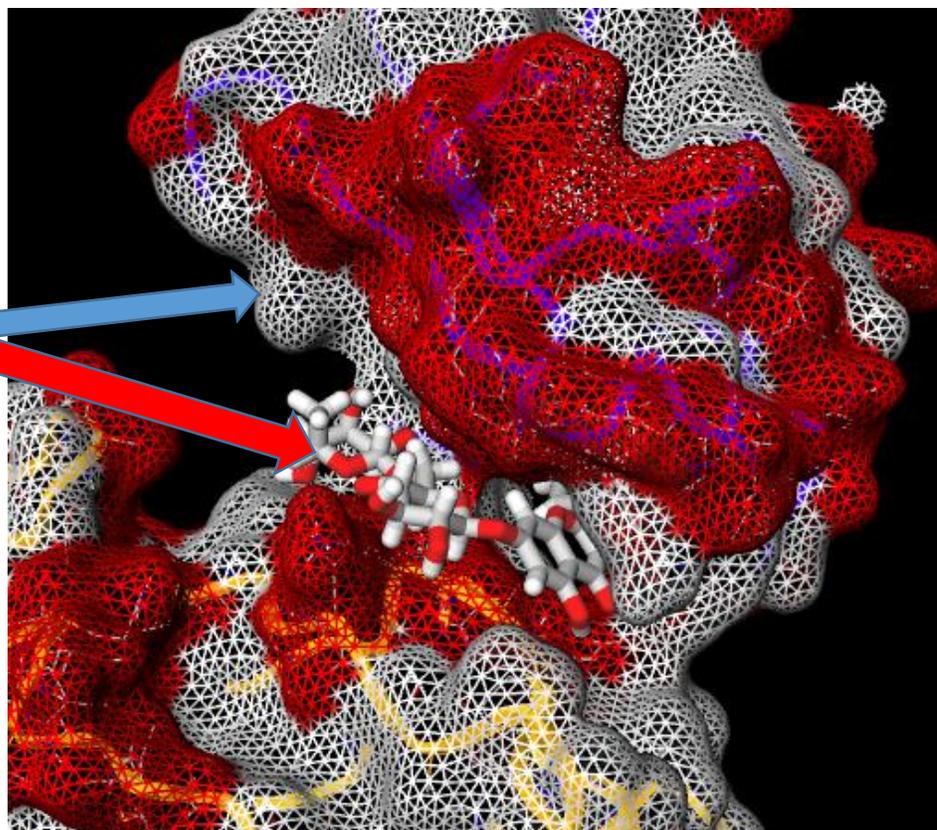
Figure 3-14 prediction the binding-conformation of small molecule ligands to the appropriate target binding site between *Fimh* and Apigenin with Docking scores (-9.4).

Various germs, including uropathogenic *Escherichia coli* (UPEC), cause urinary tract infections (UTIs). UPEC strains have unique virulence characteristics, such as type 1 fimbriae, which can exacerbate UTIs(Behzadi, 2020).

The essential step in the infection process is the colonization to the urinary tract(Di Venanzio *et al.*, 2019), so, the UPEC infection dependent mainly on *fimh* binding in uroepithelial cells, and inhibitor dependent mainly by blocking *fimh* protein.

Ordinarily, without binding to epithelium cells bacteria can be washed out by urination process(Shukla *et al.*, 2021). Type 1 fimbriae are coded in the genome of most almost 95% *E. coli* strains(Ballesteros-Monrreal *et al.*, 2021). The presence of fimbriae is required for colonization of the urinary system is: P, type 1, S, and F1C fimbriae, and it's among the stickiest organelles expressed by uropathogenic *Escherichia coli*.

Because *Fimh*'s receptor-binding site is a highly specialized mannose-binding pockets with a tyrosine gates (**Tyr.48, Ile.52, and Tyr.137**) at one side and a hydrophobic end (**Ile13, Phe1, and Phe142**) surrounding its entry, a *Fimh* inhibitor should be overlaid onto the *Fimh* lectin domain combination with oligomannose-3(Touaibia *et al.*, 2017). The affinity (grid) maps sized at 60°x60°x60° were generated using an auto grid algorithm, with the goal of targeting the grid coordinates in the target protein's catalytic site (Apigenin and *Fimh*). The *Fimh* protein targeting the catalytic region had x, y, and z coordinate values of (-16.519, 50.643, and 27.017), respectively. The ligands' initial positions, direction, and torsions were chosen at random(Murugan *et al.*, 2022)

Figures (3-13) & (3-14), Show the molecular docking of binding between *Fimh* and Apigenin.

3.11 Study of Apigenin as *Fimh* inhibitor

Table (3-7). Different result on animals group treated with Apigenin

Animals group	Description	Bacterial Cell Count in rats urine after 3 days n.(%) Cfu/ml
1	**G1- Control (without drug ,without infection)**	**20(100%) All negative $<10^4$**
2	**G2 Animals taking Drug before infection by 2 days**	**15 from 20(75%)-ve$<10^4$ 5 from 20(25%)+ve$>10^7$**
3	**G3 Animals taking Drug after infection by 2 days**	**10 from 20(50%) -ve$<10^4$ 10 from 20(50%) +ve$>10^7$**
4	**G4 Animals Infected , without drug given**	**20(100%) $> 10^7$**

* Negative (-ve) = No infection

**Positive (+ve) = Infection

Table (3-7). Show's the experimental result on rats. Four groups of rats will be taken; each group contains 20 animals of 150-200 gm in weight. The principle of the experiment is dependent on activated infection on animals with/without feeding with our drug (Apigenin) and then counted *E.coli* in their urine. The first group serves as a control; these rats are not inoculated by bacteria, not feeding with Apigenin, so it's healthy, with no disease, and all laboratory tests reveal the negative infection. The second group is pre-feeding with Apigenin for two days and then inoculated with 10^7-10^9CFU of UPEC by intraurethral catheterization. The concentration of Apigenin that was used in this experiment was prepared by adding 5 drops of 1.2% liquid extract in 100ml of water(Hanafy & El-Kemary, 2022), and animals would be fed by this solution throughout the day without being

given water. The result showed from 20 animals, 15(75%) of rats would be not infected with UTI, and 5(25%) of rats getting an infection. The third group was infected before two days of feeding with Apigenin, the result shows about 10(50%) of rats getting an infection and 10(50%) not infected (figure 3-7). We notice the infection would be increased, when Apigenin is not given before infection, this is due to the ability of *E.coli* to bind on bladder epithelial cells by *Fimh* in the absence of drug. And when the concentration of the drug would be increased after two days, we can notice the healing effect of Apigenin on the one-third number of animals group in the third group.

In The fourth group, all animals would be treatment infected without any, so all animals getting an infection showed more than 10^8 CFU in their urine, because no *Fimh* antagonist (Apigenin) was present.

Two ways were applied to test, 1st by using chamomile as a crude material, 2nd occurred by using pure Apigenin, the yield result was the same and no difference in the effect, this means that the Apigenin and crud chamomile casn be used to get the same effect and all the effect is due to the presence of Apigenin.

Conclusions:

In this study, it is included that:

1- The type 1 fimbria *Fimh* adhesion is a well-studied bacterial adhesion. 2-The profile created by combining all dinucleotides resulted in species-specific patterns that were distinct to each species.

2- *Escherichia coli* had resistance to many antibiotics, because of many causes like , uncontrolled and overused of antibiotics

3- Dinucleotide studies provide a 'fingerprint' for many organisms, because it has a constant rhythm for each specific species.

4- Good effect of chamomile (Apigenin) in UTI treatment, and Apigenin may consider as a novel inhibitor to *Escherichia coli* attachment to urinary tract epithelia.

5- *Escherichia coli* isolates showed highly sequence similarity except two nucleotide sub-situation without effecting on protein active site.

6- Apigenine may have a strong capacity to inhibitor FimH function.

7-little variance found among *FimH* protein sequences from local and international sequences.

8- Mutation in the *Fimh* gene sequences from local isolates, may not affect in *Fimh* active site.

Recommendations:

Depending on the finding of this study, the recommended subjects include:

1- Dinucleotide signature can be used in the future as a distinctive fingerprint indicating the type of the gene, and in terms of the value of Dinucleotide signature, it is possible to infer the knowledge of the gene and the organisms.

2-Further Dinucleotide signature can be made for other *Escherichia coli* genes and can make comparative studies with the same gene in different organism.

3- We recommended using Apigenin as a natural herbal product in adjuvant therapy with other drugs or products in UTI treatment.

4- Recommended to use Apigenin as prophylaxis in high-risk factors for UTI.

5- A Suggestion to used Apigenin in UTIs treatment, with/without other suitable drugs.

6- A suggestion of other agents by using the docking technique to design novel inhibitors of fimH of *Escherichia coli*.

7- Because of *Escherichia coli* showed some antibiotics resistance, so, we Suggest to lookout on "antibiotic-like" activity, Apigenine or others good *fimh* antagonist.

References

Abebe, W. (2002). Herbal medication: potential for adverse interactions with analgesic drugs. Journal of clinical pharmacy and therapeutics, 27(6), 391-401.

