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EVALUATION OF *MYCOPLASMA HOMINIS* PROTEOME TO IDENTIFY THE POTENTIAL VACCINE CANDIDATE PROTEINS

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ABSTRACT: Objective: *Mycoplasma hominis*is a gram-negative bacteria belonging to class Mollicutes that is commonly present in men and women of reproductive age. At present, there is no effective prophylaxis for *Mycoplasma hominis*, as it has gained resistance for the drugs hence an *in-silico* approach was undertaken to find out the peptide-based vaccine for the pathogen. **Methods:** In women, it affects the genital tract and involved in causing pelvic inflammatory disease, ectopic pregnancy, early delivery, miscarriage and prolonged infection may lead to infertility. *M. hominis* can also be transmitted from mother during childbirth and cause fever and infection in the new-born baby. All the 529 protein sequences of *Mycoplasma hominis* were taken in FASTA format from the UniProt proteome database. Antigenicity study was done for all the proteins of the pathogen using VaxiJen v2.0 and the proteins with high antigenicity scores were taken for further analysis like structural and functional analysis using InterPro, molecular docking using Autodock and molecular simulation study was carried out using Charmm. **Results:** The study identifies 50S ribosomal protein L28, Potassium transporter KtrB, Cobalt ABC transporter permease and Membrane protein as the putative vaccine candidates which are membrane-bound with high antigenicity properties and show good molecular docking and simulations results. **Conclusion:** From the study and analysis of the results, the proteins identified might work as a vaccine against the pathogen as they have passed all the necessary *in-silico* screenings. However, the *in-silico* results have to be validated by *in-vitro* studies.

INTRODUCTION: *Mycoplasma hominis*is a gram-negative bacterium often found in genito-vaginal tracts of women and in sexually active adult males [1]. The human pathogen is transmitted by direct contact during the intercourse and vertically from mother to offspring either during birth or in uterus [2].

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The pathogenicity of mycoplasmas in the female genital tract was previously confirmed by the presence of anti-mycoplasma antibodies among women with intra-amniotic infection and postpartum fevers [3]. The symptoms of the bacterium involve vaginal discharge, genital warts and other inflammatory responses. Some of the studies link the pathogen to Pelvic Inflammatory disease and preterm labor [4].

The scientific classification of the pathogen is given in **Table 1**. The epidemiology of the pathogen is associated with colonization in the genitourinary tract and is seen in the patients of young age who are sexually active.

The clinical manifestations of the pathogen infection include Pelvic Inflammatory Disease, cervicitis, urethritis, post-partum fever, stillbirth, brain abscess, pyelonephritis. Pelvic inflammatory disease has minimal symptoms like fever, pelvic and abdominal pain and tenderness of the tissues of uterine, cervix and adnexal. Cervicitis and urethritis lead to vaginal discharge in women and urethral discharge in men and irritation during the urine pass. Epididymitis is the swelling of epididymis underlying the testis within the scrotum[5].

The pathogen in some cases, is associated with the other pathogen *U. urealyticum* [6]. However, a significant relationship exists between *U. urealyticum* and *M. hominis* and male infertility [7]. There are several pathogens known to contribute to male infertility; the two types that most commonly occur are genital urea plasma and mycoplasma. They are ubiquitous resulting in colonization of the genitalia by sexual contact [8]. Several studies have demonstrated that *U. urealyticum* and *M. hominis* play an etiologic role in male infertility, with these infections changing parameters of semen such as spermatozoa density and motility [9]. The current treatment options include commonly prescribed antibiotics, and very few are effective against bacterial infections.

Nowadays, bacteria have gained resistance for antibiotics therefore vaccination can be a more effective way of treating bacterial infections [10]. With the data from sequencing projects and advances in proteomics and genomics, the field of vaccine design and development have emerged to be more promising [11].

Particularly the epitope-based vaccines elicit specific and accurate immune responses [12]. Hence in the current work, various *in-silico* tools are used to obtain the immunogenic proteins from the bacteria which significantly reduces the time and cost in the developmental process. All the proteome of the bacteria was collected and checked for antigenicity, allergenicity, structural and functional analysis for identifying the more immunogenic proteins within the bacterial proteome. Finally, an attempt was made to design an effective peptide-based vaccine. The results of the study identify novel vaccine candidate proteins for development of a vaccine against *Mycoplasma hominis*.

