Ciprofloxacin Resistance in *Staphylococcus aureus* and *Pseudomonas aeruginosa* Isolated from Patients in Baghdad

Mohammed F. AL-Marjani

Address: Baghdad /Iraq
Job address: Department of Biology/College of Science/Al- Mustansiriyah University/ Baghdad – Iraq
Email: marjani20012001@yahoo.com
Mobile: 009647901756021

Kadhim A. Kadhim

Address: Baghdad/Iraq
Job address: Department of Clinical Pharmacy/College of Pharmacy/Al- Mustansiriyah University/ Baghdad – Iraq.
Email: kadhimali_2011@yahoo.com
Mobile: 009647700365655

Afraa A. Kadhim

Sumayah Abdul-Hussein Ibraheem
Job address: Department of Biology/College of Science/Al- Mustansiriyah University/Baghdad – Iraq
Address: Baghdad/Iraq

Ahmad AB Yosef Kinani

Job address: Department of pharmaceutics /Baghdad College of pharmacy
Address: Pune 411028 /India
Mobile: 00919503118174

Abstract

Total of 50 isolates of *Pseudomonas aeruginosa* and 50 isolates of *Staphylococcus aureus* were collected to detect the gyrA and qnrs among *S aureus and Ps. aeruginosa* isolates respectively from patients in Baghdad. The susceptibility to different antibiotics was evaluated by disk diffusion method and MICs of ciprofloxacin were determined. Among 50 *Pseudomonas aeruginosa* isolates, resistance was observed most often to Nalidixic acid (54%), followed by Levofloxacin (38%) and Norfloxacin (36%). The resistance patterns of S. aureus were Levofloxacin (20 %) , Norfloxacin (16 %) , Ofloxacin (18 %) ,Ciprofloxacin (16 %), Lomofloxacin (14 %) and Nalidixic acid (50 %). Pseudomonas aeruginosa isolates had MICs between (2-512) µg/ml and S. aureus isolates had MICs between (4-265) µg/ml. All *S. aureus* and *Pseudomonas aeruginosa* isolates were tested for the presence of the gyrA and *qnrS genes* by PCR, 36% of S. aureus and 24% of Pseudomonas aeruginosa isolates were positive respectively.

Key Words: Staphylococcus aureus, Pseudomonas aeruginosa, Ciprofloxacin, Resistance

Introduction

Historically, the development of antimicrobial resistance in *Staphylococcus aureus* has been rapid. Resistance to penicillin in *S. aureus* was noted only a year after its introduction, and, in the early 1950s, three-quarters of S. aureus strains in large hospitals in many countries had become penicillin resistant [1]. *Staphylococcus aureus* isolates resistant to ciprofloxacin were described shortly after introduction of the agent into clinical practice, and presently up to 89% of isolates were resistant to the antimicrobial in some areas of the world [2].

Multidrug-resistant (MDR) strains of *P. aeruginosa* are isolated from patients suffering from nosocomial infections .Thus, the infections are particularly problematic because the organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs. *P. aeruginosa* is the main cause of mortality in cases of polymicrobial bacteraemia, and the second most common bacterium causing sepsis in the ICU.and it implicated in urinary tract infections, burn wounds, ventilator-associated pneumonia and multi-organ system failure[3].

The broad-spectrum fluoroquinolone antimicrobials were introduced in the early 1960's and are today extensively used in human and veterinary medicine [4]. Quinolone resistance was for a long time considered to be entirely mediated by mutations in chromosomal genes encoding quinolone targets (that is, DNA gyrase and topoisomerase IV) and/or in regulatory genes of outer-membrane proteins or efflux pumps .Plasmids carrying

qnr genes have been found to transmit quinolone resistance. These genes encode pentapeptide repeat proteins that block the action of ciprofloxacin on bacterial DNA gyrase and topoisomerase IV [5].

Materials and Methods

Materials

Bacterial isolates

A total of 50 isolates of *Ps.* aeruginosa and 50 *S. aureus* isolates were collected from patients in Baghdad. The isolates were identified by their colony characteristic, gram-stain and confirmed by the pattern of biochemical profiles using Vitec, 2 systems [6].

