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IN SILICO DOCKING STUDIES OF *STAPHYLOCOCCUS AUREUS* VIRULENT PROTEINS WITH ANTIMICROBIAL PEPTIDES

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ABSTRACT

Antibiotic resistance in bacteria has increased world wide which leads to inability in the treatment of human and animal infectious diseases. Resistance against antibiotics by pathogenic bacteria is a major concern in the anti-infective therapy of both humans and animals. Serious concerns about bacterial antibiotic resistance from nosocomial, community-cquired and food-borne pathogens have been growing for a number of years, and have been raised at both national and international levels. So, antimicrobial peptides which are potent, broad spectrum antimicrobial agent that demonstrate potential as well as novel therapeutic agents are used as alternatives to the current use of antibiotics. In this present study, five Staphylococcus aureus virulent proteins were docked with four antimicrobial peptides against bacteria using patchdock -an automated docking server which gave list of results of receptor and ligand complexes. The receptor-ligand interactions were visualized using pymol to study the binding interaction between them.

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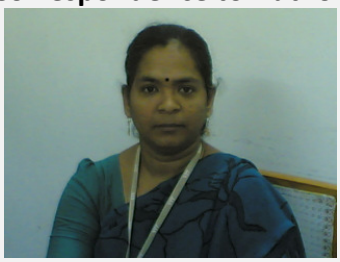

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INTRODUCTION

During the last decade, antibiotic resistance by various mechanisms has increased world-wide in bacterial pathogens leading to treatment failures in human and animal infectious diseases (1). Resistance against antibiotics by pathogenic bacteria is a major concern in the anti-infective therapy of both humans and animals. Bacteria are able to adapt rapidly to new environmental conditions such as the presence of antimicrobial molecules and, as a consequence, resistance increases with the antimicrobial use (2). Serious concerns about bacterial drug resistance from nosocomial, community acquired and food-borne pathogens have been growing for a number of years and have been raised at both national and international levels (3). So, antimicrobial peptides which have antimicrobial activity found in plants and animals are used against microorganisms like bacteria, fungi and viruses.

A large group of low molecular weight natural compounds that exhibit antimicrobial

activity has been isolated from animals and plants during the past two decades. Among them, cationic peptides are the most widespread. Interestingly, the variety and diversity of these peptides seem to be much wider than suspected. In fact, novel classes of peptides with varying chemical properties continue to be isolated from different vertebrate and invertebrate species, as well as from bacteria. To the early characterized peptides, mostly cationic in nature, anionic peptides, aromatic dipeptides, processed forms of oxygen-binding proteins and processed forms of natural structural and functional proteins can now be added, just to name a few. In spite of the astonishing diversity in structure and chemical nature displayed by these molecules, all of them present antimicrobial activity, a condition that has led researchers to consider them as "natural antibiotics" and as such a new and innovative alternative to chemical antibiotics with a promising future as biotechnological tools. A resulting new generation of antimicrobial peptides (AMP) with higher specific activity and wider microbe-range of action could be constructed, and hopefully endogenously expressed in genetically-modified organisms (4).

Antimicrobial peptides

Antimicrobial peptides are widely recognized as promising alternatives to the current use of antibiotics and fungicides. Amino acid sequences of a vast majority of AMP share cationic and amphipathic biophysical properties that allow their insertion into lipid bilayers, and can lead to alteration of biological membrane functions. Initial characterization studies linked these properties to antimicrobial killing activity. However, additional data indicate that this is not the sole mode of action and that more subtle mechanisms might mediate the interaction with and effect to target microbes, as well as the specificity and toxicity of peptides. As such, antimicrobial peptides are increasingly viewed as powerful multifunctional drugs.

Antimicrobial peptides and/or small antimicrobial proteins have been characterized from a vast number of organisms, from bacteria to insects, plants and humans. The increasing problem of antibiotic resistance in clinic and the pressure to reduce antibiotic and fungicide use in agricultural and food industry has put AMP at the edge front as promising compounds to fight microbial infections and contaminations. Over 1000 natural AMP are currently known to their amino acid sequence. The epithelial lining of our skin, gastrointestinal tract and bronchial tree produces a number of antibacterial peptides, and our phagocytic neutrophils rapidly ingest and enzymatically degrade invading organisms, as well as produce peptides and enzymes with antimicrobial activities. Some of these antimicrobial moieties also appear to alert host cells involved in both innate host defense and adaptive immune responses. The epithelial cells are a source of constitutively produced *β*-defensin (HBD1) and proinflammatory cytokine-inducible *b* defensins (HBD2 and -3) and cathelicidin (LL37). The neutrophils-derived antimicrobial peptides are released on demand from their cytoplasmic granules (5).

