Molecular Investigation of Quinolone-Resistant Genes among Clinical *Staphylococcus aureus* Isolates in Babylon Hospitals

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Abstract

Background: *Staphylococcus aureus* is considered an essential etiological factor in several human infections. Although fluoroquinolones are typically used to treat these illnesses, a growing average level of resistance to these medications has been found globally in recent years. **Objective:** *S. aureus* is considered a main etiological agent of many human infections. Fluoroquinolones are routinely used in the drugs for these infections; however, in latest years, a growing rate of resistance to these drugs has been reported worldwide. The current study aimed to investigate the molecular resistance of quinolone *(qnrA and qnrS)* genes among clinical *S. aureus* isolates in Babylon. **Materials and Methods:** During the research, 431 clinical samples were collected from hospitals in the Babylon Health Directorate between August 2022 and January 2023. Standard laboratory procedures and biochemical tests were used to identify the bacterial isolates. Antimicrobial susceptibility was determined by the standard disk diffusion method. Polymerase chain reaction (PCR) was used for detecting the *qnrA* and *qnrS* genes. **Results:** From a total of 78 *S. aureus* isolates, 18 (23.77%) and 19 (24.35%) isolates of *S. aureus* bacteria were sensitive and intermediate to quinolone compounds, respectively, whereas 41 (52.56%) isolates showed high-level quinolone resistance. The findings revealed that the majority of them were antibiotic-resistant. Most frequently, high resistance was noted for ciprofloxacin with a ratio of 75%, followed by levofloxacin with a ratio of 72%, and high sensitivity was shown for norfloxacin with an 85% and chloramphenicol with an 80%. From a total of 41 quinolones resistance *S. aureus*, the *qnrA* gene was identified in 10 (24.39%) and *qnrS* in 8 (19.51%). **Conclusion:** The results showed a high rate of *qnr* gene resistance among the isolates of *S. aureus* in the Babylon hospitals, which emphasizes the need for establishing careful policies. From a total of 41 quinolones resistance *S. aureus*, the *qnrA* gene was identified in 10 (24.39%) and *qnrS* in 8 (19.51%).

Keywords: Babylon hospitals, PCR, *qnr genes*, quinolone, *S. aureus*

Introduction

Staphylococci are Gram-positive bacteria that are seen as cocci under the microscope. According to their ability to produce coagulase, they are categorized as coagulase-positive bacteria and coagulase-negative bacteria.[1] The more closely regarding bacterium in this genus that is coagulase positive. It is one of the most significant infections in a few clinical cases involving humans. Nonetheless, coagulase-negative *Staphylococci*, specifically *Epidermidis* and *S. saprophyticus*, are two significant species that should be taken into account in some clinical conditions.[2]

Staphylococcus aureus can develop resistance to antibiotics in a variety of ways, including through horizontal gene transfer of various mobile genetic elements, such as *Staphylococcus* cassette chromosomes, pathogenicity islands, plasmids, bacteriophages, and transposons.[3] *S. aureus* is one of the most common causes of nosocomial, and hospital staff members, who act as carriers of the organism, contribute significantly to its spread among patients.[4] Up to 50% of adults are thought to be colonized, and 15% of people have *S. aureus* in their anterior nares permanently.[5,6] The prevalence of *S. aureus* colonization

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can reach up to 80% in certain populations, including healthcare workers, those who frequently used needles (such as diabetics and intravenous [IV] medication users), hospitalized patients, and people with impaired immune systems. Through direct contact or by fomites, *S. aureus* can spread from person to person.[7-9] In addition to humans and domestic animals, livestock and termites may also serve as additional reservoirs for this bacterial illness, which has a substantial influence on veterinary care.[8,10,11] Despite the close relationship between people and the bacteria, it has long been regarded as a major human pathogen and is one of the Gram-positive bacteria most frequently associated with human disease globally.[9,12]

Less effectiveness against positive pathogens, such as *S. aureus*, has become a problem because the present members of this chemical have the greatest efficacy against aerobic negative bacteria.[13,14] Even though these organizations may be active, it is concerning because some organisms started showing signs of increasing antimicrobial resistance within a short while after they were first used in clinical settings.[15,16] Significant morbidity and mortality have been linked to serious illnesses brought on by these resistant pathogens.[17]

Usually, changes in the target enzymes (DNA gyrase and topoisomerase IV) as well as adjustments to drug entry and efflux cause fluoroquinolone resistance to rise. The more sensitive target is chosen for mutation selection first: Gram-negative bacteria use DNA gyrase, whereas Gram-positive bacteria use topoisomerase IV.[18] The most resistant isolates have mutations in several genes due to further changes in the next most susceptible target as well as in genes governing drug accumulation. Quinolone resistance can also be transmitted by plasmids that produce the Qnr protein, which protects quinolone targets from inhibition,[19,20] The current study aimed to isolate and identify *S. aureus* from samples to evaluate the sensibility of the bacterial samples versus a number of antibiotics, in adding to the finding of some resistance and virulence genes.

