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Research Article

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In silco Potential Analysis of gene tsst1, eno, & clfA (*S. aureus*) for candidate protein vaccine of Mastitis.

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Abstract

Staphylococcus aureus is one of the most important causes of Mastitis and community-acquired infections. The increasing incidence of multiple antibiotic-resistant *S. aureus* strains and the emergence of vancomycin resistant *S. aureus* strains have placed renewed interest on alternative means of prevention and control of infection. *S. aureus* produces a variety of virulence factors, so a multi-subunit vaccine will be more successful for preventing *S. aureus* infections than a mono-subunit vaccine. We selected three important virulence factors of *S. aureus*, tssst1 enolase &clumping factor A (ClfA), that are potential candidates for vaccine development. We designed synthetic genes encoding the tsst1, eno and clfA, used bioinformatics tools to predict structure of the synthetic construct and its stabilities. VaxiJen analysis of the protein showed a high antigenicity. Linear and conformational B-cell epitopes were identified. The proteins encoded by these genes were useful as vaccine candidates against *S. aureus* infections. In silico tools are highly suited to study, design, and evaluate vaccine strategies.

Keywords: Computer simulation, *Staphylococcus aureus*, protein Vaccines, tsst1, eno, clfa, mastitis, cow

Introduction

From the finding of worldwide study on mastitis some gene are reported as virulence gene/factor. The gene TSST, ENO and clfA are potent target gene for vaccine production study on mastitis in worldwide in mastitis the pathogenicity used mainly 3 phenomenon, cell wall adhesion second is biofilm formation & third is antigen toxicity. Most of bacterial pathogen follows this. Mastitis is defined as an inflammatory reaction of udder tissue to bacterial, chemical, thermal or mechanical injury.

Materials and Methods

Sequences, databases & structural design

Tsst, eno & clfA nucleotides sequence were obtained from publicly available sequence database primarily from the National Centre for Biotechnology Information (http://www.ncbi.nlm.cov). Multiple sequence alignments were

performed using ClustelW software of European Bioinformatics institute website (http://www.ebi.ac.uk/tools/clustalw2) to find a common fragment in all the sequences. Antigenic sequences within the genes (tsst1, eno & clfA) were selected for recombinant protein formation. Vaxijen v2 server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html.) was used to predict the immunogenicity of each protein alone & in combination.

Prediction of mRNA secondary structure

The analysis of mRNA secondary structure of gene was performed by mfold softwere. the portal for the mfold web server is http://www.bioinfo.rpi.edu/applications/mfold.

The physic-chemical parameters

The physico-chemical parameters of the protein including molecular weight, extinction coefficient, half life, instability index, theoretical isoelectric point(pI), grand average of hydropathy (GRAVY) and total number of positive and negative residues were obtained using the Expasy ProtParam (http://us.expasy.org/tools/protparam.html/).

Secondary & Tertiary structure prediction

The protein secondary structure prediction was performed by Garnier-Osguthorpe-Robson (GOR) secondary structure prediction server. AlphaFold online software (https://alphafold.ebi.ac.uk/) was used to predict the three-dimensional structure. The structure was validated to see the quality of the resulting stereochemistry of structure by Ramachandran plot in PROCHECK software (http://www.ebi.ac.uk/thornton-srv/software/PROCHECK).

Prediction of antigenic B-cell Epitopes

The amino acid sequence of protein was analyzed using the software based on B-cell epitope prediction algorithms to predict continuous and discontinuous B-cell epitopes. The first, targeted protein was analyzed for continuous B-cell epitopes using Bcepred (http://www.imtech.res.in/raghava/bcepred/).

