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Research Article

Molecular Investigation of *gyrA* Mutations in Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* Derived from Diverse Sources

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Abstract

Background: Fluoroquinolones are the most effective antibiotics against *Staphylococcus aureus* isolates. In hospitals, excessive use of antibiotics has led to the emergence of highly resistant strains of *S. aureus* isolates. ***Objective***: The aim of this study was to detect the mutations that occur in the *gyrA* gene encoding for DNA gyrase, which is one of the targets for fluoroquinolone resistance. ***Methods***: Fifty clinical isolates were diagnosed as *S. aureus* according to molecular and bacteriological methods. The susceptibility tests were performed on all bacterial isolates by the disc diffusion method using methicillin and six fluoroquinolone antibiotics. ***Results***: Out of fifty isolates, twelve were resistant to methicillin and all six antibiotics (nalidixic acid, lomefloxacin, ciprofloxacin, norfloxacin, ofloxacin, and levofloxacin). From the fifty isolates, 12 were resistant, 3 were intermediate, and 38 were sensitive to three or more tested antibiotics. The resistance of *S. aureus* isolates was also confirmed by the minimum inhibitory concentration test. The main sources of isolates were burns (10%), nose (16) wounds (8%), operation room (10%), ear (20%), urine (8%), skin (6%), and throat (22%). Twelve resistant isolates were used to examine the mutations in the *gyrA* gene. A direct sequence analysis found eight mutations in the *gyrA* gene; these mutations included 2 (25% missense mutations), 1 (12.5%) deletion mutation, and 5 (62.5%) silent mutations at various sites. ***Conclusion***: *gyrA* mutations resulting from the excessive use of antibiotics may be one of the mechanisms leading to fluoroquinolone resistance.

Keywords: Antibiotics, Fluoroquinolone, *gyrA*, Mutations, *Staphylococcus aureus*.

التحقيق الجزيئي لطفرات *gyrA* في عزلات المكورات العنقودية الذهبية السريرية المقاومة للميثيسيلين من مصادر مختلفة

الخلاصة

الخلفية: الفلوروكينولونات هي المضادات الحيوية الأكثر فعالية ضد عزلات المكورات العنقودية الذهبية. في المستشفيات، أدى الاستخدام المفرط للمضادات الحيوية إلى ظهور سلالات شديدة المقاومة من عزلات المكورات العنقودية الذهبية. **الهدف**: الكشف عن الطفرات التي تحدث في ترميز جين ***gyrA*** لجيراز الحمض النووي، وهو أحد أهداف مقاومة الفلوروكينولون. **الطريقة**: تم تشخيص خمسين عزلة سريرية على أنها **S. aureus** وفقا للطرق الجزيئية والبكتريولوجية. تم إجراء اختبارات الحساسية على جميع العزلات البكتيرية بطريقة انتشار القرص باستخدام الميثيسيلين وستة مضادات حيوية من مشتقات فلوروكينولون. النتائج: من بين خمسين عزلة، كان اثنا عشر مقاوما للميثيسيلين وجميع مشتقات الفلوروكينولون الستة. و كانت 12 مقاومة، و 3 متوسطة، و 38 كانت حساسة لثلاثة أو أكثر من المضادات الحيوية المختبرة. كما تم تأكيد مقاومة عزلات المكورات العنقودية الذهبية من خلال اختبار الحد الأدنى للتركيز المثبط. كانت المصادر الرئيسية للعزلات هي الحروق (10٪) وجروح الأنف (16) (8٪) وغرفة العمليات (10٪) والأذن (20٪) والبول (8٪) والجلد (6٪) والحلق (22٪). تم استخدام اثني عشر عزلة مقاومة لفحص الطفرات في جين جيراز. وجد تحليل التسلسل المباشر ثماني طفرات في جين جيراز، وتضمنت هذه الطفرات 2 (25٪ طفرات خاطئة) ، و 1 (12.5٪) طفرة حذف، و 5 (62.5٪) طفرات صامتة في مواقع مختلفة. الأستنتاجات: قد تكون طفرات جيراز الناتجة عن الاستخدام المفرط للمضادات الحيوية إحدى الآليات التي تؤدي إلى مقاومة الفلوروكينولون.

