Treatment of urinary tract infections that caused by Escherichia coli through the suggest fimH inhibitors from molecular docking approach

Alaa Abdul Razaq Alnuaimi [1], Mohammed Sabri Abdul Razzaq[2], Hassan M. Abo almaali [3]

[1]. Medical microbiology, college of medicine, university of Babylon, Babylon, Iraq
[2]. Medical microbiology, college of medicine, university, Babylon, Iraq
[3]. Laboratory Sciences/ College of Pharmacy, university of Karbala, Karbala, Iraq
*Corresponding Author: tocson11@yahoo.com

Abstract:

Escherichia coli are one of the most important organisms that cause urinary tract infection (UTI) in more than 95% of patients with UTI. This study aims to search for inhibitors of (fimH) by docking method using computer programs and websites specialized for this purpose. This study involved (63 samples) with positive E.coli was collected from patients with UTI during the period from February 2021 to October 2021, from an Iraqi hospital in Karbala. Full laboratory investigation for E.coli would be made to detect FimH and prediction suitable inhibitors. FimH was found in most E.coli isolates, it was found in 61(96.82%) of 63 samples. Chamomile appears to be a good FimH inhibitor, with a docking score of (-9.4), and can reduce UTI in roughly 50 percent of rats examined. Most pathogenic Type 1 fimbriae, which are hair-like appendages, can be expressed by E. coli strains. FimH protein starts out as a 300-amino-acid precursor before being transformed into a 279-amino-acid mature version. These fimbriae aid in the bacteria's colonization of numerous host tissues by mediating binding to D-mannose-containing structures. The chamomile was predicted as a suitable inhibitor of (fimH), and it was tested on rats. The results showed that it has a good inhibitory property; in addition to that, it is safe and has other benefits.

Keyword: **E.coli, molecular docking, FimH, UPEC, urinary tract infection.**

1. Introduction

Uropathogenic Escherichia coli (UPEC) is the microorganism that most frequently causes urinary tract infections and is considered responsible for more than 95 % of all urinary tract infections [1]. Most Escherichia coli strains can produce type I fimbriae, which are rod like appendages made up of roughly 1,000 FimA protein subunit and a few percent minor components. These fimbriae mediate the bacteria's attachment to D-mannose-containing structures, allowing them to bind in a variety of host tissues. D-mannose and most of its derivatives were shown to be very strong antagonist of type 1 fimbria-mediated attachment in all cases, whereas all saccharide that did not contain D-mannose had not blocking properties. Each level in the UTI pathogenesis is kicked off by adhesion[2]. Urinary tract infection (UTI) generally starts with a uropathogen infecting the periurethra in the guts, accompanied by colonization of the urethra and then transfer of the bacteria to the bladder, necessitating the usage of appendages such as flagella and pili. In the bladder, the consequences of complex host-pathogen interaction ultimately decide whether uropathogens are spread or eliminated. E.coli's capacity to bind to uro-epithelial cells is greatly dependent on fimbriae type I, which are distinguished by the presence of an adhesion called FimH at the tip of the fimbriae. FimH uses the catch–bond binding mechanism to bind to end of epitope of

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high mannosylated glycan's attached to uro-plakin 1a (UP1a), a receptor exclusively expressed at the surface of urothelial cells[3].

The use of computational analysis to anticipate the physicochemical, spectral, and biological features of a newly synthesized drug is common. The main goal of this research was to create new biologically active fimH inhibitor compounds and examine their binding affinities and interactions with E. coli FimH using computational techniques in order to identify an effective inhibitor of bacterial function [4].

Molecular docking is useful in development of drug, because it assist in the identification of active or lead compounds from a library of natural molecules. It's one of the most used virtual screening techniques, especially when the target protein's three-dimensional structure is available. Docking allows for the prediction of ligand–target binding affinity as well as the structure of the protein–ligand complex, both of which are important for lead optimization. We examined the physicochemical attributes and toxicity potential FimH antagonists before starting the molecular docking analysis [5]

After choosing some suitable medical compound and docking it by Mcule web site, We choose 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 [6].

By using the "Mcule website," the ligands were docked to the Chamomilla and FimH proteins, as reported by our study.

