Pakistan Journal of Advances in Medicine and Medical Research(PJAMMR)
ISSN: (Online) 2957-5893
Website: https://www.pjammr.com/index.php/pjammr/homepage

Open Access
Volume 4, Issue 2, 2026

Integrative Phenotypic and Structural Characterization of Ciprofloxacin Resistance in Clinical Klebsiella pneumoniae: GyrA QRDR Mutations and Predicted Drug–Target Interactions

Muhammad Amir[1], Muhammad Yaman[2], Abbas Saleem[3], Muhammad Rizwan[4], Manahil Anjum[5], Syed Ab Un Nassar[6]

[1-6] BS Microbiology Students, Center of Biotechnology and Microbiology, University of Peshawar Pakistan

Keywords: Klebsiella pneumoniae; ciprofloxacin resistance; GyrA; quinolone resistance-determining region; molecular docking; antimicrobial resistance

Article History

Received: APRIL 01, 2026
Revision: April 24, 2026
Accepted: May 05, 2026
Published: May 10, 2026

Corresponding Author: Syed Abu Un Nassar

Email: sanassar60912@gmail.com

Amir M, Yaman M, Saleem A, Rizwan M, Anjum I, Nassar SAU. Integrative phenotypic and structural characterization of ciprofloxacin resistance in clinical Klebsiella pneumoniae: GyrA QRDR mutations and predicted drug–target interactions**. Pak J Adv Med Med Res. 2026;4(2):60-65.**

ABSTRACT

Background: Ciprofloxacin resistance in *Klebsiella pneumoniae* compromises an important therapeutic option for Gram-negative bacterial infections and may arise through alterations in quinolone targets, efflux mechanisms, and plasmid-mediated resistance determinants. This study investigated the phenotypic profile and potential molecular basis of ciprofloxacin resistance in clinical K. Pneumonia isolates using complementary microbiological and computational approaches.

Methods: Five clinical *K. pneumoniae* isolates recovered from sputum, urine, and wound specimens were subjected to antimicrobial susceptibility testing. Ciprofloxacin-resistant and susceptible isolates were comparatively evaluated for mutations within the quinolone resistance-determining region (QRDR) of *GyrA* and *parC*. Detected *GyrA* substitutions were assessed computationally using I-Mutant for predicted effects on protein stability. Molecular docking with Auto Dock Vina was subsequently performed to characterize the interaction of ciprofloxacin with the *K. pneumoniae* *GyrA* target.

Results: Three of five isolates (60%) were resistant to ciprofloxacin, with resistance occurring alongside substantial resistance to other antimicrobial classes. QRDR analysis identified *GyrA* substitutions at positions 83 (Y83I) and 87 (G87N) exclusively among ciprofloxacin-resistant isolates, whereas no corresponding QRDR mutations were detected in susceptible isolates. Both substitutions were predicted to decrease *GyrA* stability. Ciprofloxacin exhibited a best predicted binding affinity of −6.0 kcal/mol, with docking interactions involving residues within the *GyrA* binding region.

Conclusion: Ciprofloxacin resistance in these clinical *K. pneumoniae* isolates was associated with QRDR alterations in *GyrA* and predicted structural effects on the drug target. These findings support continued phenotypic surveillance and molecular investigation of fluoroquinolone resistance, while larger studies incorporating MIC determination, whole-genome sequencing, and functional validation are warranted.

