ISSN: 2588-4115; Infection Epidemiology and Microbiology. 2026;12(1):15-23 10.52547/iem.12.1.15

# Molecular Mechanisms of Fluoroquinolone Resistance in *Streptococcus pneumoniae* Isolates from Iran

ARTICLE INFO

*Article Type*
**Original Article**

*Authors*
Aram Sharifi, *PhD*[1]
Amin Tarinjoo, *MSc*[2]
Samira Dahaghin, *MSc*[3]
Ali Ahmadi, *PhD*[4*]

[1] Department of Animal Science, Faculty of Agriculture, University of Kurdistan, Sanandaj, Kurdistan, Iran
[2] Islamic Azad university, Science and Research Branch, Tehran, Iran.
[3] Department of Microbiology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[4] Molecular Biology Research Center, Biomedicine Technologies Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

** Correspondence*
Molecular Biology Research Center, Biomedicine Technologies Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
E-mail: Aliahmadi@bmsu.ac.ir

**How to cite this article**
Sharifi A., Tarinjoo A., Dahaghin S., Ahmadi A. Molecular Mechanisms of Fluoroquinolone Resistance in Streptococcus pneumoniae Isolates from Iran Infection Epidemiology and Microbiology. 2026;12(1): 15-23.

*Article History*
Received: April 24, 2025
Accepted: February 19, 2026
Published: May 26, 2026

ABSTRACT

**Background:** Due to recent reports of resistance to β-lactams and macrolides, the use of fluoroquinolones (FQs) for treating *Streptococcus pneumoniae* infections has increased. This study aimed to evaluate the molecular mechanisms of FQ resistance in pneumococcal isolates.

**Materials & Methods:** This study was conducted on 131 pneumococcal isolates (67 nasopharyngeal isolates from healthy individuals and 64 clinical isolates) collected in Tehran, Iran, in 2023. Susceptibility to FQs was determined, and quinolone resistance–determining regions (QRDRs) of gyrA, gyrB, parC, and parE in resistant isolates were amplified and sequenced.

**Findings:** Disk diffusion testing showed that 23 (17.5%) and five (3.8%) isolates were resistant to norfloxacin (5µg) and ofloxacin (5µg), respectively. Minimum inhibitory concentration (MIC) test confirmed resistance in 22 of 23 norfloxacin-resistant isolates. All five ofloxacin-resistant isolates were distinct from the norfloxacin-resistant group, and their resistance was also confirmed by MIC testing. Overall, 28 non-overlapping resistant isolates were selected for sequencing. Among these isolates, mutations in *parC* and *gyrA* were detected in four (14.28%) and five (17.85%) isolates, respectively, while two (7.14%) isolates harbored simultaneous mutations in both genes. The most frequent substitutions were Ser81→Leu in *parC* and Glu85→Lys and Ser81→His/Thr in *gyrA*. No statistically significant difference was observed between nasopharyngeal (healthy flora) and clinical isolates regarding FQ resistance patterns.

**Conclusion:** This study identified key molecular mechanisms of fluoroquinolone resistance in S. pneumoniae, primarily involving mutations in parC and gyrA, including double mutations. Notably, all isolates remained susceptible to moxifloxacin, supporting its effectiveness in treating pneumococcal infections.

