Journal of Proteomics & Bioinformatics - Open Access

JPB/Vol.3 Issue 3

Research Article OPEN ACCESS Freely available online doi:10.4172/jpb.1000125

In *Silico* Identification of Dual Ability of N. *gonorrhoeae* ddl for Developing Drug and Vaccine Against Pathogenic Neisseria and Other Human Pathogens

Debmalya Barh[1]*, Amarendra Narayan Misra[2], and Anil Kumar[3]

[1]Centre for Genomics and Applied Gene Technology, Institute of Integrative Omics and Applied Biotechnology (IIOAB), Nonakuri, Purba Medinipur, WB-721172, India
[2]Department of Biosciences and Biotechnology, School of Biotechnology, Fakir Mohan University, Jnan Bigyan Vihar, Balasore-756020, Orissa, India
[3]School of Biotechnology, Devi Ahilya University, Khandwa Road Campus, Indore, MP-452001, India

Abstract

The transmission and prevention of gonococcal infection is a global health problem and an effective drug or vaccine against the pathogen is yet to be developed. In our previous studies by analyzing metabolic pathways and membrane proteome of *N. gonorrhoeae* we found that four membrane associated targets could be better option in developing anti-gonorrhoeae drugs and vaccine. Here we showed that among these putative targets, D-alanine—D-alanine ligase (ddl) is the best candidate for development of both drug and vaccine against *N. gonorrhoeae* and various other human bacterial pathogens. Using *in silico* approaches, we developed the 3-D model of the enzyme and potential epitopes are also identified that may be helpful in peptide vaccine development against multiple pathogens including pathogenic *Neisseria*. Epitopes require experimental validation for their effectiveness as peptide vaccines against these pathogens.

Keywords: Computational prediction; Epitope design; Immunoinformatics; Sexually transmitted disease; Broad spectrum target

Introduction

Gonorrhea is one of the most common sexually transmitted diseases (STD) caused by *Neisseria gonorrhoeae.* Although the gonococcal infection and transmission is mostly prevalent in underdeveloped countries, in USA, it is the second most common STD with 358 366 cases reported in 2006 that has shown a 5.5% increase incidences in 2007 (Centers for Disease Control and Prevention, 2007; Cornelissen, 2008). The disease transmission occurs from infected man to a woman. The infected male shows symptoms of urethritis, epididymitis, and prostatitis. But infected female develops polyarthralgia, tenosynovitis, arthritis, and in severe cases pelvic inflammatory disease (PID) that leads to infertility and ectopic pregnancy due to permanent blockage of the fallopian tube (Furuya and Tanaka, 2009). Due to the immergence of antibiotic resistance and lack of appropriate vaccine against the pathogen, the gonococcal transmission and infection remains a global public health problem/ menace (Tapsall, 2006; Workowski et al., 2008).

N. gonorrhoeae pili proteins have been reported as possible vaccine candidates (Rothbard et al., 1985) and are already patented (US Patent: 4443431). But pilE globular domain is reported to be non-immunogenic (Hansen et al., 2007). Other reported vaccine targets are porB (Zhu et al., 2004), opa, lipo-oligosaccharides, protein-I, lactoferrin (lbpl, lbp2) (Barbosa-Cesnik et al., 1997), phospholipase A (pldA) (Bos et al., 2005), transferrin-binding proteins (tbpA and tbpB) (Price et al., 2005; Thomas et al., 2006; Price et al., 2007), IgA1 proteases (Barbosa-Cesnik et al., 1997), and 2C7 oligosaccharide (OS) epitope (Gulati et al., 2001). The tspA and tspB of *N. meningitides* have been patented for vaccination against pathogenic *Neisseria* (US patent: 6861507). However, in perceive none of these vaccines are effective in combating the pathogen. In our recent study, to elucidate new drug target for *N. gonorrhoeae*, we found that 29% drug targets are associated with or localised in the membrane, and the transferrin-binding protein - tbpA (Price et al. 2005) is the most potent vaccine candidate (Barh and Misra, 2009). But if a single target can be utilised for dual purpose in developing an effective drug as-well-as vaccine against the pathogen, it will be most effective way for suppression of growth and development of such non-responsive pathogens. With this objective, we selected four targets from our previous works (Barh and Kumar, 2009; Barh and Misra, 2009) viz. sulfate transport permease protein C, ABC transporter iron-uptake permease inner membrane protein (afuB), competence lipoprotein (Cpl), and D-alanine—D-alanine ligase (ddl) for this study to explore the ability of any of these target(s) to be used for dual purpose of drug targeting and/or vaccine against pathogenic *Neisseria*. These targets were selected because these are non-human homologue essential enzymes, involved in pathogen specific metabolic pathways, and localized to the cell membrane or cell wall.

Materials and Methods

Identification of epitopes of *N. gonorrhoeae*

The basic epitope prediction strategy was taken as described in Barh and Misra, (2009) to get minimum number of antigenic epitopes that are able to produce both the B-cell and T-cell

***Corresponding author:** Debmalya Barh, Centre for Genomics and Applied Gene Technology, IIOAB, Nonakuri, Purba Medinipur, WB-721172, India, Tel: +91-9449 5500 32; E-mail: dr.barh@gmail.com

Received January 23, 2010; **Accepted** March 14, 2010; **Published** March 14, 2010

Citation: Barh D, Misra AN, Kumar A (2010) In *Silico* Identification of Dual Ability of N. *gonorrhoeae* ddl for Developing Drug and Vaccine Against Pathogenic Neisseria and Other Human Pathogens. J Proteomics Bioinform 3: 082-090. doi:10.4172/jpb.1000125

Copyright: © 2010 Barh D, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

mediated immunity. In addition, few other tools had also been used to confirm the method. Briefly, amino acid sequences of candidate targets from our previous studies [D-alanine—D-alanine ligase (ddl), Competence lipoprotein (NGO0277), ABC transporter iron related permease protein (NGO0216/fbpB), and Putative ABC transporter (NGO0446)] were retrieved from Swiss-Prot protein database (http://us.expasy.org/sprot) and each sequence was then analyzed for antigenicity using B-cell antigenic site prediction tool "Antigenic" (http://bio.dfci.harvard.edu/Tools/antigenic.pl). Similarly, each protein sequence was also subjected for B-cell epitope prediction using BCPreds (EL-Manzalawy et al., 2008). Both the BCPred and AAP prediction modules of BCPreds were used to identify B-cell non-overlaping epitopes and common epitopes (generated by both the prediction servers) were selected. Top scored (cut off values for "Antigenic" and BCPreds were respectively 1.2 and 1) sequences were then aligned to get an overlapping sequences to make a continuous stretch of amino acid sequence that possess both antigenic sequences as-well-as the B-cell binding sites. Each antigenic B-cell epitope sequences were then analyzed with ProPred 1 (Singh and Raghava, 2003) for MHC class I and ProPred (Singh and Raghava, 2001) for MHC class II epitope prediction using default parameters. The proteosomal cleavage sites of identified epitopes were also analyzed. Common epitopes for both the MHC classes that also can bind to maximum MHC alleles were selected. To confirm the parameters of epitopes, each epitope was further analyzed with VaxiJen v2.0 antigen prediction server (Doytchinova and Flower, 2007) for antigenicity. To verify MHC binding properties, less than 1000 nM IC_{50} scores for DRB1*0101 based on MHCPred v.2 (http://193.133.255.13/mhcpred/) (Guan et al., 2003) was used. Exo-membrane localization and fold level topology of epitopes were confirmed using TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) (Krogh et al., 2001). The finally selected epitopes were used for structural characterization.

