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Subtractive proteomics assisted therapeutic targets mining and designing ensemble vaccine against Candida auris for immune response induction

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1. Introduction

Candida auris infections, and outbreaks caused by diverse fungal groups, pose a significant threat to human health, and have affected almost 1.7 billion people worldwide. These infections are associated with the immune invasion properties of these pathogens, causing skin and mucosa diseases [1,2]. Candida species are the major source of nosocomial infections, and 0.4 million people are infected by it annually, with more than 40% of patients succumbing to it [1,2].

Candida auris was first isolated in 2009 from a female patient’s ear discharge in Japan. This species is nested within the Clavispora clade of the family Metschnikowiaceae (order Saccharomycetales), and four major lineages/clades have been reported: South Asian, South African, South American, and East Asian [2]. The members of these lineages are associated with the spread of C. auris infections and outbreaks [3–5].

With the discovery of C. auris, the rapid expansion of its lineages across

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the globe has been evident from the infection cases reported from 6 continents [6]. These nosocomial outbreaks can be linked to the prolific spread of *C. auris* across health facilities, with its persistence in the human host and on surfaces [3,7,8]. *C. auris* linked nosocomial outbreaks were first observed in South Korea, followed by reports from India, South Africa, Kuwait, Venezuela, the USA, and many European countries [9–15].

*C. auris* has been isolated from patients admitted to the hospitals, and it has been reported that this pathogen favors colonizing the skin [16]. According to one study, 5% of nosocomial infections reported in intensive care units (ICUs) in India are linked with *C. auris* [17]. This pathogen has caused multiple infection outbreaks in hospitals [16] and is considered one of the most prolific pathogens to colonize ICUs, and therefore plays a vital role in the transmission of nosocomial infections [18].

After December 2019, due to the fast expansion of SARS-CoV-2 (COVID19), hospitals have made additional efforts to accommodate the huge influx of patients. Of these patients, 5–30% were critically ill and were provided mechanical ventilators in ICUs [19,20]. Due to the influx of patients, proper compliance of guidelines was not followed, leading to substantial contamination of ICUs and the development of co-infections. *C. auris* co-infection has been associated with 50% of deaths caused by COVID19, making it a major contributor to the death rate of critically ill COVID patients [21,22]. Moreover, as *C. auris* is one of the most capable colonizers of the ICU environment, it has an increased chance of co-infection in critically ill COVID patients. Fungal co-infection was reported in SARS patients treated with heavy doses of corticosteroids resulting in immunosuppression and the establishment of mycosis. The ability of *C. auris* to develop multidrug resistance and to grow at high temperature (42°C), with rapid expansion and high mortality rate, makes it an emerging and deadly pathogen.

*C. auris* genome size ranges from 12.1 Mb to 12.7 Mb and consists of over 5500 genes [23,24]. Subtractive proteomics approaches aided by computational algorithms are used to select the most suitable proteins, among this large pool of data, to design suitable vaccines and to combat nosocomial infections caused by *C. auris*. Computational vaccine design is an effective approach to check all required parameters, such as antigenicity, allergenicity, physicochemical properties, and thermostability, making this approach cost-effective, thermodynamically stable, and safe [25]. In this study, outer membrane proteins which are considered highly virulent, antigenic, and essential for host-pathogen interactions were investigated [26]. The selected proteins were then used to predict cytotoxic T lymphocyte (CTL), helper T lymphocyte (HTL), and B-cell epitopes, and we have designed protein-specific and whole proteome-wide multi-epitope subunit vaccines against *C. auris*. This study may help to combat nosocomial infections and reduce the chances of co-infection with COVID. The designed candidate vaccines were also evaluated using molecular docking, and immune simulation strategies. However, further experimental validations are required to confirm the efficacy and safety of the proposed vaccine designs.

2. Materials and methods

2.1. Retrieval of Candida auris proteome and subtractive proteomics

*Candida auris* standard reference proteome (UniProt ID: UP000230249) containing 5409 proteins was retrieved from UniProtKB (https://www.uniprot.org/) [27] and analyzed by using the subtractive proteomics to identify proteins that might be used in vaccine development against *Candida auris* infection. Firstly, the BLASTp tool using non-redundant protein sequences (nr) database with default parameters (e-value: 10^-5) was used to identify the human host (*Homo sapiens*, ID: 9606) and pathogen homologous proteins. To avoid the autoimmune response of the host, the non-homologous proteins were utilized while the homologous proteins were eliminated [28]. To prevent overlapping’s in the epitope selection owing to sequence similarity, the Cluster Data Base with High Tolerance (CD-HIT) suite (http://weizhonggli-lab.org/cd-hit/) was used to remove the duplicated proteins with an 80% percent identity (cut-off value: 0.8) [29]. This cut-off number has already been utilized by many researchers and has been submitted to the database to report the extremely high-quality matches [30]. The methodological workflow adopted in this study is shown in Fig. 1.