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INTRODUCTION

Antibiotic resistance indicates the ability of bacteria to survive the effects of drug treatment. Bacterial resistance was developed naturally due to genetic mutations or natural selection. The bacteria might evolve a mechanism to eliminate them and grow in the presence of antibiotics [1]. *Staphylococcus aureus* is a gram-positive, round-shaped bacterium that is found in the natural, normal flora of the human body. It is located in the skin, nose, and respiratory tract of humans [2]. Many of the coding genes for antibiotic resistance were located on mobile genetic elements (MGEs), considering the horizontal transfer of these genes between *Staphylococcus aureus* and other *staphylococci* species [3]. Generally, for the time being, *S. aureus* strains were resistant to penicillin groups and popular antibiotics such as tetracycline, fluoroquinolones, macrolides, aminoglycosides, and chloramphenicol. The mucosal surfaces were the favorite sites for *S. aureus*, like the throat, gastrointestinal tract, and vaginal wall [4]. Transportation of the bacterium from the nose occurs due to nose picking dispersing to other hosts or mucosal surfaces of the other body. The fluoroquinolone family is a synthetic compound containing a core that has a bicyclic structure used to treat many types of bacterial Gram-negative, Gram-positive, and other types of infections with anaerobic microorganisms [5]. The primary actions of fluoroquinolones were to inhibit the nucleic acid synthesis of bacteria by causing damage to the active sites of DNA gyrase and topoisomerase IV enzymes or the separation of chromosomes [6]. The resistance of *S. aureus* to fluoroquinolones has been acquired like other types of antibiotics due to two types of mechanisms: the first mechanism is mutations that occur in the bacterial encoding genes for DNA gyrase or topoisomerase IV by alterations in antibiotic genes; the second mechanism is acquisition; mutations of resistance genes come from many sources, like plasmid acquisition from resistant strains, which have many pathways for resistance, or from other environments [7]. The aim of the study is to detect the mutations in the *gyrA gene that* may be leading to fluoroquinolone resistance in *S. aureus* strains.

METHODS

Isolation and identification of Staphylococcus aureus

A total of 185 bacterial specimens of burns, wounds, urine, skin infections, operation room noise, ear, and throat were collected aseptically from various clinical sources, and swap specimens were transferred to the laboratory under cooling conditions. Specimens were cultured in mannitol salt agar (MSA) and chrome agar and incubated at 37°C for 24 hours under aerobic conditions (Table 1). All the *S. aureus* isolates are subjected to different biochemical and morphological tests, in addition to the VITEK2 system, to ensure their identity [8].

Table 1: Sources and percentages of *Staphylococcus aureus* samples and isolates

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

Antibiotic susceptibility of Staphylococcus aureus

Kirby-Bauer disc diffusion was the basic method used for bacterial investigation for susceptibility testing. It was used to detect the most effective bacterial therapy technique on Muller-Hinton agar media. In this study, disc diffusion was used for fifty *S. aureus* isolates against methicillin and fluoroquinolone antibiotics (Nalidixic Acid, Ciprofloxacin, Norfloxacin, Ofloxacin, Lomefloxacin, and Levofloxacin) [9,10]. Fifty *Staphylococcus aureus* isolates identified previously were cultured in nutrient broth to get turbidity equal to McFarland and incubated for 24 hours at 37°C. Then immerse a sterile cotton swab in a nutrient broth tube. Streaked the cotton swab on Muller-Hinton agar media in several directions from the plates. Place the plates at room temperature and wait for 10 minutes for complete absorption. Then, via antibiotic forceps, press discs of antibiotics on agar. Only four discs were placed in the plate. After fifteen minutes of discs being applied, incubate the plates for 24 hours at 37°C. The inhibition zone diameters of isolates were compared with CLSI 2021 [11].

Determination of minimum inhibitory concentrations

Based on the CLSI, 2021 [11], the agar dilution method was used to find the minimum inhibitory concentration (MIC) for S. aureus isolates that were resistant. Nine-fold dilutions (0.25 to 64 µg/ml) were prepared for Ciprofloxacin, Ofloxacin, and Levofloxacin; nine-fold dilutions (0.5 to 128 µg/ml) were prepared for Cefoxitin and Lomefloxacin; nine-fold dilutions from 1 to 256 µg/ml were prepared for Norfloxacin; and nine-fold dilutions from 2 to 512 µg/ml were prepared for Nalidixic acid using D.W. Prepare Muller-Hinton agar (MHA) and sterilize in an autoclave, then cool to 45°C. After mixing the media well, pour it into Petri dishes. Take three colonies by using a sterile loop and transfer them into a tube of Brain Heart infusion broth. Incubate at 37°C for 24 hours; it should be equal to the 0.5 McFarland standard. Add 1 ml of broth and dilute with normal saline (1:10), then place 100 microliters from each inoculum on the agar surface via a micropipette. Leave plates to dry for 10 minutes, and make wells on each agar plate by using Cork Borer agar. Label the tubes with the concentration of diluted antibiotics on each agar plate to identify the activity of diluted

antibiotics and their concentration in every well by placing 75 µl of each dilution in each well. The plates were basically incubated neatly overnight. incubate for 24 hours at 37°C and use one plate of MHA without antibiotics as a control [12].

