Biol. Stud. 2026, 20(2), 81–96 • https://doi.org/10.30970/sbi.2002.881
www.https://publications.lnu.edu.ua/journals/index.php/biology

UDC: 575.224:579.253.4:579.86]:577.181.7]:616-092-06

CORRELATION BETWEEN MUTATIONAL PROFILES IN FLUOROQUINOLONE RESISTANCE GENES (*gyrA* AND *grlA*) AND PHENOTYPIC ANTIBIOTIC SUSCEPTIBILITY IN CLINICAL ISOLATES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

Riyam Hasan Tuama [1], ***Lujain Ali Ghannawi*** [1], ***Jihad Anad Khalaf*** [2], ***Safaa Ehssan Atta*** [3], ***Omar Yasir Shakir*** [1], ***Mohammed Amer Thamer*** [1], ***Hanan Ibrahim Abdulwahid*** [1]

[1] *National Diabetes Center, Mustansiriyah University, Al-Qadisiyah, 10011 Baghdad, Iraq*

[2] *College of Medicine, Al-Iraqia University, Al-Adhamia, Baghdad, Iraq*

[3] *Continuing Education Center, Mustansiriyah University, Al-Qadisiyah, 10011 Baghdad, Iraq*

Tuama, R. H., Ghannawi, L. A., Khalaf, J. A., Atta, S. E., Shakir, O. Y., Thamer, M. A., & Abdulwahid, H. I. (2026). Correlation between mutational profiles in fluoroquinolone resistance genes (*gyrA* and *grlA*) and phenotypic antibiotic susceptibility in clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). *Studia Biologica*, 20(2), 81–96. doi:10.30970/sbi.2002.881

Background. Methicillin-resistant *Staphylococcus aureus* (MRSA) is recognized as a highly significant multidrug-resistant pathogen that can lead to severe fetal infections in both humans and animals. Fluoroquinolones (FQs) are considered among the antibiotics of choice used to manage MRSA infections. This study addresses rising fluoroquinolone resistance that limits treatment options. Resistance mechanisms typically involve mutations in the *gyrA* and *grlA* genes, which encode the drug's targets. **Objective:** the aim of this research is to identify the genetic basis for resistance in clinical MRSA isolates. Specifically, the research focused on investigating the distribution of mutations in the *gyrA* and *grlA* genes responsible for fluoroquinolone resistance in MRSA isolates obtained from different clinical sources and assessing the correlation of these mutations with phenotypic antibiotic resistance.

Materials and Methods. Fifty MRSA isolates collected from various clinical specimens (burn, wound, nose, throat, urine, skin, ear, and operating room samples) were used in the study. Bacteriological methods and PCR detection of the *nuc* gene confirmed the identification of the isolates. Then, all of the isolates were tested against seven different antibiotics (methicillin, ciprofloxacin, levofloxacin, norfloxacin, ofloxacin, lomefloxacin, and nalidixic acid) using the disk diffusion method and Minimum Inhibitory

© 2026 Riyam Hasan Tuama *et al*. Published by the Ivan Franko National University of Lviv on behalf of Біологічні Студії / Studia Biologica. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Concentration (MIC) tests. Twelve isolates exhibiting antibiotic resistance were selected for direct sequence analysis of the *gyrA*, *grlA*, and *mecA* gene regions. The relationship between mutations and resistance was analyzed statistically.

Results. All isolates (100 %) carried the *mecA* gene, and no mutations were detected in this gene. The prevalence of fluoroquinolone resistance was 24 % (12 isolates). Sequence analysis revealed mutations at eight different positions in the *gyrA* gene (two missense, one deletion, and five silent) and seven different positions in the *grlA* gene (three missense, one silent, and three insertions). Statistical analysis revealed a significant positive correlation between mutant isolates in the *gyrA* and *grlA* genes and fluoroquinolone resistance ($p < 0.0001$). A significant correlation was also found between nalidixic acid resistance and the presence of mutations ($p < 0.009$).

Conclusion. The findings of this study indicate that a major mechanism of fluoroquinolone resistance in clinical MRSA isolates is the accumulation of mutations in the *gyrA* and *grlA* genes, which encode target enzymes, and that these mutations are strongly associated with high-level phenotypic resistance. It has been confirmed that methicillin resistance Are rely to the presence of the mecA gene and does not require a mutation in the gene itself. These results provide deeper insight into the underlying mechanisms of antimicrobial resistance.

Keywords: MRSA, fluoroquinolone resistance, mutation analysis, sequence analysis, antibiotic resistance

INTRODUCTION

Antibiotic resistance is one of the most urgent global health threats facing modern medicine. According to the World Health Organization (WHO, 2024), antimicrobial resistance is directly responsible for approximately 1.27 million deaths worldwide annually, and this number is expected to increase exponentially in the coming years (WHO, 2024; Hetta *et al.*, 2025). The key contributor to this crisis is *Staphylococcus aureus*, and especially Methicillin-Resistant *S. aureus* (MRSA), which is recognized as the leading cause of both hospital- and community-acquired infections (Yuan *et al.*, 2025; Dhaif *et al.,* 2025; Dakheel *et al.*, 2025).

MRSA has the ability to acquire resistance not only to beta-lactam antibiotics but also to other antibiotic classes, exhibiting a multidrug resistance (MDR) profile (Ali Alghamdi *et al.*, 2023; Atta & Salman, 2020). Among these antibiotics, fluoroquinolones, broad-spectrum synthetic agents, are of critical importance. Fluoroquinolones act by interfering with bacterial DNA replication through inhibition of the key enzymes called DNA gyrase and topoisomerase IV. However, the widespread and sometimes inappropriate use of these antibiotics has led to the selection and spread of resistant bacterial strains (Tang & Zhao, 2023; Collins & Osheroff, 2024).