Antimicrobial peptides are small molecular weight proteins with broad spectrum antimicrobial activity against bacteria, viruses, and fungi. These evolutionarily conserved peptides are usually positively charged and have both a hydrophobic and hydrophilic side that enables the molecule to be soluble in aqueous environments yet also enter lipid-rich membranes. Once in a target microbial membrane, the peptide kills target cells through diverse mechanisms. In addition to important antimicrobial properties, growing evidence indicates that AMPs alter the host immune response through receptor-dependent interactions (6**).** Staphylococcus aureus is a common cause of disease, giving rise to uncomplicated as well as life threatening infections in humans. Although most staphylococcal infections can be successfully treated with antibiotics, reports of strains resistant to most available treatments are of great concern (7, 8). The development of alternative therapies is of great importance. Thus, antimicrobial peptides which are alternatives to current antibiotics are used as therapeutic agents to treat Staphylococcus infections.

In this study, we investigated the interaction and binding energies of five *Staphylococcus aureus* virulent proteins with four antimicrobial peptides which are against bacteria by patchdock server-an automatic server for molecular docking. The interactions between the receptor-ligand complexes were visualized through Pymol.

Molecular docking

Binding of a small molecule (ligand) with a large molecule (protein) is called docking. Docking is the process by which two molecules fit together in 3D space. The objective of computational docking is to determine how two molecules will interact which will aid the interaction studies in bio-molecules. Molecular docking is often employed to aid in determining how a particular drug lead will interact to form a binding pocket.

MATERIALS AND METHODS

Patchdock

Patchdock algorithm (9) is inspired by object recognition and image segmentation techniques that are used in computer vision. Given two molecules, their surfaces are divided into patches according to the surface shape. All possible patches concave, convex or flat surface patches which can be visually seen are detected using segmentation algorithm. The patches are then filtered, so that only patches with hot spot residues are retained.

Once the patches are identified, they are superimposed using shape matching algorithm. Shape matching algorithm uses hybrid of the geometric hashing (10) and pose clustering matching techniques to match the patches detected by segmentation algorithm. Concave patches are matched with convex patches and flat patches with any type of patches to obtain complexes. All the candidate possible complexes are examined.

Preparation of receptors

The 3D co-ordinates of *S. aureus* receptors were downloaded from the Protein Data Bank (http://www.rcsb.org/pdb/home.do). Before docking, all water molecules were removed from protein file. After removing the water molecules H-atom were added for the correct ionization and tautomeric states of amino acid residues.

Table 1: Protein receptors and ligands used in this docking study

Protein receptor PDB id	Antibacterial peptides PDB id
1STE	1KV4
2GOM	1LAV
2NTT	2MAG
3DOA	2GW9
3D5S	

Preparation of ligands

A dataset of four antimicrobial peptides namely Moricin, Sapecin, Magainin and Defensin from silkworm, Sarcophaga peregrine, Xenopus laevis and Mouse respectively were used as ligand molecules. These were retrieved from antimicrobial peptide database which had PDB link from where obtained the structures of corresponding peptides.

Docking of Staphylococcus aureus protein receptors with antimicrobial peptides

The selected protein receptors from *S. aureus* were docked with antimicrobial peptides of four different organisms by uploading the molecules in Patchdock server, an automatic server for molecular docking. Clustering RMSD was chosen as 4.0 Å. E-mail address to retrieve the result was given. Results were obtained through

the e-mail address provided and the docked complex structures were downloaded (11).

Table 2: Names of receptors and ligands used

PDB ID	Names of receptors and antimicrobial peptides
1STE	Enterotoxin C2
2GOM	Extracellular fibrinogen binding protein (Efb-C)
2NTT	Staphylococcal enterotoxin K
3DOA	Fibrinogen binding protein
3D5S	Efb-c/C3d complex
1KV4	Moricin (silkworm *Bombyx mori*)
1L4V	Sapecin (*Sarcophaga peregrina*)
2MAG	Magainin (*Xenopus laevis*)
2GW9	Defensin Cryptdin4 (Mouse)

