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Bioinformatics analysis of wild-type ParC and S79F, S79Y mutations in *Streptococcus pneumoniae*

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Abstract. – OBJECTIVE: S79Y and S79F mutations in ParC at the fluoroquinolone binding site confer resistance to quinolones in *Streptococcus pneumoniae*. The aim of this study was to perform bioinformatics analysis of wild-type and mutant ParC identified in *S. pneumoniae* strains.

MATERIALS AND METHODS: Homology models were created with Swiss Model, and the UCSF Chimera was used for the analysis and evaluation of the models. Molecular docking studies and protein-ligand interaction analysis were performed using CB-Dock developed by AutoDock Vina and Protein Ligand Interaction Profiler (PLIP), respectively.

RESULTS: According to the homology modeling results, changes were observed in the physicochemical properties. Both mutant types showed a decrease in hydrophobicity (S79F: 2.0, S79Y: 0.5 - Kyte-Doolittle) and an increase in regional volume (S79F: 95 Å3, S79Y: 100 Å3). The docking scores for the wild-type, S79F, and S79Y types were 6.3, 4.9, and 5.0, respectively. The decrease in docking scores indicates weaker binding in the mutant types. The results of the PLIP analysis indicate that new bonds were formed, and the bond types changed in both mutant types, while some bonds disappeared.

CONCLUSIONS: It is believed that the change in physicochemical properties caused by the mutation altered the conformation of the quinolone binding region and the bond types, numbers, and proximities of the amino acids involved in the interaction with the active substance. The results obtained from the modeling performed in this study with visualization and analysis of possible conformational, hydrophobic, and volumetric changes of only ParC mutations provided data and literature support to elucidate the mechanism. The molecular docking and PLIP results obtained in this study support both the homology modeling results and the fact that S79F and S79Y have been shown to be resistant in the literature.

Key Words:
Streptococcus pneumoniae, Fluoroquinolones, Homology modeling, Bioinformatics, ParC mutation, Molecular docking, Protein ligand interaction analysis.

Introduction

As one of the leading causes of lower respiratory infection and meningitis morbidity and mortality worldwide, *Streptococcus pneumoniae* also causes invasive or non-invasive infections such as bacteremia, osteomyelitis, septic arthritis, otitis media, and sinusitis.

Among the agents of lower respiratory tract infections in all age groups, *S. pneumoniae* causes 1,189,937 deaths [95% uncertainty interval (UI) 690,445-1,770,660], thus many more than the total of other most common etiological agents (Respiratory syncytial virus, Influenza virus, and *Haemophilus influenzae* type b)[1,2].

It is the most common cause of meningitis, especially in children under the age of five years. Although it is a vaccine-protected microorganism, there is close surveillance throughout the world in terms of the distribution of serotypes included or not in the vaccine content, infections caused by these serotypes, and antibiotic resistance rates[2].

Beta lactams, macrolides, tetracyclines, trimethoprim-sulfamethoxazole, glycopeptides, and fluoroquinolones are used in treatment[3]. *S. pneumoniae* has developed resistance to most of these antibiotics. Therefore, levofloxacin, moxifloxacin, gatifloxacin, and gemifloxacin are recommended as the fluoroquinolone group in treatment. Quinolones act by binding to topoisomerase (DNA

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gyrase and topoisomerase IV) enzymes and disrupting DNA synthesis[4,5].

DNA gyrase and topoisomerase IV are the enzymes that play a role in controlling the topological status of DNA[6] . Topoisomerase IV consists of ParC and ParE subunits required for catalytic activity. In the literature was shown that the genes encoding ParC and ParE are essential for bacterial DNA replication. The catalytic properties of topoisomerase IV can be distinguished from those of DNA gyrase mainly in two ways. First, while topoisomerase IV can remove positive and negative superhelical twists from DNA, it cannot actively wrap the double helix. Second, the ability of topoisomerase IV to untangle DNA tangles is much better than DNA gyrase. As a result of these differences, the physiological roles of topoisomerase IV are significantly distinct from those of DNA gyrase[7]. The primary target site of quinolones in Gram-positive bacteria is topoisomerase IV, while in Gram-negative bacteria, the primary target site is DNA gyrase[8] (Figure 1).

