

## Identification of Targetable Virulence Factor and Drug Screening For Bacterial Pneumonia

Ravikant Yadav<sup>1\*</sup>, Nikita Chordia<sup>2</sup>, Anil kumar<sup>2</sup>, Shobha Shouche<sup>1</sup>

*1*govt. Madhav science college, ujjain, madhya pradesh, india

*2* School of Biotechnology, Devi Ahilya University, Takshashila Campus, Khandwa Road,INDORE

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**Abstract:** *Pneumonia is an infectious disease. This is prevalent in pandemic proportions across the Globe; especially in developing countries. It is one of the major causes of morbidity and mortality among infants and children. It is mainly caused by bacterial or viral infection. Irrespective of the abundant occurrence of bacterial pneumonia, there is no specific antibiotic therapy available. On the other hand non-specific therapies are less effective and may induce bacterial resistance. Therefore, in present work, we explored a common but novel target for four pathogenic pneumonia causing bacteria and also revealed the most specific antibiotic(s) to this target. We observed trigger factor protein as a common targetable virulence factor present in four pathogenic bacteria viz. Streptococcus pneumoniae, Klebsiella pneumoniae, Chlamydomphila pneumoniae and Mycoplasma pneumoniae. After screening of 116 available antibacterial compounds, we found 04 most specific and potent antibacterial agents that inhibit the growth and survival of bacteria through trigger factor protein. The best efficacies showed by enoxacin, sulfamonomethoxine, carbadox and isoniazid.*

**Keywords:** *pneumonia, virulence, docking, screening, targetable, inhibitor*

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### I. Introduction

Pneumonia is a common infectious disease caused in one or both lungs. Annually pneumonia affects 450 million peoples resulting in about 7 million deaths. This deaths include either very old (more than 65) or very young people (less than 2). It can be caused mainly by bacteria or viruses. Till now the patients are treated with the hit and trial as cause of pneumonia is hard to diagnose. There are varieties of bacteria that can cause the disease viz. Streptococcus pneumonia, Klebsiella pneumonia, Chlamydomphila pneumonia, Legionella pneumophila, mycoplasma pneumonia and many more. This divergency leads to non-specific treatment of pneumonia. To treat bacterial pneumonia patients are treated with antibiotics like penicillin, doxycycline or clarithromycin. All these antibiotics are not target specific so it only helps in decreasing the survival but not the proper curement.

As already reported most cases of pneumonia is caused by Streptococcus pneumoniae then others including Klebsiella pneumoniae, Chlamydomphila pneumoniae and Mycoplasma pneumoniae [1, 2]. We have considered the four causative bacterial pathogen viz. streptococcus pneumoniae, klebsiella pneumoniae, chlamydomphila pneumoniae and mycoplasma pneumoniae. This are the main causative organism involved in causing bacterial pneumonia. If an organism specific drug for this bacterial species is designed and causative bacteria is diagnosed in the early stages then proper treatment surely helps in declining the mortality rate caused by pneumonia. As the diagnosis of pneumonia in early stages is difficult, causative organism can't be identified at the onset of the infection. This problem can be solved if the drug is targeted to a protein that is common to all the bacterial species. This target can be called as multispecies target. And this target is to be inhibited by the single inhibitor that can be used as drug to inhibit the survival of the multiple-organism. Advantage of using this multispecies targeted drug is that it can be used when pneumonia symptoms are identified even without knowing the causative organism. This early treatment may not leads patient to the severe and hospitalized cases. This helps in the easy curement of the patients.

We are using the new paradigm of targeting virulence factor as virulence factors are responsible for converting non-pathogenic to pathogenic bacteria. Virulence factors are the bacterial factors that cause damage to host tissues. Inhibiting virulence rather than growth may impose weaker selective pressure for the development of antibiotic resistance relative to current antibiotics [3]. For inhibiting virulence, a virulence factor is required that can act as target that we can called as targetable virulence factor. Then this targetable virulence factors are inhibited by the antibacterial compounds. This antibacterial compounds are mostly antibiotics that are already FDA approved that helps to fight against bacterial infection and goes through fast drug approval process. The best four antibacterial compounds that are specific for bacterial pneumonia were identified. This antibacterial compound(s) inhibit the virulence factor results in the hampering of pathogenic growth as well as survival.