Materials and Methods

Subjects and specimen collections

Our research involved 431 samples that were isolated between August 2022 and January 2023. All clinical samples were received and gathered via the Babylon hospitals' lab departments, such as AL-Sadiq Hospital, AL-Hashemite Hospital, and AL-Shomali Hospital. Standard microbial was used to identify isolates biochemically, catalase testing, Gram staining, and tube coagulase testing were all performed on a solitary colony from pure cultures. Bacterial samples were isolated from cases and hospitalized patients in numerous wards who were of both sexes and all age groups. Clinical isolates were collected from a variation of infected body sites, including burns wounds, sputum, urine, and blood.

Isolation and identification of isolates

Clinical isolates were assimilated using accepted assembly methods and inoculated into the proper bacteriological media, such as Mannitol Salt Agar and Blood Agar company of (Himedia, India). The petri plates underwent an aerobic 37°C incubation for 24 h. The value, Integrity, Teamwork, Excellence and Knowledge (VITEK 2) is an automated microbiology system utilizing growth-based technology, according to the manufacturer's instructions (BioMerieux, France).

Antibiotic susceptibility investigation

The Clinical Laboratory Standards Institute criteria were followed when using the Kirby-Bauer disk diffusion method.[21] All samples were tested for sensitivity to the following antimicrobials: ciprofloxacin (CIP, 5 mg), levofloxacin (LEV, 5 mg), moxifloxacin (MFX, 5 mg), chloramphenicol (C, 30 mg), norfloxacin (N, 10 mg), rifampin (RP, 5 mg), methicillin (MET, 5 mg), erythromycin (E, 15 mg), ofloxacin (OF, 10 mg), and augmentin (AMC, 10 mg).

DNA extraction and polymerase chain reaction

All isolates that exhibited ciprofloxacin resistance are thought to include *qnr genes*. All these isolates were subjected to DNA extraction using the Wizard Genomic DNA Purification Kit (Promeg, USA).

Genomic DNA was isolated from bacterial growth according to the protocol. Primer sets used are shown in Table 1.

The detection of *qnrA* and *qnrS*-mediated quinolone-resistance genes was performed using polymerase chain reaction (PCR) and particular primers [Table 2].[2] PCR amplifications were performed in a thermo cycler (Gene Amp PCR System 9700, Applied Bio systems, USA) as follows: 94°C for 5 min and 35 cycles of 5 min at 72°C,

Table 1: Thermal cycle conditions for PCR monoplex technology

Monoplex gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Cycle no.
		Cycling condition	Cycling condition	Cycling condition		
qnrA	94°C/5 min	95°C/45 s	53°C/45 s	72°C/1 min	72°C/7 min	32
qnrS	94°C/5 min	95°C/45 s	53°C/ 45 s	72°C/1 min	72°C/7 min	32

PCR: polymerase chain reaction

Table 2: Primers special of detect virulence genes

Gene	Primer	Sequence (5′-3′)	Size (bp)
*qnr*A	F	ATTTCTCACGCCAGGATTTG	516 bp
	R	GATCGGCAAAGGTTAGGTCA	
*qnr*S	F	GCAAGTTCATTGAACAGGGT	428 bp
	R	TCTAAACCGTCGATTCGGCG	

Table 3: Distributing microorganisms from various sources

Specimen origin	No. specimen	Specimen with growth	Isolates staphylococcus sp.	*S. aureus*
				No (%)
Burns	80	55	45	12 (21.81)
Wounds	40	35	26	20 (57.14)
Sputum	130	95	54	25 (26.31)
Urine infection	181	130	95	21 (16.15)
Total	431	315	220	78

1 min at specific annealing temperature for each primer, and 1 min at 72°C. A final extension phase lasting 7 min at 72°C was completed.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 1987 (including the number and the data on July 6, 2022, to get this approval).