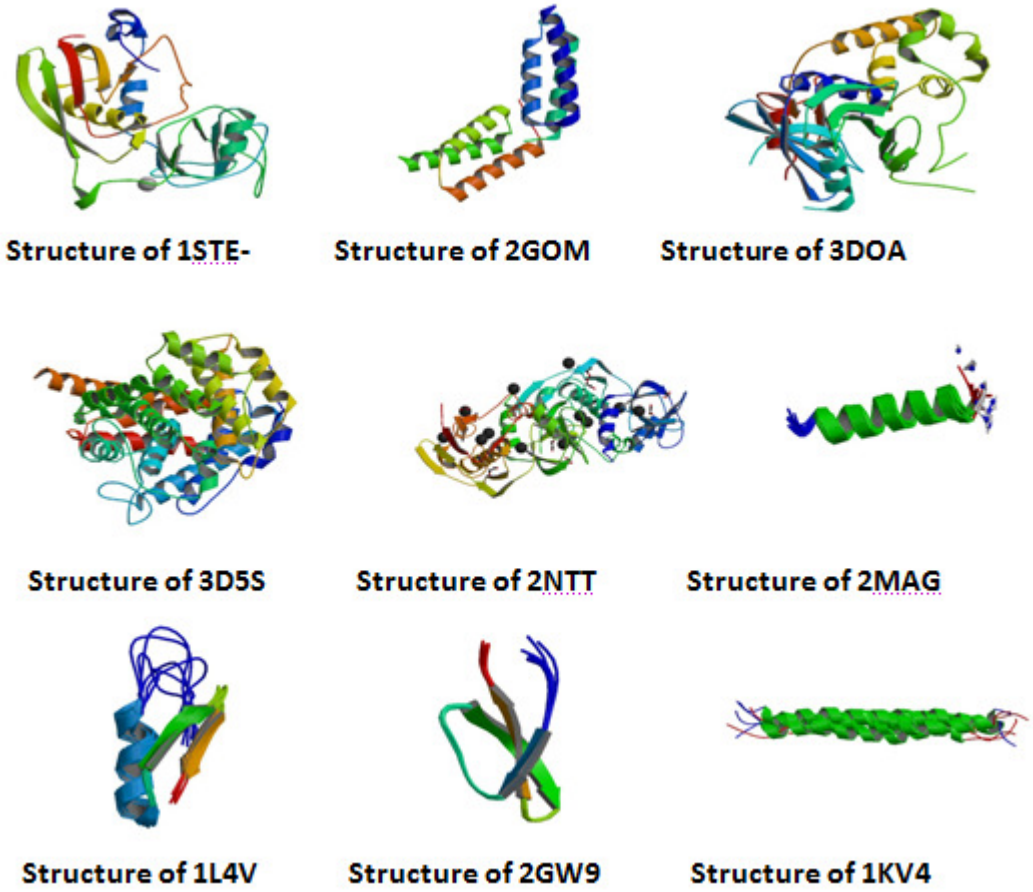

Fig.1: Structures of bacterial protein receptors and AMP ligands used in this study

Analysis of docking results

Analysis of docking interaction of the bacterial receptors with the four different antimicrobial peptides was done to identify the original binding mode between the antimicrobial peptides and Staphylococcal protein receptors.

RESULT AND DISCUSSION

The output of Patchdock was a list of candidate complexes between receptor and ligand molecule. The list was presented in the format of a table. Each table line represented one candidate complex. Solution No. represented the number of the solution. Score corresponded to geometric shape complementarity score (9). The solutions were sorted according to this score. Area stands for the approximate interface area of the complex. ACE indicates Atomic Contact Energy (12). Transformations represented are 3D transformations that include 3 rotational angles and 3 translational parameters. These transformations are applied on the ligand molecule. PDB file of the complex denoted the predicted complex structure in PDB format. High scoring solution structure of docked protein-peptides complexes was downloaded.