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2. Materials and Methods

This study involved (63 samples) with positive Escherichia coli collected from patients with UTI, the samples collected from both sexes and different ages, who attended to hospitals of Karbala Province, during the period from February 2021 to October 2021.The age of patients ranged from 15 to 60 years.

2.1 Fast Identification System by VITEK-2 Compact System

The automated VITEK-2 compact system successfully identified E. coli. It's a gadget that uses a methodology to identify an organism based on the characteristics of information and knowledge about the microbe and reaction in question. The producers' instructions were followed in order to develop a confirmatory tool for biochemical tests[7]

2.2 DNA extraction and PCR

DNA was extracted from clinical isolates. Each isolate's colony was grown overnight at 37oC after being cultivated and put into five ml of BHI (Brain Heart Infusion). A Genomic DNA kit provided by the manufacturing company was used to harvest DNA from bacterial cells in these isolate cultures. The DNA acquired as templates was used in all PCR assays. The virulence gene listed in Table 1 was found in PCR utilizing nucleic acid (DNA) extracted from E.coli cells as a template. A single reaction combination contained 2,5 microliters of upstream primers, 2,5 microliters of downstream primers, 5 microliters of DNA extraction, 12,5 microliters of master mix, and 2,5 microliters of nuclease free water. After that, the PCR products were run on a 1.5 percent agarose gel.

2.3 Molecular docking

The molecular docking method are be used to describe the molecular level connection among a fine molecules and a protein, making us to notice fine molecule behavior at specific protein binding sites and deduce critical biochemical processes. Molecular docking can performed in Mcule.com, which is a web-based drug discovery service[8].

2.4 Animals study

Four groups of rats would be taken, weighted 150-200 gm, and inoculated with 10^8-10^{10} colony forming units (CFU) of UPEC bacteria directly into the bladder by transurethral catheterization[9] (by using angio-catheter of 24G, diameter=0.7mm, length =19mm) and small injection.

2.5 FimH inhibitor study

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 are usually single-housed in metabolic cages for 16 to 24 hours[10]. 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 C for 20 hrs. The colonies formed and then counted. The extent of bacteriuria was graded as follows: no excretion of bacteria (<10^3 cells/ ml), occasional excretion (10^2-10^3 cells/ml), and excretion of bacteria (10^{10} cells/ml)[11]

2.6 Bacterial cell count

The Photopette® portable spectrophotometer is used to count E.coli cells at OD600. The approach is simple and quick to execute in the bio reactor or cell culture hood. In the cell culture flask, E.coli may be directly detected at 600 nm. The procedure may be carried out anywhere and does not need the use of a laboratory[12].

Table 1. Forward and reverse primers used in this study

Primers	Sequence	Product size(bp)	Reference
FimH forward	5'-ATGAAACGAGTTATTACCCT-3’	903	(13)
FimH reverse	5'-TTATTGATAAACAAAAGTCACG-3′		

3. Results and Discussion

In this study, figure 1. Demonstrated that Uropathogenic E.coli samples are identified using biochemical test and screened using PCR technician. The FimH quality was improved with explicit preliminaries and revealed a band of around 903bp. FimH quality was found in 61 of 63 samples of the UPEC strain samples (96.82%), indicating that fimH is one of the most important virulence factors in UPEC bacteria to cause a UTI**.**