Identification of common epitope for multiple pathogens

A modified comparative and subtractive genomic approach as described by Barh and Kumar, (2009) was adopted to identify a single epitope that can be used against multiple pathogens. Briefly, full length sequence of the *N. gonorrhoeae* ddl was subjected to blastp against Database of Essential Genes (DEG) (http://tubic.tju.edu.cn/deg) (Zhang et al., 2004). Bacterial pathogens that show significant sequence homology (essential gene for the respective pathogens) were selected based on blastp scores [cut-off values: bit score (>100), *E*-value ($<E^{-10}$), and percentage of identity at amino acid level (>35%)]. Because of the subjected sequence is a non-human homologue essential protein and putative drug target for the *N. gonorrhoeae*, it is presumed that the homologous sequences also will be drug targets for the corresponding pathogens. Selected sequences of pathogens were then examined using standard human blastp in NCBI server (with same cutoff values) and pathogens that carry non-human homologs were selected. NCBI blastp was used for selection of pathogens having the common epitopes at nearly accessible region. Similarly, the selected amino acid sequences of each pathogen were aligned with multiple sequence alignment tool - COMBINE Advanced T - COFFEE (V s 7.7) (Notredame et al., 2000) to determine the sequence similarity at the epitope position among the identified pathogens. The final selection of epitopes from other human pathogen were based on TMHMM (exo-membrane localization), VaxiJen (antigenicity), and MHCPred (DRB1*0101) analysis.

Homology modelling and model validation

To carry out homology modeling, Automated, Alignment, and Project modes of Swiss model server (Arnold et al., 2006) were used. The resultant models were optimized using Swiss-PdbViewer (Guex and Peitsch, 1997) and Accelrys Discovery Studio (http://accelrys.com/). Best model was selected based on reliability assessment that was carried out using Prosa-web (Wiederstein and Sippl, 2007) and Procheck (Laskowski et al., 1993) tools at SAVS server (http://nihserver.mbi.ucla.edu/SAVS/). ProFunc (Laskowski et al., 2005) was used to predict domains, motifs, ligand binding clefts, and various other functional parameters of 3D structures.

Characterization of epitopes

Due to the short sequence (9-20 mars) of epitopes, it was difficult to find the template from PDB database and to make a homology model using Swiss-model server. Therefore, initially we used drug discovery studio to identify and determine the native site and the structure of epitopes within the protein. In another effort, the DISTILL server (Bau et al., 2006) that can predict 3-D structure of small fragments of proteins based on the similarity with PDB template was also used for epitope modeling. Resultant epitopes were then validated with ProSA-web and PROCHECK. ProFunc, Motif Scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan), and InterProScan (Quevillon et al., 2005) were use for domain, motif, and functionality assignment of epitopes. ProteinDigest (http://db.systemsbiology.net:8080/proteomicsToolkit/proteinDigest.html) was used to determine molecular weight, pI, and enzymatic degradation site (s) of epitopes.

Results

Identification of candidate peptide vaccine

The common antigenic B-cell epitopes were identified using "Antigenic" and BCPreds. This method generates a stretch of 21 mers sequence (EYSCPVLNGKGLPGIHIIPAT) spanning amino acid position 182 to 202 of the ddl protein that meets all selection criteria for the antigenic B-cell epitope (Table 1). But other three candidate vaccine targets [Competence lipoprotein (NGO0277), ABC transporter iron related permease protein (NGO0216/fbpB), and Putative ABC transporter (NGO0446)] did not have such type of common sequence. Therefore, in this study they were excluded from further analysis. TMHMM 2.0 based exo-membrane localization and topology of ddl shows that it is fully exposed to out side the cell (Table 3).

MHC binding T-cell epitopes were identified using ProPred 1 (for MHC I) and ProPred (for MHC II) with default parameters. The 21-mer antigenic B-cell epitope sequence was analyzed with these two servers and the common epitope(s) that can bind both the MHC classes and covers maximum MHC alleles were selected. In this way only one 9-mer sequence (LPGIHIIPA) spanning at amino acid position 193 to 202 of the ddl protein was selected. Another peptide (CPVLNGKGL) spanning at amino acid position 185 to 194 was found to bind 38 MHC I alleles. But this peptide contains proteosomal cleavage site and was not considered due to the fact that a peptide which contains a

ISSN:0974-276X JPB, an open access journal

Sequence	Amino acid position	Length (mers)
B-Cell antigenic site prediction with Antigenic		
GSSVGVVKVK	144	10
AEGCVRVDFL	246	10
EYSCPVLNGK	182	10
RCKLIWQALGLPVPEFAVLYDD	101	22
B-cell epitope prediction with BCPreds		
BCPred algorithm		
KSKGIDAYAFDPKETPLSEL	33	20
NGKGLPGIHIIPATEFYDYE	189	20
AAP Prediction algorithm		
LLEINTLPGMTGHSLVPKSA	264	20
YSCPVLNGKGLPGIHIIPAT	183	20
PVPEFAVLYDDTDFDAVEEK	112	20
YGEDGAVQGALELLGIPYTG	68	20
NALKSKGIDAYAFDPKETPL	30	20
Common antigenic B-cell epitope (s)		
EYSCPVLNGKGLPGIHIIPAT	182	21

Table 1: Antigenic B-cell epitopes of *N. gonorrhoeae* ddl. Epitopes are identified by “Antigenic” and two modules of BCpreds. The 21 mers sequence “EYSCPVLNGKGLPGIHIIPAT” was identified following the methode as described in text.

ProPred 1 (MHCI)		
TCL epitope sequences	**Amino acid position**	**No of MHC I binding alleles**
YSCPVLNGK	182	9
KGLPGIHII	191	15
CPVLNGKGL*	185	23
GKGLPGIHI	190	2
LPGIHIIPA	193	10
ProPred (MHCII)		
HCL epitope sequences	**Amino acid position**	**No of MHC II binding alleles**
LNGKGLPGI	188	2
LPGIHIIPA	193	4
VLNGKGLPG	187	1

Table 2: T-cell epitopes of *N. gonorrhoeae* ddl. The common antigenic B-cell epitope “EYSCPVLNGKGLPGIHIIPAT” was analyzed for its ability to bind MHC I and MHC II molecules using Propred I and Propred. A common epitope “LPGIHIIPA” (9 mers) that generates both TCL and HCL mediated immune response was selected. Peptides those contain proteosomal cleavage site are marked with an asterisk (*).