Table. 3 Docking results of Staphylococcal protein receptor and AMPs

Receptor pdb id	Ligand pdb id	Patchdock score	ACE value
1ste	1kv4	11392	126.67
1ste	1l4v	11300	-60.39
1ste	2mag	8896	309.41
1ste	2gw9	10914	354.45
2gom	1kv4	8492	383.31
2gom	1l4v	9034	148.19
2gom	2mag	7994	110.91
2gom	2gw9	8790	240.20
2ntt	1kv4	13776	417.33
2ntt	1l4v	11412	245.73
2ntt	2mag	8822	-56.61
2ntt	2gw9	10892	-105.65
3d5s	1kv4	11938	338.92
3d5s	1l4v	12298	119.97
3d5s	2mag	10594	42.31
3d5s	2gw9	11508	468.99
3doa	1kv4	11882	-15.38
3doa	1l4v	13156	46.06
3doa	2mag	9612	97.56
3doa	2gw9	10264	126.47

Molecular Docking Algorithm Based on Shape Complementarity Principles

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Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail	Receptor Site	Ligand Site	Distance Constraints
1ste.txt	2mag.txt	Default	4.0	mramdevi85@gmail.com	-	-	-

Solution No	Score	Area	ACE	Transformation	PDB file of the complex
1	8896	1028.50	309.41	0.27 -0.04 -0.82 37.90 16.26 14.12	result.1.pdb
2	8796	1161.40	-52.41	1.00 0.03 -1.51 44.89 37.93 32.87	result.2.pdb
3	8644	1229.50	-139.90	-0.81 0.07 -2.59 42.33 32.47 0.99	result.3.pdb
4	8544	1131.60	148.47	0.94 -0.27 -0.29 34.41 18.71 14.10	result.4.pdb
5	8516	1120.70	-7.19	0.99 -0.12 -1.76 43.47 34.98 34.74	result.5.pdb
6	8198	1199.60	-98.62	2.65 -0.70 2.17 47.33 42.56 26.72	result.6.pdb
7	8084	1261.00	229.51	-2.28 -1.02 -0.72 46.33 50.48 18.61	result.7.pdb
8	8022	1032.20	-303.42	-0.98 -0.05 2.89 47.85 29.48 0.67	result.8.pdb
9	8018	1011.40	-237.83	0.17 -0.62 2.64 44.52 13.71 16.02	result.9.pdb
10	8006	974.90	54.83	-1.53 -0.96 1.17 34.86 13.81 22.31	result.10.pdb
11	7980	1264.40	-125.28	-2.70 0.53 -0.44 46.57 14.17 12.60	result.11.pdb
12	7928	1168.80	180.89	1.62 -0.27 1.20 35.56 28.09 2.00	result.12.pdb
13	7904	1105.00	123.46	-0.87 1.38 2.86 46.28 50.55 20.93	result.13.pdb
14	7810	1049.70	95.21	-1.13 -0.48 1.99 31.32 18.81 18.24	result.14.pdb
15	7786	930.00	162.35	2.21 -0.22 0.87 37.57 25.65 36.77	result.15.pdb
16	7778	1312.20	-402.94	-1.67 0.56 -0.75 44.08 14.92 14.70	result.16.pdb
17	7714	923.70	162.86	2.05 -0.23 1.00 35.89 22.23 37.02	result.17.pdb
18	7666	1254.20	-59.83	-1.05 -0.44 1.87 33.08 30.79 1.59	result.18.pdb
19	7628	974.70	320.98	2.82 0.88 2.98 26.62 26.24 9.46	result.19.pdb
20	7620	1005.00	-139.28	2.95 0.60 2.84 53.24 44.09 18.09	result.20.pdb

Fig. 2: Patchdock result for 1ste and 2mag showing less score and higher ACE value which was least best result in this study.

Molecular Docking Algorithm Based on Shape Complementarity Principles

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Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail	Receptor Site	Ligand Site	Distance Constraints
2ntt.txt	1kv4.txt	Default	4.0	mramdevi85@gmail.com	-	-	-