INTRODUCTION

Klebsiella pneumoniae (K. pneumoniae) is a clinically important Gram-negative opportunistic pathogen responsible for a broad spectrum of community- and healthcare-associated infections, including pneumonia, urinary tract infections (UTIs), bloodstream infections, wound infections, and sepsis (1). It is commonly found in feces and the respiratory tract. It can also live in soil and water in the environment. Its clinical significance is amplified by its remarkable capacity to acquire and disseminate antimicrobial resistance determinants through plasmids, transposons, integrons, and other mobile genetic elements (2). Consequently, *K. pneumoniae* has emerged as an important reservoir and disseminator of antimicrobial resistance (AMR), with multidrug-resistant (MDR) and carbapenem-resistant lineages posing substantial therapeutic and public-health challenges (3).Fluoroquinolones, particularly ciprofloxacin, have historically represented important therapeutic options for infections caused by Gram-negative organisms, including *K. pneumoniae.* Ciprofloxacin exerts bactericidal activity primarily by targeting bacterial type II topoisomerases, principally DNA *Gyr*Ase and topoisomerase IV (4). In Gram-negative bacteria, DNA *Gyr*Ase is a major intracellular target. Ciprofloxacin stabilizes the enzyme–DNA cleavage complex and prevents efficient DNA strand resealing, thereby disrupting DNA replication and transcription and ultimately producing lethal DNA damage. The effectiveness of ciprofloxacin therefore(5). The progressive emergence of ciprofloxacin-resistant *K. pneumoniae* is consequently of considerable clinical importance. Fluoroquinolone resistance is multifactorial and may arise through chromosomal mutations in the quinolone resistance-determining regions (QRDRs) of genes encoding DNA *Gyr*Ase and topoisomerase IV, reduced membrane permeability, overexpression of multidrug efflux systems, and acquisition of plasmid-mediated quinolone resistance (PMQR) determinants (6). Among these mechanisms, amino acid substitutions within the QRDR of *GyrA* represent a well-established molecular pathway associated with reduced fluoroquinolone susceptibility. Alterations in critical residues can modify the local architecture and physicochemical properties of the quinolone-binding region, potentially impairing productive drug–target interactions (7).The biological consequences of resistance-associated mutations cannot necessarily be inferred solely from their genetic presence. Integrating sequence-based analysis with structural and computational approaches can provide additional insight into how amino acid substitutions may influence protein stability and antimicrobial–target interactions (8). In this context, protein-stability prediction tools such as I-Mutant can estimate the thermodynamic consequences of amino acid substitutions. In contrast, molecular docking can provide a structural framework for evaluating the predicted interaction of ciprofloxacin with its *GyrA* target (9). Such approaches are complementary rather than definitive: computational predictions can generate mechanistic hypotheses, but they do not substitute for experimental determination of minimum inhibitory concentrations (MICs), biochemical assays, gene-expression studies, or other functional validation. The present study therefore employs these approaches as supportive tools for interpreting phenotypic and molecular observations.Despite extensive evidence that fluoroquinolone resistance is increasing among *K. pneumoniae*, locally generated data integrating phenotypic susceptibility with molecular and structural characterization remain important for understanding resistance patterns in individual healthcare settings (10). In particular, characterizing QRDR alterations alongside assessing their predicted effects on *GyrA* stability and ciprofloxacin binding may provide a more coherent mechanistic framework than phenotypic susceptibility testing alone (11). In the present study, we investigated clinical K. pneumoniae isolates using an integrated laboratory and computational strategy encompassing antimicrobial susceptibility testing, analysis of *GyrA* and *parC* QRDRs, screening for selected PMQR determinants, prediction of mutation-associated changes in *GyrA* stability, and molecular docking of ciprofloxacin with the *GyrA* target.This study aimed to characterize ciprofloxacin susceptibility among clinical *K. pneumoniae* isolates and investigate the potential molecular basis of resistance, with particular emphasis on *GyrA* alterations and their predicted structural consequences. By integrating phenotypic resistance data with sequence-level and in silico structural analyses, this study seeks to contribute to a more mechanistic understanding of ciprofloxacin resistance in *K. pneumoniae* and to provide a basis for continued antimicrobial susceptibility surveillance and molecular investigation of fluoroquinolone resistance. Importantly, given the limited number of isolates examined, interpret the findings as preliminary evidence requiring validation in larger, genetically and clinically representative collections.

MATERIAL AND METHODS

2.1 Study design and clinical isolates

This cross-sectional, laboratory-based study was conducted in the Department of Microbiology at the University of Peshawar, Pakistan, from January 2025 to June 2025. It integrated phenotypic antimicrobial susceptibility testing with molecular and computational analyses of clinical K. pneumoniae isolates. Clinical specimens, including sputum, urine, and wound swabs, were processed using standard microbiological procedures (12). The study included five isolates confirmed as K. pneumoniae, comprising three from sputum, one from urine, and one from a wound specimen.

2.2 Isolation and identification of Klebsiella pneumoniae

Clinical specimens were inoculated onto MacConkey agar and blood agar and incubated at 35–37°C for 18–24 h. Presumptive *K. pneumoniae* colonies were identified based on characteristic colony morphology, Gram staining, and conventional biochemical characteristics. Colonies exhibiting typical lactose-fermenting morphology on MacConkey agar were further evaluated by standard biochemical testing (12). Species-level identification was additionally confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), in which protein spectral fingerprints generated from pure bacterial cultures were compared with reference database spectra (12).

2.3 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar according to standardized interpretive criteria. The antimicrobial panel included ciprofloxacin, cefoperazone–sulbactam, ceftazidime, ceftriaxone, amoxicillin–clavulanate, colistin, imipenem, meropenem, and tigecycline. We measured the diameter of inhibition zones after 24 hours of incubation and interpreted results according to the Clinical and Laboratory Standards Institute (CLSI) 2022 guidelines (12). Ciprofloxacin susceptibility was the principal phenotype investigated in subsequent molecular analyses.

2.4 Genomic DNA extraction and molecular analysis

Genomic DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. DNA quantity and purity were assessed using agarose gel electrophoresis and NanoDrop spectrophotometry (Thermo Fisher Scientific, Wilmington, DE, USA) (12). The quinolone resistance-determining regions (QRDRs) of *GyrA* and *parC* were amplified by polymerase chain reaction (PCR) using published primer sets targeting regions encompassing recognized fluoroquinolone resistance-associated sites (13). PCR products were purified and subjected to sequence analysis. Obtained sequences were compared with corresponding wild-type *K. pneumoniae* reference sequences to identify nucleotide substitutions and predicted amino acid changes.

2.5 Detection of plasmid-mediated quinolone resistance determinants

Selected plasmid-mediated quinolone resistance (PMQR) determinants, including *qnr*A, *qnr*B, *qnr*S, and aac(6′)-Ib-cr, were investigated by PCR using published primer sets (14). The presence or absence of these determinants was evaluated in relation to the observed ciprofloxacin susceptibility phenotype and chromosomal QRDR alterations \.

2.6 Prediction of the effect of *GyrA* substitutions on protein stability

The potential structural consequences of identified *GyrA* substitutions were assessed using I-Mutant 3.0 (15). We obtained the relevant K. pneumoniae GyrA amino acid sequence from UniProt and evaluated individual amino acid substitutions identified through QRDR sequence analysis computationally (16). The predicted change in Gibbs free energy (ΔΔG) was used as an indicator of each substitution's potential effect on protein stability. Negative ΔΔG values were interpreted as indicative of a predicted decrease in protein stability. The associated Reliability Index was recorded to indicate the confidence of the computational prediction (17).

