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Virtual Screening of Potential Inhibitors against the Penicillin-Binding Protein 1a (PBP1a) of *Streptococcus pneumoniae*

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ABSTRACT

Background: Pneumonia is an inflammatory condition of the lungs caused by the bacterium *Streptococcus pneumoniae*. It is a significant cause of mortality and morbidity, particularly among young children, adults and immunocompromised persons. Resistance against drugs is continuously evolving in nearly all pathogens. The constant need for alternative therapeutic options demands the necessity of an ongoing search for novel drugs. **Objective:** The current study was thus designed to target the penicillin binding protein of *Streptococcus pneumoniae* (PBP1a), a protein involved in critical cellular and metabolic processes. **Method:** PBP1a sequence of *Streptococcus pneumoniae* was obtained from UniProt database and protein BLAST was performed. 3D structure of PBP1a was downloaded from RCSB and visualized using Discovery Studio Visualizer. 150 drugs were docked using PatchDock web server and protein interactions were explored using GS Viewer, LigPlot+ and Discovery Studio Visualizer. **Result:** Out of the 150 drugs chosen, Lamivudine, Dolutegravir and Loperamide showed the most interactions with *Streptococcus pneumoniae* PBP1a. These interactions included covalent bonds, hydrogen bonds and hydrophobic interactions. **Conclusion:** The drugs Lamivudine, Dolutegravir and Loperamide interacted uniquely with the target protein. These interactions may trigger metabolic changes and could inhibit the growth and kill the parasite. Further experimental study is needed to fully understand the potential of these drugs.

INTRODUCTION

Streptococcus pneumoniae is a gram-positive, capsulated, nonmotile, facultatively anaerobic coccus that tends to congregate in pairs or short chains [1]. S. *pneumoniae* is a common commensal of the nasopharynx of healthy individuals. Once the nasopharynx has been colonised, the pneumococcus can penetrate the nasal sinuses, the Eustachian tube, the trachea and the bronchial tree [2, 3]. Only in susceptible individuals or those with predisposing factors, and once local clearance mechanisms have been exceeded, it will spread to neighbouring structures and may cause acute otitis media (AOM), sinusitis or even pneumonia, which may be complicated by pleural effusion or empyema if oropharyngeal secretions colonised with this microorganism are aspirated [1, 4].

The capsule is the main virulence factor [2]. About 90 serotypes have been identified. The components of the cell wall induce the intense inflammatory response typical of pneumococcal infection [1, 3]. Infections are more frequent in children and older individuals and in people with multiple comorbidities, including human immunodeficiency virus (HIV) infection, complement deficiency, asplenia, difficulty in mucociliary clearance from the airways or immunosuppressive treatments [2, 4].

The pneumococcus has a capsule, a true virulence factor, which protects it from the action of phagocytes and, therefore, promotes invasion and multiplication in tissues, in addition to stimulating the production of protective antibodies specific to each serotype [5, 6]. Approximately 15% of the general population has

detectable levels of anticapsular antibodies against the most common serotypes, and only 30% of people with pneumococcal pneumonia develop specific antibodies against the causal serotype [2, 5, 6].

Pneumococcal infection, especially pulmonary infection, is common in situations that promote the adherence of *S. pneumoniae* to the respiratory epithelium [5, 6], or that hinder its mucociliary clearance, as occurs in bronchial asthma, COPD or smoking; even more relevant, when bronchial aspiration of oropharyngeal secretions is favoured, as occurs in the elderly, neurological diseases, altered level of consciousness, alcoholism, etc [2, 6].

Drug resistance in *Streptococcus pneumonia* has been reported widely [7]. In the search for new antimicrobials, special attention is being paid to surface-associated proteins [8]. Protein Binding Proteins (PBPs) in *Streptococcus pneumoniae* are great targets for drug design because of their role in bacterial cell wall synthesis and their susceptibility to specific antibiotics [9, 10]. These proteins catalyze essential steps in peptidoglycan biosynthesis which keeps the bacterial cell wall intact. Since the cell wall is essential for bacterial survival, disrupting PBP function causes the weakening and eventual bursting of the bacterial cell. Beta-lactam antibiotics like penicillin's target PBPs by binding to their active sites, inhibit their enzymatic activity and cause cell lysis [10-12].