Aglawe, S., Gayke, A., Sharma, K., Jadhav, S., Gore, S., Pandit, B., Valate, S., & Wagh, M. (2020). Chamomile: A Review. *Research journal of Pharmacology and Pharmacodynamics*, *12*(1), 12-14.

Agrawal A; Brendel VP; Huang X (2013).Pairwise statistical significance and empirical determination of effective gap opening penalties for protein local sequence alignment. International Journal of Computational Biology and Drug Design. 1 (4): 347–367.

Akour, A., Abuloha, S., Mulakhudair, A. R., Kasabri, V., & Ala'a, B. (2021). Complementary and alternative medicine for urinary tract illnesses: A cross-sectional survey in Jordan. *Complementary Therapies in Clinical Practice*, *43*, 101321.

AL_Okhedi, M. J. I., Najim, T. M., Al-shammari, B. F. M., Hasan, M. S., & Jead, M. R. (2020). Effects of E. Coli Infection on Kidney Function Tests in Experimentally Inoculated Rats. Prof.(Dr) RK Sharma, 20(4), 41354.

Alexi Lienard, Michel Hosny, Joanne Jneid, Sophie Schuldiner, Nicolas Cellier, Albert Sotto, Bernard La Scola, Jean-Philippe Lavigne, and Alix Pantel (2021). *Escherichia coli* Isolated from Diabetic Foot Osteomyelitis: Clonal Diversity, Resistance Profile, Virulence Potential, and Genome Adaptation. Microorganisms. 9(2): 380.

Alfredo Montes-Robledo, Rosa Baldiris-Avila, and Johan Fabian Galindo (2021). D-Mannoside FimH Inhibitors as Non-Antibiotic Alternatives for Uropathogenic *Escherichia coli* .Antibiotics (Basel). 10(9): 1072.

Alkandari, F. A. H. (2017). Characterisation of *Escherichia coli* in poultry and their interaction with phytochemicals (Doctoral dissertation, University of Reading).

Alshara, M. (2011).Antimicrobial Resistant Pattern of *Escherichia coli* Strains Isolated from Pediatric Patients in Jordan. *Acta Medica Iranica* . 49(5): 293-295.

Anderson G., Dodson K., Hooton T., Hultgren J.(2004). Intracellular bacterial communities of uropathogenic *Escherichia coli* in urinary tract pathogenesis. Elsevier . 12(9): 424-430.

Andrew J. Gentles and Samuel Karlin(2001).Genome-Scale Compositional Comparisons in Eukaryotes.Genome Res. 11: 540-546.

Ardila-Leal, L.D.; Monterey-Gutiérrez, P.A.; Poutou-Piñales, R.A.; Quevedo-Hidalgo, B.E.; Galindo, J.F.; Pedroza-Rodríguez, A.M.(2021). Recombinant laccase rPOXA 1B real-time, accelerated and molecular dynamics stability study. BMC Biotechnol. 2021, 21, 1–18.

Aronson, M., Medalia, O., Schori, L., Mirelman, D., Sharon, N., & Ofek, I. (1979). Prevention of colonization of the urinary tract of mice with *Escherichia coli* by blocking of bacterial adherence with methyl α-D-mannopyranoside. Journal of Infectious Diseases, 139(3), 329-332.

Ashwell M, Gunn P, S G,(2012).Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardio metabolic risk factors: systematic review and meta-analysis. Obesity Reviews .13(3):275-86.

Azarkan, Mohamed & Feller, Georges & Vandenameele, Julie & Herman, Raphaël & Mahyaoui, Rachida & Sauvage, Eric & Vanden Broeck, Arnaud & Matagne, Andre & Charlier, Paulette & Kerff, Frédéric. (2018). Biochemical and structural characterization of a mannose binding jacalin-related lectin with two-sugar binding sites from pineapple (Ananas comosus) stem. Scientific Reports. 8. 10.1038/s41598-018-29439-x.

Baldiris-Avila, R.; Montes-Robledo, A.; Buelvas-Montes, Y.(2020). Phylogenetic Classification, Biofilm-Forming Capacity, Virulence Factors, and Antimicrobial Resistance in Uropathogenic *Escherichia coli* (UPEC). Curr. Microbiol., 3, 1–10.

Ballesteros-Monrreal, M., Arenas-Hernández, M., Barrios-Villa, E., Juarez, J., Álvarez-Ainza, M., Taboada, P., De la Rosa-López, R., Bolado-Martínez, E., & Valencia, D. (2021). Bacterial Morphotypes as Important Trait for Uropathogenic E. coli Diagnostic; a Virulence-Phenotype-Phylogeny Study. Microorganisms 2021, 9, 2381. *Virulence Factors and Antibiotic Resistance of Enterobacterales*, 19.

Banik, A., Ghosh, K., Patil, U. K., & Gayen, S. (2021). Identification of molecular fingerprints of natural products for the inhibition of breast cancer resistance protein (BCRP). *Phytomedicine*, *85*, 153523.

Barák I, Muchová K (2018) The positioning of the asymmetric septum during sporulation in Bacillus subtilis. PLoS ONE 13(8): e0201979.

Bawono, P., Dijkstra, M., Pirovano, W., Feenstra, A., Abeln, S., & Heringa, J. (2017). Multiple sequence alignment. In Bioinformatics (pp. 167-189). Springer.

Bazaid, A. S., Aldarhami, A., Gattan, H., Barnawi, H., Qanash, H., Alsaif, G., ... & Eldrehmy, E. H. (2022). Antibiogram of Urinary Tract Infections and Sepsis among Infants in Neonatal Intensive Care Unit. Children, 9(5), 629.

Behzadi, P. (2020). Classical chaperone-usher (CU) adhesive fimbriome: uropathogenic *Escherichia coli* (UPEC) and urinary tract infections (UTIs). *Folia microbiologica*, *65*(1), 45-65.

Belfield, E. J., Ding, Z. J., Jamieson, F. J., Visscher, A. M., Zheng, S. J., Mithani, A., & Harberd, N. P. (2018). DNA mismatch repair preferentially protects genes from mutation. *Genome research*, *28*(1), 66-74.

Benson, M. (2001). Microbiological applications laboratory Manual. Eight edition.

Berry R, Klummp D, Schaeffer A.,(2009) Urothelial cultures support Intracellular Bacterial Community Formation by uropathogenic Escherichia coli. Journal Infect Inmun. 77(7):2762-72.

Bessaiah, H., Anamalé, C., Sung, J., & Dozois, C. M. (2021). What Flips the Switch? Signals and Stress Regulating Extraintestinal Pathogenic *Escherichia coli* Type 1 Fimbriae (Pili). *Microorganisms*, *10*(1), 5.

Bhargavi, P. S., Rao, G. T., Mukkanti, K., Kumar, D. B., & Krishna, T. P. (2010). Increasing emergence of antibacterial resistance mainly in uropathogens: southeast part of India. International journal of microbiology research, 2(1), 1.

Bien, J., Sokolova, O., & Bozko, P. (2012). Role of uropathogenic *Escherichia coli* virulence factors in development of urinary tract

infection and kidney damage. International journal of nephrology, 2012.

Blahna, M. T., C. A. Zalewski, J. Reuer, G. Kahlmeter, Foxman, B., & Marrs, C. F. (2006) The role of horizontal gene transfer in the spread of trimethoprim-sulfamethoxazole resistance among uropathogenic *Escherichia coli* in Europe and Canada. J Antimicrob Chemother 57: 666-672.

Bouckaert, J.; Mackenzie, J.; de Paz, J.L.; Chipwaza, B.; Choudhury, D.; Zavialov, A.; Mannerstedt, K.; Anderson, J.; Piérard, D.; Wyns, L.; et al.(2006). The affinity of the FimH fimbrial adhesin is receptor-driven and quasi-independent of *Escherichia coli* pathotypes. Mol. Microbiol., 61, 1556–1568.

Brierley, L., & Fowler, A. (2021). Predicting the animal hosts of coronaviruses from compositional biases of spike protein and whole genome sequences through machine learning. PLoS Pathogens, 17(4), e1009149.

Brooks, B. W., James, S. V., Valenti, Jr, T. W., Urena-Boeck, F., Serrano, C., Berninger, J. P., ... & Roelke, D. L. (2010). Comparative toxicity of Prymnesium parvum in inland waters 1. JAWRA Journal of the American Water Resources Association, 46(1), 45-62.

Bulbul, M. Z., Hosen, M. A., Ferdous, J., Chowdhury, T. S., Misbah, M. M., & Kawsar, S. (2021). DFT study, physicochemical, molecular docking, and ADMET predictions of some modified uridine derivatives. *International Journal of New Chemistry*, 8(1), 88-110.

Busch, A., & Waksman, G. (2012). Chaperone-usher pathways: diversity and pilus assembly mechanism. Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 367(1592), 1112–1122.

Bush, N. G., Diez-Santos, I., Abbott, L. R., & Maxwell, A. (2020). Quinolones: mechanism, lethality and their contributions to antibiotic resistance. Molecules, 25(23), 5662.

Cai, Z., Liu, S., Xue, Y., Quan, H., Zhang, L., & Gao, Y. Q. (2020). Genome Wide Analysis of Dinucleotide Distribution along the Genomes of

Species and its Biological Implication. *bioRxiv*, 2020.2002.2020.957142.