2.2. Prioritisation of the essential proteins

The DEG (database of essential genes) (http://tubic.tju.edu.cn/deg/) contains information on key proteins and genes that pathogens need to survive. These essential proteins are promising candidates for vaccine development. Therefore, the important protein of *Candida auris* was found by using the DEG online tools [31]. However, the CELLO online
server (http://cello.life.nctu.edu.tw/cello.html) having 88% accuracy and based on SVM was used for the identification of subcellular localization of important proteins [32]. According to scientific logic, the extracellular proteins are measured as highly appropriate for vaccine development because of their critical role in the attachments of the pathogen to the host cell surface and induction of pathogenicity [33]. Selected pathogenic proteins were also tested for antigenicity using the webserver VaxiJen [http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html] by selecting fungi as the target organism at a threshold of 0.4 and used for further investigations [34]. The ProtParam weight calculator program was used to compute the molecular weight of chosen proteins [35].

2.3. Mapping immunogenic peptides in the target proteins

An online web server NetCTL 1.2 (http://www.cbs.dtu.dk/services/NetCTL/) [36] was used to predict Cytotoxic T-lymphocytes. The key values used by the server for the prediction of CTL are transportation efficiency, TAP (Transport Associated with Antigen Processing), MHC I binding peptides prediction, and proteasomal C terminal cleavage. The threshold value was set to 0.75 for the prediction of CTL epitopes. The IEDB online server (http://www.iedb.org/) was used to predict HTL epitopes using the seven reference alleles set of human HLAs [37,38]. The IC50 value determines the affinity score of each peptide. Binding affinity is strong for peptides with an IC50 value less than 50 nM. Peptides with an IC50 of less than 500 nM are intermediates, whereas peptides with an IC50 of fewer than 5000 nM indicate poor epitope binding affinity. The peptides’ affinity scores and percentile rankings are inversely related, which indicates that the higher the binding affinity, the lower the percentile rank, and vice versa. The Algpred server (http://www.imtech.res.in/raghava/algpred2/) [39,40] was used to evaluate the Allergenicity of each MHC-II predicted epitopes. Similarly, the VaxiJen server [http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html] was used to assess the antigenicity of each epitope [34]. As a result, non-allergenic epitopes with high antigenicity were chosen for putative vaccine development. Next, we used the ABCPred [http://webs.iiitd.edu.in/raghava/abcpred/] online server to predict the linear B-cell epitopes. The mentioned server has >65% accuracy, 0.49 sensitivity and 0.75 specificity [41]. For linear B-cell epitope prediction, the user employs the Kernel Method by analyzing SVM (Support Vector Machine) and uses AAP (amino acid pair) antigenicity. The Ellipro service (http://tools.iedb.org/ellipro/) was also used to predict discontinuous B-cell epitopes, with findings based on Thornton’s method and a residue clustering algorithm [42]. Additionally, IFN-epitope (http://crdd.osdd.net/raghava/ifnpeptide/) server was used to investigate interferon-gamma triggering MHC-II epitopes. This online tool uses the SVM score and motif for each input epitope to predict interferon-inducing properties [43].

2.4. Construction of multi epitopes subunit vaccine

The final putative vaccine constructs were designed using B cell, CTL, and HTL epitopes with high binding affinity, non-allergenicity, and antigenicity. Based on published data [37,39], the selected epitopes were linked together using different linkers. This included the use of epitopes specific AAY, GPGPG, and KK linkers to join the putative CTL, HTL, and B-cell epitopes, respectively. The strategy was used in the target proteins specific vaccine design. Additionally, a proteome-wide vaccine was also constructed in this study. These linkers are very important in epitope presentation as well as prevention of neoepitopes and enhance immunogenicity by stabilizing the protein structure. The EAAK linker was used to link the adjuvant to the N-terminal of the final candidate vaccine constructs [44].

2.5. Prediction of physicochemical properties

The ProtParam online tool (http://web.expasy.org/protparam/) was used to estimate the physicochemical features of constructed vaccines such as instability index, theoretical PI, in vivo and invitro half-life, amino acid composition, and GRAVY [35].

2.6. Prediction of 3D structure of the designed vaccines

The Robetta online server (http://robeta.bakerlab.org) was used to predict the tertiary structure of the putative vaccine [45]. Robetta server uses a comparative modeling approach based on BLAST, FFAS03, or 3d-jury to identify templates for the particular amino acid sequence. The Robetta server has been rated as one of the most correct and stable servers since 2014. It makes use of Continuous Automated Model Evaluation (CAMEO) to compute up to 20 pre-released PDB targets per week. This server’s average LDDT (Local Distance Difference Test) score is around 69, indicating a highly independent score [45].