**Keywords:** *Streptococcus pneumoniae*, Fluoroquinolone resistance, QRDRs, Iran

CITATION LINKS

[1] Shi W, Zhou K, Yuan L, Meng Q, Dong F, Gao W, et al. Serotype... [2] Sharifi A, Kavoosi F, Hosseini SM, Mosavat A, Ahmadi A. Prevalence of... [3] Fuller J, McGeer A, Low D. Drug-resistant... [4] Li L, Ma J, Yu Z, Li M, Zhang W, Sun H. Epidemiological... [5] Patel SN, Melano R, McGeer A, Green K, Low DE. Characterization... [6] Bast DJ, Low DE, Duncan CL, Kilburn L, Mandell LA, Davidson RJ, et al. Fluoroquinolone... [7] Hawkey PM. Mechanisms of... [8] Ceyssens PJ, Van Bambeke F, Mattheus W, Bertrand S, Fux F, Van Bossuyt E, et al. Molecular... [9] Korzheva N, Davies TA, Goldschmidt R. Novel Ser79Leu and... [10] Bokaeian M, Khazaei H, Javadimehr M. Nasopharyngeal... [11] Aligholi M, Emaneini M, Jabalameli F, Shahsavan S, Abdolmaleki Z, Sedaghat H, et al. Antibiotic... [12] Sandoval MM, et al. Antimicrobial resistance of... [13] Kohanteb J, Sadeghi E. Penicillin-resistant... [14] Kargar M, Jahromi FM, Doosti A, Handali S. Molecular... [15] Mosleh MN, Gharibi M, Alikhani MY, Saidijam M, Vakhshiteh F. Antimicrobial... [16] Azadegan A, Ahmadi A, Lari AR, Talebi M. Detection of... [17] Clinical and Laboratory Standards Institute... [18] Jacobs MR, et al. Determination of... [19] Sharew B, et al. Antimicrobial... [20] Janoir C, Zeller V, Kitzis MD, Moreau NJ, Gutmann L. High-level... [21] Linares J, et al. Changes in... [22] Broskey J, et al. Efflux and target... [23] Fogarty C, et al. Efficacy of... [24] Naba MR, et al. Emergence of... [25] Yamamoto K, Yanagihara K, Sugahara K, Imamura Y, Seki M, Izumikawa K, et al. In vitro... [26] Beheshti M, et al. Molecular characterization... [27] Ramakrishnan R, et al. Comparative in-vitro... [28] Adam HJ, Schurek KN, Nichol KA, Hoban CJ, Baudry TJ, Laing NM, et al. Molecular... [29] Jorgensen J, Weigel L, Ferraro M, Swenson J, Tenover F. Activities of... [30] Pan XS, et al. Involvement of... [31] Tankovic J, et al. Contribution of... [32] Weigel L, Anderson G, Facklam R, Tenover F. Genetic analyses of... [33] Robertson GT, Doyle TB, Lynch AS. Use of... [34] Gill MJ, Brenwald NP, Wise R. Identification of... [35] González I, et al. Fluoroquinolone resistance...

## Introduction

*Streptococcus pneumoniae* (pneumococcus) is an opportunistic pathogen responsible for severe infections, such as septicemia, meningitis, and pneumonia [1, 2]. Since resistance to β-lactams and macrolide antibiotics has become increasingly prevalent among pneumococci worldwide, fluoroquinolones (FQs) with enhanced activity against Gram-positive bacteria have been increasingly used to treat lower respiratory tract infections [2, 3]. Although the overall rate of FQ resistance in pneumococci is relatively low, ranging from 1-7%, epidemiological studies have recently shown increasing FQ resistance rates, especially in countries with high levels of FQ consumption [4]. In Iran, several recent reports have also documented a concerning upward trend, underscoring the need for closer surveillance and research in this region [4, 5]. The mechanism of FQs is inhibition of DNA gyrase and topoisomerase IV and subsequently inhibition of DNA synthesis. These are two heterotetrameric enzymes composed of two subunits; DNA gyrase is encoded by the *gyrA* and *gyrB* genes, and topoisomerase IV is encoded by the *parC* and *parE* genes [6].

FQ resistance could arise through chromosomally-mediated mutations in quinolone resistance-determining regions (QRDRs) of *gyrA*, *gyrB*, *parC*, and *parE* [7] as well as through the activity of efflux pumps (including PmrA and PatAB). Importantly, these mechanisms may act sequentially or in combination, with efflux pumps often providing an initial low-level resistance that facilitates subsequent selection of stable QRDR mutations [8].

Previous studies have shown that pneumococci with substitutions at positions 81 and 85 of *gyrA* and 79 and 83 of *parC* are most frequently found in isolates with reduced susceptibility to FQs [9]. Understanding the prevalence rate and molecular mechanism of FQ resistance in pneumococci will help design strategies to minimize the emergence of FQ resistant strains. In addition, comparing resistance rates between normal flora and invasive pneumococcal isolates is essential to determine the distribution pattern of FQ resistance. Most studies in Iran have evaluated the susceptibility of pneumococcal isolates to ciprofloxacin only by antibiogram testing. Although previous studies have reported various resistance rates, including 1.5 [10], 30 [11], 10 [12], and 7.8% [13], only a few of them have evaluated the corresponding minimum inhibitory concentrations (MICs) and associated gene mutations [14, 15], highlighting the need for further molecular epidemiological studies in Iran.

**Objectives:** Therefore, this study investigated the prevalence of fluoroquinolone resistance among nasopharyngeal (normal flora) and clinical *S. pneumoniae* isolates and identified mutations in the QRDRs of fluoroquinolone-related genes in resistant isolates.

## Materials and Methods

**S. pneumoniae isolates:** A total of 131 *S. pneumoniae* isolates were analyzed in this study. The isolates originated from Tehran (Iran) and included 67 isolates recovered from Dacron nasopharyngeal swabs collected from healthy volunteers and 64 clinical isolates collected from two university-affiliated hospitals (Baqiyatallah and Milad hospitals) in 2023. Information regarding prior antibiotic exposure within the preceding 90 days was not available for patients with clinical infections.

