Identification of vaccine candidate proteins in Ureaplasma urealyticum causing infertility

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Abstract

Background: Ureaplasma urealyticum has gained resistance to number of antibiotics and has been of the greatest concerns nowadays. The treatment options remain extremely low due to the increased levels of intrinsic resistance gained by the pathogen. Aim: The present study focuses on designing a peptide-based vaccine as there is no vaccine available for the pathogen. Materials and Methods: All the protein sequences of pathogen were collected and examined using various in silico methods to identify the most immunogenic proteins. The study identifies the proteins which are antigenic in nature which induce the immune response, which lends to quick response of immune system on reinfection. The study describes peptide-based vaccine against U. urealyticum using molecular docking and molecular dynamics simulation approach. Results: The study identifies novel putative vaccine candidate proteins that are antigenic, membrane bound and non-allergic. Conclusion: The results of the study imply that the vaccine candidate proteins identified may bring about vigorous enduring defensive immunity against U. urealyticum.

Key words: Docking, epitope, Ureaplasma urealyticum, vaccine

INTRODUCTION

Ureaplasma urealyticum is a Gram-negative bacterium frequently found in urogenital tracts of humans that is usually associated with mycoplasmas. The unique feature of it is the ability to generate adenosine triphosphate (ATP) by hydrolysis of urea. The bacterium is often a part of normal flora in the reproductive tract of sexually active men and women. Many a times women with this infection experience fertility problems, the untreated infections can render to infertility. Ureaplasma species can cause acute urethritis and have been associated with bacterial vaginosis, preterm birth, and neonatal respiratory disease. A number of reports discuss the association of U. urealyticum with prematurity-linked conditions such as preterm labor, premature rupture of the fetal membranes, placental invasion, and intra-amniotic infection as well as chorioamnionitis, postpartum, and postabortal fever. The infected mothers can transmit the pathogen to their babies, and the newborns which get infected are prone to diseases such as meningitis, pneumonia, and respiratory tract disorders. The untreated infections move towards the upper genital tract and cause cervical discharge and may result in secondary infertility. Ureaplasma are self-replicating organisms that colonize in human and have the capacity to utilize urea for generation of ATP.
However, a significant relationship existed between \textit{U. urealyticum} and \textit{Mycoplasma hominis} and male infertility.\cite{9} There are a number of pathogens known to contribute to male infertility; the two types that most commonly occur are genital \textit{Ureaplasma}\cite{10} and \textit{Mycoplasma}. They are ubiquitous resulting in colonization of the genitalia by sexual contact. Several studies have demonstrated that \textit{U. urealyticum} and \textit{M. hominis} play an etiologic role in male infertility, with these infections changing parameters of semen such as spermatozoa density and motility.\cite{11}

The current treatment options include commonly prescribed antibiotics, and very few are effective against the bacterial infections. Nowadays, bacteria have gained resistance for the antibiotics; therefore, vaccination can be more effective for treating the bacterial infections.\cite{12} With the data from sequencing projects and advances in proteomics and genomics, the field of vaccine design and development has emerged to be more promising.\cite{13} Hence, in the current work, various in-silico tools are used to obtain the immunogenic proteins from the bacteria which significantly reduce the time and cost in developmental process.\cite{14} All the proteins of the bacteria under study were collected and screened for antigenicity, surface accessibility and allergenicity. Further structural and functional analysis of the proteins was performed as these antigenic, outer membrane proteins are important in therapeutic interventions. Finally, an attempt was made to design an effective vaccine, and the results of the study offer novel vaccine candidate proteins for development of vaccine against \textit{U. urealyticum}.

\section*{MATERIALS AND METHODS}

\textbf{Protein sequence retrieval, identification of antigenic proteins, evaluation of allergenic, subcellular localization, and functional analysis of proteins}

All the 646 protein sequences of \textit{U. urealyticum} were retrieved in FASTA format from UniProt Proteome database \url{www.uniprot.org}.\cite{15} Antigenic proteins of \textit{U. urealyticum} were identified using VaxiJen v2.0.\cite{16} Four hundred and thirty-five sequences were identified as antigenic proteins.

The overall methodology adopted for the study is shown in the Figure 1. The antigenic proteins were further evaluated for their allergenicity using AlgPred.\cite{17} The subcellular localization predictions of \textit{U. urealyticum} protein sequences were done using SOSUI-GramN tool\cite{18} as the pathogen lacks cell wall. Then, the structural and functional analysis was performed for the sequences by ProtParam and InterPro\cite{19} for obtaining the physical and chemical parameters of the proteins and identification of the conserved domains and important sites in the proteins.

\section*{Two-dimensional, three-dimensional protein structure modeling and validation of the models}

The two-dimensional structure prediction for the sequences was performed using Self-Optimized Prediction Method with Alignment. Moreover, the three-dimensional (3D) structures were obtained by protein modeling using Swiss-Model. The models were then subjected to energy minimization using Swiss-PDB Viewer. The quality of the homology model was then validated using online tools such as Rampage and Procheck.