2.7. TLR2-vaccine docking

Using the HawkDock server (http://cadd.sju.edu.cn/hawkdock/), the designed vaccine was docked with the TLR2 (Human Toll-like receptor-2). The server employs a hybrid docking method and requires an amino acid sequence as input for analysis, setting it apart from other similar servers. The HawkDock server provides experimental information on the protein-protein binding site and small-angle x-ray drip quickly and authentically [46]. The putative vaccine design was modeled using Robetta before protein-protein docking and the TLR-2 structure was acquired from the ALPHAFOLD-2 using ID: AF-O60603-F1 [47]. The structure was prepared before the docking by removing water molecules, heteroatom, and other atoms.

2.8. Immune simulation

To create an immunogenic profile of the designed vaccine, we performed the immune simulation by using the online server called C-ImmSim (http://150.146.2.1/C-IMMSIM/index.php) [48]. C-ImmSim is a dynamic agent-based simulator for assessing the immunological responses of the body to antigens. C-ImmSim uses an agent-based approach and the specific scoring matrix PSSM to predict immune interactions and epitopes. Following the submission of the designed vaccine to the online server with the default simulation settings, the production of antibodies, interferon, and cytokines were measured; however, the same server was also used to check the reactions of both Th1 and Th2 [49].

2.9. In-silico cloning and codon optimization

We used the Java codon adaptation tool (JCat tool) for codon optimization and reversed translation to produce the multi-epitope subunit vaccine in an appropriate vector [50]. The Jcat program is also used to
Table 1
Putative vaccine target proteins shortlisted through subtractive proteomics pipeline.

| UniProt ID | Protein Name                          | Activity/Function       |
|------------|---------------------------------------|-------------------------|
| A0A2H0Z3E44 | Putative mitochondrial import receptor subunit | Ion transport, Protein transport |
| A0A2H0ZCD43 | Putative beta-glucanase/Beta-glucan synthetase | Carbohydrate metabolic process |
| A0A2H1AY1794 | 1,3-beta-glucan synthetase | Transferase activity |
| A0A2H1AY1800 | 1,3-beta-glucan synthetase | Transferase activity |
| A0A2H1AY1803 | Putative beta-glucanase/Beta-glucan synthetase | Cell wall biogenesis |
| A0A2H1AY1806 | Uricase                               | Urate oxidase activity |
| A0A2H1AY1810 | Putative SUN family protein           | Mitochondrial and cell wall functions |

Table 2
Predicted CTL (Cytotoxic T Lymphocytes) epitopes, their position, combined scores, antigenicity, and allergenicity profiling.

| UniProt ID/Putative vaccine name | CTL Epitopes | Residues Start | Residues End | Combined score (Binding Affinity) | MHC binder | Antigenicity Score | Antigenicity Status | Allergenicity |
|----------------------------------|--------------|----------------|--------------|----------------------------------|------------|-------------------|---------------------|--------------|
| A0A2H0ZE14/A0A2H0Z3E44/MEVC-1   | LSVSVGKGY    | 137            | 145          | 1.1                              | YES        | 0.85              | Antigen             | Non          |
|                                  | SAISFABY     | 229            | 237          | 1                                | YES        | 0.84              | Antigen             | Allergens     |
| A0A2H0ZCD43/A0A2H1AY1794/MEVC-2 | ETDTNTMKL    | 468            | 476          | 1.7                              | YES        | 2.8               | Antigen             | Non          |
|                                  | SSSRITSVL    | 263            | 271          | 1.2                              | YES        | 1                 | Antigen             | Allergens     |
| A0A2H1AY1700/A0A2H0ZCD43/MEVC-3 | CNSEKLSY     | 422            | 430          | 2.6                              | YES        | 1.5               | Antigen             | Non          |
|                                  | SVMDFAMY     | 142            | 150          | 0.9                              | YES        | 0.9               | Antigen             | Allergens     |
| A0A2H1AY1700/A0A2H0ZCD43/MEVC-4 | YSAADDY      | 187            | 195          | 3.3                              | YES        | 0.7               | Antigen             | Non          |
|                                  | GVDVKGKRY    | 377            | 385          | 1.9                              | YES        | 2                 | Antigen             | Allergens     |
| A0A2H1AY1700/A0A2H0ZCD43/MEVC-5 | STITPGWDY    | 224            | 232          | 2.6                              | YES        | 2.1               | Antigen             | Non          |
| A0A2H1AY1700/A0A2H0ZCD43/MEVC-6 | VAGYTFTEY    | 243            | 251          | 1                                | YES        | 2.9               | Antigen             | Allergens     |
| A0A2H1AY1700/A0A2H0ZCD43/MEVC-7 | SSDPPLIK     | 281            | 289          | 1.7                              | YES        | 1.2               | Antigen             | Non          |
| A0A2H1AY1700/A0A2H0ZCD43/MEVC-8 | IQVQIVWTKY   | 102            | 110          | 1                                | YES        | 2.9               | Antigen             | Allergens     |
| A0A2H1AY1700/A0A2H0ZCD43/MEVC-9 | EIDPSDLGY    | 241            | 249          | 1.4                              | YES        | 1.4               | Antigen             | Non          |
| A0A2H1AY1700/A0A2H0ZCD43/MEVC-10| NTDSDGRTF    | 197            | 205          | 1.3                              | YES        | 2.3               | Antigen             | Allergens     |

guarantee that the vaccination sequence is expressed at a high level in an appropriate vector. Three choices, such as bacterial ribosome binding sites, Rho-independent transcription termination, and restriction enzyme cleavage sites, were selected in this tool. Jcat determines the GC content and CAI score of the constructed vaccine to optimized the reversely transcribed vaccine construct to a bacterial expression system [31]. The XhoI and EcoRI restriction enzymes were manually inserted into the putative vaccine sequence, and the sequence was subsequently cloned onto the pET-28a (+) plasmid using Snapgene software [26,52].