2.7 *GyrA* structural modeling and preparation

The DNA *Gyr*ase subunit (*GyrA*) of *K. pneumoniae* was selected as the molecular target because DNA *Gyr*ase represents a major target of fluoroquinolone antibiotics in Gram-negative bacteria. The corresponding protein sequence was obtained from UniProt (18). Where an experimentally determined structure was unavailable, a structural model was generated using an available predicted or homology-based structure. The resulting protein model was prepared for molecular docking by removing non-protein components, adding hydrogen atoms, and assigning appropriate atomic charges. Structural quality was assessed using standard protein-model evaluation procedures (18).

2.8 Ciprofloxacin preparation and molecular docking

The three-dimensional structure of ciprofloxacin was retrieved from PubChem (CID 2764) and prepared for docking analysis (19). The ligand structure was energy-minimized using the Universal Force Field (UFF) to obtain an energetically optimized conformation. Molecular docking was performed using AutoDock Vina or an equivalent docking platform. The docking grid was positioned around the relevant quinolone-binding region of *GyrA*, and multiple ligand poses were generated. Docking poses were ranked by predicted binding affinity, expressed as binding energy in kcal/mol (20). Root-mean-square deviation (RMSD) values were used to assess the spatial similarity among predicted ligand conformations.

2.9 Analysis of ciprofloxacin–*GyrA* interactions

The highest-ranked ciprofloxacin–*GyrA* docking poses were examined to characterize the predicted interaction of ciprofloxacin with amino acid residues within or surrounding the binding region. Two- and three-dimensional representations of the docked complex were generated to visualize ligand positioning and potential hydrogen-bonding, hydrophobic, electrostatic, and other non-covalent interactions. Particular attention was given to residues located within the functionally relevant region of *GyrA* and to the potential structural implications of resistance-associated amino acid substitutions (21).

2.10 Data analysis

Phenotypic susceptibility results were summarized as the number and proportion of isolates categorized as resistant or susceptible to each tested antimicrobial. Ciprofloxacin resistance was calculated as the proportion of isolates classified as resistant among the five *K. pneumoniae* isolates. Molecular findings were summarized according to the presence or absence of *GyrA* and *parC* QRDR substitutions and PMQR determinants. I-Mutant predictions were reported using ΔΔG values and Reliability Index scores, whereas molecular docking results were summarized according to predicted binding affinity and RMSD. Because of the small sample size, the analysis was primarily descriptive, and no inferential statistical testing was undertaken.

Results

3.1 Distribution of *K. pneumoniae* isolates

Five clinical *K. pneumoniae* isolates were included in the study. Sputum was the predominant specimen source, accounting for three isolates (60.0%), whereas urine and wound swab specimens contributed one isolate each (20.0%).

3.2 Antimicrobial susceptibility profile

The antimicrobial susceptibility profile demonstrated resistance to several clinically relevant agents (**Table 1**). Ciprofloxacin resistance was identified in three of five isolates (60.0%; 95% CI: 14.7–94.7%), while two isolates (40.0%) remained susceptible. The highest resistance rates were observed for ceftriaxone and co-amoxiclav, with four isolates each (80.0%; 95% CI: 28.4–99.5%) showing resistance. Ceftazidime resistance was detected in three isolates (60.0%; 95% CI: 14.7–94.7%). Resistance to cefoperazone–sulbactam and meropenem was observed in two isolates each (40.0%; 95% CI: 5.3–85.3%). Imipenem and tigecycline resistance were each detected in one isolate (20.0%; 95% CI: 0.5–71.6%). All five isolates were susceptible to colistin, corresponding to a resistance rate of 0.0% (95% CI: 0.0–52.2%).

Table 1. Antimicrobial susceptibility profile of clinical *K. pneumoniae* isolates (n = 5)

Antimicrobial	Resistant, n (%)	Susceptible, n (%)	95% CI for resistance
cefotaxima–sulbacta m	2 (40.0)	3 (60.0)	5.3–85.3
Ceftazidime	3 (60.0)	2 (40.0)	14.7–94.7
Ceftriaxone	4 (80.0)	1 (20.0)	28.4–99.5
Ciprofloxacin	3 (60.0)	2 (40.0)	14.7–94.7
Co-amoxiclav	4 (80.0)	1 (20.0)	28.4–99.5
Colistin	0 (0.0)	5 (100.0)	0.0–52.2
Imipenem	1 (20.0)	4 (80.0)	0.5–71.6
Meropenem	2 (40.0)	3 (60.0)	5.3–85.3
Tigecycline	1 (20.0)	4 (80.0)	0.5–71.6

3.3 Ciprofloxacin resistance according to specimen type

Ciprofloxacin resistance was identified in isolates recovered from both respiratory and non-respiratory specimens. Among the three sputum isolates, one (33.3%) was resistant to ciprofloxacin, and two (66.7%) were susceptible. Both the urine and wound swab isolates were resistant, corresponding to 100% resistance within each single-isolate specimen category. Because of the limited number of isolates, these distributions were interpreted descriptively, and no statistical comparison between specimen types was undertaken.

3.4 *GyrA* quinolone resistance-associated mutations

Molecular analysis of the quinolone resistance-determining regions (QRDRs) identified amino acid substitutions in *GyrA* among the ciprofloxacin-resistant isolates. The reported substitutions occurred at positions 83 and 87, namely Y83I and G87N, respectively. These substitutions were detected among ciprofloxacin-resistant isolates but were not identified in the ciprofloxacin-susceptible isolates. No corresponding resistance-associated QRDR alterations were reported among the susceptible isolates.The potential effects of the identified *GyrA* substitutions on protein stability were subsequently evaluated using I-Mutant. Both substitutions were predicted to decrease *GyrA* protein stability. The Y83I substitution had a Reliability Index of 2, whereas G87N yielded a Reliability Index of 8 (**Table 2**). The higher Reliability Index for G87N indicates greater confidence in the computational prediction; however, these results are in silico predictions and do not constitute experimental evidence of altered protein stability.