The predominant and most significant mechanism of fluoroquinolone resistance in *S. aureus* is point mutations in the "quinolone resistance determining regions" (QRDR) of the *gyrA* and *grlA* genes, which encode the A subunits of the target enzymes. Such mutations decrease the ability of antibiotics from binding to the enzyme-DNA complex, thereby promoting resistance (Huynh *et al.*, 2023a; Huynh, 2023b; Kadham *et al.*, 2022). During the development of resistance, mutations initially accumulate in *grlA* (topoisomerase IV), followed by *gyrA* (DNA gyrase). This gradual accumulation of mutations correlates with increasing minimum inhibitory concentration (MIC) values, leading to high-level resistance (Ebrahimi *et al.*, 2025).

Recent molecular epidemiology studies indicate that MRSA isolates from different geographic regions may exhibit different mutational patterns, and these patterns are critical for shaping regional treatment guidelines (Kumar *et al.,* 2021). However, comprehensive studies that systematically characterize the *gyrA* and *grlA* gene mutations linked to fluoroquinolone resistance in clinical MRSA isolates from Baghdad region in Iraq, and statistically correlate these genotypic changes with phenotypic resistance profiles, are limited (Qader *et al.,* 2025).

Accordingly, the present study aims to assess phenotypic resistance profiles to fluoroquinolones in clinical MRSA isolates from the Baghdad region and to identify QRDR mutations in the *gyrA* and *grlA* genes by direct sequence analysis. In addition, the study aimed to statistically evaluate the correlation between the detected genotypic mutation patterns and phenotypic antibiotic susceptibility results (MIC values and susceptibility categories).

It is anticipated that the results of this study will contribute to understanding the regional resistance epidemiology, the development of rapid molecular methods (diagnostic), and the establishment of effective empirical treatment strategies. Furthermore, the obtained data are expected to make a valuable contribution to the global understanding of the molecular mechanisms underlying fluoroquinolone resistance in MRSA and to antimicrobial resistance monitoring efforts.

MATERIALS AND METHODS

Study design. This cross-sectional, experimental study was conducted on clinical samples collected from three different healthcare centers in Baghdad (Al-zafrania Hospital, Al-alwia Children's Hospital, and a private pathological analysis laboratory) between March and June 2019.

Clinical samples and inclusion criteria. A total of 185 clinical samples (burn, wound swabs, nasal swabs, throat swabs, urine, skin swabs, ear swabs, and operating room surface swabs) were included in the study. Inclusion criteria included visibility of Gram-positive cocci on Gram staining, the formation of yellow/golden colonies on Mannitol Salt Agar (MSA), and a positive coagulase test.

Bacterial isolation and identification

Culture and morphological identification. Samples were plated on Mannitol Salt Agar (MSA, Himedia), Blood Agar (BA, Himedia), and Hi-Crome Rapid MRSA Agar Base (Himedia) and incubated at 37 °C for 24–48 h. The morphological criteria were evaluated for *S. aureus* identification, including the formation of yellow colonies due to mannitol fermentation on MSA, Beta-hemolysis on Blood Agar, and the formation of greenish-yellow colonies on Hi-Crome Rapid MRSA Agar.

Biochemical tests. Suspected isolates were confirmed using the following biochemical tests: catalase test (using 3 % H_2O_2), coagulase test (slide and tube method), oxidase test, methyl red test, and Voges–Proskauer test.

Automated identification system. The identification of all isolates was confirmed using a GP card with the VITEK 2 Compact system (Bio-Mérieux, France).

Antibiotic susceptibility tests

Disk diffusion method. The Kirby–Bauer disk diffusion method was performed according to CLSI 2018 criteria. Bacterial suspensions adjusted to a 0.5 McFarland standard were plated on Mueller–Hinton agar (Hi-media). The antibiotic discs (Mast

Diagnostics, UK) were applied, using the following antibiotics [ciprofloxacin (5 µg), levofloxacin (5 µg), lomefloxacin (10 µg), nalidixic acid (30 µg), norfloxacin (30 µg), ofloxacin (5 µg), and methicillin (5 µg)]. After incubating the plates at 37 °C for 24 hours, the diameters of the inhibition zone were measured and classified as "susceptible," "intermediately susceptible," or "resistant" depending on the CLSI criteria.

Minimum inhibitory concentration (MIC) determination. MIC values of 12 isolates exhibiting fluoroquinolone resistance were determined using the agar dilution method. Antibiotic concentration ranges (0.25–512 µg/mL) were prepared depending on the CLSI 2025 guidelines. MIC represents the lowest antibiotic application that gives no bacterial growth (Schuetz *et al.*, 2025).

Molecular analyses

Genomic DNA extraction. Genomic DNA from a total of 50 MRSA isolates was isolated using the Presto™ Mini gDNA Bacteria kit (Geneaid, USA) in line with the manufacturer's instructions. DNA concentration and purity of the DNA were tested and determined using a NanoDrop spectrophotometer (260/280 nm absorbance ratio) (Thermo Scientific, USA).

Polymerase chain reaction (PCR). The following primers (Alpha DNA, USA) were used for *S. aureus* identification and detection of resistance genes:

Table 1. **PCR primer sequences and amplification conditions**

Gene	Primer sequence (5′–3′)	Product size (bp)	Amplification conditions
nuc	F:GCGATTGATGGTGATACGGTT R:GAGCCGAAGTCTTGGGTAAAAAC	276	94 °C for 5 min; 35 cycles of [94 °C for 30 s, 54°C for 30 s, 72 °C for 30 s]; final extension at 72 °C for 10 min
mecA	F:TGCAGTACCGGATTTGCC R:TCGATGGTAAAGGTGGC	525	94 °C for 5 min; 30 cycles of [94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s]; final extension at 72 °C for 10 min
grlA	F:AGGTGATCGCTTTGGAAGA R:CGTCCATTGCGTAAGTTTC	770	94 °C for 5 min; 30 cycles of [94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min]; final extension at 72 °C for 10 min
gyrA	F:GAACAAGGTATGACACCGGA R:AATACGTTGACGTCGCC	660	94 °C for 5 min; 30 cycles of [94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min]; final extension at 72 °C for 10 min

Note: PCR reactions were performed in a final volume of 25 µL using GoTaq® Green Master Mix (Promega, USA)

Gel electrophoresis. PCR products were run on a 2 % agarose gel at a voltage of 70 V (constant voltage) and for 60–70 min. Gel visualization was performed using ethidium bromide staining under a UV transilluminator (Flowgen, UK).