Clinical isolates were collected from blood (n=18), eye (n=15), cerebrospinal fluid (n=9), sputum (n=8), sinus secretion (n=7), tracheal aspirate (n=4), urine (n=2), and pleural fluid (n=1) specimens. Each isolate- either clinical or nasopharyngeal- was

derived from a distinct individual. Healthy volunteers were defined as individuals without respiratory symptoms, fever, or antibiotic use in the preceding four weeks. The age range of participants was 1-80 years, and individuals with any chronic illness or recent infection were excluded. Participants were recruited from the statistical population of Tehran (Iran) and provided written informed consent prior to sample collection. All nasopharyngeal and clinical specimens were transported separately to the laboratory at 4 °C and immediately cultured on Columbia blood agar plates. The cultures were incubated at 37 °C in a 5% $CO_2$ atmosphere. Colonies showing α-hemolysis were subjected to standard identification tests, including optochin susceptibility, bile solubility, and PCR detection of the *lytA* gene as a species-specific marker [16]. Confirmed isolates were preserved at -70 °C in skim milk for further analysis.

**Antibiotic susceptibility determination:** Antimicrobial susceptibility testing was conducted using the disk diffusion technique following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2024) [17]. The procedure was carried out on Mueller–Hinton agar supplemented with 5% defibrinated sheep blood. Plates were incubated at 35 °C in a 5% $CO_2$ atmosphere for 20-24 hours, and inhibition zone diameters were measured after incubation. Each *S. pneumoniae* isolate was tested against six fluoroquinolone antibiotics: ciprofloxacin (5 µg), moxifloxacin (5 µg), norfloxacin (5 µg), gatifloxacin (5 µg), ofloxacin (5 µg), and levofloxacin (5 µg). All antibiotic discs were obtained from Mast Diagnostics Ltd. (Merseyside, UK).

MICs were determined for isolates showing intermediate or resistant phenotypes using the broth microdilution method. This assay was performed in accordance with CLSI recommendations using cation-adjusted Mueller–Hinton broth supplemented with 5% lysed horse blood. The antibiotic concentration range tested was 0.05-64 µg/mL. Bacterial suspensions were prepared from overnight blood agar cultures, adjusted to a turbidity equivalent to a 0.5 McFarland standard, and then diluted to yield a final inoculum of 4–5×$10^5$ CFU/mL. All MIC determinations were conducted in duplicate to verify reproducibility.

Microdilution plates were incubated at 35 °C for 24 hours under ambient conditions [18, 19]. Results were interpreted according to CLSI (2024) breakpoints for pneumococci [17]. *S. pneumoniae* ATCC 49619 served as a quality control strain, with its MIC values consistently falling within the reference range established by CLSI.

**DNA extraction, PCR amplification, and sequencing analysis:** Genomic DNA was extracted from 10 mL of overnight cultures grown in brain heart infusion (BHI) broth using the phenol–chloroform method as previously described [19]. The QRDRs of the *gyrA*, *gyrB*, *parC*, and *parE* genes of *S. pneumoniae* were amplified by PCR using specific primer sets listed in Table 1. Each 25 µL PCR mixture consisted of 3-5 µL of template DNA, 2.5 µL of 10× PCR buffer, 0.75 µL of 50 mM $MgCl_2$, 0.5 µL of 10 mM dNTP mix, 0.25 µL of Taq DNA polymerase (5 U/µL), and 25 pmol of each primer.

Amplification was carried out under the following conditions: an initial denaturation step at 95 °C for 5 min; followed by 30 cycles of denaturation at 94 °C for 30 s, annealing for 1 min (at 55 °C for *gyrA*, 51 °C for *gyrB*, and 58 °C for *parC* and *pare*), and extension at 72 °C for 1 min; with a final extension step at 72 °C for 10 min. PCR products were visualized by electrophoresis on 1.5% agarose gels containing ethidium bromide (0.5 µg/mL).