Results and Discussion

Sequences, databases & structural design

Enolases are metallo enzymes typically localized in cytosol & participate in glycolysis pathway also involved in its regulation. The sequence available on NCBI database has 1205 bps. Sequence between 169bps to 465bps have immunological importance so we targeted this sequence its 279bps long. (Pal-bhowmick et.al. 2007)

Toxic shock syndrome toxin-1 is super antigen produced by 5to 25% of *S. aureus* bacteria. Toxin is not produced by growing in blood, rather it is produced at the local site of an infection. So it has pathogenic importance for vaccine development. This gene has 705bps in size. In this study it was found that DNA sequence from 169 bps to 465bps (297bps) have important region for vaccine developing point of view. (Jognk Mccormick et.al. 2003) (Jennifer L.Wahlsten et.al. 1998)

The 3rd targeted gene of this study wasclfA, coded cell-surface associated protein that promoted bacterial attachment to the gama chain of host animal & also help in clump formation. The DNA sequence available on online data source of this gene is 2856 bps long. In this 2 cluster have immunological importance one is -117 to 681 & second is 661 to 1677. That mines -117 to 1677 bps have pathological domain. (Rebecca A brady et. al. 2018)

Antigen index by Vaxijen server for Tst1, eno & clfA gene was 0.8898, 0.4376, 1.1660 respectively.

Structure prediction ofmRNA

The minimum free energy for secondary structures formed by each RNA molecule was appointed. tsst1 formed 4 structure, best prediction have ΔG= -23.40, eno formed 10 prediction, best prediction have ΔG= -74.20 and clfA formed 35 structure, best prediction have ΔG= -302.30. (Picture shown)

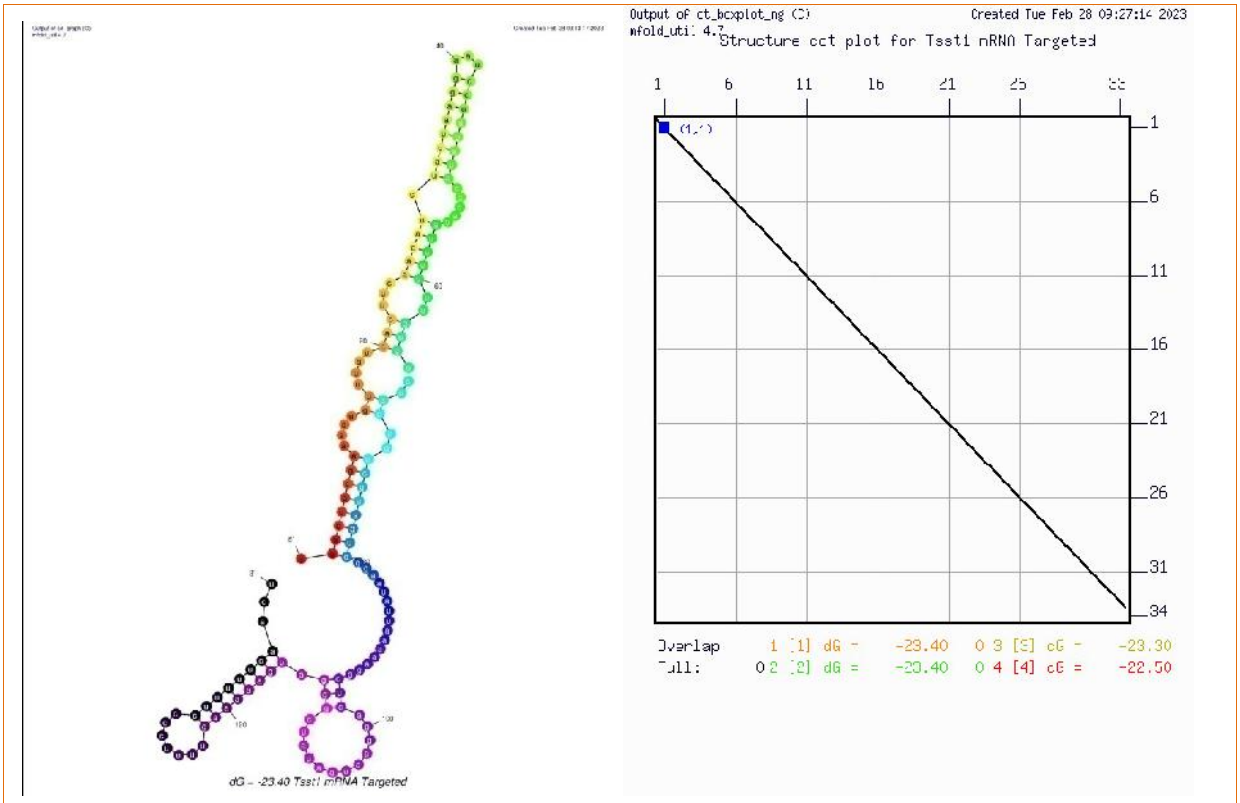


Figure 1:- 3D structure DOT plot of mRNA prediction of Tsst1 gene from mFOLD.