TABLE 1: SCIENTIFIC CLASSIFICATION OF THE PATHOGEN *M. HOMINIS*

Domain	Bacteria
Division	Firmicutes
Class	Mollicutes
Order	Mycoplasmatales
Family	Mycoplasmataceae
Genus	*Mycoplasma*
Species	*M. hominis*

MATERIALS AND METHODS:

Retrieval of all the Proteins of *Mycoplasma hominis*: The proteome of the *M. hominis* contains 529 proteins. All the 529 proteins were retrieved from UniProt [13]. Proteome database in FASTA format.

Structural and Functional Analysis of Proteins: Protein structural and functional analysis is important to know their role in the organism's survival. The physic-chemical properties of proteins were studied using ProtParam [14]. Interpro [15] was used to get the number of conserved domains and other important sites within the proteins.

Localization, Antigenicity and Allergy Study of the Proteins: *M. hominis* lacks cell wall hence is a gram-negative bacterium. Sub-cellular localization prediction and Antigenicity study for all the retrieved proteins was done using SOSUIGramN [16] and VaxiJen v2.0 [17]. AlgPred [18] was used to eliminate the allergic proteins and the non-allergic proteins were considered for further analysis.

Protein Modelling, Validation and Protein Optimization: Due to the unavailability of the protein structures in the database, the three-dimensional structure of the proteins was generated using Swiss Model [19]. The protein models generated were then validated for the correctness using Ramchandran Plot analysis tools Rampage [20] and Procheck [21]. The models with more than 90% accuracy were then subjected to energy minimization to obtain a stable structure before docking using Swiss PDB Viewer [22].

Molecular Docking and Molecular Simulation study: The docking studies were performed using AutoDock [23]. To obtain the specific interactions between the drug and proteins the active binding sites for proteins were predicted using Metapocket [24]. As the study focuses on designing the *in-silico*

vaccine for the pathogen, the drug azithromycin [25] was used for docking to know the stability and sustainability of the proteins against the drug. To further know the suitability of the proteins to be the vaccine candidates, Molecular dynamics and simulation studies were performed using CHARMM [26]. VMD and NAMD are files compatible with CHARMM. 100000 molecular dynamic steps were run for getting intense results.

RESULTS AND DISCUSSION: 529 proteins of the *M. hominis* were collected in fasta format from the UniProt Proteome database.

The parameters like molecular weight, theoretical pI, number of amino acids, amino acid composition, atomic composition, extinction coefficient, estimated half-life, aliphatic index, instability index, and grand average of hydropathicity (GRAVY) were obtained from the ProtParam, and the functional analysis of proteins was done using InterPro by classifying them into families, predicting domains and other important sites.

Table 2 below gives the details of the protein parameters.

TABLE 2: PROTPARAM RESULTS FOR THE FINAL VACCINE CANDIDATE PROTEINS

Protein	Number of amino acids	Molecular weight	Theoretical pI	Extinction coefficients	Estimated half-life	Instability index	Aliphatic index	(GRAVY)
50S ribosomal protein L28	65	7112.27	11.17	1031	30 hours	37.06	79.54	-0.654
Potassium transporter KtrB	515	58433.89	9.6	62230	30 hours	33.29	109.42	0.485
Cobalt ABC transporter permease	315	35998.12	9.85	48360	30 hours	24.41	313.21	0.602
Membrane protein	174	20769.85	10.47	40910	30 hours	21.14	120.46	0.156

The protein screening was done based on the Vaxijen scores with a threshold of 0.4. The proteins with antigenicity scores more than 0.5 were considered for further investigation. Then the proteins were checked for the localization using SOSUIGramN. Non-allergenic proteins were then identified using AlgPred, the mapping of protein with the IgE epitopes is done to predict the allergen score. **Table 3** gives the vaxijen score and the localization and allergen scores for the vaccine candidate proteins.

TABLE 3: VAXIJEN SCORE AND THE LOCALIZATION PREDICTION FOR THE PROTEINS

Protein name	Vaxijen score	Localization
50S ribosomal protein L28	0.8157	Cytoplasmic
Potassium transporter KtrB	0.5165	Membrane
Cobalt ABC transporter permease	0.5623	Membrane
Membrane protein	0.8286	Membrane

Due to the lack of the 3D structure of the proteins in the database, homology modeling was performed using SwissModel using Alignment Mode. All the models with good Qmean scores were then validated for the accuracy of the structures with RAMPAGE and PROCHECK, **Table 4** gives the validation scores. The predicted models were subjected to energy minimization using SwissPDB Viewer. The XYZ coordinates or the active sites were predicted using the prediction tool Metapocket to get the site-specific interactions.

