# Molecular detection of antimicrobial resistance genes among ciprofloxacin-resistant *Pseudomonas aeruginosa* isolates

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**Abstract.** *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic pathogen that is considered one of the most important causes of nosocomial infections, especially in burns and immunocompromised individuals. So this study was aimed to detection of quinolone-resistant genes among ciprofloxacin-resistant *Pseudomonas aeruginosa* isolates. Data showed out of 168 specimens obtained from burns patients the rate women and men in this study were 107(63..69%) and 61(36.3%) respectively, positive bacterial growth were 159 (94.64 %) while 9(5.3%) of specimens were no growth. According to result of the vitek-2 system recorded 75 isolates as *P. aeruginosa*. Results of ciprofloxacin susceptibility recorded 29(38.67%) of *P.auroginosa* was resistance to ciprofloxacin , while was 34(45.33%), and 12(16%) of isolates were intermediate and sensitive respectively. Results of antibiotic susceptibility showed that the highest bacterial resistance was imipenem 29(100%) , while the least resistance were meropenem and Piperacillin-Tazobactam reached 22(75.8%). Results of Polymerase chain reaction (PCR) showed that 29(100%), 28 (96.06%), 26(89.65%), 23(79.31%) and 21(72.41%) of ciprofloxacin-resistant Pseudomonas aeruginosa isolates were harbored aac(6')-Ib, parC, qnrS, qnrB and qnrVC respectively, while qnrA, qnrC, qnrD, and qepA genes were not detect in present study. Sequence results for qnrB, qnrvc showed that they are identical to qnrB2, qnrvc1 when compared with international NCBI isolates. **Keywords:** Molecular, antimicrobial resistance, ciprofloxacin-resistant, *Pseudomonas aeruginosa*

## 1 Introduction

*Pseudomonas aeruginosa* is an important Gram-negative opportunistic pathogen which causes many severe acute and chronic infections with high morbidity, and mortality rates as high as 40%[1] *Pseudomonas aeruginosa* became a significant cause in wound and burn infections as well as severe mortality in burn patients [2]. The burn infections commonly caused by *P. aeruginosa* which colonized by the patient's microflora or from the environment in burn wounds [ 3 ].This bacterium has shown high resistance to a wide range of antibiotics, including beta-lactams, aminoglycoside , monobactam . Fluoroquinolones are widely used in the treatment of burn infections. Fluoroquinolones

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inhibit DNA replication by inhibiting gyrase and preventing bacterial growth [ 4] *peudomonas aeruginosa* has the ability to form biofilmswhich consist of aggregates of bacterial cells that adhere to one another and/or a surface and embed within an extracellular polymeric substance (EPS) such as DNA, polysaccharides, and proteins make up EPS [5]. Major contributors to ciprofloxacin resistance in *P. aeruginosa* are mutations in the ciprofloxacin target-encoding genes *gyrAB* and *parCE* that decrease the affinity of DNA gyrase or topoisomerase for ciprofloxacin [6].

## 2 Material and Method

The present study involved in the burns unit center as well as some chief clinical laboratories in Al-Najaf City. 168 clinical specimens get from non-duplicate patients suffering burn infection were randomly collected and checked to recognize P. aeruginosa isolates. All isolates of *P. aeruginosa* were identified by Gram staining some biochemical tests, oxidase test, and culture characterization [7],and finally comfirm by Vitek-2 compact system.

### 2.1 Detection of Ciprofloxacin resistance among *p.aeruginosa* isolates and antimicrobial susceptibility testing

Firstly antibiotic susceptibility testing of P. aeruginosa was performed according to the Kirby-Bauer method using disk diffusion[8] ,against ciprofloxacin antibiotic .then only ciprofloxacin resistance p. aeruginosa were done antibiotic susceptibility for 16 different drugs Piperacillin-Tazobactam 100/10mg,Ticarcillin-Clavulanic acid 75/10 mg,Piperacillin 100 mg , Cefepime 30 mg, Ceftazidime 30 mg, Imipenem 10mg, Meropenem 10mg , Ofloxacin 5mg , Levofloxacin 5mg, Norfloxacin 10mg, Gemifloxacin 5mg , Aztreonam 30mg , Netilmicin (30 mg), Tobramycin (30 mg), Amikacin (30 mg) and Gentamicin (10 mg). The zone diameter were applied base on instructions of the Clinical and Laboratory Standards Institute [9].