Solution No	Score	Area	ACE	Transformation	PDB file of the complex
1	13776	1672.80	417.33	-0.78 0.64 -2.59 14.37 16.84 18.20	result.1.pdb
2	11196	1402.30	274.04	2.99 -0.56 0.32 15.47 16.37 21.64	result.2.pdb
3	11104	2473.50	131.92	-1.43 -0.60 0.38 29.06 -13.42 32.98	result.3.pdb
4	11034	1633.60	409.36	2.32 -0.64 0.07 38.14 13.33 28.98	result.4.pdb
5	10990	1592.70	284.10	2.07 1.18 -0.20 29.08 -8.46 15.24	result.5.pdb
6	10914	1290.10	122.88	0.21 0.38 0.52 16.91 -16.54 22.40	result.6.pdb
7	10878	1768.30	85.47	2.74 0.54 -2.67 15.46 -19.84 40.61	result.7.pdb
8	10862	1499.20	42.13	0.17 0.51 -2.65 2.94 10.51 27.99	result.8.pdb
9	10792	1452.00	383.00	-1.98 1.18 -2.75 36.46 -7.77 1.49	result.9.pdb
10	10750	1873.60	472.79	-1.83 0.09 -2.65 28.52 -9.44 32.21	result.10.pdb
11	10688	1289.60	329.68	-0.07 -0.65 -2.98 12.80 -12.66 16.13	result.11.pdb
12	10630	2032.70	-276.93	0.24 0.26 0.52 25.97 -10.00 25.13	result.12.pdb
13	10584	1430.50	139.90	1.67 -0.60 0.55 16.02 17.96 17.21	result.13.pdb
14	10562	1939.50	459.95	1.73 -0.65 0.20 43.07 12.58 23.75	result.14.pdb
15	10556	1582.30	-129.49	0.42 0.23 0.53 29.90 -9.40 26.09	result.15.pdb
16	10492	1597.00	202.02	-1.96 0.05 0.35 30.73 -9.88 29.03	result.16.pdb
17	10466	1336.10	73.80	1.46 -0.87 0.60 15.45 17.17 12.21	result.17.pdb
18	10446	1724.10	427.40	0.43 -0.64 -0.44 44.14 2.68 24.72	result.18.pdb
19	10402	1491.90	84.72	3.05 0.18 -2.59 14.50 -19.72 30.77	result.19.pdb
20	10332	1412.30	281.26	-2.33 -0.97 0.28 14.73 16.47 12.72	result.20.pdb