TABLE 4: PROTEIN MODEL VALIDATION RESULTS FOR THE PROTEINS USING RAMPAGE

Protein name	Validation results (%)
50S ribosomal protein L28	92.3
Potassium transporter KtrB	93.8
Cobalt ABC transporter permease	90.9
Membrane protein	92.3

TABLE 5: XYZ COORDINATES USED FOR AUTODOCK AND THE BINDING ENERGIES FOR THE PROTEINS

Protein name	X	Y	Z	Binding Energy
50S ribosomal protein L28	-8.86	-9.139	5.022	-7.53
Potassium transporter KtrB	-15.102	-0.031	97.555	-9.92
Cobalt ABC transporter permease	-19.216	77.078	-21.568	-5.49
Membrane protein	-69.99	9.488	-6.571	-9.31

The drug was used for docking azithromycin. The protein-drug docking was performed with AutoDock, the binding energies for the protein-drug complexes were noted and used for further

investigation. **Table 5** gives the binding energies. The interactions the drug azithromycin with proteins is shown in **Fig. 1**. The complexes with minimum binding energy were then taken for the molecular dynamics simulation with 100000 steps. CHARMM simulation package was used for molecular simulation studies. **Fig. 2** shows the distance and histogram graphs of simulation.

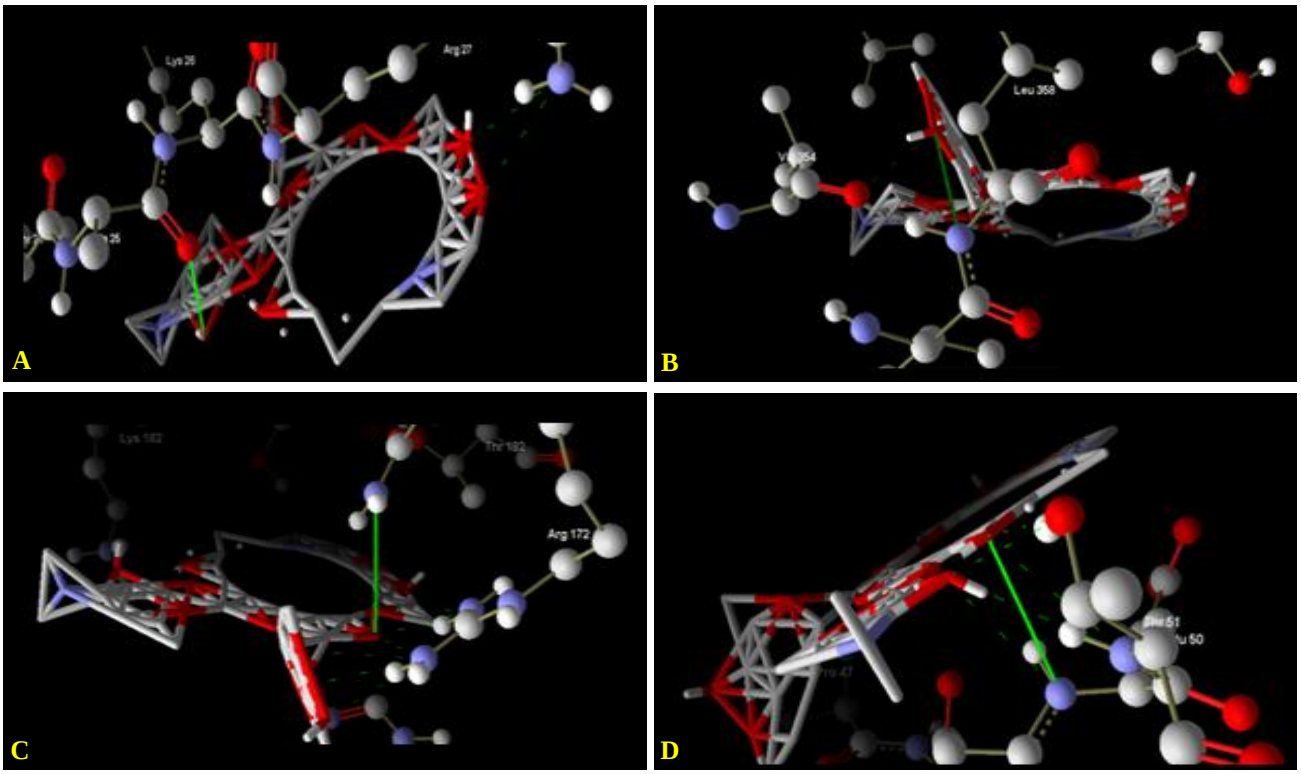
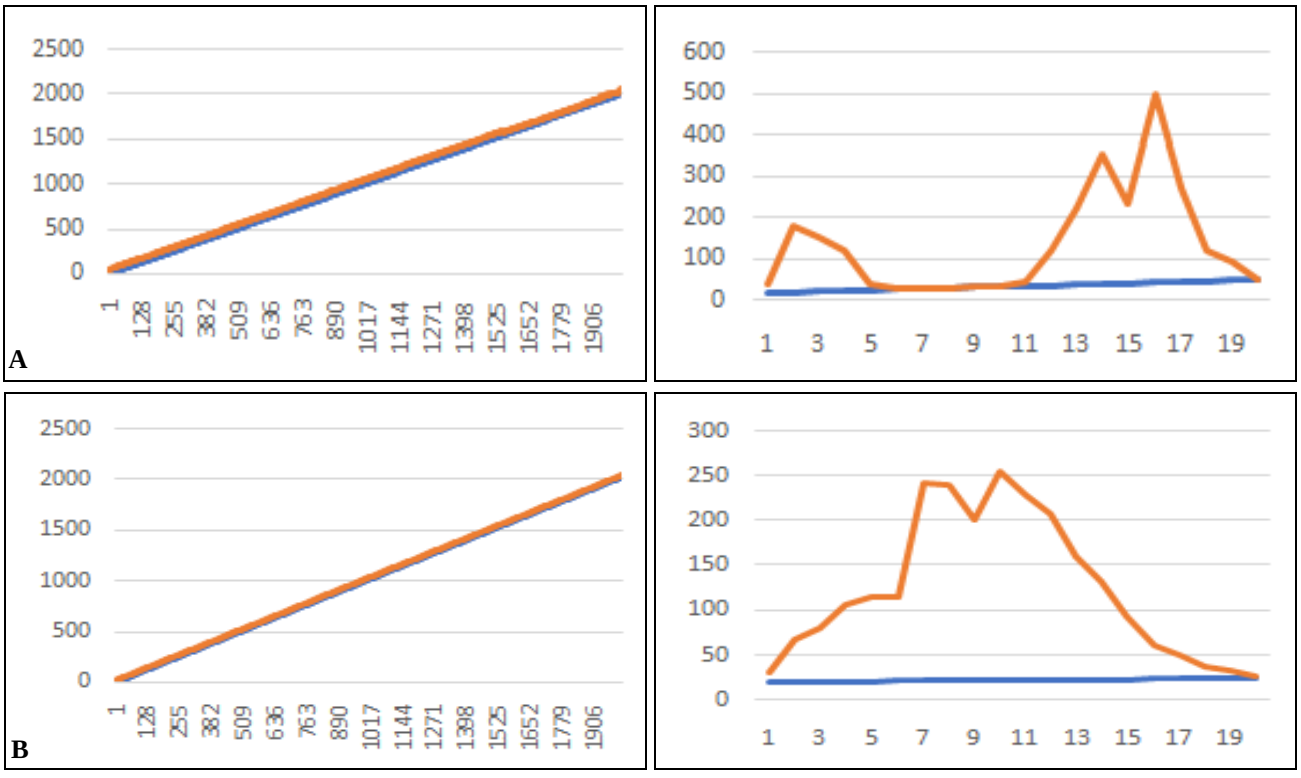


FIG. 1: THE INTERACTIONS THE DRUG AZITHROMYCIN WITH PROTEINS A. 50S RIBOSOMAL PROTEIN L28 B. POTASSIUM TRANSPORTER KTRB C. COBALT ABC TRANSPORTER PERMEASE D. MEMBRANE PROTEIN