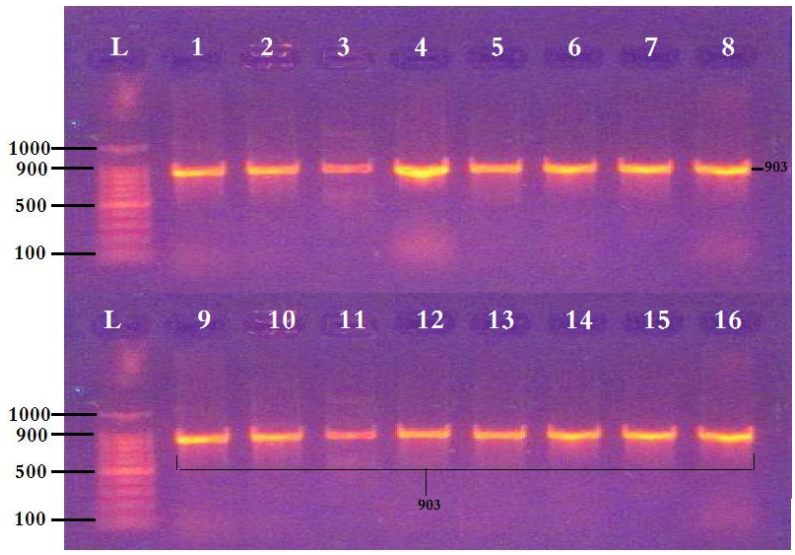

Figure 1. FimH PCR results are seen in Ultraviolet light at 300 nm after staining with E.B stain on a one percent of agarose gel electrophoresis at 71 volts for 51 minutes. (L): 1000 bp ladder; for these genes, lane (1-16) was positive; the product size is 903 bp.

Figure 2. Shown Electron density map for oligomannose-3 in the FimH receptor-binding site with Chamomilla to blocking FimH active site with docking scores (-9.4).

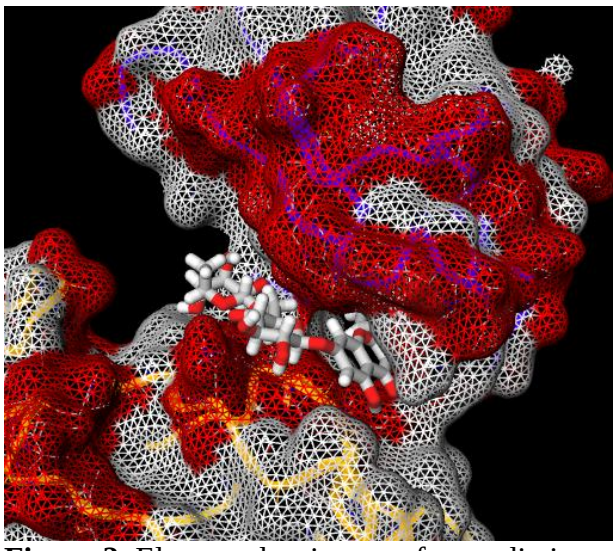

Figure 2. Electron density map for prediction of FimH-target binding

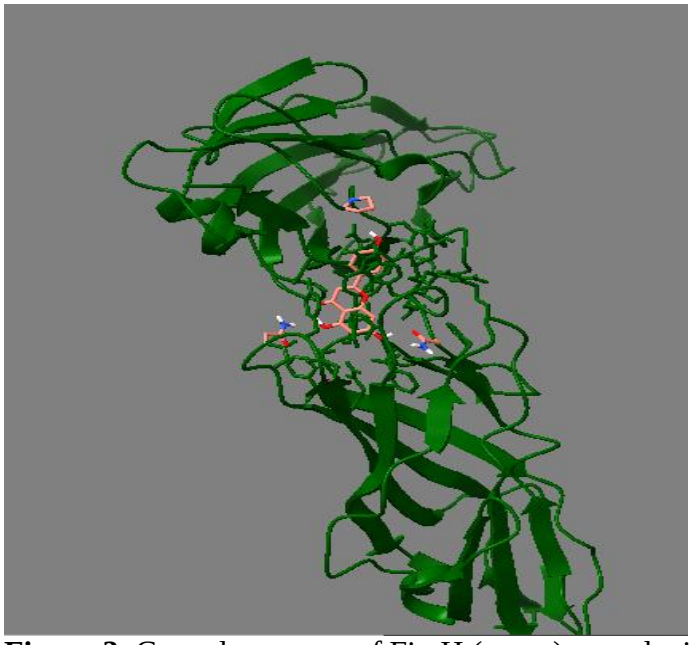

Figure 3. Crystal structure of FimH (green) complexing with Chamomilla.

In table 2 ,a groups of animal show different result before and after giving chamomile.