Amplification of gyrA gene

The extraction of bacterial DNA (template) was carried out for fifty *S. aureus* isolates with the Wizard® Genomic DNA Purification Kit (Promega, USA). In PCR amplification, 25 µl was performed by using 3µl of DNA template, 9.1 µl nuclease-free water, 12.5 µl of PCR 2X Master Mix kits, and 0.2 µl of each oligonucleotide (10 µM) primers that we designed for the *gyrA* gene with the following sequence: *gyrA* F: GAACAAGGTATGACACCGGA *gyrA* R: AATACGTTGACGTCCGCC. A thermo-cycler device (Eppendorf, Germany) was used with the appropriate conditions (Table 2). After that, 5 µl of PCR product for the sample was analyzed using gel electrophoresis on 1% (w/v) Tris-acetate buffer agarose gel containing 0.5 µg/mL of ethidium bromide.

Table 2: The optimal conditions of PCR amplification

Step	No. of Cycles	Temperature (°C)	Time (Sec)
Initial Denaturation	1	94	300
Denaturation	30	94	30
Annealing		58	30
Extension		72	30
Final-Extension	1	72	600

Note: the annealing temperature for fluoroquinolone resistance gene (*gyrA*) was 58°C.

DNA Sequencing

Twenty microliters of PCR products that screened the *gyrA* gene and primers were sent for DNA sequencing to Macrogen Company in Korea. The resulting sequences of *S. aureus* isolates were aligned by BioEdit software and compared with NCBI databases to test the presence of mutations in the *gyrA* gene [13].

RESULTS

One hundred eighty-five bacterial samples were gathered from different clinical sources. Of these, only fifty samples, or 27% of the total isolates, were subjected to the standard biochemical tests and morphology characteristics of S. aureus strains. There were fifty isolates that turned phenol red to golden yellow and formed colonies that were yellow. This was because they were able to handle the high salt levels in the MSA-selective medium (Mannitol salt agar). These biochemical reactions gave the typical characteristics of *S. aureus* strain morphology [14]. The basic biochemical tests used for specimens showed a positive reaction for catalase, coagulase, MR-VP, and Voges-Proskauer tests. However, it gave a negative reaction for oxidase tests. To ensure identification of *Staphylococcus aureus, we* used the VITEK2 system [8]. The main sources of *S. aureus* isolates were burns (10%), nose (16%) wounds (8%), operation room (10%), ear (20%), urine (8%), skin (6%), and throat (22%). All *S. aureus* isolates were subjected to primary identification tests using different biological methods (gram staining, cultural characteristics, and biochemical tests). All bacterial isolates were subjected to the VITEK2 system to identify suspected bacteria at the species level. In this study, antibiotic disc diffusion was used in fifty *S. aureus* isolates against methicillin and fluoroquinolone antibiotics. The tested *S. aureus* isolates revealed various ranges of susceptibility and resistance to six antibiotics. The inhibition zones were compared with the CLSI (2021) diameter of the isolate (Table 3) [11].

Table 3: Antibiotics susceptibility of *S. aureus* isolates to Fluoroquinolones antibiotics and percentage of each antibiotic

Antibiotic	S	%	I	%	R	%
Ciprofloxacin	38	76	0	0	12	24
Levofloxacin	38	76	0	0	12	24
Lomefloxacin	34	68	4	8	12	24
Nalidixic acid	20	40	2	4	28	56
Norfloxacin	38	76	0	0	12	24
Ofloxacin	38	76	0	0	12	24

Note: S = Sensitive, I = Intermediate, R = Resistance.

Of the fifty *S. aureus* isolates, 12 were resistant to all selected antibiotics in this study. These isolates were subjected to MIC tests in accordance with antibiotic disc diffusion, depending on the CLSI recommendations [14]. Take into account the diameter inhibition zones of each antibiotic by comparing them to the standard diameters of CLSI 2021 (Table 4).