Pathogen	Epitope from N. gonorrhoeae and homologous epitopes	Seq position	VaxiJen >0.4= probable antigen	IC50 (nM) (DRB1*0101)	TMHMM exo-membrane topology
N. gonorrhoeae	**LPGIHIIPA**	193-201	0.4102	839.46	1-304 aa
N. meningitidis	**LPGIHIIPA**	193-201	0.4102	839.46	1-304 aa
H. influenzae	**LPAIRIVPE**	198-206	0.9334	704.69	1-306 aa
B. pseudomallei	**LPLIRIVPA**	203-211	0.8761	69.66	1-313 aa
A. baumannii	**LPVIRLQPP**	194-202	1.6436	56.23	1-308 aa

Table 3: Epitopes from ddl of various pathogens that are homologous to *N. gonorrhoeae* ddl. All epitopes have potentiality to be used as vaccine for corresponding pathogen and other listed pathogens.

proteosomal cleavage site may be degraded during antigen processing. So it is not suitable for peptide vaccine design (Toes et al., 2001). Thus, only the epitope peptide sequence “LPGIHIIPA” was taken for further analysis as this can potentially induce both the B- cell and T-cell mediated immunity. Predicted T-cell epitopes of dll are represented in Table 2. VaxiJen and MHCPred v.2 analysis of the epitope (LPGIHIIPA) further confirm that the epitope is antigenic (VaxiJen score = 0.4102) and can bind to DRB1*0101 allele (MHCPred nM IC_{50} score =839.46) (Table 3).

“LPGIHIIPA” is common epitope for multiple pathogens

The blastp analysis showed that the ddl protein is also a putative drug target in pathogens such as *N. gonorrhoeae, N. meningitides, Haemophilus influenzae*, Salmonella typhimurium, *Staphylococcus aureus, Helicobacter pylori, Burkholderia pseudomallei, Vibrio cholerae,* and *Acinetobacter baumannii* (data not shown). Ddl was also reported as potential drug target in *A. hydrophila* (Sharma et al., 2008). The NCBI blastp with *N. gonorrhoeae* ddl and multiple alignments of ddl protein sequences of these identified pathogens show that the epitope sequence (LPGIHIIPA) is highly conserved in *N. meningitides* and moderately conserved in *H. influenzae, B. pseudomallei,* and *A. baumannii.* Therefore, the identified epitope may be a candidate vaccine against these pathogens also. The NCBI blastp and T-COFFEE (Notredame et al., 2000) multiple alignments are represented in Figure 1. Further to demonstrate the possibility of the native homologous epitope sequences of “LPGIHIIPA” in these bacteria that can potentially be used as peptide vaccine, we used VaxiJen, MHCPred, and TMHMM. As shown in Table-3, all homologous epitopes have potentiality to be used as peptide vaccine against the respective bacteria.

3-D modeling of *N. gonorrhoeae* ddl

The 3D structure of *N. gonorrhoeae* ddl is not available and in this study, we have focused 3D modelling of ddl only for *N.*

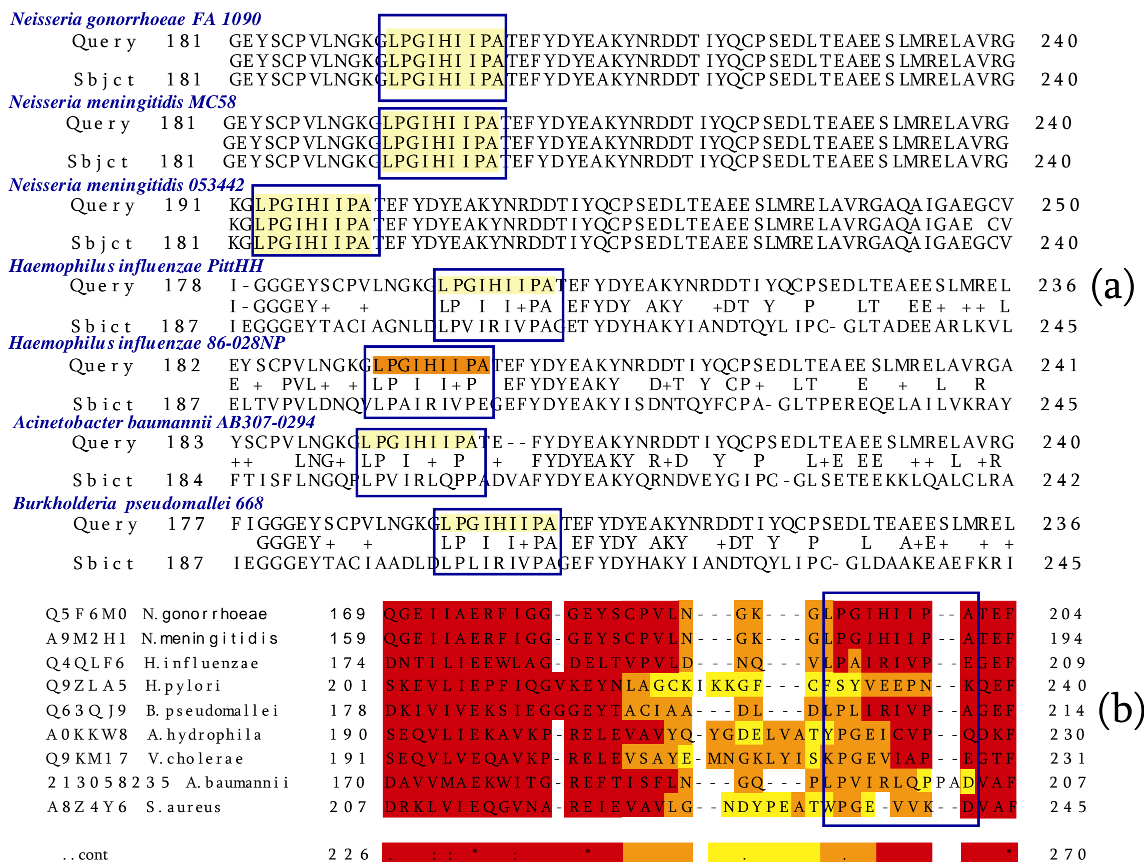

Figure 1: NCBI blastp with *N. gonorrhoeae* ddl showing conserved epitope sequences "LPGIHIIPA" in other human pathogens. (b) The multiple alignment of ddl from identified other human pathogens using T-Coffee.