Table 2. Predicted effects of identified *GyrA* substitutions on protein stability

GyrA position	Wild-type	Mutant	Predicted effect	Reliability Index	ΔΔG (kcal/mol)
83	Y (Tyr)	I (Ile)	Decreased stability	2	(–)
87	G (Gly)	N (22)	Decreased stability	8	(–)

3.5 Plasmid-mediated quinolone resistance determinants

Screening for the investigated plasmid-mediated quinolone resistance determinants did not detect *qnr* genes in the study isolates. Thus, within this limited collection, detectable *GyrA* QRDR alterations were observed among ciprofloxacin-resistant isolates, whereas the investigated *qnr* determinants were not identified. This study did not experimentally evaluate other potential contributors to fluoroquinolone resistance, including efflux-pump overexpression and altered membrane permeability.

3.6 Molecular docking of ciprofloxacin with *GyrA*

Molecular docking was performed to investigate the predicted interaction between ciprofloxacin and the *K. pneumoniae* DNA *GyrA*se A subunit. Nine docking poses were evaluated, with predicted binding affinities ranging from −5.2 to −6.0 kcal/mol (**Table 3; Figure 1**). The highest-ranked pose showed a predicted binding affinity of −6.0 kcal/mol and an RMSD of 0 Å. A second pose also exhibited a binding affinity of −6.0 kcal/mol but had an RMSD of 18.16 Å. The remaining poses showed binding affinities between −5.2 and −5.8 kcal/mol, with RMSD values ranging from 2.77 to 19.13 Å.

Table 3. Molecular docking results for ciprofloxacin–*GyrA* interaction

Pose	Binding affinity (kcal/mol)	RMSD (Å)
1	−6.0	0
2	−6.0	18.16
3	−5.8	7.36
4	−5.7	3.17
5	−5.5	4.55
6	−5.4	2.77
7	−5.3	19.13
8	−5.3	2.79
9	−5.2	6.3

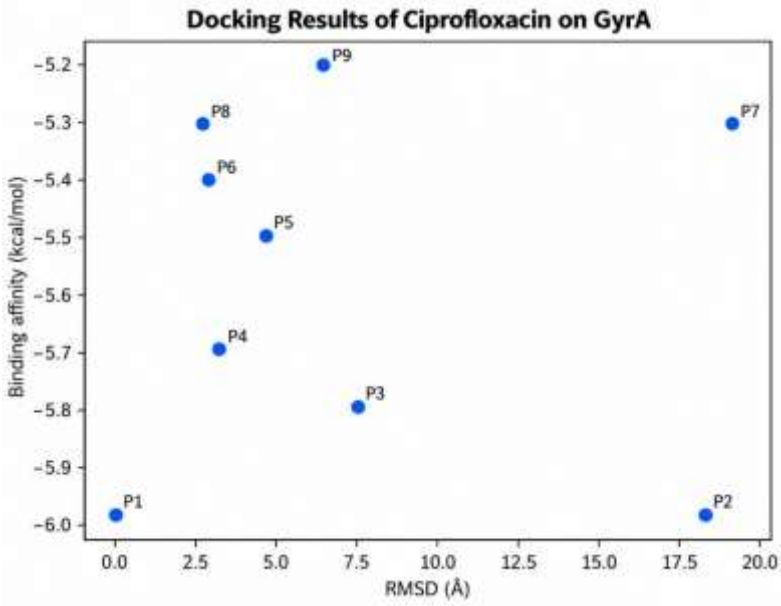

Figure 1: Docking results. Scatter plot of binding affinity versus RMSD for ciprofloxacin poses on Three-dimensional visualization demonstrated that ciprofloxacin occupied the predicted ligand-binding region of *GyrA* (**Figure 2A**). The

tw dimensional docked complex showed the ligand positioned within the protein structure, with several surrounding residues, including His19, Val14, Val26, Glu94, Arg95, Ile96, Pro97, and Tyr18. Tyr18 was reported to exhibit a close interaction with ciprofloxacin, while the surrounding residues potentially contributed to stabilization of the docked ligand through non-covalent interactions (**Figure 2B**).

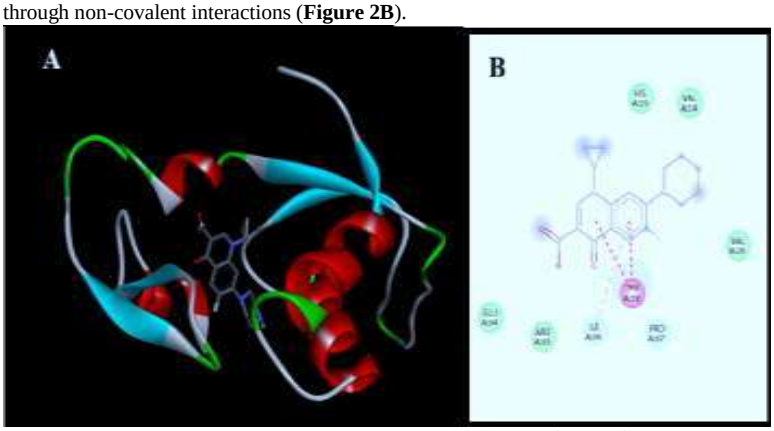

Figure 2. Molecular docking and interaction analysis of ciprofloxacin with *K. pneumoniae GyrA*. **(A)** Three-dimensional representation of the predicted ciprofloxacin–*GyrA* docked complex, showing the spatial positioning of ciprofloxacin within the *GyrA* protein structure. **(B)** Two-dimensional representation of the predicted ligand–protein interactions, illustrating ciprofloxacin and the surrounding amino acid residues within the putative binding region.