### 2.2 DNA extraction and PCR assay

The DNA extraction micro kit (Favorgen, South Korea) was used to collect all of the nucleic acid for 29 clinical isolates of *p*.aeruginosa. This was done in accordance with the manufacturer's protocol. After ensuring the integrity of the whole DNA sample by storing it in a deep freezer set to -20 degrees Celsius, a PCR analysis was carried out in order to test for the genes listed in Table 1. The equipment for gel documentation was employed for the migration of PCR amplification at 1% agarose, which were dyed with ethidium bromide at a concentration of 0.5 g/ml [10].

**Table 1.** Primer Sequence and condition of thermocycles

| Primer | Sequence (5' to 3') | Annealing (°C) | Product (bp) | Reference |
|---|---|---|---|---|
| qnrA-F | GATAAAGTTTTTCAGCAAGAGG | 57 | 593 | [22] |
| qnrA-R | ATCCAGATCCGCAAAGGTTA | | | |
| *qnrB-F* | ATGACGCCATTACTGTATAA | 53 | 560 | [23] |
| *qnrB-R* | GATCGCAATGTGTGAAGTTT | | | |
| *qnrC-F* | GGGTTGTACATTTATTGAATC | 50 | 447 | [24] |
| *qnrC-R* | TCCACTTTACGAGGTTCT | | | |
| *qnrS-F* | ATGGAAACCTACAATCATAC | 55 | 492 | [25] |
| *qnrS-R* | AAAAACACCTCGACTTAAGT | | | |
| qnrD-F | TATTCCCCGTAAATTGATCTCG | 53 | 2200 | [26]. |

| | | | | |
|---|---|---|---|---|
| qnrD-R | CAGGCGCTTCAGCTTGTT | | | |
| qnrVC-F | GGGTGYGATTTTTCTTAYKCKGATCTT | 58 | 447 | [27] |
| qnrVC-R | CTGYTGCCACGARCAKATTTTTACACC | | | |
| ParC-F | CTGAATGCCAGCGCCAAATT | 58 | 389 | [28] |
| ParC-R | TGCGGTGGAATATCGGTCGC | | | |
| aac(6')-Ib -cr-F | TTGCGATGCTCTATGAGTGGCTA | 55 | 482 | [29] |
| aac(6')-Ib -cr-R | CTCGAATGCCTGGCGTGTTT | | | |
| *qepA-F* | GCAGGTCCAGCAGCGGGTAG | 60 | 217 | [30] |
| *qepA-R* | CTTCCTGCCCGAGTATCGTG | | | |

### 2.3.Sequence analysis of qnrS and qnrVC genes

The positive band of PCR amplification for qnrS and qnrVC genes were sent to company of Macrogen (Korea) to analyzed the sequence of nucleotides. The data of sequence were compared with the results in the GenBank database through using BLAST at the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov/). as well as ExPASy translate tool was applied to abtained sequence of amino acids for these genes. Forward DNA strands of target gene was sequenced according to procedure of Sanger method (the dideoxy chain termination).

## 3 Results

Results of this study showed among 168 burn patient were 132(78.5%) and 27 (16.0%) of positive bacterial growth return to gram-negative and gram-positive bacteria respectively, while 9(5.3%) of specimens were no growth , (table, 2). At same respect, according to culture characteristics, some biochemical tests and finally using Vititec-2 system, the results according total rate of *P. aeruginosa* isolates were 75(44.64%) At same as following 34 (20.24) in men compared with 41 (24.40)in women (table, 3).

Burns are among the commonest types of trauma worldwide and burn injury is one of the top leading causes of disease burden in many countries of the world [11] .A study by [12].pointed that admitted to the Burns and Plastic surgery Hospital in Sulaimani–Kurdistan region of Iraq in 2009 as following 338 (38.2%) were male and 546 (61.8%) were female.

**Table 2.** Results of culture media of 68 specimens obtained from burn patients

| **Status** | **Number** | **Percentage %** | **Total Bacterial growth** |
|---|---|---|---|
| **Gram negative** | 132 | 78.5 | 159(94.64) |
| **Gram positive** | 27 | 16.0 | |
| **Non growth** | 9 | 5.3% | |
| **Total patients** | 168 (100%) | | |

**Table 3.** Spreading of *P. aeruginosa* in bacterial growth specimens according the Sex

| **Sex** | **Number (%) of specimens growth** | ***P. aeruginosa*** |
|---|---|---|
| **Men** | 61(36.31%) | 34 (20.24%) |
| **Women** | 107(63.69%) | 41 (24.40%) |
| **Total** | 168(100%) | 75(44.64%) |