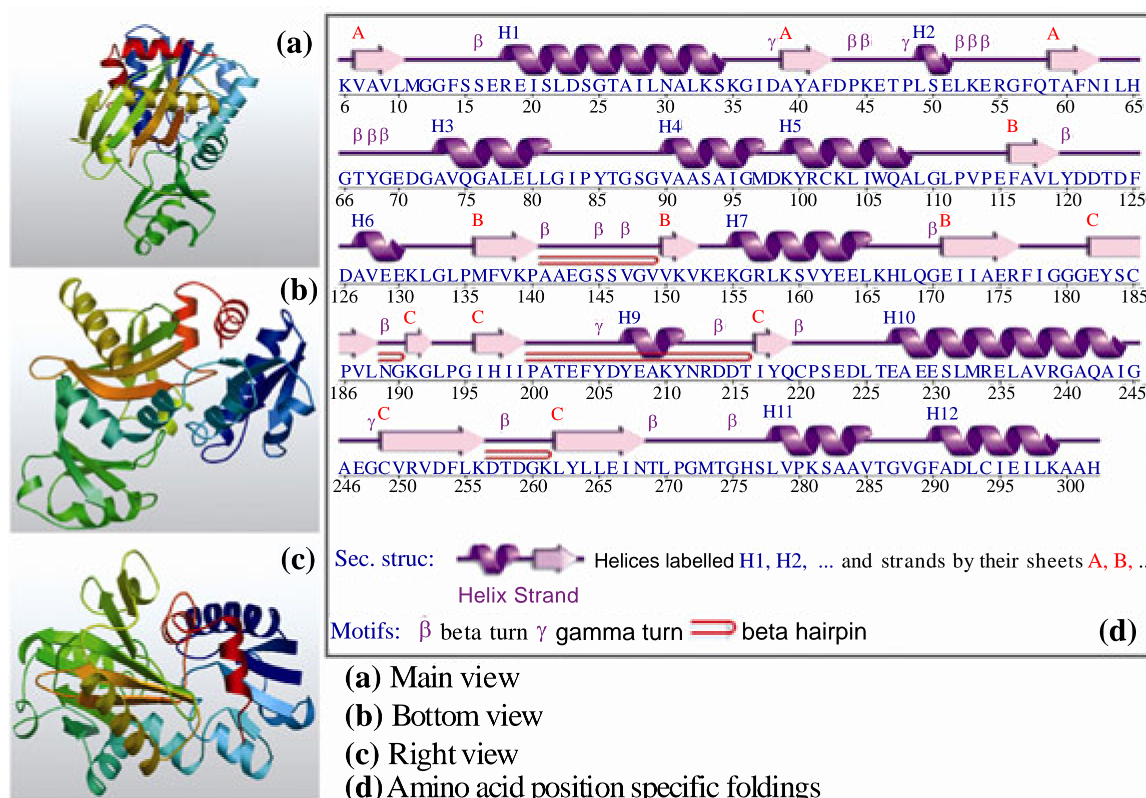

Figure 2: 3-D model of *N. gonorrhoeae* ddl from various angles. (b) The secondary structure the ddl showing various foldings at corresponding amino acid positions.

ISSN:0974-276X JPB, an open access journal

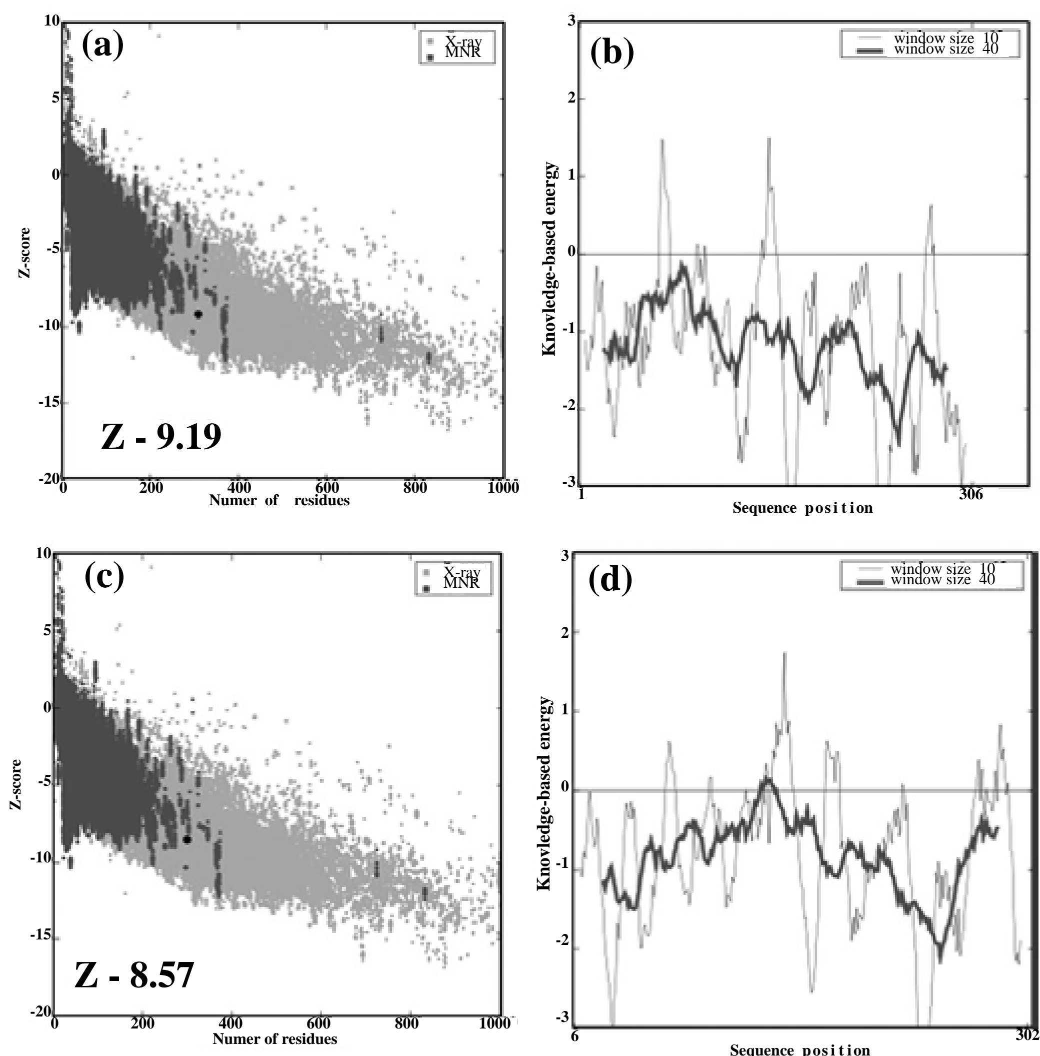

Figure 3: Validation of the 3-D model of *N. gonorrhoeae* ddl with ProSa-web. The upper panel is template (PDB id: 1iowA) and lower is ddl. (a) Overall model quality of 1iowA (Z=-9.19). (b) Local model quality of 1iowA. (c) Overall model quality of ddl (Z=-8.57). (d) Local model quality of ddl.