Table 4: Actual MIC ranges for *S. aureus* resistance isolates

Resistant Isolates		Actual MIC range (µg/ml)						
Source	No.	NA	CIP	NOR	OFX	LOM	LEV	FOX
Burn	1	64	0.5	16	32	512	16	128
	4	512	2	32	64	512	32	128
Ear	9	512	1	32	128	256	32	32
Nose	16	512	1	32	32	256	32	128
	17	512	2	32	32	512	32	64
Operation room	24	512	1	32	64	512	32	64
Skin	29	512	2	32	64	512	32	128
Throat	32	1024	1	64	32	64	16	64
	42	512	2	32	32	512	32	256
Urine	43	32	2	64	64	128	32	64
Wound	47	128	1	16	32	32	32	128
	50	1024	1	8	32	1024	16	64

The fluoroquinolone-resistant isolates were associated with the *gyrA* 660 bp gene. They were amplified by conventional PCR techniques based on annealing temperature. In agarose gel electrophoresis, the PCR product of this gene appears as clear bands with the same molecular size as the gene (660 for *gyrA*) (Figure 1).

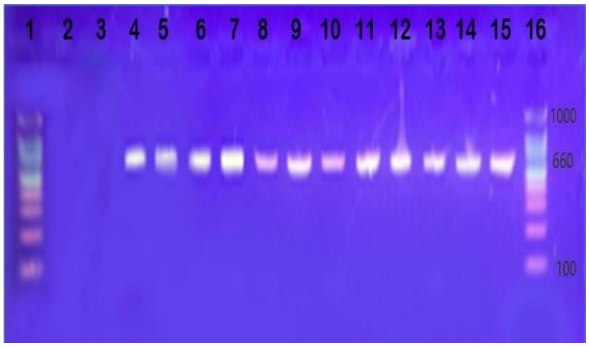

Figure 1: An agarose gel stained with Ethidium Bromide dye showing PCR products with *gyrA* gene 660bp primers for an extracted DNA. The electrophoresis process resulted at 70 volts for 70 min. Lane1 and lane16 for DNA marker (100-1000 bp), Line 2, Lane3 : Negative control, Lane 4-15: represented for a band of the positive results of amplified PCR product (660 bp).

The *gyrA* nucleotide sequences were analyzed using program software (Bio-Edit) when using the toggle translation option from the alignment menu. The sequence alignment was used to detect the alterations or any changes in the nucleotides of *S. aureus* isolates (Figure 2). The mutations occurred in many amino acids and nucleotides due to alterations or substitutions, as mentioned in Table 5. In *gyrA* alignment experiments on amino acid sequences of *S. aureus* resistant isolates between mutant and wild-type (RF122), many positions of mutation were detected, including Phe110Ser, Ala119Ser, and Ala119X [15]. Silent mutations (Leu167Leu, Glu211Glu, Pro219Pro,Thr132Thr, and Arg323Arg) (Figure 3).

DISCUSSION

Catalase is a catalytic enzyme that decomposes hydrogen peroxide into water and oxygen. Then, it prevents the toxic metabolites from accumulating. The *S. aureus* isolates differentiated from other *Streptococcus* genera by giving a positive catalase reaction [16]. *S. aureus* gave a negative reaction to the oxidase enzyme, which differentiated it from other *Micrococcus* genera. They were subjected to coagulase reactions that differentiated between *S. aureus* species (positive coagulase) and other *Staphylococci* species (negative coagulase) due to the reaction of coagulase enzymes of bacteria with prothrombin of human blood to form staphylothrombin (clot of blood), which led to converting the fibrinogen into fibrin [17]. *Staphylococcus aureus* isolates gave positive results in the methyl red reaction and the Voges-Proskauer reaction [18]. In this study, the percentage of resistance isolates to Ciprofloxacin, Norfloxacin, ofloxacin, and levofloxacin antibiotics was 12% of the total fifty *S. aureus* isolates. These results did not relate to the antibiotic sensitivity of Al-Marjani *et al.