3.7 Integrated phenotypic and molecular findings

Overall, ciprofloxacin resistance was observed in three of five clinical *K. pneumoniae* isolates. The resistant phenotype occurred alongside resistance to several other antimicrobial agents. The resistant isolates carried the reported *GyrA* QRDR substitutions Y83I and G87N, whereas susceptible isolates lacked these alterations. Both substitutions were predicted to reduce *GyrA* protein stability, and molecular docking demonstrated a predicted interaction between ciprofloxacin and the *GyrA* target, with a maximum predicted binding affinity of −6.0 kcal/mol. Taken together, these findings provide complementary phenotypic, molecular, and structural observations concerning ciprofloxacin resistance in the study isolates.

Discussion

Ciprofloxacin resistance among clinical *K. pneumoniae* represents an important component of the broader antimicrobial resistance problem because fluoroquinolone resistance can substantially constrain therapeutic options for infections caused by this pathogen (23). In the present study, 3 of 5 clinical *K. pneumoniae* isolates (60%) were resistant to ciprofloxacin. Although the small sample size precludes estimation of a precise population-level prevalence, the finding is consistent with the established global burden of fluoroquinolone resistance in *K. pneumoniae*. The clinical importance of this phenotype is further underscored by the broader resistance profile observed in the present isolates, particularly the high resistance to ceftriaxone and co-amoxiclav. *K. pneumoniae* is recognized internationally as an important antimicrobial-resistant pathogen, and the 2024 WHO Bacterial Priority Pathogens List specifically includes third-generation cephalosporin- and carbapenem-resistant *K. pneumoniae* among priority threats (24).The 60% ciprofloxacin resistance observed in this study is broadly comparable with the substantial fluoroquinolone resistance reported in previous *K. pneumoniae* investigations. However, direct comparisons should be interpreted cautiously because resistance estimates vary by geographical setting, patient population, specimen source, antimicrobial susceptibility methodology, and inclusion criteria (25). For example, an Iranian study of 215 clinical *K. pneumoniae* isolates reported ciprofloxacin resistance in 22.7% of isolates by disk diffusion and confirmed resistance in 40 isolates using E-test-based MIC assessment (26). Conversely, earlier investigations have documented considerably higher ciprofloxacin resistance in selected hospital populations. Such heterogeneity emphasizes that ciprofloxacin susceptibility is strongly context-dependent and supports the value of local surveillance rather than reliance on resistance estimates derived from geographically distinct populations (27).The accompanying resistance profile provides additional evidence that ciprofloxacin resistance in the present isolates occurred within a broader MDR phenotype. Resistance to ceftriaxone and co-amoxiclav was observed in 80% of isolates, while 60% were resistant to ceftazidime. In contrast, all isolates remained susceptible to colistin, and most remained susceptible to imipenem and tigecycline. Although these observations cannot be generalized because of the limited sample size, the coexistence of fluoroquinolone and β-lactam resistance is clinically relevant. *K. pneumoniae* possesses a highly plastic genome capable of accumulating chromosomal mutations and horizontally acquired resistance determinants, allowing resistance to multiple antimicrobial classes to emerge within the same bacterial population (28). This pattern is particularly concerning because fluoroquinolone resistance may occur alongside β-lactam resistance, progressively narrowing empirically effective treatment options (28).The molecular findings provide a plausible mechanistic framework for the observed ciprofloxacin phenotype. All ciprofloxacin-resistant isolates carried the reported *GyrA* QRDR substitutions Y83I and G87N, whereas these alterations were not identified in ciprofloxacin-susceptible isolates (29). The GyrA QRDR is a well-established hotspot for fluoroquinolone resistance in *K. pneumoniae.* Experimental and epidemiological studies have repeatedly implicated substitutions at critical *GyrA* residues, particularly positions corresponding to Ser83 and Asp87, in reduced ciprofloxacin susceptibility (12). In a large Chinese investigation, substitutions involving *GyrA* Ser83 and Asp87 were strongly associated with ciprofloxacin resistance, with Ser83→Ile and several double substitutions involving Ser83 and Asp87 representing important resistance-associated patterns(30) (31). More recently, a 2024 study of clinical *K. pneumoniae* isolates similarly identified frequent mutations in *GyrA* and *parC* among ciprofloxacin-resistant isolates, with mutations at *GyrA* codon 83 being particularly prominent (32).The concordance between the ciprofloxacin-resistant phenotype and the reported *GyrA* alterations in the present study is therefore biologically plausible. Nevertheless, the relationship should be interpreted as an association rather than definitive evidence of causation. QRDR mutations can occur in isolates with different levels of fluoroquinolone susceptibility, and resistance expression may depend on the combination of target-site alterations, efflux activity, membrane permeability, and acquired PMQR determinants. Previous studies have demonstrated that *K. pneumoniae* fluoroquinolone resistance is frequently genetically heterogeneous rather than attributable to a single mechanism (33). Consequently, the absence of *qnr*s determinants in the present isolates does not establish that *GyrA* alterations were the only mechanisms responsible for resistance (34).Indeed, the failure to detect the investigated *qnr* determinants is an informative finding but should not be interpreted as evidence that PMQR mechanisms are generally absent from ciprofloxacin-resistant *K. pneumoniae*. A recent study from Iran identified PMQR determinants in all 40 ciprofloxacin-resistant isolates examined, including *oqxA, qnrB, qnrS, and aac(6')-Ib-cr*, and also found mutations in GyrA and parC to be highly prevalent (35). These findings illustrate the mechanistic diversity of fluoroquinolone resistance and contrast with the present observation of no detectable investigated *qnr* genes. The discrepancy may reflect geographical differences, clonal composition, sample size, or differences in the resistance determinants screened. Moreover, the present study did not experimentally quantify efflux-pump activity or assess porin alterations; therefore, additional chromosomal or regulatory mechanisms cannot be excluded (36).The computational analysis provided additional structural context for the observed *GyrA* substitutions. I-Mutant predicted decreased protein stability for both Y83I and G87N, with Reliability Index values of 2 and 8, respectively. These predictions suggest that the substitutions may influence GyrA conformational stability; however, protein-stability prediction algorithms estimate thermodynamic effects computationally and cannot establish whether a mutation alters catalytic activity, drug-binding geometry, or cellular susceptibility. Thus, the principal value of these results is hypothesis generation and mechanistic interpretation rather than functional confirmation.Molecular docking similarly demonstrated that ciprofloxacin could occupy the predicted *GyrA* binding region, with the best-ranked pose exhibiting a predicted binding affinity of −6.0 kcal/mol. The docked ligand was positioned within the predicted binding region and surrounded by several amino acid residues. These observations are consistent with the established pharmacological mechanism whereby fluoroquinolones interact with the DNA *Gyr*ase–DNA complex. However, docking scores should not be interpreted as direct measurements of experimental binding affinity. In particular, the docking model did not reproduce the complete physiological drug–enzyme–DNA cleavage complex, and therefore cannot independently demonstrate that the identified substitutions reduce ciprofloxacin binding or confer resistance. The study appropriately recognizes this limitation, particularly because the structural analysis relied on computational modeling rather than biochemical validation.An important methodological consideration concerns how the reported residue substitutions are interpreted. The present study describes Y83I and G87N, whereas much of the established *K. pneumoniae* fluoroquinolone-resistance literature refers to the canonical QRDR residues Ser83 and Asp87. This distinction matters because amino-acid numbering and reference-sequence annotation must be consistent when comparing mutations across studies. This study has several strengths. First, it integrates conventional antimicrobial susceptibility testing with molecular characterization and computational structural analysis, thereby examining ciprofloxacin resistance at multiple biological levels. Second, the comparison between ciprofloxacin-resistant and susceptible isolates