DNA sequence analysis and mutation detection. PCR products of the *gyrA*, *grlA*, and *mecA* genes from 12 isolates exhibiting fluoroquinolone resistance were sent to Macrogen Inc. (South Korea) for sequence analysis using forward primers. Raw sequence data were aligned using BioEdit v7.2.6 software and compared with

the sequences of the reference *S. aureus* strain RF122 in NCBI GenBank. Amino acid substitutions were determined using MEGA7 software.

Statistical analysis. SPSS (version 25) was used to analyze the data we collected. Categorical variables were analyzed using the Chi-square test, as the expected cell counts were ≥ 5. Spearman's rank correlation coefficient was used to assess correlations between variables. Any result in which the probability of error was less than 5 % (p-value < 0.05) was considered statistically significant. The statistical findings were interpreted with caution in view of the relatively small sample size.

RESULTS

Bacteriological identification and isolate distribution. Of the 185 clinical samples, 50 (27 %) were identified as *S. aureus*. The distribution of isolates by clinical source is shown in **Table 2**.

All isolates showed mannitol fermentation on MSA, beta-hemolysis on Blood Agar, and characteristic colony morphology on Hi-Crome Rapid MRSA Agar. In biochemical tests, all isolates were positive for catalase, coagulase, methyl red, and Voges–Proskauer tests, and negative for oxidase tests.

Table 2. **Distribution of *Staphylococcus aureus* isolates by clinical source**

Clinical source	Sample number	Number of isolates	Percentage (%)
Throat	23	11	22
Ear	25	10	20
Nose	21	8	16
Burn	23	5	10
Operating room	20	5	10
Urine	18	4	8
Wound	27	4	8
Skin	28	3	6
Total	185	50	100

Antibiotic susceptibility profiles

Antibiotic susceptibility profiles determined by the disk diffusion method are summarized in **Table 3**.

Table 3. **Antibiotic susceptibility test results of *Staphylococcus aureus* isolates**

Antibiotic	Susceptible (%)	Intermediate Susceptible (%)	Resistant (%)
Methicillin	0 (0)	0 (0)	50 (100)
Nalidixic acid	20 (40)	2 (4)	28 (56)
Ciprofloxacin	38 (76)	0 (0)	12 (24)
Levofloxacin	38 (76)	0 (0)	12 (24)
Norfloxacin	38 (76)	0 (0)	12 (24)
Ofloxacin	38 (76)	0 (0)	12 (24)
Lomefloxacin	34 (68)	4 (8)	12 (24)

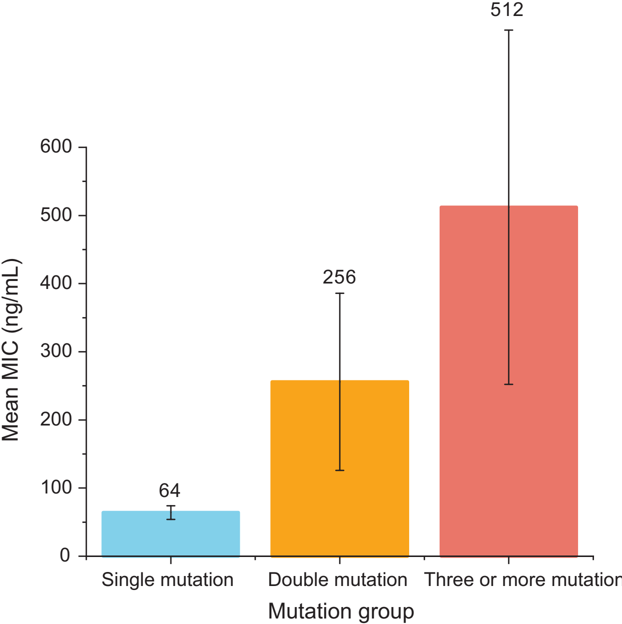
All isolates (100 %) were identified as methicillin-resistant (MRSA). The resistance rate to fluoroquinolone antibiotics was found to be 24 % (12 isolates).

Minimum inhibitory concentration (MIC) results. The MIC values of the 12 isolates showing fluoroquinolone resistance are shown in **Table 4**. Also, see **Figure.**

Table 4. **Minimum inhibitory concentrations (MICs) of fluoroquinolones (μg/mL) against *Staphylococcus aureus* isolates**

Isolate No.	Source	CIP	LEV	NOR	OFX	LOM	NA
1	Burn	0.5	16	16	32	128	64
4	Burn	2	32	32	64	512	512
9	Ear	1	32	32	128	256	512
16	Nose	1	32	32	32	256	512
17	Nose	2	32	32	32	512	512
24	Operating room	1	32	32	64	512	512
29	Skin	2	32	32	64	512	512
32	Throat	1	16	64	32	64	1024
42	Throat	2	32	32	32	512	512
43	Urine	2	32	64	64	128	32
47	Wound	1	32	16	32	32	128
50	Wound	1	16	8	32	1024	1024

Note: CIP – ciprofloxacin; LEV – levofloxacin; NOR – norfloxacin; OFX – ofloxacin; LOM – lomefloxacin; NA – nalidixic acid



Mean MIC values of fluoroquinolones according to the number of *gyrA* and *grlA* mutations in MRSA isolates. Error bars represent standard deviation (SD)

The statistical analysis for MIC distribution revealed a clear gradient in resistance levels among the tested fluoroquinolones. The highest mean MIC was observed for nalidixic acid (488 ± 302.21 μg/mL), followed by lomefloxacin (370.67 ± 270.73 μg/mL), ofloxacin (50.67 ± 27.58 μg/mL), norfloxacin (32.67 ± 16.15 μg/mL), and levofloxacin (28 ± 6.93 μg/mL), while ciprofloxacin showed the lowest mean MIC (1.38 ± 0.54 μg/mL). These findings indicate a marked variability in resistance intensity among the fluoroquinolone group, with particularly elevated MIC values for lomefloxacin and nalidixic acid. Several isolates demonstrated extremely high MIC values (up to 1024 μg/mL), indicating high-level resistance.