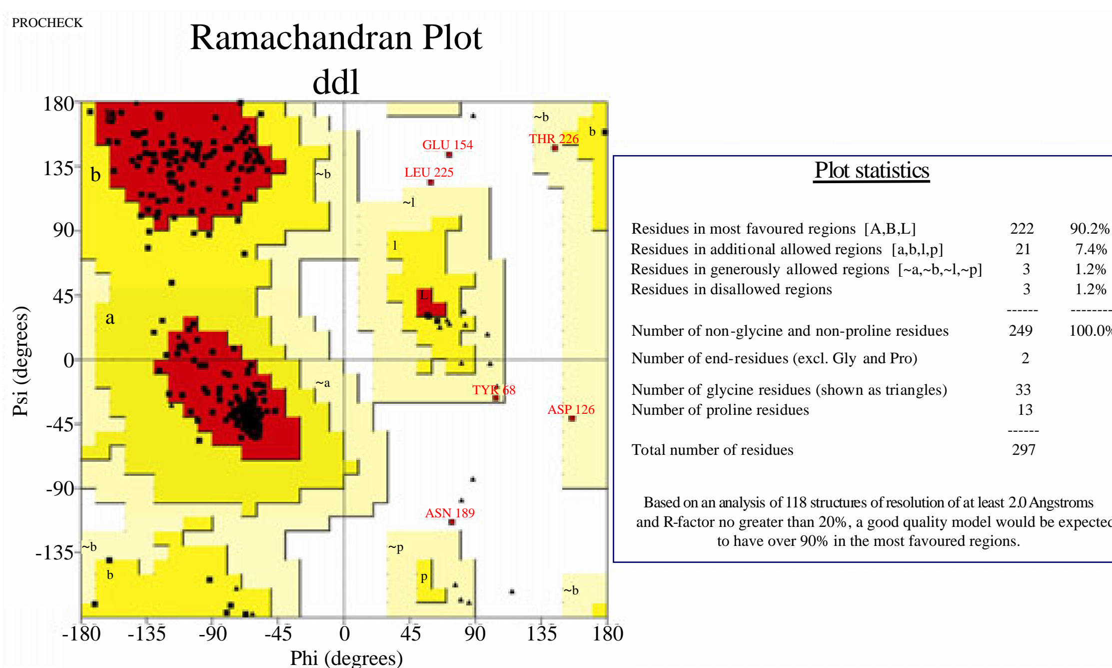

Figure 4: The Ramachandran plot for *N. gonorrhoeae* ddl. The plot shows the acceptability of the model.

gonorrhoeae using homology modelling. Based on blast parameters, the *E. coli* D-alanine-D-alanine ligase (PDB id: 1iowA) having 46% sequence identity with an E- value of 2e^{-69} was selected as template. The X-Ray diffraction structure has 1.90 Å resolutions. There are overall 2% gaps in sequence alignment and the epitope sequence was LP+I+I+P+ against LPGIHIIPA. The optimized final 3-D model of *N. gonorrhoeae* ddl (Figure 2a-c) consists of 3 sheets, 1 beta alpha beta unit, 4 beta hairpins, 3 beta bulges, 13 strands (3 parallel and 10 anti- parallel barrels), 12 helices (ten alpha and two 3, 10), 8 helix-helix interacs, 20 beta turns, and 4 gamma turns (Figure 2d). The molecular weight and total energy of the model were estimated as 31.9 KD, -7391.5 KJ/mol, respectively. There are Glutathione synthetase ATP-binding domain-like motiff and PreATP-grasp domain but no potential Helix-turn-helix DNA-binding motif found. The protein contains only one enzyme active site (D-alanine-D-alanine ligase) and 12 significant ligand-binding sites (data not shown).

Validation of the model

To validate the model, initially ProSA-web was used that compares and analyzes the energy distribution in protein structure as a function of sequence position to determine a structure as native like or fault. As shown in Figure 3 and the Z-scores, the model is of good quality of structure. The Procheck of SAVA master server was used for assessment of stereochemical quality of the model. According to the Ramchandran plot, residues in most favoured regions, residues in additional allowed regions, residues in generously allowed regions, and residues in disallowed regions were respectively, 90.2%, 7.4%, 1.2%, and 1.2% that ensures the geometrically acceptable quality of the model (Figure 4).

Characterization of the epitope

The epitope position within the *N. gonorrhoeae* ddl protein was determined using Accelrys Discovery Studio Visualizer (v1.7). The native position of the epitope (LPGIHIIPA) from various angles is shown in Figure 5a-c (marked with a white arrow) and the amino acid position is shown in Figure 5d (marked with a circle). Combining the results from the 3D model and TMHMM 2.0 based topology analysis, it is evident that the epitope is exposed to the surface of the protein and therefore it also supports that the predicted sequence is a potential candidate peptide vaccine. Due to the very short length (9mars) of the epitope, instead of Swiss model server, the DISTILL server was used to generate 3-D structure (Figure 6). But both the validation tools (ProSA-web and Procheck) show that those models are highly unusual (data not shown) and no domain or motif could be assigned using ProFunc, Motif Scan, or InterProScan for this 9 mers epitope. Calculated molecular weight and pI of the 9 mers epitope are respectively, 930.16 and 6.74, and is found to be undigested by Trypsin, Chymotrypsin, Cyanogen bromide, Clostripain, Iodoso Benzoate, Staph Protease, and AspN, as determined by ProteinDigest. As the focus of this study is on *N. gonorrhoeae* ddl, we have excluded 3D characterization of epitopes from other pathogens. But based on sequence homol-