\section*{Molecular docking and molecular dynamics simulation studies}

Protein–drug interaction study was performed using AutoDock, and MetaPocket\cite{19} was used to get the active sites of the protein to obtain site-specific interactions. The binding-free energy of the protein–drug complexes was noted, and the extent of interactions between them was viewed with UCSF Chimera.\cite{20} The entire molecular dynamics simulation study for the complexes was accomplished in CHRAMM. NAMD and VMD are file compatible with CHARMM.\cite{21} Both are popular molecular dynamics programs with high-performance simulation for large biomolecules.

\section*{RESULTS}

A total of 646 protein sequences of \textit{U. ureaplasma} were collected from the UniProt Proteome database. The sequences were set to series of screening for obtaining efficient vaccine candidates. Out of all proteins of the pathogen, 435 protein sequences in the study were screened out based on the antigenicity score. All the proteins with higher prediction scores were considered as antigenic and were selected for further analysis. AlgPred allows prediction of allergens based on similarity and mapping of IgE epitopes with any region of protein. SOSUI-GramN tool was used for screening the protein sequences; the tool predicted outer and inner membrane proteins and extracellular proteins. Table 1 gives the localization, antigenicity, and allergenicity scores for the proteins.

Due to the lack of crystal structure of the proteins, 3D structures were predicted using Swiss-Model and the
structures were validated using Rampage and Procheck which produce the PostScript Ramachandran plots given in Figure 2; the results of validation are given in Table 2. Figure 3 shows 3D structure prediction results using Swiss-Model with QMEAN-3.52.

The drug, doxycycline, which is usually given for the urea plasma infection treatment was used for the molecular docking analysis. To obtain the site-specific interactions between protein and doxycycline, the active sites for the proteins were predicted using MetaPocket. Table 2 gives the values of XYZ coordinates, binding energy, and inhibition constant for the vaccine candidate proteins. The AutoDock results were then subjected for molecular dynamic simulations studies using CHARMM. Figure 4 gives the distance and histogram graphs of simulation studies for the vaccine candidate protein molecules. The straight lines in the distance graphs indicate good interactions between proteins and drug molecule. MHC-I and MHC-II was done using the ProPred, as shown in Figure 5.

**DISCUSSION**

50S ribosomal protein L2 is evolutionarily highly conserved protein, which is involved in catalysis of peptide bond formation during transcription. Furthermore, it brings about association between 50S and 30S subunits and helps in binding of tRNA to active and peptide formation sites in the ribosomes. Another ribosomal protein, the 30S ribosomal subunit protein, performs the two basic
functions during protein synthesis, and it ensures the accuracy during translation by avoiding the mismatch codon between aminoacyl-tRNA and mRNA. It also helps in translocation by moving the tRNA to the next codon that is associated with mRNA.\textsuperscript{[25]} Translation initiation factor IF1 is a highly conserved protein and is an important element in prokaryotic translation as it stimulates the initiation phase of protein synthesis. IF1 enhances the rates of 70S ribosome dissociation and subunit association but does not change the equilibrium position. IF1 is also involved in enhancing the association and dissociation of the ribosomal subunits; hence, the protein is essential for keeping the organism viable.\textsuperscript{[26]}

The mitochondrial inner membrane harbors three protein translocases. Presequence translocase and carrier translocase are essential for importing nuclear-encoded proteins. Biogenesis of numerous metabolite carriers depends on OXA, although they are not imported by the presequence pathway.\textsuperscript{[27]}

**CONCLUSION**

Adequate treatment options for the infectious pathogens help in timely cure of the patients. The current study identifies the potential vaccine candidates for the pathogen that brings about effective immune responses against the pathogen. The current treatment options include antibiotics but the pathogen has gained resistance to the available drugs and may reinfect. Thus, vaccination would be an effective way of treating the STIs. In the current study, the effort is made to design the vaccine against the pathogen *U. urealyticum*.

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**Conflicts of interest**

There are no conflicts of interest.
Table 2: Three-dimensional model validation, active sites of proteins with their binding energy, and inhibition constants

| Protein name               | Percentage validation | X       | Y       | Z       | Estimated free energy of binding (kcal/mol) | Estimated inhibition constant (KnM) |
|----------------------------|-----------------------|---------|---------|---------|------------------------------------------|-----------------------------------|
| 50S ribosomal protein L2   | 90.02                 | 45.46   | −8.665  | −40.484 | −8.98                                    | 262.54                            |
| 30S ribosomal subunit S3   | 93.2                  | 13.62   | 19.337  | −38.135 | −7.97                                    | 1.45                              |
| Translation initiation factor IF-1 | 91.4           | 0.321   | 10.105  | −0.543  | −9.23                                    | 170.82                            |
| Membrane protein OxaA      | 90.6                  | 10.735  | 47.345  | −124.311| −6.81                                    | 10.26                             |

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