Results

From the 431 clinical samples isolated from different parts of the body, such as burns, wounds, urine, and sputum, 315 (73.08%) showed microorganism growth, and between them, 220 (69.84%) were *Staphylococcus spp*. Based on the results of culture on the various selective media (catalase, coagulase, and Gram staining), 78 (35.4%) of 220 samples showed the morphological and biochemical characteristics of *S. aureus* [Table 3].

In total, 18 (23.77%) and 19 (24.35%) isolates of *S. aureus* bacteria showed sensitivity and intermediate, respectively, to quinolone compounds, whereas 41 (52.56%) isolates showed high-level quinolone resistance.

- From 55 bacterial growth in burns, 21.81% was *S. aureus*
- From 35 bacterial growth in wounds, 20 (57.14%) were *S. aureus*
- From 95 bacterial growth in sputum, 25 (26.31%) were *S. aureus*
- From 130 bacterial growth in urine infection, 21 (16.15%) were *S. aureus*.

Table 4: Antibiotic susceptibility pattern of *S. aureus*

Antibiotics	Resistant	Intermediate	Sensitive
	N (%)	*N* (%)	*N* (%)
Methicillin	71	10	19
Ofloxacin	51	10	39
Erythromycin	60	15	25
Levofloxacin	72	8	20
Augmentin	60	6	34
Rifampin	61	0	39
Moxifloxacin	62	0	38
Norfloxacin	16	0	84
Ciprofloxacin	75	0	15
Chloramphenicol	15	5	80

The study indicated that the common of them were resistant to most antimicrobials. High resistance was experiential greatest frequently for ciprofloxacin with rate of 75%, followed by levofloxacin with a ratio of 72%, and high sensitivity was shown for norfloxacin with rate of 84% and chloramphenicol with an 80% [Table 4].

The PCR analysis showed that among the 41 (52.56%) isolates from *S. aureus* showed that the range of DNA concentration was 50 ng/μL and the DNA purity was 1.8–2.0.

The current study's screening for the *qnr* genes turned up a total of 41 (52.56%). The *qnrA* gene was identified in 10 (24.39%) and *qnrS* in 9 (21.95%) [Figures 1 and 2].

Discussion

The importance of demonstrating this pathogen between clinical isolates has grown as a result of multi-drugs resistance (MDR). The pathological relevance of MDR *S. aureus* has increased the importance of screening for this pathogen among clinical isolates. It is screening to look for and show the significance of *S. aureus* as the typical isolated human bacterial pathogen in both society-based and hospital-based illnesses has been widely recognized.[22,23]

The results of the current study revealed that *S. aureus* was common in burns (21.81%), wounds (57.14%), sputum (26.31%), and the urinary tract (16.15%). According to a changed study, 80% of *S. aureus* infections in hospitals and the general population are endogenous, because they are caused by the colonizing when it arrives the body through any route.[15] It is the most common reason for nosocomial pneumonia and surgical wound infections.[16]

Fluoroquinolones resistance pattern of Quinolones comprises an important and widely utilized class of antimicrobial medications for a variety of microbiological infections in both human and veterinary medicine. Due to widespread clinical use, fluoroquinolone and

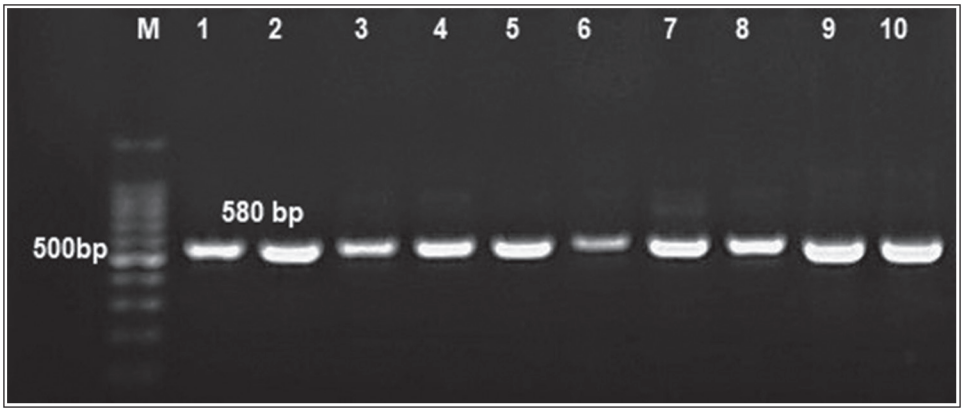

Figure 1: Gel electrophoresis of PCR product of *qnrA* gene (580 bp) positive samples (No. 1–10 *S. aureus*) L = ladder (100–1500). The electric current was allowed at 80 V for 1 h. PCR: polymerase chain reaction