3. Results and discussion

3.1. Proteome subtraction

Annotation of pathogen proteomes substantially assist mining for therapeutic targets and the development of therapeutic strategies. Identification and characterization of target proteins are important to aid vaccine target prioritization and for designing strategies for the treatment of different diseases. As such, proteome- and genome-wide, and functional genomics-based identification and validation of therapeutic biomarkers are widely used for different diseases [38,53]. In this study, we used a subtractive proteomics pipeline to identify the putative vaccine targets for the multiple drug-resistant fungi, C. auris. This determined that only 13 of these proteins are essential and have a critical role in pathogenesis. Next, we evaluated the antigenic and allergenic properties of these shortlisted targets, which showed that only 7 proteins were determined as highly antigenic and non-allergic, shown in Table 1. These target proteins include a mitochondrial import receptor subunit, two putative beta-glucanase/Beta-glucan synthetases, two 1,3-beta-glucanosyltransferases, a uricase, and a putative SUN family protein. These identified proteins are known to be critical for C. auris pathogenesis. The putative mitochondrial import receptor subunit protein, with an antigenicity score 0.665 and classified as non-allergic, is a key mediator for regulating ion and protein transport. Similarly, the two beta-glucanase/Beta-glucan synthetase proteins, with antigenicity scores of 0.4504 and 0.9645 and also classified as non-allergic, are involved in carbohydrate metabolism and cell wall synthesis. The two 1,3-beta-glucanosyltransferase proteins, with high antigenicity scores of 0.7396 and 0.6666, play a significant role in transerase activity and the uricase protein, with an antigenicity score of 0.5435, is important in the regulation of urate oxidase activity. Finally, the SUN family protein, with an antigenicity score of 0.7738, is also essential for regulating mitochondrial and cell wall functions. All seven target proteins sequences retrieved from Uniprot [54] were then subjected to epitope screening to find CTL, HTL, and B Cell epitopes.

3.2. Immune-based epitopes screening

T-lymphocytes and B-cells play an important role in the recognition of the antigenic components of pathogens. T-cells are equipped with cell surface T-cell receptors (TCRs), which help to recognise antigens. The T-cell epitope presentation is performed by MHC molecules and is vital to their recognition of antigens. Contrastingly, B-cell differentiation results in the soluble secretion of immunoglobulins which work as antibodies to mediate adaptive immunity. These B-cell-produced antibodies perform several functions when triggered by antigen-specific binding. Other vital functions, including neutralization and targeted destruction of pathogens, are also performed by B-Cells. T- and B-cell epitope prediction aims for the specific inclusion of highly antigenic protein components for enhanced antibody production and vaccine design. Identification of antigenic immune epitopes is a prerequisite to designing peptide-based ensemble vaccines.

Recent advancements in the field of computational modeling, and the evolution of structural vaccinology-specific algorithms for epitope predictions, are a great alternative to rational vaccine design [37,38].
These methods are cost-effective and less time-consuming as compared to experimental methods. Specifically, CTLs and B-cells are key to the regulation of cell-mediated and humoral immunity. Similarly, HTL epitopes are essential for inducing and maintaining effective antibody response. Here, we screened antigenicity of the seven shortlisted proteins in *C. auris* potential CTL, B-Cell, and HTL epitopes. Among the screen of CTL epitopes, a total of 6, 32, 29, 17, 9, 10, and 9 MHC binders were identified for each protein, respectively. Similarly, the number of B-Cell epitopes identified for each protein were 36, 65, 59, 46, 29, 33, and 30, and there were 2583, 4634, 3752, 3094, 1806, 2002, and 1883 HTL epitopes. These epitopes were further analyzed by subjecting them to antigenicity and allergenicity checks. The epitopes with high antigenic potential and non-allergenic status then proceeded to the next step of vaccine design. During the CTL epitope prediction for protein A0A2H02Z14, epitopes “LSVSGKINY” and “SAISYFARY”, with high antigenicity scores (0.85, 0.84), and a combined score >1 (showing higher binding affinity), were selected. For protein A0A2H02Z14, two epitopes, “ETDTNTMKL and SSIRSITLV” at corresponding positions of 468–476 and 263–271 were selected. Similarly, the remaining five proteins with accession IDs A0A2H1A5Q4, A0A2H1A279, A0A2H1A7U2, A0A2H0ZDW4 and A0A2H1A5R4, having corresponding positions of 422–430, 142–150, 187–197, 277–385, 224–232, 243–251, 281–289, 102–110, 241–249, 197–205, had high combined scores ranging from 0.9 to 3.3. High antigenicity scores above 3.3 were shortlisted. All T-Cell epitopes selected for each protein were also screened for allergenicity status before inclusion in the candidate vaccine designs, as shown in Table 2.