Molecular identification and detection of resistance genes. PCR analysis of all isolates confirmed the identification of *S. aureus* at the molecular level, with the *nuc* gene (276 bp) positive. Furthermore, all isolates were genotypically confirmed as MRSA phenotypes, with the *mecA* gene (525 bp) positive.

The *gyrA* (660 bp) and *grlA* (770 bp) genes were amplified by PCR in all 12 isolates exhibiting fluoroquinolone resistance. In this study, these PCR products represent diagnostic fragments rather than the full-length genes.

DNA sequence analysis and mutation profiles

***gyrA* gene mutations.** Silent mutations were recorded for descriptive purposes and excluded from the association analysis, as they do not alter amino acid sequences and are unlikely to influence fluoroquinolone resistance. A total of eight different mutations were identified in the QRDR region of the *gyrA* gene (**Table 5**).

Table 5. **Detected mutations in the *gyrA* gene of *Staphylococcus aureus* isolates**

Nucleotide position	Codon change	Amino acid change	Mutation type	Number of isolates
329	TTT → TCT	Phe110 → Ser	Missense	1
355	GCA → TCA	Ala119 → Ser	Missense	1
355	GCA → –	Ala119 → Deletion	Deletion	1
396	ACA → ACT	Thr132 → Thr	Silent	12
501	TTA → TTG	Leu167 → Leu	Silent	12
633	GAA → GAG	Glu211 → Glu	Silent	12
657	CCA → CCT	Pro219 → Pro	Silent	12
696	CGC → CGT	Arg232 → Arg	Silent	12

***grlA* gene mutations.** A total of seven different mutations were identified in the QRDR region of the *grlA* gene (**Table 6**).

***mecA* gene sequence analysis.** No mutations were detected in the *mecA* gene sequence analysis of all isolates.

Statistical analysis results. The relationship between the presence of mutations and antibiotic resistance was statistically evaluated (**Table 7**).

A statistically significant, strong positive correlation was found between fluoroquinolone resistance and the presence of the *gyrA*/*grlA* mutation (Spearman's r = 0.896,

p < 0.0001). Other resistance mechanisms, such as efflux pump overexpression, were not investigated in this study, and therefore their possible contribution to fluoroquinolone resistance cannot be excluded.

Table 6. **Detected mutations in the *grlA* gene of *Staphylococcus aureus* isolates**

Nucleotide position	Codon change	Amino acid change	Mutation type	Number of isolates
108	– → GAA	X36 → Glu	Insertion	1
115	– → TAA	Stop39 → Stop	Insertion	1
122	CCG → CAG	Pro41 → Gln	Missense	12
173	– → AGT	X58 → Ser	Insertion	1
242	TCC → TTC	Ser81 → Phe	Missense	1
597	CCA → CCG	Pro199 → Pro	Silent	12
670	ATT → GTT	Ile224 → Val	Missense	3

Table 7. **Mutation and antibiotic resistance correlation analysis**

Antibiotic group	Statistical test	p-value	Correlation coefficient (r)
Fluoroquinolones*	Pearson Chi-square	< 0.0001	–
Fluoroquinolones*	Spearman correlation	< 0.0001	0.896
Nalidixic acid	Pearson Chi-square	0.009	–
Nalidixic acid	Spearman correlation	0.017	-0.337
Lomefloxacin	Pearson Chi-square	< 0.0001	–
Lomefloxacin	Spearman correlation	< 0.0001	0.663

Note: Fluoroquinolones include ciprofloxacin, levofloxacin, norfloxacin, and ofloxacin. * – Chi-square test was used for comparison

Distribution of MIC values according to mutation profiles. Statistical analysis was performed using one-way ANOVA to compare the mean MIC values among the groups. The results are summarized in **Table 8**.

Table 8. **Distribution of minimum inhibitory concentration (MIC) values of fluoroquinolones according to the number of the *gyrA* and *grlA* mutations in MRSA isolates**

Mutation group	n	Mean MIC (µg/ML)	SD	Range (µg/ML)
Single mutation	4	64	8	16–128
Double mutation	5	256	128	32–512
Three or more mutations	3	512	256	128–1024

We measured MICs using the agar dilution method following CLSI 2025 guidelines. The highest MIC observed was 1024 μg/mL, markedly exceeding the CLSI cutoff for resistance in *S. aureus* (≤1 μg/mL is considered susceptible). Very few studies have reported such high MICs before, which suggests that multiple mutations can substantially enhance resistance levels. Statistical analysis using one-way ANOVA showed a significant difference in MIC values between the groups ($p < 0.05$), indicating that the accumulation of mutations is associated with higher levels of fluoroquinolone resistance. Post-hoc analysis (Tukey's test) confirmed that each increase in the number of mutations significantly elevated MIC values compared to the previous group ($p < 0.05$). Thus, isolates that have multiple mutations in *gyrA* and *grlA* genes showed the highest MIC values, confirming the "stepwise accumulation" model of resistance development.

DISCUSSION

Amid the global crisis, Methicillin-Resistant *Staphylococcus aureus* (MRSA) stands out as a particularly concerning pathogen (Ahmed *et al*., 2024). It continues to be a significant cause of morbidity and mortality in both hospital- and community-acquired infections (Kumar *et al*., 2025; Li *et al*., 2025). Only fluoroquinolone-resistant isolates were sequenced; therefore, mutation specificity could not be assessed.

The most notable result in this study is the identification of the Pro41 → Gln missense mutation in the *grlA* gene across all (100 %) fluoroquinolone-resistant MRSA isolates collected from Baghdad region. This observation differs from mutation patterns that have been commonly described in the global literature. According to a comprehensive analysis by N. A. Turner *et al*., the most common mutations in the *grlA* gene are traditionally Ser80 → Phe and Glu84 → Lys (Turner *et al*., 2019). Similarly, a multicenter study by D. J. Diekema *et al*. and depending on data from the SENTRY Antimicrobial Surveillance Program confirms that these classic mutations are the dominant resistance mechanism in many regions of North America, Europe, and Asia (Diekema *et al.,* 2019).