All 28 fluoroquinolone-resistant *S. pneumoniae* isolates were subjected to PCR amplification of the QRDRs of the *gyrA*, *gyrB*,

**Table 1)** Primers used for PCR assay in the present study

| Gene | Sequence (5' → 3') | Annealing Temperature (°C) | Product Size (bp) | Reference |
|---|---|---|---|---|
| *lytR* | GTTTCAATCGTCAAGCCGTT<br>CGGACTACCGCCTTTATATCG | 55 | 250 | [35] |
| *gyrA* | TGTTCACCGTCGCATTCTCT<br>ATACCAGTTGCTCCATTAACC | 55 | 393 | [33] |
| *gyrB* | TTCTCCGATTTCCTCATG<br>AGAAGGGTACGAATGTGG | 51 | 485 | [35] |
| *parC* | TGGGTTGAAGCCGGTTCA<br>TGCTGGCAAGACCGTTGG | 58 | 367 | [9] |
| *parE* | AAGGCGCGTGATGAGAGC<br>TCTGCTCCAACACCCGCA | 58 | 290 | [35] |

*parC*, and *parE* genes, and the PCR products were purified and sequenced bidirectionally using Sanger sequencing (Macrogen Inc., Seoul, Korea).

The obtained sequences were aligned and compared with the reference genome of *S. pneumoniae* R6 (GenBank accession No. NC_003098) using MEGA4 and Gene Runner software. Mutations were identified as nucleotide substitutions that led to amino acid replacements within the QRDRs, and all sequence alignments were manually verified to confirm mutation accuracy.

**Statistical analysis:** Statistical analysis was performed using SPSS software (Version 26; IBM Corp., Armonk, NY, USA). Categorical variables, such as resistance rates of clinical and nasopharyngeal isolates, were compared using Chi-square test or Fisher's exact test when the expected cell counts were <5. There was no significant difference in FQ continuous variables, including mean MIC values between groups, which were compared using Student's t-test. Resistance rates were presented as percentages; due to the exploratory nature of the study and the limited sample size, 95% confidence intervals were not calculated. A *p*-value of <.05 was considered statistically significant.

## Findings

**Demographic data:** A total of 131 isolates were collected during 2023, of which 67 (51.14%) isolates were recovered from the nasopharynx of a healthy population, and 64 (48.85) isolates were obtained from clinical samples in two university hospitals, including blood (n=18, 28.1%), eye (n=15, 23.43%), CSF (n=9, 14%), sputum (n=8, 12.5%), sinus secretion (n=7, 10.9%), tracheal aspirate (n=4, 6.25%), urine (n=2, 3.1%), and pleural fluid (n=1, 1.56%) specimens. Among different age groups, the age group 0-15 years had the highest prevalence (59.5%). Also, 97 (74%) isolates were taken from males, and 34 (26%) isolates were obtained from females (Table 2).

**FQ susceptibility:** In both nasopharyngeal and clinical isolates, the highest resistance rate was reported to norfloxacin (23 of 131, 17.55%), followed by ofloxacin (5 of 131, 3.81%). Besides, all isolates were susceptible to moxifloxacin, ciprofloxacin, levofloxacin, and gatifloxacin. The results of antibiotic susceptibility testing are presented in Table 3. There was no significant difference in FQ resistance between nasopharyngeal and clinical isolates ($p$= .008, Chi-square test). MIC testing confirmed resistance in 22 of

23 norfloxacin-resistant isolates in the disk diffusion test (MIC≥ 8 µg/mL), whereas one isolate showed intermediate susceptibility level (MIC= 4 µg/mL). Regarding ofloxacin, all five isolates resistant in the disk diffusion test were also confirmed as resistant (MIC ≥ 8 µg/mL) in the MIC determination test (Table 4).

**DNA sequence analysis of the QRDRs**: Of the 28 fluoroquinolone-resistant isolates sequenced, 11 (39.28%) carried mutations in the QRDRs. Mutations were identified in *parC* (four isolates) and *gyrA* (five isolates), while two isolates simultaneously exhibited mutations in both genes.

Among the isolates harboring only a single mutation, amino acid substitutions in the *parC* gene were detected at several positions. Specifically, the Ser81→Leu substitution was identified in two isolates, while Asp83→Gly, Asp78→Asn, and Ser79→Pro were each observed in one isolate.

Similarly, among single-mutation isolates with *gyrA* alterations, the detected substitutions included Glu85→Lys, Glu85→Pro, Ser81→His, Ser81→Thr, and Ser114→Val. No mutations were identified in the *gyrB* and *parE* genes. The distribution of both single- and double-mutation isolates is summarized in Table 5.

