Al-Rafidain J Med Sci. 2023;5(Suppl 1):S64-70. DOI: https://doi.org/10.54133/ajms.v5i1S.282



Research Article

Online ISSN (2789-3219)

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

Safaa Ehssan Atta* 🔍, Lujain Ghannawi 🔍, Omar Yasir Shakir, Karam Mazin Gharab 🔍

National Diabetes Center, Mustansiriyah University, Baghdad, Iraq

Received: 1 September 2023; Revised: 16 October 2023; Accepted: 22 October 2023

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: الفلور وكينولونات هي المضادات الحيوية الأكثر فعالية ضد عز لات المكورات العنقودية الذهبية. في المستشفيات، أدى الاستخدام المفرط للمضادات الحيوية إلى ظهور سلالات شديدة المقاومة من عز لات المكورات العنقودية الذهبية. الهدف: الكشف عن الطفرات التي تحدث في ترميز جين gyrA لجيراز الحمض النووي، و هو أحد أهداف مقاومة الفلور وكينولون. الطريقة: تم تشخيص خمسين عز لة سريرية على أنها **S. aureus** في ترميز جين gyrA والبكتر يولوجية. تم إداوي، و هو أحد أهداف مقاومة الفلور وكينولون. الطريقة: تم تشخيص خمسين عز لة سريرية على أنها **S. aureus** في ترميز جين الكتريولوجية. تم إجراء اختبارات الحساسية على جميع العز لات البكتيرية بطريقة انتشار القرص باستخدام الميثيسيلين وستة مضادات حيوية من مشتقات فلور وكينولون. النتائج: من بين خمسين عز لة، كان اثنا عشر مقاوما للميثيسيلين وجميع مشتقات الفلور وكينولون السرية. و كانت 12 مقاومة، و والبكتريولوجية. تم إحراء اختبارات الحساسية على جميع العز لات البكتيرية بطريقة انتشار القرص باستخدام الميثيسيلين وستة مضادات حيوية من مشتقات فلور وكينولون. النتائج: من بين خمسين عزلة، كان اثنا عشر مقاوما للميثيسيلين وجميع مشتقات الفلور وكينولون الستقر و كانت 12 مقاومة، و مقوسطة، و 38 كانت حساسة لثلاثة أو أكثر من المضادات الحيوية المختبرة. كما تم تأكيد مقاومة عز لات المكورات العنودية الذهبية من خلال اختبار عن الحد الخبيرة. كما تم تأكيد مقاومة عز لات المكورات العمليات (10٪) و الأذن (20٪) والأدن للتركيز المثبط. كانت المصادر الرئيسية للعز لات هي الحروق (10٪) وجروح الأنف (16) (8٪) وغرفة العمليات (10٪) والأذن (20٪) والدنى للتركيز المثبط. كانت المصادر الرئيسية للعز لات هي عشر عزلة مقاومة لفحص الطفرات في جين و إلى (20٪) والأذن (20٪) والأذن (20٪) والذن للتركيز المثبط. كانت المصاد الاغيسية للعز لات هي الحروق (10٪) وجروح الأنوس و حرف من ورد العاليات المتعليات (10٪) والأذن (20٪) والذن للتركيز المثبط. كانت المصاد (20٪) والأدن (20٪) وجروح الأنو (10) (8٪) وخرفة العمليات (20٪) والأذن (20٪) وغرول (30) وقرول (8٪) وجروح الأفرات في جين جير از. والمان مو (20٪) ولمن علوم والم أول في والور العرات مو حمين عيراز. وعمنات هذه الطفرات 2 (25٪ طفرات خاطئة) ، و 1 (201٪) طفرة حذف، و 2 (20٪) طفرات صامنة في مواقع مختلفة. الطفرات في جين جير از

* *Corresponding author*: Safaa E. Atta, National Diabetes Center, Mustansiriyah University, Baghdad, Iraq; Email: <u>safaaehssan@uomustansiriyah.edu.iq</u>

Article citation: Atta SE, Ghannawi L, Shakir OY, Gharab KM. Molecular investigation of gyrA mutations in clinical methicillin-resistance Staphylococcus aureus isolates from various sources. Al-Rafidain J Med Sci. 2023;5(Suppl 1):S5164-70. doi: https://doi.org/10.54133/ajms.v5i1S.282

© 2023 The Author(s). Published by Al-Rafidain University College. This is an open access journal issued under the CC BY-NC-SA 4.0 license (https://creativecommons.org/licenses/by-nc-sa/4.0/).