Moreover, PBPs are involved in antibiotic resistance especially through genetic mutations that alter their structure and reduce the binding affinity to beta-lactam drugs [9]. This has made PBPs a hot spot in drug design because targeting the specific mechanisms of resistance can lead to the development of new antibiotics that bypass existing resistance pathways [11, 13]. Drug design efforts aim to create compounds that can either restore the effect of old antibiotics or develop new class of drugs that can overcome the mutations that confer resistance to PBPs [10, 12]. Given the global issue of antibiotic resistance, targeting PBPs in *Streptococcus pneumoniae* is a good approach to develop better treatment. The present work aims to target PBPs of *S. pneumoniae* using FDA approved drugs.

MATERIALS AND METHODS

Data Preparation and Visualization

The PBP1a sequence of *Streptococcus pneumoniae* was downloaded from the UniProt database (http://www.uniprot.org) [14, 15]. A protein BLAST was performed to get homologous protein and select the best structural protein sequence. The 3D structure of PBP1a with a resolution of 2.16 Å was retrieved from the RCSB Protein Data Bank (PDB) under the ID 2C6W in PDB format. DS Visualizer [16] was used to visualize the protein structure which showed one protein chain (A) and 2,035 water molecules.

The active site of the protein was defined for docking studies which included the following residues: ASP279, HIS309, PYR319, SER370, HIS395, GLU379, SER428, GLU435, GLY441, ASN443, ARG444, HIS460, GLU514, THR558, GLU566, and HIS571. The 3D structures of the ligands were downloaded from ChemSpider [17] and saved in PDB format.

For ligand-protein docking, PatchDock [14] was used. Ligand-protein interactions were analyzed using LigPlot+ [14] to see the interaction pattern between the ligands and the active site residues.

RESULTS

A total of one hundred and fifty different drugs were docked with the Penicillin Binding Protein 1a (PBP1a) of *Streptococcus pneumoniae*. Many drugs showed interaction with PBP1a, but only those with enough interactions were selected. The details of each drug-ligand interactions are as under.

Docking of Lamivudine with PBP1a

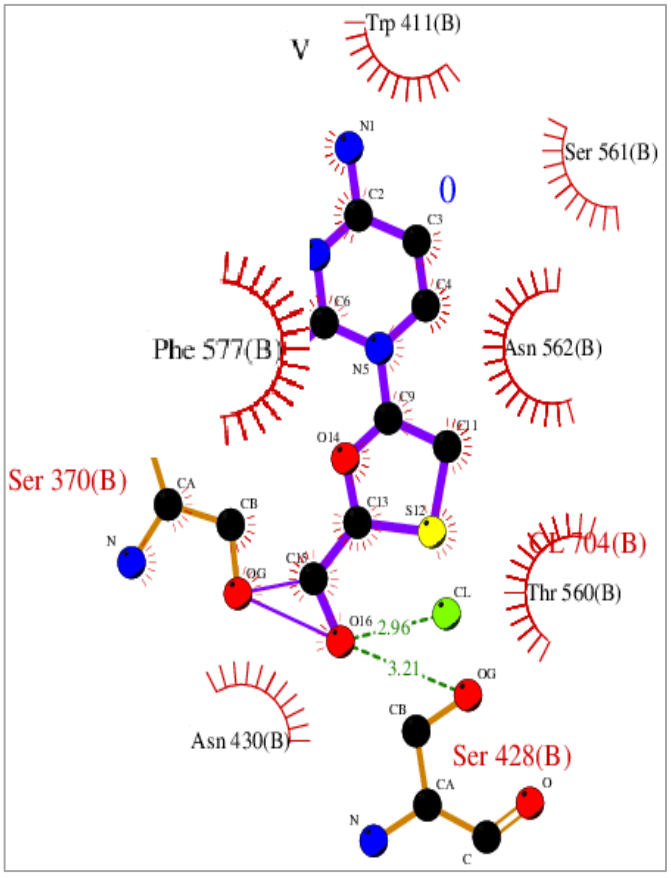
The docking study of Lamivudine with PBP1a, showed sixteen various interactions: including covalent bonds, hydrogen bonds and hydrophobic interactions (Table 1). The residue SER 428 formed two hydrogen bonds, while the residue SER 370, formed two covalent bonds. Additionally, five hydrophobic interactions were also observed, with one occurring at the active site (Figure 1).