provides an internally coherent observation: the reported *GyrA* alterations were identified in resistant but not susceptible isolates. Third, screening for PMQR determinants complements QRDR analysis and reduces the likelihood of attributing the phenotype exclusively to chromosomal target alterations without considering plasmid-mediated mechanisms. Finally, the docking analysis provides a structural framework for interpreting the possible relationship between the target protein and ciprofloxacin.Nevertheless, the findings require cautious interpretation. The most important limitation is the extremely small sample size of five isolates, which results in wide confidence intervals and substantially limits statistical precision and external validity. The observed 60% ciprofloxacin resistance rate should therefore be considered a preliminary estimate rather than a population prevalence. The study also relied on disk-diffusion susceptibility testing without quantitative ciprofloxacin MIC determination, limiting the ability to distinguish low- from high-level resistance or examine genotype–MIC relationships. Furthermore, the study investigated only selected PMQR determinants, and it did not comprehensively characterize efflux-pump expression, porin alterations, parC contributions, or other potential mechanisms. The computational stability and docking analyses provide supportive structural hypotheses but require experimental validation.Taken together, the findings indicate that ciprofloxacin resistance was common among the small collection of clinical *K. pneumoniae* isolates examined and occurred alongside resistance to several other antimicrobial agents. The detection of the reported *GyrA* QRDR substitutions exclusively among ciprofloxacin-resistant isolates, together with predicted effects on *GyrA* stability and the observed ciprofloxacin–*GyrA* docking interaction, provides a coherent preliminary model in which alterations of the fluoroquinolone target may contribute to resistance. However, the evidence does not establish that these mutations are sufficient or solely responsible for the phenotype. Larger studies incorporating quantitative MIC determination, comprehensive *GyrA*/*parC* sequencing, whole-genome sequencing, systematic characterization of PMQR determinants, efflux and porin analysis, and experimental validation of mutation-specific phenotypes are warranted. Such integrated surveillance would provide a stronger basis for defining the molecular epidemiology of ciprofloxacin-resistant *K. pneumoniae* and informing antimicrobial stewardship and infection-control strategies.

CONCLUSION

This study provides preliminary evidence of substantial ciprofloxacin resistance among the clinical *K. pneumoniae* isolates examined, occurring within a broader pattern of antimicrobial resistance. The detection of the reported *GyrA* QRDR substitutions among ciprofloxacin-resistant isolates, together with their predicted effects on *GyrA* protein stability and the demonstrated in silico interaction of ciprofloxacin with the *GyrA* target, supports a potential contribution of target-site alterations to fluoroquinolone resistance. However, these findings should be interpreted cautiously because of the small sample size and the absence of functional validation of the identified mutations. Larger, well-characterized studies incorporating quantitative MIC determination, comprehensive resistance-genome analysis, and experimental validation are warranted to clarify the molecular determinants and epidemiological burden of ciprofloxacin-resistant *K. pneumoniae*.