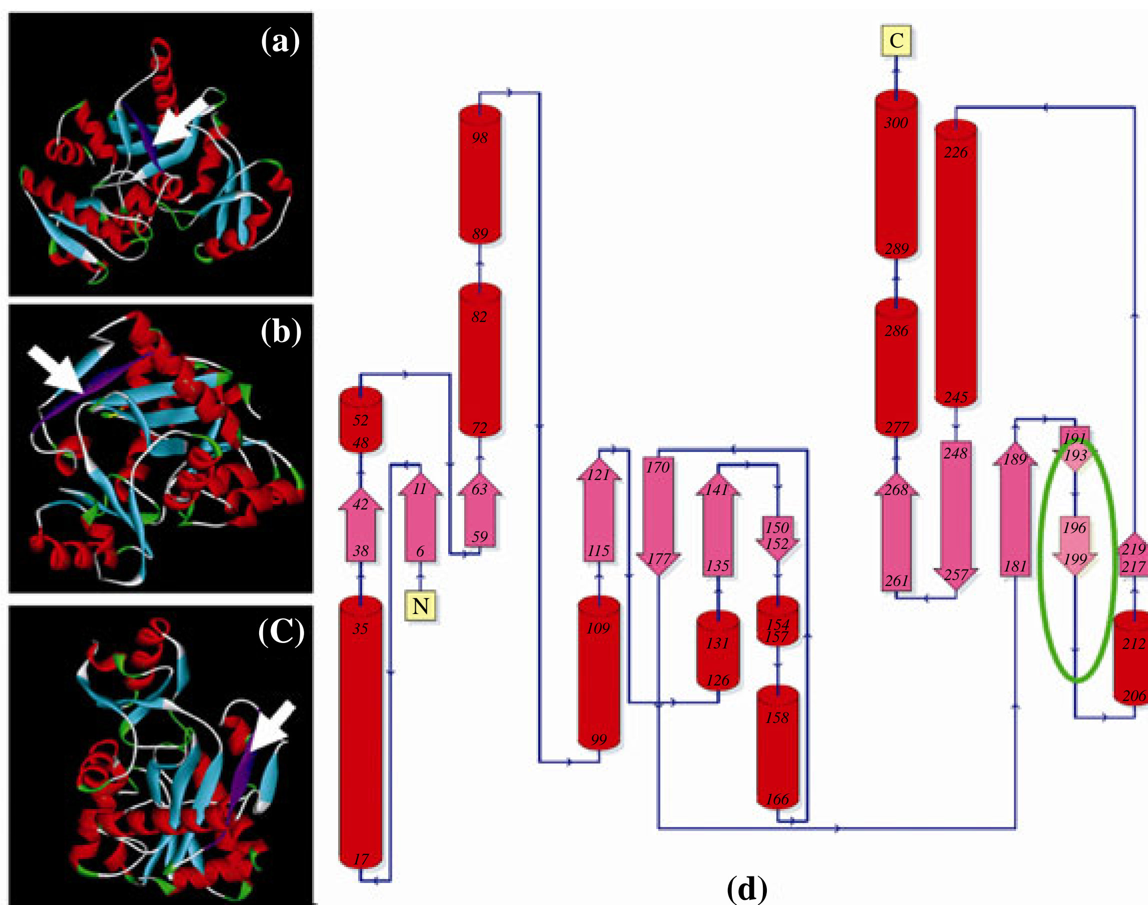

Figure 5: The native position of the epitope within *N. gonorrhoeae* ddl from various angles (a-c). The graphical view of the epitope position at corresponding amino acid sequence (d).

ISSN:0974-276X JPB, an open access journal

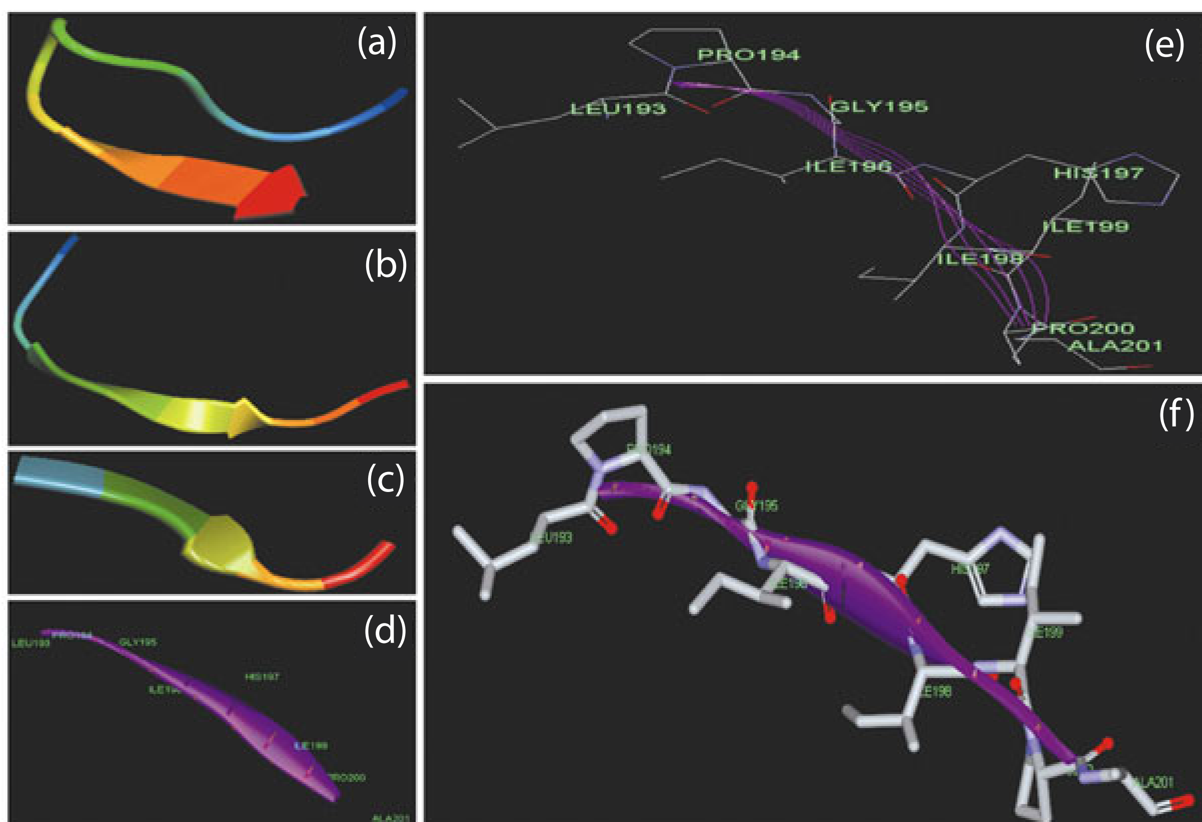

Figure 6: 3-D structures of 21 mers (a), 17 mers (b), and 9 mers (c) epitopes created by DISTILL. (d-f) Folding and amino acids positions of 9 mers epitope in detail.

ogy and topology analysis, it is found that, eiptopes identified from other pathogens are also antigenic, MHC II (DRB1*0101) binding, located at nearly same accessable region, and exposed to cell surface similar to the *N. gonorrhoeae* ddl. Therefore they are also potential vaccine candidates.

Discussion

D-alanine-alanine ligase (ddl) is an essential enzyme that acts in bacterial peptidoglycan biosynthetic pathway and is also a non-human homolog. Therefore, it is an important target for developing new antibiotics. In this current study, we demonstrated that among the selected four drug targets of *N. gonorrhoeae*, ddl is one of the potential and preferred candidates to develop either anti-gonorrhoeal drug or vaccine in isolation or in tandem. This study also showed that ddl may also be a good drug target in *N. meningitides, Haemophilus influenzae, Staphylococcus aureus, Helicobacter pylori, Burkholderia pseudomallei, Vibrio cholerae,* and *Acinetobacter baumannii* along with *A. hydrophila* as identified by Sharma et al. (2008).

Crystal structures of ddl from *E. coli* (Fan et al., 1997) and *Staphylococcus aureus* (Liu et al., 2006) are available. Similarly, 3-D modeling of the enzyme in *Enterococcus faecalis* (Prévost et al., 2000) and *Enterococcus faecium* (Gholizadeh et al., 2001) have been developed to identify active ligand binding sites in the enzyme for development of appropriate inhibitor and to understand the mechanism of drug resistance in respective pathogens. In this study, we characterized 3-D structure of the *N. gonorrhoeae* ddl to serve the same purpose. Using conventional drug development methods and structure based rational drug discovery strategy several ddl inhibitors namely D-cycloserine (Zawadzke et al., 1991; McCoy and Maurelli, 2005), diazenedicarboxamides (Kovac et al., 2007), quercetin, apigenin (Wu et al., 2008), Brutons's tyrosine kinase inhibitor LFM-A13 (Triola et al., 2009) etc have been identified. In our docking study, we found that apigenin is one of the best ligands for *N. gonorrhoeae* ddl (data not shown).

It is well known that two amino acid sequences are functionally similar if they share >40% sequence identity at amino acid level. In this analysis, our identified ddl sequences from pathogens that are homologous to *N. gonorrhoeae* ddl qualify this cut off value. Hence, it is presumed that those ddls may have similar structure and therefore identified inhibitors for *N. gonorrhoeae* ddl may inhibit those ddls. Hence we excluded 3D and docking studies for ddls from other pathogens.

