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Original Research Article

Presence of *qnr* gene in Environmental and Clinical *Pseudomonas aeruginosa* isolates in Baghdad

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Keywords

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A B S T R A C T

A total of 78 *Pseudomonas aeruginosa* isolates (40 environmental and 38 clinical) showing a multidrug resistance phenotype including resistance to fluoroquinolones were included in the current study. Ciprofloxacin was the most active drug against *P. aeruginosa* followed by Lomofloxacin. The clinical isolates of *P. aeruginosa* showed higher resistance than Environmental isolates. Minimum Inhibitory concentrations(MICs) for Ciprofloxacin were determined, *P .aeruginosa* clinical isolates had MICs between (4-256) µg/ml, while Environmental isolates had MICs ≤ (64) µg/ml. By polymerase chain reaction amplification and sequencing, A *qnrS* gene was present in 21% of clinical *Pseudomonas aeruginosa* isolates and only one environmental isolate , 13.1 % of clinical isolates with 516 bp amplified product of *qnrA* isolates. The data of the sequencing of PCR products were revealed (99 -100 %) homology with *qnr* genotype .

Introduction

Multidrug - resistant *Pseudomonas aeruginosa* is a serious challenge for antimicrobial therapy of nosocomial infections, it also has many ways of resistance to antibiotics (1).

The broad-spectrum fluoroquinolone antibiotics were introduced in the early 1960's and are today widely used in human and veterinary medicine (2) . In Gram-negative bacteria, 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 (3) . 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 (4).

The *qnrA1 gene consider the* first plasmid-mediated quinolone resistance (PMQR), found in a *K. pneumoniae* strain isolated in 1994. The *qnrB8*-like and *qnrB9*-like alleles were later present in enterobacterial isolates from 1988 (5). The extensive use of

quinolones, together with the mobility at the molecular and cellular level of most quinolone resistance determinants, has contributed to the spread of these genes among almost all clinical enterobacteria across the world , and their incidence seems to have increased in recent years(6).

The distribution of *qnrA* genes in different area is known to be wide , but those of the newer *qnr* types have not been studied (7 , 8) . The aims of this study were to detection of *qnr* gene in Quinolon - resistant *Pseudomonas aeruginosa* isolates (environmental and Clinical) in Baghdad.

Materials and Methods

Bacterial isolates and identification

A total of 78 *Pseudomonas aeruginosa* (38 clinical isolates and 40 environmental isolates), collected between August/2013 to December/2013 in Baghdad, were studied. These isolates were identified by conventional biochemical reactions according to the criteria established by (9).

Antimicrobial susceptibility test

Antimicrobial susceptibility of the isolates were tested by using Kirby-Bauer disk diffusion method following CLSI guidelines (10) , using commercially available 6mm discs (Bioanalyse /Turkey).

The susceptibility of the isolates was determined against 7 antibacterial agents, They include: Ciprofloxacin (Cip), Levofloxacin (Lev), Norfloxacin (Nor), Ofloxacin (Ofx), Lomofloxacin (Lom) , Enorfloxacin (Enr) and Nalidixic acid (NA) on Mueller Hinton agar Plate (Lab M Limited Topley House, United Kingdom), using overnight culture at a 0.5 McFarland standard followed by incubation at 35 ^{o}c for 16 to 18 h.

Minimal Inhibitory Concentrations

The MICs of Ciprofloxacin were determined by a broth dilution method. We used Mueller-Hinton broth (Oxoid, England) with Ciprofloxacin concentrations (2-512) µg/ml according to the guidelines recommended by the CLSI document.

DNA Preparation and PCR

A PCR reactions with specific primers were performed to identify *qnrA* and *qnrS* gene of each ciprofloxacin resistant isolate (Table 1).DNA template was prepared as described by (11) . (25µl) of PCR amplification mixture contained deionized sterile water (12.5)µl Green Go *Taq* Master Mix pH (8) (Promega,USA).

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).

Amplification products were provisionally identified by their size in ethidium bromide-stained agarose gels.

Sequencing of PCR products

DNA sequencing was performed for the identification of the *qnr* .The products were purified with a PCR kit and sequenced with primers by Macrogen (USA). The program (BioEdit Pro.version: 7.0.0) was used for bioinformatic analysis of nucleotide sequences.

Results and Discussion

A total of 78 *Pseudomonas aeruginosa* isolates , (38) clinical isolates were isolated from different samples including urine, blood samples and swaps of otitis media, burns and wounds from different hospitals in

Baghdad, in addition to 40 environmental isolates were isolated from swage and Taps water.