Fig. 3: Docking between 2ntt and 1kv4 showing highest docking score of 13776

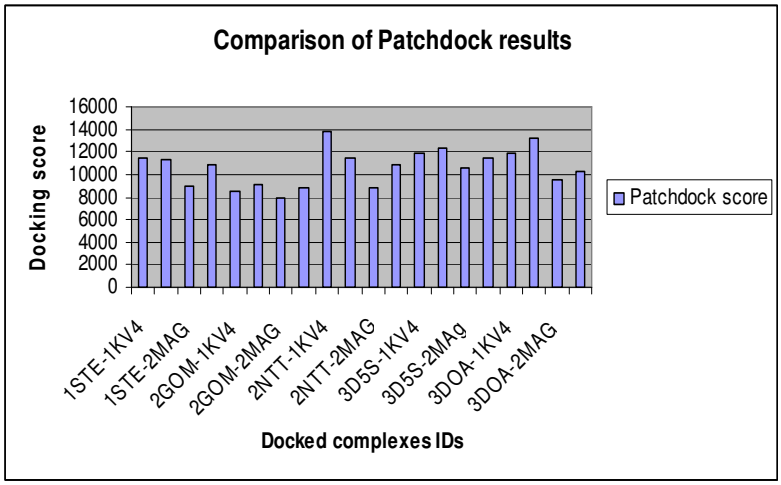

Fig. 4: Overall comparison of patchdock scores of docked complexes

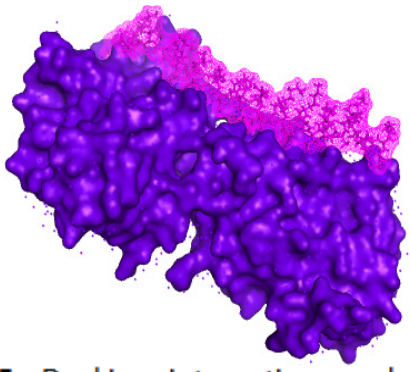

Fig.5: Docking interaction mode of 2NTT with 1KV4 visualised through pymol

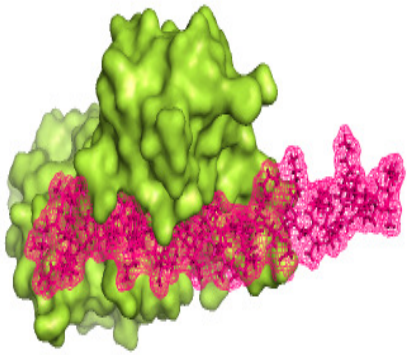

Fig. 6: Binding of 3doa with 1kv4 viewed through pymol

The patchdock result of the protein receptor 1ste and ligand 1kv4 had docking score of 11392 with ACE 126.67, and the receptor 1ste and ligand 1lav had docking score of 11300 with ACE value -60. The patchdock result for 1ste and 2mag had docking score of 8896 with ACE value 309.41. The 1ste and 2gw9 had docking score of 10914 and ace value was 354.45. The patchdock result of 2gom and 1kv4 had docking score of 8492 with ace value 383.31, 2gom and 1lav showed docking score of 9034 with ace value 148.19, 2gom and 2mag had 7994 score with ace value 110.91, and 2gom and 2gw9 had docking score of 8790 with ace value 240.20. The patchdock result of receptor 2ntt with ligands 1kv4, 1lav, 2mag and 2gw9 showed docking score of 13776, 11412, 8822 and 10892 respectively. The patchdock result of 3d5s with the four ligands showed docking score of 11938, 12298, 10594 and 11508. Docking of 3doa with the four ligands showed docking score of 10264, 13156, 9612 and 11882.

Of the five *S. aureus* virulent proteins docked with four antimicrobial peptides, docking with 2ntt and lkv4 showed highest score which was 13776 with ACE value 417.33. The result which showed good docking score with less atomic contact energy was the result docked with 2ntt and 2gw9 in which score was 10892 and ACE value was -105.65 and also docking with 3doa and 1lav had score with 13156 with less ACE value of 46.06. The score for 3doa and 1kv4 showed good docking score of 11882 with better less ACE value of -15.38.

CONCLUSION

In this study, antimicrobial peptides which are alternatives to antibiotics and are against

pathogenic microorganisms were used for docking-binding interactions studies with Staphylococcal virulent proteins. Five virulent proteins from *staphylococcus aureus* and four antimicrobial peptides against bacteria were randomly selected and their structures were obtained from PDB database. The proteins and AMPs were docked in patchdock server. The results showed that the docking between 2NTT and 1KV4 had good interaction having docking score of 13776. The docked structures were visualized through pymol.

REFERENCES

1) Harbarth S, Samore MH. Antimicrobial resistance determinants and future control. Emerg Infect Dis 2005; 11:794-801.
2) Falagas ME, Bliziotis IA. Pandrug-resistant Gram-negative bacteria: the dawn of the post-antibiotic era? Int J Antimicrob Agents 2007; 29:630-6.
3) Jansen WT, van der Bruggen JT, Verhoef J, Fluit AC. Bacterial resistance: a sensitive issue complexity of the challenge and containment strategy in Europe. Drug Resist Updat 2006; 9:123-33.
4) Sergio H. Marshall**,** Antimicrobial peptides: A natural alternative to chemical antibiotics and a potential for applied biotechnology. Electronic Journal of Biotechnology ISSN: 0717- 3458 Vol.6 No.2, Issue of August 15, 2003.
5) Marcos, Jose F, Gandía, Mónica , Antimicrobial peptides: to membranes and beyond. Expert Opinion on Drug Discovery, 2009.
6) Arash Izadpanah, Richard L. Gallo, Antimicrobial peptides *Journal of the American Academy of Dermatology*, *Volume 52, Issue 3*, *March 2005*, *Pages 391-392.*
7) Groom AV, Wolsey DH, Naimi TS et al. Community acquired methicillin resistant Staphylococcus aureus in a rural American Indian community. Jama 2001; 286: 1201±5.
8) Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin-resistant Staphylococcus aureus. Trends Microbiol 2001; 9: 486±93.
9) Duhovny D, Nussinov R, Wolfson HJ. Efficient Unbound Docking of Rigid Molecules. In Gusfield et al., Ed. Proceedings of the 2'nd Workshop on Algorithms in Bioinformatics(WABI) Rome, Italy, Lecture Notes in Computer Science 2452, pp. 185-200, Springer Verlag, 2002.
10) Schneidman-Duhovny D, Inbar Y, Polak V, Shatsky M, Halperin I, Benyamini H, Barzilai A, Dror O, Haspel N, Nussinov R, Wolfson H. J. Taking geometry to its edge fast unbound rigid (and hinge-bent) docking. Proteins. 2003 Jul 1; 52(1): 107-12.
11) Uma Maheswari , Molecular docking study of tripeptides VPP and IPP inhibiting angiotensin I converting enzyme to alleviate high altitude pulmonary edema. Int. Jour. of Genetics and Molecular Biology Vol. 3(1), pp. 1-6.
12) Zhang C, Vasmatzis G, Cornette JL, DeLisi C (1997). Determination of atomic desolvation energies from the structures of crystallized proteins. J. Mol. Biol., 267(3): 707-726.

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