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, Grampositive, 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 auren	ıs
samples and isolates	

Sample source	Number of	Number of isolates
	samples	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 Ciprofloxacin, (Nalidixic Acid, 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; ninefold 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 μ l 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 thermocycler 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

Tuble 2. The optimal conditions of FCR amplification							
Step	No. of	Temperature	Time				
	Cycles	(°C)	(Sec)				
Initial	1	94	300				
Denaturation	1						
Denaturation		94	30				
Annealing	20	58	30				
Extension	30	72	30				
Final-Extension	n 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 **Table 4**: Actual MIC ranges for *S. aureus* resistance isolates

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
Fluoro	auir	olones antibi	iotics and perce	ntas	ze o	f each an	tibiotic	

Fluoroquinoiones antibiotics and percentage of each antibiotic								
Antibiotic	S	%	Ι	%	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 = Interm	ediate,	$\mathbf{R} = \mathbf{R}\mathbf{e}$	esistanc	e.			

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).

Resistant Isc	olates	Actual MIC range (µg/ml)						
Source	No.	NA	CIP	NOR	OFX	LOM	LEV	FOX
D	1	64	0.5	16	32	512	16	128
Burn	4	512	2	32	64	512	32	128
Ear	9	512	1	32	128	256	32	32
N	16	512	1	32	32	256	32	128
Nose	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
TTI (32	1024	1	64	32	64	16	64
Throat	42	512	2	32	32	512	32	256
Urine	43	32	2	64	64	128	32	64
XX 7 1	47	128	1	16	32	32	32	128
Wound	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).

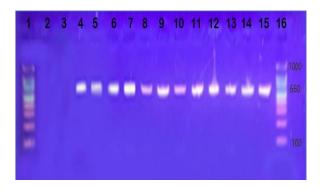


Figure 1: An agarose gel stained with Ethidium Bromide dye showing PCR products with *gyrA* gene 660bp primers for an extracted DNA. The electrophoresis process resulted at 70 volts for 70 min. Lane1 and lane16 for DNA marker (100-1000 bp), Line 2, Lane3 : Negative control, Lane 4-15: represented for 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 μ g/ml, and the highest MIC in wound and throat (1024 μ g/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 $8-512 \,\mu\text{g/mL}$, 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 Leu167Leu, Glu211Glu, resistance isolate. 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 gvrA 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	N	lucleotide		Amino Acid	Mutation trms	
isolates	Position Changed codon		Position	Changed amino acids	- Mutation type	
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	