**Table 2)** Demographic characteristics of the study population with *Streptococcus pneumoniae* isolates

| Age Group (Year) | Male | Female | Total (%) |
|---|---|---|---|
| 0-15 | 57 | 21 | 78 (59.5) |
| 15-30 | 10 | 2 | 12 (9.1) |
| 30-60 | 9 | 2 | 11 (8.4) |
| 60-100 | 21 | 9 | 30 (22.9) |
| Total (%) | 97 (74) | 34 (26) | 131 (100) |

**Table 3)** Fluoroquinolone susceptibility patterns of clinical and normal flora *Streptococcus pneumoniae* isolates determined by disk diffusion

| Antibiotic | No. (%) of Intermediate | | | No. (%) of Resistant | | |
|---|---|---|---|---|---|---|
| | Normal Flora | Clinical Isolates | Total | Normal Flora | Clinical Isolates | Total |
| Norfloxacin (5 µg) | 38 (56.7) | 33 (51.5) | 71 (54.2) | 10 (14.9) | 13 (20.3) | 23 (17.5) |
| Ofloxacin (5 µg) | 18 (26.8) | 9 (14) | 27 (20.6) | 1 (1.5) | 4 (6.2) | 5(3.8) |
| Ciprofloxacin (5 µg) | 2 (3) | 1 (1.5) | 3 (2.3) | 0 | 0 | 0 |
| Levofloxacin (5 µg) | 1 (1.49) | 1 (1.56) | 2 (1.5) | 0 | 0 | 0 |
| Gatifloxacin (5 µg) | 1 (1.5) | 0 | 1(0.7) | 0 | 0 | 0 |
| Moxifloxacin (5 µg) | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 4)** Minimum inhibitory concentration (MIC) values of 28 fluoroquinolone-resistant *Streptococcus pneumoniae* isolates

| Antibiotic | No. of Intermediate Isolates | | | No. of Resistant Isolates | | |
|---|---|---|---|---|---|---|
| | Normal Flora | Clinical Isolates | Total | Normal Flora | Clinical Isolates | Total |
| Norfloxacin | 1 (out of 10) | 0 (out of 13) | 1 | 9 (out of 10) | 13 (out of 13) | 22 |
| Ofloxacin | 0 (out of 1) | 0 (out of 5) | 0 | 1 (out of 1) | 4 (out of 5) | 5 |

**Table 5)** Distribution of *gyrA*, *gyrB*, *parC*, and *parE* gene mutations among 28 fluoroquinolone-resistant *Streptococcus pneumoniae* isolates

| Isolate ID | *gyrA* | | | Isolate ID | *parC* | | | Isolate ID | Both Genes | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mutation | MIC (µg/mL) | | | Mutation | MIC (µg/mL) | | | Mutation | MIC (µg/mL) | |
| | | Nor | Ofl | | | Nor | Ofl | | | Nor | Ofl |
| 16C | Glu-85-Lys | 8 | 16 | 7C | Ser-81-Leu | 32 | 0 | 88 | Glu-85-Asn (*gyrA*) Ser-81-His (*gyrA*) Asp-83-Thr (*parC*) | 16 | 2 |
| 37C | Glu-85-Pro | 8 | 2 | 26F | Ser-81-Leu | 16 | 2 | 304 | Ser-114-Gly (*gyrA*) Ser-55-Arg (*parC*) | 16 | 4 |
| 348 | Ser-114-Val | 8 | 2 | 70H | Ser-79-Pro Asp-78-Asn | 32 | 0 | | | | |
| P93 | Ser-81-Thr | 16 | 0 | 83F | Asp-83-Gly | 64 | 4 | | | | |
| 60C | Ser-81-His | 64 | 32 | | | | | | | | |

Nor: Norfloxacin; Ofl: Ofloxacin

## Discussion

Although FQs remain highly effective against pneumococcal infections, their widespread use has facilitated the emergence of resistant strains in several regions, highlighting the importance of continuous molecular surveillance [2, 5]. In the present study, resistance was most frequently detected against norfloxacin (17.5%) and ofloxacin (3.8%), two early-generation FQs with limited potency against pneumococci due to preferential targeting of ParC [20]. Consistent with global observations, moxifloxacin with an $MIC_{90}$ value of 0.25 µg/mL displayed the highest activity, confirming its role as the most reliable FQ in pneumococcal therapy [21–23].

Compared to studies conducted in Asia and the Middle East, the resistance rates in this study appear to be lower than those reported in Hong Kong (14.3%) and the Philippines (9.1%); however, increasing resistance to levofloxacin in countries such as Qatar, Kuwait, and Lebanon remains a concern [24, 25]. Data from Iran are limited; however, Kargar et al. (2014) [14] reported higher resistance rates (ciprofloxacin 73.3%, ofloxacin 53.3%, norfloxacin 48.9%, levofloxacin 42.2%), which may reflect regional differences in antibiotic use, sampling sites, and clinical versus commensal origins of isolates. Similar to this study results, another Iranian study also confirmed low resistance to levofloxacin (2%) despite high resistance to several other antibiotics [26].