## **II. Method**

### **Sequence retrieval**

Complete proteome of all four species of bacteria viz. *Streptococcus pneumoniae* strain 670-6B (Accession No. NC\_014498), *Klebsiella pneumoniae* strain 342 (Accession No. NC\_011281), *Chlamydomphila pneumoniae* strain AR39 (Accession No. NC\_002179) and *Mycoplasma pneumoniae* (Accession No. NC\_016807) were downloaded from NCBI ftp site (<ftp://ftp.ncbi.nlm.nih.gov/genome>).

### **Orthologs Prediction**

All four bacterial proteome were compared to find the orthologs protein. This was done using an internally developed method called reciprocal BLAST using *Streptococcus pneumoniae* as reference organism. In reciprocal BLAST, blastp [4] is done for a query protein against *Streptococcus pneumoniae* the resulting protein of *Streptococcus pneumoniae* was again blastp against the query protein's organism. If the same targeted protein is the result then it is considered to be orthologs.

### **Identification of virulence factors**

Virulence factors were identified among all orthologs protein identified. This was done using a database called mvirDB [5] also known as LLNL Virulence Database available at <http://mvirdb.llnl.gov/>. This database contains microbial virulence factor from various sources. Mvir's blast with default parameters was used to find the virulence factor among orthologs.

### **Targetable virulence factors**

Predicted virulence factors were then checked for their targetability using Therapeutic Target Database (TTD) [6]. This database contains information about explored therapeutic targets. Target similarity search was used to find the targetable virulence factor. Among all the results, one with the best score was selected as target for pneumonia.

### **Structure Prediction**

Target structure is modeled using online tool called Phyre2 server [7]. This server provides a validated model for the target protein that can be used for the drug screening.

### **Drug Screening**

Identified targetable virulence factor was then screened for the best inhibitor that can be used as a drug. 116 antibacterial compounds were downloaded from Pubchem [8]. Target was then docked with the antibacterial compounds taken from PubChem using AutoDock 4.2 [9]. Binding cavity for docking is identified using Q-SiteFinder [10]. Docking is done taking whole protein as a grid so that ligand can be allowed to bind at any of the site of the protein. Based on the interaction of ligand and protein, ligand which interacts at the active site are taken for further consideration. Thereafter, results were analyzed based on their binding energy, ligand efficiency and hydrogen bonds formed. Compounds with the best binding at the active site of the target are considered as the best inhibitor that can be used as a drug to treat pneumonia.

## **III. Result and Discussion**

### **Retrieval of orthologs**

Among four genomes of pneumonia causing organism viz. *Streptococcus pneumoniae* (strain 6706B), *Klebsiella pneumoniae* (strain 342), *Chlamydomphila pneumoniae* (strain AR39) and *Mycoplasma pneumoniae* (strain 309), a total number of 192 sequences were found to be orthologs. This orthologs sequences are evolved from the common ancestral gene and are retaining the same function. By finding all the common protein from all bacterial species we are attempting to short list the proteins that are necessary in causing the disease. This 192 protein may play a significant role in causing the disease as this proteins are present in all the four causative organism of the pneumonia. Other proteins that are excluded from orthologs must not have significant role in causing the disease as they are not present in all the four virulent organism. So we exclude all uncommon protein from further studies.

### **Prediction of Virulence factors**

All 192 orthologs protein were checked for their virulency using mvirDB blast. This results in 07 protein sequences which were virulent. Virulent proteins are responsible for converting non-pathogenic bacteria to pathogenic bacteria. We are using mvirDb as it contains virulence factors for several pathogenic organisms collected from many other databases [5]. This database is providing a blast tool that compares the query protein to database protein and declares the query protein to be virulent or non-virulent. This comparison is based on the concept that function is transferred by sequence similarity [11]. So Mvir's blast compares the protein

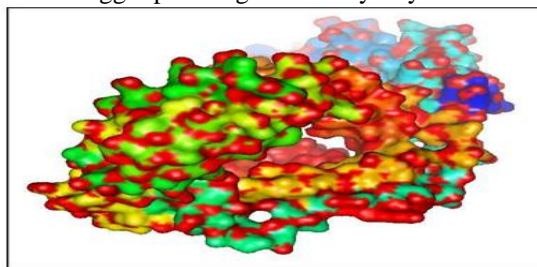
sequence with database and shortlist the virulent proteins. This virulent protein are the factors that helps in the survival of the organism by immunosuppression of the host, obtaining nutrition from the host and colonization. Thus targeting virulence factor does not allow organism to survive.