	250	260	270	280	290	300	310	320
ref.			TGAAGCAATG				TATCCGCTTG	
S1				0				
52								
S 3								
54								
S 5								
S6								
S7								
58								
S9								
S10								
S11								
S12								
	330							
2.52								
ref.	AGGTAACTTT	GGTTCAATGG	ATGGAGATGG	CGCAGCAGCA	ATGCGTTATA	CTGAAGCGCG	TATGACTAAA	ATCACACTTG
S1							* * * * * * * * * *	
52								
S3	· · · · · · · · · · ·			· · · · T · · · · ·				T
S4								
S5								
S6 S7								
58								
59								
510								
S11								
512								
SIL								
	410							
_						1		
ref.	AACTGTTACG	TGATATTAAT	AAAGATACAA	TAGATTTTAT	CGATAACTAT	GATGGTAATG	AAAGAGAGCC	GTCAGTCTTA
S1								
S2								
53								
S4								
S5								
56						· · · · · · · · · · · · ·		
S7	· · · · · · · · · · · · · · ·						· · · · · · · · · · · ·	
S8								
S9 S10								
S11								
S12								
312								
	490	500	510	520	530	540	550	560
					1		1	
ref.	CCTGCTCGAT	TCCCTAACTT	ATTAGCCAAT	GGTGCATCAG	GTATCGCGGT	AGGTATGGCA	ACGAATATTC	CACCACATAA
S1			G					
52			G					
53			G					
S4			G					
S5			G					
S6			G					
			G					
S7			G					
S7 S8			G G					
S7 S8 S9			G G G					
S7 S8 S9 S10			G G G G					
S7 S8 S9 S10 S11			G G G G G					
S7 S8 S9 S10			G G G G					
S7 S8 S9 S10 S11	57	0 590	G G G G G		610	620	630	640
S7 S8 S9 S10 S11	57		G G G G G G 590	600	610		630	640
S7 S8 S9 S10 S11			G G G G G 590		610			640
S7 S8 S9 S10 S11 S12 ref.			G G G G G G 590					
\$7 \$8 \$9 \$10 \$11 \$12 ref. \$1 \$2			G G G G G 590					GAAGATATTG
57 58 59 510 511 512 ref. 51 52 53			G G G G G 590					GAAGATATTG
57 58 59 510 511 512 ref. 51 52 53 54			G G G G G 590					GAAGATATTG G G G G
\$7 \$8 \$9 \$10 \$11 \$12 ref. \$1 \$2 \$3 \$4 \$5			G G G G G 590					GAAGATATTG
s7 s8 s9 s10 s11 s12 ref. s1 s2 s3 s4 s5 s6			G G G G G 590					GAAGATATTG G G G G
s7 s8 s9 s10 s11 s12 ref. s1 s2 s3 s4 s5 s6 s7			G G G G G 590					GAAGATATTG
s7 s8 s9 s10 s11 s12 ref. s2 s3 s4 s5 s6 s7 s8			G G G G G 590					GAAGATATTG
s7 s8 s9 s10 s12 ref. s1 s2 s3 s4 s5 s7 s9 s9			G G G G G 590					
s7 s8 s9 s10 s11 s12 ref. s1 s2 s3 s4 s5 s6 s7 s8 s9 s10			G G G G G 590					
s7 s8 s9 s10 s12 ref. s1 s2 s3 s4 s5 s7 s9 s7 s9			G G G G G 590					
S7 S8 S9 S10 S11 S12 Fef. S1 S2 S3 S4 S5 S5 S6 S7 S8 S9 S10 S11			G. G. G. G. G. G. G. G. G. G. G. G. G. G	CTTAAGTAAG	AACCCTGATA	TTTCAATTGC	TGAGTTAATG	G
S7 S8 S9 S10 S11 S12 Fef. S1 S2 S3 S4 S5 S5 S6 S7 S8 S9 S10 S11	CTTAACAGAA	TTAATCAATG	G. G. G. G. G. G. G. G. G. G. G. G. G. G	CTTAAGTAAG	AACCCTGATA	TTTCAATTGC	TGAGTTAATG	G. G. G. G. G. G. G. G. G. G. G. G. G. G