Interestingly, no ciprofloxacin resistance was observed in the isolates, which contrasts with the findings of Kargar et al. (2014) [14] and Ramakrishnan et al. (2010) [27]. These discrepancies may stem from variations in clinical sources, antibiotic exposure patterns, or local prescribing practices. Notably, 54.2 and 20.6% of the isolates exhibited intermediate resistance to norfloxacin and ofloxacin, respectively. Although these isolates were not fully resistant, they may act as precursors to high-level resistance as previ-

ously reported in the literature [5, 28]. Therefore, vigilance is required to prevent their progression into more resistant clones.

Molecular analysis revealed that the most frequent mutations were located in *parC* (Ser-79, Ser-81, Asp-83), consistent with prior reports linking these sites to reduced FQ susceptibility [28-32]. Mutations in *gyrA*, particularly at Ser-81 and Glu-85, were also detected, with some isolates carrying double mutations (positions 81 and 85) that markedly elevated their MICs for norfloxacin and ofloxacin. These findings corroborate those of Patel et al. (2010) [5], who reported Ser-81 substitutions in *gyrA* as critical determinants of ciprofloxacin resistance. However, studies have shown that resistance levels are not always correlated directly with the number of mutations; for example, some triple mutants may show lower MICs than isolates with single or double substitutions, emphasizing the positional rather than numerical importance of amino acid changes [32].

Of note, 42.8% (12/28) of fluoroquinolone-resistant isolates did not harbor mutations in the QRDRs, suggesting that alternative mechanisms, such as efflux pumps, may contribute to resistance. Previous studies have reported the involvement of the PmrA and PatAB efflux systems in mediating low-level fluoroquinolone resistance and facilitating stepwise QRDR mutations [8, 33, 34]. A limitation of the present study was that no formal sample size or power calculation was performed, as this investigation was designed as an exploratory study based on all available isolates. In addition, isolates were collected from hospitals within a single city, which may limit the generalizability of the findings to other geographical regions. Furthermore, efflux-related assays were not conducted due to resource constraints; however, the preserved isolates allow future investigations to assess efflux pump expression and its potential interplay with mutational resistance.

Taken together, our data show that although pneumococcal resistance to newer FQs such as moxifloxacin and levofloxacin remains relatively low in Iran, the presence of mutations in *parC* and *gyrA*, coupled with intermediate resistance phenotypes, indicates a risk of emerging high-level resistance. Integration of phenotypic MIC results with genotypic data underscores that both mutational and non-mutational pathways must be considered in resistance surveillance. Future work should incorporate efflux pump analysis, larger sample sizes, and correlation with clinical treatment outcomes to provide a more complete picture of fluoroquinolone resistance in *S. pneumoniae*.

## Conclusion

The results of this study show that the overall rate of FQ resistance in pneumococci is still low, and there is no significant correlation between FQ resistance and the source of pneumococcal isolates. However, the frequent mutations and high MIC levels to primary FQs reported here are alarming. Although all isolates were susceptible to moxifloxacin in our study, the sample size (131 isolates) may not fully represent the broader epidemiological situation of *S. pneumoniae* in Iran. Therefore, larger multicenter studies are warranted to draw more generalizable conclusions.

## Acknowledgments

The authors would like to thank all the staff of the Baqiyatallah clinical research laboratory for their kind assistance.

**Ethical permissions:** The present study was approved by the Institutional Review Board (IRB) of Islamic Azad University, Tehran, Iran, under the ethical code IR.IAU.TNB.REC.1396.138. All procedures involving human participants were conducted in

accordance with the ethical standards of the institutional and/or national research committee and the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all participants or their legal guardians, as applicable.

**Authors' contributions:** Study design: AT and AS, data collection and analysis: AT and SD, manuscript preparation: AS and MK.

**Conflicts of Interests:** The authors declare that there is no conflict of interests.

**Funding:** None declared.

**Data availability statement:** All data generated or analyzed during this study are included in this article. Additional information is available from the corresponding author upon reasonable request.