Caitlin N Spaulding, Henry Louis Schreiber, Weili Zheng , Karen W Dodson, Jennie E Hazen, Matt S Conover, Fengbin Wang , Pontus Svenmarker , Areli Luna-Rico , Olivera Francetic , Magnus Andersson , Scott Hultgren, Edward H Egelman,(2018). Functional role of the type 1 pilus rod structure in mediating host-pathogen interactions. eLife .7:e31662.

Castanheira, M., Simner, P. J., & Bradford, P. A. (2021). Extended-spectrum β-lactamases: An update on their characteristics, epidemiology and detection. JAC-antimicrobial resistance, 3(3), dlab092.

Chatzou, M., Magis, C., Chang, J.-M., Kemena, C., Bussotti, G., Erb, I., & Notredame, C. (2016). Multiple sequence alignment modeling: methods and applications. *Briefings in bioinformatics*, *17*(6), 1009-1023.

Chhaya Shah, Ratna Baral, Bijay Bartaula & Lok Bahadur Shrestha (2019). Virulence factors of uropathogenic *Escherichia coli* (UPEC) and correlation with antimicrobial resistance.BMC Microbiology volume 19, Article number: 204.

Chockalingam, A., Xu, L., Stewart, S., LeMerdy, M., Tsakalozou, E., Fan, J., ... & Rouse, R. (2019). Protocol for evaluation of topical ophthalmic drug products in different compartments of fresh eye tissues in a rabbit model. Journal of Pharmacological and Toxicological Methods, 96, 9-14.

Choudhury, D., Thompson, A., Stojanoff, V., Langermann, S., Pinkner, J., Hultgren, S. J., & Knight, S. D. (1999). X-ray structure of the FimC-FimH chaperone-adhesin complex from uropathogenic Escherichia coli. Science (New York, N.Y.), 285(5430), 1061–1066.

CHRIS BURGEt, ALLAN M. CAMPBELLO, AND SAMUEL KARLINt (1991).Over- and under-representation of short oligonucleotides in DNA sequences.Proc. Natd. Acad. Sci. USA Vol. 89, pp. 1358-1362.

Clark, J. R., & Maresso, A. M. (2021). Comparative Pathogenomics of Escherichia Coli: Polyvalent Vaccine Target Identification through Virulome Analysis. Infection and immunity, 89(8), e00115-21.

CLSI, C. (2012). Performance standards for antimicrobial disk susceptibility tests; approved standard. CLSI document M02-A11, 950.

Cusumano, C. K., Pinkner, J. S., Han, Z., Greene, S. E., Ford, B. A., Crowley, J. R., ... & Hultgren, S. J. (2011). Treatment and prevention of urinary tract infection with orally active FimH inhibitors. Science translational medicine, 3(109), 109ra115-109ra115.

Dadi, B. R., Abebe, T., Zhang, L., Mihret, A., Abebe, W., & Amogne, W. (2020). Distribution of virulence genes and phylogenetics of uropathogenic *Escherichia coli* among urinary tract infection patients in Addis Ababa, Ethiopia. BMC infectious diseases, 20(1), 1-12.

Daniela Scribano, Meysam Sarshar, Carla Prezioso,1 Marco Lucarelli, Antonio Angeloni, Carlo Zagaglia,1 Anna Teresa Palamara, and Cecilia Ambrosi (2020). d-Mannose Treatment neither Affects Uropathogenic *Escherichia coli* Properties nor Induces Stable FimH Modifications. Molecules, 25(2): 316.

Darnton, N. C., Turner, L., Rojevsky, S., & Berg, H. C. (2007). On torque and tumbling in swimming Escherichia coli. Journal of bacteriology, 189(5), 1756-1764.

De La Cadena, E.; Mojica, M.F.; Castillo, N.; Correa, A.; Appel, T.M.; García-Betancur, J.C.; Villegas, M.V.(2020). Genomic analysis of ctx-m-group-1-producing extraintestinal pathogenic E. coli (ExPEC) from patients with urinary tract infections (UTI) from Colombia. Antibiotics, 9, 899.

De Nisco, N. J., Neugent, M., Mull, J., Chen, L., Kuprasertkul, A., de Souza Santos, M., ... & Orth, K. (2019). Direct detection of tissue-resident bacteria and chronic inflammation in the bladder wall of postmenopausal women with recurrent urinary tract infection. Journal of molecular biology, 431(21), 4368-4379.

Di Conza, J. A., Badaracco, A., Ayala, J., Rodriguez, C., Famiglietti, Á., & Gutkind, G. O. (2014). β-lactamases produced by amoxicillin-clavulanate-resistant enterobacteria isolated in Buenos Aires, Argentina: a new blaTEM gene. Revista Argentina de microbiologia, 46(3), 210-217.

Di Venanzio, G., Flores-Mireles, A. L., Calix, J. J., Haurat, M. F., Scott, N. E., Palmer, L. D., Potter, R. F., Hibbing, M. E., Friedman, L., & Wang, B. (2019). Urinary tract colonization is enhanced by a plasmid that regulates uropathogenic Acinetobacter baumannii chromosomal genes. *Nature Communications*, *10*(1), 1-13.

Díaz, B. F., Villamar, M. V., & Alarcón, N. C.,(2016). Identificación Molecular de Factores de Virulencia de cepas de *Escherichia coli* uropatógenas. Encuentro de Jóvenes en la Investigación de Bachillerato-CONACYT. Volume 7 Special Issue 1.

Dlamini, G. S., Muller, S. J., Meraba, R. L., Young, R. A., Mashiyane, J., Chiwewe, T., & Mapiye, D. S. (2020). Classification of COVID-19 and Other Pathogenic Sequences: A Dinucleotide Frequency and Machine Learning Approach. *IEEE access : practical innovations, open solutions*, *8*, 195263-195273.

Douafer, H., Andrieu, V., Phanstiel IV, O., & Brunel, J. M. (2019). Antibiotic adjuvants: make antibiotics great again! Journal of medicinal chemistry, 62(19), 8665-8681.

Duedu, K. O., & French, C. E. (2017). Two-colour fluorescence fluorimetric analysis for direct quantification of bacteria and an application in monitoring bacterial growth in cellulose degradation systems. Journal of microbiological methods. 135, 85-92.

Dumych, T.; Bridot, C.; Gouin, S.G.; Lensink, M.F.; Paryzhak, S.; Szunerits, S.; Krammer, E.M.(2018). A novel integrated way for deciphering the glycan code for the FimH lectin. Molecules, 23, 2794.

El-Hachem, N., Haibe-Kains, B., Khalil, A., Kobeissy, F. H., & Nemer, G. (2017). AutoDock and AutoDockTools for protein-ligand docking: beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) as a case study. In Neuroproteomics (pp. 391-403). Springer.

Evgeni V. Sokurenko, Viola Vogel, and Wendy E. Thomas (2008). Catch bond mechanism of force-enhanced adhesion: counter-intuitive, elusive but … widespread? Cell Host Microbe. 16; 4(4): 314–323.

Faria, R., Triant, D., Perdomo-Sabogal, A., Overduin, B., Bleidorn, C., Santana, C. I. B., ... & Nowick, K. (2018). Introducing evolutionary biologists to the analysis of big data: guidelines to organize extended

bioinformatics training courses. Evolution: Education and Outreach, 11(1), 1-10.

Fatriansyah, J. F., Rizqillah, R. K., Yandi, M. Y., Fadilah, & Sahlan, M. (2022). Molecular docking and dynamics studies on propolis sulabiroin-A as a potential inhibitor of SARS-CoV-2. *Journal of King Saud University. Science*, *34*(1), 101707-101707.

Feenstra, T., Thøgersen, M. S., Wieser, E., Peschel, A., Ball, M. J., Brandes, R., ... & Kain, R. (2017). Adhesion of *Escherichia coli* under flow conditions reveals potential novel effects of FimH mutations. European Journal of Clinical Microbiology & Infectious Diseases, 36(3), 467-478.

Fimmel, E., & Strüngmann, L. (2018). Mathematical fundamentals for the noise immunity of the genetic code. Biosystems, 164, 186-198.

Flament-Simon, S. C., Duprilot, M., Mayer, N., García, V., Alonso, M. P., Blanco, J., & Nicolas-Chanoine, M. H. (2019). Association between kinetics of early biofilm formation and clonal lineage in Escherichia coli. Frontiers in microbiology, 10, 1183.

Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature reviews microbiology, 13(5), 269-284.

Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). Diagnostic microbiology (pp. 288-302). St Louis: Mosby.

Franco Scaldaferri, Viviana Gerardi, Francesca Mangiola, Loris Riccardo Lopetuso, Marco Pizzoferrato, Valentina Petito, Alfredo Papa, Jovana Stojanovic, Andrea Poscia, Giovanni Cammarota, and Antonio Gasbarrini(2016).Role and mechanisms of action of *Escherichia coli* Nissle 1917 in the maintenance of remission in ulcerative colitis patients: An update.World J Gastroenterol. 22(24): 5505–5511.

Francoeur, P. G., Masuda, T., Sunseri, J., Jia, A., Iovanisci, R. B., Snyder, I., & Koes, D. R. (2020). Three-dimensional convolutional neural networks and a cross-docked data set for structure-based drug design. Journal of Chemical Information and Modeling, 60(9), 4200-4215.