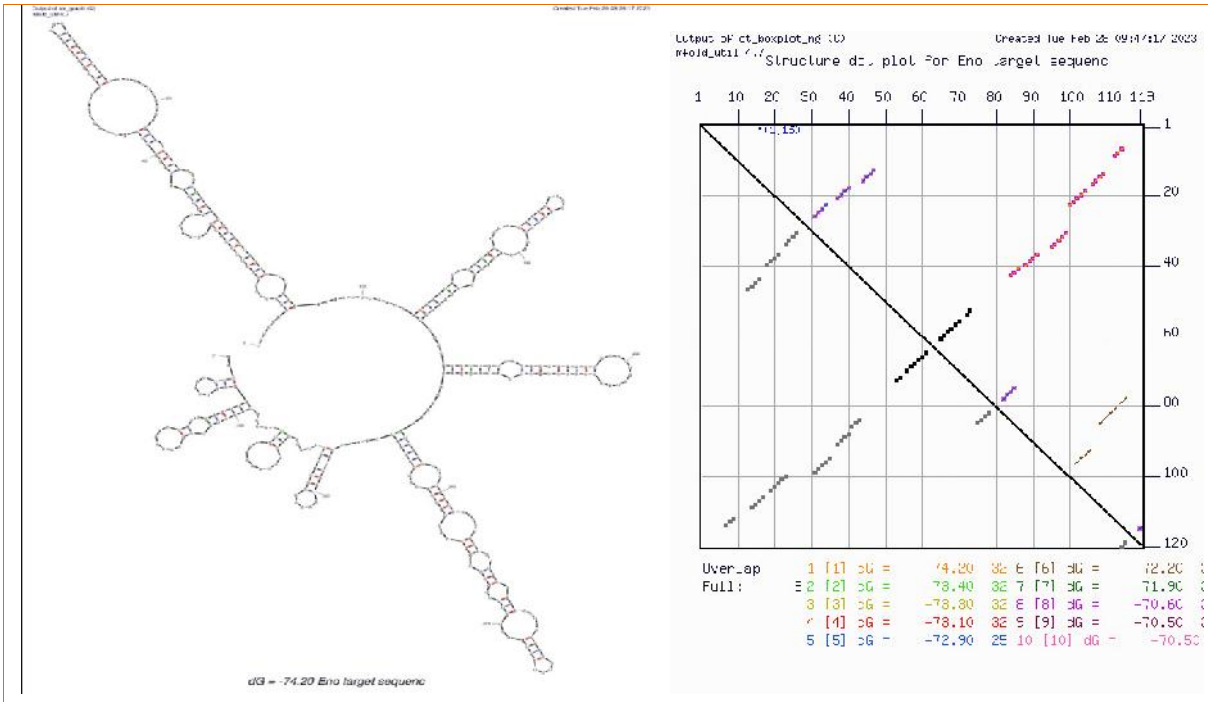


Figure 2:- 3D structure DOT plot of mRNA prediction of enolase gene from mFOLD.