S7 S8 S9 S10 S11 S12 S12 S12 S12 S2 S3 S4 S5 S6 S7 S7 S6 S7 S10 S11 S12	CTTAACAGAA	TTAATCAATG	G. G. G. G. G. G. G. G. G. G. G. G. G. G	CTTAAGTAAG	AACCCTGATA	TTTCAATTGC	7100	G
S7 S8 S9 S10 S11 S12 ref. S1 S2 S3 S4 S5 S5 S5 S5 S5 S5 S5 S10 S11 S12 ref. Tef.	CTTAACAGAA	TTAATCAATG	G. G. G. G. G. G. G. G. G. G. G. G. G. G	CTTAAGTAAG	AACCCTGATA	TTTCAATTGC	TGAGTTAATG	G
S7 S8 S9 S10 S11 S12 Fef. S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S12 Fef. S1	CTTAACAGAA	TTAATCAATG	G. G. G. G. G. G. G. G. G. G. G. G. G. G	CTTAAGTAAG	AACCCTGATA	TTTCAATTGC	7100	G G G G G G G G.
S7 S8 S9 S10 S11 S12 ref. S1 S2 S3 S4 S5 S5 S6 S10 S11 S12 ref. S1 S2 S2	CTTAACAGAA	TTAATCAATG	G. G. G. G. G. G. G. G. G. G. G. G. G. G	CTTAAGTAAG	AACCCTGATA	TTTCAATTGC	7100	G G G G G G G G.
S7 S8 S9 S10 S11 S12 Fef. S1 S3 S4 S5 S6 S7 S8 S7 S8 S7 S8 S11 S12 Fef. S1 S3	CTTAACAGAA	TTAATCAATG	G. G. G. G. G. G. G. G. G. G. G. G. G. G	CTTAAGTAAG	AACCCTGATA	TTTCAATTGC	7100	G G G G G G G G.
s7 s8 s9 s10 s11 s12 ref. s1 s2 s3 s3 s5 s5 s5 s5 s6 s7 s7 s1 s1 s1 s2 s3 s4 s5 s5 s5 s5 s5 s5 s6 s5 s7 s1 s1 s1 s1 s1 s1 s1 s1 s1 s1 s1 s1 s1	CTTAACAGAA	TTAATCAATG	G. G. G. G. G. G. G. G. G. G. G. G. G. G	CTTAAGTAAG	AACCCTGATA	TTTCAATTGC	7100	G G G G G G G G.
S7 S8 S9 S10 S11 S12 S3 S2 S3 S4 S5 S6 S7 S7 S7 S8 S9 S10 S12 Fef. S12 Fef. S3 S3 S4 S5	CTTAACAGAA	TTAATCAATG	G. G. G. G. G. G. G. G. G. G. G. G. G. G	CTTAAGTAAG	AACCCTGATA	TTTCAATTGC	7100	G G G G G G G G.
s7 s8 s9 s10 s11 s12 ref. s1 s2 s3 s5 s5 s6 s7 s7 s7 s11 s12 ref. s1 s2 s3 s4 s5 s5 s5 s6 s5 s5 s5 s5 s5 s6 s1 s1 s1 s1 s1 s1 s1 s1 s1 s1 s1 s1 s1	CTTAACAGAA	TTAATCAATG	G. G. G. G. G. G. G. G. G. G. G. G. G. G	CTTAAGTAAG	AACCCTGATA	TTTCAATTGC	7100	G G G G G G G G.
S7 S8 S9 S10 S11 S12 S1 S2 S34 S52 S34 S54 S57 S10 S12 Fef. S12 Fef. S3 S4 S5 S5 S5 S5 S5 S5 S5 S5 S5 S5	CTTAACAGAA	TTAATCAATG	G. G. G. G. G. G. G. G. G. G. G. G. G. G	CTTAAGTAAG	AACCCTGATA	TTTCAATTGC	7100	G G G G G G G G.
s7 s8 s9 s10 s11 s12 ref. s1 s2 s3 s5 s5 s5 s6 s7 s11 s12 s3 s4 s5 s6 s5 s5 s5 s5 s5 s5 s5 s5 s5 s5 s6 s10 s1 s1 s1 s1 s1 s1 s1 s1 s1 s1 s1 s1 s1	CTTAACAGAA	TTAATCAATG	G. G. G. G. G. G. G. G. G. G. G. G. G. G	CTTAAGTAAG	AACCCTGATA	TTTCAATTGC	7100	G G G G G G G G.
S7 S8 S9 S10 S11 S12 S1 S2 S3 S5 S5 S6 S11 S12 Fef. S12 Fef. S12 S3 S5 S5 S5 S5 S5 S5 S5 S5 S5 S5	ctradicadad 650	TTAATCAATG	G	CTTAAGTAAG CTTAAGTAAG 680 CTTTAGGTAA	69 GAGTGGTATT	0 700 AGAGCGCAT T T T T T T T T T T T	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 S12 S2 S3 S4 S5 S5 S5 S5 S5 S5 S5 S5 S5 S5	ctradicadad 650	TTAATCAATG	G	CTTAAGTAAG CTTAAGTAAG 680 CTTTAGGTAA	69 GAGTGGTATT	0 700 