Fraunfelder, F. T., Fraunfelder, F. W., & Chambers, W. A. (2014). Drug-induced ocular side effects: clinical ocular toxicology E-Book. Elsevier Health Sciences.

Giani, A. M., Gallo, G. R., Gianfranceschi, L., & Formenti, G. (2020). Long walk to genomics: History and current approaches to genome sequencing and assembly. Computational and Structural Biotechnology Journal, 18, 9-19.

Girlich, D., Bonnin, R. A., Dortet, L., & Naas, T. (2020). Genetics of Acquired Antibiotic Resistance Genes in Proteus spp [Review]. Frontiers in Microbiology, 11.

Gomes, M. N., Muratov, E. N., Pereira, M., Peixoto, J. C., Rosseto, L. P., Cravo, P. V., Andrade, C. H., & Neves, B. J. (2017). Chalcone derivatives: promising starting points for drug design. *Molecules*, *22*(8), 1210.

Gonçalves, G., & Paiva, R. (2017). Gene therapy: advances, challenges and perspectives. Einstein (Sao Paulo, Brazil), 15(3), 369–375.

Goswami, R., Bal, C. S., Tejaswi, S., Punjabi, G. V., Kapil, A., & Kochupillai, N. (2001). Prevalence of urinary tract infection and renal scars in patients with diabetes mellitus. Diabetes research and clinical practice, 53(3), 181-186.

Gupta V, Mittal P, Bansal P, Khokra SL, Kaushik D(2010). Pharmacological potential of Matricaria recutita-A review. Int J Pharm Sci Drug Res.2:12–6.

Gupta, K., Donnola, S. B., Sadeghi, Z., Lu, L., Erokwu, B. O., Kavran, M., ... & Flask, C. A. (2017). Intrarenal injection of *Escherichia coli* in a rat model of pyelonephritis. Journal of visualized experiments: JoVE, (125).

Gustavo Bodelón, Carmen Palomino, Luis Ángel Fernández (2013). Immunoglobulin domains in *Escherichia coli* and other enterobacteria: from pathogenesis to applications in antibody technologies. FEMS Microbiology Reviews, Volume 37, Issue 2, Pages 204–250.

Hanafy, N. A., & El-Kemary, M. A. (2022). Silymarin/curcumin loaded albumin nanoparticles coated by chitosan as muco-inhalable delivery

system observing anti-inflammatory and anti COVID-19 characterizations in oleic acid triggered lung injury and in vitro COVID-19 experiment. International Journal of Biological Macromolecules, 198, 101-110.

Hanna T, Totsika M, Mansfield K.,(2012). Host-Pathogen Checkpoints and Population Bottlenecks in Persistent and Intracellular Uropathogenic E. coli Bladder Infection. FEMS Microbiol Rev. 36(3):616-648.

Hannan, T. J., & Hunstad, D. A. (2016). A murine model for *Escherichia coli* urinary tract infection. In Bacterial Persistence (pp. 159-175). Springer.

Hardison, R. L., Heimlich, D. R., Harrison, A., Beatty, W. L., Rains, S., Moseley, M. A., ... & Mason, K. M. (2018). Transient nutrient deprivation promotes macropinocytosis-dependent intracellular bacterial community development. MSphere, 3(5), e00286-18.

Healy, M. D. (2007). Using BLAST for performing sequence alignment. Current Protocols in Human Genetics, 52(1), 6-8.

Heinrich, M., Williamson, E. M., Gibbons, S., Barnes, J., & Prieto-Garcia, J. (2017). *Fundamentals of pharmacognosy and phytotherapy E-BOOK*. Elsevier Health Sciences.

Herrera, S., Reyes-Herrera, P. H., & Shank, T. M. (2015). Predicting genome sizes and restriction enzyme recognition-sequence probabilities across the eukaryotic tree of life. *bioRxiv*, 007781.

Hibbing, M. E., Dodson, K. W., Kalas, V., Chen, S. L., & Hultgren, S. J. (2020). Adaptation of arginine synthesis among uropathogenic branches of the *Escherichia coli* phylogeny reveals adjustment to the urinary tract habitat. MBio, 11(5), e02318-20.

Hidron, A. I., Edwards, J. R., Patel, J., Horan, T. C., Sievert, D. M., Pollock, D. A., & Fridkin, S. K. (2008). Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infection Control & Hospital Epidemiology, 29(11), 996-1011.

Hilbert, D. W. (2011). Antibiotic resistance in urinary tract infections: current issues and future solutions. *Urinary Tract Infections. Rijeka: INTECH*, 193-206.

Hiroshi NAKASHIMA, Ken NISHIKAWA, and Tatsuo Ooi (1997). Differences in Dinucleotide Frequencies of Human, Yeast, and *Escherichia coli* Genes. DNA RESEARCH 4, 185-192.

Hogeweg, Paulien (2011). "The Roots of Bioinformatics in Theoretical Biology". PLOS Computational Biology.7 (3): e1002021.

Huang, J.; Tao, G.; Liu, J.; Cai, J.; Huang, Z.; Chen, J.X(2020). Current Prevention of COVID-19: Natural Products and Herbal Medicine. Front. Pharm.11, 588508.

Huey, R., Morris, G. M., & Forli, S. (2012). Using AutoDock 4 and AutoDock vina with AutoDockTools: a tutorial. The Scripps Research Institute Molecular Graphics Laboratory, 10550, 92037.

Huwaitat, R., McCloskey, A. P., Gilmore, B. F., & Laverty, G. (2016). Potential strategies for the eradication of multidrug-resistant Gram-negative bacterial infections. *Future microbiology*, *11*(7), 955-972.

Isolde Le Trong,1,‡ Pavel Aprikian,2,‡ Brian A. Kidd,3 Manu Forero-Shelton,4,5 Veronika Tchesnokova,2 Ponni Rajagopal,6 Victoria Rodriguez,3 Gianluca Interlandi,3 Rachel Klevit,6 Viola Vogel,5 Ronald E. Stenkamp, Sokurenko and Wendy E. Thomas.(2010).Structural Basis for Mechanical Force Regulation of the Adhesin FimH via Finger Trap-like Sheet Twisting. Cell. 141(4): 645–655.

Jaktaji, R. P., & Mohiti, E. (2010). Study of mutations in the DNA gyrase gyrA gene of Escherichia coli. Iranian Journal of Pharmaceutical Research: IJPR, 9(1), 43.

Jiang, Y., Oron, T. R., Clark, W. T., Bankapur, A. R., D'Andrea, D., Lepore, R., ... & Radivojac, P. (2016). An expanded evaluation of protein function prediction methods shows an improvement in accuracy. Genome biology, 17(1), 1-19.

John R. Brannon,corresponding author1 Taryn L. Dunigan,1 Connor J. Beebout,1 Tamia Ross,1 Michelle A. Wiebe,1 William S. Reynolds,2 and Maria Hadjifrangiskou (2020).Invasion of vaginal epithelial cells by uropathogenic Escherichia coli.Nat Commun. 11: 2803.

Johnson, J. R., Johnston, B. D., Porter, S. B., Clabots, C., Bender, T. L., Thuras, P., ... & Banerjee, R. (2019). Rapid emergence, subsidence, and molecular detection of *Escherichia coli* sequence type 1193-fimH64, a new disseminated multidrug-resistant commensal and extraintestinal pathogen. Journal of clinical microbiology, 57(5), e01664-18.

Johnson, J.R.; Russo, T.A.(2005). Molecular epidemiology of extraintestinal pathogenic (Uropathogenic) Escherichia coli. Int. J. Med. Microbiol., 295, 383–404.

Jonas S Almeida and Susana Vinga (2009). Biological sequences as pictures – a generic two dimensional solution for iterated maps. BMC Bioinformatics. 10: 100.

Jones, J., Murphy, C. P., Sleator, R. D., & Culligan, E. P. (2021). The urobiome, urinary tract infections, and the need for alternative therapeutics. Microbial pathogenesis, 161, 105295.

Jørgensen, S. L., Stegger, M., Kudirkiene, E., Lilje, B., Poulsen, L. L., Ronco, T., Pires Dos Santos, T., Kiil, K., Bisgaard, M., & Pedersen, K. (2019). Diversity and population overlap between avian and human *Escherichia coli* belonging to sequence type 95. MSphere, 4(1), e00333-00318.

Kamng'ona, A. W. (2014). Understanding the nasopharyngeal carriage dynamics of Streptococcus pneumoniae and other microbiota in Malawian children and adults. The University of Liverpool (United Kingdom).

Kapalka, G. M. (2010). Practical Resources for the Mental Health Professional, Nutritional and Herbal Therapies for Children and Adolescents.

Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic escherichia coli. Nature reviews microbiology, 2(2), 123-140.

Karlin, S., & Mrázek, J. (1997). Compositional differences within and between eukaryotic genomes. Proceedings of the National Academy of Sciences, 94(19), 10227-10232.

Karlin,S. and Burge,C. (1995) Dinucleotide relative abundance extremes—a genomic signature. Trends Genet., 11, 283–290.