| UniProt ID/Putative Vaccine name | B-Cell Epitopes | Start position | Binding Score | Antigenicity score | Antigenicity Status | Allergenicity |
|---------------------------------|----------------|---------------|---------------|--------------------|--------------------|---------------|
| A0A2H0ZE14/MEVC-1               | CQWEQDNANDFSIN | 167           | 0.9           | 1.9                | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-2               | AFSGDLPYAFSAL  | 106           | 0.88          | 0.8                | Antigen            | Non Allergens |
| A0A2H1A5Q4/MEVC-3               | NGHILTWYVGDPITLV | 554         | 0.92          | 1.8                | Antigen            | Non Allergens |
| A0A2H1A5R4/MEVC-4               | IEAETDTNTMKLGLAS | 465        | 0.92          | 2                  | Antigen            | Non Allergens |
| A0A2H1A279/MEVC-4               | IGDYPACGDDBAKADF | 209       | 0.91          | 0.8                | Antigen            | Non Allergens |
| A0A2H1A279/MEVC-5               | ACKDVXLYADAKTNW | 73         | 0.88          | 1.5                | Antigen            | Non Allergens |
| A0A2H1A279/MEVC-6               | YIKGDYQPQGSSVTS | 31         | 0.86          | 0.8                | Antigen            | Non Allergens |
| A0A2H1A279/MEVC-7               | LYSYDYTRPVPFSL | 238         | 0.86          | 1.3                | Antigen            | Non Allergens |
| A0A2H1A279/MEVC-8               | TITPGWDYTVSKVNS | 225       | 0.95          | 0.8                | Antigen            | Non Allergens |
| A0A2H1A279/MEVC-9               | GHSYTYRNFLKLDMK | 119       | 0.92          | 0.5                | Antigen            | Non Allergens |
| A0A2H1A279/MEVC-10              | KFLKVRKRDHNPKVQR | 14         | 0.88          | 0.8                | Antigen            | Non Allergens |
| A0A2H1A279/MEVC-11              | RRTLYTKPNPSKVTI | 129       | 0.86          | 1.3                | Antigen            | Non Allergens |
| A0A2H1A279/MEVC-12              | DKSVGNWAVPYVAGANT | 183     | 0.93          | 0.8                | Antigen            | Non Allergens |
| A0A2H1A279/MEVC-13              | DDGELSKPSDKYC | 91          | 0.87          | 1                  | Antigen            | Non Allergens |

Table 3
Predicted B cell epitopes, their position, scores, antigenicity, and allergenicity profiling.

| UniProt ID/Putative Vaccine name | HTL-Epitopes | Method | IFN | Antigenicity score | Antigenicity Status | Allergenicity |
|---------------------------------|--------------|--------|-----|--------------------|--------------------|---------------|
| A0A2H0ZE14/MEVC-1               | AHPQTMQEQWEQDYN | SVM   | Positive | 0.81                | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-2               | KQSSKVGGVGGVQETA | SVM   | Positive | 2.6                 | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-3               | SRNSNSSTSSDQQE | SVM   | Positive | 0.8                 | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-4               | ENKGDGEKWLFSVSD | SVM   | Positive | 0.5                 | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-5               | GSGSGSGSGGSS | SVM   | Positive | 3.1                 | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-6               | PTNNVTGTSPITVGT | SVM | Positive | 2                   | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-7               | TSQNPKLPSLDPIC | SVM | Positive | 0.9                 | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-8               | DPLSDLPKDCDIAL | SVM | Positive | 1.1                 | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-9               | KPSVTGESFSAASGSS | SVM | Positive | 1.8                 | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-10              | SRVRQATLQAOQRRR | SVM | Positive | 0.5                 | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-11              | LRGGDFVSYTEDANS | SVM | Positive | 0.5                 | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-12              | PSSDPNLKISTVGR | SVM | Positive | 0.9                 | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-13              | NDDGELSKPSDKDY | SVM | Positive | 0.8                 | Antigen            | Non Allergens |
| A0A2H0ZXY4/MEVC-14              | SGSGSGSGSGSGS | SVM | Positive | 3.8                 | Antigen            | Non Allergens |

Table 4
Predicted HTL (Helper T Lymphocytes) epitopes, their position, scores, IFN status, antigenicity and allergenicity profiling.