### **Prediction of target**

Out of this 07 orthologs virulent factor, one protein is identified as targetable virulence factor using TTD. This targetable virulent factor is the trigger factor protein involved in many functions. This protein helps other protein in performing their function by providing assistance in proper folding and forms functional three dimensional structures [12]. If activity of this protein is altered it affects many other important protein of the organism that arrests its normal functioning. This hinders the growth and survival of the organism. So by targeting trigger factor which is common in all four pathogenic bacteria and is a virulent factor certainly helps in treating pneumonia caused by either of the bacterial organism. As trigger factor is present in all the four causative bacteria so trigger factor can be a drug target in all cases of bacterial pneumonia.

As structure of target is required so that further study on the interaction of target and inhibitor can be done. But structure of *Streptococcus pneumoniae* trigger factor is not known so we modeled the structure with the help of Phyre2 server. This server generates the structure taking 1W26 as template with the help of homology modeling. The structure is modeled with 100 percent confidence and 97 percent query coverage. Complete structure of trigger factor is shown in Fig.1. The modeled structure is modeled with good quality and is stable so there is no need of dynamics studies.

**Figure 1:** Modeled structure of trigger protein generated by Phyre2 server with 97% query coverage.

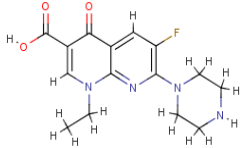
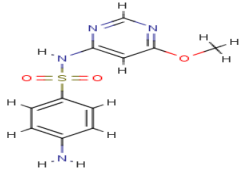
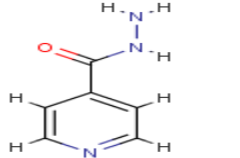
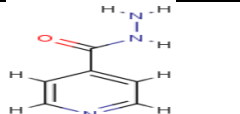