Kebira, A. N., Ochola, P., & Khamadi, S. (2009). Isolation and antimicrobial susceptibility testing of *Escherichia coli* causing urinary tract nfections. Journal of Applied Biosciences, 22, 1320-1325.

Khan, A. S., Kniep, B., Oelschlaeger, T. A., Van Die, I., Korhonen, T., & Hacker, J. (2000). Receptor structure for F1C fimbriae of uropathogenic Escherichia coli. *Infection and immunity*, *68*(6), 3541-3547.

Kim D, Subhadra B, Kang H, Woo K, Kim J, Son Y, et al(2018). Virulence properties of uropathogenic *Escherichia coli* isolated from children with urinary tract infection in Korea. Genes Genomics.40(6):625-34.

Kisiela, D. I., Magala, P., Interlandi, G., Carlucci, L. A., Ramos, A., Tchesnokova, V., Basanta, B., Yarov-Yarovoy, V., Avagyan, H., & Hovhannisyan, A. (2021). Toggle switch residues control allosteric transitions in bacterial adhesins by participating in a concerted repacking of the protein core. PLoS pathogens, 17(4), e1009440.

Klein, R. D., & Hultgren, S. J. (2020). Urinary tract infections: microbial pathogenesis, host–pathogen interactions and new treatment strategies. *Nature Reviews Microbiology*, *18*(4), 211-226.

Kolanos, R., & Stice, S. A. (2021). German chamomile. In Nutraceuticals (pp. 757-772). Academic Press.

Komala, M., & Kumar, K. S. (2013). Urinary tract infection: causes, symptoms, diagnosis and it's management. Indian Journal of Research in Pharmacy and Biotechnology, 1(2), 226.

Konstantinos Apostolou-Karampelis , Dimitris Polychronopoulos, Yannis Almirantis. Introduction of 'Generalized Genomic Signatures' for the quantification of neighbor preferences leads to taxonomy- and functionality-based distinction among sequences. Sci Rep.9 (1):1700.

Krawczyk, P. S., Lipinski, L., & Dziembowski, A. (2018). PlasFlow: predicting plasmid sequences in metagenomic data using genome signatures. Nucleic acids research, 46(6), e35-e35.

Kumar, S., Tamura, K., & Nei, M. (1994). MEGA: molecular evolutionary genetics analysis software for microcomputers. Bioinformatics, 10(2), 189-191.

Kurien, B. T., Everds, N. E., & Scofield, R. H. (2004). Experimental animal urine collection: a review. Laboratory animals, 38(4), 333-361.

Kuźmińska-Bajor, M., Grzymajło, K., & Ugorski, M. (2015). Type 1 fimbriae are important factors limiting the dissemination and colonization of mice by Salmonella Enteritidis and contribute to the induction of intestinal inflammation during Salmonella invasion [Original Research]. Frontiers in Microbiology, 6.

Le Trong, I., Aprikian, P., Kidd, B. A., Forero-Shelton, M., Tchesnokova, V., Rajagopal, P., Rodriguez, V., Interlandi, G., Klevit, R., Vogel, V., Stenkamp, R. E., Sokurenko, E. V., & Thomas, W. E. (2010). Structural basis for mechanical force regulation of the adhesin FimH via finger trap-like beta sheet twisting. Cell, 141(4), 645–655.

Leach, Matthew J.; Page, Amy T. (2015). "Herbal medicine for insomnia: A systematic review and meta-analysis". Sleep Medicine Reviews. 24: 1–12.

Leimbach, A., Hacker, J., & Dobrindt, U. (2013). E. coli as an all-rounder: the thin line between commensalism and pathogenicity. Between pathogenicity and commensalism, 3-32.

Lene K. Vestby, Torstein Grønseth, Roger Simm, and Live L. Nesse (2020). Bacterial Biofilm and its Role in the Pathogenesis of Disease. Antibiotics (Basel). 9(2): 59.

Litster, A., Moss, S. M., Honnery, M., Rees, B., & Trott, D. J. (2007). Prevalence of bacterial species in cats with clinical signs of lower urinary tract disease: recognition of Staphylococcus felis as a possible feline urinary tract pathogen. Veterinary Microbiology, 121(1-2), 182-188.

Liu, S.-c., Han, X.-m., Shi, M., & Pang, Z.-l. (2016). Persistence of uropathogenic *Escherichia coli* in the bladders of female patients with sterile urine after antibiotic therapies. Journal of Huazhong University of Science and Technology [Medical Sciences], 36(5), 710-715.

Lodish, H., A. Berk, P. A. Matsudaira, C. Kaiser, M.P. Krieger, M. Scott, S.L. Zipursky and J. Darnell (2004).Molecular Cell Biology. 5th

Edition. Printed in the United states of America. W.H.Freeman and Company

Longhi, C., Maurizi, L., Conte, A. L., Marazzato, M., Comanducci, A., Nicoletti, M., & Zagaglia, C. (2022). Extraintestinal Pathogenic Escherichia coli: Beta-Lactam Antibiotic and Heavy Metal Resistance. Antibiotics, 11(3), 328.

Luscombe NM, Greenbaum D, Gerstein M. What is bioinformatics? A proposed definition and overview of the field. Methods Inf Med. 2001;40(4):346-358.

M Haghi, A., & M Taheri, K. (2007). Etiology and antibacterial resistance of bacterial urinary tract infections in children's medical center, Tehran, Iran.

MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria, williams and wilkins. Philadelphia, PA, 113.

Mageiros, L., Méric, G., Bayliss, S. C., Pensar, J., Pascoe, B., Mourkas, E., Calland, J. K., Yahara, K., Murray, S., & Wilkinson, T. S. (2021). Genome evolution and the emergence of pathogenicity in avian Escherichia coli. Nature Communications, 12(1), 1-13.

Mapatac, L. C. (2015). Antibacterial, Histochemical and Phytochemical Screening and Cytotoxicity Activity of Tubog, Ficus nota (Blanco) Merr Leaf and Fruit Extracts. Recoletos Multidisciplinary Research Journal, 3(2).

Maria E. Terlizzi, Giorgio Gribaudo, and Massimo E. Maffei(2017).UroPathogenic *Escherichia coli* (UPEC) Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-antibiotic Antimicrobial Strategies. Front Microbiol. 8: 1566.

Mark A. Schembri, Evgeni V. Sokurenko, and Per Klemm(2000).Functional Flexibility of the FimH Adhesin: Insights from a Random Mutant Library. Infect Immun. 68(5): 2638–2646.

Mashraqi, M. M., Chaturvedi, N., Alam, Q., Alshamrani, S., Bahnass, M. M., Ahmad, K., Alqosaibi, A. I., Alnamshan, M. M., Ahmad, S. S., & Beg, M. M. A. (2021). Biocomputational Prediction Approach Targeting FimH by Natural SGLT2 Inhibitors: A Possible Way to Overcome the

Uropathogenic Effect of SGLT2 Inhibitor Drugs. Molecules, 26(3), 582.

Mathers, A.J.; Peirano, G.; Pitout, J.D.(2015). *Escherichia coli* ST131: The quintessential example of an international multiresistant high-risk clone. Adv. Appl. Microbiol., 90, 109–154.

Matuschek, E., Longshaw, C., Takemura, M., Yamano, Y., & Kahlmeter, G. (2022). Cefiderocol: EUCAST criteria for disc diffusion and broth microdilution for antimicrobial susceptibility testing. Journal of Antimicrobial Chemotherapy, 77(6), 1662-1669.

McKay, D. L.; Blumberg, J. B. (2006). "A review of the bioactivity and potential health benefits of chamomile tea (Matricaria recutita L.)". Phytotherapy Research. 20 (7): 519–30.

McLellan, L. K., & Hunstad, D. A. (2016). Urinary tract infection: pathogenesis and outlook. Trends in molecular medicine, 22(11), 946-957.

McLellan, L. K., McAllaster, M. R., Kim, A. S., Tóthová, Ľ., Olson, P. D., Pinkner, J. S., Daugherty, A. L., Hreha, T. N., Janetka, J. W., & Fremont, D. H. (2021). A host receptor enables type 1 pilus-mediated pathogenesis of *Escherichia coli* pyelonephritis. PLoS pathogens, 17(1), e1009314.

Mei-an Sung, Keiran Fleming, Ho An Chen, and Stephen Matthews (2001).The solution structure of PapGII from uropathogenic *Escherichia coli* and its recognition of glycolipid receptors. EMBO Rep. 2(7): 621–627.

Meier A, Söding J (2015) Automatic Prediction of Protein 3D Structures by Probabilistic Multi-template Homology Modeling. PLoS Comput Biol 11(10): e1004343.

Meng, E. C., Pettersen, E. F., Couch, G. S., Huang, C. C., & Ferrin, T. E. (2006). Tools for integrated sequence-structure analysis with UCSF Chimera. BMC bioinformatics, 7(1), 1-10.

Million, Á., Angelakis, E., Maraninchi, M., Henry, M., Giorgi, R., Valero, R., ... & Raoult, D. (2013). Correlation between body mass index and gut concentrations of Lactobacillus reuteri, Bifidobacterium animalis,

Methanobrevi bacter smithii and *Escherichia coli*. International journal of obesity, 37(11), 1460-1466.