AGAGCGCAT T T T T T T T T T T T	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 Fef. S1 S3 S5 S5 S7 S3 S12 S12 S12 S12 S12 S12 S12 S12	ctradicadad 650	TTAATCAATG	G	CTTAAGTAAG CTTAAGTAAG 680 CTTTAGGTAA	69 GAGTGGTATT	0 700 AGAGCGCAT T T T T T T T T T T T	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 S12 S2 S3 S4 S5 S5 S5 S5 S5 S5 S5 S5 S5 S5	650 AAGGTCCTGA	TTAATCAATG	G	CTTAAGTAAG CTTAAGTAAG 680 CTTACGTAA	AACCCTGATA	TTTCAATTGC	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 S12 S2 S3 S4 S5 S5 S5 S5 S5 S5 S5 S5 S5 S5	650 AAGGTCCTGA	TTAATCAATG	G	CTTAAGTAAG CTTAAGTAAG 680 CTTACGTAA	AACCCTGATA	TTTCAATTGC	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 S34 S52 S34 S52 S34 S52 S34 S55 S10 S11 S12 Fef. S1 S1 S1 S1 S1 S1 S2 S34 S56 S10 S11 S12 S34 S56 S10 S11 S12 S34 S56 S10 S11 S12 S34 S56 S10 S11 S12 S34 S57 S10 S11 S12 S34 S56 S10 S11 S12 S12 S34 S57 S16 S11 S12 S14 S12 S14 S12 S14 S12 S14 S12 S14 S12 S14 S12 S14 S12 S14 S12 S14 S12 S14 S12 S14 S16 S16 S17 S16 S17 S17 S17 S17 S17 S17 S17 S17	650 AAGGTCCTGA	TTAATCAATG	G	CTTAAGTAAG CTTAAGTAAG 680 CTTACGTAA	AACCCTGATA	TTTCAATTGC	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 S2 S3 S3 S5 S5 S5 S7 S3 S10 S11 S12 S2 S11 S12 S2 S3 S4 S5 S5 S5 S5 S6 S10 S11 S12 S12 S12 S12 S12 S12 S12 S12 S12	ctrtal.cagaa 650 AaggTccTga 730	TTAATCAATG	ее. е	680 TTTTAGGTAA 680 TTTTAGGTAA	AACCCTGATA	TTTCAATTGC	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 S12 S2 S3 S4 S5 S5 S6 S10 S11 S12 Fef. S1 S1 S1 S1 S1 S1 S2 S3 S4 S5 S5 S10 S11 S12 S12 S12 S14 S14 S12 S14 S14 S14 S14 S14 S14 S14 S14	650 	TTAATCAATG	а а	ettaagtaag ese ese ttttaggtaa tttttaggtaa	GAGTGGTATT	TTTCAATTGC	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 ref. S3 S5 S5 S7 S8 S9 S10 S12 ref. S1 S2 S5 S5 S5 S5 S5 S5 S7 S1 S12 S12 S12 S12 S12 S12 S12	ctrtalcagaa ctrtalcagaa 650 aacgtccrga aacgtccrga 730 artcaaatgc	TTAATCAATG	ее. е	680 TTTTAGGTAA 680 TTTTAGGTAA	AACCCTGATA	TTTCAATTGC	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 S1 S2 S3 S4 S5 S6 S7 S8 S9 S101 S12 S3 S4 S5 S6 S7 S8 S9 S101 S12 S1 S1 S1 S1 S1 S1 S1 S1 S1 S1 S1 S1 S1	650 AAGGTCCTGA	TTAATCAATG	С	680 TTTTAGGTAA GAACGTGGAG	GAGTGGTATT	DO 700 AGACGCCCAT T T T T T T T T T T T T T	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 S2 S3 S4 S5 S5 S5 S5 S5 S5 S5 S5 S5 S5	cttradeagaa 650 AAGGTCCTGA 738 ATTCAAATGC	TTAATCAATG	G	GBCC GBCC GBCC GBCC GBCC GBCC GBCC GBCC	aaccergata 65 Gagriggtatt Gagriggtatt Gecglacgtet Gecglacgtet	o 700 AGGATTGT	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 ref. S1 S3 S4 S5 S6 S7 S8 S9 S101 