Apart from regional differences, some of our results are similar to those around the world. Mutations in both *gyrA* and *grlA* have been detected in resistant isolates, a well-known mechanism for high fluoroquinolone resistance. We also found that the MIC values increased with the number of mutations, a pattern reported in several previous studies. This confirms the important role of QRDR mutations in MRSA, which is resistant to fluoroquinolone.

Several factors may explain this regional variation, including the potential dissemination of a dominant MRSA clone harboring Pro41 → Gln mutation. However, no molecular typing (MLST or spa typing) was performed to confirm this hypothesis. A genomic study by S. Baker *et al.*, published in Science, demonstrated that specific antibiotic resistance mutations can become fixed within successful bacterial clones and spread geographically (Roer *et al*., 2025; Abebe & Birhanu, 2023; Baker *et al*., 2018). Furthermore, the varying selective pressures created by regional fluoroquinolone use patterns may have also played a role in the selection of this mutation. At the molecular level, the proximity of Pro41 position to the classical quinolone resistance-determining region (QRDR) suggests that this mutation may play a critical role in the stability of the enzyme-DNA-quinolone ternary complex (Ostrer *et al*., 2018; Tang & Zhao, 2023).

Another important finding of our study is that it provides data that quantitatively confirms the "gradual development of resistance" hypothesis in clinical isolates (Kaul *et al*., 2025). The statistically significant differences in minimum inhibitory concentration (MIC) values observed between isolates carrying single, double, and multiple mutations clearly demonstrate that each new mutation confers higher levels of resistance to the bacteria. Modern studies revealed that simultaneous mutations in DNA gyrase and topoisomerase IV cause an exponential decrease in drug binding affinities, resulting in high MIC values (Collins & Osheroff 2024; Li *et al.,* 2022). Specifically, isolates carrying mutations in both *grlA* and *gyrA* genes reached MIC values up to 512 µg/mL, indicating that the combination of these two mutations creates a particularly effective resistance profile.

The strong positive correlation observed between resistance to ciprofloxacin, levofloxacin, norfloxacin, and ofloxacin also the mutations in *gyrA/grlA* genes (Spearman r = 0.896) indicates that these genetic alterations play a major role in determining resistance pattern (Fu *et al.*, 2013). This finding indicates that other resistance mechanisms, such as efflux pumps and permeability changes, play a secondary role for these particular antibiotics (Mlynarczyk-Bonikowska *et al*., 2022). This strong correlation carries significant implications for clinical microbiology and infectious disease management. A recent study demonstrated that predicting resistance based on rapid molecular testing can improve clinical outcomes and optimize antibiotic management compared to the treatment based on traditional culture and susceptibility testing results (Banerjee *et al*., 2015; Kim *et al*., 2022).

In contrast, the weak negative correlation between nalidixic acid resistance and the presence of the QRDR mutation is likely due to the pharmacological properties of this drug. Recent studies indicated that nalidixic acid binds to target enzymes with lower affinity than modern fluoroquinolones and is a better substrate for efflux pumps such as *NorA* (de Morais Oliveira-Tintino *et al.,* 2023; Mahey *et al*., 2021). These pharmacokinetic differences clearly demonstrate that nalidixic acid resistance alone cannot be used as a reliable marker for modern fluoroquinolone resistance.

Our study contributes significantly to the molecular epidemiology of fluoroquinolone resistance in the MRSA population in the Baghdad region. The dominant presence of the Pro41 → Gln mutation highlights the geographical heterogeneity in global resistance patterns. In contrast, the gradual relationship between mutation accumulation and MIC values reveals the dynamic nature of resistance development (Windels *et al.,* 2019). The strong genotype-phenotype correlation supports the clinical applicability of molecular diagnostic methods. These findings provide valuable data for developing personalized treatment strategies and regional surveillance programs to combat antimicrobial resistance, elucidating the way in which bacteria change their composition in order to neutralize the effect of antibiotics.

CONCLUSION

This comprehensive study provides insight into the molecular basis of fluoroquinolone resistance in clinical MRSA isolates from the Baghdad region. Our study demonstrated that the Pro41 → Gln mutation in the grlA gene is a major contributor, with 100 % prevalence, in fluoroquinolone-resistant MRSA isolates from the region. This suggests

a possible region-specific resistance pattern distinct from mutations more frequently reported in the literature. A direct correlation was found between mutation accumulation and MIC values, with the mean MIC values being 64 µg/mL for isolates carrying a single mutation, rising to 256 µg/mL in those carrying double mutations, and up to 512 µg/mL in those carrying three (or more) mutations. Furthermore, the strong positive correlation (r = 0.896) between resistance to ciprofloxacin, levofloxacin, norfloxacin, and ofloxacin and the presence of the *gyrA*/*grlA* mutation indicates that molecular diagnostic methods may serve as a reliable tool for predicting fluoroquinolone resistance in our region. Our study demonstrates that fluoroquinolone resistance is a significant public health problem in the Baghdad region and that the rational use of this antibiotic group should be carefully reconsidered. The prevalence of the Pro41 → Gln mutation, in particular, highlights the potential importance of considering this mutation in regional resistance surveillance programs and rapid diagnostic tests.

ACKNOWLEDGEMENTS

We extend our sincere gratitude to all individuals and institutions whose collaboration was vital for this study. We are deeply thankful to the administration and technical staff of Al-zafrania Hospital, Al-alwia Children's Hospital, and the Private Pathological Analysis Laboratory in Baghdad for their crucial support during the collection of clinical samples.

CONFLICT OF INTEREST

The researchers emphasize that there is no conflict of interest regarding the conduct or publication of this research.

FUNDING

The study confirms that no external financial support was received, as researchers covered all research costs at their own expense.