In *S. pneumoniae*, fluoroquinolone resistance develops with decreased binding to quinolone resistance-determining regions (QRDRs) as a result of mutations in the genes (*parC* and *gyrA*) encoding the subunits of both ParC and GyrA enzymes. Gradually developing resistance is low-level when it occurs in either of the ParC or GyrA subunits, followed by high-level resistance when it occurs in the other. Increased activity of efflux pumps, another cause of quinolone resistance, does not increase the minimum inhibitory concentrations (MIC) as much as topoisomerase enzymes[4,5]. ParC is an important target site in quinolone resistance. It has been reported in the literature that the conversion of the 79th amino acid, serine, of the *parC* gene, to tyrosine (S79Y) and phenylalanine (S79F) amino acids causes low and intermediate quinolone resistance[9-12].

Bioinformatics studies are one of the fields of study used both to reduce *in-vitro* and *in-silico* experimental costs and to elucidate molecular mechanisms. The homology modeling technique is used for mutation analysis in proteins for which the three-dimensional structure has not been analyzed as yet, or if the three-dimensional structure is known and is based on the creation of hypothetical models using sequence similarity of proteins with previously analyzed three-dimensional structure. Homology modeling is one of the basic methods used for the prediction of three-dimensional structures of proteins, protein-protein interactions, protein-ligand interactions, modeling drug-resistance mechanisms, and drug development studies[13,14]. The Swiss Model is one of the leading bioinformatics tools used for the creation of homology models[15].

The use of molecular docking provides an atomic-level understanding of the interaction between a protein and a small molecule. This approach allows for the characterization of small molecule behavior in the binding site of target

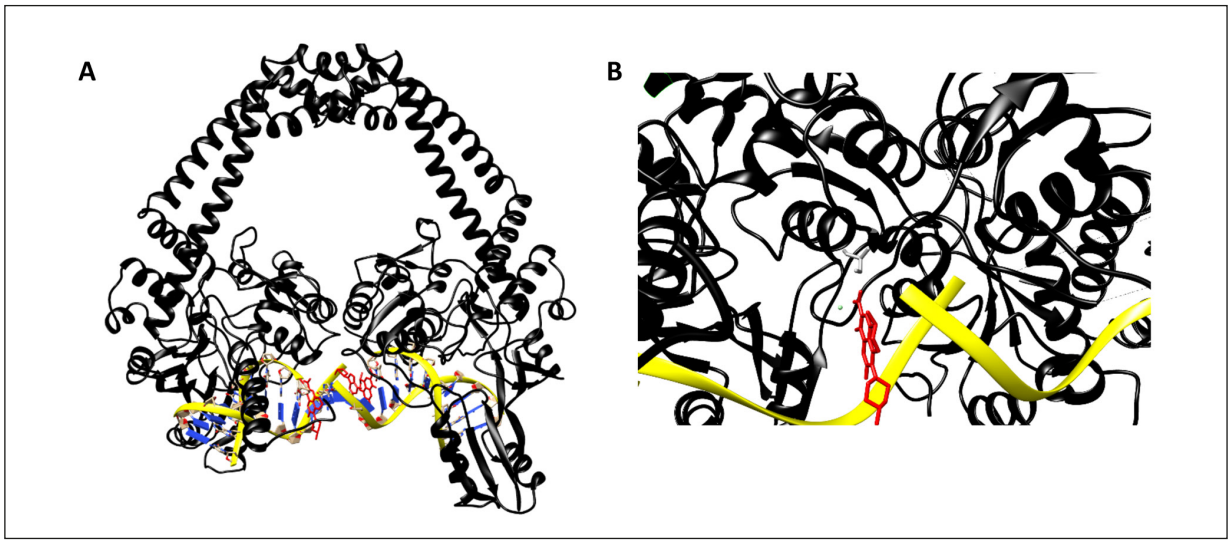


Figure 1. A, Wild type ParC (*black, ribbon*) with DNA (*yellow, ribbon*). **B**, The quinolone molecule (*red, atomic*) inserted between DNA and ParC.

proteins and the elucidation of fundamental biochemical processes. The docking process is accomplished through two steps: a conformational sampling of the ligand in the active site of the protein, followed by ranking these conformations using a scoring function. To achieve optimal results, sampling algorithms should be able to reproduce the experimental binding mode, and the scoring function should rank it highest among all generated conformations[16,17].

