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Phenotypic and genotypic evaluation of fluoroquinolone resistance in clinical isolates of *Staphylococcus aureus* in Tehran

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Fluoroquinolones are broad-spectrum antibiotics widely used in the treatment of bacterial infections such as *Staphylococcus aureus* isolates. Resistance to these antibiotics is increasing.

Material/Methods: The occurrence of mutations in the *grlA* and *gyrA loci* were evaluated in 69 fluoroquinolone-resistant *S. aureus* isolates from 2 teaching hospitals of Tehran University of Medical Sciences.

Results: Out of the 165 *S. aureus* isolates, 87 (52.7%) were resistant to methicillin and 69 (41.8%) were resistant to fluoroquinolone. Fluoroquinolone-resistant *S. aureus* isolates had a mutation at codon 80 in the *grlA* gene and different mutational combinations in the *gyrA* gene. These mutational combinations included 45 isolates at codons 84 and 86, 23 isolates at codons 84, 86 and 106 and 1 isolate at codons 84, 86 and 90. Fluoroquinolone-resistant *S. aureus* isolates were clustered into 33 PFGE types.

Conclusions: The findings of this study show that the fluoroquinolone-resistant *S. aureus* strains isolated in the teaching hospitals in Tehran had multiple mutations in the QRDRs region of both *grlA* and *gyr*A genes.

key words: **fluoroquinolone resistance • *Staphylococcus aureus* • QRDR**

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Background

Staphylococcus aureus, particularly methicillin-resistant *S. aureus* (MRSA) is of great global concern as it can cause serious infections in both hospitals and the community [1–3]. Increasing resistance to antibiotics among staphylococcal isolates limits the choices of antibiotics available to treat infections caused by these bacteria [4]. Fluoroquinolones are broad-spectrum antibiotics widely used in the treatment of bacterial infections such as gram-positive cocci; however, resistance to these antibiotics has significantly increased world-wide [5,6]. Three different mechanisms of fluoroquinolones resistance have been described in staphylococci. The first is the mutation in the *grlA* and *grlB* genes that encodes the subunits of DNA topoisomerase IV, the second is the mutation in the *gyrA* and *gyrB* genes that encode the subunits of DNA gyrase, and the third is an active efflux pump mediated by mutations in the *norA* gene [5–9]. In most cases, mutations occur in the highly conserved quinolone resistance-determining regions (QRDRs) of the *grlA* and *gyrA* genes [10].

Despite the high incidence of fluoroquinolones resistance among staphylococci, especially among MRSA, there is currently little information available on the incidence and the types of these resistances in many countries. This lack of information is of particular concern in the Persian Gulf region, where the prevalence of MRSA is high [9,11]. The aim of the present study was to provide information regarding the prevalence of fluoroquinolones resistance among *S. aureus* isolates in Tehran, Iran.

Material and Methods

Bacterial strains

A total of 165 *S. aureus* clinical isolates were cultured from patients attending 2 teaching hospitals of Tehran University of Medical Sciences between April 2009 and September 2010 (155 isolates from Imam Khomeini hospital and 10 isolates from Amir Alam hospital). They were cultured from wounds, blood, CSF, body fluid, and urine. Only 1 isolate per patient was included. Isolates were identified to species level using standard biochemical methods including Gram stain, catalase test, tube coagulase, DNase, and fermentation of mannitol [12,13].

Antimicrobial susceptibility test

Antibiotic-containing disks (Mast, UK) were used to determine the susceptibility of *S. aureus* isolates to several antibiotics according to the criteria established by the Clinical and Laboratory Standards Institute (CLSI) [14]. The antibiotics tested were ciprofloxacin, gatifloxacin, levofloxacin, norfloxacin, ofloxacin, and oxacillin. The minimum inhibitory concentrations (MIC) of ciprofloxacin, ofloxacin and oxacillin were determined using the microbroth dilution method as recommended by the CLSI guidelines. *S. aureus* (ATCC 29213) was used as control.

Amplification of *mecA, grlA,* and *gyrA* genes

Chromosomal DNA was extracted from the clinical *S. aureus* isolate as previously described [2]. The *mecA, grlA,* and *gyrA* genes were amplified by PCR-based methods, using specific primers [10,15,16]. PCR reactions were performed in a 50 µL volume consisting of 1X PCR buffer, 3 mM $MgCl_2$, 0.4 µg/mL of each primer, 1.5 U *Taq* DNA polymerase, 0.2 mM dNTP Mix and 5 µL of DNA template. The PCR conditions consisted of a pre-denaturation step at 94ºC for 5 min, followed by 30 cycles of at 94°C for 40 sec, 51°C for 40 sec and 72°C for 45 sec. A final extension step was performed at 72°C for 5 min. To determine the QRDRs sequences, the PCR products of *grlA* and *gyrA* genes were sequenced with both forward and reverse strands at Macrogen (Seoul, South Korea). Sequences were compared with wild-type sequences of *grlA* and *gyrA* genes with no mutations [17].

Pulsed Field Gel Electrophoresis (PFGE)

All fluoroquinolone-resistant *S. aureus* isolates were analyzed by PFGE. The entire genomic DNA was prepared as described previously [15]. After digestion with *Sma* I endonuclease, DNAs were separated by PFGE (APZoha, Tehran, Iran) for 24 h at 15°C, with an electric field of 6 V/cm^3 in 0.5× TBE buffer. The pulse time increased from 1 to 30 sec for 11 h and 1 to 3 sec for 13 h. The gels were stained with ethidium bromide (1 µg/ml) and visualized by UV illumination. DNA from *S. aureus* NCTC8325 was prepared in the same way and run as molecular size standard.