For protein A0A2H02Z14, specific B cell epitopes were also predicted and two of these epitopes, “CQWEQDNANDFSIN” and “AFSGDLPYAFSAL”, were selected based on high binding scores (0.9, 0.8) and antigenicity scores (1.9, 0.8). Similarly, for the protein A0A2H02ZXY4, two B cell epitopes, “NGHILTWYVGDPITLV” and “IEAETDTNTMKLGLAS”, having start positions (167, 106) and high antigenicity scores (1.8, 2), were selected. Furthermore, for the remaining five proteins, A0A2H1A5Q4, A0A2H1A279, A0A2H1A7U2, A0A2H0ZDW4, and A0A2H1A5R4, high binding scores in the range 0.5–1.4 and high antigenicity scores up to 1.5 were selected. Further analysis was performed for all the studied proteins and corresponding B-cell epitopes were examined for allergenicity status as shown in Table 3.

For potential HTL epitope selection, SVM-based analysis was performed for the protein A0A2H02Z14 and two Interferon γ (IFN) inducing HTL epitopes, “AHGQPTMCQEQWEQDYN” and “KQSSKVGGVGGVQETA”, were selected. Similarly, the protein A0A2H02ZXY4 returned two HTL epitopes, “CIVGVYLYQRPRTFLL” and “YVSVYLYQRPRTFLL”, with high antigenicity scores (0.8–0.5) and potential IFN induction. The analysis for the remaining five studied proteins (A0A2H1A5Q4, A0A2H1A279, A0A2H1A7U2, A0A2H0ZDW4, and A0A2H1A5R4) possessing high antigenic potential (ranging 0.5–3.1) helped in the identification of putative IFN inducing HTL epitopes and were classified against each protein as shown in Table 4. The final shortlisted epitopes (CTL, B cell, and HTL) from Tables 2–4 were included in further MEVC design against each target protein.
3.3. Putative vaccine designing

Identification of antigenic epitopes through proteome-wide screening is vital in designing multi-epitope-based vaccines during immunoinformatic approaches. The mapped CTL, B-cell, and HTL epitopes for the selected seven essential proteins of *C. auris* were further subjected to in silico vaccine design. The shortlisted epitopes (Tables 2–4) were used in the full-length MEVC designs and joined together using different linkers (i.e., EAAK, AAY, GPGPG, and KK) against each target protein as shown in Table 5. This also included MEVC design based on the whole proteome, including epitopes from all the seven target proteins.

These linkers are vital to enabling epitope display, maintaining epitope separation, and preventing folding, to ensure an effective immune response. Moreover, the addition of an adjuvant enhances the immunogenic potential of MEVC peptide vaccine designs [39]. All the designed MEVC constructs were therefore subjected to the addition of a non-toxic adjuvant called human beta defensin-2 (hBD-2) following an EAAAK linker. The hBD-2 possesses the property of self-production with expression levels that enable it to induce a robust immune response against the attached antigen [55,56].

| UniProt ID/Vaccine Name | Protein Specific and Proteome-wide MEVC Constructs | Number of Amino Acids | Antigenicity score | Antigenicity Status | Allergenicity |
|-------------------------|---------------------------------------------------|-----------------------|--------------------|---------------------|--------------|
| A0A2H0ZE14/MEVC-1       | MRVLYLLFLFIFEMPQVGFDGPVLKCIWGSAC                | 165                   | 1                  | Antigen             | Non-Allergen |
| A0A2H0ZXV4/MEVC-2       | MRVLYLLFLFIFEMPQVGFDGPVLKCIWGSAC                | 165                   | 0.96               | Antigen             | Non-Allergen |
| A0A2H1A5Q4/MEVC-3       | MRVLYLLFLFIFEMPQVGFDGPVLKCIWGSAC                | 165                   | 0.98               | Antigen             | Non-Allergen |
| A0A2H1A279/MEVC-4       | MRVLYLLFLFIFEMPQVGFDGPVLKCIWGSAC                | 165                   | 1                  | Antigen             | Non-Allergen |
| A0A2H1A7U2/MEVC-5       | MRVLYLLFLFIFEMPQVGFDGPVLKCIWGSAC                | 165                   | 0.72               | Antigen             | Non-Allergen |
| A0A2H0ZDW4/MEVC-6       | MRVLYLLFLFIFEMPQVGFDGPVLKCIWGSAC                | 165                   | 1.1                | Antigen             | Non-Allergen |
| A0A2H1A5R4/MEVC-7       | MRVLYLLFLFIFEMPQVGFDGPVLKCIWGSAC                | 165                   | 1.09               | Antigen             | Non-Allergen |
| MEVC-WP                 | MRVLYLLFLFIFEMPQVGFDGPVLKCIWGSAC                | 475                   | 1.09               | Antigen             | Non-Allergen |
3.4. Protein specific vaccine designs

For each of the seven proteins, MEVCs 165 amino acids in length were designed and processed for calculation of antigenic and allergenic potential to confirm its experimental feasibility. Of the final putative vaccine constructs (MEVC-1, MEVC-2, MEVC-3, MEVC-4, MEVC-5, MEVC-6, MEVC-7) for the individual proteins A0A2H0ZE1, A0A2H0ZXY4, A0A2H1A5Q4, A0A2H1A279, A0A2H1A7U2, A0A2H0ZDW4, A0A2H1A5R4, the lowest antigenicity score was 0.72 and the highest 1.1.