The aim of this study was to perform bioinformatics analyses of S79F and S79Y mutant ParC enzymes identified in wild-type and *S. pneumoniae* strains using the homology modeling technique, molecular docking, protein interaction analysis, and to clarify the molecular causes of mutation resistance as a result of the analyses.

Materials and Methods

Homology Modeling and Visualization Analysis of Models

Wild-type and mutant-type homology models were created using the Swiss-Model (www.swissmodel.expasy.org) bioinformatics homology modeling program. Model levofloxacin presented with LFX [(3S)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid] ligand. The main structure of the model used is the 4I3H (pdb doi number: 10.2210/pdb4I3H/pdb) model. All edited amino acid sequence sets, including wild type, were loaded into the system separately, and hypothetically formed three-dimensional structures were obtained. The model quality of the obtained three-dimensional structures was selected based on sequence parity[18]. Sequence parity and sequence similarity are consistent with model accuracy.

The UCSF Chimera (1.14) program (Resource for Biocomputing, Visualization, and Informatics, University of California, San Francisco, America) visualizes three-dimensional structures according to x-y-z coordinates. The ribbon is one of the atomic and surface representation visualization tools. The UCSF Chimera program can calculate global and regional hydrophobicity, interatomic distance and angles. Using UCSF Chimera tools, all models are visualized with ribbon display, local atomic display, and hydrophobic surface analysis display. The wild-type and mutant amino acids were atomically superimposed and visualized. The region where the active substance was located was visualized using hydrophobic surface analysis[19].

Molecular Docking and Protein-Ligand Interaction Analysis

Molecular docking studies were performed using CB-Dock developed by AutoDock Vina. CB-Dock is a method of docking protein-ligand complexes that involve the automatic identification and analysis of binding sites, followed by the calculation of the center and size of the binding site. The method then customizes the size of the docking box based on the properties of the ligand being analyzed and performs the molecular docking using AutoDock Vina. Extensive testing has demonstrated that the use of cavity-focused docking can improve the accuracy and hit ratio of blind docking. Therefore, CB-Dock can assist in the docking process and enhance the accuracy by predicting the binding sites of target proteins through its curvature-based cavity detection approach (CurPocket) and determining the binding poses of query ligands using AutoDock Vina[20].

Protein-Ligand Interaction Profiler (PLIP) is a tool that enables easy identification of non-covalent interactions between biological macromolecules and their ligands, providing detailed information on binding characteristics as well as customizable output files and publication-quality visualizations. Although initially designed for the analysis of interactions with proteins, PLIP is suitable for both the analysis and visualization of docking results and the detailed study of existing structures. With PLIP, hydrogen bonds, hydrophobic contacts, π-stacking, π-cation interactions, salt bridges, water bridges, metal complexes, and halogen bonds can be detected between ligands and targets[21].

No statistical analysis was performed in this study.

Results

Homology Modeling Visualization Analysis of Models Results

Wild-type, S79Y and S79F were determined to have 99.59%, 99.39% and 99.39% sequence parity, respectively. No major changes were observed in the structure of proteins on a global scale. A change was observed at the microscale mutation site, at the location of amino acid 79. When the wild type was examined, the regions where the DNA helix and ParC protein interact