Mohutsky, M. A. (2002). Anti-salmonella adhesion activity of Saccharomyces boulardii: Effects of Ginkgo biloba on activities of cytochromes P-450. University of Washington.

Mojaz-Dalfardi, N.; Kalantar-Neyestanaki, D.; Hashemizadeh, Z.; Mansouri, S.(2020). Comparison of virulence genes and phylogenetic groups of *Escherichia coli* isolates from urinary tract infections and normal fecal flora. Gene Rep., 100709.

Montes-Robledo, A., Baldiris-Avila, R., & Galindo, J. F. (2021). D-Mannoside FimH Inhibitors as Non-Antibiotic Alternatives for Uropathogenic Escherichia coli. Antibiotics, 10(9), 1072.

Mood, E. H., Japoni-Nejad, A., Karam, M. A., Pooya, M., Bouzari, S., & Shahrokhi, N. (2021). Evaluation of accessible regions of *Escherichia coli* fimH mRNA through computational prediction and experimental investigation. Iranian Journal of Microbiology, 13(5), 653.

Müller, D., Greune, L., Heusipp, G., Karch, H., Fruth, A., Tschäpe, H., & Schmidt, M. A. (2007). Identification of unconventional intestinal pathogenic *Escherichia coli* isolates expressing intermediate virulence factor profiles by using a novel single-step multiplex PCR. Applied and environmental microbiology, 73(10), 3380-3390.

Mullins, R. J., & Heddle, R. (2004). Adverse reactions associated with Echinacea and other Asteraceae (pp. 249-260). CRC Press.

Muniesa, M., Hammerl, J. A., Hertwig, S., Appel, B., & Brüssow, H. (2012). Shiga toxin-producing *Escherichia coli* O104: H4: a new challenge for microbiology. Applied and environmental microbiology, 78(12), 4065-4073.

Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), (2003). Manual of Clinical Microbiology, ASM, Washington, D.C. 8th Ed.

Murugan, N. A., Podobas, A., Gadioli, D., Vitali, E., Palermo, G., & Markidis, S. (2022). A Review on Parallel Virtual Screening Softwares for High-Performance Computers. Pharmaceuticals, 15(1), 63.

Néha Sihra, Anna Goodman, Rhana Zakri, Arun Sahai & Sachin Malde(2021). Nonantibiotic prevention and management of recurrent urinary tract infection. Nature Reviews Urology. 15,750–776.

Némethy, S., Takács, T., Szemethy, L., Lagerqvist, B., Barócsi, Z., Dinya, A., & Peterffy, I. (2020). Collection, cultivation and processing of medical plants, herbs and spices in the Balaton Ecomuseum–herbal medicine as intangible cultural heritage. Ecocycles, 6(1), 52-87.

Niranjan, V., & Malini, A. (2014). Antimicrobial resistance pattern in *Escherichia coli* causing urinary tract infection among inpatients. The Indian journal of medical research, 139(6), 945-948.

Nyamwamu, N., Okari, O., & Gisesa, W. (2020). A survey of medicinal plants used by the gusii community in the treatment of digestive disorders and other inflammatory conditions. Journal of Medicinal Plants, 8(3), 21-33.

Oliveira, L. M., Long, A. S., Brown, T., Fox, K. R., & Weber, G. (2020). Melting temperature measurement and mesoscopic evaluation of single, double and triple DNA mismatches. Chemical science, 11(31), 8273-8287.

Olson, P. D., & Hunstad, D. A. (2018). Appendix two subversion of host innate immunity by uropathogenic escherichia coli. Sex differences in Uropathogenic *Escherichia coli* Urinary Tract Infection, 217.

Ompal Singh, Zakia Khanam,1 Neelam Misra, and Manoj Kumar Srivastava (2011).Chamomile (Matricaria chamomilla L.): An overview.Pharmacogn Rev. 5(9): 82–95.

Paggi, J. M., Belk, J. A., Hollingsworth, S. A., Villanueva, N., Powers, A. S., Clark, M. J., Chemparathy, A. G., Tynan, J. E., Lau, T. K., Sunahara, R. K., & Dror, R. O. (2021). Leveraging nonstructural data to predict structures and affinities of protein & ligand complexes. Proceedings of the National Academy of Sciences, 118(51), e2112621118.

Park, J. T., & Uehara, T. (2008). How bacteria consume their own exoskeletons (turnover and recycling of cell wall peptidoglycan). Microbiology and Molecular Biology Reviews, 72(2), 211-227.

Poovendran, P., & Ramanathan, N. (2014). In vitro study on antibiotic susceptibility pattern of biofilm producing uropathogenic *Escherichia coli* isolates and their molecular characterization. *Asian J. Pharm. Clin. Res*, *7*(3), 181-185.

Puay Yen Yap and Dieter Trau, (2019). Direct *E.coli* cell count at OD600. *Application Note*. Version 1.0 , Page 2 of 3.

Qiangde Duan, Rahul Nandre, Mingxu Zhou & Guoqiang Zhu (2017).Type I fimbriae mediate in vitro adherence of porcine F18ac+ enterotoxigenic *Escherichia coli* (ETEC).Annals of Microbiology volume 67, pages793–799.

Qiu, Y., Li, X., He, X., Pu, J., Zhang, J., & Lu, S. (2020). Computational methods-guided design of modulators targeting protein-protein interactions (PPIs). *European Journal of Medicinal Chemistry*, 112764.

Rahdar M, Rashki A, Miri HR, Rashki Ghalehnoo M,(2015). Detection of pap, sfa, afa, foc, and fim Adhesin-Encoding Operons in Uropathogenic *Escherichia coli* Isolates Collected From Patients With Urinary Tract Infection. Jundishapur J Microbiol. 8(8):e22647.

Raheema, R. H., & Alsaidi, M. A. Protective effect of chamomile recutita flowers extract against Urinary tract infection induced by Pseudomonas aeruginosa in Experimental mice models.

Ralf Rabus, Theo A. Hansen, Friedrich Widdel(2006); Dissimilatory Sulfate- and Sulfur-Reducing Prokaryotes. The Prokaryotes pp 659-768.

Randhawa, G. S., Soltysiak, M. P., El Roz, H., de Souza, C. P., Hill, K. A., & Kari, L. (2020). Machine learning using intrinsic genomic signatures for rapid classification of novel pathogens: COVID-19 case study. PloS one, 15(4), e0232391.

Ratna Prabha, D. P. Singh(2014). Analysis of Dinucleotide Bias and Genomic Signatures Across Cyanobacterial Genomes. JOURNAL OF ADVANCES IN BIOTECHNOLOGY.3(3).

Ren, Y.; Palusiak, A.; Wang, W.; Wang, Y.; Li, X.; Wei, H.; Wang, Q.(2016). A high-resolution typing assay for uropathogenic *Escherichia coli* based on fimbrial diversity. Front. Microbiol., 7, 623.

Rice, A. M., Morales, A. C., Ho, A. T., Mordstein, C., Mühlhausen, S., Watson, S., Cano, L., Young, B., Kudla, G., & Hurst, L. D. (2020). Evidence for strong mutation bias towards, and selection against, T/U content in SARS-CoV2: implications for attenuated vaccine design. *bioRxiv*.

Robert H. Baran and Hanseok Ko (2008). Detecting Horizontally Transferred and Essential Genes Based on Dinucleotide Relative Abundance. DNA Res. 15(5): 267–276.

Rosen D., Hooton T., Stamm W., Humphrey P., Hultgren S.(2007). Detection of intracellular bacterial communities in human urinary tract Infection. PLoS Med . 4(12):1949-1958.

Ruiz, J., Bertran, S., Sauca, G., Julià, A., Vila, X., Gómez, E., ... & Vila, J. (2005). Isolation of an amikacin-resistant *Escherichia coli* strain after tobramycin treatment of previous recurrent episodes of respiratory tract infections caused by Pseudomonas aeruginosa. Clinical microbiology and infection, 11(1), 71-73.

S. Karlin(1998).Global dinucleotide signatures and analysis of genomic heterogeneity.Curr Opin Microbiol . 1(5):598-610.

Sambrook, J., & Russell, D. W. (2001). Molecular Cloning-Sambrook & Russel-Vol. 1, 2, 3. Cold Springs Harbor Lab Press: Long Island, NY, USA.

Sanz-García, F., Gil-Gil, T., Laborda, P., Ochoa-Sánchez, L. E., Martínez, J. L., & Hernando-Amado, S. (2021). Coming from the Wild: Multidrug Resistant Opportunistic Pathogens Presenting a Primary, Not Human-Linked, Environmental Habitat. *International journal of molecular sciences*, *22*(15), 8080.

Sarowska, J., Futoma-Koloch, B., Jama-Kmiecik, A., Frej-Madrzak, M., Ksiazczyk, M., Bugla-Ploskonska, G., & Choroszy-Krol, I. (2019). Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. Gut pathogens, 11(1), 1-16.

Sarshar, M., Behzadi, P., Ambrosi, C., Zagaglia, C., Palamara, A. T., & Scribano, D. (2020). FimH and anti-adhesive therapeutics: a disarming strategy against uropathogens. Antibiotics, 9(7), 397.