3.5. Whole proteome (WP) based vaccine design

The whole proteome-based final MEVC (MEVC-WP) was constructed with 14 CTL, 14 HTL, and 14 B-Cell epitopes linked together. The length of the final construct was 475 amino acids with a high antigenicity score of $>1$, demonstrating high experimental feasibility. The general schematic diagram of the putative vaccine construct with included epitopes from different proteins and suitable added linkers is presented in Fig. 2, where each mapped vaccine and its components are presented in different colors.

3.6. Physiochemical properties

The putative vaccine constructs were then analyzed through the ProtParam server to verify several physiochemical parameters vital to confirming the stability of the vaccine designs. These parameters included calculation of theoretical pI (7.4–9.9), molecular weight (17–50 kd), and other parameters determining the feasibility and structural stability of the vaccine for further experimental designs. Moreover, the demonstrated half-life, aliphatic index (thermos-stability), and GRAVY (Grand average of hydropathicity) were also explored. These results reflected the hydrophilic and stable nature of the designed MEVCs. The different physiochemical properties for protein-specific and proteome-wide putative vaccines are given in Table 6.

3.7. Structural modelling of the designed MEVC

The widely used Robetta server generates highly efficacious structural models through parsing of potent domains and by utilizing different strategies including comparative modeling and de novo sequence-based structural prediction. Based on the confidence levels of available matching protein structures, templates are used to produce a comparative model. In contrast, the de novo Robetta fragment insertion method is used for proteins with no available matching structures. 3D structural modeling for each of the constructed MEVCs was performed with the utilities of the Robetta server. All generated models were visually evaluated using PyMOL software and the refined best models, as shown in Fig. 3, were included for further analysis.

3.8. Interaction of MEVCs with the immune receptor (TLR-2)

The HAWKDOCK server was employed to evaluate vaccine-TLR-2 interaction. The top ten docking models of vaccine-TLR-2 were generated by the server, ranked based on docking complex score. The docking scores acquired for each of the docking complexes are given in Table 7. For MEVC1 the docking score was $-5832.94$ kcal/mol, with three hydrogen bonds and 1 salt bridge reported. Similarly, the MEVC4 also formed three hydrogen bonds and 1 salt bridge with a docking score $-4261.51$ kcal/mol. Unlike MEVC1 and MEVC4 the total number of hydrogen bonds were higher in MEVC2, with six hydrogen bonds and 1 salt bridge, and a docking score of $-5260.80$ kcal/mol. MEVC5 and MEVC7 formed 5 hydrogen bonds, with MEVC5 also having 2 salt bridges. The docking score for MEVC5 and MEVC7 were reported to be $-5263.43$ and $-4600.89$ kcal/mol. On the other hand, MEVC6 formed a single hydrogen bond and two salt bridges with a docking score of $-3449.97$ kcal/mol. Unlike MEVC1 and MEVC4 the total number of hydrogen bonds were higher in MEVC2, with six hydrogen bonds and 1 salt bridge reported. The results decisively suggest that the designed vaccine candidates robustly interact with the immune receptor. The best complex (lowest energy score) for each of the designed vaccine candidates were subjected to interaction analysis by utilizing PDBsum, a web tool for the graphical representation of macromolecular complexes. The docking complexes, hydrogen bonds,
salt bridges, and non-bonding contacts between putative vaccines and TLR-2 are shown in Fig. 4 and Fig. S1 (A-G).

### 3.9. Codon optimization and in-silico cloning

All constructed vaccine sequences were improved by utilizing the Jcat server for optimal expression in *E. coli* strain K-12. For each of the vaccine constructs, the codons were optimized, and reverse translated to obtain the corresponding nucleotides. The codon adaptation index (CAI) values were also calculated through the Jcat server for each of the vaccine constructs. The higher calculated CAI scores, in the range 0.98–1, and optimal percentage of GC contents (51–53%) (Table 6) indicated that the vaccine protein is expressed at a high level in *E. coli*.

The 5′ and 3′ ends restriction enzymes selected for cloning of the gene sequence of each vaccine into pET28a (+) were XhoI and EcoRI. The software package Snapgene was utilized for the insertion of putative vaccine sequences into the pET28a (+) plasmids to obtain the cloning designs (Fig. 5).