**Consent to participate:** Not applicable.

## References

1. Shi W, Zhou K, Yuan L, Meng Q, Dong F, Gao W, et al. Serotype distribution, antibiotic resistance patterns, and molecular characteristics of serogroup 6 Streptococcus pneumoniae isolates collected from Chinese children before the introduction of PCV13. J Glob Antimicrob Resist. 2018;14:23-8.
2. Sharifi A, Kavoosi F, Hosseini SM, Mosavat A, Ahmadi A. Prevalence of Streptococcus pneumoniae in ventilator-associated pneumonia by real-time PCR. Arch Clin Infect Dis. 2019;14(3):e86416.
3. Fuller J, McGeer A, Low D. Drug-resistant pneumococcal pneumonia: Clinical relevance and approach to management. Eur J Clin Microbiol Infect Dis. 2005;24(12):780-8.
4. Li L, Ma J ,Yu Z, Li M, Zhang W, Sun H. Epidemiological characteristics and antibiotic resistance mechanisms of Streptococcus pneumoniae: An updated review. Microbiol Res. 2022;266:127221.
5. Patel SN, Melano R, McGeer A, Green K, Low DE. Characterization of the quinolone resistant determining regions in clinical isolates of pneumococci collected in Canada. Ann Clin Microbiol Antimicrob. 2010;9(1):1-6.
6. Bast DJ, Low DE, Duncan CL, Kilburn L, Mandell LA, Davidson RJ, et al. Fluoroquinolone resistance in clinical isolates of Streptococcus pneumoniae: Contributions of type II topoisomerase mutations and efflux to levels of resistance. Antimicrob Agents Chemother. 2000;44(11):3049-54.
7. Hawkey PM. Mechanisms of quinolone action and microbial response. J Antimicrob Chemother. 2003;51(Suppl 1):29-35.
8. Ceyssens PJ, Van Bambeke F, Mattheus W, Bertrand S, Fux F, Van Bossuyt E, et al. Molecular analysis of rising fluoroquinolone resistance in Belgian non-invasive Streptococcus pneumoniae isolates (1995-2014). PloS One. 2016;11(5):e0154816.
9. Korzheva N, Davies TA, Goldschmidt R. Novel Ser79Leu and Ser81Ile substitutions in the quinolone resistance-determining regions of ParC topoisomerase IV and GyrA DNA gyrase subunits from recent fluoroquinolone-resistant Streptococcus pneumoniae clinical isolates. Antimicrob Agents Chemother. 2005;49(6):2479-86.
10. Bokaeian M, Khazaei H, Javadimehr M. Nasopharyngeal carriage, antibiotic resistance, and serotype distribution of Streptococcus pneumoniae among healthy adolescents in Zahedan. Iranian Red Crescent Med J. 2011;13(5):328-33.
11. Aligholi M, Emaneini M, Jabalameli F, Shahsavan S, Abdolmaleki Z, Sedaghat H, et al. Antibiotic susceptibility pattern of Gram-positive cocci cultured from patients in three university hospitals in Tehran, Iran during 2001-2005. Acta Med Iran. 2009;47(4):329-34.
12. Sandoval MM, Ruvinsky S, Palermo MC, Alconada T, Brizuela ME, Wierzbicki ER, et al. Antimicrobial resistance of Streptococcus pneumoniae from invasive pneumococcal diseases in Latin American countries: a systematic review and meta-analysis. Front Public Health. 2024;22(12):1337276.
13. Kohanteb J, Sadeghi E. Penicillin-resistant Streptococcus pneumoniae in Iran. Med Princ Pract. 2006;16(1):29-33.
14. Kargar M, Jahromi FM, Doosti A, Handali S. Molecular investigation of quinolone resistance of quinolone resistance-determining region in Streptococcus pneumoniae strains isolated from Iran using polymerase chain reaction–restriction fragment length polymorphism method. Osong Public Health Res Perspect. 2014;5(5):245-50.
15. Mosleh MN, Gharibi M, Alikhani MY, Saidijam M, Vakhshiteh F. Antimicrobial susceptibility and analysis of macrolide resistance genes in Streptococcus pneumoniae isolated in Hamadan. Iran J Basic Med Sci. 2014;17(8):595-9.
16. Azadegan A, Ahmadi A, Lari AR, Talebi M. Detection of the efflux-mediated erythromycin resistance transposon in Streptococcus pneumoniae. Ann Lab Med. 2015;35(1):57-61.
17. Clinical and Laboratory Standards Institute. CLSI supplement M100: Performance standards for antimicrobial susceptibility testing. 34th ed. Wayne PA: Clinical and Laboratory Standards