Results

S. aureus strains were isolated from wounds (76), blood (42), respiratory tract (20), joint fluid (15), urine (11) and CSF (1) in this study.

Out of the165 *S. aureus* isolates, 87 (52.7%) and 69 (41.8%) were resistant to methicillin and tested fluoroquinolone antibiotics, respectively. All MRSA isolates contained *mecA* gene and 67 (77%) isolates were resistant to fluoroquinolones. Overall, the oxacillin MIC_{50} and MIC_{90} levels among isolates of MRSA were 128 µg/ml and 512 µg/ml, respectively.

The results of mutations in the QRDRs of the *gyrA* and *grlA* genes and the MIC of ciprofloxacin and ofloxacin among fluoroquinolone-resistant *S. aureus* isolates examined in this study are shown in Table 1. In all the assessed isolates, a mutation in the *grlA* gene was observed at codon 80. Fluoroquinolone-resistant *S. aureus* isolates had different combinations of mutations in the *gyrA* gene. These mutational combinations included 45 isolates at codons 84 and 86, 23 isolates at codons 84, 86 and 106, and 1 isolate at codons 84, 86 and 90.

Among the 69 fluoroquinolone-resistant *S. aureus* isolates, 42 PFGE patterns were generated by digestion with *SmaI*, which clustered into 33 PFGE types. The majority of the isolates (52) were clustered in 16 pulsotypes (Figure 1), and 4 PFGE types displayed subtypes. Seventeen PFGE types contained a single isolate.

Discussion

The spread of antimicrobial resistance among *S. aureus* isolates is a worldwide problem. Since fluoroquinolones, particularly ciprofloxacin and gatifloxacin, are widely used in Iran to treat a variety of infections such as staphylococcal

Table1. Mutations in *grlA* and *gyrA* genes and susceptibilities in fluoroquinolone resistant *Staphylococcus aureus* isolates.

Gene	codon	Mutation	No of isolates	MIC of CIP* (µg/ml)			MIC of OFX (µg/ml)		
				Range	50%	90%	Range	50%	90%
grlA	80	TCC→TTC	69	16–128	16	128	8–32	8	32
gyrA	84 + 86	TCA→TTA + ATT→ATC	45	16–64	16	32	8–32	8	16
	84 + 86 + 90	TCA→TTA + ATT→ATC + ATG→AGG	1	16	16	16	8	8	8
	84 + 86 + 106	TCA→TTA + ATT→ATC + GGC→GAC	23	16–128	32	128	8–32	16	32

* MIC – Minimum Inhibitory Concentration; CIP – ciprofloxacin; OFX – ofloxacin.

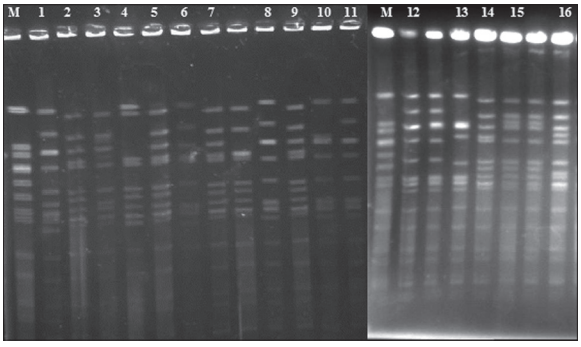

Figure 1. Pulsed-field gel electrophoresis (PFGE) of *SmaI* macro-restriction fragments of fluoroquinolone-resistant *Staphylococcus aureus* isolates. Lanes 1–16: Common PFGE types among tested fluoroquinolone-resistant *S. aureus*. Lane M. *S. aureus* NCTC 8325.

infections [18], fluoroquinolone resistance has posed a serious challenge to the Iranian medical community. This study aimed to characterize the phenotypic and genotypic resistance to fluoroquinolone among *S. aureus* isolates in Tehran, Iran.

The high prevalence rate of MRSA (52.7%) in the present study is alarming and indicates an increase in the prevalence of MRSA isolates in Tehran. The previous prevalence rate reported in Tehran teaching hospitals in 2008 was 36% [15]. This finding highlights the importance of modified empiric therapy and infection control policies among hospitals in Iran.

The results of this study show a fluoroquinolone resistance rate of 41.8% among *S. aureus* isolates, which was higher than the 13% to 20.7% reported by similar investigations [19, 20]. Many studies have shown that 1 or 2 point mutations in the QRDRs region of both *grlA* and *gyr*A genes are the main mechanisms of fluoroquinolone resistance, in particular ciprofloxacin resistance, among *S. aureus* isolates [6,16,21,22]. In agreement with many other studies, the results showed that all the fluoroquinolone-resistant isolates had a single mutation at codon 80 in the *grlA* gene and a mutation at codons 84 in the *gyrA* gene [16,17,23]. Among the 69 tested isolates, 23 had an additional mutation at codon 106 and 1 had a mutation at codon 90 in the *gyrA* gene. The mutation at codon 106 has also been reported in a few studies, but its effect on resistance was reported to be unknown [17,21]. According to our literature review, the point mutation at codon 90 (Tyr to Ser) in the *gyrA* gene has not been reported previously and further studies are needed to determine its effect on fluoroquinolone resistance. In the current study, all the fluoroquinolone-resistant isolates had a mutation at codon 86 in the *gyrA* gene; this point mutation is a silent mutation and has already been reported by others [8,24].

Our results are consistent with those of others who found that the same mutations in the QRDRs were detected in different PFGE types, and different combinations of mutations were also found in the isolates of the same PFGE type [10,16,25].

Conclusions

In conclusion, our findings show that fluoroquinolone-resistant *S. aureus* strains isolated from teaching hospitals in Tehran have multiple mutations in the QRDRs region of both *grlA* and *gyr*A genes.

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