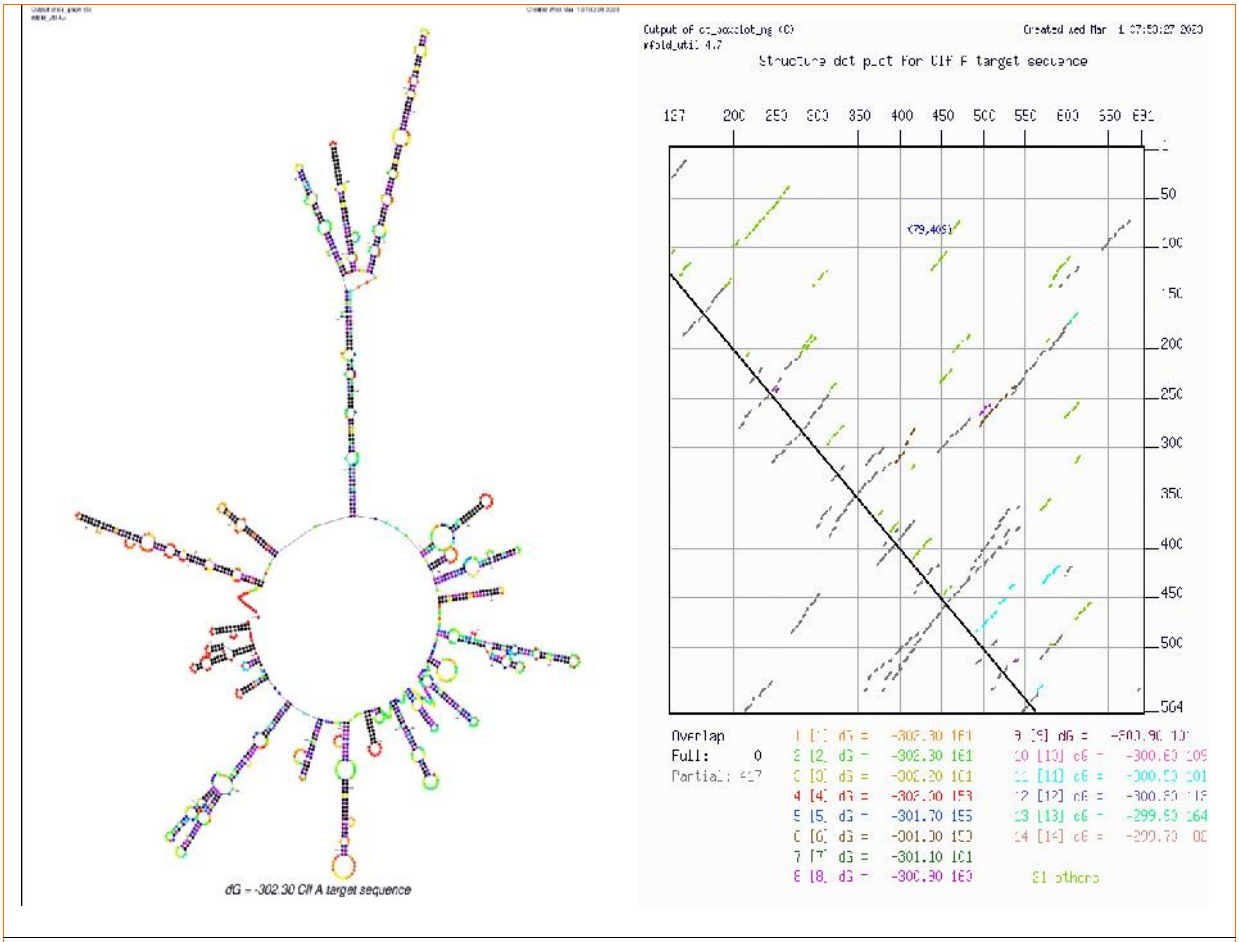


Figure 3:- 3D structure DOT plot of mRNA prediction of clfA gene from mFOLD.

The physico-chemical parameters

Physiochemical properties of a vaccine protein refer to the characteristics that describe its chemical and physical nature. These properties play a crucial role in determining the efficacy, stability, and safety of the vaccine. These physiochemical properties collectively influence the vaccine's efficacy, safety, and stability, and they are carefully considered during the development and manufacturing processes. The specific properties of a vaccine protein will depend on the type of vaccine (e.g., viral, bacterial, subunit, etc.) and the characteristics of the target pathogen.

The all 3 Targeted amino acids sequences physicochemical parameter as following chart.

Parameter in expesy	TSST1	ENO	CLF A
1. Molecular weight	11036.55D	14749.28D	986360.35D
2. Isoelectric point	PH =9.22	PH =4.14	PH =3.59
3. Extinction coefficient at 280nm	9970	19940	41830
4. Half life in *E.coli*	Less than 10hrs	3 minute	10hrs
5. Instability Index	28.59 (stable)	31.81 (stable)	50.35 (stable)
6. Aliphatic Index	78.69	85.68	45.44
7. GRAVY	-0.543	-0.389	-1.086