### 3.1 Ciprofloxacin Susceptibility among *P. aeruginosa* isolates

Results showed that among 75 (100%) *P. aeruginosa* isolates using disk diffusion According to Kaurby-baur methods for ciprofloxacin antibiotic revealed that only 29(38.67%), of isolates were resistant to ciprofloxacin, while 34(45.33%), and 12(16%) of isolates were intermediate and sensitive to this antibiotic respectively (table 4). A local done by Alkhulaifi[13]. they recorded that P. aeruginosa isolates were resistance to ciprofloxacin antibiotics at rate 71.6%. While another in Baghdad by AL-Fridawy [14 ].pointed the resistance of *P. aeruginosa* isolates to ciprofloxacin antibiotics was 60%.Ciprofloxacin resistance can arise through the acquisition of mutations in genes encoding the target proteins of ciprofloxacin and regulators of efflux pumps, which leads to overexpression of these pumps [15].

**Table 4.** Ciprofloxacin susceptibility of *P. aeruginosa* isolates

| ***Pseudomonas aeruginosa* isolates** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Total** | | **Resistance** | | **Intermediate** | | **Sensitive** | |
| **75(100%)** | | 29(38.67%) | | 34(45.33%) | | 12(16%) | |
| **Male** | Female | Male | Female | Male | Female | Male | Female |
| **34 (45.33%)** | 41 (54.66%) | 13 (17.33%) | 16 (21.33%) | 16 (21.33%) | 18 (24%) | 5 (6.66%) | 7(9.33%) |

### 3.2 Antibiotic Susceptibility of ciprofloxacin-resistant *P. aeruginosa* isolates

The results in table 5 revealed 22(75.8%) of ciprofloxacin-resistance *P. aeruginosa* isolates were resistance to Piperacillin-tazobactam drug while 27(93.1%) of isolates were resistance to ticarcilin-cluvlanic acid, cefepime, amikacin and ofloxacin. This pathogens showed rate of resistance reached 28(96.5%) against ceftazidime , aztreonam levofloxacin and norfloxacin while imipenem and meropenem antibiotics were 29(100%), and 22(75.8%) respectively. However, 23(79.3%) of isolates were resistance to both gentamicin and tobramycin drugs . while 25(86.2) of isolates were resistance to netilmicin. Gatifloxacin recorded resistance reached 26(89.6%).

A local study achieved in baghad by AL-Fridawy[14]. they pointed the resistance of *P. aeruginosa* isolates to different classes of antibiotics were as following, The rate of resistance for ticarcillin-clavulanic acid and piperacillin-tazobactam, meropenem, cefepime, amikacin and gentamicin were 70%, while ceftazidime and imipenem were 40%, 50% respectively.

A work achieved by Vo [16]. they pointed the resistance of P. aeruginosa isolates to beta-lactam antibiotics were as following, piperacillin was 28.8%, ticarcillin-clavulanic acid was 39.6%, piperacillin-tazobactam was 25.5%, while ceftazidime , cefepime,

imipenem and meropenem were 27.3%, 27.4%, 31.7% and 30.2% respectively, while tobramycin, gentamicin and levofloxacin were 24.1%, 25% and 32.4% respectively.

**Table 5.** Antibacterial agent susceptibility of ciprofloxacin-resistant *P.auroginosa* isolates.

| Antibacterial agents | Resistance (%) | Intermediate (%) | Sensitive (%) |
|---|---|---|---|
| Piperacillin-Tazobactam 100/10mg | 22(75.8) | 4(13.7) | 3(10.3) |
| Ticarcillin-Clavulanic acid75/10 mg | 27(93.1) | 1(3.4) | 1(3.4) |
| Piperacillin 100 mg | 28(96.5) | 0(0%) | 1(3.4) |
| Cefepime 30 mg | 27(93.1) | 0(0%) | 2(6.8) |
| Ceftazidime 30 mg | 28(96.5) | 0(0%) | 1(3.4) |
| Imipenem 10mg | 29(100) | 0(0%) | 0(0%) |
| Meropenem 10mg | 22(75.8) | 4(13.7) | 3(10.3) |
| Ofloxacin 5mg | 27(93.1) | 0(0%) | 2(6.8) |
| Levofloxacin 5mg | 28(96.5) | 0(0%) | 1(3.4) |
| Norfloxacin 10mg | 28(96.5) | 0(0%) | 1(3.4) |
| Gemifloxacin 5mg | 26(89.6) | 1(3.4) | 2(6.8) |
| Aztreonam 30mg | 28(96.5) | 1(3.4) | 0(0%) |
| Netilmicin 30mg | 25(86.2) | 0(0%) | 4(13.7) |
| Tobramycin 30 mg | 23(79.3) | 6(20.6) | 0(0%) |
| Amikacin 30mg | 27(93.1) | 1(3.4) | 1(3.4) |
| Gentamicin 10 mg | 23(79.3) | 0(0%) | 6(20.6) |