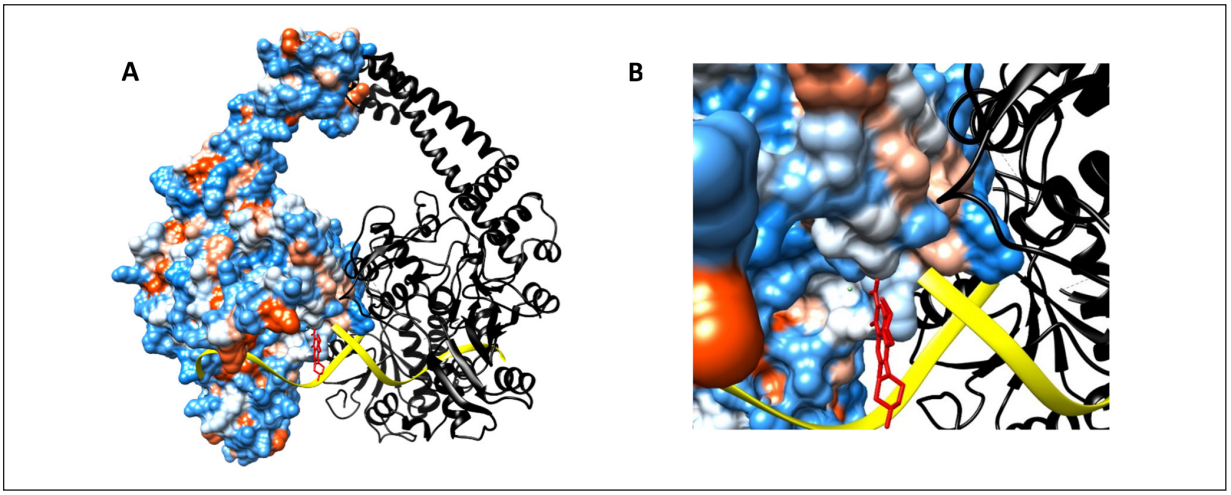


Figure 2. **A**, Wild-type ParC right ribbon representation (*black*), left superficial hydropathy representation (*white neutral, red hydrophobic, blue hydrophilic*) with DNA (*yellow*). **B**, The quinolone (*red, atomic*) inserted between hydropathy representation ParC and DNA molecule.

were seen to change to hydrophobic-neutral and hydrophilic (Figure 2A). The 79th amino acid, with which quinolone and ParC protein interact, was in a neutral structure (Figure 2B). When the S79Y mutant type was examined, a mean volumetric increase of 100 Å^3 at the mutation site was analyzed with the conversion of the amino acid serine (89.0 Å^3) to the amino