METHODS

Antibiotic susceptibility testing:

The antimicrobial susceptibility was done by using Kirby-Bauer disc diffusion technique on Mueller Hinton agar (Oxoid, England) following Clinical and Laboratory Standards Institute (CLSI) with commercially available antimicrobial discs. Isolates were tested against the following antimicrobial agents: Levofloxacin, Norfloxacin , Ofloxacin, Ciprofloxacin, Lomofloxacin and Nalidixic acid [7].

1-Minimal inhibitory concentrations (MIC):

The MICs of ciprofloxacin were determined by using Mueller-Hinton agar with antibiotic concentrations (2-512) µg/ml according to the guidelines recommended by the CLSI document.

2-DNA Preparation and PCR:

PCR reactions with specific primers were performed to identify gyrA and qnrA gene of each ciprofloxacin resistant isolate shown in (Table 1).DNA template was prepared as described by Olsvik [9]. (25µl) of PCR amplification mixture contained deionized sterile water (12.5) µl Green Go Taq Master Mix pH (8) (Promega, USA).

Table 1: Sequence of forward and reverse primers used for detecting gyrA and *qnrs* among *S aureus* and *Ps. aeruginosa* isolates respectively.

Primer type	Primer sequence	Product size bp
Forward primer gyrA	3-CCAGATGTTCGTGACGGTT-5	258
Reverse primer gyrA	3-ATTGCTGCTGCGCCATCTCC-5	
Forward primer qnrS	5′-ACGACATTCGTCAACTGCAA-3	417
Reverse primer qnrS	5′-TAAATTGGCACCCTGTAGGC-3	

The protocol for the PCR condition was: 94°C for 45 s, 53°C for 45 s, and 72°C for 60 s, with a cycle number of 32, Gradient PCR (TechNet – 500, USA)**.**

Results

A total of 50 isolates of Ps. aeruginosa and 50 isolates of S. aureus were isolated from different clinical samples from different hospitals in Baghdad.

The resistance patterns of isolates were determined, the S.aureus isolates showed a varied levels of resistance to; Levofloxacin (20 %) , Norfloxacin (16 %) , Ofloxacin (18 %) ,Ciprofloxacin (16 %), Lomofloxacin (14 %) and Nalidixic acid (50 %). The resistance patterns of *P. aeruginosa* isolates were :Levofloxacin (38 %) , Norfloxacin (36 %) , Ofloxacin (32 %) ,Ciprofloxacin (30 %), Lomofloxacin (24 %) and Nalidixic acid (54 %) (Table2). Minimum Inhibitory concentrations (MICs) for Ciprofloxacin were determined, Ps .aeruginosa isolates had MICs between (2-512) µg/ml and S. aureus isolates had MICs between (4-265) µg/ml.

The result of our study is agreed with result obtained by Al-Taei (2012) in Iraq, who found that (9%) of P. aeruginosa isolates were resistant to Ciprofloxacin, showed that clinical isolates of P. aeruginosa were resistant to Ciprofloxacin (31%). MDR nosocomial infections by P. aeruginosa are increasing worldwide. The development of MDR bacteria can be attributed to the extensively use of antibiotics in hospitals and the community.

Table 2: Susceptibility of *S. aureus* and *Ps .aeruginosa* isolates to Quinolones antibiotics.

Antimicrobial Agents	*S.aureus isolates* No. per million	Resistance %	*Ps.aeruginosa* isolates No. per million	Resistance %
Levofloxacin	10	20	19	38
Norfloxacin	8	16	18	36
Ofloxacin	9	18	16	36
Ciprofloxacin	8	16	15	30
Lomofloxacin	7	14	12	24
Nalidixic acid	25	50	27	54

In the USA, a 40% increase in the use of fluoroquinolones led to a increase in the percentage of resistance to ciprofloxacin among Gram-negative bacilli isolated from hospital intensive care units [8].

Throughout the 1990s, S. aureus acquired more resistance to the commonly prescribed antibiotics. The fourth generation fluoroquinolones have *in-vitro* activity similar to that of ciprofloxacin and ofloxacin against gram-negative bacteria but enhanced activity against *S. aureus* (gram-positive bacteria). Levofloxacin has activity against gram-negative bacteria similar to that of ciprofloxacin or ofloxacin and has a greater potency in vitro against gram-positive bacteria.