### **Prediction of inhibitor**

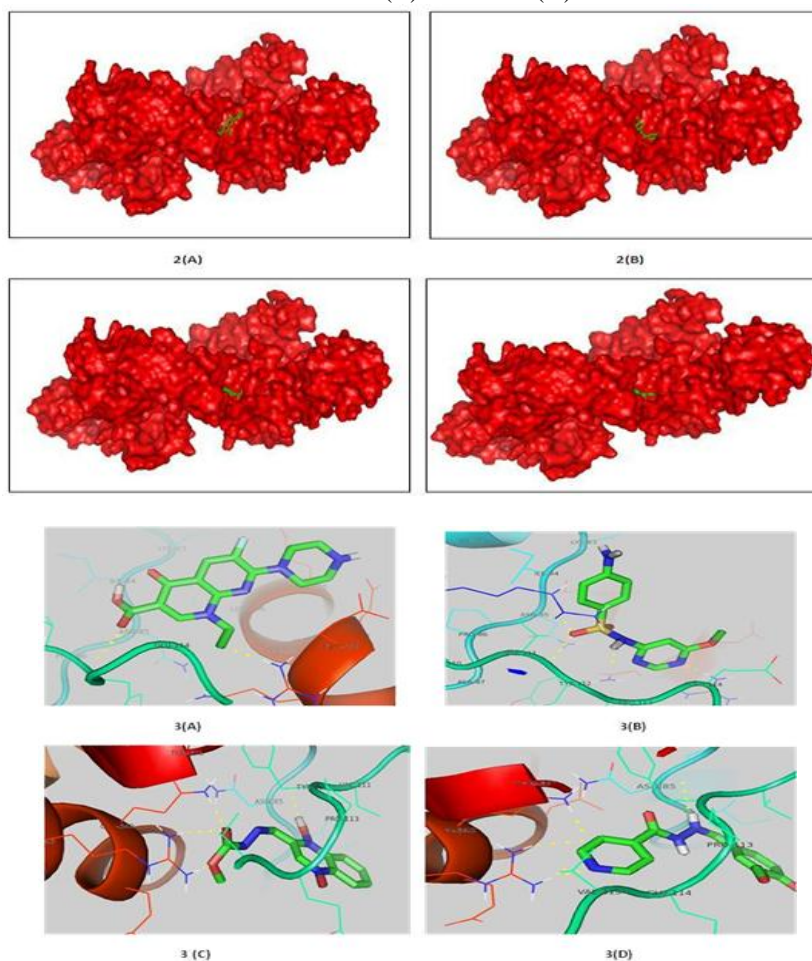
Trigger factor protein as a target is then docked with 116 antibacterial compounds taken from Pubchem library that can be used as a drug to treat pneumonia. This antibacterial compounds are mostly antibiotics that are already FDA approved and can be used against bacterial diseases. Using these compounds as drug against pneumonia will fast track the clinical trial and drug approval process. These compounds are docked with trigger factor by using AutoDock. This screening resulted in giving 04 antibacterial compounds that shows significant binding with the trigger factor. As it blocked the binding site, this binding inhibits the protein in its proper functioning. This hinders the growth and survival of the organism thus disease is cured. Significant binding is analyzed based on their binding energy, hydrogen bonds and ligand efficiency. Their binding pocket is identified by Q SiteFinder. It gives area and residues of all the cavities present in the protein. Deepest and biggest cavity is considered for binding and considered to be its active site. During docking no grid area is defined for the binding as it is considered that suitable candidate will automatically binds to the active site. On analysis of all the docking results of 116 compounds, 04 compounds with good docking score are considered as shown in table 1. Table 1 shows the selected four compounds their structure and score. Fig. 2 shows that all four compounds bind at the same active site so can be able to inhibit the protein in its activity. But their interactions are on same or different residues shown in Fig.3. It is based on their binding ligand as enoxacin interact with residues Asn-85, Glu-114, Phe-337 and Glu-339 and forms two hydrogen bonds. sulfamonomethoxine interacts at residues Ile-84, Asn-85, Val - 111 and Glu-114 and forms two hydrogen bonds. Carbadox interacts at Asn-85, Val - 111, Tyr-112, Gln-340 and Arg-343 and forms two hydrogen bonds. Isoniazid Asn-85, Val-115, Gln-340 and Thr-372. All these four compounds have common Asn-85 in their interaction so we can consider that this residue is an active residue involve in interaction with other compounds. This residue may involve in binding the factor that activates this protein for its activity. On binding the inhibitor to this residue definitely blocks the protein to bind the other molecule required for its activity thus its activity is blocked by anyone of this inhibitor.

Our significant finding is also supported by available literature as Wood M.J. et al.[13], Philip-Joet F et al. [14], Thadepalli H et al. [15] and Scarpazza G. et al. [16]. shows clinical efficiency of enoxacin in respiratory tract infections. Use of Sulfamonomethoxine in pneumonia is reported by Matveeva SA [17]. Our study doesn't conclude on the single molecule as we have done only in silico studies. Further wet lab work is required to find a single molecule either from any of this four or a combination of this molecule that can act as a drug to cure pneumonia.

**Table 1:** Selected four compounds their structure, Pubchem ID and docking score.

Name	Structure	Pubchem id	Binding energy	Ligand Efficiency	Hydrogen bond
Enoxacin		3229	-5.0	-0.22	02
Sulfamonomethoxine		5332	-4.53	-0.24	02
Carbadox		5353472	-6.38	-0.34	02
Isoniazid		3767	-4.88	-0.24	03

**Figure 2:** Figure shows that all four compounds bind at the same active site of the protein. (A) Enoxacin (B) Sulfamonomethoxine (C) Carbadox (D) Isoniazid.



**Figure 3:** Interaction of trigger factor with (A) Enoxacin (B) Sulfamonomethoxine (C) Carbadox (D) Isoniazid. Yellow dashed lines shows the interaction of protein and ligand.

#### IV. Conclusion

The present study finds trigger factor protein as the common targetable virulence factor from four bacterial pneumonia pathogens. This target is then docked with the available antibacterial compounds taken from PubChem library. This results in four compounds that can inhibit the target and helps hampering of pathogenic growth as well as survival. This four compounds needs to be validated by the wet lab studies that either may give a combination of four compounds (pharmacophore hybridization) or any one compound from this.

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