S12 ref. S1 S1 S1 S1 S1 S1 S1 S1 S1 S1	cttradeagaa 650 AAGGTCCTGA 738 ATTCAAATGC	TTAATCAATG	G	GBCC GBCC GBCC GBCC GBCC GBCC GBCC GBCC	aaccergata 65 Gagriggtatt Gagriggtatt Gecglacgtet Gecglacgtet	o 700 AGGATTGT	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 S2 S3 S4 S5 S5 S7 S8 S10 S12 S3 S4 S5 S7 S8 S10 S12 S12 S12 S12 S12 S12 S12 S12	cttradeagaa eso aaggteetga aaggteetga atteaaarge	TTAATCAATG	G	GBC GBC CTTAAGTAAG GBC CTTAAGTAA GBC CTTAAGTAA	aaccergata 65 Gagriggtatt Gagriggtatt Gecglacgtel	o 700 AGGATTGT	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 ref. S1 S3 S4 S5 S5 S7 S8 S9 S101 S12 S1 S12 S3 S4 S5 S5 S5 S5 S10 S11 S12 S12 S12 S12 S12 S12 S12	650 AAGGTCCTGA 730 ATTCAAATGC	TTAATCAATG	6	ettaagtaag ettaagtaag eest eest eest eest eest eest eest ees	aaccergata 65 Gagriggtatt Gagriggtatt Gecglacgtel	o 700 AGGATTGT	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S7 S8 S9 S10 S11 S12 S2 S34 S52 S34 S12 Fef. S12 Fef. S12 S12 Fef. S12 S12 S12 S12 S12 S12 S12 S12	ctrialcagaa 650 AAGGTCCTGA 734 ATTCAAATGC	TTAATCAATG	G	GRACGTCCAG	aaccergata 65 Gagriggtatt Gagriggtatt Gecglacgtel	o 700 AGGATTGT	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 Fef. S3 S5 S5 S5 S5 S7 S8 S9 S10 S12 Fef. S12 S12 S12 S12 S12 S12 S12 S12	650 AAGGCCCTGA AAGGCCCTGA	TTAATCAATG	с	680 	aaccergata 65 Gagriggtatt Gagriggtatt Gecglacgtel	o 700 AGGATTGT	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 Fef. S12 S3 S4 S5 S5 S5 S5 S5 S5 S5 S5 S5 S5	ctraacadaa 650 AAGGTCCTGA 730 ATTCAAATGC	TTAATCAATG	с с	GENERAL GENERA	aaccergata 65 Gagriggtatt Gagriggtatt Gecglacgtel	o 700 AGGATTGT	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 S2 S3 S4 S5 S5 S5 S7 S8 S9 S10 S12 S1 S12 S3 S4 S5 S5 S5 S5 S7 S8 S9 S10 S12 S12 S12 S12 S12 S12 S12 S12	650 AAGGCCCTGA 730 ATTCAAATGC	TTAATCAATG	е	GETTAAGTAAG GEOGRAFIA GEOGRAFIA TITTAGGTAA GEOGRAFIA GAACGTGGAG	aaccergata 65 Gagriggtatt Gagriggtatt Gecglacgtel	o 700 AGGATTGT	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 Fef. S12 S3 S4 S5 S5 S5 S5 S5 S5 S5 S5 S5 S5	650 AAGGCCCTGA 730 ATTCAAATGC	TTAATCAATG	с с	GETTAAGTAAG GEOGRAFIA GEOGRAFIA TITTAGGTAA GEOGRAFIA GAACGTGGAG	aaccergata 65 Gagriggtatt Gagriggtatt Gecglacgtel	o 700 AGGATTGT	TGAGTTAATG 710 ATGAAACAGG	G. G
S7 S8 S9 S10 S11 S12 Fef. S3 S5 S5 S5 S7 S8 S9 S10 S12 Fef. S1 S2 S3 S5 S5 S5 S7 S8 S9 S10 S12 S12 S12 S12 S12 S12 S12 S12	650 AAGGCCCTGA 730 ATTCAAATGC	TTAATCAATG	е	GETTAAGTAAG GEOGRAFIA GEOGRAFIA TITTAGGTAA GEOGRAFIA GAACGTGGAG	aaccergata 65 Gagriggtatt Gagriggtatt Gecglacgtel	o 700 AGGATTGT	TGAGTTAATG 710 ATGAAACAGG	G. G