Scribano, D., Sarshar, M., Prezioso, C., Lucarelli, M., Angeloni, A., Zagaglia, C., Palamara, A. T., & Ambrosi, C. (2020). D-Mannose treatment neither affects uropathogenic *Escherichia coli* properties nor induces stable fimh modifications. *Molecules*, *25*(2), 316.

Sharma, K., Thacker, V. V., Dhar, N., Signorino-Gelo, F., Cabrer, M. C., Dubois, A, & McKinney, J. D. (2021). Early invasion of uropathogenic *Escherichia coli* into the bladder wall by solitary bacteria that are protected from antibiotics and neutrophil swarms in an organoid model. bioRxiv, 2020-10.

Shepherd, A. K., & Pottinger, P. S. (2013). Management of urinary tract infections in the era of increasing antimicrobial resistance. Medical Clinics, 97(4), 737-757.

Shinomiya K, Inoue T, Utsu Y, Tokunaga S, Masuoka T, Ohmori A, Kamei C. Effects of kava-kava extract on the sleep-wake cycle in sleep-disturbed rats. Psychopharmacology. 2005;180:564–569.

Shukla, G., Subrahmanyam, C., Khess, A. S., & Kumar, C. S. (2021). CYSTOCRAN Tablet: A Natural Antibiotic Safeguard in Urinary Tract Infections. *Mediterranean Journal of Basic and Applied Sciences (MJBAS)*, *5*(2), 71-80.

Shuvra, S. K. (2017). Isolation of stx1 and stx2 genes from clinical and environmental samples of *Escherichia coli* (Doctoral dissertation, BRAC University).

Sin, M., Yoon, S., Kim, Y. B., Noh, E. B., Seo, K. W., & Lee, Y. J. (2020). Molecular characteristics of antimicrobial resistance determinants and integrons in Salmonella isolated from chicken meat in Korea. Journal of Applied Poultry Research, 29(2), 502-514.

Singaravelu, M.; Selvan, A.; Anishetty, S.(2014). Molecular dynamics simulations of lectin domain of FimH and immunoinformatics for the design of potential vaccine candidates. Comput. Biol. Chem., 52, 18–24.

Singh, O., Khanam, Z., Misra, N., & Srivastava, M. K. (2011). Chamomile (Matricaria chamomilla L.): An overview. Pharmacognosy reviews, 5(9), 82–95.

Skeparnias, I., & Zhang, J. (2021). Cooperativity and Interdependency between RNA Structure and RNA–RNA Interactions. *Non-coding RNA*, *7*(4), 81.

Slavchev G., Pisareva E., Markova N.(2009). Virulence of uropathogenic Escherichia coli. Journal of culture collections . 6(1):61-68.

Šmarda P, Bureš P (2012) The variation of base composition in plant genomes. Plant Genome Diversity, eds Wendel F, Greilhuber J, Doležel J, Leitch IJ (Springer, Vienna), Vol 1, pp 209–235

Soltani S, Emamie AD, Dastranj M, Farahani A, Davoodabadi A, Mohajeri P,(2018). Role of toxins of uropathogenic *Escherichia coli* in development of urinary tract infection. British Journal of Pharmaceutical Research. 21(1):e.JPRI.39188.

Soman N. Abraham and Yuxuan Miao (2015). The nature of immune responses to urinary tract infections. Nat Rev Immunol.; 15(10): 655–663.

Sora VM, Meroni G, Martino PA, Soggiu A, Bonizzi L, Zecconi A.(2021) Extraintestinal Pathogenic Escherichia coli: Virulence Factors and Antibiotic Resistance. Pathogens ;10(11):1355.

Soutis, C., Mohamed, G., & Hodzic, A. (2011). Modelling the structural response of GLARE panels to blast load. Composite Structures, 94(1), 267-276.

Spurbeck, R.R.; Dinh, P.C.; Walk, S.T.; Stapleton, A.E.; Hooton, T.M.; Nolan, L.K.; Mobley, H.L.(2012). *Escherichia coli* that carry vat, fyuA, chuA, and yfcV efficiently colonize the urinary tract. Infect. Immun., 80, 4115–4122.

Starks, C.M.; Miller, M.M.; Broglie, P.M.; Cubbison, J.; Martin, S.M.; Eldridge, G.R.(2021). Optimization and qualification of an assay that demonstrates that a FimH vaccine induces functional antibody responses in women with histories of urinary tract infections. Hum. Vaccines Immunother., 17, 283–292.

Supriya, S, Suresh, V, Sarfraz, A, et al. (2004).Evaluation of ESBL in urinary isolates. Indian J.Med. Res. 120(6): 553-559.

Svetlov, M. S., Plessa, E., Chen, C. W., Bougas, A., Krokidis, M. G., Dinos, G. P., & Polikanov, Y. S. (2019). High-resolution crystal structures of ribosome-bound chloramphenicol and erythromycin provide the ultimate basis for their competition. Rna, 25(5), 600-606.

Syed Awais Attique , Muhammad Hassan , Muhammad Usman , Rana Muhammad Atif, Shahid Mahboob , Khalid A. Al-Ghanim , Muhammad Bilal , Muhammad Zohaib Nawaz(2019) .A Molecular Docking Approach to Evaluate the Pharmacological Properties of Natural and Synthetic Treatment Candidates for Use against Hypertension. Int. J. Environ. Res. Public Health 16, 923.

Tabasi M.(2016). Genotypic Characterization of Virulence Factors in *Escherichia coli* Isolated from Patients with Acute Cystitis, Pyelonephritis and Asymptomatic Bacteriuria. J Clin Diagn Res.;10(2):1-7.

Tabasi, M., Karam, M. R. A., Habibi, M., Yekaninejad, M. S., & Bouzari, S. (2015). Phenotypic assays to determine virulence factors of uropathogenic *Escherichia coli* (UPEC) isolates and their correlation with antibiotic resistance pattern. *Osong public health and research perspectives*, 6(4), 261-268.

Tamadonfar, K. O., Omattage, N. S., Spaulding, C. N., & Hultgren, S. J. (2019). Reaching the end of the line: urinary tract infections. Microbiology spectrum, 7(3), 7-3.

Tayel, AA; El-Tras, WF (2009). "Possibility of fighting food borne bacteria by egyptian folk medicinal herbs and spices extracts". J Egypt Public Health Assoc. 84 (1–2): 21–32.

Terlizzi, M. E., Gribaudo, G., & Maffei, M. E. (2017). UroPathogenic *Escherichia coli* (UPEC) infections: virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. Frontiers in Microbiology, 8, 1566.

Thomas, W. E. (2007). Understanding the counterintuitive phenomenon of catch bonds. Current Nanoscience, 3(1), 63-77.

Tomašič, T.; Rabbani, S.; Jakob, R.P.; Reisner, A.; Jakopin, Ž.; Maier, T.; Anderluh, M.(2021). Does targeting Arg98 of FimH lead to high affinity antagonists? Eur. J. Med. Chem., 211, 113093.

Tooke, C. L., Hinchliffe, P., Bragginton, E. C., Colenso, C. K., Hirvonen, V. H. A., Takebayashi, Y., & Spencer, J. (2019). β-Lactamases and β-Lactamase Inhibitors in the 21st Century. *Journal of molecular biology*, *431*(18), 3472-3500.

Toombs-Ruane, L. J., Benschop, J., French, N. P., Biggs, P. J., Midwinter, A. C., Marshall, J. C., ... & Burgess, S. A. (2020). Carriage of extended-spectrum-beta-lactamase-and AmpC beta-lactamase-producing *Escherichia coli* strains from humans and pets in the same households. Applied and environmental microbiology, 86(24), e01613-20.

Toscano, Y.G.; Támara, M.F.; Urbina, M.C.; Rodriguez, L.G.; Martínez, A.B.(2020). Perfiles de los fenotipos de resistencia en *Escherichia coli* y Klebsiella pneumoniae en Barranquilla, Colombia. J. Biomed. Sci., 9, 15–24.

Totsika, M., Kostakioti, M., Hannan, T. J., Upton, M., Beatson, S. A., Janetka, J. W., ... & Schembri, M. A. (2013). A FimH inhibitor prevents acute bladder infection and treats chronic cystitis caused by multidrug-resistant uropathogenic *Escherichia coli* ST131. The Journal of infectious diseases, 208(6), 921-928.

Touaibia, M., Krammer, E.-M., Shiao, T. C., Yamakawa, N., Wang, Q., Glinschert, A., Papadopoulos, A., Mousavifar, L., Maes, E., Oscarson, S., Vergoten, G., Lensink, M. F., Roy, R., & Bouckaert, J. (2017). Sites for Dynamic Protein-Carbohydrate Interactions of O- and C-Linked Mannosides on the E. coli FimH Adhesin. *Molecules*, *22*(7), 1101.

Troy CS, MacHugh DE, Bailey JF, Magee DA, Loftus RT, Cunningham P, Chamberlain AT, Sykes BC, Bradley DG(2001). Genetic evidence for Near-Eastern origins of European cattle. Nature. vol. 410, p. 1091.

V. wintzingerode, F., Göbel, U. B., & Stackebrandt, E. (1997). Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS microbiology reviews, 21(3), 213-229.