In general, earlier studies have reported either T-cell or B-cell based epitope designing for a given pathogen (Arockiasamy and Krishnaswamy, 1995; Arévalo-Herrera et al., 2002; Sollner et al., 2008). Similarly, some vaccines can only activate helper T-lymphocytes (HTL)/CD4^{+} / MHC II. But activation of CD8^{+} cytotoxic T- lymphocytes (CTLs)/ MHC I is also required in many cases (Pancre et al., 1996). Therefore, an epitope that can produce both the B-cell and T-cell (MHC I and MHC II) mediated immunity is highly useful in developing peptide-based vaccines. In this study, to map epitopes from *N. gonorrhoeae* ddl, we have used the strategy as described by Barh and Misra, (2009) where the identified epitopes have high probability to produce both the B- and T- cell mediated immunity. In addition to our previously described method, we have also used VaxiJen and MHCPred to support the efficacy of epitopes. Similarly, in this study, using subtractive genomics and homology analysis we have also tried to identify a single epitope within a nearly same accessible region of corresponding ddl proteins in various other human pathogens so that the identified epitope may be useful against a wide range of pathogens. The identified peptide

ISSN:0974-276X JPB, an open access journal

(LPGIHIIPA) from *N. gonorrhoeae* ddl may induce B-cell and both the CD4+ and CD8+ T-cell mediated immunity. The sequence homology analysis demonstrates that this epitope sequence is conserved in ddls of *N. meningitides, H. influenzae, B. pseudomallei,* and *A. baumannii* and at nearly same accessible region. Therefore, this peptide might be useful in designing vaccine against all these human pathogens. Similarly, homologous and native sequences of the peptide (LPGIHIIPA) in these pathogens also can serve the same purpose in developing broad spectrum peptide vaccine for all these pathogens (Table-3).

Conclusions

To conclude, ddl protein can be a good target for developing effective antibiotic and vaccine for pathogenic *Neisseria, H. influenzae, B. pseudomallei,* and *A. baumannii.* Effective inhibitor screening for ddl is required. Similarly, the identified epitope(s) require proper design and subsequent validation for their uses as peptide vaccine against these human pathogens.

Financial Support

This research was carried out without any financial support or grant.

Acknowledgement

We are thankful to all IIOAB members for their support and encouragement while carrying out the analysis. ANM and AK acknowledge the infrastructures and facilities of DBT's Bioinformatics sub-centers hosted at respective University Departments used in this work. We also thank Dr. Suvro Chatterjee, Mr. Syamantak Majumder and Mr. Rajadhurai for their valuable inputs.

References

1. Arockiasamy A, Krishnaswamy S (1995) Prediction of B-cell epitopes for Salmonella typhi OmpC. J Biosci 20: 235-243. » CrossRef » PubMed » Google Scholar

2. Arévalo-Herrera M, Valencia AZ, Vergara J, Bonelo A, Fleischhauer K, et al. (2002) Identification of HLA-A2 restricted CD8(+) T-lymphocyte responses to Plasmodium vivax circumsporozoite protein in individuals naturally exposed to malaria. Parasite Immunol 24: 161-169. » CrossRef » PubMed » Google Scholar

3. Arnold K, Bordoli L, Kopp J, Schwede T (2006) The SWISS-MODEL Workspace: A web-based environment for protein structure homology modelling. Bioinformatics 22: 195-201. » CrossRef » PubMed » Google Scholar

4. Barbosa-Cesnik CT, Gerbase A, Heymann D (1997) STD vaccines an overview. Genitourin Med 73: 336-342. » CrossRef » PubMed » Google Scholar

5. Barh D, Kumar A (2009) *In silico* identification of candidate drug and vaccine targets from various pathways in *Neisseria gonorrhoeae.* In Silico Biol 9: 0019. » CrossRef » PubMed » Google Scholar

6. Barh D, Misra AN (2009) *In silico* Identification of membrane associated candidate drug targets in *Neisseria gonorrhoeae.* International Journal of Integrative Biology 6: 65-67. » CrossRef » PubMed » Google Scholar

7. Baú D, Martin AJ, Mooney C, Vullo A, Walsh I, et al. (2006) Distill: A suite of web servers for the prediction of one-, two- and three-dimensional structural features of proteins. BMC Bioinformatics 7: 402. » CrossRef » PubMed » Google Scholar

8. Bos MP, Tefsen B, Voet P, Weynants V, van Putten JP, et al. (2005) Function of neisserial outer membrane phospholipase a in autolysis and assessment of its vaccine potential. Infect Immun 73: 2222-2231. » CrossRef » PubMed » Google Scholar

9. Centers for Disease Control and Prevention (CDC): Sexually transmitted disease surveillance 2006. Atlanta: U.S. Department of Health and Human Services, 2007. » CrossRef » PubMed » Google Scholar

10. Cornelissen CN (2008) Identification and characterization of gonococcal iron transport systems as potential vaccine antigens. Future Microbiol 3: 287-298. » CrossRef » PubMed » Google Scholar

11. Doytchinova IA, Flower DR (2007) VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics 8: 4. » CrossRef » PubMed » Google Scholar

12. El-Manzalawy Y, Dobbs D, Honavar V (2008) Predicting linear B-cell epitopes using string kernels. J Mol Recognit 21: 243-255. » CrossRef » PubMed » Google Scholar

13. Fan C, Park IS, Walsh CT, Knox JR (1997) D-alanine:D-alanine ligase: phosphonate and phosphinate intermediates with wild type and the Y216F mutant. Biochemistry 36: 2531-2538. » CrossRef » PubMed » Google Scholar

14. Furuya R, Tanaka M (2009) *Neisseria gonorrhoeae* infections. Nippon Rinsho 67: 129-135. » CrossRef » PubMed » Google Scholar

15. Gholizadeh Y, Prevost M, Van Bambeke F, Casadewall B, Tulkens PM, Courvalin P (2001) Sequencing of the ddl gene and modeling of the mutated D-alanine:D-alanine ligase in glycopeptide-dependent strains of *Enterococcus faecium*. Protein Sci 10: 836-844. » CrossRef » PubMed » Google Scholar

16. Guan P, Doytchinova IA, Zygouri C, Flower DR (2003) MHCPred: A server for quantitative prediction of peptide-MHC binding. Nucleic Acids Res 31: 3621-3624. » CrossRef » PubMed » Google Scholar

17. Guex N, Peitsch MC (1997) SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. Electrophoresis 18: 2714-2723. » CrossRef » PubMed » Google Scholar

18. Gulati S, Ngampasutadol J, Yamasaki R, McQuillen DP, Rice PA (2001) Strategies for mimicking Neisserial saccharide epitopes as vaccines. Int Rev Immunol 20: 229-250. » CrossRef » PubMed » Google Scholar

19. Hansen JK, Demick KP, Mansfield JM, Forest KT (2007) Conserved regions from *Neisseria gonorrhoeae* pilin are immunosilent and not immunosuppressive. Infect Immun 75: 4138-4147. » CrossRef » PubMed » Google Scholar