COMPLIANCE WITH ETHICAL STANDARDS

Human Rights: The research adhered to the Bioethics Protocol when collecting samples from Zafaraniya Hospital, Al-Alawi Children's Hospital, and private pathological analysis laboratories; where patients' privacy was ensured and their prior consent was obtained in full compliance with ethical standards. All samples were collected after obtaining the necessary approvals, following strict guidelines in handling and processing. The protocol also focused on privacy, safety, and transparency throughout the research period, while maintaining professional responsibility in laboratory practices. Furthermore, all procedures involving human participants were subject to the standards of institutional and national research committees, and were in accordance with the 1964 Helsinki Declaration and its subsequent amendments, or equivalent ethically approved standards.

AUTHOR CONTRIBUTIONS

Conceptualization, [R.H.T.]; methodology, [L.A.G.]; validation, [S.E.A.]; formal analysis, [J.A.K.].; investigation, [H.I.A.; S.E.A]; resources, [M.A.T.]; data curation, [L.A.G.];

writing – original draft preparation, [L.A.G.]; writing – review and editing, [H.I.A.]; visualization, [M.A.T.; O.Y.S.] supervision, [S.E.A]; project administration, [R.H.T.].

All authors have read the final version of the research and agreed to publish it.

REFERENCES

Abebe, A. A., & Birhanu, A. G. (2023). Methicillin resistant *Staphylococcus aureus*: molecular mechanisms underlying drug resistance development and novel strategies to combat. *Infection and Drug Resistance,* 16, 7641–7662. doi:10.2147/idr.s428103
Crossref • PubMed • PMC • Google Scholar

Ahmed, S. K., Hussein, S., Qurbani, K., Ibrahim, R. H., Fareeq, A., Mahmood, K. A., & Mohamed, M. G. (2024). Antimicrobial resistance: impacts, challenges, and future prospects. *Journal of Medicine, Surgery, and Public Health*, 2, 100081. doi:10.1016/j.glmedi.2024.100081
Crossref • Google Scholar

Ali Alghamdi, B., Al-Johani, I., Al-Shamrani, J. M., Musamed Alshamrani, H., Al-Otaibi, B. G., Almazmomi, K., & Yusnoraini Yusof, N. (2023). Antimicrobial resistance in methicillin-resistant *Staphylococcus aureus*. *Saudi Journal of Biological Sciences*, 30(4), 103604. doi:10.1016/j.sjbs.2023.103604
Crossref • PubMed • PMC • Google Scholar

Atta, S. E., & Salman, E. D. (2020). Molecular study of fluoroquinolones resistance *Staphylococcus aureus* isolated from different clinical sources. *International Journal of Pharmaceutical Research*, 12(3), 814–820. doi:10.31838/ijpr/2020.12.03.118
Crossref • Google Scholar

Baker, S., Thomson, N., Weill, F.-X., & Holt, K. E. (2018). Genomic insights into the emergence and spread of antimicrobial-resistant bacterial pathogens. *Science*, 360(6390), 733–738. doi:10.1126/science.aar3777
Crossref • PubMed • PMC • Google Scholar

Banerjee, R., Teng, C. B., Cunningham, S. A., Ihde, S. M., Steckelberg, J. M., Moriarty, J. P., Shah, N. D., Mandrekar, J. N., & Patel, R. (2015). Randomized trial of rapid multiplex polymerase chain reaction-based blood culture identification and susceptibility testing. *Clinical Infectious Diseases*, 61(7), 1071–1080. doi:10.1093/cid/civ447
Crossref • PubMed • PMC • Google Scholar

Collins, J. A., & Osheroff, N. (2024). Gyrase and topoisomerase IV: recycling old targets for new antibacterials to combat fluoroquinolone resistance. *ACS Infectious Diseases*, 10(4), 1097–1115. doi:10.1021/acsinfecdis.4c00128
Crossref • PubMed • PMC • Google Scholar

Dakheel, K. H., Rahim, R. A., Al-Obaidi, J. R., Razali, N., Neela, V. K., Hun, T. G., & Yusoff, K. (2025). Proteomic analysis reveals phage-driven metabolic shifts and biofilm disruption in methicillin-resistant *Staphylococcus aureus* (MRSA). *World Journal of Microbiology and Biotechnology*, 41(7), 230. doi:10.1007/s11274-025-04397-5
Crossref • PubMed • PMC • Google Scholar

de Morais Oliveira-Tintino, C. D., Muniz, D. F., dos Santos Barbosa, C. R., Silva Pereira, R. L., Begnini, I. M., Rebelo, R. A., ... & da Silva, T. G. (2023). NorA, Tet(K), MepA, and MsrA efflux pumps in *Staphylococcus aureus*, their Inhibitors and 1,8-naphthyridine sulfonamides. *Current Pharmaceutical Design*, 29(5), 323–355. doi:10.2174/1381612829666221212101501
Crossref • PubMed • Google Scholar

Diekema, D. J., Hsueh, P.-R., Mendes, R. E., Pfaller, M. A., Rolston, K. V., Sader, H. S., & Jones, R. N. (2019). The microbiology of bloodstream Infection: 20-year trends from the SENTRY antimicrobial surveillance program. *Antimicrobial Agents and Chemotherapy*, 63(7). doi:10.1128/aac.00355-19
Crossref • PubMed • PMC • Google Scholar

Ebrahimi, E., Hadi, Z., Farsioo, S., Hasani, B., Badmasti, F., Beig, M., & Sholeh, M. (2025). Global genomic and antimicrobial resistance profiling of *Neisseria gonorrhoeae*: insights from whole genome sequencing and minimum inhibitory concentration analysis. *PLOS Neglected Tropical Diseases*, 19(10), e0013505. doi:10.1371/journal.pntd.0013505
Crossref • PubMed • PMC • Google Scholar

Fu, Y., Zhang, W., Wang, H., Zhao, S., Chen, Y., Meng, F., Zhang, Y., Xu, H., Chen, X., & Zhang, F. (2013). Specific patterns of *gyr* A mutations determine the resistance difference to ciprofloxacin and levofloxacin in *Klebsiella pneumoniae* and *Escherichia coli*. *BMC Infectious Diseases*, 13(1). 8. doi:10.1186/1471-2334-13-8
Crossref • PubMed • PMC • Google Scholar