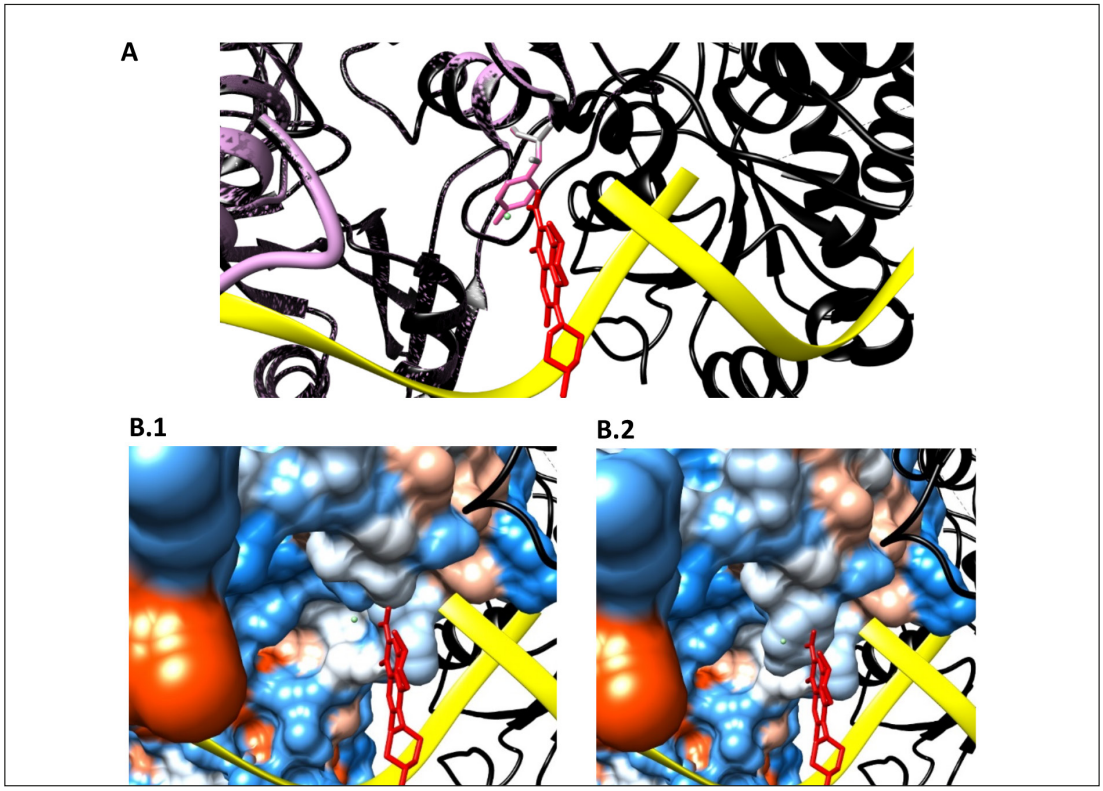


Figure 3. **A**, Representation of wild-type *(black)* S79Y mutant-type ParC models with overlapped ribbon representation of DNA (*yellow*), region 79. serine amino acid (*grey*) mutant tyrosine amino acid (*pink*). **B**, Wild-type (**B.1**) and S79Y mutant type (**B.2**) hydropathy surface display, the region where the quinolone (*red*) molecule interacts are neutral (*white)* in both models.

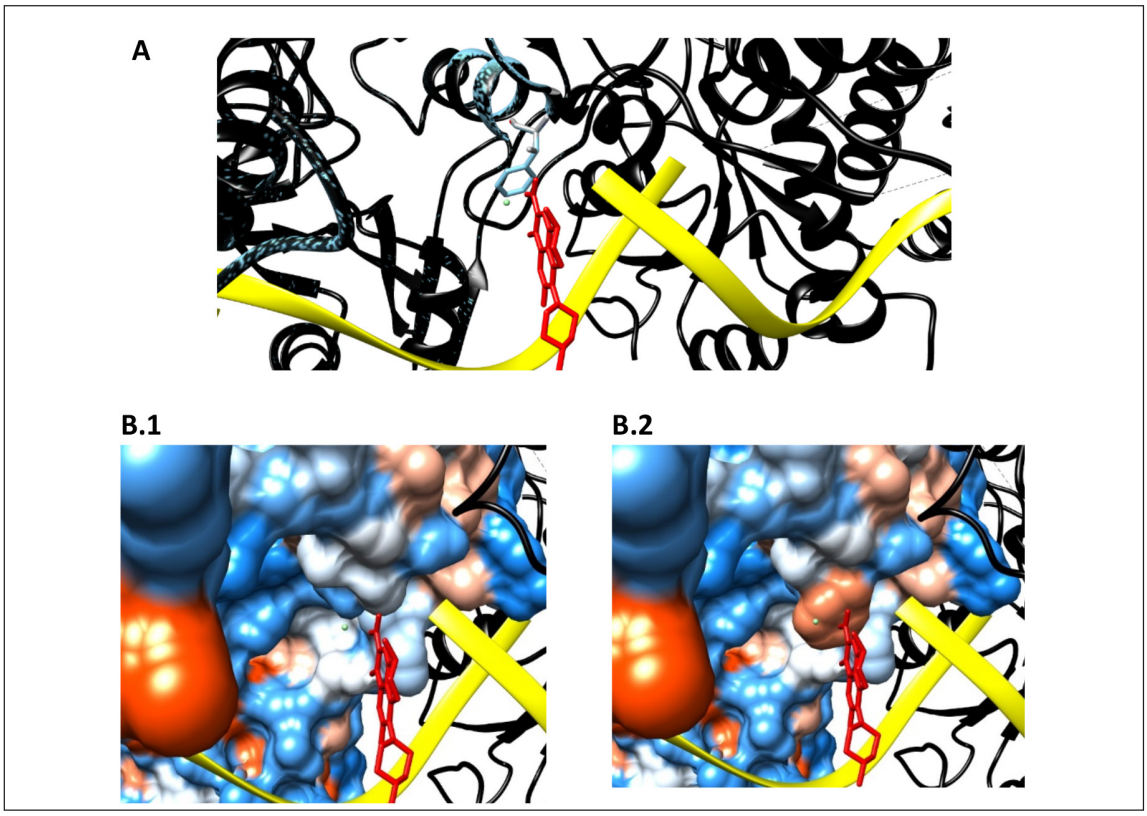


Figure 4. A, Representation of wild-type (*black*) S79F mutant-type ParC models with overlapped ribbon representation of DNA (*yellow*), region 79. serine amino acid (*grey*) mutant phenylalanine amino acid (*blue*). **B**, Wild-type (**B.1**) and S79Y mutant type (**B.2**) hydropathy surface display, the region where wild type and quinolone (red) molecules interact is neutral (**B.1**) and the region where S79Y and quinolone (*red*) molecules interact is hydrophobic (*red*).