Institute; 2024.
18. Jacobs MR, Bajaksouzian S, Palavecino-Fasola EL, Holoszyc HM, Appelbaum PC. Determination of penicillin MICs for Streptococcus pneumoniae by using a two-or three-disk diffusion procedure. J Clin Microbiol. 1998;36(1):179-83.
19. Sharew B, Moges F, Yismaw G, Abebe W, Fentaw S, Vestrheim D, et al. Antimicrobial resistance profile and multidrug resistance patterns of Streptococcus pneumoniae isolates from patients suspected of pneumococcal infections in Ethiopia. Ann Clin Microbiol Antimicrob. 2021;20(1):26.
20. Janoir C, Zeller V, Kitzis MD, Moreau NJ, Gutmann L. High-level fluoroquinolone resistance in Streptococcus pneumoniae requires mutations in parC and gyrA. Antimicrob Agents Chemother. 1996;40(12):2760-4.
21. Linares J, Ardanuy C, Pallares R, Fenoll A. Changes in antimicrobial resistance, serotypes, and genotypes in Streptococcus pneumoniae over a 30-year period. Clin Microbiol Infect. 2010;16(5):402-10.
22. Broskey J, Coleman K, Gwynn MN, McCloskey L, Traini C, Voelker L, et al. Efflux and target mutations as quinolone resistance mechanisms in clinical isolates of Streptococcus pneumoniae. J Antimicrob Chemother. 2000;45(Suppl 3):95.9-
23. Fogarty C, Torres A, Choudhri S, Haverstock D, Herrington J, Ambler J. Efficacy of moxifloxacin for treatment of penicillin-, macrolide-, and multidrug-resistant Streptococcus pneumoniae in community-acquired pneumonia. Int J Clin Pract. 2005;59(11):1253-9.
24. Naba MR, Araj GF, Baban TA, Tabbarah ZA, Awar GN, Kanj SS. Emergence of fluoroquinolone-resistant Streptococcus pneumoniae in Lebanon: A report of three cases. J Infect Public Health. 2010;3(3):113-7.
25. Yamamoto K, Yanagihara K, Sugahara K, Imamura Y, Seki M, Izumikawa K, et al. In vitro activity of garenoxacin against Streptococcus pneumoniae mutants with characterized resistance mechanisms. Antimicrob Agents Chemother. 2009;53(8):3572-5.
26. Beheshti M, Jabalameli F, Feizabadi MM, Hahsemi FB, Beigverdi R, Emaneini M. Molecular characterization, antibiotic resistance pattern, and capsular types of invasive Streptococcus pneumoniae isolated from clinical samples in Tehran, Iran. BMC Microbiol. 2020;20(1):1-9.
27. Ramakrishnan R, Ramesh S, Bharathi MJ, Amuthan M, Viswanathan S. Comparative in-vitro efficacy of fluoroquinolones against Streptococcus pneumoniae recovered from bacterial keratitis as determined by E-test. Indian J Pathol Microbiol. 2010;53(2):276-80.
28. Adam HJ, Schurek KN, Nichol KA, Hoban CJ, Baudry TJ, Laing NM, et al. Molecular characterization of increasing fluoroquinolone resistance in Streptococcus pneumoniae isolates in Canada, 1997 to 2005. Antimicrob Agents Chemother. 2007;51(1):198-207.
29. Jorgensen J, Weigel L, Ferraro M, Swenson J, Tenover F. Activities of newer fluoroquinolones against Streptococcus pneumoniae clinical isolates including those with mutations in the gyrA, parC, and parE loci. Antimicrob Agents Chemother. 1999;43(2):329-34.
30. Pan XS, Ambler J, Mehtar S, Fisher LM. Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in Streptococcus pneumoniae. Antimicrob Agents Chemother. 1996;40(10):2321-6.
31. Tankovic J, Perichon B, Duval J, Courvalin P. Contribution of mutations in gyrA and parC genes to fluoroquinolone resistance of mutants of Streptococcus pneumoniae obtained in vivo and in vitro. Antimicrob Agents Chemother. 1996;40(11):2505-10.
32. Weigel L, Anderson G, Facklam R, Tenover F. Genetic analyses of mutations contributing to fluoroquinolone resistance in clinical isolates of Streptococcus pneumoniae. Antimicrob Agents Chemother. 2001;45(12):3517-23.
33. Robertson GT, Doyle TB, Lynch AS. Use of an efflux-deficient Streptococcus pneumoniae strain panel to identify ABC-class multidrug transporters involved in intrinsic resistance to antimicrobial agents. Antimicrob Agents Chemother. 2005;49(11):4781-3.
34. Gill MJ, Brenwald NP, Wise R. Identification of an efflux pump gene, pmrA, associated with fluoroquinolone resistance in Streptococcus pneumoniae. Antimicrob Agents Chemother. 1999;43(1):187-9.
35. González I, Georgiou M, Alcaide F, Balas D, Liñares J, de la Campa AG. Fluoroquinolone resistance mutations in the parC, parE, and gyrA genes of clinical isolates of viridans group streptococci. Antimicrobial Agents and Chemotherapy. 1998;42(11):2792-8.