Table 1.
Molecular Interactions of potential drugs with different amino acids of PBP1a of S. pneumoniae

Ligand/Drug	Active site amino acid	Covalent interaction	Hydrogen bond	Hydrophobic interaction
Lamivudine	ASP 279, HIS 309, PYR 319, SER 370, HIS 395, GLU 397, SER 428, GLU 435, GLY 441, ASN 443, ARG 444, HIS 460, GLU 514, THR 558, GLU 566, HI 571,	SER 370	SER 428	TRP411, SER561, ASN 562, THR560, ASN 430, PHE 577
Dolutergravir	ASP 279, HIS 309, PYR 319, SER 370, HIS 395, GLU 397, SER 428, GLU 435, GLY 441, ASN 443, ARG 444, HIS 460,	SER 370, PHE 577,	PHE 577,	LEU 611, GLU 582, THR 560, ASN401, TRP 411, ASN562, TYR409

	GLU 514, THR 558, GLU 566, HI 571		
Loperamide	ASP 279, HIS 309, PYR 319, SER 370, HIS 395, GLU 397, SER 428, GLU 435, GLY 441, ASN 443, ARG 444, HIS 460, GLU 514, THR 558, GLU 566, HI 571	SER 370	SER 428

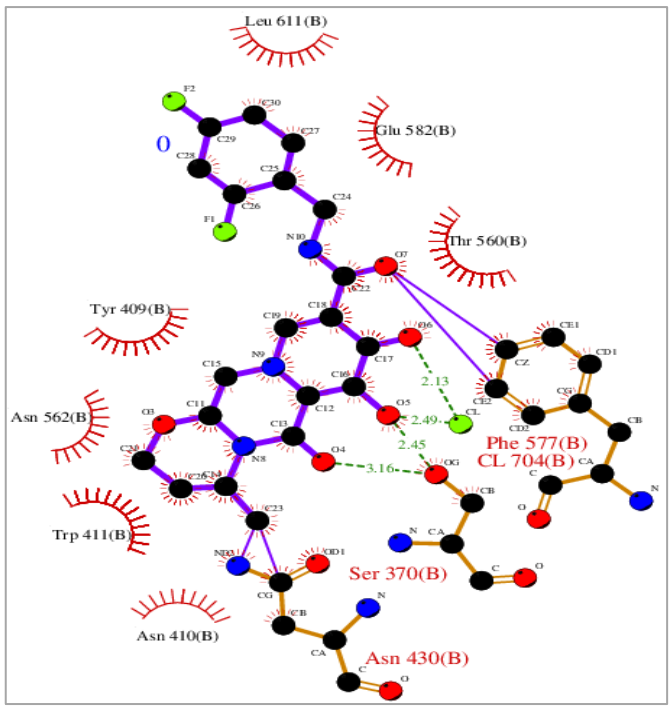
Figure 1
2D Schematic representation of Lamivudine interactions with PBP1a



Docking of Dolutegravir with PBP1a

Upon docking of dolutegravir with PBP1a, fifteen unique interactions (covalent bonds, hydrogen bonds and hydrophobic interactions) were observed (Table 1). Specifically, four covalent bonds were formed: two involving PHE 577, and two with SER 370. Moreover, the analysis revealed the formation of four hydrogen bonds and seven hydrophobic interactions (Figure 2).