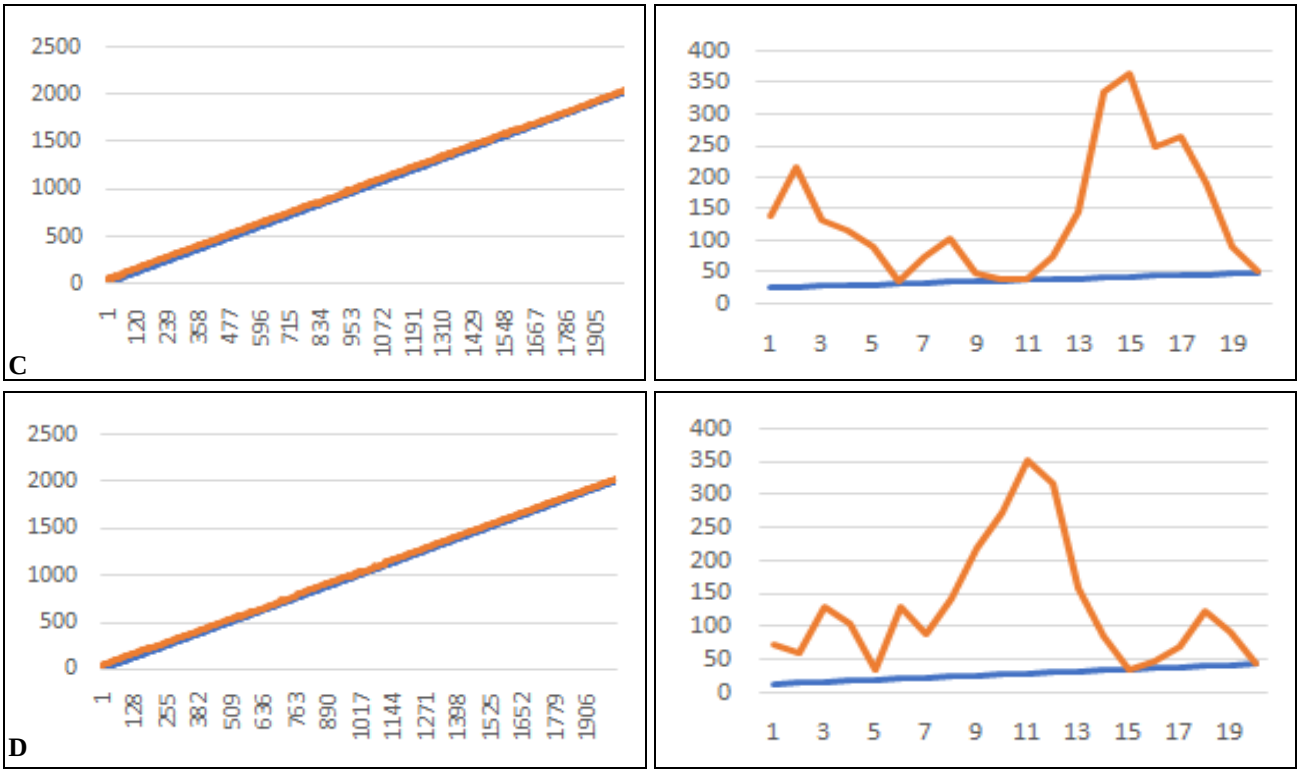


FIG. 2: DISTANCE AND HISTOGRAM GRAPHS OF SIMULATION STUDIES FOR THE VACCINE CANDIDATE PROTEIN MOLECULES PROTEINS A.50S RIBOSOMAL PROTEIN L28 B. POTASSIUM TRANSPORTER KTRB C. COBALT ABC TRANSPORTER PERMEASE D. MEMBRANE PROTEIN

50S ribosomal protein L28, Cobalt ABC transporter permease, Membrane protein, Potassium transporter KtrB are the proteins that are highly antigenic and have good docking scores.

CONCLUSION: The untreated *M. hominis* infection continues to have a negative impact on human reproductive health because of a lack of adequate treatment options. In the present study, an effort is made to design a peptide-based potential vaccine candidate proteins that are effective against the pathogen and invoke the immune responses.

With the continued growth and development of Computational tools, it is likely that drug design plays an indispensable role in the future development of sub-unit or epitope vaccines. A number of screening methods were implemented so as to obtain an effective vaccine candidate protein. Among the whole of the proteome of the *Mycoplasma homonis* the proteins 50S ribosomal protein L28, Potassium transporter KtrB, Cobalt ABC transporter permease and Membrane protein were identified as potential vaccine candidate proteins which are highly antigenic and non-allergenic with conserved domains that have good immunogenic properties.

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CONFLICTS OF INTEREST: Authors declare that they have no conflict of interest and this article does not contain any studies with human and animal subjects by any of the authors.

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