| Uniprot IDs/Vaccine name | GC-Content | CAI-Value | Molecular weight | Theoretical pl | Neg charged residues (Asp + Glu): | Positively charged residues (Arg + Lys): | Half-life | Total number of atoms | Aliphatic index | Hydrophaticity (GRAVY) |
|--------------------------|------------|-----------|------------------|----------------|-----------------------------------|------------------------------------------|-----------|------------------------|-------------------|------------------------|
| A0A2H0ZE1/MEVC-1         | 52.32      | 1         | 17845.71         | 9.11           | 9                                 | 17                                       | Mammals >30 h Yeast >20 h E.coli >10 h | 2494                 | 70.97                 | −0.072                |
| A0A2H0ZXY4/MEVC-2        | 51.11      | 1         | 17759.41         | 8.89           | 15                                | 20                                       | Mammals >30 h Yeast >20 h E.coli >10 h | 2489                 | 67.94                 | −0.293                |
| A0A2H1A5Q4/MEVC-3        | 51.51      | 1         | 17101.72         | 9.14           | 11                                | 20                                       | Mammals >30 h Yeast >20 h E.coli >10 h | 2386                 | 62.67                 | −0.098                |
| A0A2H1A279/MEVC-4        | 53.73      | 1         | 17653.41         | 8.36           | 17                                | 20                                       | Mammals >30 h Yeast >20 h E.coli >10 h | 2478                 | 76.24                 | −0.146                |
| A0A2H1A7U2/MEVC-5        | 53.53      | 0.98      | 17870.82         | 9.9            | 7                                 | 24                                       | Mammals >30 h Yeast >20 h E.coli >10 h | 2520                 | 62.67                 | −0.236                |
| A0A2H0ZDW4/MEVC-6        | 51.51      | 0.97      | 18136.25         | 9.87           | 11                                | 28                                       | Mammals >30 h Yeast >20 h E.coli >10 h | 4193                 | 72.61                 | −0.366                |
| A0A2H1A5R4/MEVC-7        | 52.12      | 1         | 17483.89         | 8.36           | 18                                | 21                                       | Mammals >30 h Yeast >20 h E.coli >10 h | 4193                 | 57.33                 | −0.395                |
| MEVC-WP                  | 51.43      | 0.98      | 50504.93         | 7.4            | 48                                | 49                                       | Mammals >30 h Yeast >20 h E.coli >10 h | 6932                 | 52.65                 | −0.547                |
3.10. Immune simulation of the proposed vaccine and immune response

To evaluate the potential antigen-based induction of the human immune response, each of the designed vaccines was examined as an injected antigen with in silico immune simulation. As shown in (Fig. 6A) the injected antigen MEVC1 (A0A2H0ZE14), after achieving the highest antigen counts at day 2, was slowly neutralized until day 5. Afterward, a strong antibody (IgM + IgG) response was observed with the highest achieved titers of >700,000 au/ml between days 10–15. Similar high antigen counts (day 2) were found for the designed vaccines MEVC2, MEVC3, MEVC4, MEVC5, MEVC6, MEVC7, and MEVCWP, following the
same pattern of neutralization (day 5) for all the designed vaccines (Fig. 6). However, variation in the antibody titers was significant between 10 and 15 days post-injection for each of the designed MEVCs. The highest titers of antibodies (IgM + IgG) for MEVC2 were observed at about 600,000 au/ml (Fig. 6B). For MEVC3, a similar higher ratio 700,000 au/ml of antibody (IgM + IgG) titers was observed (Fig. 6C). For MEVC4, the antibody titers were about 600,000 au/ml (Fig. 6D). Similarly, for MEVC5, MEVC6 and MEVC7, the observed antibody titers were ≥700,000 au/ml (Fig. 6E-G). Additionally, the MEVC designed against the whole proteome of *C. auris*, MEVCWP, also showed about 700,000 au/ml for combined (IgM + IgG) antibody titers (Fig. 6H). This was followed by IgM-specific antibody titers of about 500,000 au/ml. These results showed great immunogenic potential for each designed vaccine, all of which triggered a robust immune response. Consequently, the designed vaccine candidates may trigger the production of protective immunity against the human pathogen *C. auris* in further in vivo and in vitro models.

3.11. Limitations of the study

This study was based on in silico analysis to shortlist putative epitopes which need validation in experimental vaccine designs. Similarly, the physiochemical properties of each vaccine candidate were explored but also need further verification in the wet lab. The important step involving the expression and purification of the protein needs advanced testing using recombinant DNA technologies. Further evaluations are needed to justify the clinical manifestations associated with the vaccine designs against *Candida auris*.

4. Conclusion

In conclusion, a subtractive proteomics approach has been utilized to shortlist seven protein targets for putative vaccine design against *Candida auris*. Different epitopes for CTL, B-cell, and HTL have been explored for each of the shortlisted target proteins. Additionally, a whole proteome-based vaccine has been designed which includes each target protein-specific vaccine design. The constructed MEVCs have been modeled and evaluated for potential immunization against *C. auris*. The present study provides new and valuable epitope candidates and prompts future vaccine development against this pathogen. The suggested highly antigenic MEVCs in this study have paved a way for experimental processing of vaccines against *Candida auris*, however, despite showing immune reinforcement potential, the candidate vaccines need further validation through experimental processing to confirm their efficacy and safety.
Declaration of competing interest
Authors declare there is no declaration of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.compiobiomed.2022.105462.

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