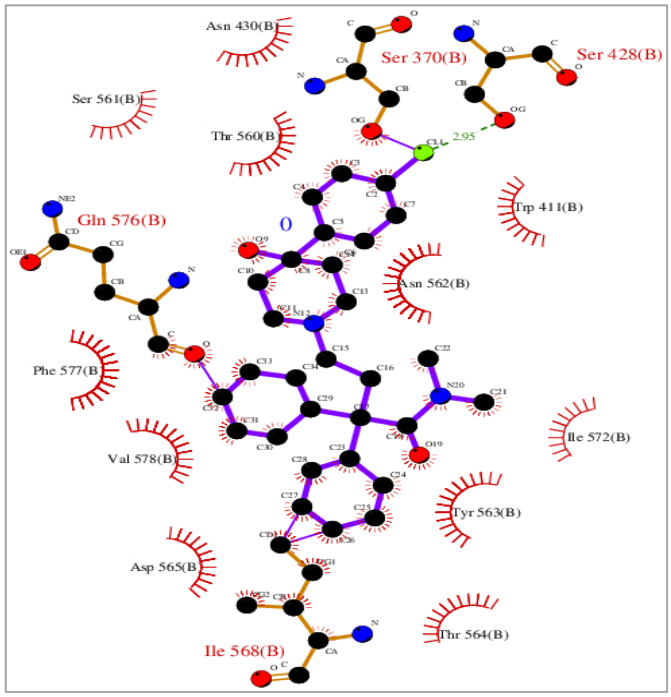
Figure 2
2D Schematic representation of Dolutegravir interactions with PBP1a



Docking of Loperamide with PBP1a

Molecular docking of Loperamide with PBP1a demonstrated fifteen different interactions, including covalent, hydrogen and hydrophobic bonds (Table 1, Figure 3). The active site residues SER 370 and SER 428 formed four covalent bonds and one hydrogen bond. Furthermore, eleven hydrophobic interactions were also observed (Figure 3).

Figure 3
2D Schematic representation of Loperamidine interactions with PBP1a



DISCUSSION

The increased levels of resistance of infectious microorganisms to traditional antimicrobials is a major problem in today's health, with elevated social and economic costs. One of the microorganisms that have such resistance is *Streptococcus pneumoniae*. In the development of new antimicrobials against these bacteria, is interesting to study the use of penicillin binding proteins (PBPs). In the current study, PBP1a of *Streptococcus pneumoniae* was targeted using FDA approved drugs. About one hundred fifty different drugs were docked with PBPa1, of which many drug showed interactions, but only those with the highest number of interactions were selected. These interactions may disturb the normal function of the protein, ultimately resulting in its death.

Lamivudine is an anti-retroviral oral drug. It is a nucleoside analogue reverse transcriptase inhibitor, chemically composed of (2R-cis)-4-Amino-1-[2-(hydroxyethyl)-1, 3-oxathiolan -5- yl] cytosine [5]. This drug is primarily used to treat AIDs and HIV by inhibiting DNA replication of virus. The docking analysis in the current study revealed various interaction of lamivudine with PBP1a (see Table 1 and Figure 1). These various interactions might bring tremendous conformational changes, which might damage the normal structure and functions of PBP1a, thereby disrupting the metabolic pathways, essential for the survival of S. pneumoniae.

Dolutegravir is antiviral drug with a unique structural composition that allows it to bind to various catalytic domains of different proteins [18]. When docked with PBP1a in the current study, various interactions were observed (see Table 1 and Figure 2). These highest number of interactions may induce conformational changes, potentially impairing the target protein structure and function, thereby disrupting the metabolic and other cellular processes of *Streptococcus pneumoniae*.

Loperamide is an FDA approved, cost effective drug, commonly used for treating diarrhoea [19, 20]. It works by reducing the gastrointestinal transit, thereby slowing peristaltic movements [21, 22].

Interestingly, findings from our recent study, suggest that loperamide can also be repurposed for treating pneumonia at all stages. Among the one hundred and fifty drugs analysed, loperamide demonstrated remarkable efficacy against pneumonia, potentially inducing conformational changes and disrupting the normal chemistry of PBP1a. Disruption of PBP1a structure and function could halt the bacterial pathogenesis.

CONCLUSION

This study aimed to identify potential alternative inhibitors for S*treptococcus pneumoniae* penicillin binding protein 1a (PBP1a) using a computational approach. Multiple drugs were docked against the target protein, and those with the highest number of interactions were selected for further analysis. Among the docked drugs, Lamivudine, Dolutegravir and Loperamide showed the highest number of interactions. These interactions may induce conformational changes in the 3D structure of the target protein, thereby disrupting its normal structure and function. Further detailed analyses are required to comprehensively assess the potential of these drugs.

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