* [19], who showed that 16% of bacterial isolates were resistant to Ciprofloxacin antibiotics, and disagreed with Al-Jebouri and Mdish [20], who showed that 40% of bacterial isolates were resistant to Ciprofloxacin, disagreed with Rong *et al.* [21], Ikeagwu *et al.* [22], who showed 6.7% resistance to norfloxacin, 35% resistance to ofloxacin, and 40% to levofloxacin. Lomefloxacin antibiotics 24% of *S. aureus* isolates were resistant; this case disagreed with Abd El-Tawab [22], who found 12% of *S. aureus* isolates were resistant to lomofloxacin. In nalidixic acid, the results showed that 56% of *S. aureus* isolates were resistant, 40% were sensitive, and 4% were intermediate. These results disagreed with Khaleel *et al.* [23], who showed all *S. aureus* isolates (100%) were resistant to nalidixic acid. The 12 resistant isolates in the disc diffusion method were subjected to MIC tests. The results presented test that of *S. aureus* isolates were resistance to nalidixic acid and Lomefloxacin at MIC ranged 32, 64, 128, 512, and 1024 µg/ml, and the highest MIC in wound and throat (1024 µg/ml). However, the lower MIC range can be seen in Ciprofloxacin antibiotics in the range of 0.5, 1.0, and 2.0 µg/ml, specifically in burn samples with 0.5 µg/ml. While *S. aureus* isolates were resistant to other types of fluoroquinolone antibiotics that ranged from 8–512 µg/mL, In this study, the PCR method was completed at a 58°C annealing temperature. The 12 resistant isolates to fluoroquinolone antibiotics group showed a clear band in agarose gel with the same molecular weight of *gyrA* primer that compared with DNA ladder (1_kb DNA Ladder, BIORON_GmbH, Germany) at 70 volts for 70 min. However, after the DNA sequencing of 12 isolates of resistance to six tested fluoroquinolone antibiotics, mutations were identified in 12 *S. aureus* isolates located with the *gyrA* gene at various positions. In the *gyrA* gene, there were identified 5 (62.5%) silent mutations, 2 (25%), missense mutations, and 1 (12.5%) deletion mutation at various positions. Eight mutations in the *gyrA* gene occurred in codons 329, 355, 355, 396, 501, 633, 657, and 696. While in *gyrA* alignment experiments on amino acid sequences of *S. aureus* resistant isolates between mutant and wild type, many positions of mutations, including Phe110Ser, Ala119Ser, Ala119X, and Thr132Thr, occurred in 12.5% of one resistance isolate. Leu167Leu, Glu211Glu, Pro219Pro, and Arg323Arg occurred in 100% of isolates in the entire DNA gyrase subunit A (*gyrA*). The changes in amino acids disagreed with Hashem *et al.* [24], who found these mutations in *gyrA* Ser84Leu, Ile86Ile, Leu103Leu, Glu88Lys, Gly106Asp, and Ser112Arg. The results were not related to McCurdy *et al.*, who found point mutations in *gyrA;* seven of these mutations were Ser84Leu, and other mutations were Glu88Lys and Ser85Pro [25].