The resistance patterns of *P. aeruginosa* isolates were determined, the clinical isolates showed a varied levels of resistance to; Lomofloxacin with percentage (34.2%); Levofloxacin and Norfloxacin (42.1% for each); Nalidix acid (39.4%). Ciprofloxacin was the most active drug against *P. aeruginosa* (Table 2). With respect to environmental isolates has the highest rate of resistance to Nalidixic acid (27.5%). Minimum Inhibitory concentrations (MICs) for Ciprofloxacin were determined, *P. aeruginosa* clinical isolates had MICs between (4-256) μg/ml, while Environmental isolates had MICs ≤ (64) μg/ml.

The result of our study is agree with result obtained by (13) in Iraq, who found that (31%) of isolates were resistant to Ciprofloxacin. A study done by Al-Taei (14), showed that clinical isolates of *P. aeruginosa* were resistant to Ciprofloxacin (9%), Nalidixic acid (100%), to Levofloxacin (45%).

Fluoroquinolones are the most active antibiotics for oral treatment of *Pseudomonas aeruginosa* infections in most countries. One of the main mechanisms of resistance are mutations in the target genes (encoding DNA gyrase (*gyrA*) and topoisomerase IV (*parC*)) (15) .

In the USA, a 40% increase in the use of fluoroquinolones led to a doubling in the percentage of ciprofloxacin resistance among Gram-negative bacilli isolated from hospital (16) . Multidrug resistant *P. aeruginosa* is found to be resistant to a wide range of antibiotics (17). The development of MDR bacteria can be attributed to the disorderly highly use of antibiotics in hospitals and in the community (18).

The detection of *qnr* genes by Polymerase Chain Reaction showed that 8 (21%) clinical isolates and only one environmental isolate with 417 bp amplified product of *qnrS* , 5 (13.1%) clinical isolates with 516 bp amplified product of *qnrA* (Fig 1).The results of present study were showed that presence of Two genes (*qnr*A and *qnr*S) together were in 2 isolates (5.2%).

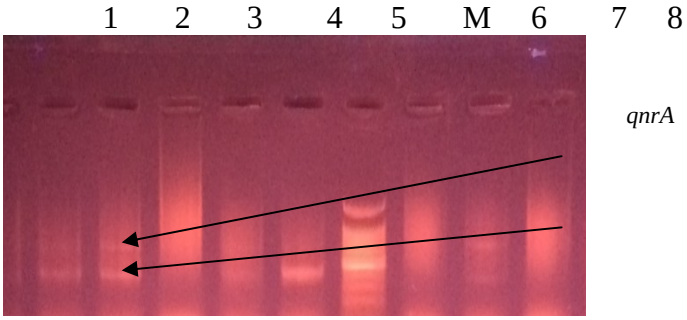
Table.1 Sequence of forward and reverse primers used for detecting *qnrA* and *qnrS* genes among *P. aeruginosa* isolates

Primer type	Primer sequence	Product size	reference
Forward primer *qnrA*	'5-ATTTCTCACGCCAGGATTTG-3'	516-bp	12
Reverse primer *qnrA*	5'-GATCGGCAAAGGTTAGGTCA-3		
Forward primer *qnrS*	5′-ACGACATTCGTCAACTGCAA-3	417-bp	12
Reverse primer *qnrS*	5′-TAAATTGGCACCCTGTAGGC		

Table.2 Susceptibility of *P .aeruginosa* isolates to Quinolones antibiotics

Antimicrobial Agents	Clinical isolates		environmental isolates	
	No.	Resistance %	No.	Resistance %
Ciprofloxacin	11	28.9	5	12.5
Levofloxacin	16	42.1	7	17.5
Norfloxacin	16	42.1	5	12.5
Ofloxacin	14	36.8	7	17.5
Lomofloxacin	13	34.2	6	15
Nalidixic acid	15	39.4	11	27.5
Enorfloxacin	14	36.8	7	17.5

Figure.1 Agarose gel electrophoresis of products from multiplex PCR, M=DNA ladder , lane 1-8 (*qnr*A and *qnrS* in *P . aeruginosa* clinical isolate)



The data of the sequencing of PCR products were revealed (99 -100 %) homology with *qnr* genotype. the wide geographical and species distribution of *qnr* genes proposes that they have been in existence for some time. The *qnrA* gene is thought to have originated in *Shewanella* algae, isolated from marine and fresh water (19). In addition, *qnrVS (1 and 2)*, from *Vibrio splendidus*, consider reservoir of *qnrS* genes. Taken together, this evidence suggests that Gram-negative aquatic bacteria may serve as the reservoir of *qnr* genes (18).

In conclusion, these results indicated that 21% of the tested clinical *Ps. aeruginosa* isolates harboured *qnrS* genes, further studies for another *qnr* genes with large numbers of isolates are needed to study the emergence these genes in *Ps. aeruginosa* in Baghdad.

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