Author Contributions

All authors contributed equally to the study. Muhammad Amir, Muhammad Yaman, Syeed Abu Un Nassar, Abbas Saleem, Muhammad Rizwan, and Manhil Anjum contributed substantially to the conception and design of the study, sample processing, laboratory investigations, data collection, data analysis, and interpretation of findings. All authors contributed to drafting the manuscript and critically revising it for important intellectual content. All authors approved the final version and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the study were appropriately investigated and resolved**.**

Conflicts of Interest

The authors declare no conflicts of interest related to this study, its findings, or its publication.

Acknowledgments

The authors are particularly grateful to the laboratory staff and technical personnel of the Center of Biotechnology and Microbiology, University of Peshawar, for assistance with specimen processing, bacterial identification, antimicrobial susceptibility testing, molecular analyses, and computational investigations. The authors also acknowledge the academic guidance and scientific support provided by the supervisory team throughout the research project.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Consent for Publication

Not applicable. The manuscript does not contain identifiable individual patient information or images.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request, subject to applicable institutional and ethical restrictions.

References

1. Rahmat Ullah S, Jamal M, Rahman A, Andleeb S. Comprehensive insights into Klebsiella pneumoniae: unraveling clinical impact, epidemiological trends and antibiotic-resistance challenges. Journal of Antimicrobial Chemotherapy. 2024;79(7):1484-92.
2. Han B, Feng C, Jiang Y, Ye C, Wei Y, Liu J, et al. Mobile genetic elements encoding antibiotic resistance genes and virulence genes in Klebsiella pneumoniae: important pathways for the acquisition of virulence and resistance. Frontiers in Microbiology. 2025;16:1529157.
3. Shah AA, Alwashmi AS, Abalkhail A, Alkahtani AM. Emerging challenges in Klebsiella pneumoniae: Antimicrobial resistance and novel approach. Microbial pathogenesis. 2025;202:107399.
4. Fukuda D, Powell D, Mulgirigama A, Vojtek I, Ozeki H, Yoshimoto D, et al. Bacterial DNA topoisomerase IV and DNA gyrase inhibitors: history of the quinolones, their clinical usage and potential alternatives for the future. Journal of Antimicrobial Chemotherapy. 2026;81(3):dkag054.
5. Rajakumari K, Aravind K, Balamugundhan M, Jagadeesan M, Somasundaram A, Devi PB, et al. Comprehensive review of DNA gyrase as enzymatic target for drug discovery and development. European Journal of Medicinal Chemistry Reports. 2024;12:100233.
6. Sani GS, Ghane M, Babaeekhou L. Fluoroquinolone-resistance mechanisms and molecular epidemiology of ciprofloxacin-resistant Klebsiella pneumoniae isolates in Iran. Folia Microbiologica. 2023;68(4):633-44.
7. Erlina L, Fadilah F, Abdelrazig OAO, Paramita RI, Prawiningrum AF, Utari WD, et al. Genomic Insights into Antimicrobial Resistance and Plasmid-Mediated Dissemination in Escherichia coli and Klebsiella pneumoniae from Pediatric Outpatients with Acute Diarrhea. Antibiotics. 2026;15(4):331.
8. Koolman L, Emenike C, Mitra D, Chattaraj S. Beyond genes: non-canonical mechanisms driving antimicrobial resistance in bacteria. Bacteria. 2025;4(4):50.
9. Gautam P, Meena LS. Revelation of point mutations effect in Mycobacterium tuberculosis MfpA protein that is involved in mycobacterial DNA supercoiling and fluoroquinolone resistance. Biotechnology and Applied Biochemistry. 2021;68(6):1357-71.
10. Tsang KK, Amke I, Aanensen DM, Abdulahi J, Aiken A, Bachman MA, et al. Ciprofloxacin resistance in Klebsiella pneumoniae: phenotype prediction from genotype and global distribution of resistance determinants. bioRxiv. 2025:2025.09. 24.678318.
11. Elbehiry A, Marzouk E, Abalkhail A. Fluoroquinolone resistance in ESKAPE pathogens: evolutionary pathways, one health transmission, and clinical surveillance. Frontiers in Microbiology. 2025;16:1719066.
12. Waheed A, Khan SA, Ahmad S, Phelan JE, Rani GF, Campino S, et al. Genomic Insights into Chromosomal Colistin Resistance and Virulence–Resistance Convergence in MDR/XDR Klebsiella pneumoniae from Tertiary Hospitals in Peshawar, Pakistan. Pathogens. 2026;15(2):218.
13. Daneshmand M, Kiani M, Zahedi M, Zare Banadkouki A, Tayefeh-Arbab MH, Pournajaf A. Biofilm Formation and Quinolone Resistance Determinants in Clinical Klebsiella pneumoniae Isolates from Babol, Northern Iran. Iranian Journal of Medical Microbiology. 2025;19(6):376-86.
14. Abdulkareem MM, Abdulrahman MA, Yassin NA. Molecular detection of plasmid-mediated quinolone resistance genes among clinical isolates of Klebsiella pneumoniae during the COVID-19 pandemic. Pharmacia. 2023;70:225-31.
15. Declercq AM, Tilleman L, Gansemans Y, De Witte C, Haesebrouck F, Van Nieuwerburgh F, et al. Comparative genomics of Flavobacterium columnare unveils novel insights in virulence and antimicrobial resistance mechanisms. Veterinary Research. 2021;52(1):18.
16. Haque TA, Urmi UL, Islam ABMMK, Ara B, Nahar S, Mosaddek ASM, et al. Detection of qnr genes and gyrA mutation to quinolone phenotypic resistance of UTI pathogens in Bangladesh and the implications: Resistance UTI pathogens Bangladesh. Journal of Applied Pharmaceutical Science. 2022:1-28.
17. Qiu Y, Huang T, Cai YD. Review of predicting protein stability changes upon variations. Proteomics. 2024;24(12-13):2300371.
18. Joshi T, Mathpal S, Kumar H, Ramaiah S, Anbarasu A. Characterization and targeting of deleterious mutations in Carbapenemase-producing Klebsiella pneumoniae: insights from variant calling, Computer-aided drug design, and DFT analysis. Archives of Microbiology. 2026;208(4):193.
19. Haryini S. Computational identification of novel natural inhibitors against triple mutant DNA gyrase A in fluoroquinolone-resistant Salmonella Typhimurium. Biochemistry and Biophysics Reports. 2025;41:101901.