### 3.3 Molecular detection quinolones-resistance genes among ciprofloxacin-resistant *Pseudomonas aeruginosa* isolates

Results of PCR about quinolones-resistance genes showed that 29(100%), 28 (96.06%) , 26(89.65%), 23(79.31%) and 21(72.41%) of ciprofloxacin-resistant *Pseudomonas aeruginosa* isolates were harbored *aac(6')-Ib*, qnrS, parC, qnrB and qnrVC respectively (figure 1,2,3,and 4). At same respect , *qnrA, qnrC, qnrD,* and *qepA* genes were not detect in present study. Results of amino acid sequence for qnrB, qnrvc showed that they are identical to qnrB2, qnrvc1 when compared with international NCBI isolates (fig. 5 and 6).

The first reported PMQR gene reducing susceptibility to ciprofloxacin in Gram-negatives was qnrA in Klebsiella pneumoniae. Subsequently, several classes of qnr genes (qnrB, qnrC, qnrD, qnrS and qnrVC) were identified that reduce susceptibility to fluoroquinolones.7,9 Qnr proteins bind to DNA gyrase and topoisomerase IV and protect the enzymes from inhibition by quinolones [17].

Globally some studies pointed that qnrVC found in P. aeruginosa , However, observed that *qnrVC* gene is strongly associated with type 1 integrons has been reported mainly in strains of clinical origin and belonging to *Vibrio* species [18]Another study done by Belotti [19 .]recorded that qnrVC1 gene among isolates of in P. aeruginosa.

A study in Tabriz, Iran done by Nouri , [20]. they revealed that the mutations in *gyrA* and *parC* were the main mechanism of fluoroquinolone resistance among the clinical isolates of *P. aeruginosa* .

In a recent study done by Venkataramana [21]. recorded that 12 (14.1%) of P. aeruginosa isolates harbored qnrB, 12 (14.1%) qnrS, 9 (10.5%) both qnrB and qnrS, and 66 (77.6%) contained aac(6′)-Ib-cr,. while, qepA gene was not detected in any of the study isolates.