20. Kovac A, Majce V, Lenarsic R, Bombek S, Bostock JM, et al. (2007) Diazenedicarboxamides as inhibitors of D-alanine-D-alanine ligase (Ddl). Bioorg Med Chem Lett 17: 2047-2054. » CrossRef » PubMed » Google Scholar

21. Krogh A, Larsson B, von Heijne G, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305: 567-80. » CrossRef » PubMed » Google Scholar

22. Laskowski RA, MacArthur MW, Moss DS, Thornton JM (1993) PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Cryst 26: 283-291. » CrossRef » PubMed » Google Scholar

23. Laskowski RA, Watson JD, Thornton JM (2005) ProFunc: a server for predicting protein function from 3D structure. Nucleic Acids Res 33: W89-W93. » CrossRef » PubMed » Google Scholar

24. Liu S, Chang JS, Herberg JT, Horng MM, Tomich PK, et al. (2006) Allosteric inhibition of *Staphylococcus aureus* D-alanine:D-alanine ligase revealed by crystallographic studies. Proc Natl Acad Sci 103: 15178-15183. » CrossRef » PubMed » Google Scholar

25. McCoy AJ, Maurelli AT (2005) Characterization of Chlamydia MurC-Ddl, a fusion protein exhibiting D-alanyl-D-alanine ligase activity involved in peptidoglycan synthesis and D-cycloserine sensitivity. Mol Microbiol 57: 41-52. » CrossRef » PubMed » Google Scholar

26. Notredame C, Higgins DG, Heringa J (2000) T-Coffee: A novel method for fast and accurate multiple sequence alignment. J Mol Biol 302: 205-217. » CrossRef » PubMed » Google Scholar

27. Pancré V, Gras-Masse H, Delanoye A, Herno J, Capron A, et al. (1996) Induction of cytotoxic T-cell activity by the protective antigen of Schistosoma mansoni Sm28GST or its derived C-terminal lipopeptide. Scand J Immunol 44: 485-492. » CrossRef » PubMed » Google Scholar

28. Prévost M, Van Belle D, Tulkens PM, Courvalin P, Van Bambeke F (2000) Modeling of *Enterococcus faecalis* D-alanine:D-alanine ligase: structure-based study of the active site in the wild-type enzyme and in glycopeptide-dependent mutants. J Mol Microbiol Biotechnol 2: 321-330. » CrossRef » PubMed » Google Scholar

29. Price GA, Masri HP, Hollander AM, Russell MW, Cornelissen CN (2007) Gonococcal transferrin binding protein chimeras induce bactericidal and growth inhibitory antibodies in mice. Vaccine 25: 7247-7260. » CrossRef » PubMed » Google Scholar

30. Price GA, Russell MW, Cornelissen CN (2005) Intranasal administration of recombinant *Neisseria gonorrhoeae* transferrin binding proteins A and B conjugated to the cholera toxin B subunit induces systemic and vaginal antibodies in mice. Infect Immun 73: 3945-3953. » CrossRef » PubMed » Google Scholar

31. Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, et al. (2005) InterProScan: protein domains identifier. Nucleic Acids Res 33: W116-120. » CrossRef » PubMed » Google Scholar

32. Rothbard JB, Fernandez R, Wang L, Teng NN, Schoolnik GK (1985) Antibodies to peptides corresponding to a conserved sequence of gonococcal pilins block bacterial adhesion. Proc Natl Acad Sci 82: 915-919. » CrossRef » PubMed » Google Scholar

33. Sharma V, Gupta P, Dixit A (2008) In silico identification of putative drug targets from different metabolic pathways of *Aeromonas hydrophila*. In Silico Biol 8: 0026. » CrossRef » PubMed » Google Scholar

34. Singh H, Raghava GP (2001) ProPred: prediction of HLA-DR binding sites. Bioinformatics 17: 1236-1237. » CrossRef » PubMed » Google Scholar

35. Singh H, Raghava GP (2003) ProPred1: prediction of promiscuous MHC Class-I binding sites. Bioinformatics 19: 1009-1014. » CrossRef » PubMed » Google Scholar

36. Sollner J, Grohmann R, Rapberger R, Perco P, Lukas A, et al. (2008) Analysis and prediction of protective continuous B-cell epitopes on pathogen proteins. Immunome Res 4: 1. » CrossRef » PubMed » Google Scholar

37. Tapsall J (2006) Antibiotic resistance in *Neisseria gonorrhoeae* is diminishing available treatment options for gonorrhea: some possible remedies. Expert Rev Anti Infect Ther 4: 619-628. » CrossRef » PubMed » Google Scholar

38. Thomas CE, Zhu W, Van Dam CN, Davis NL, Johnston RE, et al. (2006) Vaccination of mice with gonococcal TbpB expressed in vivo from Venezuelan equine encephalitis viral replicon particles. Infect Immun 74: 1612-1620. » CrossRef » PubMed » Google Scholar

39. Toes RE, Nussbaum AK, Degermann S, Schirle M, Emmerich NP, et al. (2001) Discrete cleavage motifs of constitutive and immunoproteasomes revealed by quantitative analysis of cleavage products. J Exp Med 194: 1-12. » CrossRef » PubMed » Google Scholar

40. Triola G, Wetzel S, Ellinger B, Koch MA, Hübel K, et al. (2009) ATP competitive inhibitors of D-alanine-D-alanine ligase based on protein kinase inhibitor scaffolds. Bioorg Med Chem 17: 1079-1087. » CrossRef » PubMed » Google Scholar

41. Wiederstein M, Sippl MJ (2007) ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res 35: W407-410. » CrossRef » PubMed » Google Scholar

42. Workowski KA, Berman SM, Douglas JM Jr (2008) Emerging antimicrobial resistance in *Neisseria gonorrhoeae*: urgent need to strengthen prevention strategies. Ann Intern Med 148: 606-613. » CrossRef » PubMed » Google Scholar

43. Wu D, Kong Y, Han C, Chen J, Hu L, et al. (2008) D-Alanine:D-alanine ligase as a new target for the flavonoids quercetin and apigenin. Int J Antimicrob Agents 32: 421-426. » CrossRef » PubMed » Google Scholar

44. Zawadzke LE, Bugg TD, Walsh CT (1991) Existence of two D-alanine:D-alanine ligases in *Escherichia coli*: cloning and sequencing of the ddlA gene and purification and characterization of the DdlA and DdlB enzymes. Biochemistry 30: 1673-1682. » CrossRef » PubMed » Google Scholar

45. Zhang R, Ou HY, Zhang CT (2004) DEG: A database of essential genes. Nucleic Acids Res 32: D271-D272. » CrossRef » PubMed » Google Scholar

46. Zhu W, Thomas CE, Sparling PF (2004) DNA immunization of mice with a plasmid encoding *Neisseria gonorrhea* PorB protein by intramuscular injection and epidermal particle bombardment. Vaccine 22: 660-669. » CrossRef » PubMed » Google Scholar