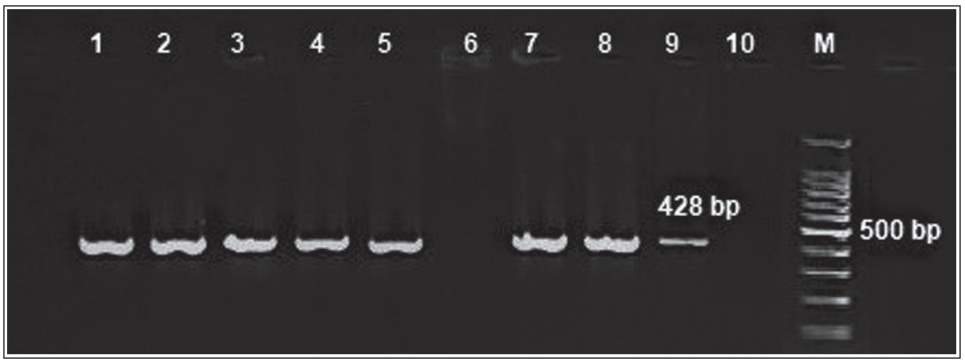

Figure 2: Gel electrophoresis of PCR product of *qnrS* gene (428 bp) the positive samples (No. 1–9 *S. aureus*) L = ladder (100–1500), the 6–10 represents some of the negative samples for this gene, The electric current was allowed at 80 V for 1 h. PCR: polymerase chain reaction

quinolone-resistant bacterial isolates, particularly in coliform bacteria, are quickly developing and spreading.

In the current study, using the disk diffusion method, the susceptibility of 78 samples of *S. aureus* to quinolone drugs was examined in order to determine the pattern of their resistance 41 (52.56%) of 78 bacterial isolates.

The susceptibility test results indicated a distinct change in the cause of the controlled bacterial segregates. Research on the antibiotics utilized indicated that the samples had the top average level of antibiotic resistance to ciprofloxacin, with a ratio of 75%.[17] As 75% of bacteria were resistant to ciprofloxacin, this raised resistance is possibly because of its unequal use and continuous use. Chloramphenicol resistance to *S. aureus* with rate 15% this study is same study,[18] with percentage 15%.[19]

The antibiotic ofloxacin logged a 51% resistance rate against drugs such as fluoroquinolones, methicillin is resistant to isolates with a ratio of 71%, and the resistance to the antibiotics levofloxacin and erythromycin is 72% and 60%, respectively.[24] Moxifloxacin resistance to *S. aureus* in the result 62% this study agree with[25] percentage 64%. The results of the nitrofurantoin antibiotic resistance study are very close to 16% of the study.[23]

The isolates showed a resistance ratio of 61% to the antibiotic rifampin, making it greater than the ratio that researchers in an Iraq study reached.[19] *S. aureus* become resistant to antibiotic due to mutation.[26]

In this study, the PCR technique was used to detect these genes that are resistant to quinolone antigens. The *qnrA* gene was identified in 10 (24.39%) and *qnrS* in 9 (21.95%).

This study showed a high prevalence rate of plasmid-mediated quinolone-resistance *qnrS* compared with other studies.[27-30] Similarly, we found the resistant genes, the *qnrA* and *qnrS* genes, in our clinical isolates. This outcome indicates that the frequency of *qnrS* genes in our study was higher than the frequency of *qnrA* and *qnrS* genes; hence, corroborating earlier studies reporting a higher frequency of *qnrS* among *S. aureus* isolates. This rate of *qnrS* is higher than those found in earlier study previously conducted among clinical isolates in same Bayelsa by Alade *et al*.[27]

Conclusion

Our findings repeatedly showed high levels of fluoroquinolone resistance but high *qnr* genes among *S. aureus* isolates from patients in the Babylon hospitals.

The *qnrA* gene was identified in 10 (24.39%) and *qnrS* in 9 (21.95%).

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Conflicts of interest
There are no conflicts of interest.

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