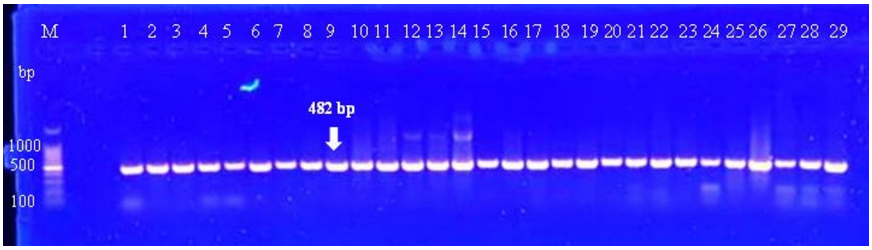


**Fig. 1.** PCR products of *aac(6')-Ib* gene for ciprofloxacin-resistant *P. aeruginosa* isolates

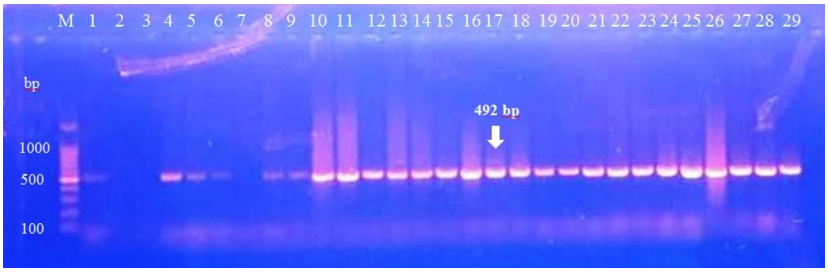


**Fig. 2.** PCR products of qenrS gene for ciprofloxacin-resistant *P. aeruginosa* isolates

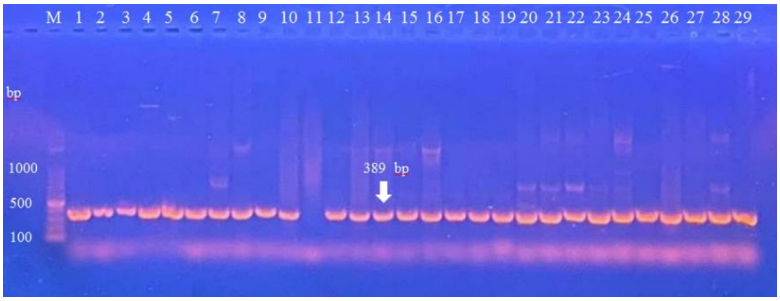


**Fig. 3.** PCR products for Par C gene of ciprofloxacin-resistant *P. aeruginosa* isolates

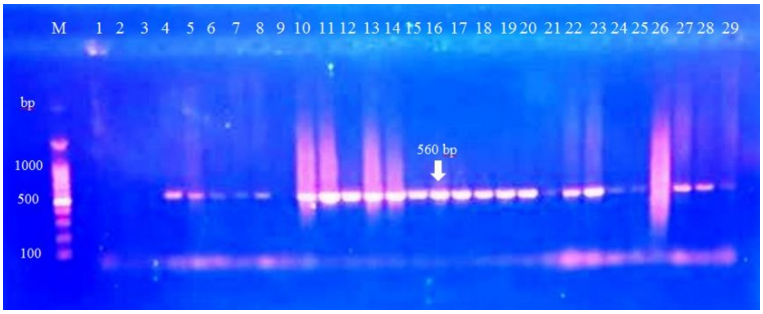


**Fig. 4.** PCR products for *qnrB* gene of ciprofloxacin-resistant *P. aeruginosa* isolates

| Score | Expect | Method | Identities | Positives | Gaps |
|---|---|---|---|---|---|
| 264 bits(674) | 6e-89 | Compositional matrix adjust. | 128/128(100%) | 128/128(100%) | 0/128(0%) |

```
Query  8    KINRNRFTGEKIENSTFFNCDFSGADLSGTEFIGCQFYDRESQKGCNFSRAMLKDAIFKS  67
            KINRNRFTGEKIENSTFFNCDFSGADLSGTEFIGCQFYDRESQKGCNFSRAMLKDAIFKS
Sbjct  9    KINRNRFTGEKIENSTFFNCDFSGADLSGTEFIGCQFYDRESQKGCNFSRAMLKDAIFKS  68

Query  68   CDLSMADFRNASALGIEIRHCRAQGADFRGASFMNMITTRTWFCSAYITNTNLSYANFSK  127
            CDLSMADFRNASALGIEIRHCRAQGADFRGASFMNMITTRTWFCSAYITNTNLSYANFSK
Sbjct  69   CDLSMADFRNASALGIEIRHCRAQGADFRGASFMNMITTRTWFCSAYITNTNLSYANFSK  128

Query  128  VVLEKCEL  135
            VVLEKCEL
Sbjct  129  VVLEKCEL  136
```

**Fig. 5**. Alignment for amino acids sequence of *qnrB* gene of ciprofloxacin-resistant *P. aeruginosa* isolates no.19 with MULTISPECIES: quinolone resistance pentapeptide repeat protein QnrB2 [Gammaproteobacteria] Sequence ID: WP_012695489.1

| Score | Expect | Method | Identities | Positives | Gaps |
|---|---|---|---|---|---|
| 218 bits(556) | 2e-71 | Compositional matrix adjust. | 104/105(99%) | 105/105(100%) | 0/105(0%) |

```
Query  7    GIELRDCDLKGANFSQVSFVNQVSNKMYFCSAYITGCNLSYANFEQQLIEKCDLFENRWI  66
            GIELRDCDLKGANFSQVSFVNQVSNKMYFCSAYITGCNLSYANFEQQLIEKCDLFENRWI
Sbjct  86   GIELRDCDLKGANFSQVSFVNQVSNKMYFCSAYITGCNLSYANFEQQLIEKCDLFENRWI  145

Query  67   GANLRGASFKESDLSRGLFSEDCWEQFRVQGCDLSHSELYGLDPR  111
            GANLRGASFKESDLSRG+FSEDCWEQFRVQGCDLSHSELYGLDPR
Sbjct  146  GANLRGASFKESDLSRGVFSEDCWEQFRVQGCDLSHSELYGLDPR  190
```

**Fig.6.** Alignment for amino acids sequence t of qnrVC gene of ciprofloxacin-resistant *P. aeruginosa* isolates no. 3 with MULTISPECIES: quinolone resistance pentapeptide repeat protein QnrVC1 [Gammaproteobacteria] Sequence ID: WP_000415714.1Length: 218

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