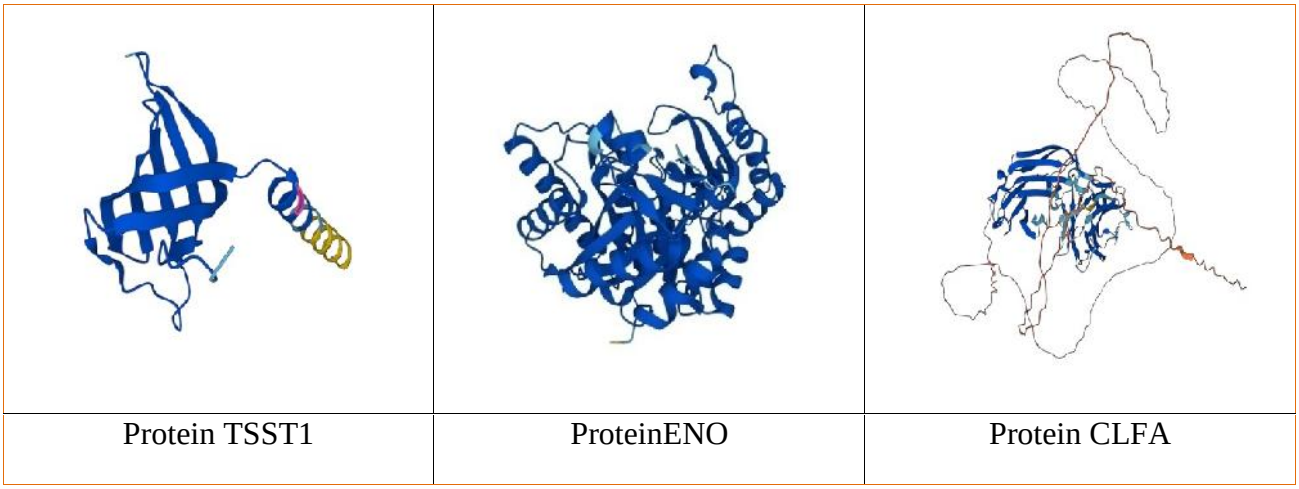
Secondary & Tertiary Structure Prediction

The all 3 proteins of secondary structure was obtained by online software. In order to validate our secondary structure prediction method, first the TSST1, ENO and CLFA were used as test sequences. We obtained predicted structural elements using software GOR IV (http://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html). The results show that, structural contents of protein include extended strand, random coil, and alpha helix. Composition of secondary structure predicted for tsst1 protein was 6.06% alpha helix, 25.25% extended strand, and 68.69% random coil.

Composition of secondary structure predicted for eno protein was 34.85% alpha helix, 17.42% extended strand, and 47.73% random coil.

Composition of secondary structure predicted for ClfA protein was 7.78% alpha helix, 15.88% extended strand, and 76.34% random coil.

The secondary structure prediction of the protein is shown in Figure 4. The three-dimensional modeled structure for protein was obtained from AlphaFold shoftwere database.



Protein TSST1	ProteinENO	Protein CLFA

Figure 4: - 3D model structure of all 3 protein, obtain from AlphaFold database.

Analysis of TSST1 targeted protein by using the Ramachandran plot showed that 91.0% of amino acid residues from the structure modeled by AlphaFold were incorporated into the favored regions of the plot. Apart from that, 9.0% of residues were in allowed regions of the plot and 0% in outlier region (Figure 5a.)

Analysis of ENO targeted protein by using the Ramachandran plot showed that 91.3% of amino acid residues from the structure modeled by AlphaFold were incorporated into the favored regions of the plot. Apart from that, 7.9% + 0.4%of residues were in allowed regions of the plot and 0.4% in Disallowed region (Figure 5b.).

Analysis of CLFA targeted protein by using the Ramachandran plot showed that 89.4% of amino acid residues from the structure modeled by AlphaFold were incorporated into the favored regions of the plot. Apart from that, 9.9% +0.7 of residues were in allowed regions of the plot and 0% in outlier region (Figure 5c).