20. Che X, Liu Q, Zhang L. An accurate and universal protein-small molecule batch docking solution using Autodock Vina. Results in Engineering. 2023;19:101335.
21. Dhara L, Tripathi A. Role of gyrase A/B double mutations along with Qnr genes in development of higher ciprofloxacin resistance among Pathogenic Escherichia coli and Klebsiella pneumoniae. Microbial Pathogenesis. 2025:107946.
22. Abdullah MA, Shaikh BT, Ashraf M, Khan SA. XDR typhoid in Pakistan: A threat to global health security and a wake-up call for antimicrobial stewardship. PLOS Neglected Tropical Diseases. 2025;19(5):e0013067.
23. Tsang KK, Amke I, Aanensen DM, Abdulahi J, Aiken A, Bachman MA, et al. Ciprofloxacin resistance in Klebsiella pneumoniae: phenotype prediction from genotype and global distribution of resistance determinants. bioRxiv. 2025:2025.09. 24.678318.
24. Kapoor A, Dey T, Kale TM, Kumar A, Tak V, Nag VL, et al. Genomic diversity in the critical priority pathogen Klebsiella pneumoniae: global and regional perspectives. BMC Microbiology. 2026;26(1):281.
25. Rezaei S, Tajbakhsh S, Naeimi B, Yousefi F. Investigation of gyrA and parC mutations and the prevalence of plasmid-mediated quinolone resistance genes in Klebsiella pneumoniae clinical isolates. BMC Microbiology. 2024;24(1):265.
26. Rezaei S, Tajbakhsh S, Naeimi B, Yousefi F. Investigation of gyrA and parC mutations and the prevalence of plasmid-mediated quinolone resistance genes in Klebsiella pneumoniae clinical isolates. BMC Microbiology. 2024;24(1):265.
27. Thomson CJ. The global epidemiology of resistance to ciprofloxacin and the changing nature of antibiotic resistance: a 10-year perspective. Journal of Antimicrobial Chemotherapy. 1999;43(suppl_1):31-40.
28. Harini A, Sundaresan AK, Ramakrishnan J. Klebsiella pneumoniae in the global AMR: resistance mechanisms and genomic adaptation. World Journal of Microbiology and Biotechnology. 2026;42(5):211.
29. Tsang KK, Amke I, Aanensen DM, Abdulahi J, Aiken A, Bachman MA, et al. Ciprofloxacin resistance in Klebsiella pneumoniae: phenotype prediction from genotype and global distribution of resistance determinants. bioRxiv. 2025:2025.09. 24.678318.
30. Fu Y, Guo L, Xu Y, Zhang W, Gu J, Xu J, et al. Alteration of GyrA amino acid required for ciprofloxacin resistance in Klebsiella pneumoniae isolates in China. Antimicrobial agents and chemotherapy. 2008;52(8):2980-3.
31. Shariati A, Arshadi M, Khosrojerdi MA, Abedinzadeh M, Ganjalishahi M, Maleki A, et al. The resistance mechanisms of bacteria against ciprofloxacin and new approaches for enhancing the efficacy of this antibiotic. Frontiers in Public Health. 2022;10:1025633.
32. Ghaffari Lashkenari E, Mir MS, Mohammadi M, Javadi K, Halaji M. Investigating the relationship between mutations in GyrA and parc genes and resistance to fluoroquinolones in uropathogenic Escherichia coli isolates. Microbial Drug Resistance. 2025;31(10):317-22.
33. Anes J, Hurley D, Martins M, Fanning S. Exploring the genome and phenotype of multi-drug resistant Klebsiella pneumoniae of clinical origin. Frontiers in Microbiology. 2017;8:1913.
34. Kareem SM, Al-Kadmy IM, Kazaal SS, Mohammed Ali AN, Aziz SN, Makharita RR, et al. Detection of gyrA and parC mutations and prevalence of plasmid-mediated quinolone resistance genes in Klebsiella pneumoniae. Infection and drug resistance. 2021:555-63.
35. Rezaei S, Tajbakhsh S, Naeimi B, Yousefi F. Investigation of gyrA and parC mutations and the prevalence of plasmid-mediated quinolone resistance genes in Klebsiella pneumoniae clinical isolates. BMC Microbiology. 2024;24(1):265.
36. Yuan P-B, Ling J-H, Zhu J-H, Peng C, Chen E-Z, Zhong Y-X, et al. Proteomics profiling of ertapenem-challenged major porin-deficient carbapenem-resistant Klebsiella pneumoniae. Journal of Proteomics. 2022;268:104715.