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.

90 100 110 120 130 140 151 ref. HCDSSTYEAM VERAGOFSYR YPLVDCGGNF GSMDGDGAAA MRYTEARMYK ITLELLRDIN KOTIDFIDNY S1	11
Pref. HGDSSIYEAM VRMAQQFSYR YPLVDGQGNF GSMDGDGAAA MRYTEARMIK ITLELLRDIN KDTIDFIDNY 51	
 S1	
 S3FSYR YPLVDGQGNF GSMDGDGAAA MRYTEARMTK ITLELLRDIN KDTIDFIDNY S4	
 S4	DGNEREPSVL
 S5	DGNEREPSVL
 S6	DGNEREPSVL
 57	DGNEREPSVL
 S8FSYR YPLVDGQGNF GSMDGDGAAA MRYTEARMTK ITLELLRDIN KDTIDFIDNY S9	DGNEREPSVL
59	DGNEREPSVL
 S10FSYR YPLVDQQNF GSMDDGAXA MRYTEARMTK ITLELLRDIN KDTIDETDNY S11	DGNEREPSVL
511	DGNEREPSVL
512	DGNEREPSVL
170180190200210220230ref.PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAELMEDIEGPDFPTAGLILCKSGIS1PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAELMEDIEGPDFPTAGLILCKSGIS2PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAELMEDIEGPDFPTAGLILCKSGIS3PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAELMEDIEGPDFPTAGLILCKSGIS4PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAELMEDIEGPDFPTAGLILCKSGIS5PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAELMEDIEGPDFPTAGLILCKSGIS6PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAELMEDIEGPDFPTAGLILCKSGIS7PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAELMEDIEGPDFPTAGLILCKSGIS8PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAELMEDIEGPDFPTAGLILCKSGIS9PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAELMEDIEGPDFPTAGLILCKSGIS11PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAELMEDIEGPDFPTAGLILCKSGIS12PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAELMEDIEGPDFPTAGLILCKSGIS11PARFPNLLANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISTAEL	DGNEREPSVL
	DGNEREPSVL
ref. PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 51 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 52 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 53 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 54 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 55 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 56 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 57 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 58 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 59 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 51 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 52 260 2	0 240
 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAEL	11
S2 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S3 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S4 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S5 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S6 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S7 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S8 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S9 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S1 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S1 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S11 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S11 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S12 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S11 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S12 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S20 260 S	RRAYETGRGS
53 PAREPNILIAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI 54 PAREPNILAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI 55 PAREPNILAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI 56 PAREPNILAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI 57 PAREPNILAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI 58 PAREPNILAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI 59 PAREPNILAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI 51 PAREPNILAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI 520 260 270 280 290 300 310 51 IQMRSRAVIE ERGCRRQRIV VTEIPFQVNK ARMIEKIAEL VRDKKIDGIT DLRDETSLRT GVRVVIDVRK 51 IQMRSRAVIE ERGX	RRAYETGRGS
S4 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S5 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S6 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S7 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S8 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S9 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S1 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S11 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S12 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S11 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S12 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S12 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGIILGKSGI S20 260 270 280 290 300 310 ref, IQMRSRAVIE ERGGRRQRIV VTEIPFQVNK ARMIEKIAEL VRDKKIDGIT DLRDETSLRT GVRVVIDVRK S1 IQMRSRAVIE ERGX	RRAYETGRGS
55 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 56 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 57 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 58 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 59 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 51 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 511 PARFPNLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 512 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 520 260 270 280 290 300 310 51 IQMRSRAVIE ERGGRRQRIV VTEIPFQVNK ARMIEKIAEL VRDKKIDGIT DLRDETSLRT GVRVVIDVRK 51 IQMRSRAVIE ERGX	RRAYETGRGS