The problem of bacterial Antibiotic resistance has achieved a global dimension, being one of the leading unresolved problems in public health. The relentless evolution of resistance, in the face of a decrease in the development of new antimicrobial agents active against resistant pathogens, has led to an increasing number of cases in which the pathogen is resistant to most, or even all, drugs available for clinical use.

A total of *S aureus* and *Ps. aeruginosa* isolates were tested for the presence of the gyrA and qnrS genes by PCR, 36% of S aureus and 24% of Ps. aeruginosa isolates respectively were positive as shown in (Fig 1) (Fig 2)

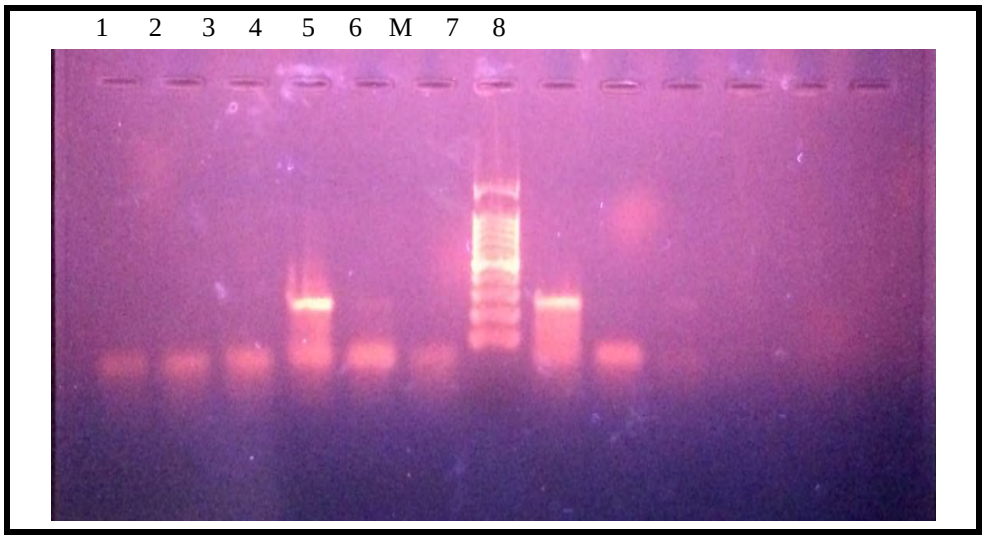

Figure 1: Detection of PCR product DNA bands of gyrA gene in S. aureus isolates, lane (4-7) positive, M=DNA marker.

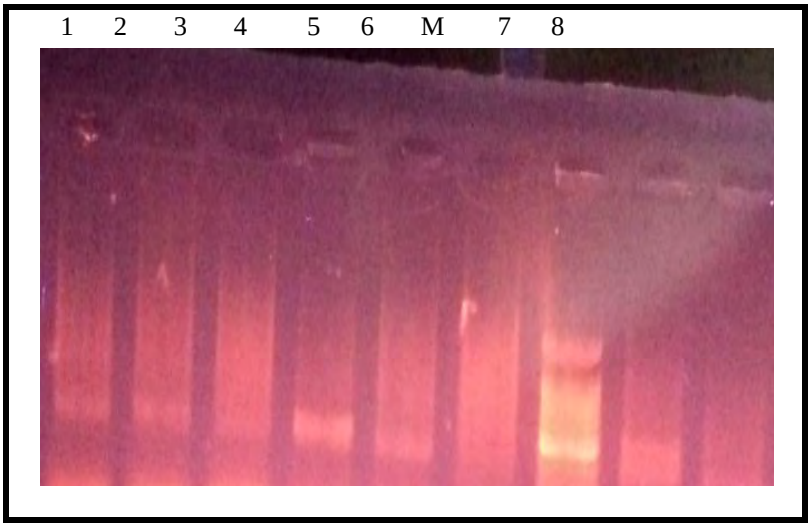

Figure 2: Detection of PCR product DNA bands of qnrS gene in Ps aeruginosa isolates, lane (1-2-4-5) positive, M=DNA marker

Conclusion

In conclusion, we report here the characterization of highly ciprofloxacin-resistance in locally *P. aeruginosa* and *S. aureus* isolates .Thus, qnrS and gyrA gene may have been spreading in those isolates in Baghdad-Iraq.

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