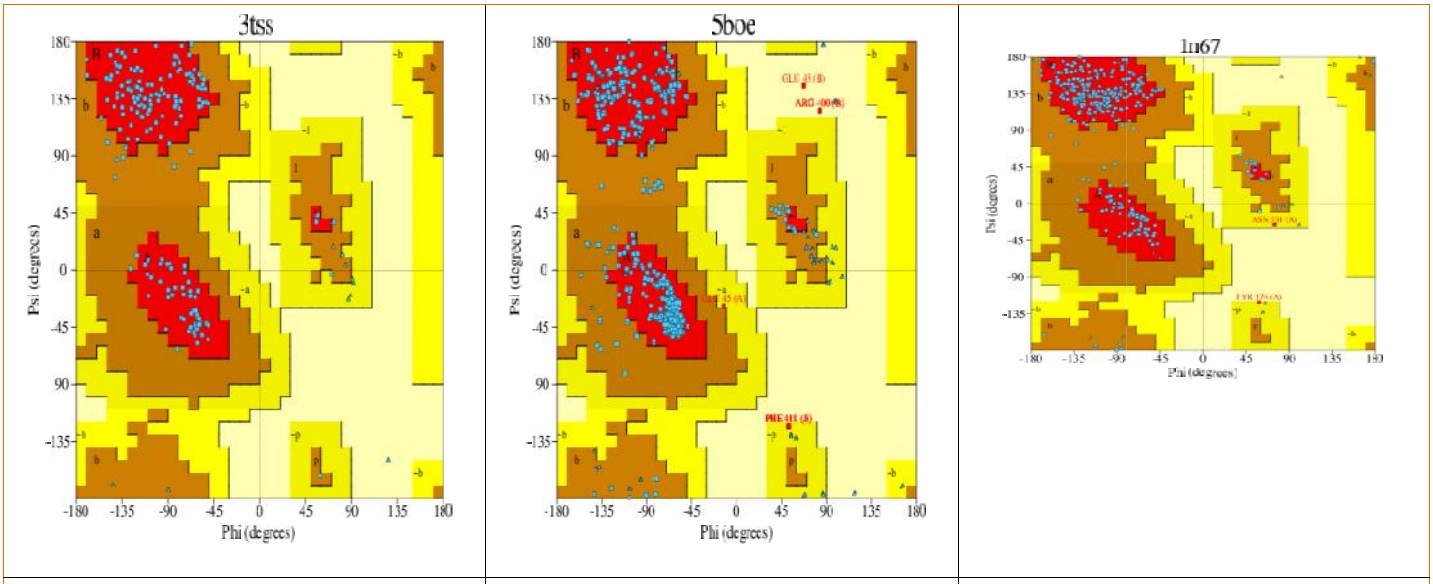


Fig. 5a:- TSST R-PLOT	Fig. 5b:- ENO R-PLOT	Fig. 5c:- CLFA R-PLOT

Figure 5:- R-plot of the 3 protein, 5a:- TSST1, 5b;- ENO & 5c:- CLFA

Prediction of antigenic B-cell Epitopes

All three proteins have several B-cell epitopes predicted in BCEPRED software. In tsst1 have 3 Antigenic propensity domains. The eno have 5 and CLFA have 10 Antigenic propensity domains.

Protein	Antigenic Propensity domain predicted by BCEPRED
TSST	GSISLIIFPSPYY, HFQISGV, IELPLKVKVHG
ENO	VFLGFDC, VYDYSKF, VDYLEQLVDKYPII, GDRVQLVGDDLFV, NSILIKV
CLFA	SVLVGTLIGFGLL, VSSVNSP, QLTNVTV, KTVLVDYEK, YRQTIYV, SIKVYKVD,LSESYFV,ITTPYIVVVNGHI, GIDKPVVPEQPD

Conclusion

Before going with wet laboratory experiment, the bioinformatical tools and software is good approach to know idea about experimental outcome & challenge. The specific attributes of a vaccine protein depend on the vaccine type and the characteristics of the targeted pathogen. In this analysis tsst1, eno & clfA gene and its protein outcome are analyzed for suitable candidate for protein vaccine of mastitis. *In silico* tools are highly suited to study, design, and evaluate vaccine strategies. VaxiJen server showed that tsst1, eno & clfA genes were immunogenic. The Expasy ProtParam tool result predict its physic-chemical property fulfill candidature for vaccine. This gens were also has been identify discontinuous b cell epitopes that are essential for antibody-antigen interaction. In final word these gene have characteristics for be a candidate of mastitis vaccine.

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