Vashist, H., & Sharma, V. (2017). INHERENT MEDICINAL USES OF PLANTS. Innovat International Journal Of Medical & Pharmaceutical Sciences, 2(5).

Vernaz, G., Malinsky, M., Svardal, H., Du, M., Tyers, A. M., Santos, M. E., Durbin, R., Genner, M. J., Turner, G. F., & Miska, E. A. (2021).

Mapping epigenetic divergence in the massive radiation of Lake Malawi cichlid fishes. Nature Communications, 12(1), 1-13.

Veronika Tchesnokova, Pavel Aprikian, Dagmara I Kisiela (2011).Type 1 Fimbrial Adhesin FimH Elicits an Immune Response That Enhances Cell Adhesion of Escherichia coli. Infection and Immunity 79(10):3895-904.

Vestby, L. K., Grønseth, T., Simm, R., & Nesse, L. L. (2020). Bacterial Biofilm and its Role in the Pathogenesis of Disease. *Antibiotics (Basel, Switzerland)*, *9*(2), 59.

Wang, Y., Zeng, Z., Liu, T. L., Sun, L., Yao, Q., & Chen, K. P. (2019). TA, GT and AC are significantly under-represented in open reading frames of prokaryotic and eukaryotic protein-coding genes. Molecular Genetics and Genomics, 294(3), 637-647.

Wellens, A., Garofalo, C., Nguyen, H., Van Gerven, N., Slättegård, R., Hernalsteens, J. P., ... & Bouckaert, J. (2008). Intervening with urinary tract infections using anti-adhesives based on the crystal structure of the FimH–oligomannose-3 complex. PloS one, 3(4), e2040.

Wellens, A., Lahmann, M., Touaibia, M., Vaucher, J., Oscarson, S., Roy, R., & Bouckaert, J. (2012). The tyrosine gate as a potential entropic lever in the receptor-binding site of the bacterial adhesin FimH. Biochemistry, 51(24), 4790-4799.

Werneburg, I., Hinz, J. K., Gumpenberger, M., Volpato, V., Natchev, N., & Joyce, W. G. (2015). Modeling neck mobility in fossil turtles. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution, 324(3), 230-243.

Winn, W., Allen, S., Janda, W., Koneman, E., Procop, G., Schreckenberger, P. and Woods, G. (2006) Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th Edition, Lippincott Williams and Wilkins, New York.

Yakovchuk P, Protozanova E, Frank-Kamenetskii MD (2006) Base-stacking and base-pairing contributions into thermal stability of the DNA double helix. Nucleic Acids Res 34(2):564–574.

Yamamoto, S. (2007). Molecular epidemiology of uropathogenic Escherichia coli. Journal of infection and Chemotherapy, 13(2), 68-73.

Yazdanpour, Z., Tadjrobehkar, O., & Shahkhah, M. (2020). Significant association between genes encoding virulence factors with antibiotic resistance and phylogenetic groups in community acquired uropathogenic *Escherichia coli* isolates. *BMC microbiology*, *20*(1), 1-9.

Yingwei Wang, Kathleen Hill, Shiva Singh, Lila Kari (2005).The spectrum of genomic signatures: from dinucleotides to chaos game representation. Gene .346:173-85.

Zaidi, S. H. (2014). Ethics and Evolution of Medicine. In Ethics in Medicine. Springer, Cham. (pp. 133-168).

Zalewska-Piątek, B., & Piątek, R. (2020). Phage therapy as a novel strategy in the treatment of urinary tract infections caused by E. coli. *Antibiotics*, *9*(6), 304.

Zeighami, H., Valadkhani, F., Shapouri, R., Samadi, E., & Haghi, F. (2019). Virulence characteristics of multidrug resistant biofilm forming Acinetobacter baumannii isolated from intensive care unit patients. BMC infectious diseases, 19(1), 1-9.

Zhi, S., Stothard, P., Banting, G., Scott, C., Huntley, K., Ryu, K.,& Neumann, N. F. (2020). Characterization of water treatment-resistant and multidrug-resistant urinary pathogenic *Escherichia coli* in treated wastewater. Water research, 182, 115827.

Zhu, J., & Hill, J. E. (2013). Detection of *Escherichia coli* via VOC profiling using secondary electrospray ionization-mass spectrometry (SESI-MS). Food microbiology, 34(2), 412-417.

الخلاصة:

تعد التهابات المسالك البولية ، واحدة من أكثر الحالات المرضية شيوعًا في كل من المجتمع والمستشفيات. وتشير التقديرات إلى أن حوالي 150 مليون شخص في جميع أنحاء العالم يصابون بالتهاب المسالك البولية كل عام ، مع تكاليف اجتماعية عالية من حيث العلاج في المستشفيات والنفقات الطبية.

من بين مسببات الأمراض البولية الشائعة المرتبطة بتطور عدوى المسالك البولية ، كانت الإشريشية القولونية المسببة للأمراض البولية ، هي السبب الرئيسي.

تمتلك سلالات الاشريشية القولونية UPEC عددًا كبيرًا من عوامل الضراوة (مثل الاسواط والاهداب والسموم ،و أنظمة اكتساب الحديد) التي تساهم في قدرتها على التسبب في المرض ، وكذلك لها القدرة على الالتصاق بالخلايا الظهارية في المسالك البولية يمثل أهم عوامل المحددات الإمراضية.

في هذه الدراسة ، تم الحصول على إجمالي 100 عينة من مرضى يعانون من التهابات المسالك البولية الذين توجهوا إلى مدينة الامام الحسين الطبية بمحافظة كربلاء المقدسة لفترة نتراوح من فبراير 2021 إلى أكتوبر 2021. تراوحت أعمار المريضى، بين (15 عامًا - 78 عامًا).

في هذه الدراسة ، من بين 89 مسببات أمراض المسالك البولية ، وجد أن 63 عينة فقط (70.79٪) هي بسبب الإشريشية القولونية و 26 (29.21٪) من الكائنات الحية الأخرى.

تم إخضاع العزلات لإختبار الحساسية للمضادات الحيوية بطريقة إنتشار قرص كيربي باور المعدلة ، و أظهرت النتائج أن كل هذه العزلات حساسة تماماً (97%) للامبينيم و 94% للكلورامفينيكول. أظهرت العزلات أيضًا حساسية إلى حد أقل لكل من النورفلوكساسين(72%) والجنتاميسين(45%). كما أظهرت الدراسة أن العزلات كانت مقاومة لمضادات الاميكاسين(11%) والببراسيلين/تازوباكتام(15%) ، و الكو- ترايموكسازول (13%) و الاموكسيكلاف(15%).

بالمقابل أظهرت العزلات مقاومة أقل لكل من الاريثروميسين(35%) والتتراسيكلين (45%) والتوبراميسين(41%) والأمبيسلين(28%).

تم إجراء الكشف الجزيئي عن جينات *Fimh* باستخدام بادئة PCR محددة للتحقق من وجود هذه الجينات في جميع العزلات. كذلك تم اختبار تسلسل الحمض النووي على 63 عينة سريرية كانت إيجابية للإشريبية القولونية. تم إجراء تفاعلات التسلسل على نواتج تفاعلات البلمرة لجين ال*Fimh*.

تم كذلك تحديد كيفية تنظم قواعد ثنائي النوكليوتيد في جزء صغير من الحمض النووي من خلال تسلسل الحمض النووي ، حيث كان مدى قيمة توقيع ثنائي النوكليوتيد هي (0.14-0.06) لمعظم عينات *fimH* من الإشريكية القولونية.

تظهر نتائجنا التشابه في ثنائية التوقيعات في جميع أنحاء الجينوم ، وهذا يعني أن جميع العينات لها تجانس وراثي ولديها أنماط فريدة خاصة بالأنواع. ويستفاد منه استخدامه كبصمة تميزية بين الكائنات الحية.

كذلك يتشابه نطاق C + G % لجميع العينات تقريبًا، حيث تروحت قيمها بين (7.44% - 8.15%)، مما يعطي علاقة عالية بين العينات.

أظهرت النتائج أن تكرار GC و CG هو الأكبر من بين تواقيع النوكليوتيدات الأخرى ، وأن تكرار AG و TC هو الأصغر بين ثنائي النوكليوتيدات الأخرى على التوالي.

كما تم استخدام نهج الالتحام للعثور على مثبط مناسب لل *Fimh* ، تم عمل التحام الأربطة بين الابجنين وبروتين *Fimh* ،حيث اعطت درجة عالية من الالتحام ، لذلك ، يمكن أن يوفر الابجنين الحماية من التهاب المسالك البولية في الفئران بنسبة 75% عند تناوله كوسيلة وقائية ، وحماية حوالي 50% من الفئران بعد الإصابة بالعدوى ، لذلك أوصينا في دراستنا باستخدام الابجنين الموجود في البابونك كمنتج عشبي طبيعي (غير مضاد حيوي) في العلاج المساعد مع أدوية أو منتجات أخرى في علاج المسالك البولية. وأيضا نوصي بتطوير مثبطات أخرى لل *fimh* تكون امنة وفعالة لعلاج حالات التهاب المجاري البولية وخصوصا بعد تفاقم حالات المقاومة البكترية للمضادات الحيوية.