Table 5: All amino acids and nucleotides with alterations in the *gyrA* gene

Number of mutant isolates	Nucleotide		Amino Acid		Mutation type
	Position	Changed codon	Position	Changed amino acids	
42	329	TTT-TCT	110	F-S	Missense
50	355	GCA-TCA	119	A-S	Missense
50	355	GCA	119	A-X	Deletion
50	396	ACA-ACT	132	T-T	Silent
1-50	501	TTA-TTG	167	L-L	Silent
1-50	633	GAA-GAG	211	E-E	Silent
1-50	657	CCA-CCT	219	P-P	Silent
1-50	696	CGC-CGT	232	R-R	Silent

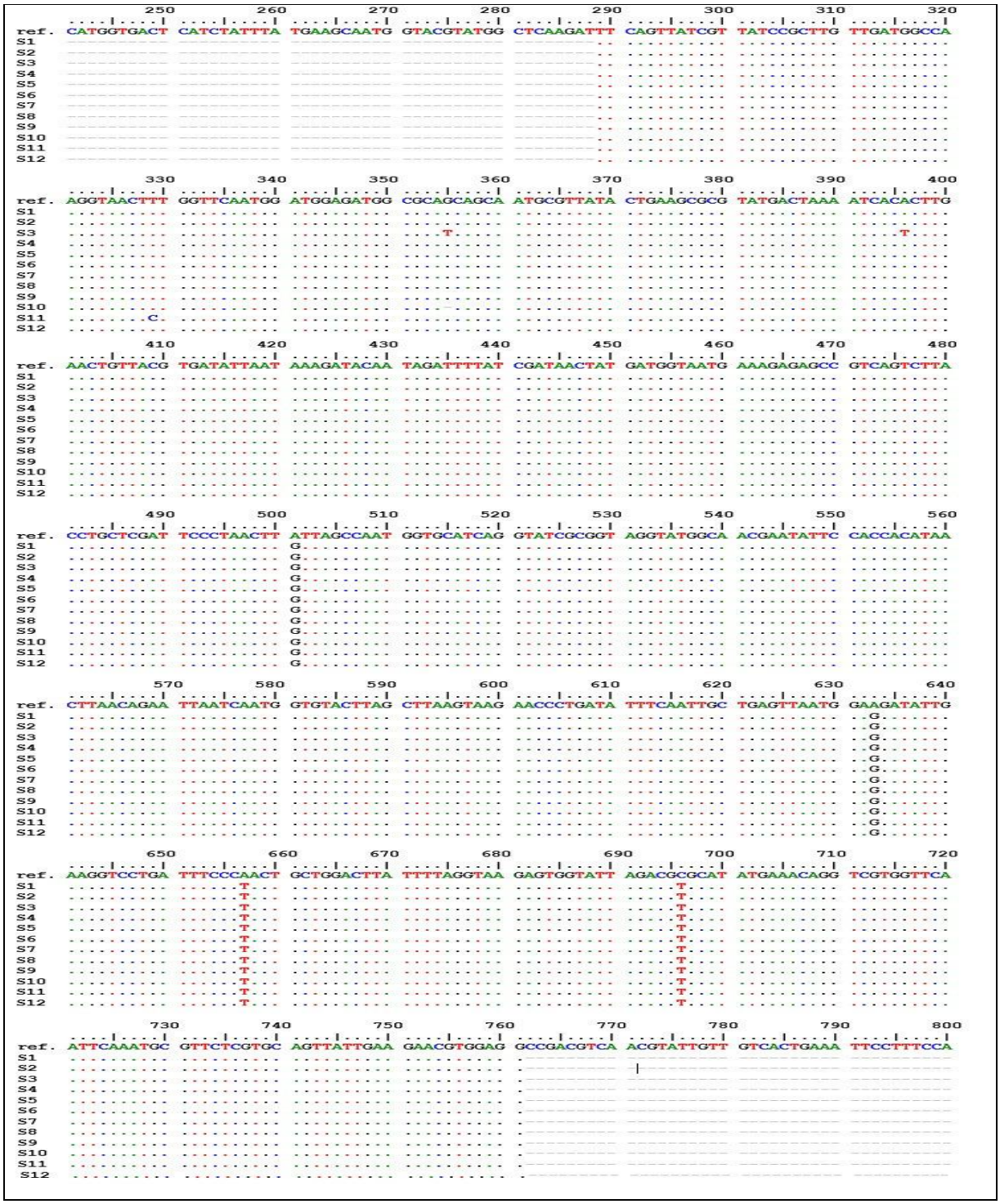

Figure 2: DNA Nucelotide sequences alignment of *S. aureus* isolates with its corresponding reference sequence of the *gyrA* gene by BioEdit software, with alterations in each isolate (Table 5). Ref= Reference sequence of *gyrA* gene of *S. aureus* strain RF122 (Wild type). The symbol "s" indicates to resistant isolates.