acid tyrosine (193.6 Å^3) (Figure 3A). The hydropathy change in the mutation site increased by 0.5 valence according to the Kyte-Doolittle scale, but there was no significant change in hydropathy since both amino acids were neutral (Figure 3B).

When the S79F mutant type was examined, the conversion of the amino acid serine (89.0 Å^3) to the amino acid phenylalanine (189.9 Å^3) showed a mean volumetric increase of 95 Å^3 at the mutation site (Figure 4A). The hydropathy change in the mutation region showed a 2.0 value decrease according to the Kyte-Doolittle scale. This change shows the transition from the regional neutral feature to the hydrophobic feature (Figure 4B).

Molecular Docking and Protein-Ligand Interaction Analysis of Results

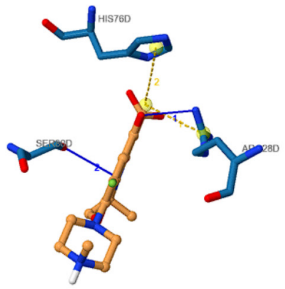
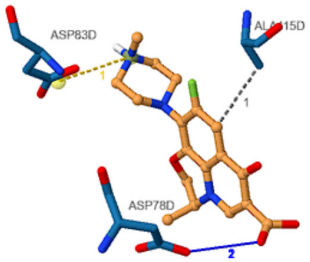
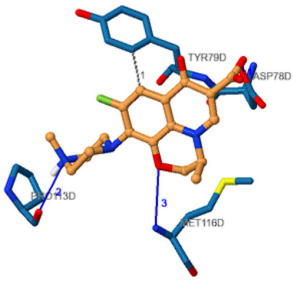
Based on the I3H model, homology models were created, and a molecular docking study was conducted using the CB-Dock for the LFX ligand (DB0113), and the results are presented in Table I. The binding score of the wild-type is higher than that of the mutant types. Analysis of the molecular docking results determined that mutant types showed lower binding. The results of molecular docking support the homology modeling results.

The results of the protein-ligand interaction analysis conducted with PLIP are presented in Table II. According to the analysis results, hydrogen bonds established between the wild type Arg28-Ser80 and the ligand have disappeared in both mutant types. In addition, in the S79F mutation, two hydrogen bonds were established between Asp78 and the ligand, and

Table I. Molecular docking results.

Model	Docking Score
Wild Type	6.3
S79F	4.9
S79Y	5.0

Table II. The results of the protein-ligand interaction analysis conducted with protein ligand interaction profiler.

 Wild Type			 S79F			 S79Y		
Hydrogen Bonds			**Hydrogen Bonds**			**Hydrogen Bonds**		
Residue	**AA**	**Distance**	**Residue**	**AA**	**Distance**	**Residue**	**AA**	**Distance**
28	Arg	2.62 Å	78	Asp	3.00 Å	78	Asp	2.36
80	Ser	2.53 Å	78	Asp	2.57 Å	113	Pro	2.28
-	-	-	-	-	-	116	Met	3.07
Salt Bridges			**Salt Bridges**			**Salt Bridges**		
28	Arg	3.99 Å	83	Asp	4.30 Å	-	-	-
76	His	4.57 Å	-	-	-	-	-	-
Hydrophobic Interac.			**Hydrophobic Interac.**			**Hydrophobic Interac.**		
-	-	-	115	Ala	3.80 Å	79	Tyr	3.70
-	-	-	-	-	-	-	-	-

in the S79Y mutation, hydrogen bonds were established between Asp78, Pro113, Met116, and the ligand.