S6 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S7 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S8 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S9 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S1 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S11 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S12 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGI'	RRAYETGRGS
S7 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S8 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S9 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S1 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S11 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S12 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S12 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S12 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGIILGKSGI S12 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGIILGKSGI S14 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGIILGKSGI S15 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGIILGKSGI S20 260 270 280 290 300 310	RRAYETGRGS
S8 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S9 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S1 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S11 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI S12 PARFPNLLAN GASGIAVGMA TNIPPHNLTE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGI'	RRAVETGRGS
59 PAREPNILIAN GASGIAVGMA TNIPPHNITE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 51 PAREPNILAN GASGIAVGMA TNIPPHNITE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 511 PAREPNILAN GASGIAVGMA TNIPPHNITE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 512 PAREPNILAN GASGIAVGMA TNIPPHNITE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGLILGKSGI 512 PAREPNILAN GASGIAVGMA TNIPPHNITE LINGVLSLSK NPDISIAELM EDIEGPDFPT AGI* 520 260 270 280 290 300 310 51 IQMRSRAVIE ERGGRRQRIV VTEIPFQVNK ARMIEKIAEL VRDKKIDGIT DLRDETSLRT GVRVVIDVRK 51 IQMRSRAVIE ERGX	RRAYETGRGS
S1 PAREPNILIAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI S11 PAREPNILIAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI S12 PAREPNILIAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI 250 260 270 280 290 300 310	RRAYETGRGS
S11 PAREPNILIAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI S12 PAREPNILIAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGLILGKSGI 250 260 270 280 290 300 310	RRAYETGRGS
S12 PAREPNILIAN GASGIAVGMA TNIPPHNITE LINGVLSISK NPDISIAELM EDIEGPDEPT AGI **	RRAYETGRGS
250 260 270 280 290 300 310	RRAYETGROS
ref. IQMRSRAVIE ERGGRRQRIV VTEIPFQVNK ARMIEKIAEL VRDKKIDGIT DLRDETSLRT GVRVVIDVRK 51 IQMRSRAVIE ERGX	RRAYETGRGS
ref. IQMRSRAVIE ERGGRRQRIV VTEIPFQVNK ARMIEKIAEL VRDKKIDGIT DLRDETSLRT GVRVVIDVRK 51 IQMRSRAVIE ERGX	320
S1 IQMRSRAVIE ERGX S2 IQMRSRAVIE ERGX	11
52 IQMRSRAVIE ERGX	DANASVILNN
S3 IQMRSRAVIE ERGX	
S4 IQMRSRAVIE ERGX	
S5 IQMRSRAVIE ERGX	
S6 IQMRSRAVIE ERGX	
S7 IQMRSRAVIE ERGX	
S8 IQMRSRAVIE ERGX	
S9 IQMRSRAVIE ERGX	
510 IQMRSRAVIE ERGX	
S11 IQMRSRAVIE ERGX	
S12 IQMRSRAVIE ERGX	

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*.

ACKNOWLEDGEMENT

The authors thank assistant instructors Haneen Ali for the information and advice during the study.

Conflict of interests

No conflict of interest was declared by the authors

Funding source

The authors did not receive any source of fund.

Data sharing statement

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

REFERENCES

- 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.
- 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.

- 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.
- 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 1,8naphthyridine sulfonamides. *Curr Pharm Des*. 2023;29(5):323-355. doi: 10.2174/1381612829666221212101501.
- Pham TDM, Ziora ZM, Blaskovich MAT. Quinolone Antibiotics. 2019;10(10):1719–1739. doi: 10.1039/c9md00120d.
- 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.
- 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.
- Abd FB. Characterization of multidrud resistant Staphylococcus aureus isolated from various sources. Univers J Res Rev Arch. 2022;1(2):94-101.
- 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.
- 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
- 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.
- 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.
- 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.

- 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
- 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
- Kadhum HH, Abood ZH. Staphylococcus aureus incidence in some patients with atopic dermatitis in Baghdad city. *Iraqi J Biotechnol.* 2022;21(2):13-20.
- 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
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.