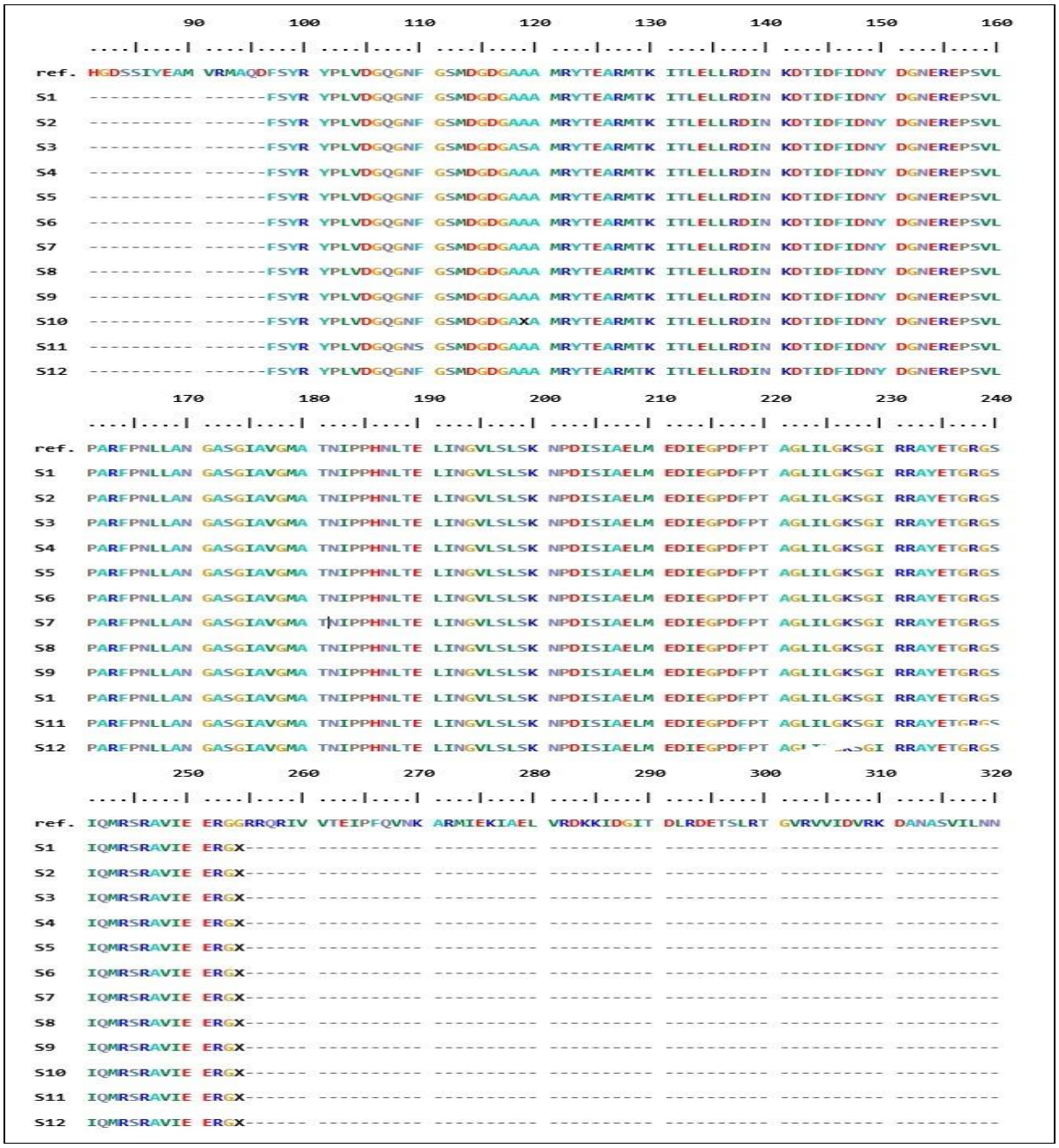

Figure 3: Amino acid alignment of the *gyrA* sequences by Bio-Edit software with alterations and substitutions in each isolate.

Conclusion

The resistant isolates to fluoroquinolones were selected to check the mutation occurrence by a direct sequence that identified 8 mutations in *gyrA* gene mutations at various positions. Some missense and deletion mutations may be related to antibiotic resistance in *S. aureus*.

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Conflict of interests

No conflict of interest was declared by the authors

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Chinnambedu RS, Marimuthu RR, Sunil SS, Amrose P, Ramachandran V, Pachamuthu B. Changing antibiotic resistance profile of Staphylococcus aureus isolated from HIV patients (2012–2017) in Southern *India. J Infect Public Health*. 2020;13(1):75-79. doi: 10.1016/j.jiph.2019.06.015.
2. Rana A, Kumar NR, Kaur J. Therapeutic effect of propolis on Staphylococcus aureus induced oxidative stress in spleen of Balb/c mice: A biochemical and histopathological study. Indian J Nat Prod Resource. 2022;13(3):346-355. doi: 10.56042/ijeb.v60i08.54823.