The salt bridges established between the wild type Arg28-His79 and the ligand have disappeared in both mutant types, but a new salt bridge was established between Asp83 and the ligand in the S79F mutant type.

Hydrophobic interactions not present between the wild type and the ligand were established in the S79F mutant type between Ala115 and the ligand, and the S79Y mutant type between Tyr79 and the ligand.

Discussion

In *S. pneumoniae*, the *parC* mutation causes resistance to ciprofloxacin, while resistance to levofloxacin and moxifloxacin does not develop. Although the single mutation of *parC* results in low-level resistance, the importance of this target region is that it is a step that allows for the addition of a second *gyrA* mutation during fluoroquinolone therapy, thus causing high-level resistance leading to fluoroquinolone therapy failure[22,23].

The 79th amino acid mutations are not close to the disulfide bridges or catalytic sites of the protein. Therefore, it is thought that mutations in this region do not affect the formation or destruction of new disulfides, and do not trigger a global-scale change, as the analyzed volumetric increase is not in the main backbone or the catalytic region, due to the conversion of the serine amino acid to phenylalanine or tyrosine amino acid in the 79th region, and there is no disruption of structures such as alpha helix and beta folding.

Quinolones used in the treatment of *S. pneumoniae* infections show a bactericidal effect by inhibiting topoisomerase activity with the interference of the 79th amino acid in the ParC subunit and its surroundings and DNA. The mutations used in the current study are seen in the 79th amino acid.

The 79th amino acid region is associated not only with the binding of the quinolone, but also with the binding of DNA (Uniprot, P72525). The hydropathy change at the mutation site of the S79Y mutated ParC protein is small-scale (0.5). However, the volumetric change (100 Å^3

increase) in the mutation site is thought to affect DNA binding. In a study by Pletz et al[24], the coexistence of the S79Y mutant strain and the *gyrA* Q118K mutation and the decrease in the reproductive rate confirmed that this mutation changes its relationship with DNA as a result of regional variation. It is also thought that the volumetric change of the S79Y mutant type not only changes its interaction with DNA, but also its interaction with the quinolone, considering the MIC values of this mutation, thereby leading to low-level resistance[9-12]. The disappearance of salt bridges that were present in the wild-type, changes in the location of hydrogen bonds, and the formation of hydrophobic interactions due to the mutation indicate that the binding site of ParC and the ligand have changed. It is thought that as a result of these changes, the molecular docking score has decreased.

The presence of phenylalanine amino acid instead of serine amino acid as a result of S79F mutation reduced hydropathy and made the region hydrophobic. Considering the MIC values, it can be thought that the quinolone cannot bind to the 79th amino acid region due to the hydrophobic feature of the region, and therefore, resistance occurs[9-11]. There is no study in the literature regarding the S79F mutation changing the reproductive rate. It is thought that the 2.0 valence decrease on the Kyte Doolittle scale and the 95 Å^3 volume increase occurring at the mutation site of the S79F mutant type lead to low levels of quinolone resistance[9-12]. Three-dimensional proteins that were hypothetically formed by in-silico methods should be analyzed using X diffraction and NMR methods to confirm their three-dimensional structure. In the continuation of our studies, it is planned to create three-dimensional structures of mutations that cause antimicrobial resistance in the ParC subunit using *in-silico* and *in-vitro* methods.

Conclusions

In conclusion, the S79F mutation in the ParC subunit of the topoisomerase IV enzyme, which is vital for *S. pneumoniae*, did not change the life cycle of the bacterium, but caused the development of resistance to quinolones, while the S79Y mutation reduced the growth rate of the bacteria and led to a low level of resistance to quinolones. The results obtained by visualizing and analyzing possible conformational, hydrophobic and volumetric changes as a result of only ParC mutations with the modeling performed in this study provided data and literature support to elucidate the mechanism. In this study, the molecular docking and PLIP results support both our homology modeling results and the fact that S79F and S79Y have been shown to be resistant in the literature.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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Ethics Approval
Not applicable.

Informed Consent
Not applicable.

Authors' Contribution
All of the authors contributed to the design of the study, data collection, analysis, and manuscript preparation.

Availability of Data and Materials
The data presented in this study are available on request from the corresponding author.

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