Hetta, H. F., Ramadan, Y. N., & Al-Kadmy, I. M. S. (2025). Editorial for special issue “antibiotic combination therapy: a strategy to overcome bacterial resistance”. *Biomedicines*, 13(1), 129. doi:10.3390/biomedicines13010129
Crossref • PubMed • PMC • Google Scholar

Huynh, T. Q., Tran, V. N., Thai, V. C., Nguyen, H. A., Nguyen, N. T. G., Surian, N. U., Chen, S., & Nguyen, T. T. H. (2023a). Analyzing genomic alterations involved in fluoroquinolone-resistant development in *Staphylococcus aureus*. *bioRxiv*, 2023-02. doi:10.1101/2023.02.26.530158
Crossref • Google Scholar

Huynh, T. Q., Tran, V. N., Thai, V. C., Nguyen, H. A., Nguyen, N. T. G., Tran, M. K., ... & Nguyen, T. T. H. (2023b). Genomic alterations involved in fluoroquinolone resistance development in *Staphylococcus aureus*. *PLoS One*, 18(7), e0287973. doi:10.1371/journal.pone.0287973
Crossref • PubMed • PMC • Google Scholar

Kadham, Z. A. A.-K. (2022). Detection of *gyrA* and *parC* genes in clinical *Acinetobacter baumannii* isolates. *Al-Mustansiriyah Journal of Science*, 33(4), 57–62. doi:10.23851/mjs.v33i4.1188
Crossref • Google Scholar

Kaul, A., Souque, C., Holland, M., & Baym, M. (2025). Genomic resistance in historical clinical isolates increased in frequency and mobility after the age of antibiotics. *Microbial Genomics*, 11(9), 001474. doi:10.1099/mgen.0.001474
Crossref • PubMed • PMC • Google Scholar

Kim, J. I., Maguire, F., Tsang, K. K., Gouliouris, T., Peacock, S. J., McAllister, T. A., McArthur, A. G., & Beiko, R. G. (2022). Machine learning for antimicrobial resistance prediction: current practice, limitations, and clinical perspective. *Clinical Microbiology Reviews*, 35(3), e0017921. doi:10.1128/cmr.00179-21
Crossref • PubMed • PMC • Google Scholar

Kumar, S., Anwer, R., Yadav, M., Sehrawat, N., Singh, M., & Kumar, V. (2021). Molecular typing and global epidemiology of *Staphylococcus aureus*. *Current Pharmacology Reports*, 7(5), 179–186. doi:10.1007/s40495-021-00264-7
Crossref • Google Scholar

Kumar, S., Mahato, R. P., Ch, S., & Kumbham, S. (2025). Current strategies against multidrug-resistant *Staphylococcus aureus* and advances toward future therapy. *The Microbe*, 6, 100281. doi:10.1016/j.microb.2025.100281
Crossref • Google Scholar

Li, J., Wei, Y., Wang, J., Li, Y., Shao, G., Feng, Z., & Xiong, Q. (2022). Characterization of mutations in DNA gyrase and topoisomerase IV in field strains and *in vitro* selected quinolone-resistant *Mycoplasma hyorhinis* mutants. *Antibiotics*, 11(4), 494. doi:10.3390/antibiotics11040494
Crossref • PubMed • PMC • Google Scholar

Li, J., Cheng, F., Wei, X., Bai, Y., Wang, Q., Li, B., Zhou, Y., Zhai, B., Zhou, X., Wang, W., & Zhang, J. (2025). Methicillin-resistant *Staphylococcus aureus* (MRSA): resistance, prevalence, and coping strategies. *Antibiotics*, 14(8), 771. doi:10.3390/antibiotics14080771
Crossref • PubMed • PMC • Google Scholar

Mahey, N., Tambat, R., Chandal, N., Verma, D. K., Thakur, K. G., & Nandanwar, H. (2021). Repurposing approved drugs as fluoroquinolone potentiators to overcome efflux pump resistance in *Staphylococcus aureus*. *Microbiology Spectrum*, 9(3), e00951-21. doi:10.1128/spectrum.00951-21
Crossref ● PubMed ● PMC ● Google Scholar

Mlynarczyk-Bonikowska, B., Kowalewski, C., Krolak-Ulinska, A., & Marusza, W. (2022). Molecular mechanisms of drug resistance in *Staphylococcus aureus*. *International Journal of Molecular Sciences*, 23(15), 8088. doi:10.3390/ijms23158088
Crossref ● PubMed ● PMC ● Google Scholar

Ostrer, L., Khodursky, R. F., Johnson, J. R., Hiasa, H., & Khodursky, A. (2019). Analysis of mutational patterns in quinolone resistance-determining regions of GyrA and ParC of clinical isolates. *International Journal of Antimicrobial Agents*, 53(3), 318–324. doi:10.1016/j.ijantimicag.2018.12.004
Crossref ● PubMed ● Google Scholar

Qader, T. A., Ali, M. R., & Alsakini, A. H. (2025). Genomic investigation and biofilm characterization of methicillin-resistant *Staphylococcus aureus* in Baghdad province. *Iraqi Journal of Medical Sciences*, 23(1), 75–82. doi:10.22578/ijms.23.1.9
Crossref ● Google Scholar

Roer, L., Yin, N., Denis, O., Vendrik, K. E., Zwittink, R. D., Notermans, D. W., ... & Petersen, A. (2025). Spread of the FAR-MRSA clone, a fusidic acid- and meticillin-resistant *Staphylococcus aureus* ST121, Europe, 2014 to 2024. *Eurosurveillance*, 30(28), 2500452. doi:10.2807/1560-7917.es.2025.30.28.2500452
Crossref ● Google Scholar

Schuetz, A. N., Ferrell, A., Hindler, J. A., Humphries, R., & Bobenchik, A. M. (2025). Overview of changes in the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing: M100 32nd and 33rd editions. *Journal of Clinical Microbiology*, 63(9), e0162323. doi:10.1128/jcm.01623-23
Crossref ● PubMed ● PMC ● Google Scholar