Table 2. Different result on animals group treated with chamomile

Test / Animals group	General Urine Examination of rats	Bacterial Cell Count in rats urine
G1- Control (without drug ,without infection)	20(100%) All negative*	20(100%) All negative $<10^4$
G2 Animals taking Drug before infec-tion by 2 days	10 from 20(50%) negative 10 from 20(50%) positive**	10 from 20(50%)-ve$<10^4$ cfu 10 from 20(50%)+ve$>10^7$ cfu
G3 Animals taking Drug after infection	7 from 20(35%) negative 13 from 20(65%) positive	7 from 20(35%) -ve$<10^4$ cfu 13 from 20(65%) +ve$>10^7$ cfu

by 2 days		
G4 Animals Infected , without drug given	20(100%) all positive	20(100%) > 10^7 cfu

*** Negative (-ve) = No infection**

****Positive (+ve) = Infection**

Discussion

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[14].

Ordinarily, without binding to epithelium cells bacteria can be washed out by urination process. Type 1 fimbriae are coded in the genome of most almost 95% E. coli strains[15]. The presence of fimbriae is required for colonization of the urinary system. P, type 1, S, and F1C fimbriae are among the stickiest organelles expressed by uropathogenic Escherichia coli. More than 90% of all uropathogenic E. coli develop type I, or mannose sensitive fimbriae[16]. 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 ridge (Ile13, Phe1, and Phe142) surrounding its entry, a FimH inhibitor should be overlaid onto the FimH lectin domain combination with oligomannose-3[18]. The affinity (grid) maps sized at 60 60 60° were generated using an auto grid algorithm, with the goal of targeting the grid coordinates in the target protein's catalytic site (Chamomilla 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[18]

Figures 2. And 3. Show the molecular docking of binding between FimH and chamomile. Chamomile, technically known as Matricaria recutita, is a medicinal plant that is native to Western Europe and North Asia. It is distinguished by its herbaceous bearing and flowering [19].

In systemic molecular genetics and computer-assisted drug design, molecular docking is an important technique [20]. 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[21]. 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 [22]. Molecular docking between fimH and chamomile give high degree of binding (docking score -9.4), which mean low energy needed for molecule binding (ligand and target) to form stable complex [(23].

Table 2. 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 (chamomile) 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 chamomile, so it's healthy, with no disease, and all laboratory tests reveal the negative infection. The second group is pre-feeding with chamomile for three days and then inoculated with 10^7-10^9CFU of UPEC by intraurethral catheterization. The concentration of chamomile that was used in this experiment was prepared by adding 5 drops of 1.2% liquid extract in 100ml of water [24] and animals would be fed by this solution throughout the day without being given water. The result showed from 20 animals, 10(50%) of rats would be not infected with UTI, and 10(50%) of rats getting an infection [25] . The third group was infected before two days of feeding with chamomile, the result shows about 13(65%) of rats getting an infection and 7(35%) not infected. We notice the infection would be increased, when chamomile 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 [3]. And when the concentration of the drug would be increased after two days, we can notice the healing effect of chamomile on the one-third number of animals group in the third group[25].

In The fourth group, all animals would be infected without any treatment, so all animals getting an infection showed more than 10^8 CFU in their urine, because no FimH antagonist (chamomile) was present. The previous result shows that type 1 fimbria is very important for E.coli in UTI be-

cause of its ability to attach with uroepithelial cell by FimH protein and render elimination by urine and facilitate colonization and pathogenicity. Also, this result explained a good effect of chamomile in UTI treatment. Also, chamomile is safer therapy and can be given in treatment for many diseases such as cold and chest disease and can be given as prophylaxis in high-risk factors for UTI. Chamomile can provide protection from UTI in rats by 50% when it's taken as prophylaxis, and protect about 35% of rats after getting an infection, so in our study, we recommended using chamomile as a natural herbal product in adjuvant therapy with other drugs or products in UTI treatment.

Authors' Contribution

Study concept and design: H.M.A
Acquisition of data: A.A.R.
Analysis and interpretation of data: A.A.R.
Drafting of the manuscript: M.S.A
Critical revision of the manuscript for important intellectual content: A.A.R.
Statistical analysis: H.M.A
Administrative, technical, and material support: M.S.A

Ethics

This is to certify that the above mentioned title was considered by the Scientific and Ethical Committee of College of Pharmacy, University of Kerbala meets the requirements of the Helsinki Declaration and was approved on September 1, 2020, under the project number 2022AN2

Conflict of Interest

The authors declare that they have no conflict of interest.

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