3. Maree M, Thi Nguyen LT, Ohniwa RL, Higashide M, Msadek T, Morikawa K. Natural transformation allows transfer of SCCmec-mediated methicillin resistance in Staphylococcus aureus biofilms. *Nat Commun*. 2022;13(1):2477. doi: 10.1038/s41467-022-29877-2.
4. de Morais Oliveira-Tintino CD, Muniz DF, Dos Santos Barbosa CR, Silva Pereira RL, Begnini IM, Rebelo RA, et al. NorA, Tet(K), MepA, and MsrA efflux pumps in *Staphylococcus aureus*, their inhibitors and 1,8-naphthyridine sulfonamides. *Curr Pharm Des*. 2023;29(5):323-355. doi: 10.2174/1381612829666221212101501.
5. Pham TDM, Ziora ZM, Blaskovich MAT. *Quinolone Antibiotics*. 2019;10(10):1719–1739. doi: 10.1039/c9md00120d.
6. Bush NG, Diez-Santos I, Abbott LR, Maxwell A. Quinolones: Mechanism, lethality and their contributions to antibiotic resistance. *Molecules*. 2020;25(23):5662. doi: 10.3390/molecules25235662.
7. Das B, Verma J, Kumar P, Ghosh A, Ramamurthy T. Antibiotic resistance in Vibrio cholerae: Understanding the ecology of resistance genes and mechanisms. *Vaccine*. 2020;38 Suppl 1:A83-A92. doi: 10.1016/j.vaccine.2019.06.031.
8. Abd FB. Characterization of multidrud resistant Staphylococcus aureus isolated from various sources. *Univers J Res Rev Arch*. 2022;1(2):94-101.
9. Mhana SMY, Aljanaby AAJ. Bacteriological Investigation of Pathogenic Bacteria Causing Urinary Tract Infections: A cross-Sectional Study. *IOP Conf Ser: Earth Environ Sci*. 2023;1215:012067. doi 10.1088/1755-1315/1215/1/012067.
10. Abd Al-Mayali M, Salman ED. Bacteriological and Molecular Study of Fluoroquinolones Resistance in Pseudomonas aeruginosa Isolated From Different Clinical Sources. *Iraqi J Sci*. 2020;61(9):2204-2214. doi 10.24996/ijs.2020.61.9.7
11. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100, 31st edition. J Clin Microbiol. 2021;59(12):e0021321. doi: 10.1128/JCM.00213-21.
12. Qamar S, Shaheen N, Shakoor S, Farooqi J, Jabeen K, Hasan R. Frequency of colistin and fosfomycin resistance in carbapenem-resistant Enterobacteriaceae from a tertiary care hospital in Karachi. *Infect Drug Resist*. 2017;10:231-236. doi 10.2147/IDR.S136777
13. Turki TG, Hashim NM, Abed BA, Al-Shuhaib MBS. Association of the growth hormone releasing hormone (rs368475481) polymorphism with acromegaly disorder in Iraqi patient. *J N Z Herpetol*. 2023;12:3112-3122.
14. Orfali RL, Silva Oliveira D, Lima D. *Staphylococcus aureus* enterotoxins modulate IL-22-secreting cells in adults with atopic dermatitis. *Sci Rep*. 2018;8(1):1–10. doi: 10.1038/s41598-018-25125-0.
15. Sanfilippo CM, Hesje CK, Haas W, Morris TW. Topoisomerase mutations that are associated with high-level resistance to earlier fluoroquinolones in Staphylococcus aureus have less effect on the antibacterial activity of besifloxacin. *Chemotherapy*. 2012;57(5):363-71. doi 10.1159/000330858
16. Ahmadi SH, Tabatabaie T, Ramavandi B, Hashemi SE. Amoxicillin enzymatic decomposition via H2O2-stimulation of catalase-positive bacteria in a sequential batch reactor. *Environ Technol Innov*. 2023;32:103352. doi: 10.1016/j.eti.2023.103352
17. Kadhum HH, Abood ZH. Staphylococcus aureus incidence in some patients with atopic dermatitis in Baghdad city. *Iraqi J Biotechnol*. 2022;21(2):13-20.
18. Parmar AS, Panwar AS. Antimicrobial Study of Chitosan-Based Crosslinked Hydrogel Against Staphylococcus aureus, Porphyromonas gingivalis, Pseudomonas aeruginosa, and Streptococcus mutans. *Current Trends in Biotechnology and Pharmacy*. 2023;17(3s):1170-83. doi: 10.5530/ctbp.2023.3s.54
19. Al-Marjani MF, Kadhim KA, Kadhim AA, Kinani A. Ciprofloxacin Resistance in Staphylococcus aureus and Pseudomonas aeruginosa Isolated from Patients in Baghdad. *Int Int J Pharm Sci Res*. 2015;6(2):382–385.
20. Al-Jebouri MM, Mdish SA. Antibiotic resistance pattern of bacteria isolated from patients of urinary tract infections in Iraq. *Open J Urol*. 2013;03(02):124–131. doi: 10.4236/oju.2013.32024.
21. Rong D, Wu Q, Xu M, Zhang J, Yu S. Prevalence, virulence genes, antimicrobial susceptibility, and genetic diversity of staphylococcus aureus from retail aquatic products in China. *Front Microbiol*. 2017;8:714. doi: 10.3389/fmicb.2017.00714.
22. El-Tawab A, Ashraf A, Hofy FI, Mohamed SR, Amin SH. Characterization of methicillin resistance staphylococcus aureus isolated from chicken and human. *Benha Vet Med J*. 2017;32(1):132–137.
23. Khaleel D, Othman R, Khudaier B. Plasmid transformation and curing of nalidixic acid gene in Staphylococcus aureus isolated from buffaloes mastitis and worker's hands. *Iraqi J Vet Sci*. 2019;32(2):167–74. doi: 10.33899/ijvs.2019.153845.
24. Hashem RA, Yassin AS, Zedan HH, Amin MA. Fluoroquinolone resistant mechanisms in methicillin-resistant Staphylococcus aureus clinical isolates in Cairo, Egypt. *J Infect Dev Ctries*. 2013;7(11):796–803. doi: 10.3855/jidc.3105.
25. McCurdy S, Lawrence L, Quintas M, Woosley L, Flamm R, Tseng C, et al. In vitro activity of delafloxacin and microbiological response against fluoroquinolone-susceptible and nonsusceptible Staphylococcus aureus isolates from two phase 3 studies of acute bacterial skin and skin structure infections. *Antimicrob Agents Chemother*. 2017;61(9). doi.org/10.1128/AAC.00772-17.