Tang, K., & Zhao, H. (2023). Quinolone antibiotics: resistance and therapy. *Infection and Drug Resistance,* 16, 811–820. doi:10.2147/idr.s401663
Crossref ● PubMed ● PMC ● Google Scholar

Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., Eichenberger, E. M., Shah, P. P., Carugati, M., Holland, T. L., & Fowler, V. G. (2019). Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. *Nature Reviews Microbiology*, 17(4), 203–218. doi:10.1038/s41579-018-0147-4
Crossref ● PubMed ● PMC ● Google Scholar

Windels, E. M., Michiels, J. E., Fauvart, M., Wenseleers, T., Van den Bergh, B., & Michiels, J. (2019). Bacterial persistence promotes the evolution of antibiotic resistance by increasing survival and mutation rates. *The ISME Journal*, 13(5), 1239–1251. doi:10.1038/s41396-019-0344-9
Crossref ● PubMed ● PMC ● Google Scholar

World Health Organization (WHO). (2025). *Global research agenda for antimicrobial resistance in human health*. World Health Organization. https://www.who.int/publications/i/item/9789240102309

Yuan, H., Xu, J., Wang, Y., Li, Y., Hao, Y., Long, J., Liu, F., Zhu, J., & Yang, H. (2025). The global antimicrobial resistance trends of *Staphylococcus aureus* and influencing factors. *Microbiology Research*, 16(6), 118. doi:10.3390/microbiolres16060118
Crossref ● Google Scholar

КОРЕЛЯЦІЯ МІЖ МУТАЦІЙНИМИ ПРОФІЛЯМИ У ГЕНАХ РЕЗИСТЕНТНОСТІ ДО ФТОРХІНОЛОНІВ (*gyrA* ТА *grlA*) ТА ФЕНОТИПОВОЮ ЧУТЛИВІСТЮ ДО АНТИБІОТИКІВ У КЛІНІЧНИХ ІЗОЛЯТАХ МЕТИЦИЛІН-РЕЗИСТЕНТНОГО *STAPHYLOCOCCUS AUREUS* (MRSA)

***Riyam Hasan Tuama*[1], *Lujain Ali Ghannawi*[1], *Jihad Anad Khalaf*[2], *Safaa Ehssan Atta*[3], *Omar Yasir Shakir*[1], *Mohammed Amer Thamer*[1], *Hanan Ibrahim Abdulwahid*[1]**

[1] *Національний центр дослідження діабету Університет Аль-Мустансирія, Аль-Кадісія, 10011, Багдад, Ірак*

[2] *Медичний коледж, Університет Аль-Іракія, Аль-Адхамія, Багдад, Ірак*

[3] *Центр післядипломної освіти Університет Аль-Мустансирія, Аль-Кадісія, 10011, Багдад, Ірак*

Обґрунтування. Метицилін-резистентний *Staphylococcus aureus* (MRSA) є критично важливим мультирезистентним патогеном, здатним спричиняти небезпечні для життя захворювання у людей і тварин. Фторхінолони (FQ) вважаються одними з препаратів вибору для лікування інфекцій, спричинених MRSA. Зростання резистентності до фторхінолонів обмежує можливості терапії. Механізми резистентності зазвичай пов'язані з мутаціями в генах *gyrA* та *grlA*, які кодують мішені дії цих препаратів. Метою дослідження було визначити генетичні основи резистентності у клінічних ізолятах MRSA, зокрема, дослідити розподіл мутацій у генах *gyrA* та *grlA*, відповідальних за резистентність до фторхінолонів, у ізолятах *Staphylococcus aureus*, резистентних до метициліну, отриманих із різних клінічних джерел, а також оцінити кореляцію цих мутацій із фенотиповою антибіотикорезистентністю.

Матеріали та методи. У дослідженні використано 50 ізолятів MRSA, отриманих із різних клінічних зразків (мазки з опіків, ран, носа, горла, шкіри, вуха, а також зі сечі та змивів з поверхонь операційної). Ідентифікацію ізолятів підтверджували за допомогою бактеріологічних методів і ПЛР-детекції гена *nuc*. Усі ізоляти тестували на чутливість до семи антибіотиків (метицилін, ципрофлоксацин, левофлоксацин, норфлоксацин, офлоксацин, ломефлоксацин і налідиксова кислота) методом дифузії в агарі та визначенням мінімальної інгібуючої концентрації (МІК). Дванадцять резистентних ізолятів було відібрано для прямого секвенування ділянок генів *gyrA*, *grlA* та *mecA*. Зв'язок між мутаціями та резистентністю аналізували статистично.

Результати. Усі ізоляти (100 %) містили ген *mecA*, однак мутацій у цьому гені не виявлено. Рівень резистентності до фторхінолонів становив 24 % (12 ізолятів). За результатами секвенування встановлено наявність мутацій у восьми позиціях гена *gyrA* (дві міссенс-мутації, одна делеція та п'ять "тихих") і у семи позиціях гена *grlA* (три міссенс-мутації, одна "тиха" і три інсерції). Статистичний аналіз виявив достовірну позитивну кореляцію між наявністю мутацій у генах *gyrA* та *grlA* і резистентністю до фторхінолонів ($p < 0,0001$). Також встановлено достовірний зв'язок між резистентністю до налідиксової кислоти і наявністю мутацій ($p < 0,009$).

Висновки. Результати дослідження свідчать, що основним механізмом резистентності до фторхінолонів у клінічних ізолятах MRSA є накопичення мутацій у генах *gyrA* та *grlA*, які кодують цільові ферменти. Цікаво, що ці мутації тісно пов'язані

з високим рівнем фенотипової резистентності. Підтверджено, що резистентність до метициліну зумовлена наявністю гена *mecA* і не потребує мутацій у самому гені. Отримані результати сприяють кращому розумінню механізмів резистентності та мають важливе значення для регіональних програм моніторингу.

Ключові слова: MRSA, резистентність до фторхінолонів, аналіз мутацій, секвенування, антибіотикорезистентність

Received / Одержано	Revision / Доопрацьовано	Accepted / Прийнято	Published / Опубліковано
22 October, 2025	02 March, 2026	22 June, 2026	25 June, 2026